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ENHANCED MEMBRANE BIOREACTOR (MBR) OPERATION BY OPTIMISATION OF BACTERIAL POPULATION

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ENHANCED MEMBRANE BIOREACTOR (MBR) OPERATION BY OPTIMISATION OF BACTERIAL POPULATION

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This is a journey of a girl……

She is the girl who has many dreams. Some of dreams are just like unrealistic stories never occurred in her life, but others really happened, for example, pursuing Ph.D in Singapore.

Back to the autumn of 2002, she met Professor Anthony Gorden Fane and Associate Professor Stephen Tay Tiong Lee, who brought her into the membrane science and environmental biotechnology world, completely new fields for her. She was very fortunate to be a student of Professor Fane, whose guidance, help and support give her encouragement, hope and confidence to finish her study. When she felt satisfied with her work, Tony always gave his advice to push her ahead; when she felt at a loss, Tony often said to her, "I know the difficulties in the project; your work is well done." The way is just like father's. Dr. Tay's pursuit of perfection and continuous demand for scientific excellence was a major driving force for her. She still remembers the words Dr. Tay used to say, "Good plan is the half success; you should believe in yourself; what you did is good." She hopes Dr. Tay can read this thesis, who now lives in the heaven. God bless him for ever. She also would like to extend her gratitude to Associate Professor Darren Sun, who gave her help in the last stage of her work.

No matter what happened, friendship is a strong power to break down the difficulties and hold every hope. In these three years, the girl makes friends with Yi Shan and Zhuang Weiqin, who always advice her in her living and studying as well as give her a big hand when she is in difficulties. Someone maybe just stay with you a short time, but a deep memory will always stay in your minds. The girl is grateful to Dr. Wong Fook-Sin, Dr. Li Hongyu, Dr. Ken Chiang, Dr. Geng Anli, Dr. Chua Hwee Chuan, Dr. Maszenan Bin Abdul Majid, Dr. Peng Zanguo, Dr. Filicia Wicaksana, Dr. Chan Mya Tun, Dr. Jia Yue, Ms Jodie Chin Sze Sze, Mr Ng Choon

Aun, Mr Chong Tzyy Haur, Mr Wei Xi, Dr. Zhang Jinsong, Mr Zhang Yanpeng, Mr Adrian Yeo Piah Song, Mr Zhang Zhenpeng, Ms Shen Qin Ping, Ms Zhang Yi, Ms Zeng Ping with their assistance, care and encouragement in her study. She is also happy to meet these youths, Mr. Yang Zhan, Ms Rachel Tham Chin Xin, Mr Chua Han Wei, Mr Ng Wei Ren, Mr Chua Boon Qing, Mr Chiew Zhi Ming and Mr Lau Kim Hui, who made their exceptional effort during their final year projects and their contributions in her research. She thinks she is fortunate because she gets many helps from staffs in Institute of Environmental Science and Engineering (IESE) and technicians in CEE of NTU when she faces to trouble in her research work.

There is a straightforward procedure and guideline of finding out the truth in scientific research, but living is another case. You need more patience to deal with these complex situations, the girl told to herself. Fortunately, her parents, a warmhearted and virtuous couple not only provide happy and beatific living for her, but also always encourage and support her with their selfless love, especially in these years of her pursuing Ph.D. Regardless of the thousand miles between them, she was always in their hearts and minds. The girl also appreciates to a boy and cherishes all the happiness as well as the misunderstandings with him. Perhaps this trip of growth is not easy for them, but it is an inevitable step in their lives to become more mature.

The girl has to say farewell to these times full of smiles and tears, which never come back to her. Although it is a tough road, she feels so satisfied that she is learning how to build up a bridge above the river full of difficulties. This is the end, also a new start for her. This girl is me.

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SUMMARY

This research program aimed to identify the conditions in a MBR that would help to reduce membrane fouling. A secondary objective was to identify specialized biomass which were membrane friendly under typical MBR operating conditions. Initially, a lab-scale submerged MBR was operated under periodic and sequential changes of operating parameters, such as substrate loading, sludge retention time (SRT), aeration rate and hydraulic retention time (HRT). Significant and rapid increases in EPS production and protein production occurred at the beginning of changes in operating parameters. Increasing F/M ratios, decreasing SRT as well as shortening HRTs resulted in an increasing trend in EPS production. Increases in aeration rate over the range studied insignificantly influenced EPS production.

Parallel MBR systems were set up and operated for a long period to investigate the effect of different substrate loadings (Phase 1), substrate compositions (Phase 2) and SRTs (Phase 3) on EPS production, bacterial community structure and membrane fouling tendency. Under steady state conditions, an increase in substrate loading resulted in a decreasing trend in EPS production. Increasing the nitrogen concentration in the substrate caused less EPS production, while increasing the phosphorus amount had a negligible effect on EPS production. When the SRT was extended from 10 days, to 30 days to infinity, a significant drop in EPS production was observed. Similarity index analysis of the bacterial community structures in denaturing gradient gel electrophoresis (DGGE) profiles showed the EPS production was related with the population shifts under the three phases. At the beginning of operation, the higher similarity index and increase in EPS production suggests that as the bacteria acclimatised to the new living environment they experienced metabolic stress causing the production of more EPS. Furthermore, constant bacterial diversity and EPS production were observed when the reactor performance stabilized. In this study, it was found that membrane fouling tendencies were associated with EPS characteristics, which correlates with previous studies that EPS is a major foulant in MBRs.

Dissolved solids played an important role in the membrane fouling in the lab-scale and pilot-plant MBRs considering specific resistance of EPS loading. Especially, after removing colloids with greater molecular weight, the membrane fouling tendency of the dissolved solids increased due to decline in the particle sizes. Molecular weight distribution analysis showed that components whose molecular weights were close to the nominal membrane pore cut-off influenced membrane fouling tendency significantly, possibly caused by the components accumulating in the pores leading to restriction and plugging.

Further, 16S rRNA DGGE was used to provide information in the bacterial community structure in order to compare bacterial community structures on the membrane surface and in the mixed liquor. Pure cultures were obtained by traditional isolation methods and the functions of isolated bacteria were evaluated by investigating EPS characteristics and membrane fouling tendency. The 'membrane-fouling' bacteria and 'membrane-friendly' bacteria were selected based on their membrane fouling tendencies. The results revealed that in pure culture systems, membrane fouling tendency is more associated with cell characteristics (surface charge, hydrophobicity and particle size) rather than EPS characteristics.

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NOMENCLATURE

BSA	Bovine serum albumin
BOD	Biochemical oxygen demand
CA	Cellulose Acetate
COD	Chemical oxygen demand
CLMS	Confocal light microscope
DGGE	Denaturing gradient gel electrophoresis
DLVO	Derjaugin, Landau, Verwey and Overbeek
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
EPS	Extracellular polymeric substances
F/M	Food/microorganism, day^{-1}
FISH	Fluorescence in situ hybridization
HPSEC	High performance size exclusion chromatography
HRT	Hydraulic residence time
ICP	Inductively coupled plasma
J	Permeate flux, L/m^2 hr
MBR	Membrane bioreactor
MWCO	Molecular weight cut off
OD	Optical density
PAC	Powdered activated carbon
PAN	Polyacrlonitrile
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PES	Polyethersulphone
PHB	Polyhydroxybutyrate
PP	Polypropylene
PVDF	Polyvinylidene Fluoride
rDNA	Ribosomal deoxyribonucleic acid
RNA	Ribonucleic acid
$\mathbf{R_m}$	Clean membrane resistance, m^{-1}
$\mathbf{R_t}$	Total membrane resistance, m^{-1}
$\mathbf{R_c}$	Cake fouling resistance, m^{-1}
$\mathbf{R_i}$	Irreversible resistance, m^{-1}
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SRT	Sludge retention time
SS	Suspended solids
SVI	Sludge volume index
TGGE	Temperature gradient gel electrophoresis
TMP	Transmembrane pressure, kPa
TOC	Total organic carbon, mg/L
TSS	Total suspended solids, mg/L
UF	Ultrafiltration
$\mathbf{V_L}$	Lift velocity, m/s
μ	Permeate viscosity, Pa S

CHAPTER 1

INTRODUCTION

1.1 Background

The activated sludge process has been developed and applied in the treatment of municipal and industrial wastewater since 1914. The well-known conventional activated sludge process contains two main compartments: aeration tank and settling tank. In the aeration tank, the activated sludge mixture is used to convert organic/inorganic substances in wastewater into carbon dioxide. In the other compartment, activated sludge can be separated from the liquid phase through sedimentation of the activated sludge. A part of sludge is removed from the settling tank and most of sludge is recycled to the aeration tank. Due to high concentration of biomass in the aeration tank and relatively slow process of gravitational sedimentation in the settling tank, the conventional activated sludge process requires a large basin.

Membrane filtration was initially employed as tertiary treatment or as a refining process in the activated sludge system for separating solid from liquid (Metcalf & Eddy, 1991). With the development of less expensive and more reliable membrane modules, the concept of membrane bioreactors (MBRs) was adopted. MBRs involve a combination of the activated sludge process and the membrane filtration process which replaces the settling tank in the conventional activated sludge process. As an efficient technology for wastewater treatment and reuse, MBRs have recently been applied in many municipal and industrial wastewater treatment plants (Cicek et al., 2001). With respect to the location of the membrane unit, there are two main configurations: submerged MBRs and side-stream MBRs, in which the membrane unit is mounted directly within the bioreactor and placed external to the bioreactor, respectively (Cicek et al., 1999; Gander et al., 2000). With the innovation of the membrane modules, more hybrid MBR systems which can meet different requirements of wastewater treatment have been implemented.

The widespread application of MBRs is derived from their advantages in treating wastewater, which can possibly overcome the current problems associated with the conventional activated sludge processes (Resenberger and Kraume, 2002). These include not only complete decoupling of sludge retention time (SRT) and hydraulic retention time (HRT), but also effective treatment of the influent with reduced sludge production in a limited space. These features ultimately benefit the operational reliability and efficiency of MBRs. However, the universal appeal of MBR technology is limited by membrane fouling, because it reduces productivity and increases operating and energy costs of the systems.

In general, membrane fouling is attributed to the larger soluble molecules plugging and narrowing the pores of membranes or the particles depositing on the membrane surfaces to form a cake layer. Therefore, unfavorable hydrodynamic conditions can lead to concentration polarization and unfavorable solute-membrane interactions will happen along the membrane surfaces. These can lead to flux decline, rejection changes and feed pressure increase (Fane, 2002). Once severe membrane fouling has occurred in MBRs, it results in an increase in hydraulic resistance and waste of energy, and the basic operation can not be maintained. Recently, various techniques have been employed to control membrane fouling, such as modifying membrane materials, optimizing module design, controlling filtration flux, improving aeration rate, chelating with chemical reagents and periodic permeate backpulsing, gas backpulsing, crossflushing and chemical cleaning. Although these approaches appear to ameliorate membrane fouling to some extent, productivity is impaired and additional cost is required.

In MBRs, extracellular polymeric substances (EPS) are considered as the major foulant responsible for the membrane fouling. Normally, their common components are neutral and acidic polysaccharides, proteins with a small amount of nucleic acids and lipids. EPS are defined as excreted substances by the microorganisms, as well as the hydrolysis substances from dead cells. Due to the many groups associated with EPS and forces (such as electrostatic interaction, hydrogen bonds and London dispersion forces) between EPS molecules, EPS can have important

functions in the wastewater treatment processes, such as adhesion to surfaces, aggregation of bacterial cells in the flocs, stabilization of the floc structure, formation of a protective barrier that provides resistance to biocides or other harmful effects, retention of water, sorption of exogenous organic compounds from the environment, and accumulation of enzymatic activities, such as digestion of exogenous macromolecules for nutrient acquisition (Flemming et al., 2000a; Laspidou and Rittmann, 2002; Wingender et al., 1999). On the other hand, because of these functional groups on their surface, EPS can easily attach on the membrane surface under the hydraulic conditions in MBRs. Hence, the variation of EPS amounts and components due to changes in the bioreactor will impact on the properties of the activated sludge and the performance of the membrane units in MBRs. This is especially implicated in affecting the floc size, floc density, viscosity and also forming, flocculating, settling and hydrophobic properties of the flocs. This will further lead to properties that influence the reactor performance and filtration resistance in MBRs.

Since production and composition of EPS are mainly related with the growth of microorganisms, their culturing environmental conditions are crucial to stimulate or inhibit EPS production and alter their composition. In MBRs, it is believed that the types of microorganism species present, compositions of nutrients fed and other operating parameters such as food/microorganism (F/M), SRT, aeration rate and HRT will influence the EPS production and the main components. Hence, clearly understanding the relationships between EPS characteristics and MBR operating conditions will provide knowledge on how to reduce EPS production and control membrane fouling by optimization of operating parameters.

With respect to the fact that EPS are the secreted and autolyzed substances produced by microorganisms, it is important to analyze the bacterial community structure in the MBR in order to demonstrate how operating parameters influence EPS production and composition. Culture-independent methods, such as DGGE (Denaturing Gradient Gel Electrophoresis), have been employed to study the bacterial community structures in MBRs, while a culture-dependent method is used

to isolate the live and cultivable species, especially functional populations, in a complex microbial community. However, far less attention has been paid on the diversity, the dynamics as well as the functions of the microorganisms, which are associated with EPS production. Thus, investigations in this area have been induced in order to clearly explain the changes in EPS production under different operating parameters and the resultant membrane biofouling mechanisms in MBRs.

1.2 Research objectives

The objectives of this study aimed to identify the conditions in the MBR that would help to reduce membrane fouling. A secondary objective was to identify specialized biomass which were membrane friendly under typical MBR operating conditions. An extensive characterization of the activated sludge, such as EPS properties, bacterial community structure and membrane fouling tendency, was performed under various operating conditions to obtain a fundamental understanding of the relationship among them. The functions of the microbial populations were analyzed by culture-dependent and culture-independent methods.

The components of the project research which were carried out are as follow:

1. To investigate the dynamic EPS characteristics when the operating parameters such as substrate loading, SRT, HRT and aeration rate, have a significant shock in a MBR system.
2. To monitor reactor performance, EPS characteristics, bacterial community structure and membrane fouling tendency for different operating parameters (substrate loading, substrate composition and SRT) in parallel MBR systems.
3. To clarify the relationship between EPS properties, bacterial community structure and membrane fouling tendency.
4. To compare the contributions of dissolved solids and suspended solids from activated sludge to membrane fouling and explain the membrane fouling mechanism caused by dissolved solids.
5. To compare the bacterial diversity on the membrane surface and in the mixed liquor under unstable and stable conditions in the MBR system.
6. To isolate the bacteria from the membrane surface as well as the mixed liquor and evaluate their EPS characteristics and membrane fouling tendency.

7. To explain the membrane fouling mechanisms of 'membrane-fouling' bacteria and 'membrane-friendly' bacteria.

1.3 Dissertation organization flow chart

The organization of this dissertation is illustrated in the following flow chart:

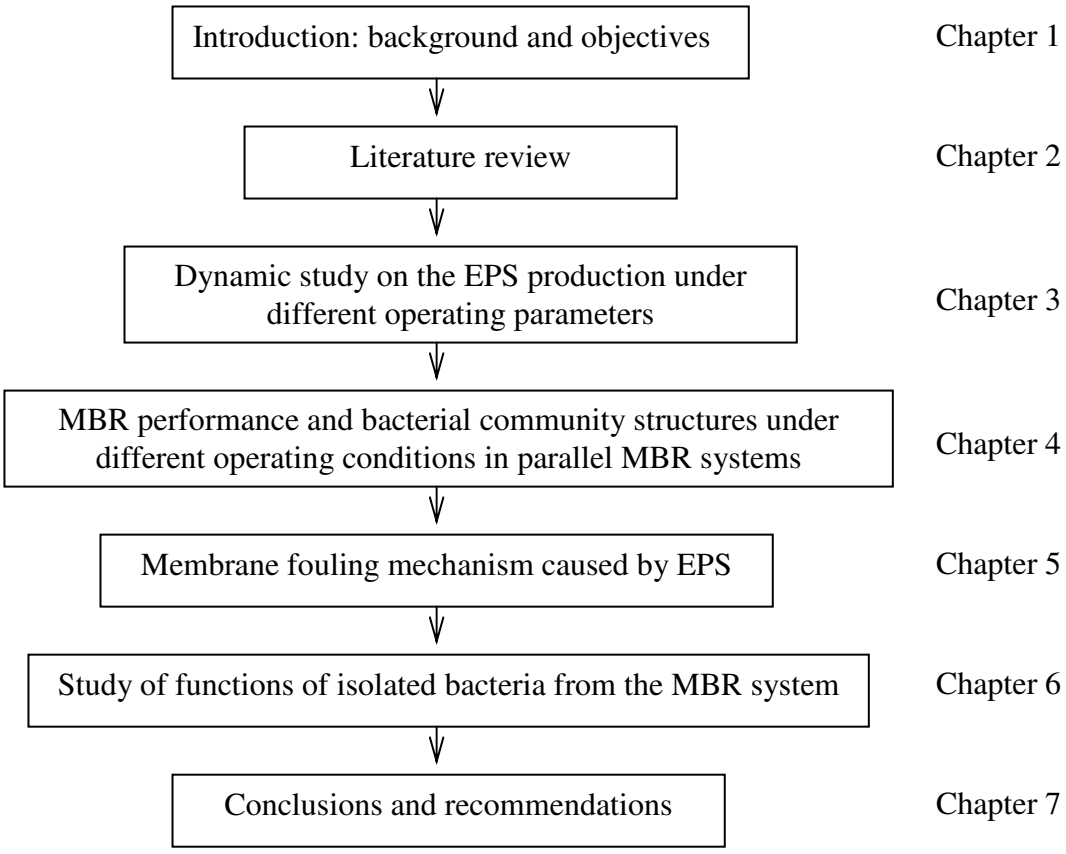


Figure 1.1 Dissertation organization flow chart

CHAPTER 2

LITERATURE REVIEW

2.1 MBR review

2.1.1 Introduction to MBRs

The membrane bioreactor (MBR) for wastewater treatment is a bioreactor having a membrane separator which replaces the secondary clarifier in the conventional activated sludge process (CASP). In the MBR system, the key role of bacteria inside the reactor is to decompose organic matters in the influent; the membrane separates the retained materials, such as microorganisms and some macro-molecules and allows the water and solute species to go through. Since the concept of combining membrane filtration with activated sludge process was commercialized by the Dorr-Oliver company in the 1960s (Fane, 1996), more than 2200 MBR installations have been in operation or under construction worldwide and several generations of MBRs have evolved (Yang et al., 2005). The application areas of MBRs have been increasing sharply with technical innovations and significant cost reductions of membrane modules. Initially, MBRs were developed for industrial and commercial application in order to obtain high quality water which could be reused. Increasingly, MBRs have been incorporated into municipal wastewater treatment to meet the discharge requirements of low biochemical oxygen demand (BOD) and total suspended solids (TSS) (He et al., 2005). MBRs have also been employed in landfill leachate treatment and drinking water reclamation (Cicek et al., 2001).

2.1.2 MBR types

MBR systems can be classified into two major configurations according to the membrane module locations: submerged MBRs and side-stream MBRs (Figure 2.1). In a submerged MBR, the membrane module is mounted directly within the bioreactor. The filtration is achieved on the permeate side by pressure force or

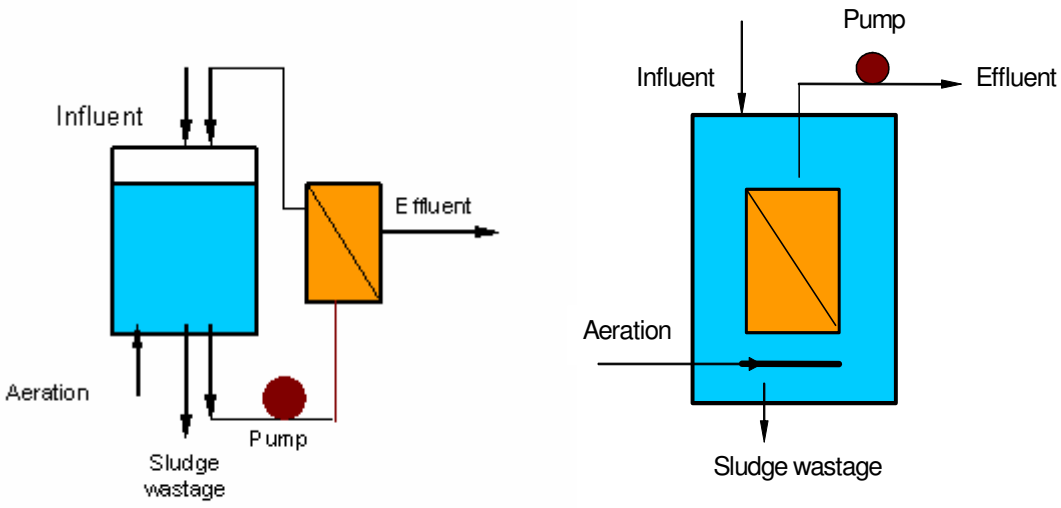
suction force; A side-stream MBR, whose membrane module is located external to the bioreactor, involves the recirculation of the mixed liquor through the membrane module. The driving force is the pressure created by hydraulic head through the membrane module (Cicek et al., 1999).

In the early phase, MBRs were constructed with the external configuration, in which biomass is re-circulated through a filtration loop (Yang et al., 2005). Nowadays, external MBRs are considered to be more suitable for wastewater streams characterized by high temperature, high organic strength, extreme pH, high toxicity and low filterability due to a broader choice of membrane and module materials with enhanced mechanical properties and resistance to chemicals (Lesjean et al., 2004). Also, drinking water and groundwater treatment mostly involve external MBRs with regard to pesticide removal and denitrification although no external MBRs had been employed in the real pilot-plants so far (Yang et al., 2005). Since Yamamoto et al. (1989) first introduced submerged membranes in an activated sludge aeration tank for direct solid-liquid separation, the applications of submerged MBRs for industrial and municipal wastewater treatment have been of interest to researchers. The internal membrane MBR configuration is attractive at high wastewater flow rates with respect to operating economics. It has been shown that the side-stream system has a higher total cost than the submerged system considering energy consumption, cleaning requirements and operational cost like aeration (Gander et al., 2000). However, the permeability of submerged membranes in MBRs is reduced slowly (transmembrane pressure rises) due to the fouling on the surface of the membrane, which is caused by the accumulation of foulants from the mixed liquor (Nagaoka et al., 1996).

There is a wide range of membranes available with different characteristics (shown as Table 2.1), such as membrane materials, surface properties, membrane pore size and module shapes. As a result, many MBRs with different configurations of membrane modules have been developed and employed in wastewater treatment processes.

2.1.3 Advantages of MBR

MBR technology offers several benefits over the traditional activated sludge processes, as the MBR technology has two unique properties related to the roles of the membrane separation unit. Firstly, the absolute retention of all microorganisms insures a complete separation of the hydraulic residence time (HRT) and sludge retention time (SRT), which can result in a high biomass concentration in the bioreactor. Since the MBR system is not involved in the settling of the suspended solids as in the conventional activated sludge clarifier, very high SRTs can be maintained. Longer sludge ages favour the development of slow-growing microorganisms, which leads to more efficient removal of refractory organic matter and makes the system more robust to load variations and toxic shocks (Lesjean et al., 2004). Therefore, although the wastewater may be highly concentrated, better treatment efficiencies can be achieved in MBRs. Complete sludge separation and cell recycle also eliminate the need for the formation of settleable flocs or aggregates, which simultaneously increases the treatment capacity of the system (Klatt and LaPara, 2003). Secondly, the retention of microorganisms keeps slowly



(a) **(b)**

Figure 2.1: Schematic presentation of the two types of MBRs

(a) Side-stream MBRs (b) Submerged MBRs

biodegradable species within the bioreactor (by adsorption etc) and non-biodegradable constituents discharged with the sludge, rather than with the treated water (Winnen et al. 1996).

Table 2.1 Relevant membrane characteristics for MBRs

Items	Membrane characteristics
Material	polysulphone, polyethylene, polyethersulfone, polyolefin, ceramic, etc
Surface property	hydrophobic, hydrophilic
Pore size	from 0.01 μm to 0.4 μm
Module shape	tubular, plate and frame, rotary disk, hollow fiber, etc

Also, MBRs can effectively treat influent with reduced sludge production in a limited space. In particular, submerged MBRs, commonly only take half the land area of a conventional sludge process under the same sludge production (Grander et al., 2000). The sludge production in the MBR system is normally lower, but their capabilities for some biochemical functions are still powerful. Moreover, the MBR technology has the ability to disinfect the effluent due to the membrane sieving or adsorbing of the pathogenic microorganisms, which is an important consideration for water reuse. Furthermore, the MBR can achieve a higher rate of nitrification because large amounts of slow-growing nitrifying microorganisms can be retained in the reactor (Chang et al., 1999). Importantly, the MBR system does not suffer from problems associated with hydraulic fluctuations, such as sludge bulking.

Therefore, it is concluded that the main advantages of the MBR process are independent control of SRT and HRT, effective treatment of wastewater, complete retention of microorganisms, successful maintenance of high biomass concentration and the relatively small footprint (Cicek et al., 2001; Kishino et al., 1996; Roberts et al., 2000; Van der Roest et al., 2001).

2.1.4 Limitations of MBRs

Membrane fouling is a serious problem inherent to the performance of the MBR system. In this hybrid system, mixed liquor species retained in the reactor tend to accumulate on the membrane surface under the combined effect of pressure or

suction force and membrane surface properties, which results in loss of permeability (fouling). This raises the overall resistance of filtration and reduces the flux or increases the suction pressure. In order to maintain the system under constant operation, fouling control strategies, such as periodic backwashing, chemical cleaning, air bubble sparging and so on, are essential requirements. These additional operational costs for energy, chemical usage and downtime are key concerns in MBR operation. It is reported that membrane cleaning and membrane replacement contributed up to 50% of the operating costs or 30% of the total cost in a typical UF membrane system (Möckel et al., 1999). So, compared with the conventional activated sludge process, the MBR technology is characterized by higher energy consumption and cost (Winnen et al., 1996). In addition, it is reported that the sludge produced by this process tends to be difficult to dewater (Fane, 2002).

2.1.5 MBR applications and future

MBRs are an attractive option for wastewater treatment processes and their performance has been significantly improved over the years. Some enhanced hybrid MBR systems have been studied and applied at the pilot-plant scale. For example, Yoon (2003) described the membrane bioreactor-sludge disintegration (MBR-SD) system and investigated the important operational parameters of the system for zero excess sludge production in order to reduce sludge discharge. Zhang et al. (2003) developed a new MBR system, in which a transverse flow membrane module and low recirculation flow rate were employed to treat sewage wastewater with lower energy compared with other MBRs. Li et al. (2005b) developed a novel membrane bioreactor system, with aerobic granular sludge, to treat municipal wastewater. Yeon et al. (2005) optimized the design of a membrane coupled higher-performance compact reactor to deal with high organic loading rates, in which a hollow-fibre membrane module with an open-type configuration was located inside the draft tube. In addition, MBRs have been shown to enhance COD, nitrogen and phosphorus removals for the treatment of swine wastewater, food processing wastewater, oily wastewater, explosive processing wastewater, and pharmaceutical wastewater containing complex organics (Vocks et al., 2005; He et al., 2005; Wyffels et al., 2004; Zhang et al., 2005). In recent years, some effort has been made on the

development of the anaerobic MBR system, which favors higher-strength wastewater and provides the production of energy from the generated biogas. For instance, Emanuelsson et al. (2003) have developed an anoxic extractive membrane bioreactor to selectively extract hydrophobic organic compounds from industrial wastewater in order to biodegrade them and thus use nitrate as an electron acceptor instead of oxygen. However, over 98% of the MBR systems use the aerobic biological process.

In the future, with stricter discharge standards and increased requirements for water re-use, the challenges for MBRs will center on scale-up, ease of operation, simplified membrane cleaning and replacement strategies, and peak flow management (Yang et al., 2005). In addition, with respect to the capital cost of the MBR system, which is mainly associated with the cost of the membrane modules, studies on membrane lifespan and innovation in membrane materials could be significant for the further development and application of MBRs in wastewater treatment plants (Lesjean et al., 2004). Overall, it is foreshadowed that MBRs will have a bright future in the wastewater treatment field (Cicek et al., 1999).

2.2 Membrane fouling in the MBR system

2.2.1 Causes and consequences of membrane fouling

In MBRs, a serious problem that has prevented this technology from reaching its potential is membrane fouling. The term ‘fouling’ is defined as ‘a process resulting in loss of performance of a membrane due to the deposition of suspended or dissolved substances on its surfaces, at its pore openings, or within its pores’ (Möckel et al., 1999). As soon as accumulated species deposit on the membrane surface and fine species enter and restrict the pores or block the pores, loss of permeability will occur due to an increase in filtration resistance (Figure 2.2). Darcy law is the basic model used for describing filtration resistance occurring during permeate transport through a membrane (Ho and Sirkar, 2001):

$$J = \frac{\Delta P}{\mu(\sum R)} = \frac{A\Delta P}{\mu} \quad [2.1]$$

where J is the permeate flux (L/m^2 h), ΔP the transmembrane pressure (TMP)(Pa), μ the viscosity of permeate (Pa s), ∑R the total filtration resistance (m^{-1}) and A is the overall permeability coefficient (m).

In MBRs, the cake layer is readily removable from the membrane if an appropriate hydrodynamic condition is achieved or a physical washing protocol is employed, thereby it is often classified as reversible fouling. On the other hand, internal fouling caused by adsorption of dissolved matter or fine colloids into the membrane pores is considered as irreversible fouling, which is generally only removed by chemical cleaning (Chang and Kim, 2005). Hence, the resistance-in-series model is used:

$$\sum R = R_m + R_{rf} + R_{if} \qquad [2.2]$$

where the total filtration resistance ∑R is composed of each filtration resistance caused by the membrane itself, $R_{m,}$ reversible fouling R_{rf} and irreversible R_{if}. If cake layer occurs before pore blocking, it is possible that the cake layer forming on the

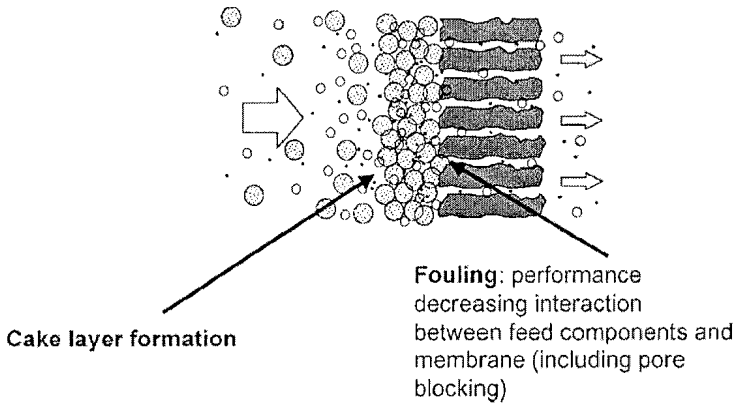


Figure 2.2 Formation mechanisms of membrane fouling (Wintgens et al., 2003)

membrane surface acts as a barrier which protects the membrane surface and pores (Bae and Tak, 2005). In order to describe the physical characteristics of the cake layer accumulated on the membrane surface, the specific cake resistance is employed, which implies the relationship between the cake resistance and the cake mass (Ho and Sirkar, 2001):

$$\alpha = R_{rf} / M \qquad [2.3]$$

where α is the specific cake resistance (m/kg) and M is the cake loading which is calculated by the cake mass (kg) divided by the membrane surface area (m^2).

In MBRs, the initial flux reduction (or rise in TMP) is thought to be caused by concentration polarization and adsorptive pore closure. Concentration polarization happens when the species accumulate on the membrane surface, where a gradient of increasing concentration occurs toward membrane. The flux will then cause an accumulation of dissolved substances and fine colloids in the pores to restrict the diameter. The Film Model of concentration polarization can be applied to depict the relationship between permeate flux (J) and concentration at the membrane wall (Porter, 1972):

$$J = k_m \ln(C_w / C_b) \quad [2.4]$$

where k_m is the mass transfer coefficient (m/s) and C_w, C_b are the species concentrations (dimensionless) along membrane wall and in the bulk. Equation (2.4) strictly applies to diffusive species, such as macromolecules and microsolutes. For colloids and particulars (size typically>0.2 μm) the back transport mechanism that limits concentration polarization could be inertial lift or shear induced diffusion (Belfort et al., 1994). These mechanisms can be incorporated into a modified Film Model as follows:

$$J = k_{si} \ln(C_w / C_b) + V_L \quad [2.5]$$

where k_{si} is the shear induced diffusivity (m/s) and V_L is the inertial lift velocity (m/s). Based on theory proposed by Green and Belfort (1980), in crossflow filtration system, if membrane permeation velocity (J) is less than lift velocity (V_L) at the membrane surface, particles would not deposit on the membrane surface. However, the deposition of particles occurs when J exceeds V_L. Since the convective transport of particles and solutes to the membrane surface is partly balanced by shear forces at the layer-solution interface, the characteristics of the system will determine the balance of forces to and away from the membrane surface (Thomas et al., 2000).

However, for long-term operation, the permeability reduction is mainly attributed to the formation of fouling layers onto the membrane surface. Once severe membrane

fouling occurs which leads to a significant increase in hydraulic resistance and a greater energy usage in the MBR system, the basic operation can not be maintained and membrane cleaning would be required. Under these conditions the Darcy's law equation (2.1) with fouling resistance (equation 2.2) applies.

2.2.2 Foulant species in MBRs

Since biofouling caused by microorganisms and their excreted substances can result in decreased productivity and problems with the stability of MBRs (Gryta, 2002), analysis of the contributions of these foulants to membrane fouling gives a clue to exploit membrane fouling mechanisms. Of particular interest are extracellular polymeric substances (EPS), which are excreted or autolyzed by microorganisms and are believed to be a major contributor in MBR fouling (Rosenberger and Kraume, 2002; Shin et al., 2001; Wisniewski and Grasmick, 1998).

EPS are not only attached on the surface of bacteria in floc, but are also present dissolved or suspended in the solution. EPS excreted from the microbial cells are considered to affect the cake resistance by filling the void spaces between the particles in the cake, which results in a drastic reduction in permeability (Hodgson et al., 1993). Also soluble EPS mainly contributes to membrane pore blocking or pore restriction in the filtration process. Thus based on the distribution of EPS, the mixed liquors are considered to contain two fractions: microbial floc and supernatant including colloids and solutes. Each fraction has its own physiocochemical and biological properties which explicitly affect membrane fouling. Recent studies have attempted to quantify the fouling caused by each fraction of the mixed liquor, such as the suspended solids (SS), colloids and solutes, although the results are inconsistent. Defrance et al. (2000) concluded that SS (floc and particulates) and colloidal particles with dissolved molecules (EPS) were predominant foulants, whose contributions to membrane fouling are 65% and 35% respectively. Lee et al. (2003) found that the relative contributions of supernatant containing colloids and solutes in the mixed liquor to membrane fouling were 37, 28 and 29% at SRTs of 20, 40 and 60 days, which reveals SS is a major foulant. Chang and Lee (1998) observed that the resistance of the cake layer formed by SS

appeared to determine the overall resistance at various SRTs. Bae and Tak (2005) also claimed that cake layer formation caused by SS constituted the main fouling mechanism. However, Wisniewski and Grasmick (1998) revealed that the soluble compounds provided up to half of the total resistance. Bouhabila et al. (2001) identified the colloids as of prime importance in causing membrane fouling in a MBR system. Chang and Kim (2005) pointed out that flux decline in the supernatant was more serious than that of the mixed liquor in a MBR system. Li et al. (2005b) found that the colloids and solutes appeared to be the main contributors to cause pore clogging or narrowing in a membrane granular-sludge bioreactor. So, it is concluded that the fouling contributions from various fractions of the mixed liquor are variable based on membrane properties, hydrodynamic conditions and physiological properties of the biomass (Bae and Tak, 2005; Bouhabila et al., 2001). Although these studies differ in quantifying the extent of fouling derived from various fractions, they do explain how foulant species contribute to biofouling, which is crucial in understanding the biofouling process and knowing how to control biofouling in MBRs. Further discussion of the properties and characterization of EPS are given in Section 2.3.

2.2.3 Techniques for fouling control

Recently, economical and effective techniques are being used to improve membrane permeability and reduce membrane fouling in MBRs. The propensity for membrane to foul is dependent on the following "fouling factors":

(i) the nature of the feed to membrane;

(ii) the properties of the membrane and module;

(iii) the hydrodynamic operating conditions.

MBRs present a unique set of characteristics in these three areas. In the following sections the various strategies to control fouling in MBRs are reviewed in the context of the three fouling factors.

(1) Optimizing operating parameters of the MBRs to alleviate membrane fouling; this deals with fouling factor (i) above. Many studies regarding membrane fouling have shown how the parameters, such as COD, biomass concentration, SRT, HRT

and aeration rate influence membrane fouling in a MBR system (Chang and Kim, 2005). For instance, Choi et al. (2005) observed that permeate flux declined faster with increasing organic feed concentration in a MBR system, which implies decreasing substrate concentration can ameliorate membrane fouling. Le-Clech et al. (2003) pointed out that no significant difference is apparent for a shift of biomass concentration from 4 to 8 g/L, but a significant increase of critical flux (defined as the flux below which the ratio of J/TMP is constant by Field et al., 1995) arises for an MLSS increase to 12 g/L. In fact, many MBR researchers have noticed that SRT is one of the most important parameters associated with membrane fouling. Numerous studies on this issue have been reported but without a clear consensus. For example, it was concluded that changes in SRT would result in variation of sludge characteristics, therefore the overall activated sludge fouling resistance was increasing as SRT was prolonged in a MBR system (Lee et al., 2003; Han et al., 2005). However, Chang and Lee (1998) observed that membrane fouling tendency was increasing as SRTs decrease in the membrane coupled activated sludge system. It is possible that fouling increases at the extremes of SRT, and that there is an optimal range of SRT for manageable fouling. In addition, Chang et al. (1999) found that membrane fouling tendency was correlated with floc structures, which indicates that bulking sludge caused more serious fouling than pinsize sludge and normal sludge. Overall, operating conditions of the MBRs appear to be very significant factors influencing membrane fouling and optimizing them is crucial in controlling the fouling in MBRs (Le-Clech et al., 2003).

(2) Modifying membrane materials in order to reduce bacterial affinity with the membrane surface by changing their interaction forces (Yang et al., 2005); this deals with fouling factor (ii). In MBRs, membrane material types, such as Polyethylene (PE), Polyvinylidene (PVDF), Polysulphone (PS), Polyethersulphone (PES), Polyaramide (PA) and Cellulose Acetate (CA) have been widely used and researchers have proved that membrane material significantly influences membrane fouling (Yamato et al., 2006; Chio et al. 2002; Jönsson and Jönsson, 1995). For example, Yamato pointed out that PE is responsible for more irreversible fouling than PVDF; Chio et al. (2002) have shown that relatively hydrophobic

Polyethersulfone (PES) membranes fouled more seriously than hydrophilic Cellulose Acetate (CA) membranes. It has been suggested that adsorptive fouling alone could account for permeability losses of up to 90% in MBRs, which indicates the potential importance of developing new materials and modify membrane surfaces (Möckel et al., 1999). Ma et al. (2001) found that desirable membrane surface characteristics such as hydrophilicity and ionic charge with less fouling potential were obtained by grafting various monomers to polypropylene (PP) base membranes. Hydrophilic membranes can be anticipated to be less fouling prone by microbial cells, soluble and insoluble metabolites, and organic and inorganic colloids because hydrophobic-hydrophobic interactions between the membrane and foulants are alleviated. Jiang et al. (1995) found that neutral PVDF membrane (treated with polyvinylic alcohol, hydrophilic) caused less membrane fouling than untreated PVDF (hydrophobic, negatively charged) and positive-charge PVDF membrane (treated with polyethylenimine, hydrophilic). In addition, surface configuration can influence membrane fouling tendency as rough surfaces are more prone to microbial attachment than smooth surfaces (He et al., 2005).

(3) Optimizing the packing density of hollow fibers or flat sheets and modifying membrane module design by regulating orientation of fibers and diameters of fibers; this also deals with fouling factor (ii). For hollow fiber membranes, it is considered that modules with thinner fibers, lower packing density and vertical orientation of fibers are better for alleviating membrane fouling (He et al., 2005). Moreover, membrane pore size and pore structure are important factors to influence membrane fouling. It was found that the rate of permeate flux decline was greater with increasing membrane pore size or porosity (Hong et al. 2002; Choi et al. 2005). He et al. (2005) further pointed out that the membrane with smaller molecular weight cutoff (MWCO) exhibited an initial flux decline (possibly pore blockage), whereas the membranes with the higher molecular weight cutoffs were observed to have relatively greater flux declines and lower recoverable fluxes during long-term operation. It should be noted that this trend may become exaggerated when experiments are run at constant pressure, rather than constant flux. This is because at constant pressure the higher MWCO membranes tend to start off with a higher

flux. Fang and Shi (2005) observed that uniform and small cylindrical membrane pore configuration made it difficult for foulants to deposit inside the pores, while, the typical sponge-like microstructures were more vulnerable to pore fouling due to their porous network.

(4) Controlling the filtration process below the nominal critical flux in order to reduce cake formation on membrane surfaces; this deals with fouling factor (iii). Fouling is generally held to persist above "critical flux", below which almost steady-state membrane permeability may be attainable (Pollice et al., 2005). At higher fluxes, convective transport of solutes, colloids and suspended matter result in rapid formation of a cake layer and internal pore blocking (Schöberl et al., 2005). When the operating flux is below the critical flux, particle accumulation in the region of membranes can be effectively prevented, as convection is outbalanced by back diffusion or tangential removal of rejected matter. Choi et al. (2005) have observed that filtration resistance by foulants adsorption was negligible in the absence of flux, as compared to total filtration resistance in the presence of permeate flux. However, it should be noted that "sub-critical" flux operation does not totally eliminate fouling in MBRs as steady deposition of EPS (see Section 2.2.2) can still occur. Flux control can slow down the process of slow fouling.

(5) Injecting air bubbles below the membranes to prevent the foulants from forming a cake layer or scouring a formed cake layer on the membrane surface; this deals with fouling factor (iii). High air aeration rates bring strong shear forces over the membrane surface and effectively reduce the deposition and adsorption of particles on the membrane surface. Several studies carried out with submerged MBRs show that increasing air flow rate across the membrane surface improves permeability and limits fouling (Le-Clech et al., 2003; Günder and Krauth, 1998; Günder and Krauth, 1999; Gander et al., 2000).

(6) Adding chemical reagents or powdered activated carbon (PAC) into the MBR to form flocculated solids by aggregating the soluble or suspended substances; this deals with fouling factors (i) and (iii). These materials play an important role in

reducing the biomass cake resistance by changing the overall particle distribution to a greater size range while sorbing and/or coagulating dissolved organics and colloidal particles in the reactor. Park et al. (1999) found that cake layer resistances decreased continuously with increasing PAC dose up to 5 g/L in an anaerobic MBR due to its incompressible nature and higher backtransport velocities as well as the scouring effect removing the deposited biomass cake from the membrane surface. Seo et al. (2004) also pointed out that high PAC concentrations (up to 40 g/L) can prevent membrane fouling in a MBR system.

(7) Periodic permeate backpulsing, gas backpulsing, crossflushing and chemical cleaning can also remove the foulants attaching on the surface of membranes or internal fouling within the membrane pores, thus leading to optimal, stable hydraulic operating conditions (Bouhabila et al., 2001). Backpulsing is a cyclic process of forward filtration followed by reverse filtration, which involves reversal of the flow through the membrane by changing transmembrane pressure (TMP). Crossflushing is accomplished by maintaining flow over the membrane with intermittent permeate flow (Ma et al., 2001). Another important step for the inhibition of membrane fouling is periodic back washing the membrane with air or operating the reactor under the intermitted mode (Ma et al., 2005). In the absence of transmembrane suction pressure, the reversible foulants attached on the membrane surface can diffuse away from the membrane surface due to the concentration gradient or be scoured by the surface shear. Large scale MBRs typically apply filtration for 10 to 15 minutes followed by 1 to 2 minutes relaxation (with cross flow). Chemical cleaning is also an essential requirement for control of 'irreversible' fouling and maintaining stable operation of MBRs. This process needs not only the special cleaning apparatus but also proper chemicals, such as NaOCl.

Although the techniques mentioned above can prevent membrane fouling to a certain extent, progress in this field has been relatively slow and more research is required to better understand membrane fouling mechanisms (Yang et al., 2005). In summary, membrane fouling control is still a key issue in the development and application of MBRs.

2.3 Extracellular polymeric substances (EPS)

2.3.1 Definition

In MBRs, most prokaryotic microorganisms and eukaryotic microorganisms can take in organic/inorganic substrates in the wastewater as their nutrient sources, which provide electrons and energy to build their components. They also excrete waste into the external environment to meet the need of biodegrading organic/inorganic substances (Spellman, 1997). At the same time, part of the active biomass undergoes endogenous decay to form residual dead cells for maintaining energy needs (Laspidou and Rittmann, 2002). These hydrolysis substrates from dead cells are coupled with those excreted substances as well as undegraded compounds from the water phase to form what is known as "EPS". Although some researchers have given EPS different meanings with respect to their respective study objectives, EPS normally stands for extracellular polymeric substances which are located at or outside the cell surface and can lead to floc formation by agglomeration of bacteria. Flemming et al. (2000b) defines EPS as 'organic polymers of microbial origin that are frequently responsible for immobilizing cells and other particulate materials together (cohesion) and to the substratum (adhesion)'. Microorganisms can move and live in this three-dimensional, gel-like, highly hydrated and charged matrix. Thus EPS are considered as the key components that determine the structural and functional integrity of microbial aggregates and biofilms (Flemming and Wingender, 2001a).

2.3.2 Composition and distribution

EPS as organic macromolecules are built up by polymerization of similar or identical building blocks, which may be arranged as repeating units within the polymer molecules (Wingender et al., 1999). In general, EPS consist of various organic substances, including polysaccharides, proteins, lipids, and also a component of nucleic acids and other bio-polymers. Therefore, their molecular masses normally range from thousands to several millions (Tsuneda et al., 2003b). Among these components, secreted polysaccharides are believed to perform mainly structural functions in forming and stabilizing the biomass floc due to their long-

chain molecule structures. The significant role of proteins is mostly considered as enzymes, which can promote the degradation of macromolecules and eventually break them down to low molecular-weight products available for microbial metabolism. Another function of extracellular proteins is that they can form electrostatic bonds much more effectively than polysaccharides with multivalent cations, a key factor in stabilizing aggregate structures because of a high content of negatively charged amino acids (Flemming et al., 2000b; Laspidou and Rittmann, 2002). In addition, proteins are thought to contribute to the hydrophobic regions within the EPS matrix. Extracellular lipids have been proposed to help bacteria to overcome the strong surface tension of surrounding water, which benefits their growth on solid surfaces (Matsuyama and Nakagawa, 1996).

Biosynthesis and discrete export mechanisms of microorganisms provide the possibility for translocation of EPS across bacterial membranes to the cell surface or into the surrounding medium. Gram-positive bacteria secrete proteins and polysaccharides mainly across the cytoplasmic membrane, while Gram-negative bacteria secrete them across the inner and outer membranes. Spontaneous liberation of integral cellular components (such as lipopolysaccharides) is another mechanism for releasing EPS. Furthermore, lysis of dead bacteria produces high molecular weight compounds also thought of as EPS (Wingender et al., 1999).

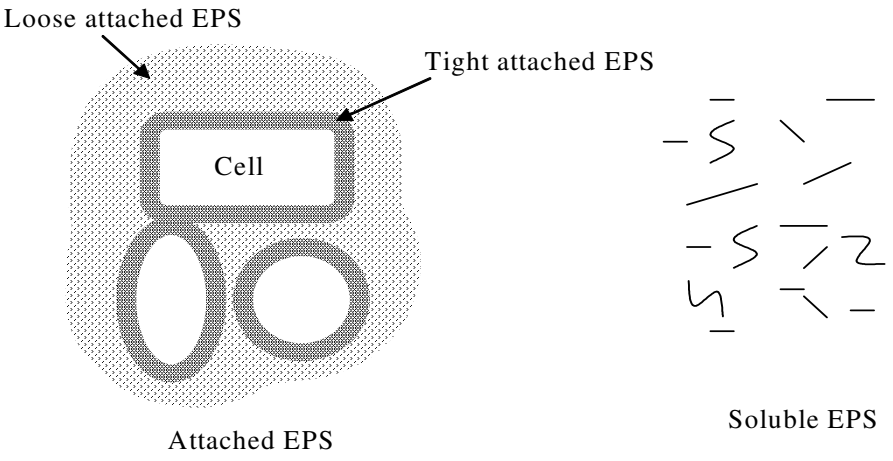


Figure 2.3 Depiction of EPS distribution (Wingender et al., 1999)

A portion of EPS is closely attached on the bacterial surface, while other EPS does not have any direct contact with the cells. The two fractions can be physically divided by centrifugation, to be defined as "Attached EPS" and "Soluble EPS". Attached EPS normally contains sheaths, capsular polymers, condensed gel, loosely bound polymers, attached organic materials, while soluble EPS includes soluble macromolecules, colloids and slimes (Wingender et al., 1999). Due to different excretion mechanisms of EPS, the composition and location of EPS depends on several processes, including active secretion, degrading activities of the bacteria, shedding of cell surface material, cell lysis, and adsorption from the environment (Wingender et al., 1999).

2.3.3 Function

Normally, EPS tends to form on the outermost surface layers of bacteria. By this boundary structures, bacteria can regulate and exchange energy with their biotic and abiotic environments (Wingender et al., 1999). Between EPS molecules there are three major kinds of forces: electrostatic interactions, hydrogen bonds and London dispersion forces (Figure 2.4). The main physico-chemical interaction roles of the forces are the adhesion and cohesion, which benefit the aggregation of

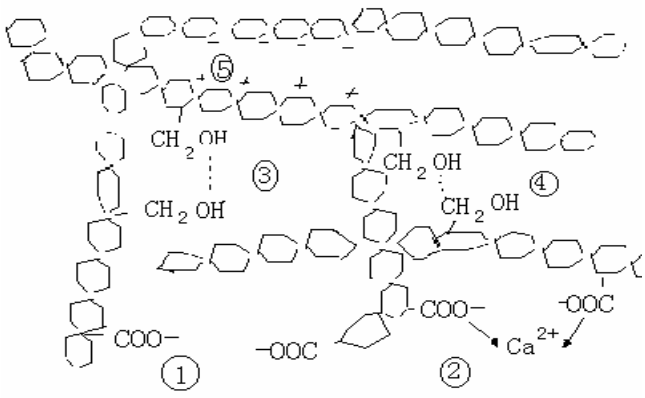


Figure 2.4 Interactions between EPS molecules (Flemming et al., 2000b)
1: Repulsion of two carboxylic groups 2: Attraction of two carboxylic groups by a divalent cation 3: Hydrogen bond 4: Dispersion force 5: Electrostatic attraction

microorganisms. The extent of contribution for each bond to the cumulative binding force depends strongly on the nature of the EPS molecules (Flemming et al., 2000b). Nevertheless, the formed EPS can not tolerate strong shear forces and structures can easily be broken due to their weak binding.

Due to complex composition of EPS, there are many active groups in the surfaces of EPS and forces between EPS molecules or EPS molecules with other molecules. Therefore, microbial cells show significant biochemical, biological, physical and physical-chemical properties in MBRs. EPS performs as a 'bridge' that bacterial cells can attach to, which results in aggregation of bacterial cells to form three-dimensioned flocs. The forces between EPS molecules also help to stabilize the floc structure. In addition, this three-dimensioned structure provides much more space to absorb exogenous organic compounds for the accumulation of nutrients and enzymatic activities, such as digestion of exogenous macromolecules for nutrient acquisition of microorganisms. The structure of the EPS matrix benefits maintenance of a higher water content in the microenvironment around microbial communities, which facilitates nutrient transportation to the cells and nutrient availability of the cells. Also, EPS can form a protective barrier that provides resistance to biocides or other harmful agents (Flemming et al., 2000a; Laspidou and Rittmann, 2002; Wingender et al., 1999).

2.3.4 Impact of EPS properties on the MBR system

In a MBR system, microorganisms exist mainly as aggregated cells due to the bonding force provided by EPS on their surfaces. By these interactions, the individual cells not only maintain their own survival but also contribute to the stability of the community (Wingender et al., 1999). Meanwhile, once the EPS covers the surface of a membrane, changes occur to the physicochemical characteristics of the surface, such as surface charge, hydrophobicity and pore structure. Therefore, the whole MBR system will be influenced by variations in EPS production and composition as this affects the properties of flocs, the membrane and effluent characteristics.

2.3.4.1 Impact of EPS on the activated sludge properties

EPS performs an important function in the floc formation by facilitating the interactions between bacterial cells. It is believed that hydrophobic interactions existing on the surfaces of EPS play a major role in the organization and the cohesion of bacterial aggregates in the activated sludge. Jorand et al. (1998) pointed out that there are two ways involved in floc cohesion by EPS, the first is through hydrophilic chains, represented by polysaccharides creating a matrix in which bacteria are embedded and the second is through glue creating bridges or reticular points between polysaccharides, represented by hydrophobic heteropolymers.

The roles of EPS for the integration of such aggregates had been demonstrated in many studies (Mikkelsen and Nielsen, 2001), which are very crucial to the biological and physico-chemical properties of the flocs. Recent studies have mainly focused on the role of EPS in floc development and have produced correlations between polymer concentration and settlability. This is especially implicated in affecting the floc size, floc density, settling and hydrophobic properties of the flocs. Shin et al. (2001) reported that the important factor in changing floc properties was the ratio of the carbohydrate to protein in the EPS. Jin et al. (2003) believed that the quantity of the EPS had a significant positive correlation with the sludge volume index (SVI). Further experiments showed that a low protein and high DNA level in EPS was associated with low negative charges in activated sludge samples and high SVI values which indicate poor settleability (Sponza, 2002). Although some scientists have devoted themselves to study the relationship between the compositions of EPS and floc properties, some detailed models and mechanisms are still not clear.

2.3.4.2 Impact of EPS on the MBR system

In the MBR system, EPS as secreted substances of microorganisms can be accumulated not only in the aeration tanks but also easily attached on the membrane surface under hydraulic operation because of their surface charges and forces. Therefore, these substances are potentially the major sources of cake resistance within the total resistance during membrane bioreactor operation (Rosenberger and

Kraume, 2002; Shin et al., 2001; Wisniewski and Grasmick, 1998). In fact, biofouling problems do not arise from micro-organisms which have suddenly invaded the system. In many cases, attachment of a layer of cells to a substratum will not cause these problems. Rather the accumulation and growth of the biofilm reaches a critical situation that leads to unacceptable membrane biofouling. It is suggested that when the EPS quantity is relatively small, cell adhesion onto the solid surfaces is inhibited by electrostatic interaction, or else, it is enhanced by polymeric interaction according to Tsuneda et al's study (2003a). On the other hand, rapid accumulation of bacterial cells and bacterial metabolic substances can directly cause an increase in viscosity of the mixed liquor, and increase in the filtration resistance and transmembrane pressure (TMP) as well as falling off for permeate flow rates according to Nagaoka et al. (1996).

In MBRs, cake layer properties on the membrane surface are associated with EPS properties. Kreft and Wimpenny (2001) found the patchiness and roughness of the biofilm decreased and the porosity increased with increasing EPS production. Some studies had shown that multiple polysaccharide synthesis, growth and exopolysaccharide production may be more prolific under attached conditions for some bacteria because attachment to solid surface may stimulate polysaccharide synthesis (Sutherland, 2001), which could intensify membrane fouling based on deposited bacteria. However, recent efforts have also shown that EPS contents were similar regardless of support media types or biofilm configuration, but EPS contents gradually increased as the film growth continued after backwashing (Flemming et al, 2000b). In addition, cationic concentration is also a very important factor to exacerbate the membrane fouling caused by EPS, as EPS can bind with membrane surfaces by cationic bridges (Wilén et al., 2002; Frank and Belfort, 2003).

However, due to the variability in EPS composition, concentration and complexity in real MBRs, membrane fouling mechanisms caused by EPS are still not well understood. Mathematical modelling is a powerful tool that allows quick and cheap evaluation of alternatives, leading to optimal solutions, which are not obviously foreseen. As a result, some researchers have applied mathematics models to display, explain and anticipate the membrane fouling situations caused by EPS in MBRs.

Ognier et al. (2002) used protein with ß-lactoglobulin as model foulants for filtration experiments and compared them with the results obtained from MBRs. Polysaccharides have been suggested by Frank and Belfort (2003) as well as Ye et al. (2005) who used alginate as a model for EPS. Their work explicitly introduce a systematic description of fouling behaviour of protein or polysaccharides, such as fouling mechanisms, specific cake resistance and compressibility with different operating parameters like pore size, pore size distribution and feed concentration. This approach may give clear clues to understand the fouling mechanisms caused by EPS in MBRs.

2.3.5 Factors influencing EPS production

The yields, compositions and properties of EPS present in the mixed liquor vary with a large number of factors, including the physiology of the species present, the nutrient situation and imposed environment created by the MBR system operation (Horn et al., 2001; Ye et al., 2005; Wilshusen et al. 2004). These operating parameters include not only wastewater compositions and availability of nutrients, but also aeration rate, HRT, SRT, temperature and so on (Abbassi et al., 1999; Lee et al., 2003; Sponza, 2003). Recently, studies on EPS production in various wastewater treatment processes have been reported, although the relationship between EPS production and some affecting factors are subject to significant controversy. The effects of various factors are discussed below.

(1) Method of extraction. EPS contain a large variety of production and chemical structures due to their different major constituents. Commonly, the compositions of EPS as analyzed largely depend upon the methods used for extraction (Flemming and Wingender, 2001b). Previous exaction methods include physical extraction methods which are mainly centrifugation, ultrasonication and heating, as well as chemical extractions which are involved in use of ammonium hydroxide, sodium hydroxide, ethylenediamine tetraacetic acid (EDTA), sulfuric acid, trichloroacetic acid, boiling benzene, Tris/HCl buffer, phosphate buffer/heat and cation exchange resin. These extraction methods provide different extraction efficiencies for each component, which result in various EPS recoveries and compositions (Azeredo et

al., 1999; Zhang et al., 1999; Liu and Fang, 2002). This thesis makes use of centrifugation and chemical extraction which are increasingly popular.

(2) Bacterial growth conditions. A large number of studies indicate EPS production is associated with bacterial growth conditions, such as suspended or sessile growth and growth rate. For instance, Evan et al. (1994) found that Staphylococcus epidermidis produced less EPS under suspended growth than their sessile growth during slow growth rates. However, when the growth rates were high, insignificant differences between EPS production by suspended cells and sessile cells was observed. Moreover, in one example of polysaccharide synthesis, its production might be more prolific under attached conditions for some bacterial strains than those found in the free-living state because solid surface may stimulate polysaccharide synthesis (Choi et al., 2001; Sutherland, 2001).

It have been reported that there is a direct correction between microbial growth rates and EPS accumulation. Because EPS production costs energy, the higher the EPS production rate, the lower the growth rates of the producers are, since less energy is available to produce more cells (Kreft and Wimpenny, 2001). It has also been recognized that autotrophic nitrifying bacteria have much slower biofilm formation rates than other heterotrophic bacteria due to lower growth rates and lack of EPS production ability in some wastewater treatment reactors (Tsuneda et al., 2001). Laspidou and Rittmann (2002) pointed to some trends, such as linear or independent relationships between EPS production and biomass growth rate. However, it seemed to depend on the kind of microorganisms involved and the operational conditions.

(3) Substrate load and compositions. MBRs have been applied to treatment of a wide range of industrial and domestic wastewaters with different chemical oxygen demand. These variations of influent concentrations inevitably affected EPS production because of the shock to bacterial growth and metabolic system. Puñal et al. (2003) have pointed out that EPS production enhancement or reduction has been associated with substrate concentration in the Upflow Anaerobic Sludge Blanket reactor and Expanded Granular Sludge Bed reactor. In Matsuda et al.'s (2003) study,

it was found that the BOD loading had significant effects on suction pressure and stability of the operation in a MBR system. Jordand et al. (1995) also pointed out that the biodegradable organic loading apparently played an important role and changed the hydrophobicity of the bacteria and the flocculation or settleability of the floc. These changes are possibly associated with the changes of EPS properties caused by substrate loadings.

EPS production and properties also respond to the nutrient composition. It was reported that different wastewater compositions would influence the EPS production and physicochemical properties in a continuously fed completely stirred tank reactor (Sponza, 2003). Taverneier et al. (1997) pointed out that there was a significant effect of culture medium composition on EPS amount and composition. In the SBR system, the C:N:P ratio of a synthetic wastewater was found to influence the hydrophobicity, surface charge and EPS composition of microbial flocs (Bura et al., 1998). Moreover, it was said that the change of the compositions of EPS was simultaneously represented by the ratio of carbohydrate to protein (C/P) when bacteria was exposed to heavy metals (Jang et al., 2001). This suggests that carbon utilization shifted towards EPS production when the C: N or C: P ratio was increased. Flemming et al. (1994) found that surfactants in the feed could also stimulate endogenous respiration, oxygen uptake and heat output in starved bacteria, thus they could modify the physiological behavior of bacteria when they adhered to these surfactants, likewise with EPS production.

(4) Sludge Retention Time (SRT). A key operational parameter in the activated sludge process is the sludge retention time, or mean 'sludge age'. This can be linked to the Food-to-Microorganism (F/M) ratio, and at steady state they are inversely related via the microbial growth coefficients (Metcalf and Eddy, 1991).

$$\frac{1}{\theta} = \mathrm{Y}\left(\frac{F}{M}\right) - K_d \quad [2.6]$$

where θ is sludge retention time (hr), Y the maximum yield coefficient measured during any finite period of logarithmic growth (hr*g/g) and K_d is endogenous decay coefficient (hr^{-1}). Hence, SRT is associated with cell metabolism, bacterial growth

and sludge production. High SRTs allow the enrichment of slowly growing bacteria and consequently, a more diverse bacterial community with broader physiological capabilities is established at lower SRTs (Clara et al., 2005). At lower SRT, higher fractions of the biomass are viable, which is attributed to younger cells and improves mass transfer conditions in the bioreactor due to dilution and floc structure (Cicek et al., 2001). However, when a MBR system is operated without sludge wastage, defined as "complete sludge retention", the biomass growth rate should balance the decay rate. This suggests equilibrium between endogenous respiration and new bacterial mass growth (Pollice et al., 2004). It is also reported that biological activity such as specific oxygen uptake rate, specific nitrification rate and specific denitrification rate was decreased with prolonged SRTs (Han et al., 2005).

Since SRT is related to bacterial growth characteristics, many researchers have found that SRT is an important factor that influences EPS production. However, a common consensus has not been drawn so far, presumably due to the complexity of the system and the different operation conditions in each study. For example, it is reported that the total amount of EPS is independent of the SRT because total EPS production is not limited only to the stationary and endogenous phases of sludge in the MBR system or sequencing batch reactor (Lee et al., 2003; Liao et al., 2000). Other studies indicated higher total EPS production occurred at lower SRTs in the membrane-coupled activated sludge process (Chang and Lee, 1998; Alavi Moghaddam, 2003; Rosenberger and Kraume, 2002; Pollice et al., 2005). Shin and Kang (2003) observed that the soluble microbial production (soluble EPS) tended to increase with SRT and then gradually decrease for a long SRT.

(5) Dissolved Oxygen (DO). Dissolved oxygen is often discussed concurrently with oxygen transfer efficiency because they both determine not only the types of organisms present (aerobic, anaerobic, or facultative) but also the rate of metabolism, the efficiency of substrate utilization, and the amount of microbial product, whether this product is the cells themselves or specific biopolymers produced by the cells (Gaudy and Gaudy, 1980). So, it is easily understood that

dissolved oxygen in MBRs will influence the EPS production based on the description of the Monod kinetic function. Chiemchaisri et al. (2001) concluded in their recent paper that the presence of an appropriate amount of oxygen is important for sustaining and regulating EPS production in soil. It is also reported that the properties of the EPS production are changed during growth at elevated oxygen concentrations in the conventional activated sludge treatment process (Abbassi et al., 1999) and in the modified sequencing batch reactors (Knoblock, 2000). Park et al. (2005) found that EPS concentration increased with increasing air recirculation rate due to the increase in oxygen transfer efficiency in a membrane coupled high performance compact reactor. They believed that the increase of the oxygen concentration will improve the deep diffusion of oxygen into the floc and provide stronger floc able to withstand shear forces. It further results in the enlargement of aerobic space inside the floc, which benefits the degradation of hydrolysed dead microorganisms and EPS production. In addition, Yun et al. (2006) had reported that at low, or approaching anoxic conditions, EPS production was lower comparing with higher DO condition.

(6) Hydraulic Retention Time (HRT). Hydraulic retention time is a crucial control parameter in the activated sludge process, which exerts a profound influence on the hydraulic conditions and the contact time between the organic/inorganic substrate and the activated sludge (Pan et al., 2004). MBR system possesses excellent solid-liquid separation ability to retain high concentrations of biomass. This unique feature favours shortening HRT from the typical range of 5-14 hours to as low as 2 hours (Tay et al., 2003). Some studies have mentioned that HRT can influence membrane filtration performance (Tay et al., 2003; Yoon et al., 2004), possibly due to changes in EPS production. Ng and Hermanowicz (2005) further confirmed that with HRT increase, EPS production also increased, and this was probably due to the increased substrate loading.

Although numerous studies on EPS production in different wastewater treatment processes have offered general guidelines to understand the factors which can influence EPS production, not all of the factors show a clear conclusion. Since EPS

are considered as the major foulants in MBRs, more emphasis is required on the best ways to control EPS production in direct response to changes in operation. Understanding how operating parameters influence microbial processes and EPS production will ultimately lead to more effective control of the biofouling problem in MBRs.

2.4 Microbial analysis of the MBR system

Microorganisms play important roles in the biological wastewater treatment process because they degrade substances by their metabolism. Metabolic products as well as degradation products co-exist in this complex system. In the development of MBRs, many studies have focused on the optimization of process efficiency in terms of effluent quality, membrane fouling control by regulating the operational parameters, plant configurations and operational practices (Pollice et al., 2004). Recently, researchers have tended to concentrate on the mechanisms of biofouling and inevitable consequence caused by microorganisms in MBRs. However, less attention has been devoted to the characteristics and role of the biological processes as well as the microbial consortia developed in MBRs (Pollice et al., 2004). Considering the influence of EPS on membrane biofouling in MBRs, which are produced by bacterial cells due to metabolism and cell auto lysis, study of the microbial population and the function of bacterial species is becoming increasingly attractive, and this should benefit optimization of design and operation of the MBR system.

It is believed that bacterial community structure (population shifts) can explain the microbial interactions that vary in relative importance both in time and space. Information on bacterial function will help to understand the contributions of the species and maintenance of the system. Unfortunately, owing to limited technology to analyze microbial community structure and function, knowledge about this issue is very limited. However, the integration of microbial ecology and molecular biology is providing new insights into studies of microbial community.

2.4.1 Methods for analysis of microbial communities

Recently many new molecular techniques have been applied to analyze and compare bacterial community diversity *in situ*. These culture-independent techniques based on precise nucleotide sequences of DNA and RNA provide more comprehensive, rapid and concise information on characterization of bacteria from the complex bacterial community (Amann et al., 1995). Techniques like DNA sequencing, Fluorescent In Situ Hybridization (FISH), Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE) offer good opportunities in community analysis, even allowing direct quantification of the presence and abundance of bacterial species.

Polymerase Chain Reaction (PCR) and DGGE is a widely used combination of techniques for describing bacterial community structure and diversity based on extracted DNA. This technique can be used not only to estimate the genetic diversity of microbial communities in natural habitats but also to infer the phylogenetic affiliation of community members (Petersen and Dahllöf, 2005; Vallaeysa et al., 1997). Truly quantitative information using molecular methods can be obtained if cell lysis and extraction efficiency, as well as biases in the PCR step are under experimental control.

PCR technology was invented by Mullis in 1985 (Saiki et al., 1985). It provides a rapid, inexpensive and simple method for detection and molecular characterization in the molecular biological field by using primers directed enzymatic amplification of specific DNA templates (Macintyre, 1989). Based on the theory that two specific oligonucleotide primers are employed to flank DNA fragments and hybridize to helix strands, PCR is carried out in the following steps: (1) Denaturing, in which the strands of DNA helix are unwound and separated to single-stranded DNA by heating to 90~95°C; (2) Hybridization or annealing at 45~55°C, in which the two primers bind to their complementary bases on the single-stranded DNA in opposite directions; (3) Extending at 70~75°C, in which starting from the primer, the polymerase can read a template strand and match it with complementary nucleotides (Figure 2.5) . The two new helixes composing of one of the original

strands plus its newly assembled complementary strand serve as a new DNA template to repeat the steps above. This results in an exponential amplification of a DNA segment (Figure 2.6) (Bartlett and Stirling, 2003; Macintyre, 1989; Lubin et al., 1991).

DGGE technology is based on the separation method first described by Fischer and Lerman (1983). DGGE is employed to separate DNA fragments of the same length but differing in base-pair sequences with a polyacrylamide gel containing a linear gradient of DNA denaturants (Gafan and Spratt, 2005). In a denaturing gradient gel, double stranded DNA is subjected to an increasing denaturant environment and will melt in discrete segments called 'melting domains'. Identical DNA molecules with a difference only in one nucleotide have different melting temperatures (Tm). Theoretically, when the melting temperature (Tm) of the lowest domain is reached,

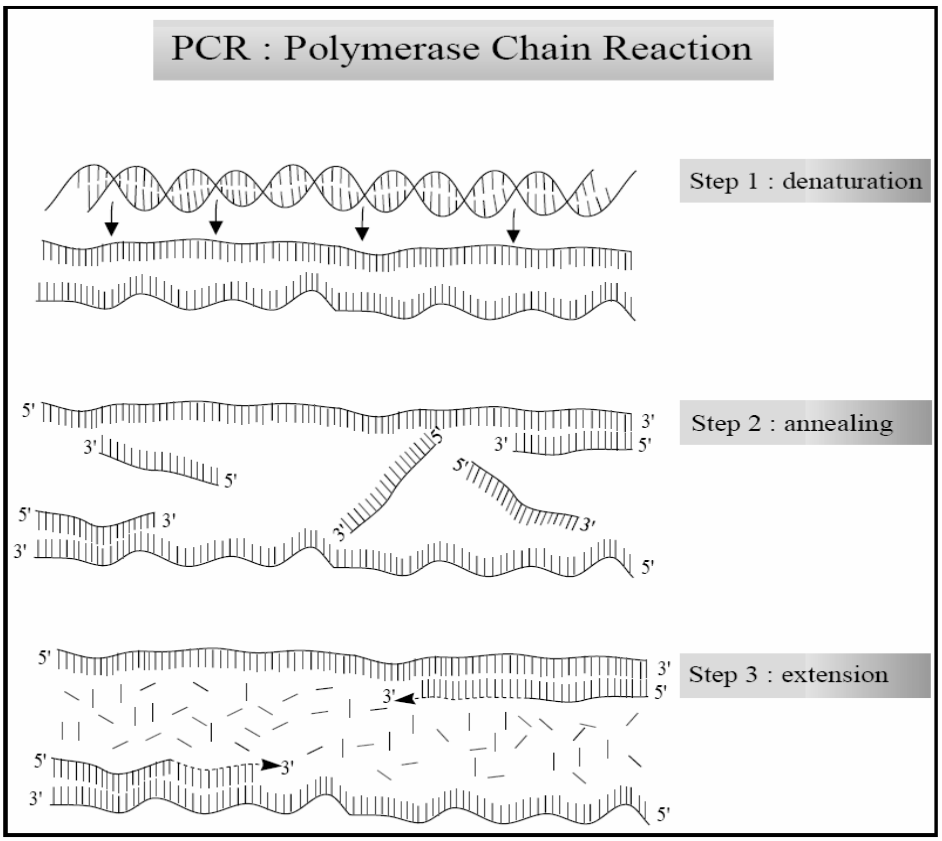


Figure 2.5 The steps of PCR amplification (Vierstraete, 1999)

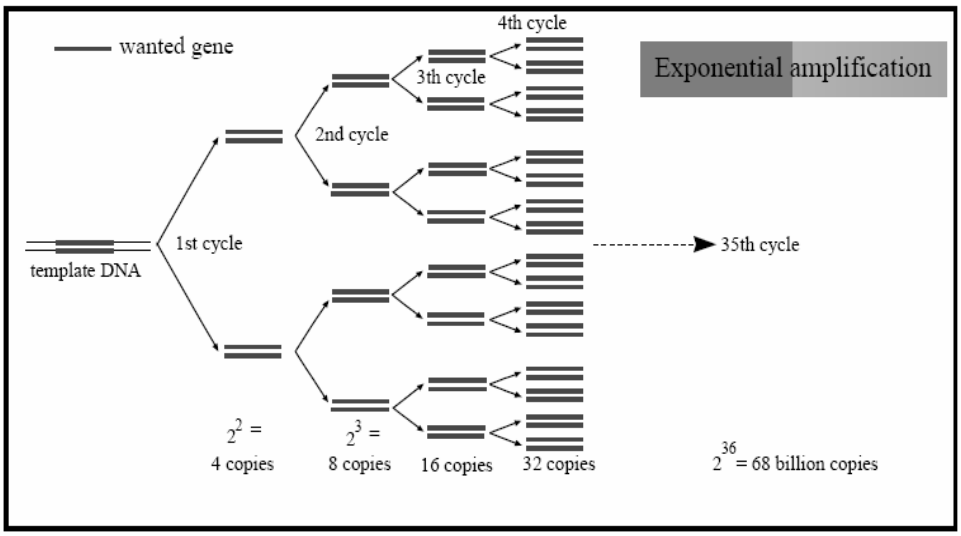


Figure 2.6 The exponential amplification of DNA in PCR (Vierstraete, 1999)

the DNA will partially melt to the branched structure of the single stranded molecule, then entangle in the gel matrix and reduce its mobility further. Therefore, different sequences within such domains have various melting temperatures, and molecules with different sequences will stop migrating at different positions in the gel, which therefore can be separated effectively by DGGE (Muyzer et al., 1993; Lerman et al., 1984). Complete melting of DNA fragments will lead to the formation of two single strands, which differ in halting position in the gradient gel. Optimal resolution is attained during PCR amplification using a PCR primer with a 5' tail consisting of a sequence of 30~40 base pair GC* clamps and that DNA will remain partially double-stranded (Muyzer et al., 1993). Before analyzing DGGE profiles of DNA fragments, it is necessary to determine the melting behaviors of the DNA fragments and proper primers which can amplify these DNA fragments. Some computer programs can be applied to predict the melting profile of a DNA sequence and precisely identify primer pairs with a proper GC clamp position. Furthermore,

*GC: A double stranded DNA is constituted of alternating units of phosphoric acid and deoxyribose, linked by cross-pieces of purine and pyrimidine bases. Each base in a strand will pair with only one kind of base to form a base pair: A (adenine) is always paired with T (thymine) and C (cytosine) is always paired to G (guanine). The amount of GC can affect the value of melting temperature (T_m) of DNA, which decides the position of DNA in DGGE. If one DNA has the same GC amount as another, but with different sequences, they will halt in the same position in DGGE.

in order to obtain the best separation of different DNA fragments, it is recommended to optimize gradient and gel running conditions, such as running voltages and times (Jiang, 2004).

PCR-DGGE technology has encountered great interest from researchers because of its merits: high detection rate and sensitivity, simple and non-radioactive, easy isolation of PCR fragments from the gel and use in sequencing reactions. In particular, this fingerprinting technique is necessary when high sample numbers are needed to understand ecological impacts on community (Petersen and Dahllöf, 2005). However, it is inevitable that some drawbacks appear in this combination of methods, such as biases in primer annealing, base pair mismatches, and limitations in DGGE-resolution. Hence, the number and intensity of bands in a DGGE gel do not always give a description of the number and abundance of corresponding species within the microbial community. One organism may produce more than one DGGE band due to multiple and heterogeneous DNA fragments. Also, one DGGE band may represent several species because of the limitation of its resolution. If DNA mixtures are present at very different concentrations, the less abundant sequences are not amplified sufficiently to be observed as bands on a DGGE gel (Muyzer et al., 1993; Boon et al., 2002). Because of these shortcomings inherent in 16S rRNA DGGE technology, therefore, further study on these issues is required to make DGGE technology more efficient.

2.4.2 Isolation approach

Isolation of the pure strains from complex bacterial community is a traditional and important method to cultivate microorganisms and study the microbial functions. However, this culture-based approach has suffered several limitations (Yi et al., 2003; Amann et al., 1995), which result in not adequately monitoring situations *in situ*. In addition, it is estimated that so far only about 10% of the extant microorganisms have been grown in pure culture and characterized (Amann et al., 1995). This is because some bacteria are viable but not culturable using conventional methods such as serial dilution and spread plate inoculation methods.

In addition, some culturable bacteria may prefer not to grow on current available media (Amann et al., 1995).

Although these disadvantages exist, culture-dependent methods appear to be more appropriate methods for microbial community analysis than culture-independent approaches in some cases (Ellis et al., 2003). Culture-independent methods, such as DGGE, can detect diverse microbial populations in the system, but can not analyze the behavior of all the individual species in a complex microbial community. Study of the isolates can provide detailed information to understand their physiologies and contributions in the system, especially on the functionally important populations. Also, this information will characterize these strains for further biotechnological application in bioaugmentation, bioengineering and DNA probe development (Jiang, 2004). Thus, the combination of both culture-independent and culture-dependent techniques can provide more complete and useful information about the bacterial community structures.

2.4.3 Microbial communities in MBRs

Information on microbial communities in MBRs could benefit the understanding of membrane fouling mechanisms and operational situations in order to design, control and optimize the process effectively. Recently advanced microbiological technologies and methods, such as Biolog Microplates, FISH and DGGE technology, have enabled us to know the detailed structure and composition of the bacterial population and compare the microbial communities in this mixed system (Kaiser et al., 1998; Luxmy et al., 2000; Witzig et al., 2002).

For example, Luxmy et al. (2000) and Li et al. (2005a) analyzed the bacterial community of a MBR system and compared them with those in a conventional activated sludge process by FISH and DGGE technology. It was found that the α- and ß- subclasses of Proteobacteria were the dominant groups in the MBR process and ammonia-oxidizers (eg. Nitrosomonas, Nitrosococcus, Nitrobacter etc) lived easily in this system. The same phenomenon was observed by Sofia et al. (2004) and Li et al. (2005a) that Nitrosospira spp. and Nitrospira spp. were the

predominant groups of the ammonia and nitrite oxidizing groups, respectively using FISH and DNA sequencing analysis in a submerged membrane bioreactor. It was also reported that the bacterial communities were constant over a long time and ß-subclass of Proterobacteria was detected as the dominant bacteria by FISH in a membrane filtration sludge process (Witzig et al., 2002). LaPara et al. (2006) employed PCR-DGGE technology to study the bacterial community structure of starch-fed MBRs and the results revealed numerous bacterial populations growing on a simple synthetic wastewater, suggesting substantial functional redundancy within the bacterial community.

However, so far very few studies have been conducted on the microorganisms in MBRs and the microbiological processes which are at the basis of the biological treatment are not yet well understood, especially, the diversity, the dynamics as well as the functions of the microorganisms. Thus, investigation in this area should be pursued in order to clearly explain EPS production and membrane biofouling mechanisms in MBRs.

2.5 Summary

Membrane bioreactors (MBRs) are suspended-growth biological reactors combined with a membrane unit process and are increasingly applied in wastewater treatment plants. However, membrane fouling in MBRs leads to higher operational cost than that in the conventional activated sludge processes although MBR investment costs can be lower due to the smaller footprint. It has been reported that extracellular polymeric substances (EPS), which are metabolic and lysis products of microorganisms, are the most critical substances contributing to membrane fouling in MBRs. Also, it was concluded by the researchers that operating parameters have a significant impact on the EPS production and composition. Therefore, reducing EPS production in MBRs by optimizing operating conditions would be a convenient way to minimize membrane fouling. Since EPS production is associated with bacterial growth, hence, further study on bacterial characteristics in MBRs will be useful to help to understand the mechanisms of membrane fouling caused by EPS and ultimately provide strategies to control the membrane fouling in MBRs.

CHAPTER 3

DYNAMIC STUDY ON THE EPS PRODUCTION UNDER DIFFERENT OPERATING PARAMETERS

3.1 Introduction

Generally, in the activated sludge treatment process, a period of time is required for microorganisms to adjust themselves to the environmental changes. This process involves the change of metabolic function of the microorganism, acclimatisation of individual species as well as adaptation of the bacterial population. If no further significant variations in the system happen, the bacterial populations and activated sludge characteristics are considered to attain a pseudo-steady state with respect to biomass concentration and effluent concentration (Gaudy and Gaudy, 1980).

In a MBR system, stable operation is desirable in order to maintain steady reactor performance and membrane filtration process, which is thought to alleviate membrane fouling. However in a real MBR plant, it is typical to treat a wide range of different industrial and domestic wastewaters with different chemical oxygen demands. This unsteady state and shock loading causes a change in the nature of the energy and carbon sources, which results in alternations of bacterial metabolic and synthesis processes (Gaudy and Gaudy, 1980). In addition, changes in operating parameters, i.e., SRT, HRT and aeration rate would occur when carrying out membrane cleaning or modifying the system. These operating parameters appear to have an effect on the state of bacterial growth. For example, SRT or mean 'sludge age' is linked to the Food-to-Microorganism (F/M) ratio, and at the steady state it is inversely related via the microbial growth coefficient (see Equation 2.6 in Chapter 2). HRT is a crucial control parameter in the activated sludge process, which exerts a profound influence on the hydraulic conditions and the contact time between the organic/inorganic substrate and activated sludge (Pan et al., 2004). The oxygen

concentration in the activated sludge process determines bacterial specific growth rate based on the Monod kinetic function. Thus, these environmental changes can easily cause severe disruption of the desirable pseudo-steady state by influencing bacterial characteristics.

Since EPS production is associated with bacterial growth characteristics, the production of EPS can be expected to be a function of the microbial population and its age, the nutrient and growth environment, and the hydrodynamic conditions including the degree of shear. Hence, it is inevitable that sudden changes of substrate concentration and operating parameters, from time to time, sometimes gradually and sometimes abruptly, would lead to variation of EPS production by the activated sludge. It has been suggested that the production of EPS is a possible survival strategy employed by bacteria to cope with fluctuations in water content (Wingender et al., 1999). Previous studies have pointed out that EPS characteristics of activated sludge in a MBR system can influence membrane filtration performance. Therefore, the effect of variations of the environmental factors on reactor operation by observing EPS production changes is an important consideration in a MBR system. However, study of the dynamic influence of operating parameters (under unsteady state) on EPS characteristics is still a field in its infancy. Thus, in this chapter, a dynamic study of EPS production (mg/gMLSS), concentration (mg/L), composition (mainly polysaccharides and protein) and distribution (attached EPS and soluble EPS) during the operation of the MBR under the effect of different parameters (substrate loading, SRT, HRT and aeration rate), together with reactor performance are presented. In subsequent chapters the effect of EPS characteristics on membrane fouling is discussed.

3.2 Materials and methods

3.2.1 Description of MBR system and operating conditions

3.2.1.1 MBR system set-up

The MBR consisted of a cylindrical vessel with a working volume of 3 L and a membrane module (Figure 3.1). The membrane module (Blue Star Pte Ltd, China) was immersed into the reactor and the design parameters are listed in Table 3.1. Air was introduced via two air diffusers below the module and also directly into the module to provide dissolved oxygen and prevent solids accumulation on the membrane surface. The synthetic wastewater was introduced into the bioreactor from the feed tank by a peristaltic pump. The liquid level was controlled by a level switch and the permeate (flux 30 L/m^2 hr) was extracted by a suction pump whose condition (on/off) was regulated by the level switch. When significant membrane fouling occurred, the membrane modules were removed and cleaned with Terg-A-Zyme (Alconox, USA) solution.

The seed sludge used in the reactor was municipal activated sludge from Jurong Wastewater Treatment and Reclamation Plant (Singapore). The seed sludge had been pre-cultivated and acclimatised in a 2 L beaker by a fill-and-draw operating mode for one week before feeding into the bioreactor. The substrate fed was the same synthetic wastewater as that in the MBR system.

Table 3.1 Design parameters of the MBR system

Reactor	Parameter	Membrane module	Parameter
Total volume	5 L	Material	Polyacrlonitrile (PAN)
Effective volume	3 L	Pore size	50,000 Dalton (≈0.045 μm)
Diameter	120 mm	Total membrane area	0.1 m^2
Height	300 mm	Module size	50 mm*150 mm

3.2.1.2 Compositions of the synthetic wastewater

The MBR system was fed with a synthetic wastewater including organic and inorganic compounds typical of real wastewater which are shown in Table 3.2. The TOC of this synthetic wastewater was 337.8 mg/L (theoretical COD was about 902 mg/L), and concentration of nitrogen and phosphorus was 78.5 mg/L and 31.1 mg/L, respectively. COD: N: P ratio was 100:8.7:3.5.

Table 3.2 Compositions of synthetic wastewater for the MBR system

Compound	Concentration (mg/L)	Compound	Concentration (mg/L)
Glucose($C_6H_{12}O_6$)	500	CH_3COONa	500
NH_4Cl	300	$MgSO_4.7H_2O$	100
KH_2PO_4	53	K_2HPO_4	107
$NaHCO_3$	40	$CaCl_2$	7.5
$FeCl_3$	0.5	COD: N: P	100:8.7:3.5

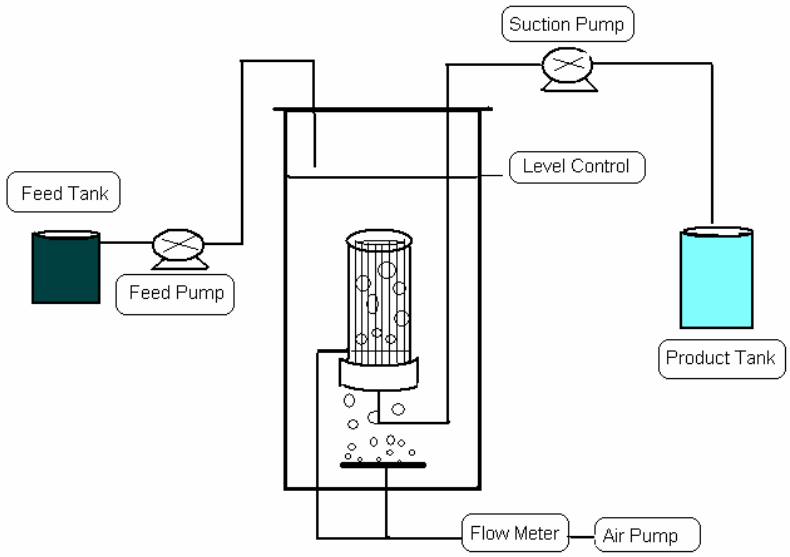


Figure 3.1 Schematic of the lab-scale MBR system

3.2.1.3 MBR operating conditions

The MBR was operated in 4 phases, as follows:

Phase 3.1: the MBR was operated at a constant SRT of 30 days by removal of 100 ml per day from the 3000 ml mixed liquor. During the period of operation, the TOC concentrations were fixed at 167 mg/L and 133 mg/L, corresponding to substrate volumetric loadings of 0.89 and 0.71 g COD L^{-1} day^{-1}. The F/M ratio varied over the range 0.3 to 0.9 day^{-1} (COD/MLSS) during the unstable stage. The HRT was fixed at 12 hours and the aeration rate was fixed at 2 L/min.

Phase 3.2: the MBR was operated at various SRTs from 10 to 60 days with fixed F/M of 0.5 day^{-1} (COD/MLSS), which was maintained by daily adjustment of feed to match the MLSS. Although this is not a common operating procedure for MBR systems, it was adopted with the specific objective of monitoring the EPS production under different SRTs without the effect of F/M. The HRT and aeration rate was maintained at 12 hours and at 2 L/min, respectively.

Phase 3.3: the HRT was regulated by a suction pump, which was solely dependent on effluent flowrate. The MBR was operated at SRT approaching infinity (about 150 to 200 days), which meant that the only sludge removed from the MBR was by sampling (about 20 mL/day). The F/M was maintained at 0.5 day^{-1} (COD/MLSS) by daily adjustment of feed to match the MLSS and the aeration rate was kept at 2 L/min.

Phase 3.4: Aeration rate was changed by regulating the flow rate of the air pump; the effect of this was to vary dissolved oxygen (see results below). The MBR was operated at SRT approaching infinity (about 150 to 200 days) and the F/M was maintained at 0.5 day^{-1} (COD/MLSS) by daily adjustment of feed to match the MLSS. The HRT was kept at 12 hours.

3.2.2 Analytical methods

The MBR was sampled periodically throughout the operating period. TOC was measured by TOC analyzer (Shimadzu, Japan). Dissolved oxygen (DO) and pH values were determined by DO meter (Fisher, USA) and pH meter (Hanna, England), respectively. MLSS was measured as proposed by Standard Methods (APHA, 1998).

3.2.2.1 EPS extraction

The EPS obtained from the surfaces of bacterial cells is defined as “attached EPS” and EPS exists in the supernatant is considered as “soluble EPS” in this study. The methods for EPS extraction introduced by Liu and Fang (2002), Azeredo et al. (1999) and Zhang et al. (1999) were considered and tested. A modified EPS

extraction method was proposed in order to determine the optimal values in this case. The microbial floc was harvested by centrifuging 10 ml samples at 4,000 rpm for 10 min (4°C). And after removing the supernatant, the microbial floc was dispersed in distilled water to keep the total volume of 10 ml. For microbial floc solution, 12 µl formaldehyde (37%) was added and kept for 1 hr at 4°C; then 0.8 ml NaOH (1 N) was added and kept for 3 hrs at 4°C. After that, the supernatant solution and microbial floc solution were centrifuged (13,200 rpm, 20 mins, 4°C) to remove the suspended solids before chemical component analysis.

3.2.2.2 EPS composition analysis

Polysaccharides and proteins are the dominant components typically in the extracted EPS. The sum of the amounts of total polysaccharides and proteins were assumed to represent the total amount of EPS in this study. Polysaccharides concentration in EPS was determined according to the phenol-sulfuric acid method with glucose as a standard by using chromatography at 490 nm (Dubois et al., 1956). The protein concentration was determined by binding of Bradford reagent to the protein with bovine serum albumin (BSA) as standard, which causes absorption maximum of the dye at 595 nm (Bradford, 1976). Considering the influence of formaldehyde in the EPS extraction process, a modified standard curve was obtained by adding the same ratio of formaldehyde into the standard BSA.

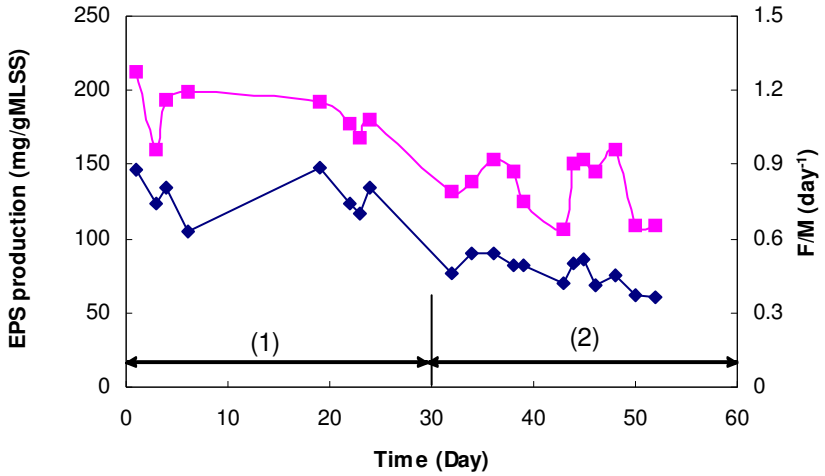
3.3 Results

3.3.1 Reactor performance

In the four phases, the MBR typically achieved 95% TOC removal capability due to the roles of biological degradation and membrane cut-off for the soluble TOC components with relatively large molecule weight. pH values registered in the reactor were about 6.0~7.0. DO values were kept at about 6.0~7.0 mg/L in Phase 3.1 and Phase 3.2. For Phase 3.3, DO was kept at about 5.0~6.0 mg/L.

3.3.2 The effect of substrate loading on EPS production

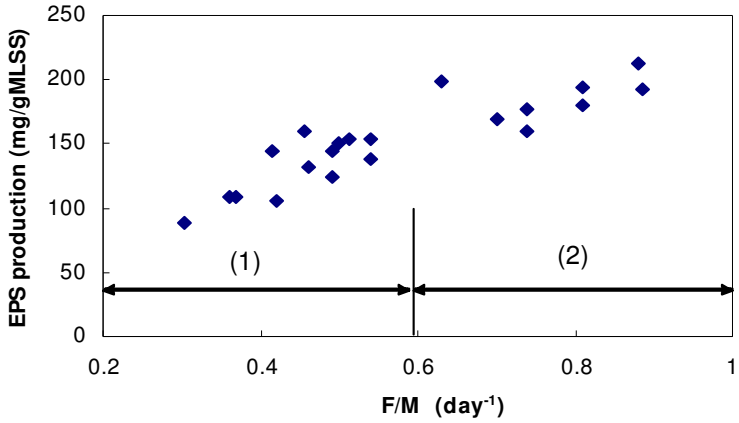
In this phase, the reactor was operated for 60 days. Two experimental periods of 30 days each were determined based on the two different substrate loadings adopted (0.89 and 0.71 g COD L^{-1} day^{-1}, respectively). When observing total EPS productions of the activated sludge (Figure 3.2), the results vary with the F/M ratios, which dropped as substrate loading decreased. Figure 3.3 shows that the total EPS production correlated strongly with the F/M ratios. This phenomenon is concurrent with the observation of Rosenberger and Kraume (2002). Hence, it suggests that F/M is a key factor influencing EPS production in a MBR system. In the lower F/M condition, it is expected that the microorganisms' allocation of the carbon source will preferentially be orientated towards their maintenance functions. Using the remaining substrate available, additional cell synthesis occurs (Low and Chase, 1999). When the energy exceeds the basic requirements for bacterial maintenance and anabolism, bacteria will take advantage of this part of surplus energy to produce EPS.



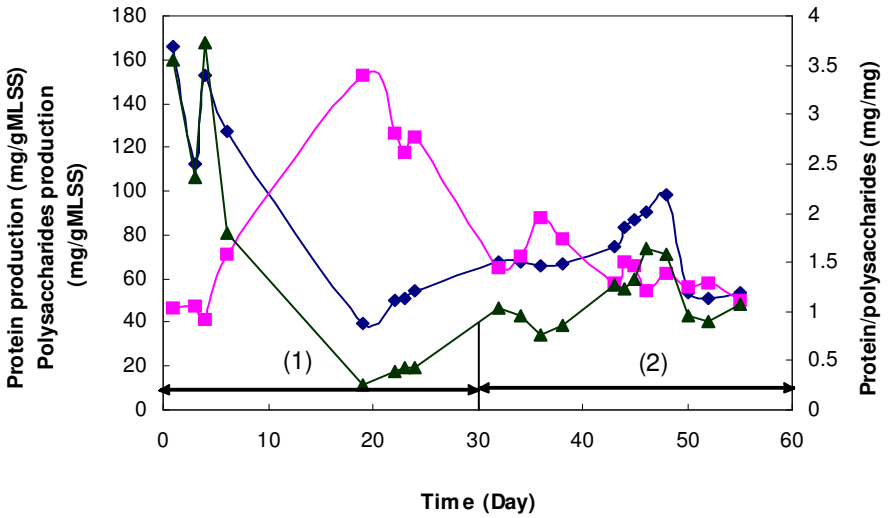
(1) TOC: 167 mg/L (2) TOC: 133 mg/L
Figure 3.2 F/M ratio and EPS production under different substrate loadings
(■) EPS production (◆) F/M ratio

As shown in Figure 3.4, at the beginning of the process, the protein production was dominant compared with the polysaccharide production. However by day 12, the total protein production had dropped below the rising polysaccharides production. Later in stage 1, the major component in EPS was polysaccharides rather than

protein. However when the substrate loading changed from 0.89 to 0.71 g COD L^{-1} day^{-1} (about day 30), the protein production increased from about 60 mg/gMLSS to 90 mg/gMLSS. It is probable that protein, in the form of enzymes excreted by microorganisms, is stimulated as a consequence of metabolic stress when the operating conditions are varied.

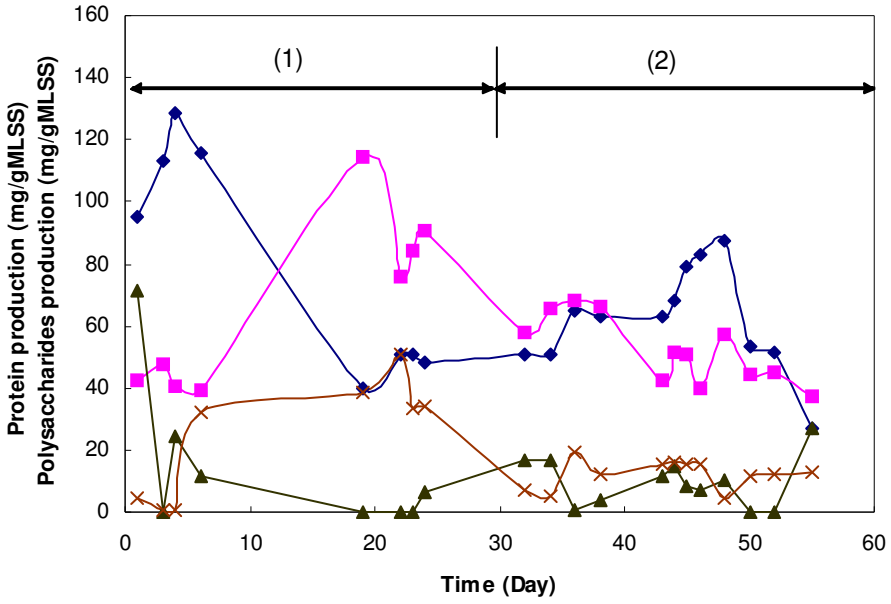


(1) TOC: 133 mg/L (2) TOC: 167mg/L
Figure 3.3 The effect of F/M ratio on EPS production



(1) TOC: 167 mg/L (2) TOC: 133 mg/L
Figure 3.4 EPS composition under different substrate loadings
(■) Polysaccharides production (◆)Protein production (▲) Protein/polysaccharides

During these two periods, the major portion of the produced EPS (typically 80 to 90 %) was attached to bacterial floc (Figure 3.5). Due to the shear force caused by aeration, a portion of the protein and polysaccharides could be detached from the bacterial floc into the liquid to form soluble EPS. In addition, a portion of the residual nondegraded glucose also existed in the reactor, and this would be measured as part of soluble EPS.

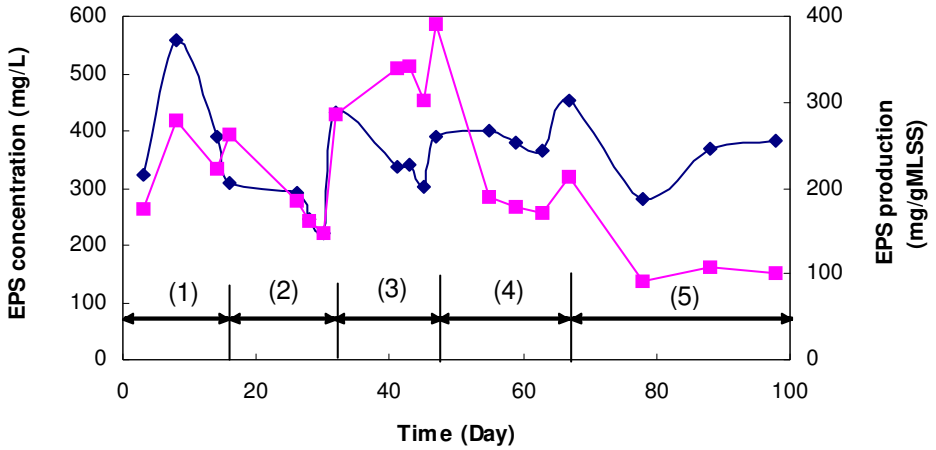


(1) TOC: 167 mg/L (2) TOC: 133 mg/L
Figure 3.5 EPS distribution under different substrate loadings
(■) Attached polysaccharides (◆) Attached protein
(×) Soluble polysaccharides (▲) Soluble protein

3.3.3 The effect of SRT on EPS production

In this phase, during 100 days' operation, five experimental periods of SRTs ranging from 10 days to infinity were employed. It should be recalled that this study focused on unsteady state responses so the usual criteria of stabilising to conditions at 3×SRT were not applied. Although SRTs were changed, by sludge wastage, the F/M was deliberately maintained at a value of 0.5 day^{-1}. As shown in Figure 3.6, a very strong and rapid increase of EPS concentration as well as production was observed immediately after changes of SRT. This variation was mainly due to protein production shown as Figure 3.8. Presumably the changes of SRT, which

changes the age distribution of microorganisms, would induce microorganisms to excrete more enzymes to adapt to the new environment. The averaged EPS production in each period was calculated and results revealed that the averaged EPS production tended to decrease with increasing SRTs (Figure 3.7). The trend agrees with the result of Rosenberger and Kraume (2002) and Chang and Lee (1998). However, EPS concentration was relatively independent with SRT in this study, possibly sharing a minimum at SRT 20 days.



(1) 10 days (2) 20 days (3) 30 days (4) 60 days (5) Infinity
Figure 3.6 EPS concentration (mg/L) and EPS production (mg/g) under different SRTs
(■) EPS production (◆) EPS concentration

The compositions of EPS at different SRTs are compared in Figure 3.8. At the shorter SRTs, the dominant component was polysaccharides, but as the SRT extended, the ratios of protein to total EPS were noticeably greater. Polysaccharides were probably excreted due to the extra carbon substrate at lower SRTs, while the protein, possibly enzymes, could be attributed to cell lysis at higher SRTs (Lee et al., 2003). It has also been reported that the changes in the ratio of protein to polysaccharides with respect to SRTs could be related to changes in both the growth rate and the microbial community of the sludge (Liao et al., 2000).

Figure 3.9 indicates that most of the produced EPS was attached on the surface of microbial floc. Under the shorter SRTs, the ratios of soluble protein and

polysaccharides to the total amount were higher than those under the longer SRTs. Reasons for this could include the effect of the lower biomass concentration at lower SRT on the viscosity and bubble rise velocity. This could cause more loosely attached protein and polysaccharides to be released from the surface of the microbial floc.

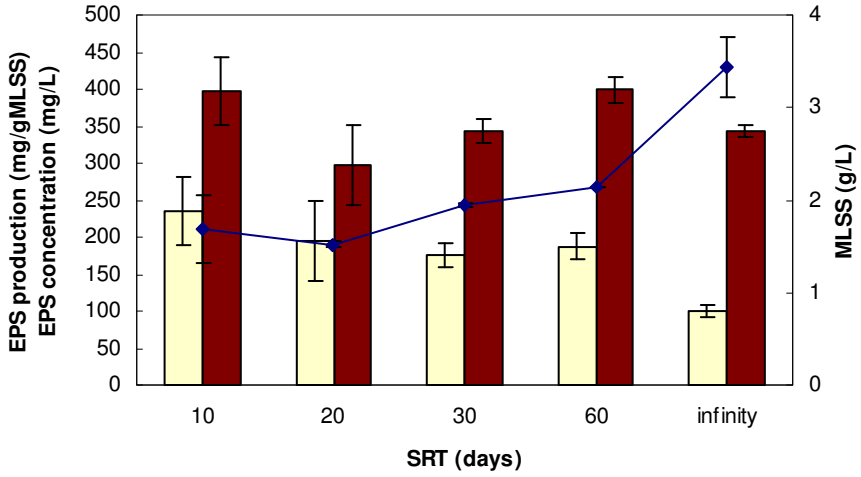
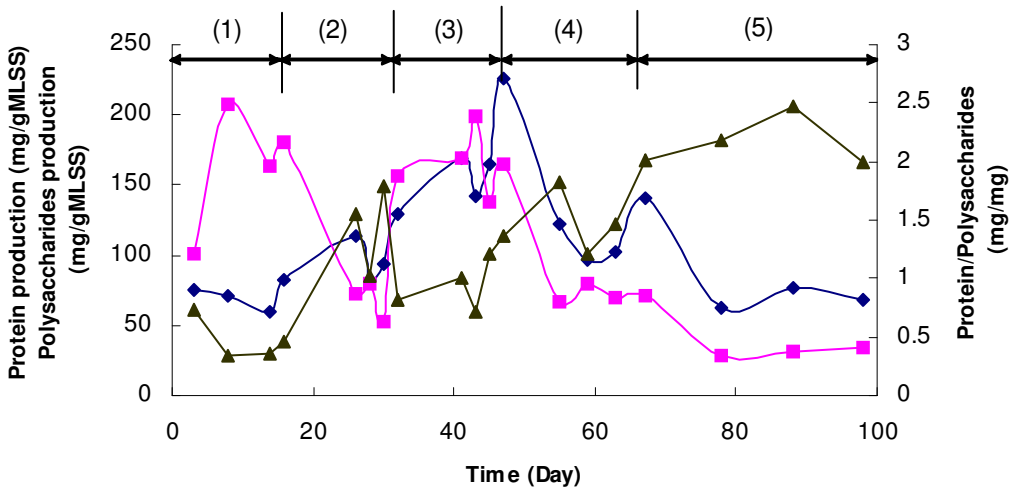
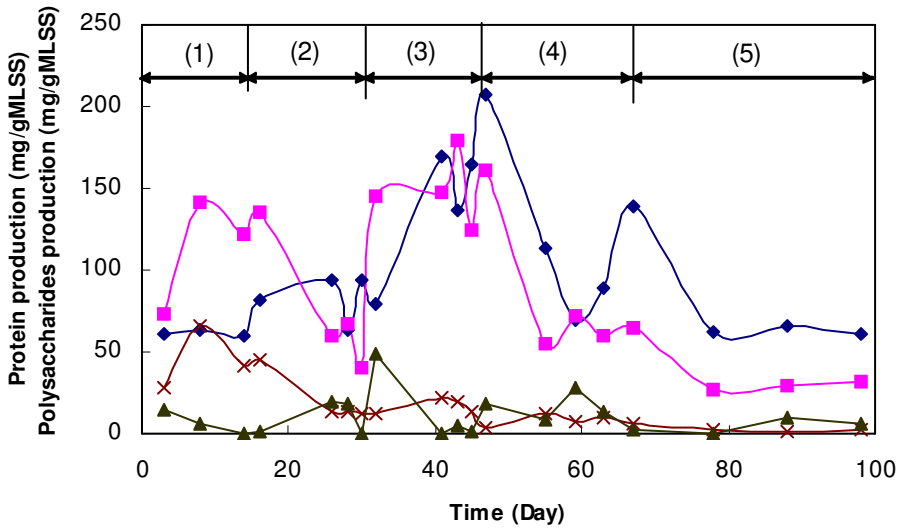


Figure 3.7 Average EPS production, EPS concentration and MLSS under different SRTs
(Light bar) EPS production (dark bar) EPS concentration (◆) MLSS



(1) 10 days (2) 20 days (3) 30 days (4) 60 days (5) Infinity
Figure 3.8 EPS composition under different SRTs
(■) Polysaccharides production (◆) Protein production
(▲) Protein/polysaccharides



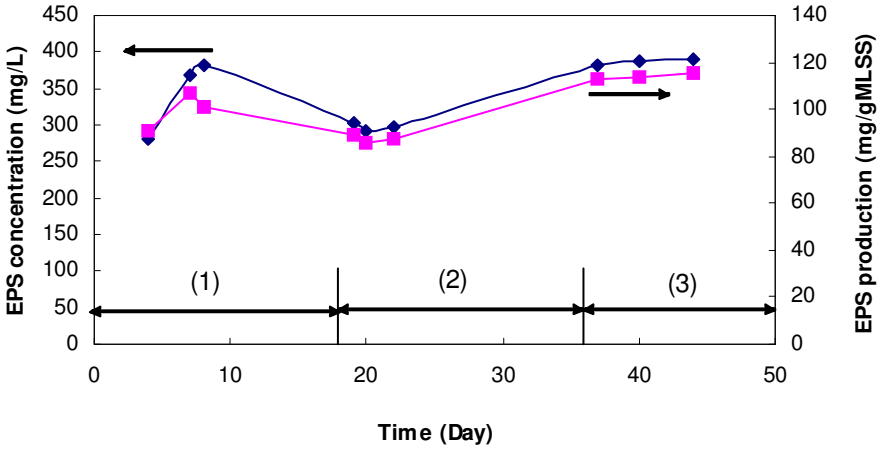
(1) 10 days (2) 20 days (3) 30 days (4) 60 days (5) Infinity
Figure 3.9 EPS distribution under different SRTs
(■) Attached polysaccharides (◆) Attached protein
(×) Soluble polysaccharides (▲) Soluble protein

3.3.4 The effect of HRT on EPS production

The experiment in this phase was performed in three periods with HRTs varying from 8 hours to 24 hours (Figure 3.10); the F/M ratio was maintained at 0.5 day^{-1}. In the initial period of the first stage, the total EPS concentration and production presented a progressive increase. However, this phenomenon was not observed in the other two stages after changes of HRTs, which illustrates that HRT was not a crucial factor to influence metabolic function of microorganism in this study. Figure 3.11 presents averaged EPS productions which were about 113.8, 99.7 and 87.6 mg/gMLSS under HRT of 8, 12 and 24 hours respectively, which indicates a slow decrease with increasing HRTs.

In terms of EPS composition, protein was more significant than polysaccharides in this phase (Figure 3.12). At HRT of 12 hours, the average ratio of protein to polysaccharides was about 2.2 g/g, which was higher than that at HRT of 24 hours (1.35 g/g) and HRT of 8 hours (1.5 g/g). Figure 3.13 shows the distribution of EPS components in the biofloc (attached) and supernatant (soluble). Apparently,

attached protein and polysaccharides production was much more than soluble protein and polysaccharides production, respectively.



(1) 12 hrs (2) 24 hrs (3) 8 hrs

Figure 3.10 EPS concentration (mg/L) and production (mg/g) under different HRTs (■) EPS production (◆) EPS concentration

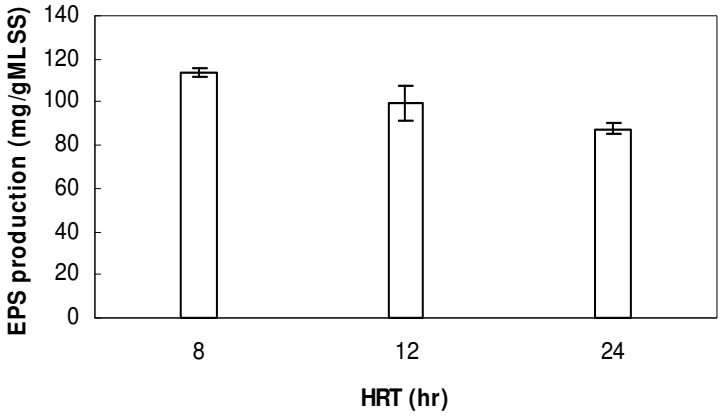
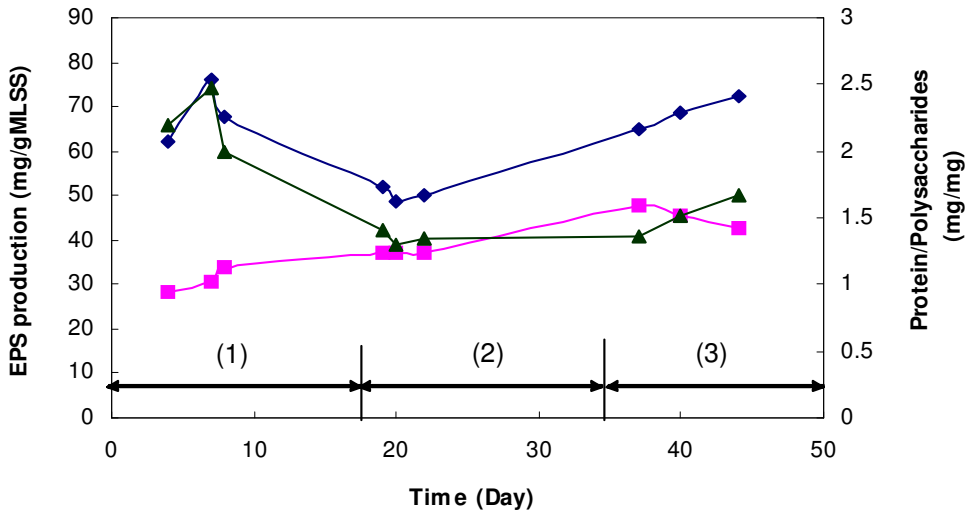
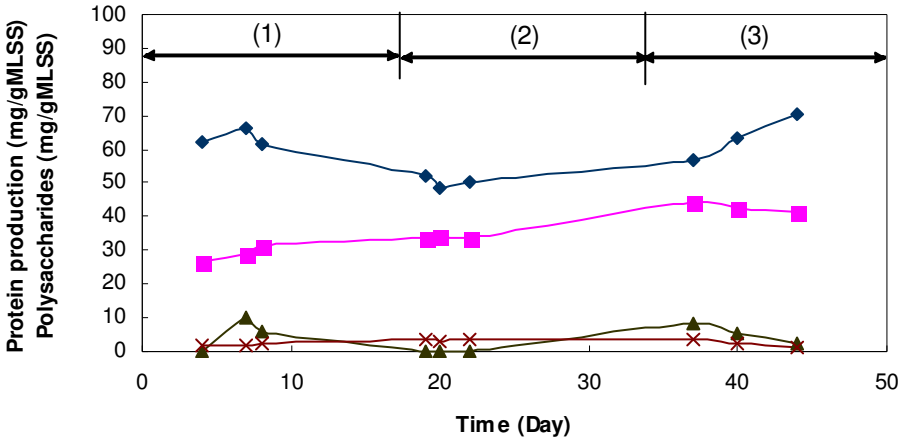


Figure 3.11 Average EPS production under different HRTs



(1) 12 hrs (2) 24 hrs (3) 8 hrs
Figure 3.12 EPS composition under different HRTs
(■) Polysaccharides production (◆) Protein production
(▲) Protein/polysaccharides

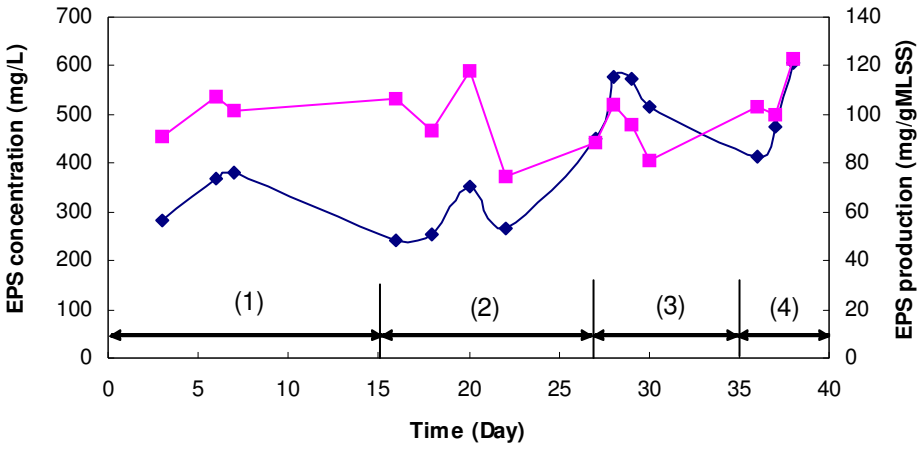


(1) 12 hrs (2) 24 hrs (3) 8 hrs
Figure 3.13 EPS distribution under different HRTs
(■) Attached polysaccharides (◆) Attached protein
(×) Soluble polysaccharides (▲) Soluble protein

3.3.5 The effect of aeration rate on EPS production

In this study (Phase 3.4), the aeration rate in the MBR system was gradually increased from 2 L/min to 6 L/min. Figure 3.14 shows that there was a sudden rising trend in total EPS production after the aeration rate shifted. However, the

averaged EPS productions under different aeration rates were quantitively similar (Figure 3.15) which illustrates that EPS production was little influenced by the aeration rate in the MBR. Theoretically, increasing aeration rate would enhance shear forces and increase the dissolved oxygen content. However the experimental results showed when aeration rate changed from 2 to 4 L/min, the dissolved oxygen amount jumped from 5.7 to 6.6 mg/L. With further increase in aeration rate from 4 to 6 L/min step by step, the dissolved oxygen values only improved 3%. Thus it was considered that the dissolved oxygen amount was adequate even for 2 L/min aeration based on the amount of substrate and biomass concentration. The surplus oxygen provided by the higher aeration rate could not be utilized by the activated sludge, which resulted in a higher dissolved oxygen concentration in the reactor (Figure 3.15). Therefore, the minor DO changes appear to have caused negligible changes in EPS production although the aeration rate increased from 2 to 6 L/min.



(1) 2 L/min (2) 4 L/min (3) 5 L/min (4) 6 L/min
Figure 3.14 EPS concentration (mg/L) and production (mg/g) under different aeration rates
(■) EPS production (◆) EPS concentration

The protein content of the EPS was highest at the lowest (2 L/min) or highest (6 L/min) aeration rates (Figure 3.16). A significant jump in the protein production was observed at the beginning of each aeration rate change, which is thought to be as the main component leading to the change in total EPS production. The EPS

distributions were relatively independent of the aeration rates as shown in Figure 3.17. Although a greater shear force would be obtained at higher aeration rate, an increase in viscosity may have counteracted this because the biomass concentration increased with time without sludge removal. Hence, the ratios of attached EPS to total EPS appeared to have less variation at different aeration rates.

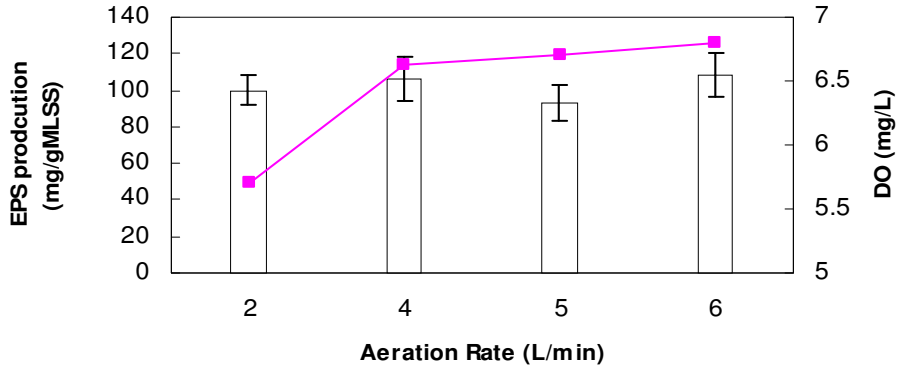
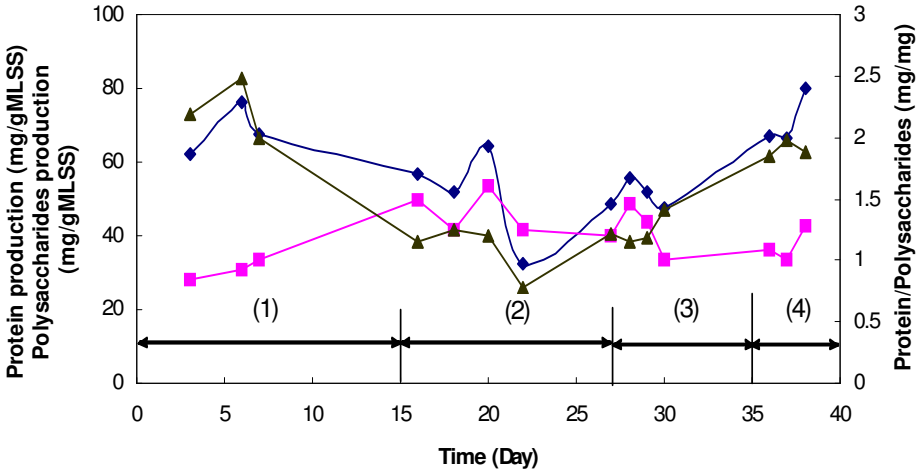
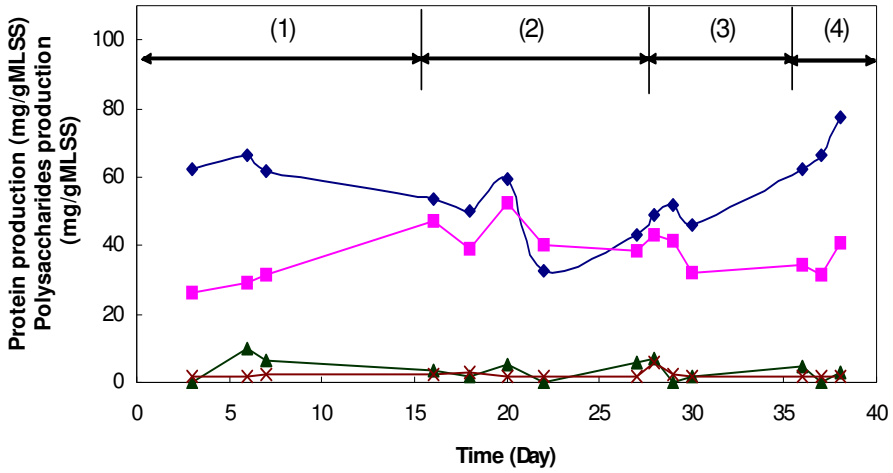


Figure 3.15 Average EPS production under different aeration rates (Light bar) EPS production, (■) Dissolved oxygen



(1) 2 L/min (2) 4 L/min (3) 5 L/min (4) 6 L/min
Figure 3.16 EPS composition under different aeration rates
(■)Polysaccharides production (◆) Protein production (▲) Protein/polysaccharides



(1) 2 L/min (2) 4 L/min (3) 5 L/min (4) 6 L/min
Figure 3.17 EPS distribution under different aeration rates
(■) Attached polysaccharides (◆) Attached protein
(×) Soluble polysaccharides (▲) Soluble protein

3.4 Discussion

In this study, variations of EPS production were demonstrated when the operating parameters (substrate loading, SRT, HRT and aeration rate) were suddenly changed. Steady operation is required in a MBR system in order to achieve stable reactor performance and biomass properties. For these conditions, the membrane resistance in the reactor gradually increases under subcritical flux operation, which should delay serious membrane fouling occurring. However, in a real MBR plant, the influent concentration is varied over time. At a result, membrane cleaning is deemed necessary to maintain sustainable MBR system operation. These unavoidable changes will directly lead to variations of microorganism metabolisms and functions in the reactor in order to adapt to the new surroundings. Such adaptation of microbial communities might arise as a result of a combination of different mechanisms, such as gene transfer or mutation, enzyme induction and population changes (Zhuang et al., 2005). Changes in EPS production might also be mediated by exposing the microbial communities to perturbations of the operating parameters. Hence, inevitably, these changes will cause destabilizing reactor performance and varying activated sludge filtration characteristics in MBRs. The

variation of membrane fouling tendency with operating parameters is examined in Chapter 4 and 5.

The results in this study also proved that the periodic and sequential changes of operating parameters in a MBR system could significantly influence EPS production and composition. In the four phases examined, in the initial period of changing operating parameters, the EPS production and concentration significantly jumped, which can be attributed to variations in protein production. In addition, the EPS distribution (attached vs soluble) in these four phases was independent of the shifts of operating parameters. In all cases the attached protein and polysaccharides contributed much more to total EPS than soluble components. Presumably enzyme activities in the mixed liquor are associated with the biofloc cells and/or other particulate materials.

Biological oxidation is the core process for eliminating the available components in the wastewater to reach the aim of degrading organic/inorganic substances to end products, such as carbon dioxide and water (Abbassi et al., 1999). The microorganisms utilize the substrate as their energy source to maintain their growth, generate new cells, and synthesize endogenous energy substances inside the cells. The state of the microorganisms is closely related to the type and the amount of substrate provided in the wastewater treatment process, which in turn indirectly impacts EPS production. In a MBR system, the F/M ratio expressed as activated sludge loading is an important factor to affect EPS production, and is calculated by dividing the amount of the treated organic carbon per day by the amount of the microorganism concentration. The results in this study clearly indicate that EPS production was positively correlated with F/M ratio. At the lower F/M condition, the energy and nutrient sources are provided primarily for maintenance of bacterial non-growth energy-demanding activities. This is the concept of maintenance energy firstly introduced by Pirt in 1965, which is regarded as the amount of biochemical energy strictly necessary for endogenous respiration (Pollice et al., 2004). Additional substrate is utilized for the net growth of microorganisms. When the energy amount provided by higher substrate loading exceeds the basic bacterial

requirement, bacteria will take advantage of this part of surplus energy to produce EPS. A similar phenomenon was also observed by Yoon (2003).

EPS production (mg/g MLSS) in Phase 3.2 appeared to be related to the activated sludge age because the same F/M ratios were employed in the five stages. In general, a greater amount of EPS might be expected at higher SRT due to the predominance of endogenous metabolism, but this was not observed. Based on our protocol an amount of sludge was removed daily from the reactor according to the SRT (ie 10% for a 10 day SRT), and following this there would be a period of time when the F/M would be higher than the 'daily average' fixed ratio, before it approached the fixed value due to the growth of biomass. The lower the SRT, the greater the incremental removal and the longer the F/M overshoot. So, it was possible that at lower SRTs biomass responded to increasing food by producing more EPS.

HRT is employed to describe the hydraulic conditions and the contact time between the substrate and microorganisms. In this study, different HRTs were realized by controlling the flowrate and concentration of influent in order to keep stable substrate volumetric loading in each stage. The results showed that EPS production slowly decreased with extending HRTs. An increase of HRT results in a lower feed flow rate, which extends retention time for substrate in the reactor and provides more chance for substrate to contact with microorganisms. It is inferred that these conditions benefit microorganism metabolism with a reduced protein production, which cause a small decrease in EPS production.

The degradation of the substrate should be more complete by the microorganisms with an optimal amount of oxygen. When the microorganisms are exposed to a high oxygen concentration, it is subjected to a condition of substrate limitation according to the description of the Monod-type kinetic for carbon source and oxygen concentration. Under this condition, the dead cells present are available as degraded substrate, which is called the auto-oxidation of the biomass. However, it has been reported that hydrolysis of the organisms appeared to be slower than either the death or synthesis processes (Abbassi et al, 1999). Thus, although stepwise increase

in aeration rate was applied, the EPS productions changed insignificantly which indicates that the dissolved oxygen was over the critical requirements of the microorganisms in the MBR at all aeration rates. Others (Yun et al., 2006; Jin et al., 2006; Ma et al., 2006) have reported an effect of DO on EPS production but this is usually at low, or approaching anoxic conditions. In the experiments reported here the DO levels were always in the high range.

In summary, this chapter has examined the effects of operating parameters on EPS production, concentration, composition and distribution. In all cases the major portion of the EPS was associated with the biofloc. Following changes in parameters (such as substrate loading, SRT and aeration rate) the total EPS production at the beginning of each stage tended to rise mainly as proteins (presumably enzymes in response to stress). The parameters most likely to cause changes in EPS production were shown to be substrate loading and SRT. In Chapters 4 and 5 the effect of operating parameters and EPS on membrane fouling are examined.

CHAPTER 4

MBR PERFORMANCE AND BACTERIAL COMMUNITY STRUCTURE UNDER DIFFERENT OPERATING CONDITIONS IN PARALLEL MBR SYSTEMS

4.1 MBR performance and bacterial community structure under different substrate loadings

4.1.1 Introduction

In the activated sludge process, the key role of bacteria is to decompose organic/inorganic materials in the influent. A portion of the organic/inorganic waste is utilized by microorganisms to obtain energy for the synthesis of cellular material, including growth of new cells and production of excreted substances. At the same time, part of the active biomass undergoes decay to form residual dead cells (Laspidou and Rittmann, 2002). MBRs allow use of relatively high MLSS and this provides good effluent quality. However, development of high biomass concentration could increase oxygen demand, decrease oxygen transfer and potentially increase fouling tendency. This means that the cost in aeration requirement and membrane cleaning could be significant operating costs of MBRs. However a low-concentration substrate in the influent will reduce treatment capability of MBRs, and also indirectly increase its operating cost. It is expected that an optimal substrate loading could be applied in MBRs, which could produce stable reactor performance and mitigate membrane fouling.

However, there is limited research in this area and a need for better understanding of the EPS characteristics and membrane fouling tendency of a MBR system fed with different substrate loading. In this work, the EPS production and composition (protein and polysaccharides) as well as membrane fouling tendency of activated sludge under different substrate loadings, together with the reactor performance are

presented. Shifts in the bacterial populations were monitored by DNA-PCR-DGGE, and help to explain the EPS production mechanism under different substrate loadings.

4.1.2 Materials and methods

4.1.2.1 MBR operating conditions

The set-up of the three MBR systems was described in section 3.2.1.1 (Chapter 3). The seed sludge was from the reactor operated in Phase 3.4 (Section 3.2.1.3, Chapter 3). The initial MLSS concentrations for the three reactors were 5 g/L. The substrate composition was shown in Table 3.2 and the feed TOC concentrations for the three reactors were fixed at 106 mg/L, 212 mg/L and 424 mg/L, respectively. The three MBR systems were operated at a SRT of 30 days. The aeration rate was kept at 3 L/min and the HRT was maintained at 12 hours for the three MBR systems.

4.1.2.2 Analytical methods

The methods for MLSS, pH, TOC and EPS extraction and composition analysis were as described in section 3.2.2 (Chapter 3).

4.1.2.3 Ionic concentration

The concentrations of ionic groups (NO_2^- , NO_3^-) were measured by Ionic Chromatography (Shimatsu, Japan).

4.1.2.4 Particle size

Microbial floc size was measured by Particle Analyzer (Mastersizer, USA), and the particle size of the supernatant was measured by ZetaPALS (Brookhaven, USA).

4.1.2.5 Microscopical examination of sludge structure

Samples for examination were taken directly from three different locations in the reactor in order to ensure a representative sample of the biomass. In order to determine details of cell morphology, sludge samples were placed on glass slides, mounted with a cover slip and examined after cell immobilization by microscope (Keyvene, Japan).

4.1.2.6 DNA extraction

The activated sludge was centrifuged (13,000 rpm, 10 min), and then the suspended cell pellet (100-200 mg wet weight) was placed into a 2 ml BeadBeater tube, to which 800 µg baked glass beads had been added. Then 50 µl 20% SDS was added to the BeadBeater tube which was topped off with saturated phenol (pH 8.0), and the samples were then lysed in a BeadBeater machine (Biospec products, USA) for 3 min (5000 oscillations/s). The tubes were placed in a 60°C water bath for 5 minutes and then spun for 10 minutes in a centrifuge machine (Eppendorf, Germany) at 4°C. The aqueous layer was extracted with saturated phenol (600 µl, pH 8.0) before being extracted twice with phenol (pH 8.0) and chloroform (4:1= 400 µl: 100 µl), and twice with chloroform (450 µl). Then 1/10 volume 20% sodium acetate and 2 volumes 95% ethanol were added for the nucleic acids to precipitate overnight. The precipitates were spun for 10 minutes in a micro-centrifuge before removing supernatant and washing the pellet twice with 80% ethanol (500 µl). Then the pellet was dried in a micro-vacuum machine (Tomy, Japan). Further DNA purity was checked by absorbance measurement at wavelengths of 260 nm and 280 nm. The pellet was suspended in 105 µl MilliQ water and frozen at -20°C until ready to use (Zhuang et al., 2005).

4.1.2.7 PCR conditions

Polymerase chain reaction (PCR) primers 357F (5'-GCC CGC CGC GCG CGG CGG GCG GGG CGG GGG CAC GGG GGG CCTCCT ACG GGA GGC AGC AG-3') and 517R (5'-ATT ACC GCG GCT GCT GG-3') were used to amplify the variable V3 region of bacterial 16S rRNA (Muyzer et al., 1993). A 50 µl mixture containing 1.25 U of *Taq* DNA polymers, 5 µl 10× Buffer B, 4 µl 25 mM $MgCl_2$ was prepared with deoxynucleoside triphosphate (including deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxythymidine triphosphate, deoxycytidine triphosphate), at a concentration of 200 µM (Promega, USA), 25 pmol of each primer and 0.5 µl of the DNA solution. PCR was performed in a thermal cycler (Eppendorf, Germany) under the following conditions: 10 min of activation of the polymerase at 94°C before two cycles consisting of 1 min at 94°C, 1 min at 65°C and 2 min at 72°C. Then the annealing temperature was decreased by

1°C for every second cycle until it reached 55°C. Subsequently, 20 additional cycles were carried out also at 55°C; finally, a 10 min extension process was performed at 72°C. In order to confirm the proper size of the PCR products, 1.5% (wt/vol) of agarose gel in 1× TAE buffer was used to run electophoresis with ethidium bromide staining (Zhuang et al., 2005).

4.1.2.8 DGGE analysis of DNA fragments

A denaturing gradient gel electrophoresis (DGGE) system (BIORAD Decode, USA) was employed to analyze the DNA fragments. A 10 % (w/v) acrylamide solution (40% acrylamide and bisaceylamide, 37.5:1 stock solution, Bio-Rad, USA) in 1× TAE buffer (Bio-Rad, USA) was used, with urea-formamide denaturing gradient gel ranging from 30-70%. Electrophoresis was performed at a constant voltage of 90 V for 15 hours. After that, the gel was stained with ethidium bromide for 20 minutes and destained in 1× TAE buffer for 1 hour. The gel was viewed and photographed by a Gel Imaging System (Kodak, USA) (Watanabe et al., 1998; Luxmy et al., 2000). GelCompar II (Applied Maths, Kortrijk, Belgium) was used to obtain the Similarity Index, which was employed to evaluate the differences and similarities of the bands observed in two lanes of the DGGE profile. The Similarity Index was automatically calculated with the Dice coefficient, without band weighting (band density) by the unweighted pair group method with arithmetic mean algorithms (UPGMA).

4.1.2.9 Membrane fouling assay

In order to study the influence of EPS and biomass concentrations on membrane fouling, a membrane fouling assay was set up as shown in Figure 4.1.1. A UF membrane (Millipore, USA, Polyethersulfone, hydrophilic, 50,000 Dalton, membrane area: $1.256*10^{-3}$ m^2) was used in the filtration cell (capacity: 110ml). A mixed sample of 20 mL was then placed in the cell and during filtration the volume was maintained by replenishing with distilled water from the reservoir (capacity: 2 L). The operating pressure was maintained at about 103 kPa by regulating nitrogen gas pressure. The transmembrane pressure changes with time were measured by the two pressure transducers (Cole-palmer, USA). The flux was kept at about 30

L/m^2*hr, which was controlled by the permeate pump and the accurate flux value was determined by weighting permeates on an electronic balance (Mettler-Toledo, Switzerland). The two pressure transducers and balance were connected to a personal computer equipped with data log system (LabVIEW, National Instruments, USA).

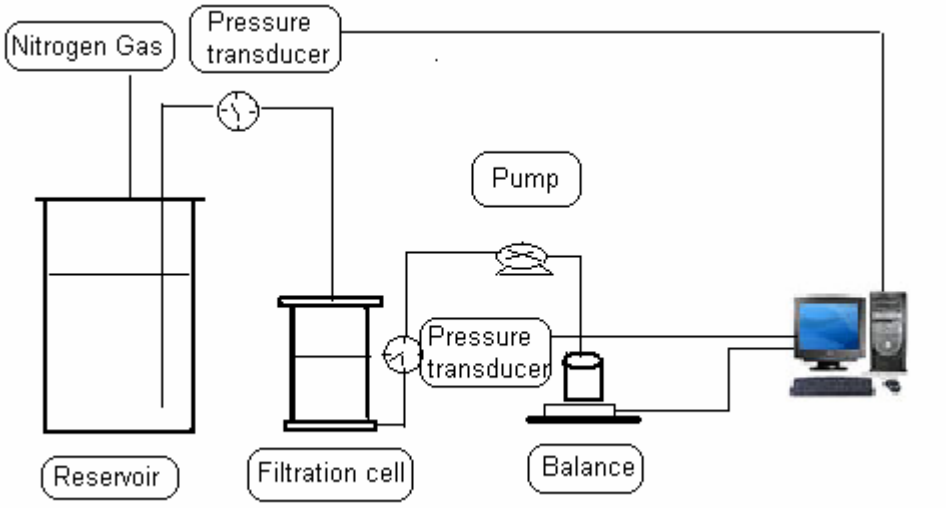


Figure 4.1.1 Schematic of the membrane fouling assay (dead-end mode)

According to the equation described by Darcy's Law (Belfort et al., 1994) as follows, the total resistance can be calculated,

$$R_t = \frac{\Delta P}{\mu \cdot J} \qquad [4.1]$$

In which, J means permeate flux (L/m^2/hr), ΔP means the transmembrane pressure (kPa), μ means the permeate viscosity (Pa S), R_t means the total resistance (m^{-1}). Here,

$$R_t = R_m + R_c \qquad [4.2]$$

R_m is the clean membrane hydraulic resistance (m^{-1}) and R_c is the cake layer resistance and irreversible resistance of the activated sludge (m^{-1}). Before the membrane fouling assay, all membranes were rinsed by floating skin-side down in distilled water and the rinsing water was changed several times to remove impurities from the membrane surface. The membrane resistances (R_m) were then measured using distilled water.

The specific resistance (α_c [m kg^{-1}]) of the foulant was calculated from equation [4.3],

$$\alpha_c = R_c / m_c \quad [4.3]$$

where Rc (m^{-1}) is the measured cake resistance at 60 minutes and m_c (kg m^{-2}) is the amount of material deposited per unit of membrane surface during the test. The membrane fouling rate (dRc/dt) was defined as the fouling resistance change over 60 minutes. The results from several samples at steady state were averaged and error bars were calculated based on their standard deviations. The rationale for these short-term fouling tests was to apply a standard constant flux filtration protocol to mixed liquor samples. Using moderate constant flux (30 L/m^2*hr) the fouling tendency was assumed to approximate longer term operation.

4.1.3 Results

4.1.3.1 MBR performance under different substrate loadings

During the operating period, the effluent TOC of the three reactors was within 10 mg/L, which indicated that the organic removal capabilities were high and stable. The three reactors had an initial biomass concentration of 5 g/L and F/M ratios of 0.11, 0.22 and 0.44 day^{-1}, respectively. Obvious variations of F/M ratios were observed for the three reactors in the start-up phase (Figure 4.1.2). After about 50 days, the biomass concentration had changed to 2.2, 4.0 and 8.4 g/L for the three reactors. It was judged that the three reactors had reached steady state as evidenced by stable organic removal and biomass concentrations. At steady-state the F/M was relatively constant at about 0.27 day^{-1} for the three reactors because increased MLSS compensated for the increased organic loading rates. There was no significant difference on pH for the three reactors, whose values were fixed at 6.0~7.0. The dissolved oxygen amounts were about 6.9, 5.4 and 0.6 mg/L for R1, R2 and R3 when the reactors were at steady state.

The activated sludge had an initial mean particle size (d_{50}) of about 120 μm (with a range of 10 ~ 800 μm) for the three reactors. A slight increase in mean particle size was observed for R1 over the whole period, rising to about 148 μm at steady state

(Figure 4.1.3). In R2, the mean particle size was almost constantly for 10 days and then dramatically jumped to about 257 μm in several days. After that, mean particle size slowly increased to about 400 μm until the end of the operation. There were similar increasing trends of mean particle size in R3 as that in R2 before 45 days, but after 45 days a sharp drop was observed and the mean particle size was averaged at about 190 μm at steady state. It is possible that when substrate loading increased, biomass concentration raised which consumed more dissolved oxygen and impeded effective oxygen transfer (Tay et al., 2003). This situation could have resulted in the aerobic condition converting temporarily to anaerobic conditions in R3. By measuring the ionic concentrations of NO_2^- and NO_3^- in the three reactors, it was confirmed that nitrate and nitrite disappeared after 45 days in R3, which implies the transformation to nitrogen under anoxic conditions.

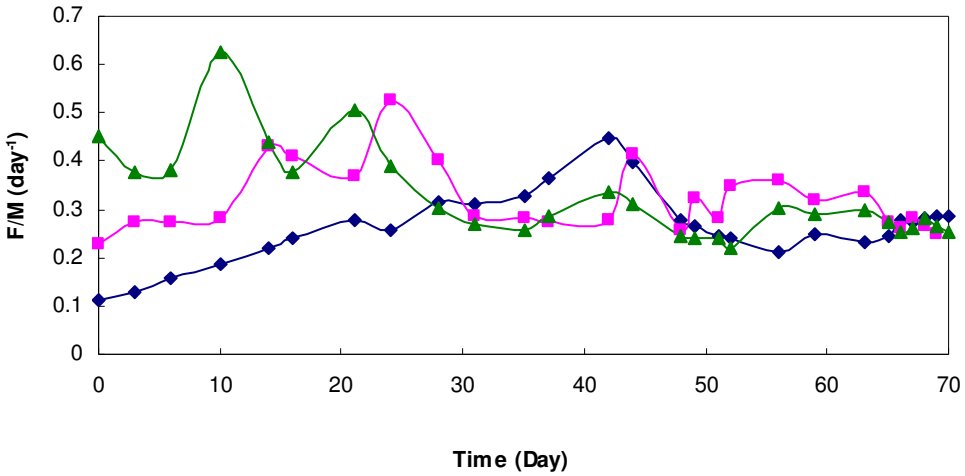


Figure 4.1.2 F/M changes under different substrate loadings
(◆) R1 (■) R2 (▲) R3

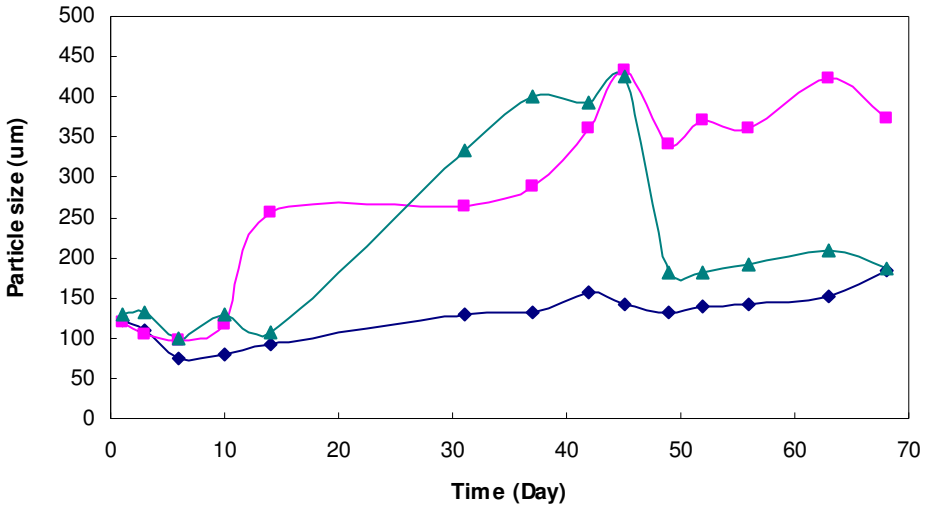


Figure 4.1.3 Particle size changes under different substrate loadings (◆) R1 (■) R2 (▲) R3

4.1.3.2 EPS analysis under different substrate loadings

As excreted and lysed substances of microorganisms, EPS consists of various organic components: polysaccharides, proteins, lipids, nucleic acids and other bio-polymers. In this study, the sum of total polysaccharides and proteins was considered to represent the total amount of EPS due to their predominant contribution to EPS (Bura et al., 1998). The trends for total EPS production (mg/g MLSS, pellet + supernatant) showed a significant increase in the initial phase of the operation for the three reactors. EPS production approached a peak of 163 mg/gMLSS on day 14 for R1. The highest EPS productions in R2 and R3 were on about day 8, with approximately 117 and 99 mg/gMLSS, respectively. After that, EPS production dropped gradually and stabilized to about 102, 66 and 58 mg/gMLSS for R1, R2 and R3, respectively (Figure 4.1.4). This indicates that EPS production (mg/g MLSS) decreased with increasing substrate loading as other operating parameters fixed. A possible explanation is that this was due to the limited dissolved oxygen amount in the R2 and R3 under a fixed aeration rate with higher biomass concentrations as compared to R1. In order to confirm the effect of DO on EPS production, purified oxygen was introduced with the air (still keeping the same aeration rate) into R3 to improve the DO value to about 6 mg/L. It was observed that at the same time the EPS production of R3 increased from about 60

g/L to 90 g/L. The findings of Nielsen et al. (1996) support this issue, by showing that prolonged exposure to low DO conditions resulted in the inhibition of EPS production as well as hydrolysis and degradation of EPS. In addition, EPS concentrations (mg/L) in the three reactors achieved stable values after 30 days, which was maintained at about 176, 221 and 601g/L for R1, R2 and R3, respectively (Figure 4.1.5). This illustrates that EPS concentrations have contrary trends to EPS production, due to the effect of increased MLSS as substrate loading increased.

A clear increase in total protein production (protein production from pellet + protein production from supernatant) and polysaccharides production (polysaccharides production from pellet + polysaccharides production from supernatant) was also observed at the beginning of the operation. Following that, obvious fluctuations of protein and polysaccharides productions as well as the ratios of protein to polysaccharides occurred as shown in Figure 4.1.6, Figure 4.1.7 and Figure 4.1.8. When the reactors were at the stable stage, protein productions averaged at 49, 28 and 31 mg/gMLSS and polysaccharides productions averaged about 53, 34 and 26 mg/gMLSS for the three reactors, respectively. This reveals that with substrate loading increasing, a significant decrease was observed in total polysaccharides production. However, substrate loadings have little influence on the ratios of total protein to total polysaccharides.

Up to this point we have discussed total EPS, proteins and polysaccharides; that is the total comprising the component associated with the biomass plus that in the supernatant. It is apparent from Figure 4.1.9 that the EPS attached on the bacterial surface was dominant compared with soluble EPS. By inspecting the compositions of EPS shown as Figure 4.1.10 and Figure 4.1.11, the results indicate the same trend, as expected that the attached protein and polysaccharides were much more than soluble protein and polysaccharides respectively. However, since MBRs typically operated below the critical flux of the biomass floc, the supernatant components could be significant. This is because the filtered liquor is predominantly supernatant.

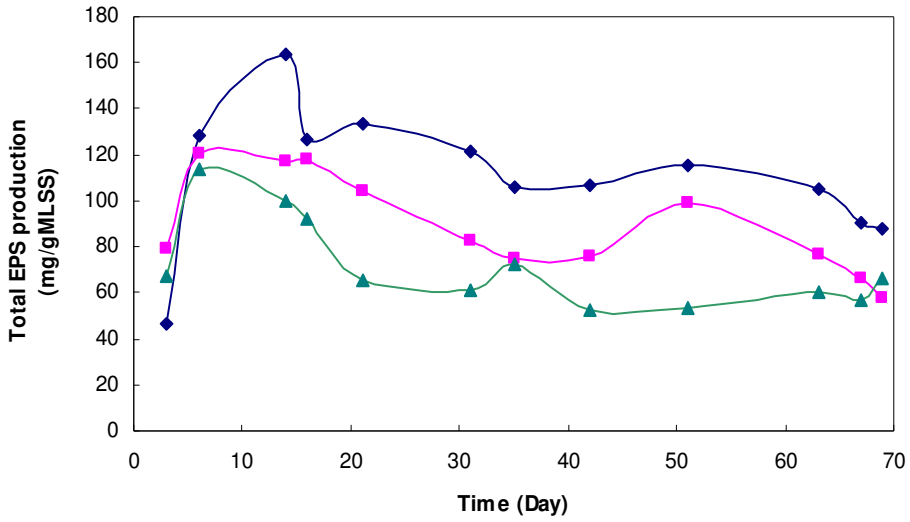


Figure 4.1.4 Total EPS production under different substrate loadings (◆) R1 (■) R2 (▲) R3

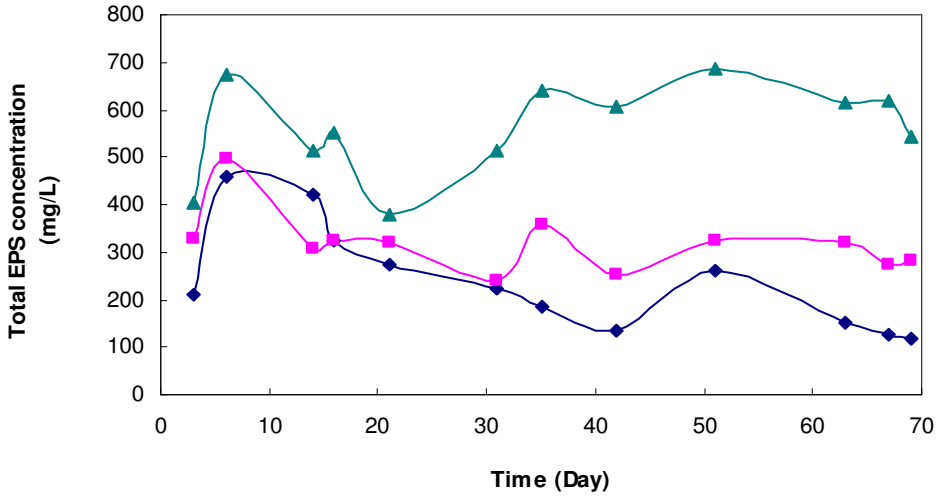


Figure 4.1.5 Total EPS concentration under different substrate loadings (◆) R1 (■) R2 (▲) R3

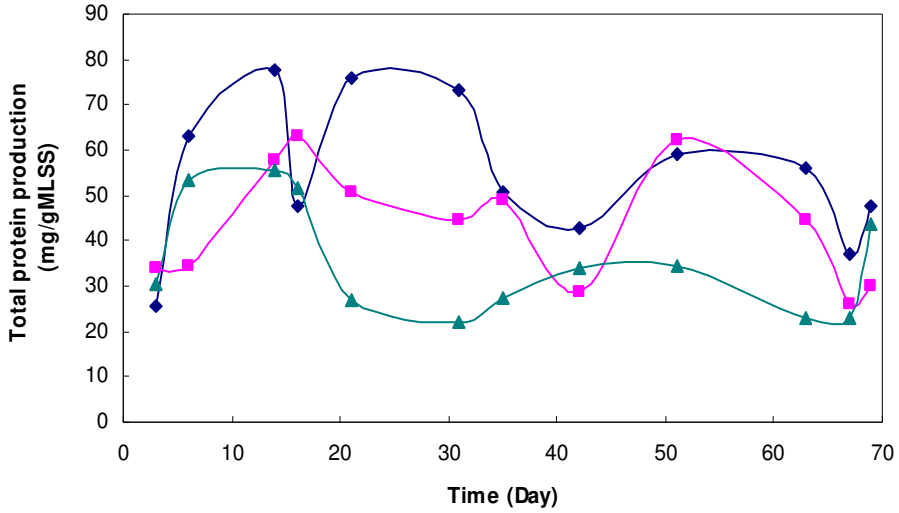


Figure 4.1.6 Total protein production under different substrate loadings (◆) R1 (■) R2 (▲) R3

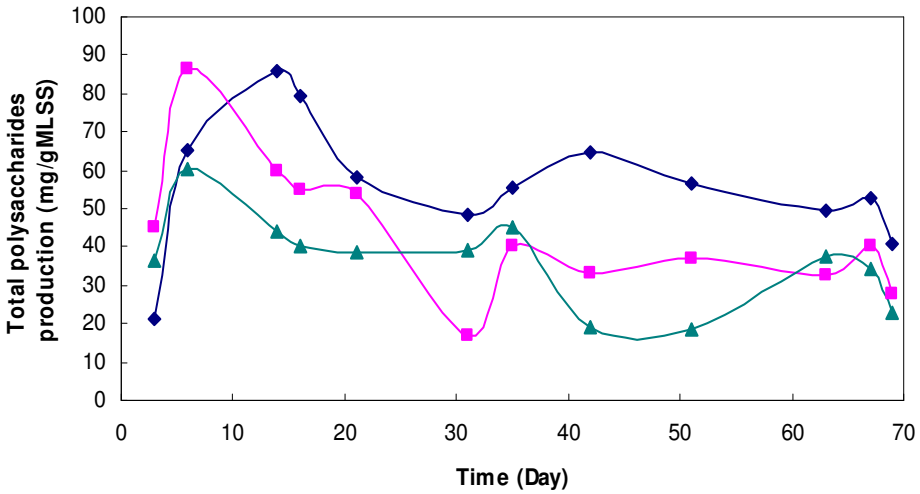


Figure 4.1.7 Total polysaccharides production under different substrate loadings (◆) R1 (■) R2 (▲) R3

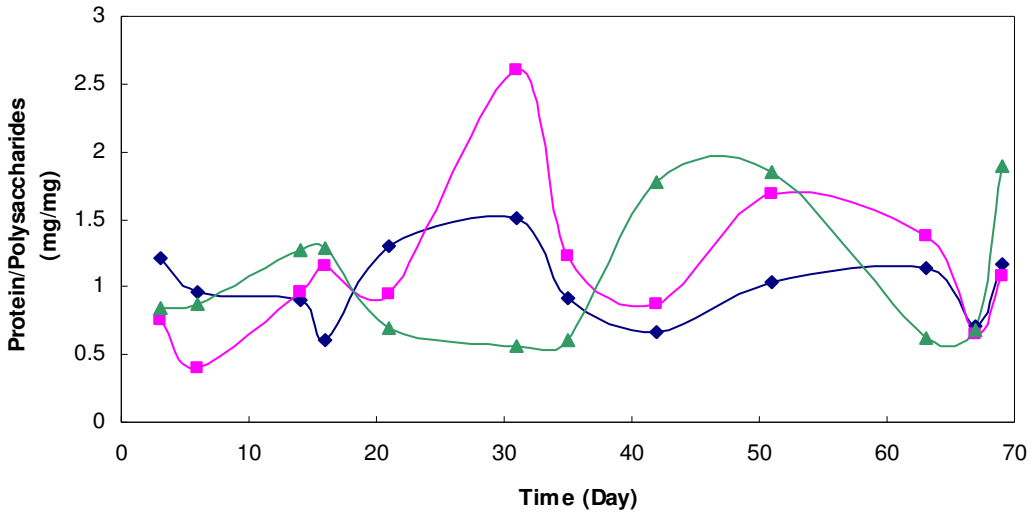


Figure 4.1.8 Ratios of protein to polysaccharides under different substrate loadings (◆) R1 (■) R2 (▲) R3

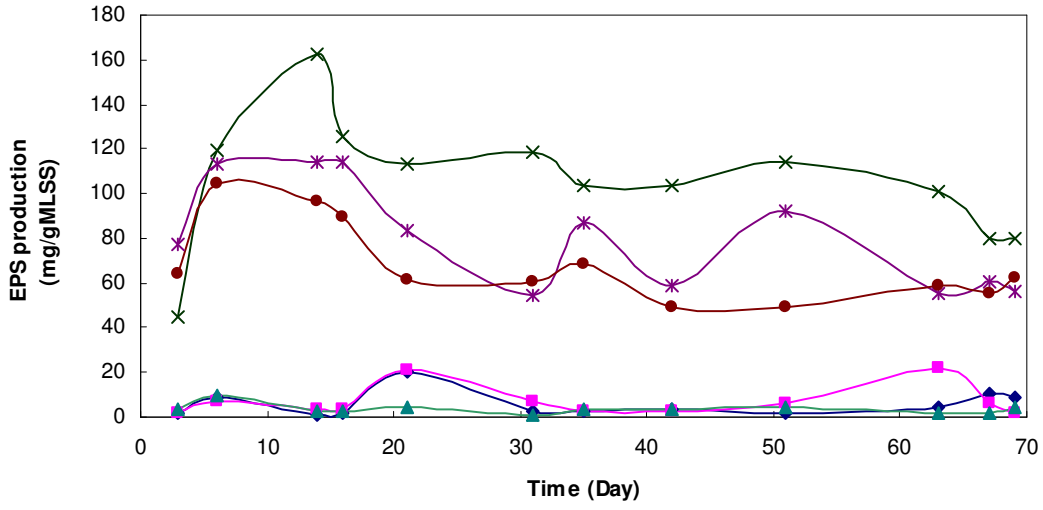


Figure 4.1.9 EPS distribution under different substrate loadings (◆) Soluble EPS (R1) (■) Soluble EPS (R2) (▲) Soluble EPS (R3) (×) Attached EPS (R1) (*) Attached EPS (R2) (●) Attached EPS (R3)

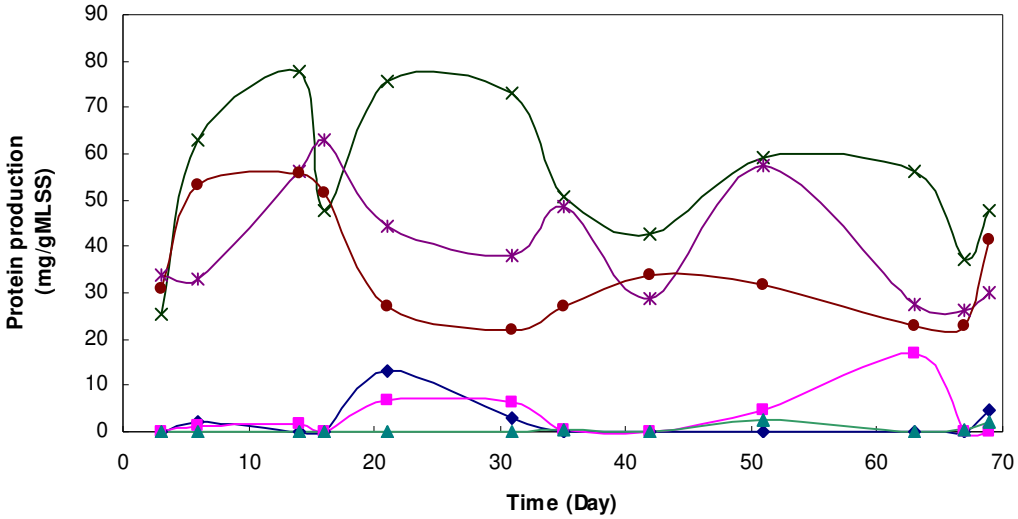


Figure 4.1.10 Protein distribution under different substrate loadings
(◆) Soluble protein (R1) (■) Soluble protein (R2) (▲) Soluble protein (R3)
(×) Attached protein (R1) (*) Attached protein (R2) (●) Attached protein (R3)

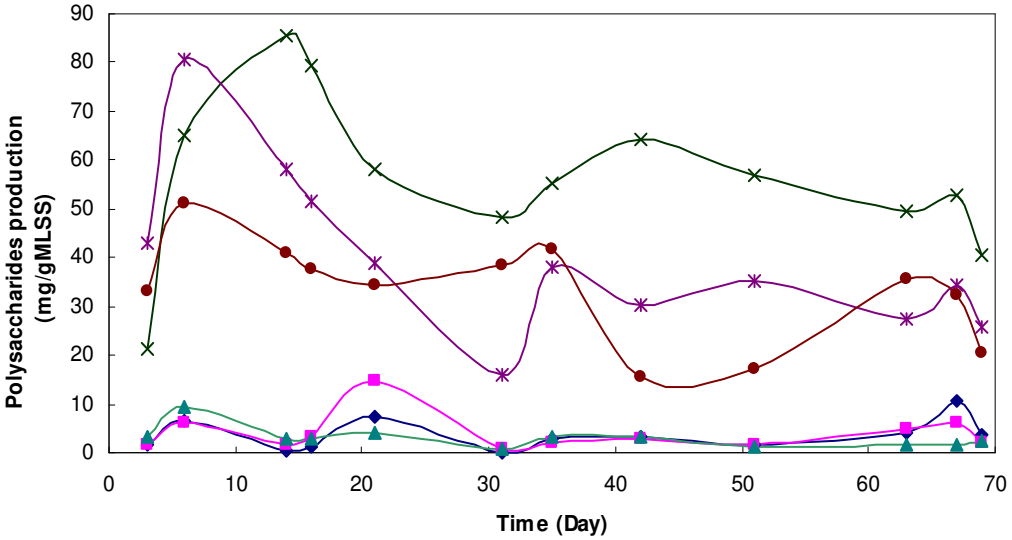


Figure 4.1.11 Polysaccharides distribution under different substrate loadings
(◆) Soluble polysaccharides (R1) (■) Soluble polysaccharides (R2)
(▲) Soluble polysaccharides (R3) (×) Attached polysaccharides (R1)
(*) Attached polysaccharides (R2) (●) Attached polysaccharides (R3)

4.1.3.3 Membrane fouling tendency under different substrate loadings

Several samples were taken from each reactor during the steady state and average values were calculated as the data for the graph (Figure 4.1.12). It was observed that

EPS concentrations in R1, R2 and R3 were 176, 221 and 601 mg/L, which indicates an increasing trend with increased substrate loadings. Correspondingly, in R1, R2 and R3 specific fouling resistances of activated sludge were 1, 1.33 and $4.70*10^{13}$ m/kg and membrane fouling rates of activated sludge were 0.53, 0.98 and $11.24*10^{10}$ m^{-1}/min, respectively. The data show that increasing substrate loading increased EPS concentration in the membrane bioreactor, which caused more serious membrane fouling. Compared with the contribution of total polysaccharides concentration to membrane fouling tendency, increase in total protein concentration was more significant (Figure 4.1.12). However, it is recognized that the data in Figure 4.1.12 can not be used to conclude that either protein or polysaccharides are the dominant foulant. Although not shown in Figure 4.1.12, soluble EPS concentration (7.1, 15.9 and 29.4 mg/L for R1, R2 and R3, respectively) also correlated with total EPS concentration and membrane fouling tendency. A more detailed analysis of fouling components is given in Chapter 5.

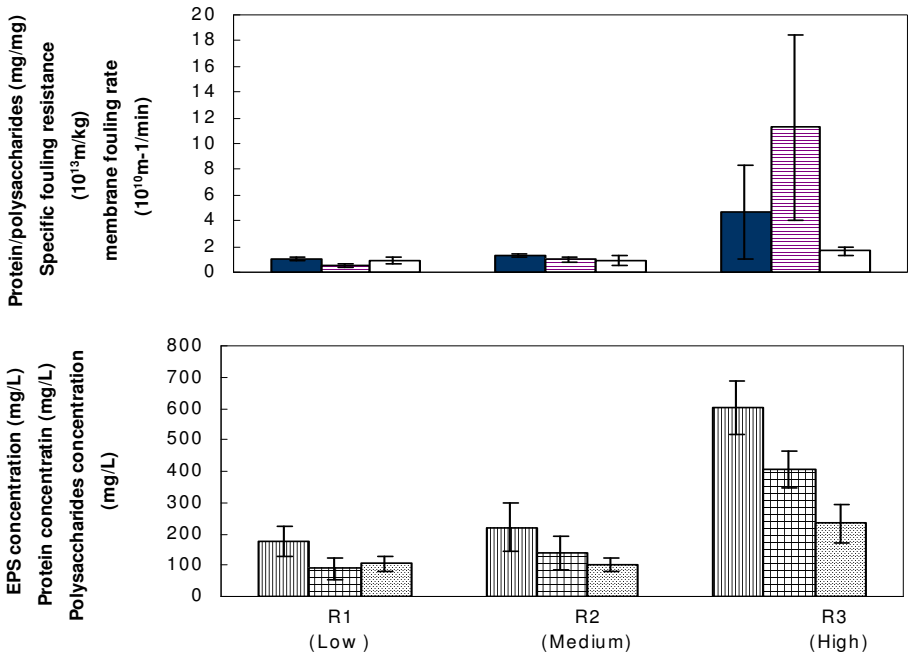


Figure 4.1.12 Relationship between membrane fouling tendency and EPS characteristics at the steady state under different substrate loadings (Dark bar) Specific fouling resistance (Horizontal stripe bar) Membrane fouling rate (Light bar) Ratio of protein to polysaccharides (Vertical stripe bar) Total EPS concentration (Square bar) Total protein concentration (Dot bar) Total polysaccharides concentration

4.1.3.4 DGGE analysis under different substrate loadings

PCR amplification and DGGE analysis of community 16S rRNA genes were performed to produce genetic fingerprints that could provide information on the composition and diversity of the microbial communities under different substrate loadings. Banding pattern similarity was analyzed by applying the Dice coefficient with Arithmetic Mean (UPGMA) algorithms (Figure 4.1.14, Figure 4.1.16 and Figure 4.1.18). DNA samples were extracted from the activated sludge of each reactor periodically.

The initial DGGE profiles were similar for the three reactors due to the same biomass seed being employed (Figure 4.1.19). From day 3 to 56, the DGGE profiles (Figure 4.1.13, Figure 4.1.15 and Figure 4.1.17) show population shifts in each reactor. It is evident that some species could propagate in the specific conditions which benefited their growth. However, their competitors were not favored and disappeared from the reactor due to the operating conditions. As the reactors approached a steady state (Figure 4.1.13, 15 and 17, day 49 onward), the bacterial

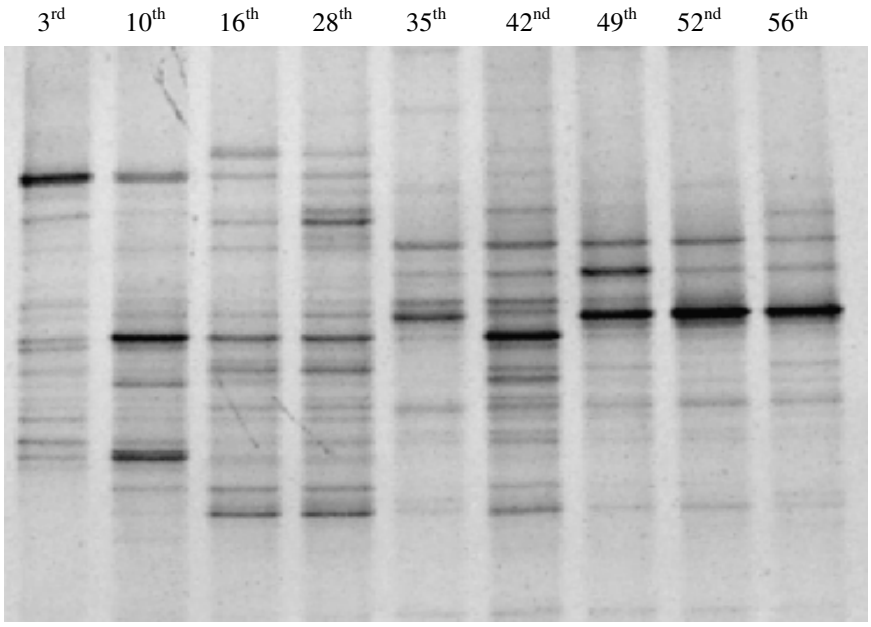


Figure 4.1.13 DGGE of PCR-amplified 16S rRNA from the bacterial communities in the Reactor 1 (samples taken on days 3 to 56, as shown)

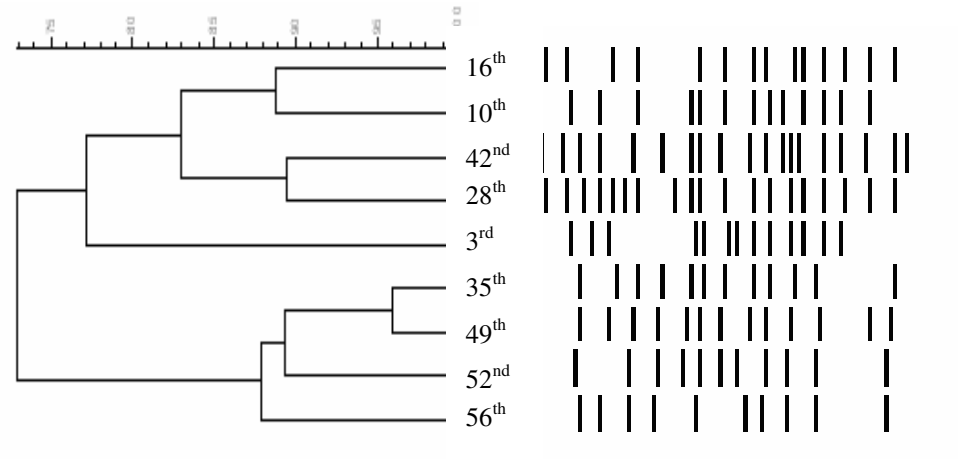


Figure 4.1.14 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns in the Reactor 1 (low substrate loading)

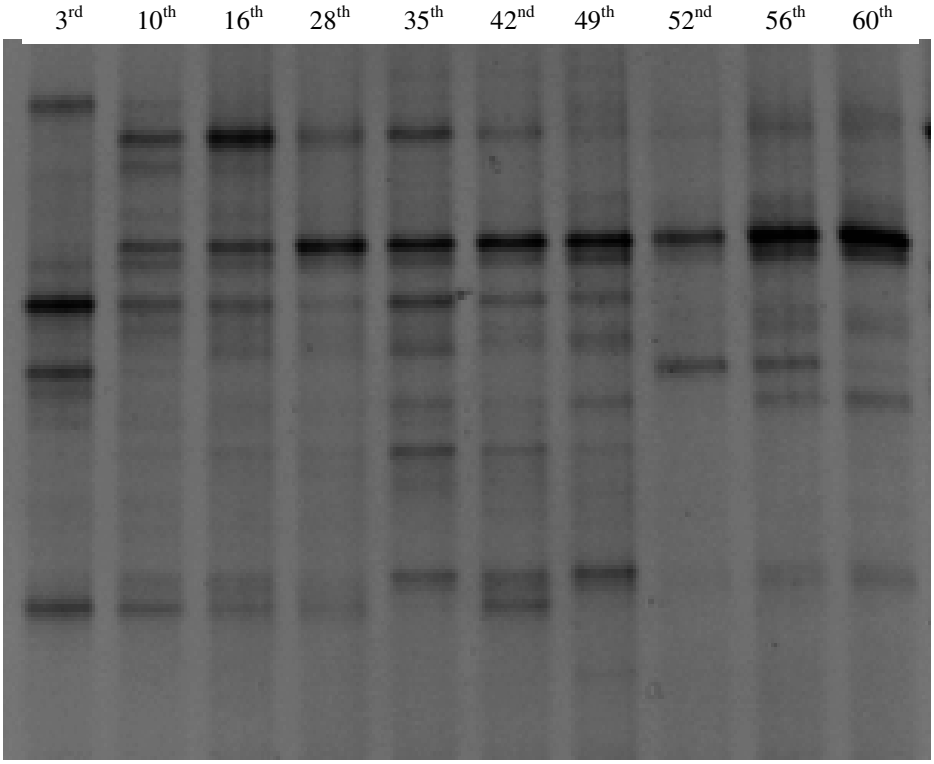


Figure 4.1.15 DGGE of PCR-amplified 16S rRNA from the bacterial communities in the Reactor 2

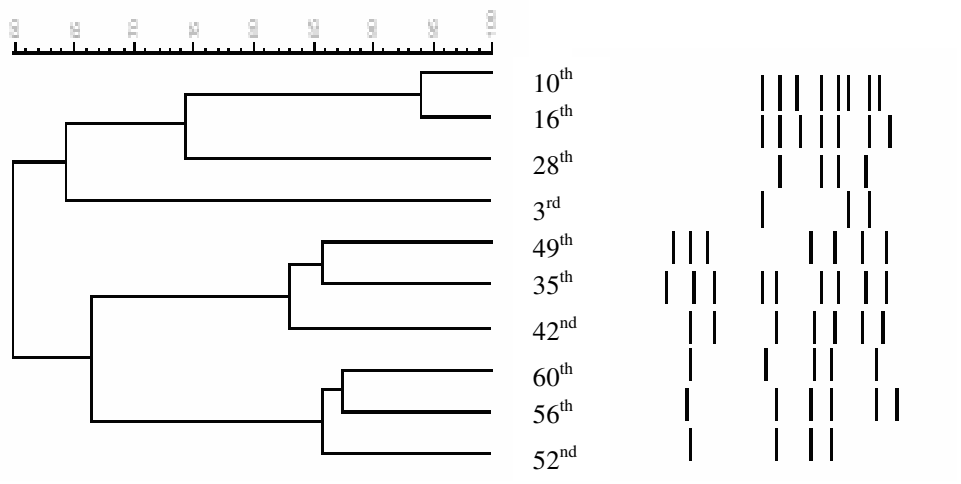


Figure 4.1.16 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns in the Reactor 2 (medium substrate loading)

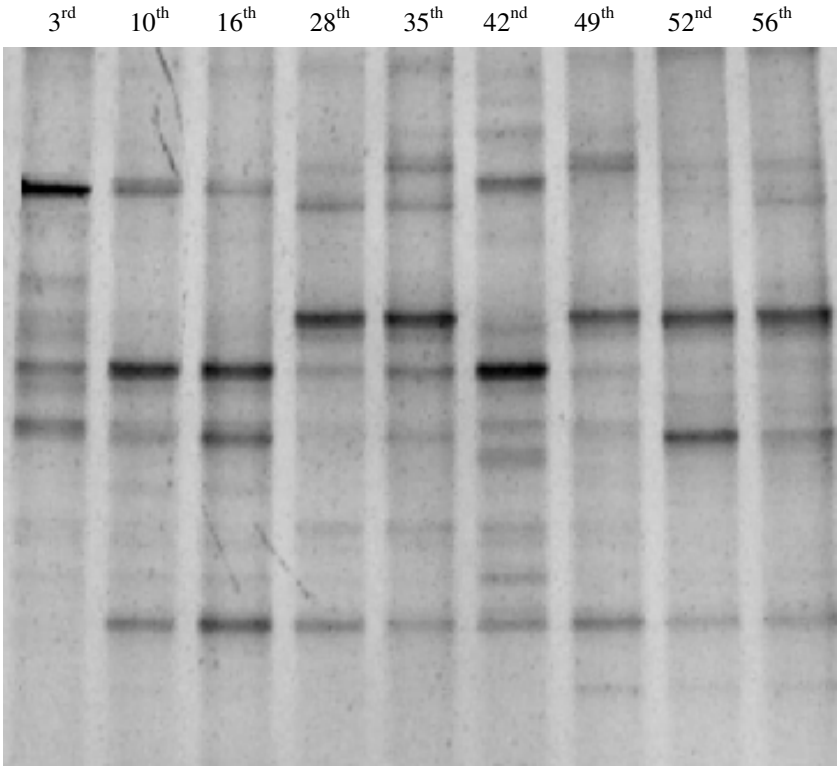


Figure 4.1.17 DGGE of PCR-amplified 16S rRNA from the bacterial communities in the Reactor 3

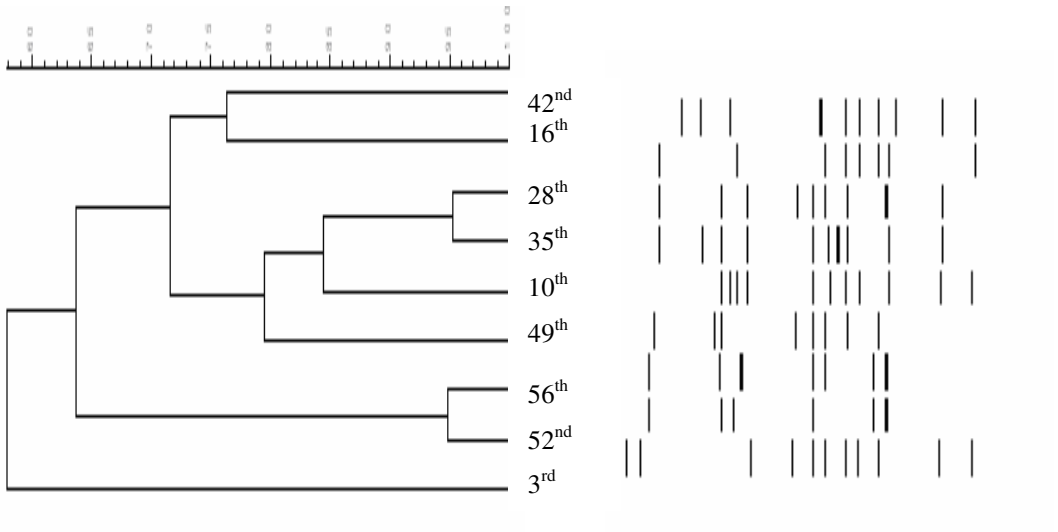
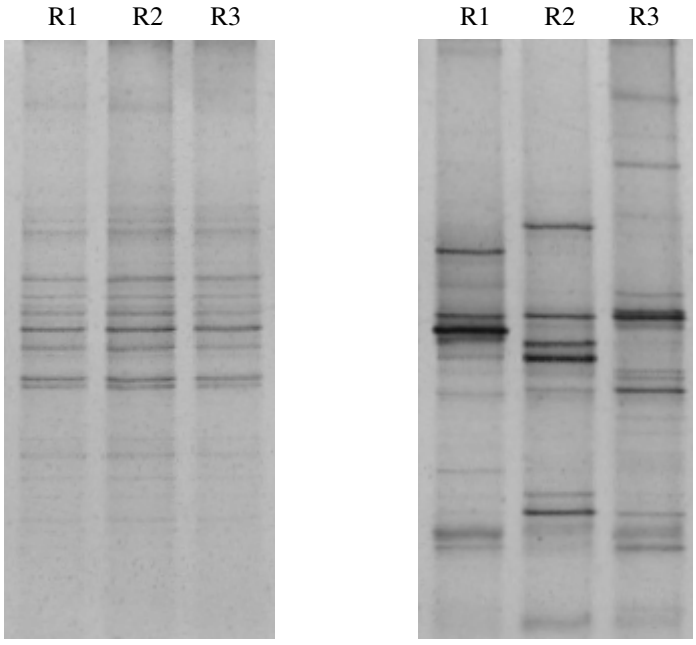


Figure 4.1.18 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns in the Reactor 3 (high substrate loading)



(1) Day 3 (2) Day 56

Figure 4.1.19 DGGE of PCR-amplified 16S rDNA from the bacterial communities under different substrate loadings

population shifts became insignificant and the dominating bacteria remained constant in each reactor. At the same time, biomass characteristics and reactor performance also showed little variations during this period. However, at steady state, comparison of the bacterial community structures of the three reactors (Figure

4.1.19) indicates that the dominating bacteria in each reactor were quite different. This shows that a change of substrate loading causes a change of dominant bacteria although the initial seed was the same in the three MBRs.

4.1.4 Discussion

This study focused on the effect of different substrate loadings on EPS production, membrane fouling tendencies as well as bacterial community structures in the MBR systems. With substrate loading increased four times from 0.57 to 2.28 g COD L^{-1} day^{-1}, biomass concentration correspondingly rose almost four times from 2.2 to 8.4 g/L at steady state. This suggests that once the volumetric loading rate has been fixed, the sludge concentration freely evolves until the F/M ratio approaches a constant value which only relates to the SRT (Pollice et al., 2004). This assumes the microbial growth coefficients (Y, K_d in Equation [2.6]) are unchanged by other parameters, such as DO.

In this study, only one activated sludge sample from each reactor was studied for DNA extraction because in reproducibility tests, the DGGE profiles of bacterial populations did not show much variation. Other researchers have also confirmed that a single sample of a reactor was sufficient for a reactor to reactor comparison (Boon et al., 2002). The results showed that the community fingerprints remained reasonably complex (more than 10 bands per lane) throughout the entire duration for the three MBRs. Figure 4.1.20 depicts the relationship between microbial community structure and EPS production in the three reactors. It was found that EPS production was loosely associated with the population shifts seen in the DGGE similarity analysis. The first EPS production spike in R1 occurred on day 14, with samples from day 3 to day 14 at 100% to 74% similarity values. From day 3 to day 6, EPS production significantly increased by 54% and 69% for R2 and R3, respectively. Correspondingly, the similarity indices dropped from 100% to 66% over these few days for R2 and R3. Hence, the changes in EPS production in the three reactors appeared to be accompanied by variations of bacterial similarity indices at the beginning of the operation. The new reactor conditions would tend to promote the presence and activity of desirable microorganisms and to discourage

the growth of unfavorable microorganisms. The favoured bacteria would acclimatise to the new living environment and tolerate the metabolic stress by producing more enzymes, which are considered as the main protein components of EPS. When the reactors approached steady state, not only the bacterial community structure but also the EPS production varied insignificantly, which indicates that these bacteria have become the dominant species in the reactor and their metabolisms have stabilized. These phenomena corresponded with steady TOC removal rates and biomass concentrations in each reactor.

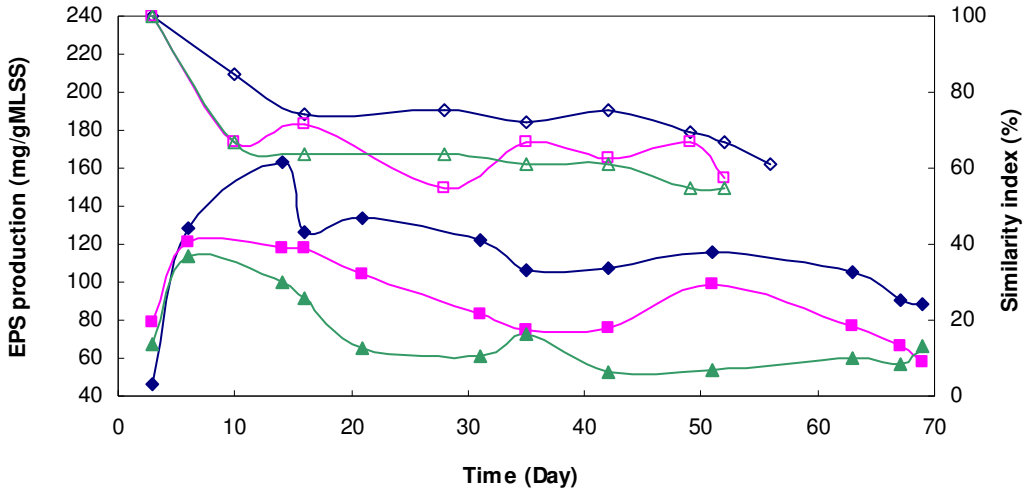


Figure 4.1.20 Relationship between bacterial community change and EPS production (◆) R1 (■) R2 (▲) R3 (◇) Similarity index of R1 (□) Similarity index of R2 (△) Similarity index of R3

Although the same seed was employed in the three reactors, after a period of operation with different substrate concentrations, the bacterial community structures in the three reactors were obviously different. The EPS production under different substrate loadings also varied significantly. The results suggest these different strains perform different functions in EPS production. In this study, EPS is considered as the substances excreted by living bacteria and hydrolyzed substances from dead bacteria. In the activated sludge process, microorganisms primarily utilize the energy supplied to satisfy their maintenance requirements. Maintenance functions include the general turnover of cell materials, continuous replacement of proteins and RNA, osmotic work to maintain concentration gradients and cell motility (Witzig et al., 2002). Only if energy is supplied in excess, will bacteria take

a part of it to grow and store another part of it inside or outside their cells. With increasing substrate loading, EPS production decreased from 102 to 58 mg/gMLSS at steady state. At the same time, F/M ratios also established similar constant values of about 0.27 day^{-1}. Hence, the phenomenon of decreased EPS production may have resulted from a distinct drop in the dissolved oxygen amount when the reactor was exposed to the higher substrate loading. Under the fixed aeration rate conditions, the dissolved oxygen in R3 dropped to 0.6 mg/L, which could have led to localize anoxic conditions in the reactor. This phenomenon has also been observed by several authors that oxygen transfer limitations occurred in MBRs operated with high biomass concentrations (Muller et al., 1995; Pollice et al., 2004). Hence at higher substrate loading, the lower dissolved oxygen condition could accelerate the growth of facultatively anaerobic bacteria, which are able to live with lower oxygen environments. According to the description of the Monod-type kinetics for carbon source and oxygen concentration, if the necessary dissolved oxygen is not provided, not only bacterial growth but also bacterial metabolisms will be inhibited. Nielsen et al. (1996) also found that EPS components, mainly proteins and polysaccharides, were to a large extent degraded during low oxygen conditions. Since adding pure oxygen would improve oxygen transfer efficiency and increase dissolved oxygen (Van Dijk and Roncken, 1997), a test was done on R3 using oxygen-enriched air from day 68 and this improved the dissolved oxygen value to about 6 mg/L. Subsequently EPS production increased, which confirms that the dissolved oxygen amount is a significant factor to influence EPS production. This result is substantiated by Wilshusen et al. (2004) and Yun et al. (2006) that at high oxygen concentration, EPS production was higher than that in low oxygen concentration. Hence, substrate loadings have an effect on EPS production by influencing bacterial diversity as well as bacterial metabolisms.

The activated sludge fed with different substrate loadings revealed various membrane fouling tendencies. Under the lower substrate loadings, the MLSS and EPS concentrations were lower, which correlated with lower membrane fouling rates and specific fouling resistances compared with those under higher substrate loadings. In a typical MBR the fouling would also relate to the properties of the

supernatant, particularly the EPS components, polysaccharide and protein. In this study the concentrations of EPS (7.1 vs 15.9 and 29.4 mg/L) in the supernatant were higher at higher substrate loading, and mirrored the trends for total EPS.

The importance of substrate loading was also evident when comparing the relative changes from R1 to R2 to R3. When the substrate loading was doubled from R1 (0.57 g COD L^{-1}) to R2 (1.14 g COD L^{-1}), the average EPS concentration only increased by 26% and the average specific fouling resistance and membrane fouling rate increased by 32% and 85% respectively. However the substrate loading in R3 (2.28 g COD L^{-1}) was double that of R2, and the average EPS concentration, specific fouling resistance and membrane fouling rates increased by 172%, 253% and 1050% compared with those of R2. This suggests that increasing substrate loading in a reasonable range (as from R1 to R2), there is not a significant increase in EPS concentration and membrane fouling tendency. This finding corresponds well with Matsuda et al.'s study (2003) in a lab-scale MBR system, where the suction pressure was constant or slightly increased with time at lower BOD loading and increased abruptly and disturbed the operation rapidly at higher BOD loading. Nagaoka et al. (1998) also pointed out that a sudden increase in the pressure and a decrease in flux occurred in the reactor fed with high substrate loading after 40 days while a low substrate loading reactor showed little increase in pressure until 120 days.

4.2 MBR performance and bacterial community structure under different substrate compositions

4.2.1 Introduction

It is common knowledge that bacterial survival depends on the availability of necessary nutrients. These nutrients not only provide basic elements required for the maintenance, growth and reproduction of all organisms, but also produce energy for their synthesizing reactions, living functions and regulating metabolisms. Besides major elements including carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and potassium, other trace elements, such as iron, calcium, chlorine and sodium,

and growth factors are considered as required nutrients. As the excreted and autolyzed substances from microorganisms, EPS components are related to bacterial species and their functions. The EPS production and composition is influenced by operating parameters as well as feed substrate compositions. Some studies have confirmed that differences in the food sources influence bacterial population structure, physical surface properties of the bacteria and chemical composition of the extracted exopolymers in the different types of activated sludge process (Table 4.2.1).

Table 4.2.1 Reported effects of food sources on EPS characteristics

Substrate characteristics	EPS characteristics	System	Reference
Synthetic/Normal/High sugar wastewater	Different polysaccharides composition and concentration	Fermentor sludge system	Boyette et al. (2001)
Chemical/Leather/Dye/Wine/Municipal wastewater	Different protein and polysaccharides production	Activated sludge process	Sponza (2003)
Increasing nitrogen concentration in the substrate	Increase in protein production; no effect on polysaccharides production	Sequencing batch reactor	Bura et al. (1998)
Increasing phosphorus concentration in the substrate	Decrease in protein and polysaccharides production	MBR with intermittent aeration	Nagaoka (1999)
High nitrogen concentration substrate	Decrease in EPS concentration		
Increasing C/N ratio	Increase in polysaccharides production and decrease in protein produciton	Biofilm	Huang et al. (1994)
Nitrogen deficient substrate	Less EPS produciton	Membrane-coupled activated sludge system	Chang and Lee (1998)

Nitrogen and phosphorus are important components in industrial and municipal wastewater due to increasing recent trends, which principally originate from fecal material, synthetic detergents and other cleaning products (Seviour et al., 2003). Particular attention is now being paid to the elimination of nitrogen-rich or

phosphorus-rich substrates due to their harmful effects on human health and eutrophication of natural waters. The nitrogen content in the raw wastewater is considered as a necessary feed constituent to synthesize new cell substances such as proteins and nucleic acids (Chang and Lee, 1998). Phosphorus is regarded as an energy source and a building block of genetic material of microorganisms (Clöte and Oosthuizen, 2001). In the activated sludge system, heterogeneous microbial communities can be composed of heterotrophs, nitrifiers, phosphate-accumulating organisms and denitrifiers. Only nitrifiers and denitrifiers are involved in nitrogen consumption and nitrification is generally a rate-limiting step in a biological nitrogen removal process due to the low growth rates and poor yield of nitrifiers. Phosphorus utilization is associated with both biological mechanisms and possible chemical precipitation (Choi et al., 2001, Gao et al., 2004). Therefore, increasing the nitrogen and phosphorus concentrations in the substrate would tend to alter the physiological state of the microorganisms, reactor performance and thereby influence membrane performance in a MBR system.

However, very little is known about the activated sludge characteristics, EPS production and membrane fouling of a MBR system under nitrogen-rich or phosphorus-rich conditions. Therefore, the object of this section is to investigate EPS production and membrane fouling in the reactors fed with nitrogen-rich and phosphorus-rich substrates. Using the DGGE technique, the relationship between microbial community structure and EPS production is also examined. The hope is that this study will be useful in understanding how the membrane fouling tendency can be affected by the substrate compositions and show whether regulating substrate compositions in MBRs can provide effective control of membrane fouling.

4.2.2 Materials and Methods

Two identical MBR systems were set up as described in Section 3.2.1.1 (Chapter 3). The seed sludge was from R1 operated in phase 4.1 (Section 4.1.2.1, Chapter 4). The initial MLSS concentrations for the two reactors were 2.5 g/L and the feed TOC concentrations were fixed at 106 mg/L. Reactor 1 was fed with nitrogen-rich substrate and Reactor 2 was fed with phosphorous-rich substrate as detailed in

Table 4.2.2. The two MBR systems were operated at a constant SRT (30 days), aeration rate (3 L/min) and HRT (12 hrs). R3 as control was run in the last phase (Phase 4.1, Chapter 4) with the same operating parameters as the other two reactors. The MBRs were sampled periodically throughout a 60-day period. The measurements were carried out following the protocols described in Section 4.1.2.

Table 4.2.2 Compositions of synthetic wastewater for the MBR systems

Compound	Concentration (mg/L)			Compound	Concentration (mg/L)		
	R 1	R 2	R3*		R 1	R 2	R3*
Glucose ($C_6H_{12}O_6$)		500		CH_3COONa		500	
NH_4Cl	600	300	300	$MgSO_4.7H_2O$		100	
KH_2PO_4	53	106	53	K_2HPO_4	107	214	107
$NaHCO_3$	40	80	40	$CaCl_2$		7.5	
$FeCl_3$		0.5		COD:N:P	100:17.4:3.5	100:8.7:7.0	100:8.7:3.5

*R3 as control was run in the last phase (Phase 4.1, Chapter 4) with the same operating parameters as the other two reactors. Theoretical COD was about 902 mg/L, and concentrations of nitrogen were 157, 78.5 and 78.5 mg/L for R1, R2 and R3; concentrations of phosphorus were 31.1, 62.2 and 31.1 mg/L, respectively.

4.2.3 Results

4.2.3.1 MBR performances under different substrate compositions

The two parallel MBR systems were operated continuously over 60 days (2×SRT). High and stable organic removal rates (above 95%) were achieved in the three reactors during the operating period. The starting sludge had a biomass concentration of 2.5 g/L and an initial F/M of about 0.23 day^{-1}. During the 60 days of operation, the F/M ratios varied widely initially but after 35 days, the F/M ratios were close to steady values of 0.26 day^{-1} for R1 (Nitrogen-rich) and 0.22 day^{-1} for R2 (Phosphorus-rich) (Figure 4.2.1). It can be assumed that the reactors reached a stable stage as evidenced by steady biomass concentrations and TOC removal rates. The two reactors had similar pH values, within a range of 6.0~7.0. The initial mean particle sizes of the activated sludge in the two reactors were about 100 μm. Significant increasing trends in mean particle size were observed in the start-up period for both MBR systems. From day 43 the systems approached a steady state, the mean particle sizes in R1 and R2 reached about 252 μm and 207 μm,

respectively (Figure 4.2.2). Over the whole operating period, the dissolved oxygen concentrations were about 6.0 mg/L for both R1 and R2.

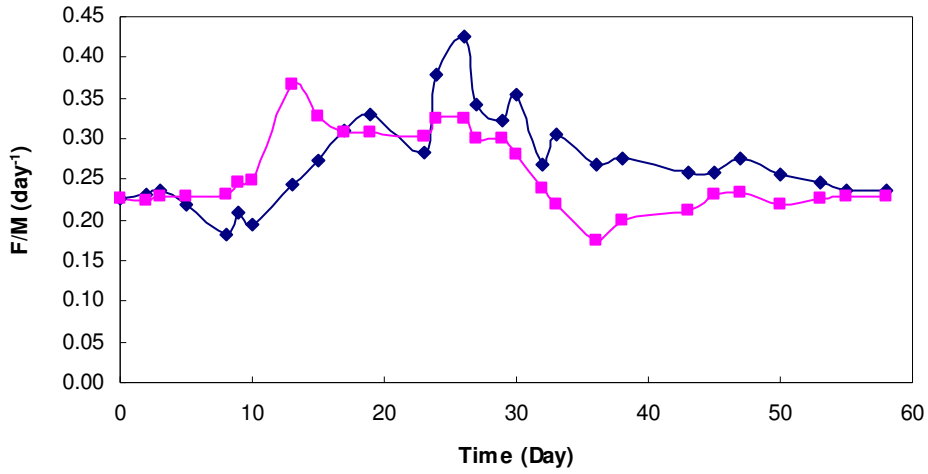


Figure 4.2.1 F/M changes under different substrate compositions (◆) R1 (N-rich) (■) R2 (P-rich)

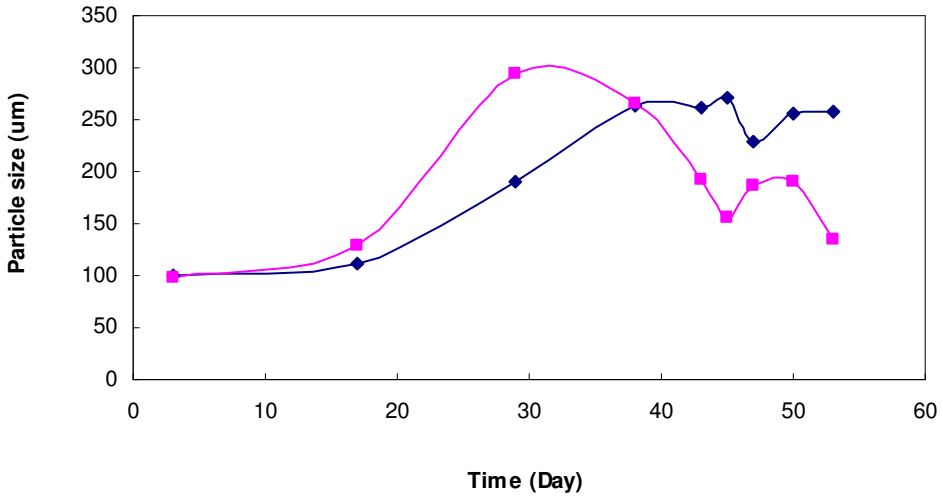


Figure 4.2.2 Particle size changes under different substrate compositions (◆) R1 (N-rich) (■) R2 (P-rich)

4.2.3.2 EPS analysis under different substrate compositions

At the beginning of the operation, the EPS production (Figure 4.2.3) showed a rapidly increasing trend up to a peak at 171 mg/g MLSS and 276 mg/g MLSS for

R1 (Nitrogen-rich) and R2 (Phosphorus-rich), respectively. This was the period of acclimatization of the biomass under metabolic stress, promoting cell lysis and liberation of intracellular products (Puñal et al., 2003). After that period a decreasing trend of EPS production occurred to reach about 80 mg/g MLSS for R1 and 106 mg/g MLSS for R2 (Phosphorus-rich), stabilizing at these levels at the end of the operation. However, in the initial phase, EPS concentration (Figure 4.2.4) in R1 (Nitrogen-rich) was higher than that in R2 (Phosphorus-rich). Averaged over the last 10 days, the EPS concentration in R1 (Nitrogen-rich) was about 180 mg/L and that in R2 (Phosphorus-rich) was about 210 mg/L.

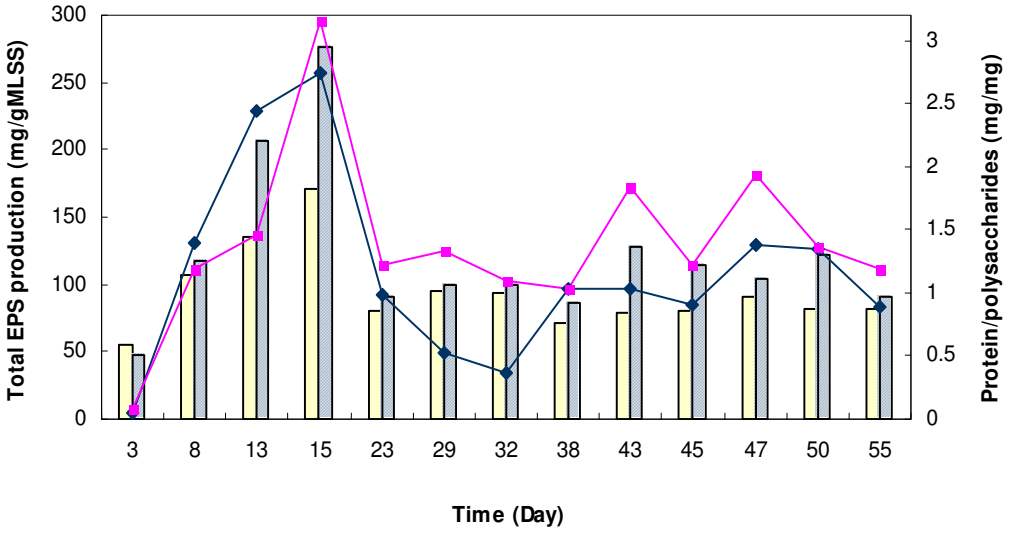


Figure 4.2.3 Total EPS production and the ratio of protein to polysaccharides under different substrate compositions
(Light bar) EPS production in R1 (N-rich) (Striped bar) EPS production in R2 (P-rich)
(◆) Protein/Polysaccharides in R1 (N-rich) (■) Protein/Polysaccharides in R2 (P-rich)

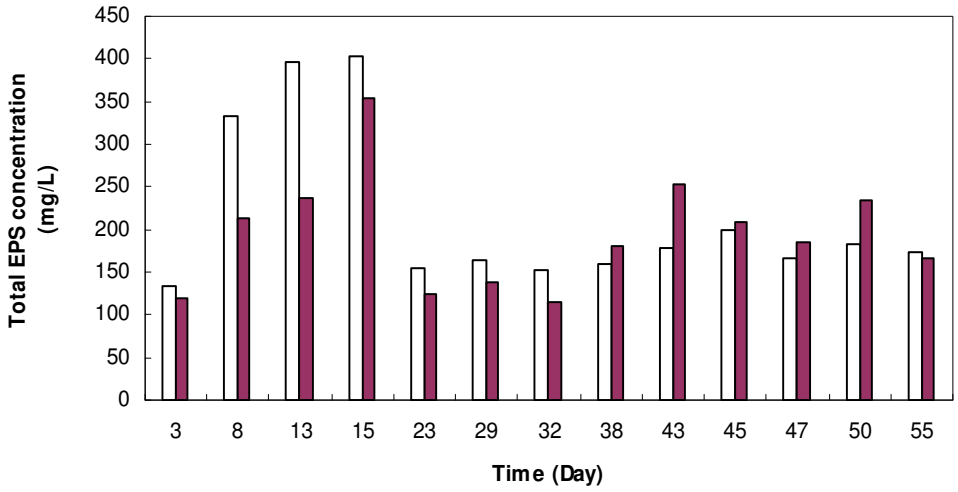


Figure 4.2.4 Total EPS concentration under different substrate compositions (Light bar) R1 (N-rich) (Dark bar) R2 (P-rich)

It was also noted that protein production shared a similar trend as EPS production (Figure 4.2.5). A reason for this is that the new operating conditions would tend to stimulate the bacteria to produce more enzymes to acclimatise to the new environment and efficiently degrade the substrate. This effect was also reported in Chapter 3 and Section 4.1 in Chapter 4. A slightly higher protein production in R2 (Phosphorus-rich) was observed compared with that of R1 (Nitrogen-rich) in the 60-day operation. Variations of polysaccharides production were less significant over the whole operating period except for a peak for R2 (Phosphorus-rich) in the initial period and average polysaccharides productions at the steady state in the R1 (Nitrogen-rich) and R2 (Phosphorus-rich) were relatively similar (Figure 4.2.6). The ratios of protein to polysaccharides (Figure 4.2.3) increased in the start-up period from 0.05 mg/mg to 2.7 mg/mg and 3.0 mg/mg in R1 and R2, respectively. After about 15 days, a sudden decline in the ratios of protein to polysaccharides appeared for both reactors due to a decrease in protein production.

Obviously, the amount of EPS attached in the bacterial floc was dominant compared with soluble EPS (Figure 4.2.7). The same trends were observed in Figure 4.2.8 and Figure 4.2.9 that the attached protein and polysaccharides were much more than soluble protein and polysaccharides respectively.

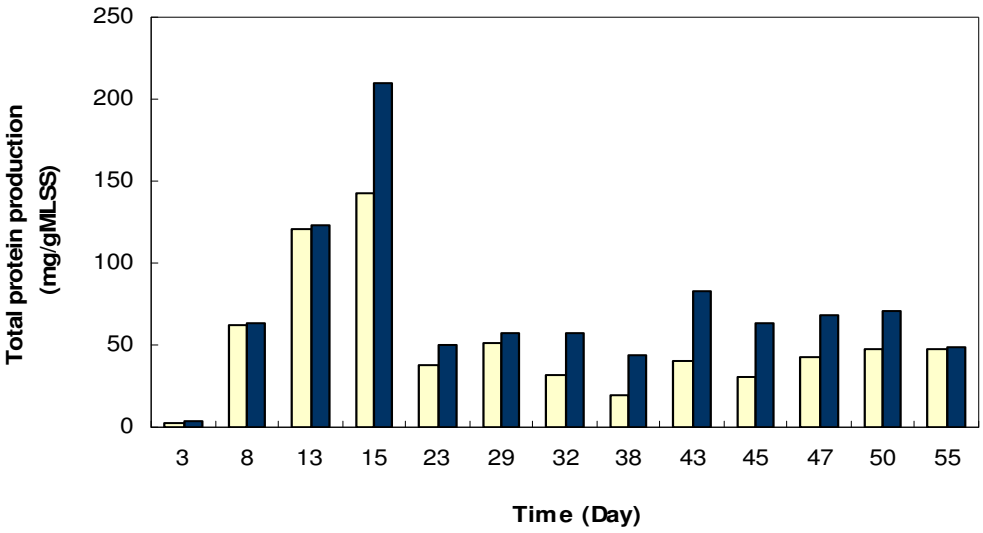


Figure 4.2.5 Total protein production under different substrate compositions
(Light bar) R1 (N-rich) (Dark bar) R2 (P-rich)

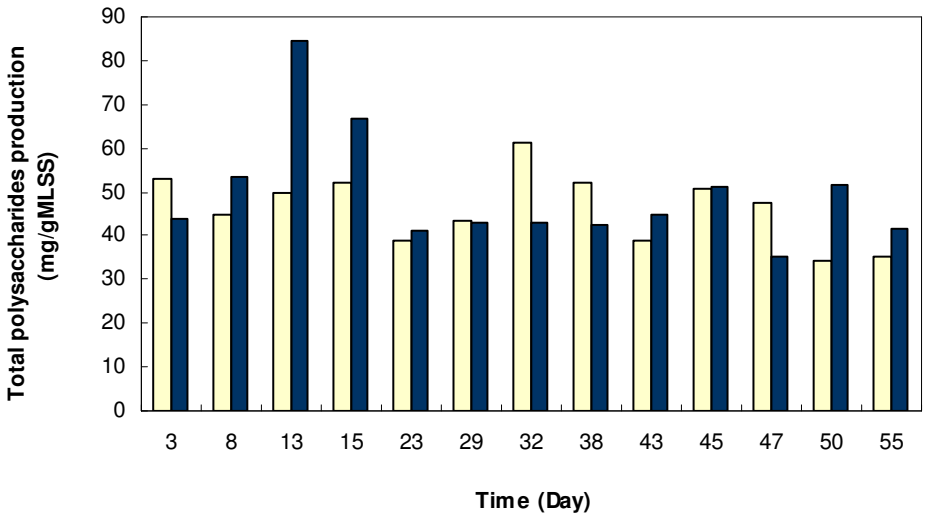


Figure 4.2.6 Total polysaccharides production of activated sludge under different substrate compositions
(Light bar) R1 (N-rich) (Dark bar) R2 (P-rich)

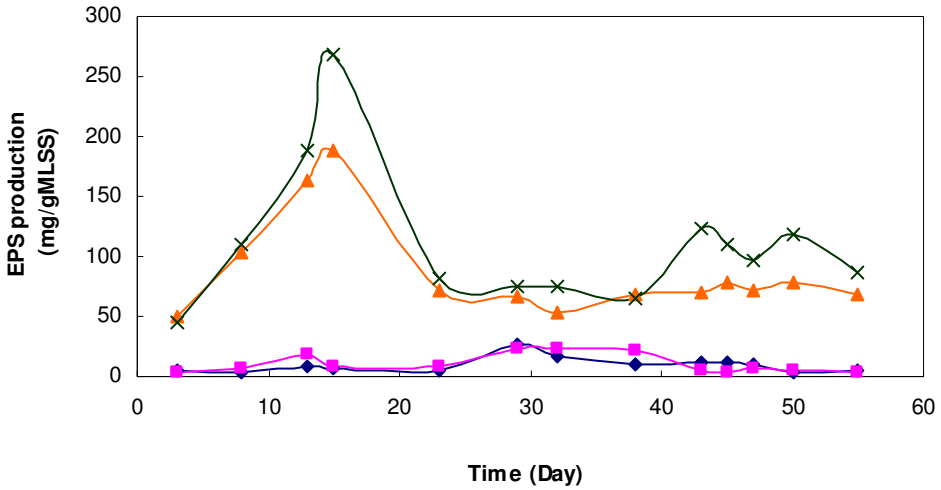


Figure 4.2.7 EPS distribution under different substrate compositions
(◆) Soluble EPS in R1(N-rich) (■) Soluble EPS in R2(P-rich)
(▲) Attached EPS in R1(N-rich) (×) Attached EPS R2(P-rich)

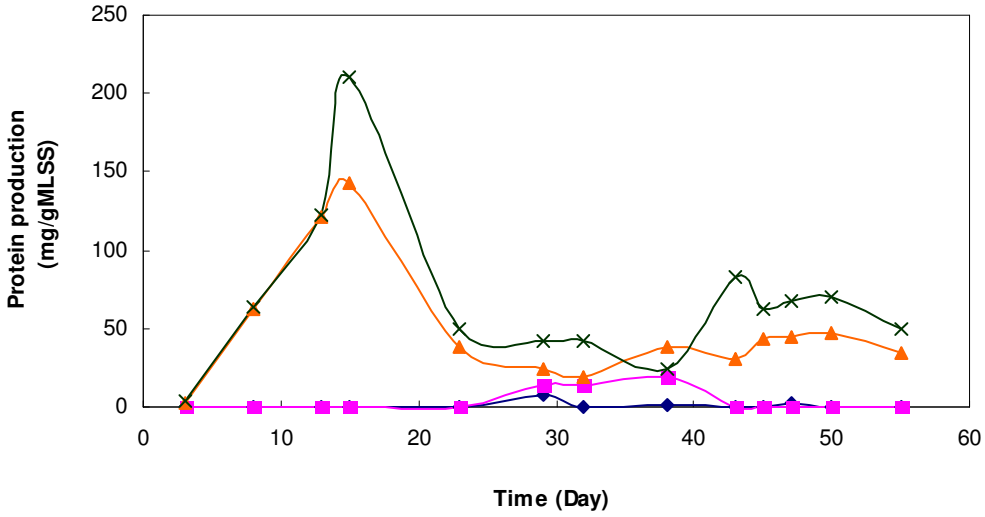


Figure 4.2.8 Protein distribution under different substrate compositions
(◆) Soluble protein in R1(N-rich) (■) Soluble protein in R2(P-rich)
(▲) Attached protein in R1(N-rich) (×) Attached protein in R2(P-rich)

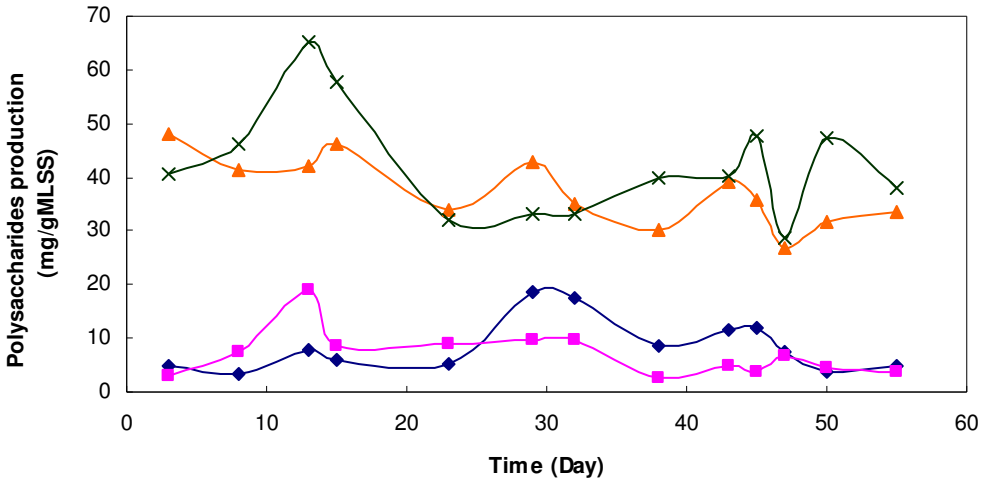


Figure 4.2.9 Polysaccharides distribution under different substrate compositions (◆) Soluble polysaccharides in R1(N-rich) (■) Soluble polysaccharides R2(P-rich) (▲)Attached polysaccharides in R1(N-rich) (×) Attached polysaccharides R2(P-rich)

4.2.3.3 Membrane fouling tendency under different substrate compositions

Several samples were taken from each reactor during steady state and the average fouling characteristic values were calculated as the data for the graphs (Figure 4.2.10). The average specific fouling resistances and membrane fouling rates for the mixed liquor in R1 (Nitrogen-rich), R2 (Phosphorus-rich) and the control were 0.63, 1.66 and $1.0*10^{13}$ m/kg and 0.26, 0.39 and $0.53*10^{10}$ m^{-1}/min, respectively. From Figure 4.2.10, it is difficult to draw a conclusion on the relationship between EPS characteristics and the membrane fouling tendencies among the three reactors. A possible reason is that the control was operated with a different initial activated sludge from R1 (Nitrogen-rich) and R2 (Phosphorus-rich), which led to significantly different biomass characteristics at steady state.

However, comparing R1 (Nitrogen-rich) and R2 (Phosphorus-rich), does indicate that the specific fouling resistance and membrane fouling rate were positively related to EPS concentration, whose values were 182 and 220 mg/L in the R1 (Nitrogen-rich) and R2 (Phosphorus-rich), respectively. Also, the protein concentration and the ratio of protein to polysaccharides were higher in R2 (Phosphorus-rich) than in R1 (Nitrogen-rich). The difference in polysaccharides

between the two MBR systems was not obvious. These results suggested that protein concentration contributed more significantly to membrane fouling tendency compared with polysaccharides concentration under the dead-end filtration test mode.

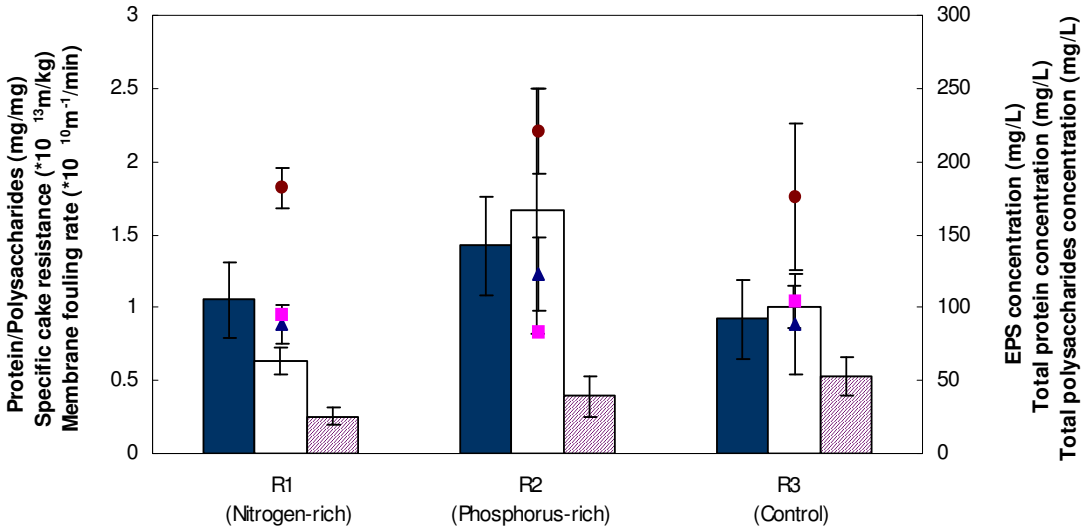


Figure 4.2.10 Relationship between membrane fouling tendency and EPS characteristics at the steady state under different substrate compositions (Dark bar) Ratio of protein to polysaccharides (Light bar) Specific fouling resistance (Striped bar) Membrane fouling rate (●) Total EPS concentration (■) Total polysaccharide concentration (▲) Total protein concentration

4.2.3.4 Microscope observation and DGGE analysis under different substrate compositions

The sludge was microscopically investigated during the start-up period and the steady state (Figure 4.2.11). At the beginning of the operation, there were no free cells or filamentous bacteria observed. However, after 15 days larger and weaker flocs with filamentous bacteria as well as protozoa in the biomass were observed in the two MBR systems. This result agrees with observations made about a MBR system by Wagner and Rosenwinkel (2000).

The DGGE technique was applied to evaluate the bacterial community structures in the MBR systems. Samples were taken from the mixed liquor in the reactors and DNA was extracted once every few days in the 60-day operating period. Figures 4.2.12 and 4.2.14 show the changes of DGGE banding patterns of the bacterial

communities in the two MBR systems with operation time. The large numbers of bands observed in each lane are indicative of a large variety of organisms (Curtis and Craine, 1998). UPGMA dendrogram were calculated based on the Dice coefficient with Arithmetic Mean algorithms by the GelCompar II software and are shown in Figures 4.2.13 and 4.2.15, respectively (the related Similarity Indices are discussed below (Section 4.2.4). In the initial stage, the two reactors shared the same bacterial community structure because an identical seed was employed (Figure 4.2.17). Within the first 10 days, the bacterial community similarity value of

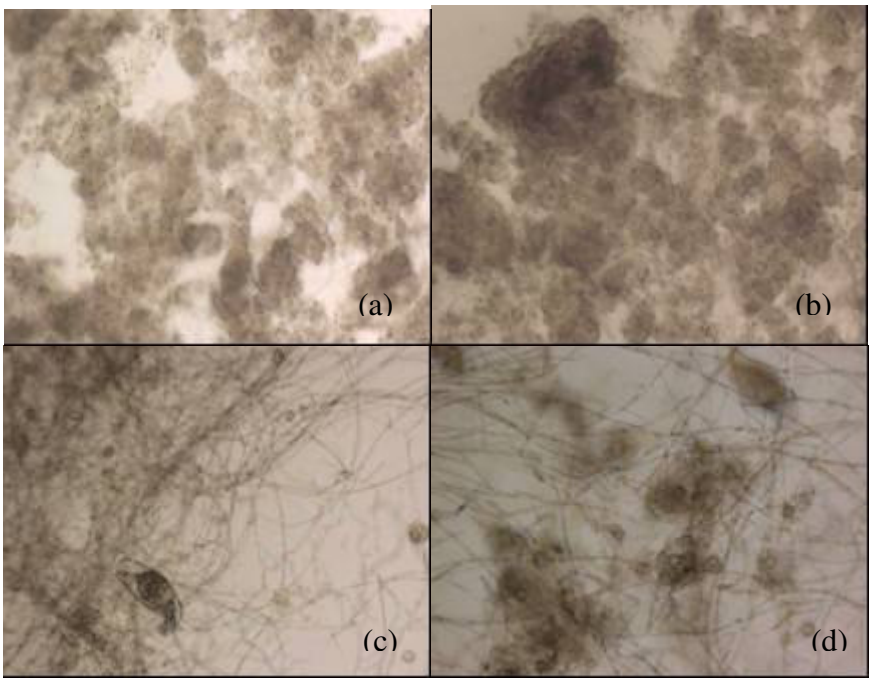


Figure 4.2.11 Microscopic photographs of activated sludge from the two reactors under the initial stage and steady state (× 450)
(a) R1 (N-rich, Day 3) (b) R2 (P-rich, Day 3)
(c) R1 (N-rich, Day 40) (d) R2 (P-rich, Day 40)

each reactor was high, which demonstrated insignificant changes in the bacterial community structures had occurred. After that, the similarity indexes in R1 (Nitrogen-rich) and R2 (Phosphorus-rich) decreased obviously which derived from shifts in bacterial community structures. After 26 days operation, the fingerprint patterns were similar for the subsequent biomass samples from R1 (Nitrogen-rich), revealing a highly stable bacterial community structure. The same phenomenon was

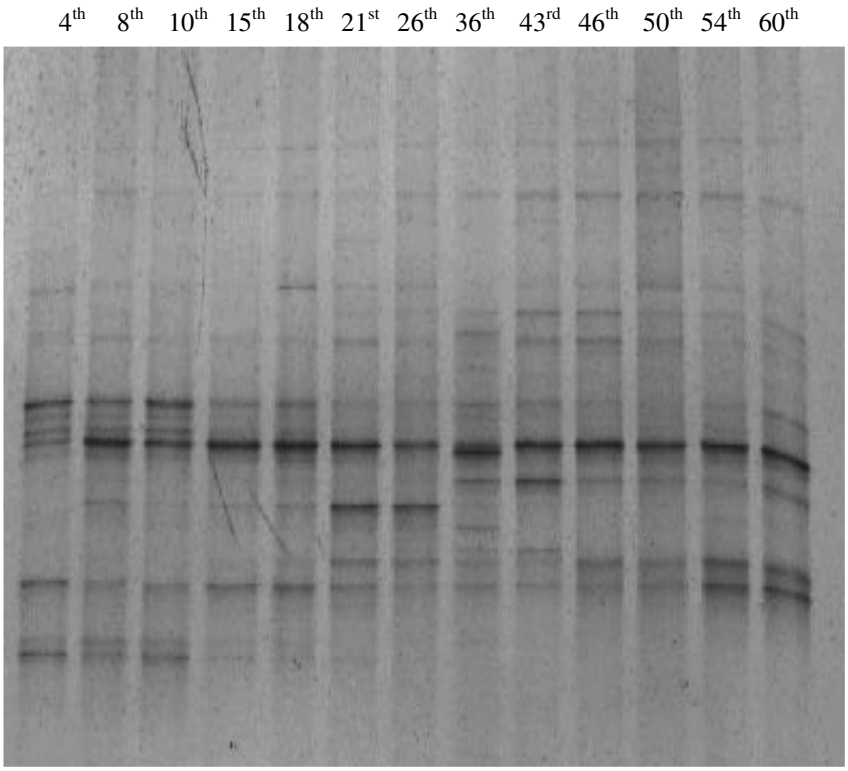


Figure 4.2.12 DGGE of PCR-amplified 16S rRNA from the bacterial communities in R1 (N-rich)

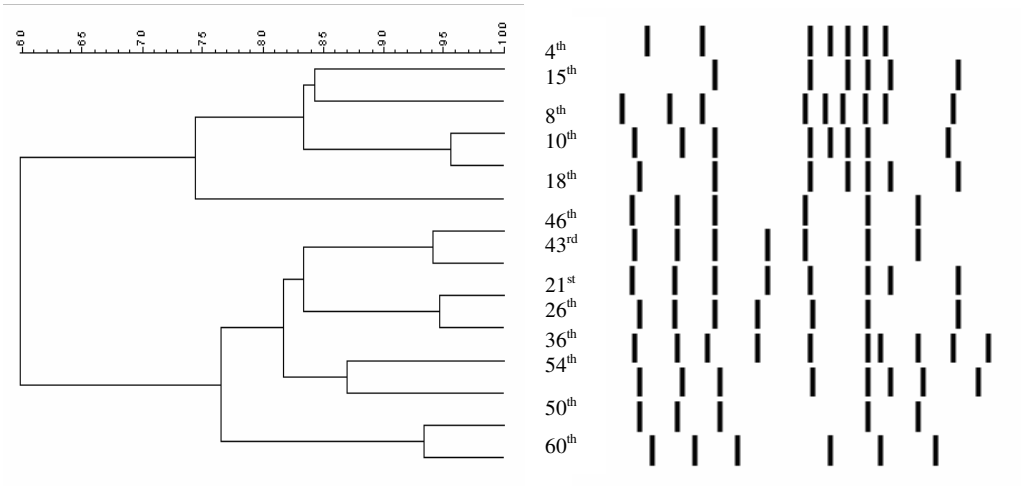


Figure 4.2.13 UPGMA dendrogram of bacterial community DGGE fingerprints in R1 (N-rich)

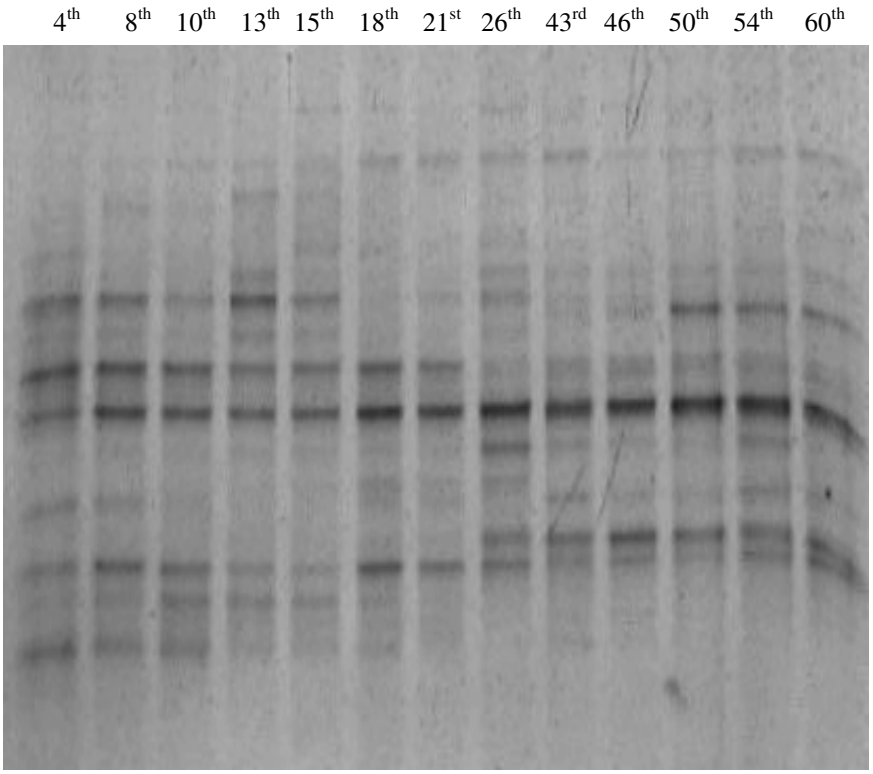


Figure 4.2.14 DGGE of PCR-amplified 16S rRNA from the bacterial communities in R2 (P-rich)

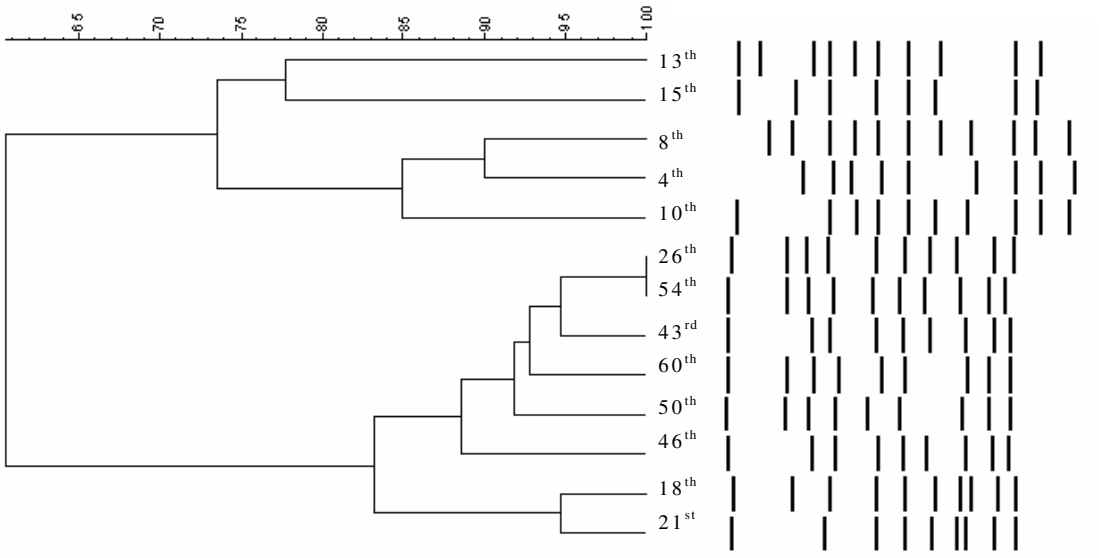


Figure 4.2.15 UPGMA dendrogram of bacterial community DGGE fingerprints in R2 (P-rich)

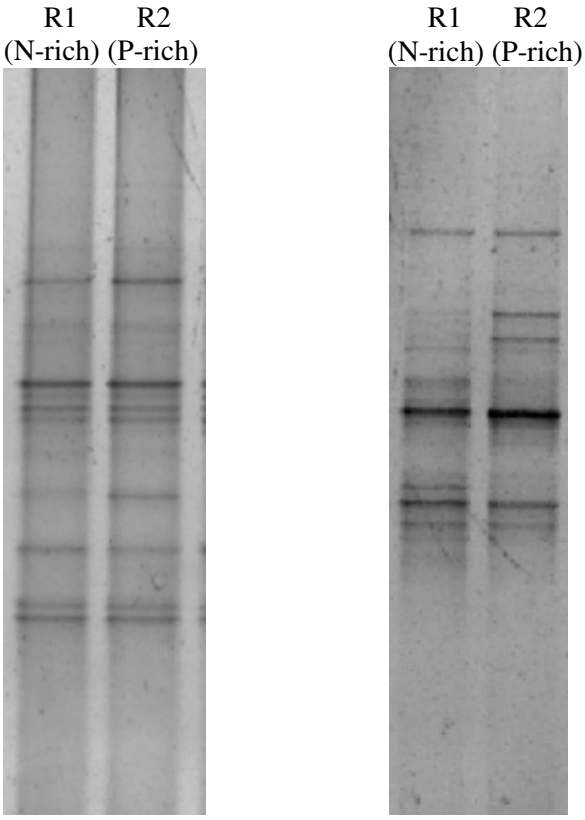


Figure 4.2.16 DGGE of PCR-amplified 16S rRNA from the bacterial communities under different substrate compositions

observed for R2 (Phosphorus-rich) after 18 days. Biomass characteristics and reactor performance also showed little variations during this period. Although there were key differences in band weighting within the bacterial community structures in the two MBRs, similar prominent bands were detected in both the communities shown in Figure 4.2.16. This suggests that the imposed changes in substrate compositions may not have significantly influenced the dominant bacterial community structures.

4.2.4 Discussion

In this study it has been demonstrated that substrate nutrient composition is an important factor that influences reactor performance, including activated sludge characteristics, bacterial community structure as well as membrane fouling tendency. Due to the change in substrate compositions for the acclimatised activated

sludge, an adaptation was required for the microorganisms in the MBR systems. Such adaptation of the microbial communities to a new substrate may arise as a result of a combination of different mechanisms, such as gene transfer or mutation, enzyme induction and population changes (Zhuang et al., 2005). At the very beginning of the operation (Phase 1, Figure 4.2.17), the bacterial community

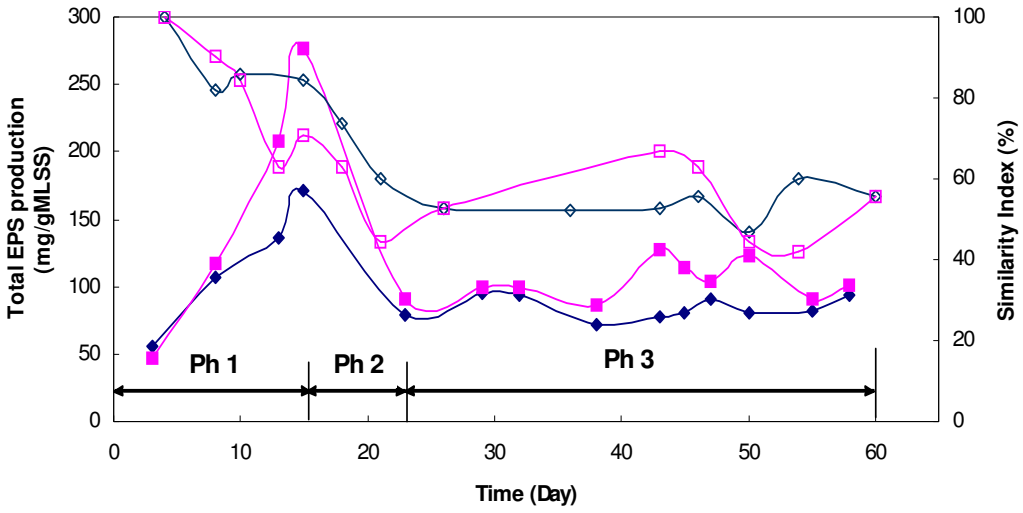


Figure 4.2.17 Relationship between bacterial community change and EPS production
(◆) Total EPS production in R1 (N-rich) (■) Total EPS production in R2 (P-rich)
(◇) Similarity index of R1 (N-rich) (□) Similarity index of R2 (P-rich)

similarity values were high which indicates there were no significant changes in bacterial community structures of either reactor. However, changes in the EPS productions of the activated sludge were significant in the both reactors, mainly as increased protein production. It is suggested that as the bacteria acclimatised to the new living environment they experienced metabolic stress causing the production of more enzymes, which are the main protein components of EPS. In Phase 2, significant bacterial population shifts occurred, which indicates that some bacteria would have been suited to the new environment and have flourished in the reactors, but others which could not tolerate the reactor conditions would disappear. These acclimatized bacterial populations would be restored to normal metabolisms with gradual adaptation to the environment, and this would lead to a slow decrease in EPS production as observed. In Phase 3, DGGE profiles and similarity indices

revealed the presence of stable bacterial populations which led to constant EPS production. Therefore, changes in EPS production with operating time resulted from shifts in the bacterial community structures in each stage of the operation in the MBR systems.

Although the compositions of the substrate fed to the two MBR systems were different, there is the evidence that the dominant bacteria appeared to be similar based on the DGGE profiles (However DGGE profiling is not a precise characteristic as noted in Section 2.4.1, Chapter 2). However, the average value of EPS production under steady state in the nitrogen-rich reactor was lower than that of the control reactor. An increasing phosphorus concentration in the substrate had an insignificant effect on EPS production. It is proposed that increasing the nitrogen concentration in the substrate could have stimulated the population of some bacterial strains, which would possibly convert the excess source and energy to intracellular storage rather than producing EPS. However, the utilization of phosphate is common to all bacteria and complete metabolism for the activated sludge cultivated in this condition would happen. This hypothesis needs to be confirmed in further study.

Because of easy adherence to the membrane surface, EPS is considered as a serious factor to increase membrane resistance and ultimately result in membrane fouling in a MBR system. The activated sludge from the phosphorus-rich MBR system contained a higher EPS content with a smaller particle size, which correlated with the more significant membrane fouling rate and specific fouling resistance. In addition, soluble EPS concentration in R2 (phosphorus-rich) was about 12.0 mg/L, and more than that in R1 (nitrogen-rich, 9.1 mg/L), which caused more serious membrane fouling.

4.3 MBR performance and bacterial community structure under different SRTs

4.3.1 Introduction

Since the MBR system does not involve the settling of the suspended solids as in the conventional activated sludge clarifier, very high SRTs can be maintained with higher biomass concentrations in the bioreactor than in the conventional activated sludge process (CASP). Although higher treatment efficiency could be expected due to the higher biomass concentration and longer SRT, in fact, the treatment efficiency is not linearly proportional to biomass concentration because specific bioactivity can be reduced at substrate deficient states (low F/M) (Han et al., 2005). Importantly, longer SRTs can change the concentration and viscosity of the activated sludge as well as the concentration and composition of the extracellular polymeric substances (EPS), which influences the hydrodynamic conditions in the MBR system (Cieck et al., 2001). Therefore, it is likely that membrane fouling in the MBR system can be affected by SRTs.

In fact, many MBR researchers have observed that SRT is one of the important parameters which influence membrane fouling in the MBR system. However there does not appear to be a clear consensus. For example, Lee et al. (2003) and Han et al. (2005) suggested that the overall activated sludge fouling resistance increased as SRT was prolonged in the MBR system. In contrast, Chang and Lee (1998) observed that membrane fouling tendency was increased as SRT decreased in the membrane coupled activated sludge system.

In order to identify the reasons for the effect of SRT on membrane fouling, the production and composition of EPS, which is considered as the crucial foulant in MBRs, has been investigated (Wisniewski and Grasmick, 1998; Bouhabia et al., 2001). However, a common view has not yet emerged on the influence of SRT on EPS characteristics, presumably due to the complexity of the system and the differences in operating conditions in each study. For example, it was reported that the total amount of EPS was independent of the SRT because there was not much difference in EPS production by bacteria in their different growth phases in the

MBR system or sequencing batch reactor (Lee et al., 2003; Liao et al., 2000). However, Ng and Hermanowicz (2005) found that a maximum EPS production was observed in the reactor with a longer SRT. Other studies indicated higher total EPS production at lower SRTs in the MBR system (Chang and Lee, 1998; Rosenberger and Kraume, 2002; Alavi Moghaddam et al., 2003). Shin and Kang (2003) also observed that the soluble microbial production (EPS) tended to increase with SRT and then gradually decrease for a long SRT.

In this study, the main objective was to compare the EPS production and membrane fouling tendency at different SRTs. The advanced microbiological technique of PCR-DGGE was used to observe the shifts in bacterial community structures. By building up the relationship between bacterial community structure, EPS production and membrane fouling tendency under different SRTs, the effect of SRT on membrane fouling in a MBR system was clarified.

4.3.2 Materials and methods

Three MBRs were operated simultaneously and in parallel, whose set-up was described in Section 3.2.1.1 (Chapter 3). The seed sludge was from R3 in Phase 4.1 (Chapter 4). The initial MLSS concentrations for the three reactors were 5 g/L. The substrate composition was as shown in Table 3.2 and the feed TOC concentration for the three reactors was fixed at 424 mg/L. The three MBR systems were operated at the same HRT of 12 hours and an airflow rate of 3 L/min at a room temperature of 25°C. The SRT was fixed at 10 days for reactor 1, 30 days for reactor 2 and (pseudo)infinity for reactor 3 (for reactor 3 the only sludge wastage was in sampling). The three systems were operated for a total of 145 days. The measurements were carried out following the description in section 4.1.2 (Chapter 4).

In order to study membrane fouling tendency in each MBR, transmembrane pressure (TMP) in the reactor was monitored when reactor performance approached steady state by the pressure transducers (SMC, Japan). These pressure transducers were connected to a personal computer equipped with data log system (LabVIEW, National Instruments, USA).

4.3.3 Results

4.3.3.1 MBR performance under different SRTs

Organics removal in the three reactors was high and stable during the operating period. The effluent TOC of the three reactors was within 10 mg/L. After about 120 days, all three reactors had reached a steady state in terms of stable COD removal rates and biomass concentrations. The activated sludge had an initial concentration of 5 g/L, F/M of 0.75 day^{-1} and mean particle size of about 300 μm. When the reactors approached stable conditions, the MLSS concentrations were steady at 4.7, 8.3 and 12.9 g/L and the ratios of F/M were at about 0.44, 0.27 and 0.17 day^{-1} for R1, R2 and R3, respectively (Figure 4.3.1). The three reactors had similar pH values (6.0~7.0). The mean particle sizes of the activated sludge flocs increased in the initial phase for the three reactors, but then decreased and reached about 246, 235 and 181 μm for R1, R2 and R3 under steady conditions (Figure 4.3.2). During this period, the dissolved oxygen concentrations were about 6.2, 3.8 and 1.8 mg/L for R1, R2 and R3, respectively. These decreasing values were probably due to the effect of increasing MLSS on viscosity and oxygen transfer efficiency.

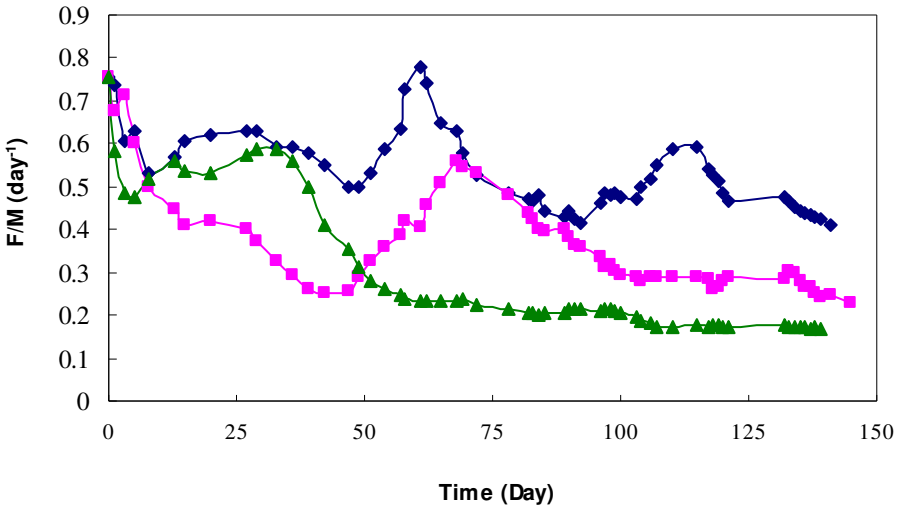


Figure 4.3.1 F/M changes under different SRTs
(◆) SRT 10 days (■) SRT 30 days (▲) SRT infinity

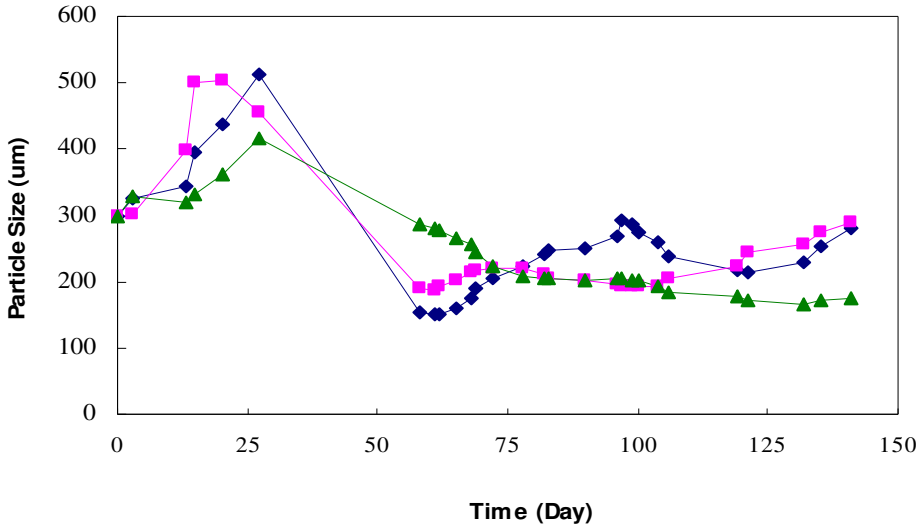


Figure 4.3.2 Particle size changes under different SRTs
(◆) SRT 10 days (■) SRT 30 days (▲) SRT infinity

4.3.3.2 EPS analysis under different SRTs

It can be seen from Figure 4.3.3 that in the start-up period of the operation there were increasing trends of EPS production for all the reactors. This phenomenon also

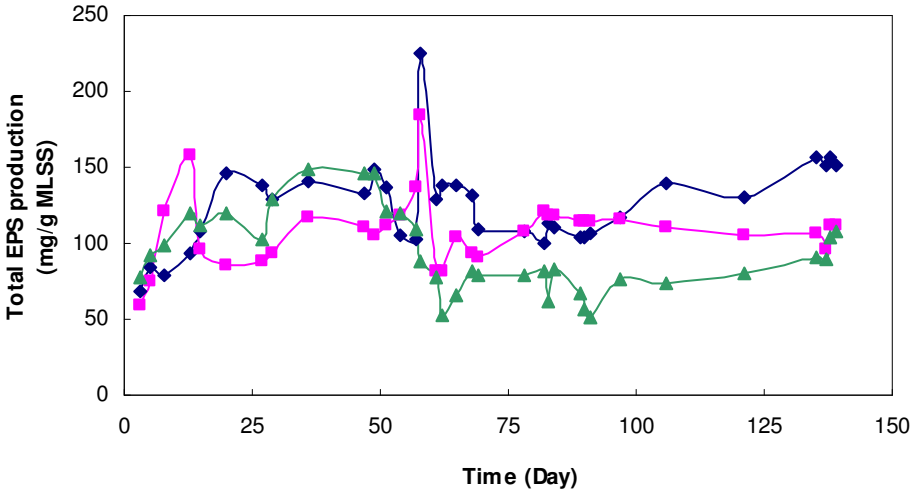


Figure 4.3.3 Total EPS production under different SRTs
(◆) SRT 10 days (■) SRT 30 days (▲) SRT infinity

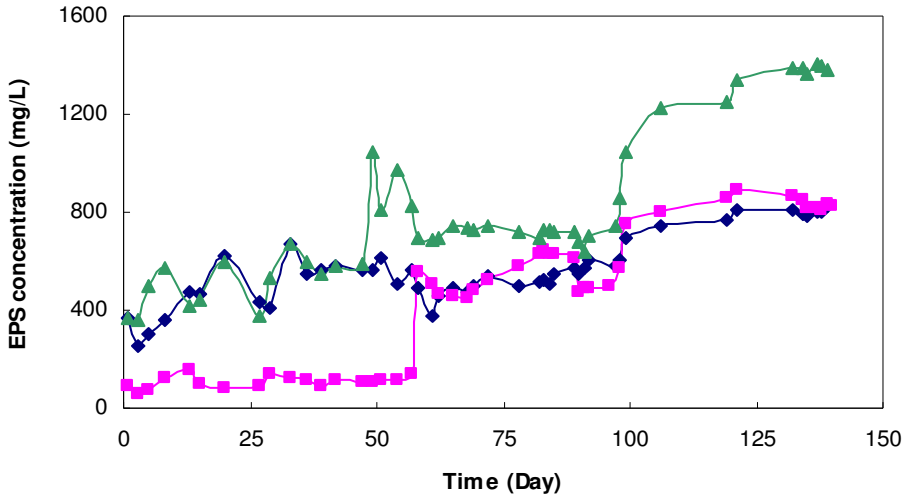


Figure 4.3.4 Total EPS concentration under different SRTs
(◆) SRT 10 days (■) SRT 30 days (▲) SRT infinity

was observed after a process interruption (day 58). Therefore, it is concluded that the biomass acclimatization process causes metabolic stress, and not only increasing cell lysis and liberation of intracellular products (Puñal et al., 2003) into the liquor phase, but also promoting the bacteria to excrete more enzymes (proteins) to adapt to the new environment. Similar trends were observed in Chapter 3 and Sections 4.1 and 4.2. When the reactors approached the steady stage, EPS productions were relatively steady at about 130, 111 and 80 mg/ gMLSS for R1, R2 and R3, which indicates that EPS production per unit biomass dropped with the extending of SRTs. However, the total EPS concentrations (mg/L) in the reactors were 783, 848 and 1345 for R1, R2 and R3, respectively.

The EPS composition was analyzed by measuring the protein and polysaccharides production (Figure 4.3.5 and Figure 4.3.6). Proteins mainly occur in the extracellular polymer network due to the excretion of intracellular polymers or cell lysis. Polysaccharides are synthesized extracellularly for a specific function (Bura et al., 1998). In the initial phase, sharp increases in the protein productions were observed for the three reactors. After a process interruption (day 58), the protein production jumped to a maximum of 185 and 154 mg/ gMLSS in R1 and R2. However, after slight variations of protein productions in R1 and R2, it decreased to

about 89 and 70 mg/ gMLSS during the final steady-state. For R3 there was an insignificant increasing trend after the interruption and the protein production was steady at about 44 mg/ gMLSS. Overall, the protein production followed the same trends as EPS production. As noted earlier, a possible reason is that the new operating conditions stimulated the bacteria to produce more enzymes to acclimatise to the environment and efficiently degrade the substrate. More obvious fluctuations of polysaccharide production with operation time were observed in the three reactors (Figure 4.3.6). However, there was no clear trend for polysaccharide production when comparing the three reactors at steady state. Figure 4.3.7 shows the ratios of protein to polysaccharides in R1, R2 and R3 during the final steady state were about 1.81, 1.35 and 0.92 mg/mg, which indicates a decreasing trend with extending SRTs. A possible reason for this was that the dissolved oxygen concentration decreased due to the increasing MLSS concentration at the same aeration rate. However, as shown later, the protein per volume of liquor (mg/L) had an increasing trend with SRT.

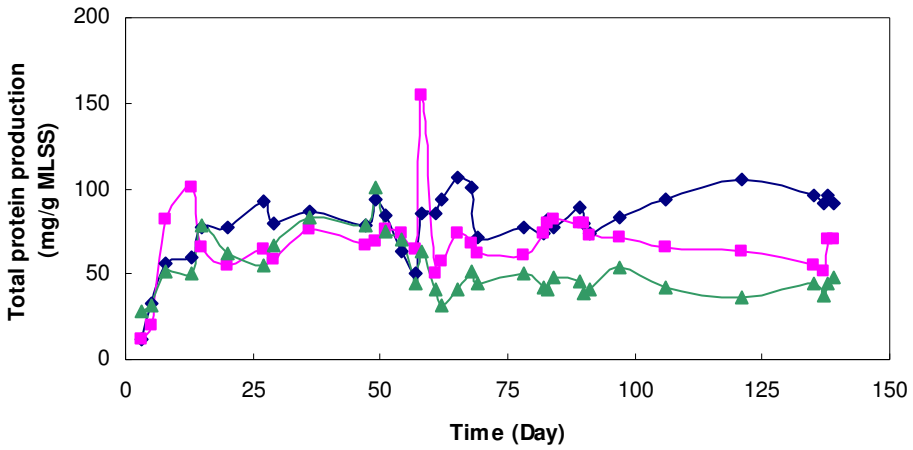


Figure 4.3.5 Total protein production under different SRTs (◆) SRT 10 days (■) SRT 30 days (▲) SRT infinity

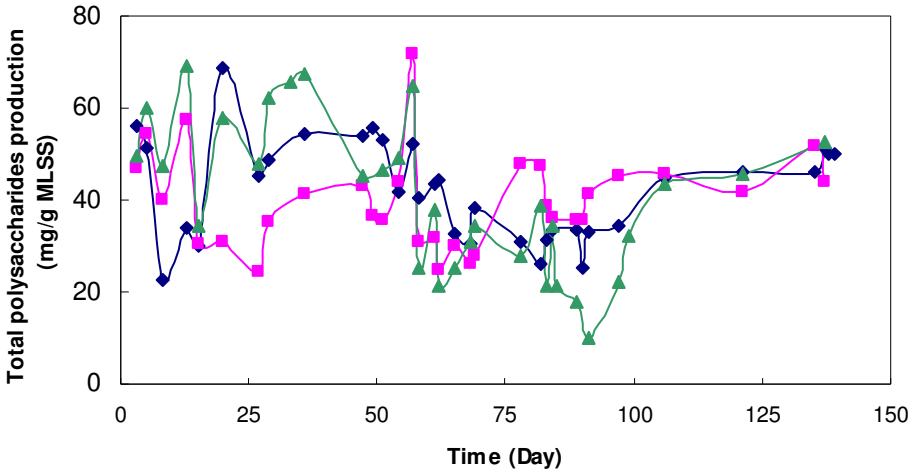


Figure 4.3.6 Total polysaccharides production under different SRTs (◆) SRT 10 days (■) SRT 30 days (▲) SRT infinity

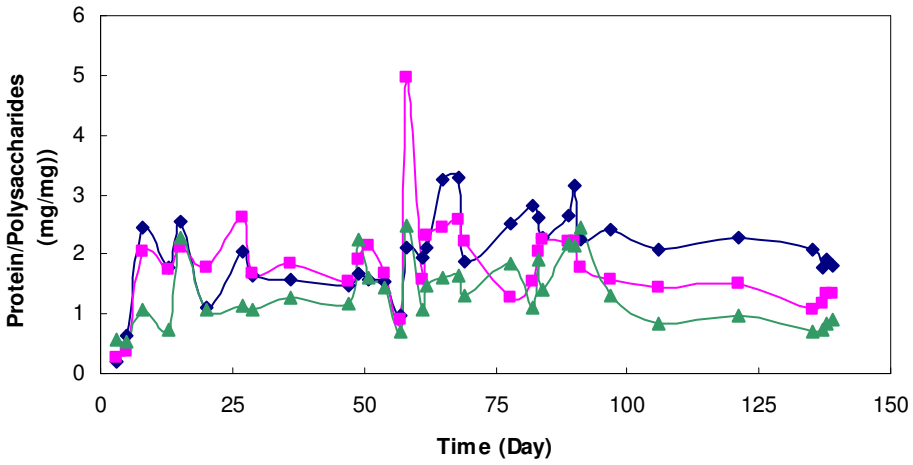


Figure 4.3.7 Ratios of protein to polysaccharides under different SRTs (◆) SRT 10 days (■) SRT 30 days (▲) SRT infinity

It was observed that of this total EPS, which was extracted from both biofloc and supernatant, less than 10% was from the supernatant (Figure 4.3.8). This suggests that the EPS attached on the bacterial surface was dominating compared with the soluble EPS. However the soluble EPS may be particularly important in terms of fouling in the MBR. During the start-up period, the soluble EPS production (including soluble protein production and polysaccharides production) was a little

higher than that at the steady state. Protein and polysaccharides followed similar trends as EPS production (Figure 4.3.9 and Figure 4.3.10).

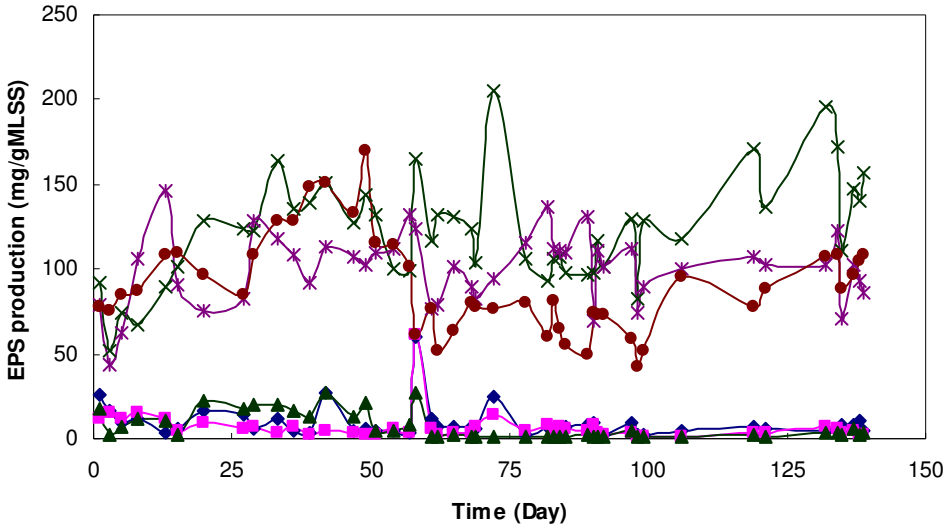


Figure 4.3.8 EPS distribution under different SRTs
(◆) Soluble EPS (SRT 10 days) (■) Soluble EPS (SRT 30 days)
(▲) Soluble EPS (SRT infinity) (×) Attached EPS (SRT 10 days)
(*) Attached EPS (SRT 30 days) (●) Attached EPS (SRT infinity)

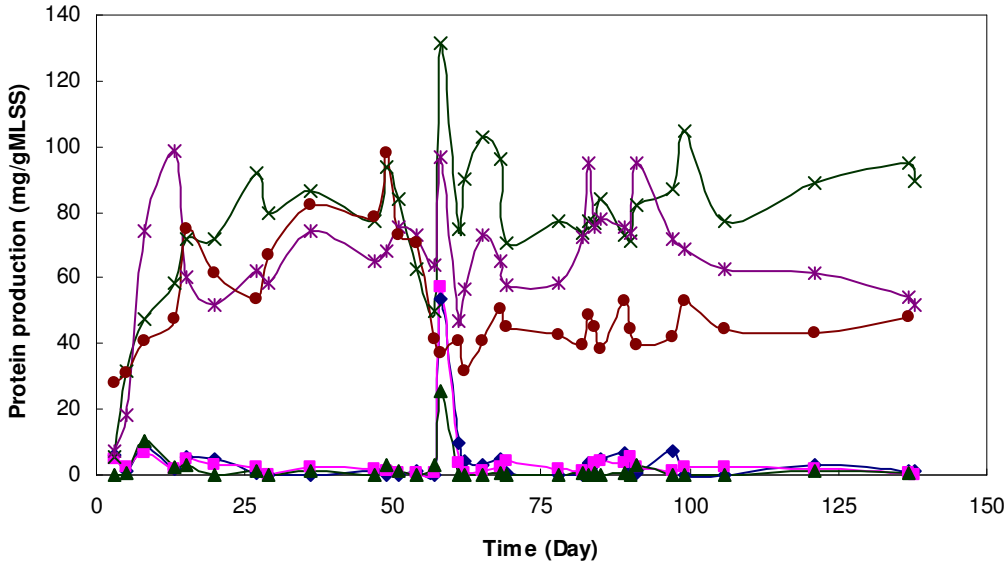


Figure 4.3.9 Protein distribution under different SRTs
(◆) Soluble protein (SRT 10 days) (■) Soluble protein (SRT 30 days)
(▲) Soluble protein (SRT infinity) (×) Attached protein (SRT 10 days)
(*) Attached protein (SRT 30 days) (●) Attached protein (SRT infinity)

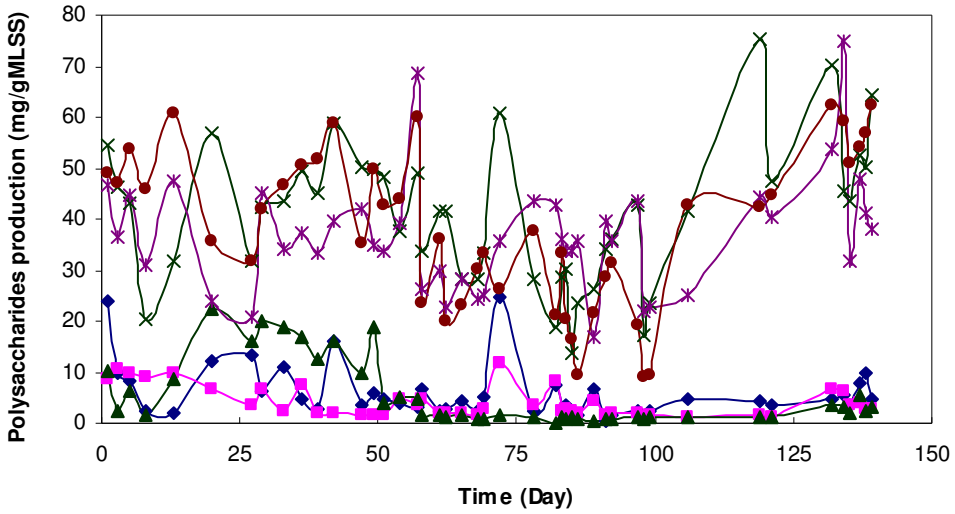


Figure 4.3.10 Polysaccharides distribution under different SRTs
(◆) Soluble polysaccharides (SRT 10 days) (■) Soluble polysaccharides (SRT 30 days) (▲) Soluble polysaccharides (SRT infinity)
(×) Attached polysaccharides (SRT 10 days) (*) Attached polysaccharides (SRT 30 days) (●) Attached polysaccharides (SRT infinity)

4.3.3.3 Membrane fouling tendency under different SRTs

Figure 4.3.11 summarizes the data for EPS concentration and composition, membrane fouling rate and specific fouling resistance of the mixed liquors at the

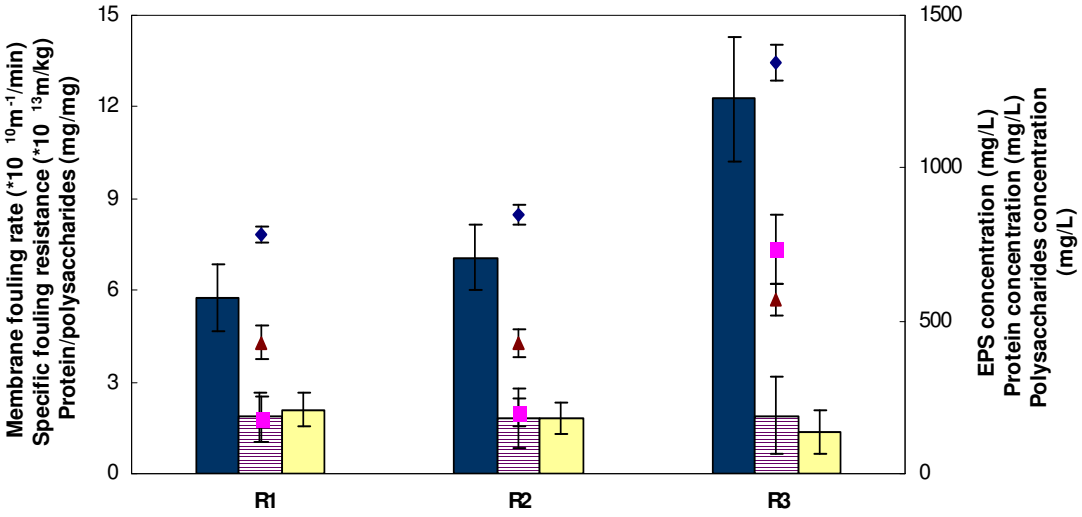


Figure 4.3.11 Relationship between membrane fouling tendency and EPS characteristics at the steady state under different SRTs
(Dark bar) Membrane fouling rate (Striped bars) Specific fouling resistance (Light bars) Ratio of protein to polysaccharides (◆) EPS concentration (■) Total protein concentration (▲) Total polysaccharides concentration

different SRTs when the reactors were at steady state. EPS concentration and protein concentrations (as mg/L of liquor) were similar at SRT 10 days and SRT 30 days, and were about half of those at SRT of infinity. On the other hand, the membrane fouling rate of the mixed liquor at SRT of infinity was about two times that at SRT 10 days and 30 days. These data suggest that the membrane fouling rate is related to the EPS concentration when the reactors are stabilized. However, the results showed that the specific fouling resistances of the mixed liquors from the three reactors were not significantly different. This suggests that fouling was more influenced by foulant load rather than by different fouling structures.

In order to confirm the suitability of the short-term fouling assays, the long-term TMP profiles from the three reactors were examined. The features of the profile as the initial TMP rise (dTMP/dt) and the approximate time to the rapid TMP rise (TMP jump). Table 4.3.1 compares the short-term fouling parameters with the long-term fouling observed for the three reactors. The results in Table 4.3.1 confirm that the short-term fouling assays of samples taken from each reactor correlated satisfactorily with the long-term TMP profiles.

Table 4.3.1 Comparison of membrane fouling tendency under different SRTs by short-term and long-term filtration tests

SRT (days)	Short-term parameters		Long-term parameters	
	Fouling rate ($*10^{10}m^{-1}$/min)	Specific fouling resistance (10^{13}m/kg)	$(dTMP/dt)_{initial}$ (m^{-1}/hr)	Time to TMP jump (hr)
10	5.73	1.86	0.0269	105~116
30	7.07	1.80	0.0201	85~100
infinity	12.27	1.89	0.0439	65~84

4.3.3.4 DGGE analysis under different SRTs

The activated sludge samples were taken from each reactor and DNA was extracted periodically over the 150-day operation. DGGE analysis of the microbial communities demonstrated the presence of many characteristic bands in the separation pattern, most likely derived from the predominant bacterial species among the population (Figures 4.3.12, 4.3.14 and 4.3.16).

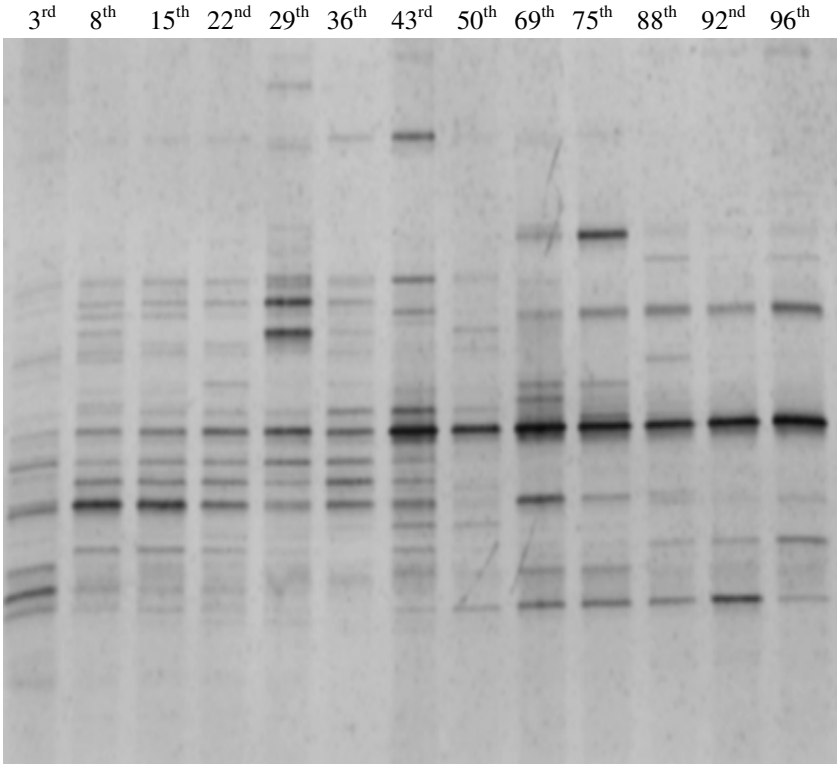


Figure 4.3.12 DGGE of PCR-amplified 16S rRNA from the bacterial communities under SRT 10 days

As shown in Figure 4.3.18, similar banding patterns were found in the initial period of R1, R2 and R3 due to the same seed being employed in the three reactors. However, at steady state, comparison of the bacterial community structure of the three reactors indicated that each reactor had different dominating bacterial consortia. This suggests that the changes in SRT explicitly caused the changes of the dominant bacteria although the initial seed was the same for each MBR system. It should be recalled that the different SRTs are associated with different F/M ratios

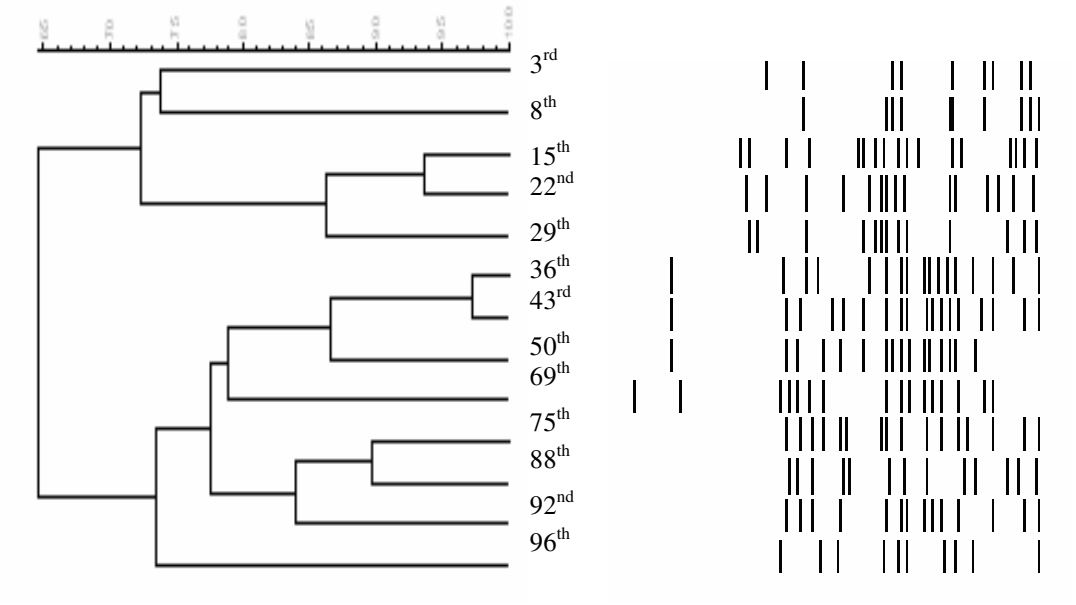


Figure 4.3.13 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns under SRT 10 days

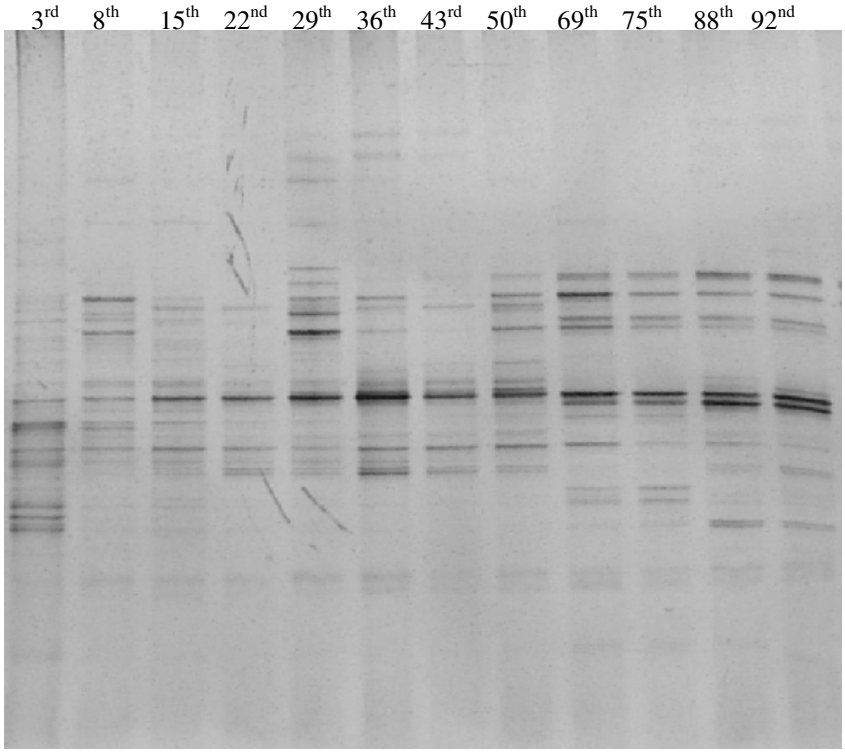


Figure 4.3.14 DGGE of PCR-amplified 16S rRNA from the bacterial communities under SRT 30 days

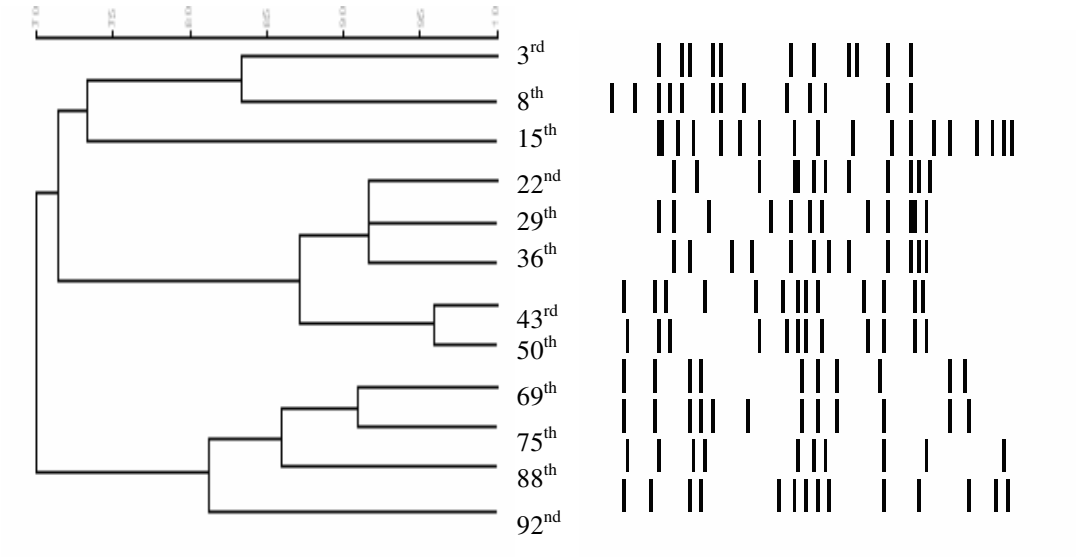


Figure 4.3.15 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns under SRT 30 days

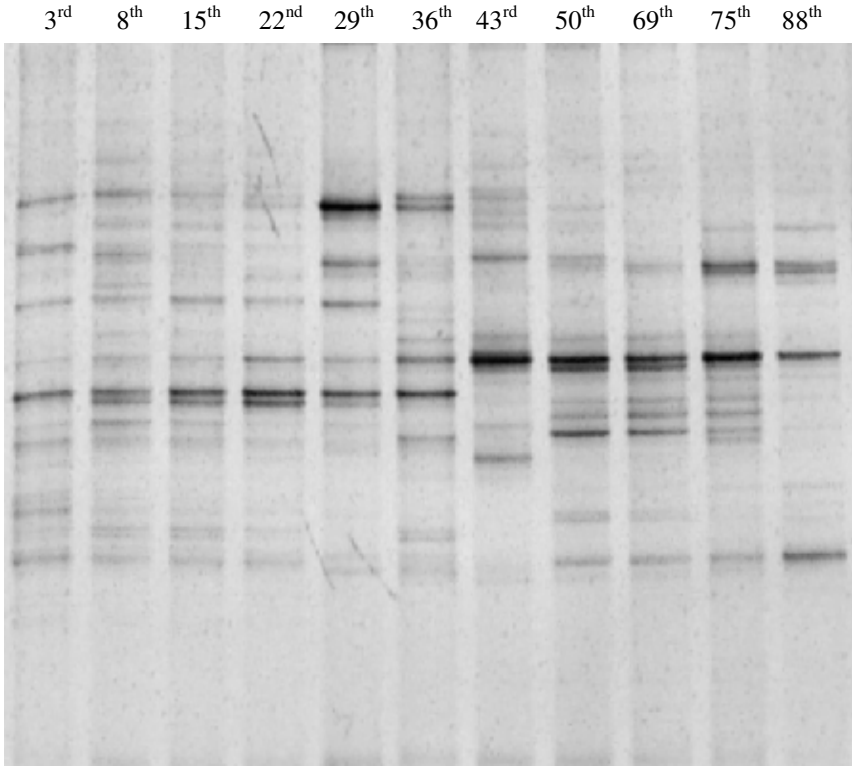


Figure 4.3.16 DGGE of PCR-amplified 16S rRNA from the bacterial communities under SRT infinity

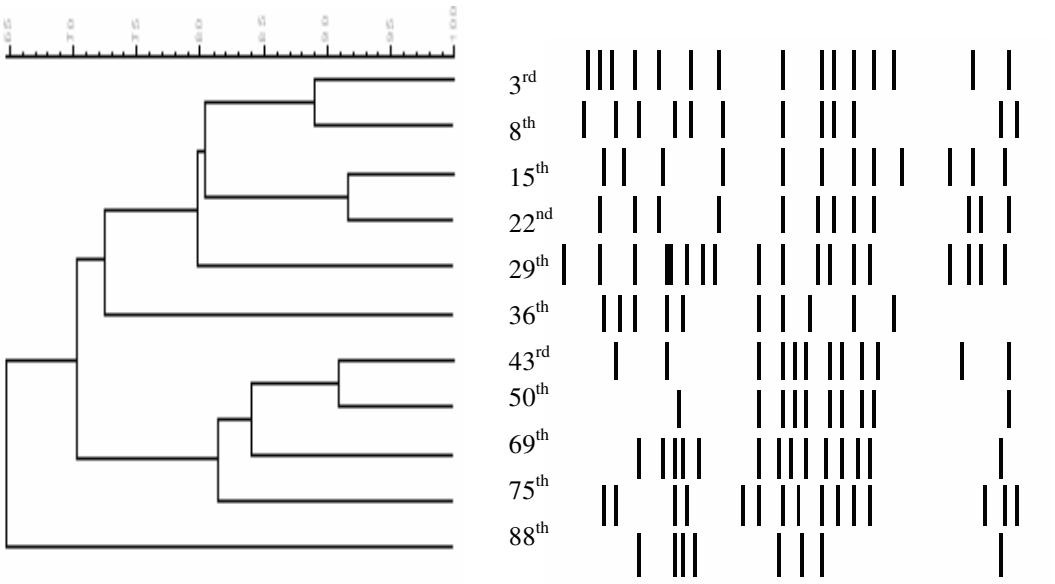
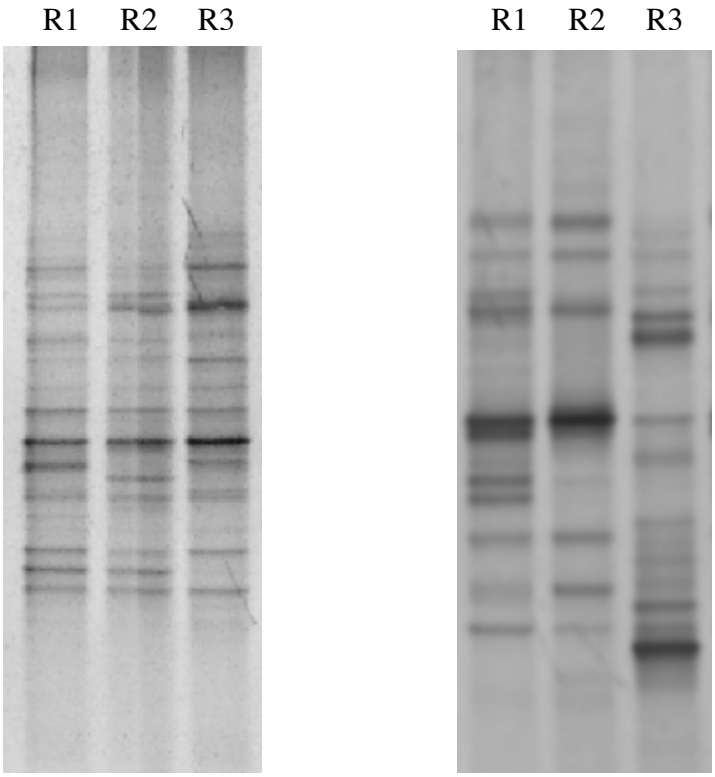


Figure 4.3.17 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns under SRT infinity



(1) Day 3 (2) Day 120

Figure 4.3.18 DGGE of PCR-amplified 16S rRNA from the bacterial communities under different SRTs

and oxygen concentrations. Either or both of these environmental conditions could cause shifts in bacterial community structure. The UPGMA dendrogram are calculated based on the Dice coefficient with Arithmetic Mean algorithms by the GelCompar II software shown in Figures 4.3.13, 4.3.15 and 4.3.17 for each reactor, respectively.

4.3.4 Discussion

SRT is employed to describe the average sludge age in an activated sludge process, which is associated with biomass growth and microbial behavior. With extended SRT, limited sludge production and low biomass activity occur, as well as heterotrophic bacteria per MLSS greatly decrease (Pollice et al., 2004; Liu et al., 2005). This study confirms that SRT is an important factor in influencing the performance of the MBR systems. SRT is linked to the activated sludge characteristics, bacterial community structure as well as membrane fouling tendency. At the same time, as noted above, SRT also determines environmental factors, F/M and dissolved oxygen.

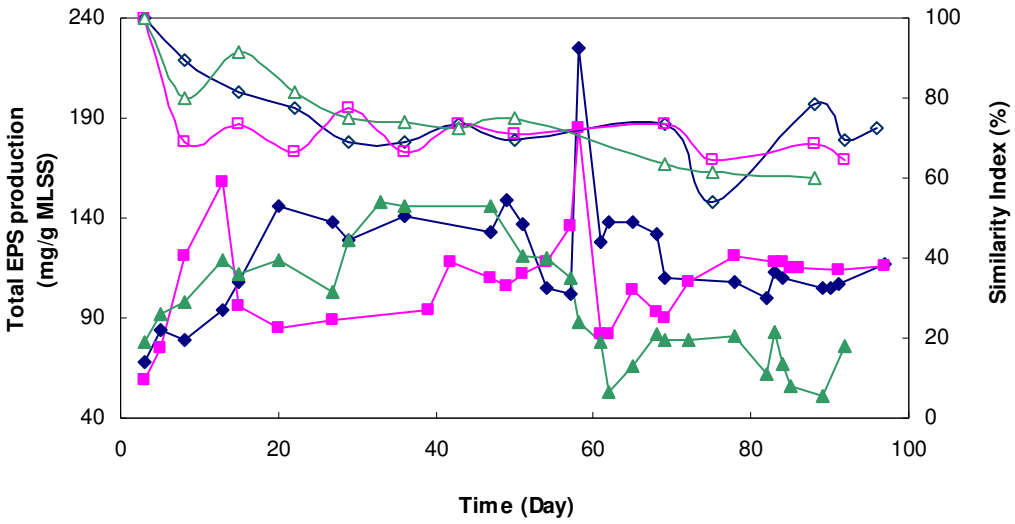


Figure 4.3.19 Relationship between bacterial community change and EPS production
(◆) SRT 10 day (■) SRT 30 day (▲) SRT infinity
(◇) Similarity index under SRT 10 day, (□) Similarity index under SRT 30 day, (△) Similarity index under SRT infinity

Previous studies have suggested that a shift in the composition of the microbial community might be expected to account for the change of EPS production in the activated sludge process (Liao et al., 2000). From this study, the relationship between EPS production and microbial population shifts can be seen in Figure 4.3.19. The observed trend for EPS production is a decline, apart from the rise at start up and the peak at day 58 due to disturbance. The population shifts seen in Figure 4.3.19 also show a trend to a new population composition over time. Thus as population approaches a new "steady state", the EPS production declines.

By examining the similarity indices of the DGGE profiles, it was observed that at the beginning of operation (day 0-10), the bacterial community structure in each reactor had shifted quite rapidly. Changes in EPS production of the activated sludge also occurred in the reactors, mainly via protein production (Figure 4.3.5). This could be because the bacteria would tend to induce more enzymes in order to acclimatize to the new living environment and experience metabolic stress. These enzymes are considered to be the main protein components of EPS. Further shifts in the bacterial population as well as fluctuating EPS production were observed in the three reactors. A reason for this is that reactor conditions promote the presence and activity of desirable microorganisms and discourage the growth of the unfavorable microorganisms. An interesting feature of the DGGE profiles is a trend to fewer bands and the dominance of one or two bands. This suggests a less diverse community, dominated by some of the microorganisms. These population changes cause obvious changes of EPS production for each MBR system. When the system approached a "steady state", the fingerprint patterns of DGGE profiles were similar for the different biomass samples, indicating a highly stable microbial community in each reactor. During this period, the change in EPS production in each reactor was also not significant. Therefore, it appears that shifts of bacterial community structures in each stage lead to changes of EPS production over time in the MBR system.

The DGGE profiles revealed that the dominant bacteria under the different SRTs were explicitly various species after a long period of acclimatization, which would

play different functions in the MBR system. It is also seen that the bacterial community was more diverse at longer SRT compared with shorter SRT. SRT is related to the growth rate of microorganisms, which are able to reproduce themselves and can be detained and enriched in the system. So, high SRTs allow the enrichment of slowly growing bacteria and hence, a more diverse bacterial community with broader physiological capabilities compared to low SRTs (Clara et al., 2005).

For shorter SRT (10 days), the MLSS concentration in the reactor was lower which resulted in a higher ratio of food to microorganisms (F/M) under the same feed conditions. In addition, the dissolved oxygen concentration was higher compared with the longer SRT MBRs; the more efficient oxygen transfer was probably due to decreased fluid resistance with the lower MLSS concentration and viscosity. This surplus substrate and energy utilized by microorganisms (mainly aerobic bacteria) would contribute to form intracellular storage granules and extracellular polymers that accumulated as EPS (Liao et al., 2000). Thus the EPS production (mg/g MLSS) would be higher at shorter SRT, as observed here. For prolonged SRT (infinity), it has been reported that biological activity such as specific oxygen uptake rate, specific nitrification rate and specific denitrification rate was decreased (Han et al., 2005). It is evident that a comparatively large amount of cell lysis could occur due to lower microbial growth rates and a higher level of endogenous metabolism at higher SRTs. However, although cell lysis would be predominant and contribute much more to the total EPS production, the limited substrate, and lower dissolved oxygen concentration and inefficient oxygen transfer would not only decrease the sludge yield coefficient (Tay et al., 2003) but also retard EPS production by microorganisms (mainly facultatively anaerobic or anaerobic bacteria). Therefore, EPS production as a characteristic of microorganisms is dependent on the living condition of the microorganisms, such as F/M, dissolved oxygen or sludge age. A simple model is suggested to describe the balance between the excreted substances and cell lysis of microorganisms, both of which are major contributors to EPS production (Figure 4.3.20). An optimal SRT range with lower EPS production can be inferred from this model, although more work is needed to confirm this.

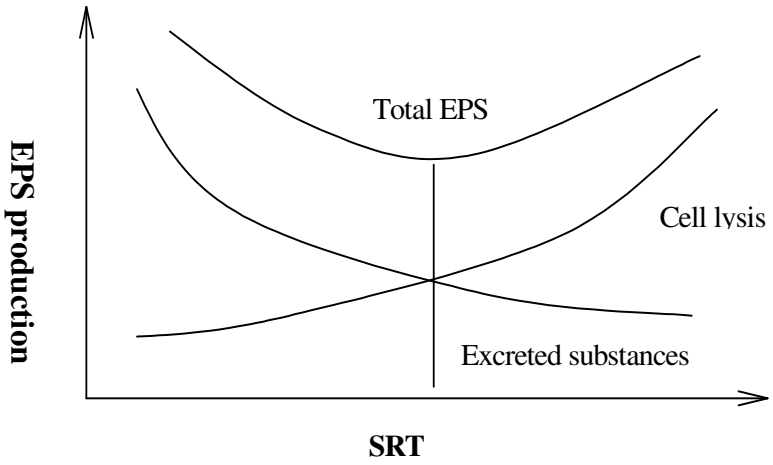


Figure 4.3.20 EPS production model under different SRTs

EPS as excreted substances and cell lysis substances easily adhere to the membrane surface. In addition, extracellular matrix can fill the void spaces between the cell particles in a deposited cake to affect the cake resistance (Hodgson et al., 1993). Thus, EPS concentration can be a major factor to cause membrane fouling in a MBR system. The results show that several factors could explain the greater fouling rate characteristics at prolonged SRT. These include the greater MLSS concentration (12.9 g/L vs 4.7 and 8.3 at SRT 10 and 30 days), the relatively smaller floc size (d_{50} of 181 μm vs 246 and 235 μm), the greater amount of EPS (as mg/L), polysaccharides and proteins. In a typical MBR the flux is adjusted to avoid substantial cake formation, so the effect of MLSS concentrations per se is unlikely to be the factor of interest. The most likely fouling factors are those that relate to the supernatant, particularly the EPS components, polysaccharide and protein. Notably the concentrations of EPS (36.1 mg/L vs 31.5 and 32.3 mg/L), polysaccharides (32.1 mg/L vs 25.4 and 27.9 mg/L) in the supernatant were higher at prolonged SRT.

In summary, this chapter has investigated the effects of operating parameters (substrate loading, substrate composition and SRT) on EPS characteristics, membrane fouling tendency and bacterial populations. In all cases, bacterial populations shifted significantly in the start-up period, accompanied by an obvious

change in EPS production in each reactor. When the reactors approached the stable stage, the bacterial community structure and EPS production varied insignificantly over time. However, different operating parameters had a significant (substrate loading and SRT) or slight (substrate composition) influence on the dominant bacterial population at steady state by affecting the reactor properties, such as F/M and dissolved oxygen. Different functions were performed by these different dominant bacteria, which led to a change in EPS production under different operating parameters. Membrane fouling tendency was associated with EPS characteristics, such as EPS concentration, polysaccharides and protein concentration. The most likely fouling factors are those that relate to the supernatant, particularly the soluble EPS concentration.

CHAPTER 5

MEMBRANE FOULING MECHANISMS CAUSED BY EPS

5.1 Introduction

Activated sludge is a complex system in which three different solute fractions can be distinguished: suspended solids consisting mainly of bacterial floc with a concentration depending on the sludge age, and dissolved solids including colloids (polymers, fragments of lysed cells) and dissolved molecules (Defrance et al., 2000). In Chapter 3 it was confirmed that most of the EPS is attached on the surface of microbial floc, and a minor amount of the EPS is dissolved in the solution. Hence with EPS regarded as a major contributor to membrane fouling, each fraction with its own physiocochemical and biological properties performs various functions in the fouling process. Investigations of MBR membrane fouling contribution caused by each fraction have been evaluated, although common conclusions have not been drawn so far. Some researchers observed that the resistance of the cake layer formed by suspended solids appeared to determine the overall resistance, which suggests that suspended solids play an important role in membrane fouling. (Defrance et al., 2000; Lee et al., 2003; Chang and Lee, 1998; Bae and Tak, 2005). However, other studies have shown that the colloids and dissolved solids are to be considered of prime importance in causing membrane fouling in a MBR system (Bouhabila et al., 2001; Chang and Kim, 2005; Le-Clech et al., 2005). In their studies, Wisniewski and Grasmick (1998) showed that the soluble compounds provided half of the total resistance, which indicates that both fractions can contribute similarly to membrane fouling. This range of findings may be explained by the complex nature of fouling which depends on many factors including membrane properties, imposed flux, hydrodynamic environment as well as the physiological state of the sludge which is determined by the biological operating parameters. Obviously, these conflicting results can not give a clear picture of

membrane fouling factors in the activated sludge process. Thus, in this chapter, the contributions of suspended solids and dissolved solids (including colloids) from a lab-scale MBR and the Bedok MBR plant to membrane fouling will be examined considering EPS characteristics. Further, the mechanisms of fouling by dissolved solids with regard to the effect of cation and colloids will be illustrated.

5.2 Materials and methods

5.2.1 MBR set-up

The description of the MBR is given in Section 3.2.1 (Chapter 3).

5.2.2 Sampling

Phase 5.1: The activated sludge samples were taken from a lab-scale MBR system and a large-scale MBR system for studying the contributions of suspended solids and dissolved solids to membrane fouling. Samples were taken when the lab-scale MBR system was operated in phase 3.2 (Section 3.2.1 in Chapter 3). The large-scale MBR samples were obtained from the Kubota MBR system and Mitsubishi MBR system located at the Bedok MBR demonstration plant in Singapore. Some details of the operation of those MBRs have been provided by Tao et al. (2005). The separation of suspended solids and dissolved solids was achieved by centrifuging at 4,000 rpm for 10 minutes. Then the suspended solids were resuspended in the same amount of distilled water as the decanted supernatant.

Phase 5.2: Three samples were obtained from the lab-scale MBR system. Dissolved solids were separated from the activated sludge by centrifuging at 4,000 rpm for 10 minutes. Cations were removed from the dissolved solids by a centrifugal filter device with a regenerated cellulose membrane (3,000 MWCO, Millipore Pte Ltd., USA) at 4,000 rpm for 30 minutes. Removal of colloids were fulfilled by employing 250 mg/L $Al_2(SO_4)_3$ solution to flocculate the colloids for 30 minutes after the dissolved solids had passed through a 0.45 μm membrane filter. Finally, the colloids were removed by centrifuging at 4,000 rpm for 10 minutes (Defrance et al., 2000).

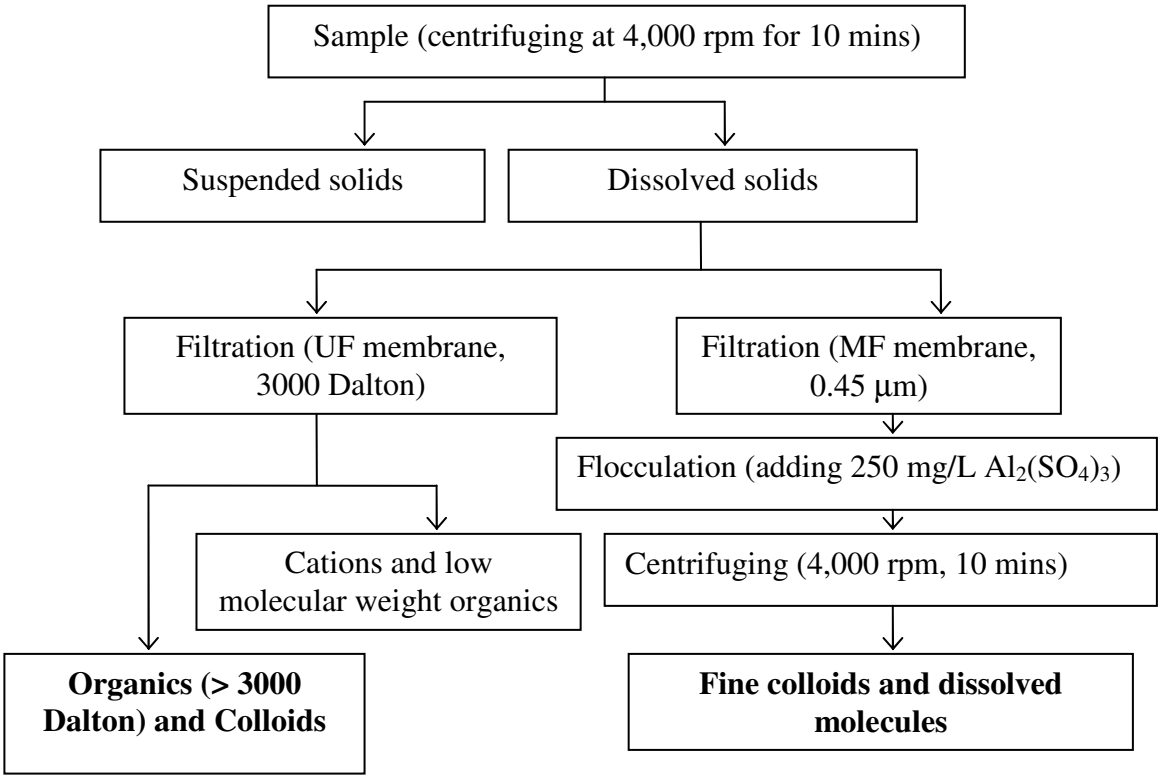


Figure 5.1 Pathway of the methods used for removing colloids and cations

5.2.3 Analytical methods

Cationic concentrations (Ca^{2+}, Na^{+}, Mg^{2+}, K^{+}, Fe^{3+}) were measured by Inductively Coupled Plasma (ICP) analyzer (Perkin Elmer, USA). Molecular weight distribution of the dissolved solids was determined by using High Performance Size Exclusion Chromatography (HPSEC) (Waters, USA). Deionized water was used as a mobile phase. The Altra hydrogel HPSEC columns 120, 250, 500 (7.8 mm × 30 cm, Waters, USA) were used in series and a refractive index detector was employed. Analysis was performed at 40ºC using Shodex standards (Waters, USA). Samples prepared for HPSEC analysis were filtered through 0.45 µm microfiltration membranes to remove large particles and large colloids.

5.2.4 Membrane fouling assay

The membrane fouling test was performed as described in section 4.1.2 (Chapter 4). Cross-flow mode was fulfilled with an internal magnetic bar (40 mm long & 8.7

mm diameter), which was suspended 9 mm above the membrane surface in the membrane fouling test set-up (Figure 5.2). The magnetic stirrer was operated with a stirring speed of about 80 rpm. Permeate flux was controlled by a peristaltic pump at 30 L/m^2 hr. The fouling properties were characterized by two parameters. The fouling resistance (Rc) after 60 minutes filtration was based on flux and permeate data and application of equations [4.1] and [4.2]. The specific cake (or fouling) resistance (α) was calculated according to equation [5.1].

$$\alpha = \frac{R_c}{m_c} \quad [5.1]$$

where α is the specific resistance of foulant (m/g), Rc is fouling resistance (m^{-1}) and m_c is the foulant load on the membrane (g/m^2). Because EPS are considered as the major foulant in MBRs, the value of m_c was estimated by EPS loading (g/m^2) deposited on the membrane surface. EPS loading was calculated by the EPS amount (g) (equals concentration (mg/L) multiplied by sample volume (L)) in the filtration test divided by the membrane area (m^2).

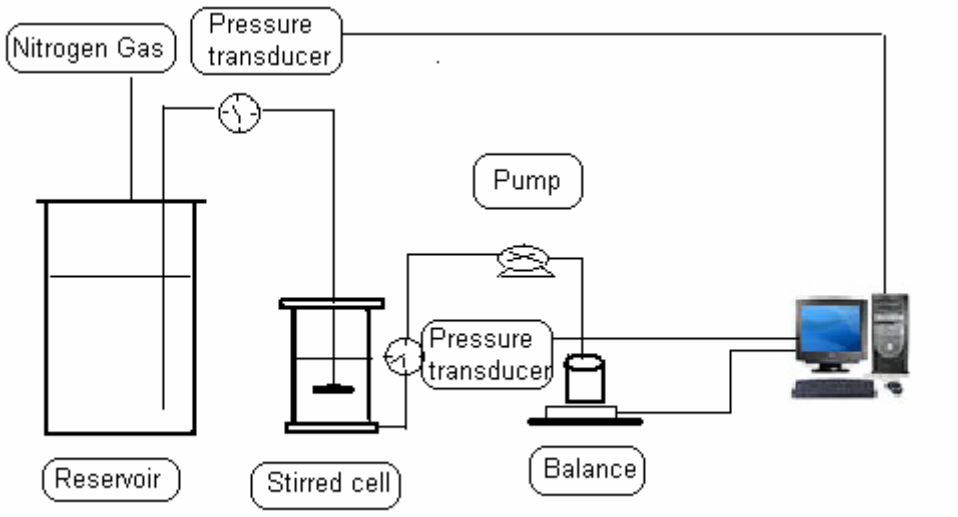


Figure 5.2 Schematic of the membrane fouling assay (stirred cross-flow mode)

5.3 Results

5.3.1 The contributions of suspended solids and dissolved solids to membrane fouling

Figure 5.3 presents the membrane fouling resistances of the dissolved solids and suspended solids compared with those of activated sludge under dead-end (unstirred) and cross-flow (stirred) conditions. The three activated sludge samples were taken from the lab-scale MBR operated in Phase 3.2. Dissolved solids and suspended solids were separated by centrifuging (4000 rpm, 10 minutes). Using this protocol the dissolved solids include colloids. Data for the graphs were derived from the average values of triplicate results. Overall the resistances of the dissolved solids had slightly higher values compared with the suspended solids, implying a marginally greater contribution to fouling. However it was immediately obvious

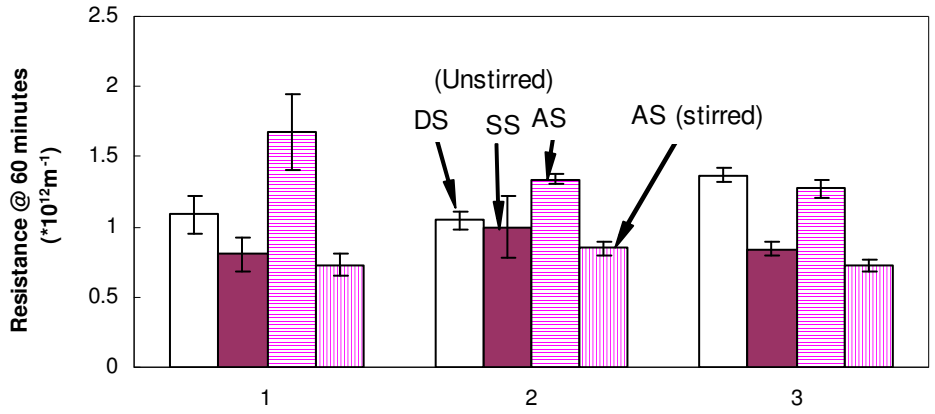


Figure 5.3 Contributions of suspended solids and dissolved solids to membrane fouling in the lab-scale MBR system
(Light bar) Dissolved solids without stirring (Dark bar) Suspended solids without stirring (Horizontal striped bar) Activated sludge without stirring (Vertical striped bar) Activated sludge with stirring

that the sums of the resistances of the dissolved solids and suspended solids were more than that of the combined activated sludge, which might not be surprising as the resistances are not 'in series' in the mixed activated sludge. The colloids and macrosolutes of the supernatant would be present in the cake layer mixed with the suspended solids and would not form a separate layer. Thus, in the mixed feed their contribution to the overall resistance is probably diminished (Defrance et al., 2000). Under stirred conditions, the resistance would be decreasing compared to that without stirring because shear forces along the membrane surface would limit cake layer formation.

Figure 5.4 indicates the contributions of suspended solids and dissolved solids in the activated sludge from the Bedok MBR demonstration plant to membrane fouling. Samples 1 and 2 came from the Kubota MBR system and samples 3 and 4 were taken from the Mitsubishi MBR system at different times. Overall the magnitudes of total resistance were in the same range ($1\sim5\times10^{12}$ m^{-1}) as the lab-scale MBR. However, the results show that for the pilot-plant MBR samples, the suspended solids played a more dominant role in the membrane fouling assay compared with the dissolved solids. These trends do not correspond to that from the lab-scale MBR. A possible reason is related to the characteristics of the suspended solids in the two systems. The suspended solids had a biomass concentration of about 1.5~4.0 g/L in the lab-scale MBR system and above 10 g/L in the pilot MBR systems. In addition, there was a significant difference in the particle size for both systems. Evidently, the mean particle sizes of the activated sludge from the lab-scale MBR system were 10~20 times larger than those from the pilot MBR system (Figure 5.5).

Since EPS are considered as major substances that increase the viscosity of mixed liquor and cause an increase in transmembrane pressure in MBRs (Nagaoka et al., 1996), the specific membrane fouling resistance per unit EPS loading (Equation 5.1) is employed to compare the contributions of suspended solids and dissolved solids*. Figures 5.6 and 5.7 illustrate that the dissolved solids performed substantial functions in the membrane fouling in the two systems with regard to specific fouling resistance of EPS loading although the amounts of the dissolved solids were less compared with suspended solids.

*In order to attain the specific fouling resistance per unit EPS, the Rc values for Figures 5.3 and 5.4 were divided by the estimate loadings of EPS on the membrane. For example, in order to estimate sample 1 data for the dissolved solids in Figure 5.6, the resistance $Rc=1.09\times10^{12}$ m^{-1} (Figure 5.3) and the amount of EPS filtered in the supernatant $=0.84$ g/m^2 were used. This gives $\alpha=1.30\times10^{12}$ m/g (Figure 5.6). Similarly for suspended solids is $Rc=0.81\times10^{12}$ m^{-1}(Figure 5.3) and the EPS amount in the filtered suspended solids is 3.89 g/m^2, so $\alpha=0.21\times10^{12}$ m/g (Figure 5.6).

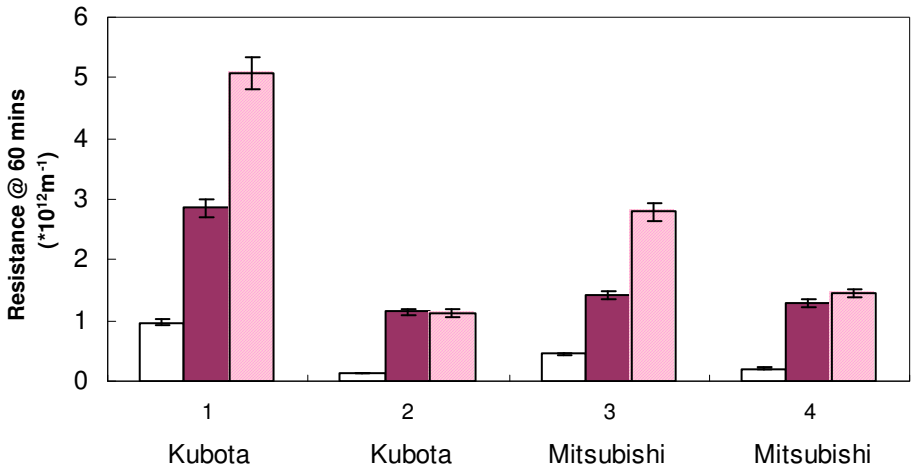


Figure 5.4 Contributions of suspended solids and dissolved solids to membrane fouling in the Bedok MBR system
(Light bar) Dissolved solids without stirring (Dark bar) Suspended solids without stirring (Striped bar) Activated sludge without stirring

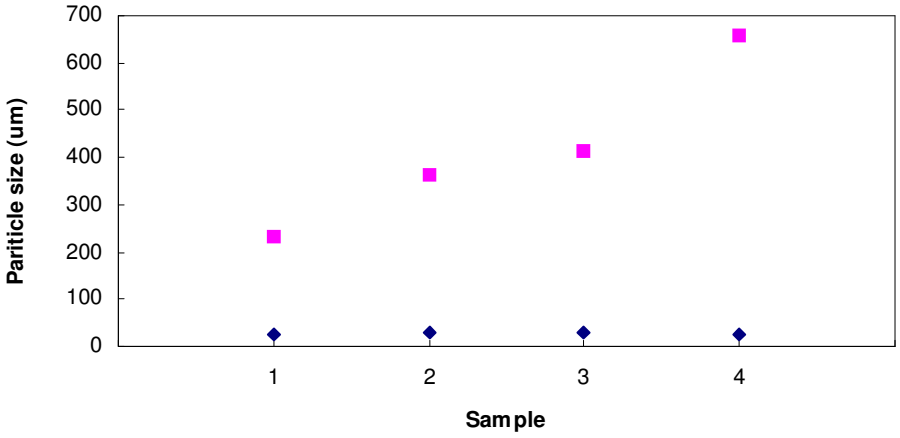


Figure 5.5 Particle sizes (d_{50}) of activated sludge from the lab-scale MBR system and the Bedok MBR system
(◆) Bedok MBR (■) Lab-scale MBR

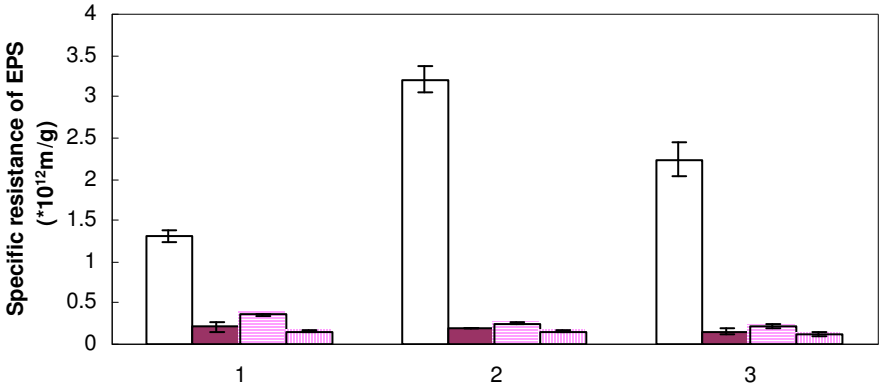


Figure 5.6 Contributions of suspended solids and dissolved solids to membrane fouling in the lab-scale MBR system with regard to EPS loading (Light bar) Dissolved solids without stirring (Dark bar) Suspended solids without stirring (Horizontal striped bar) Activated sludge without stirring (Vertical striped bar) Activated sludge with stirring

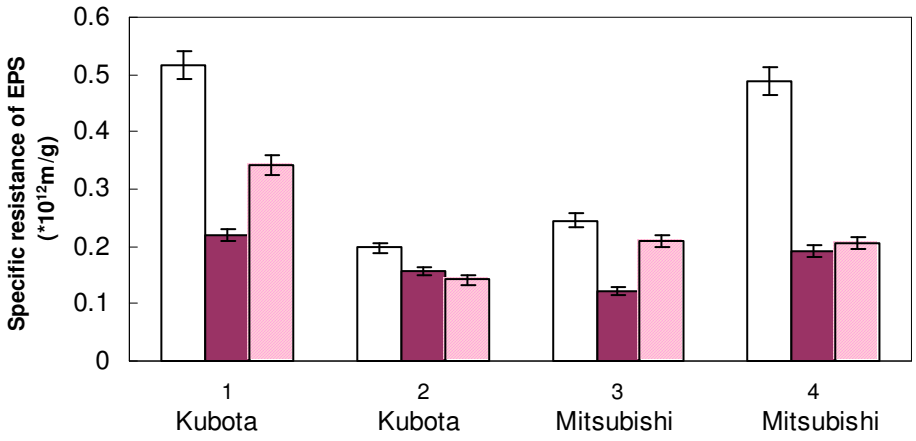


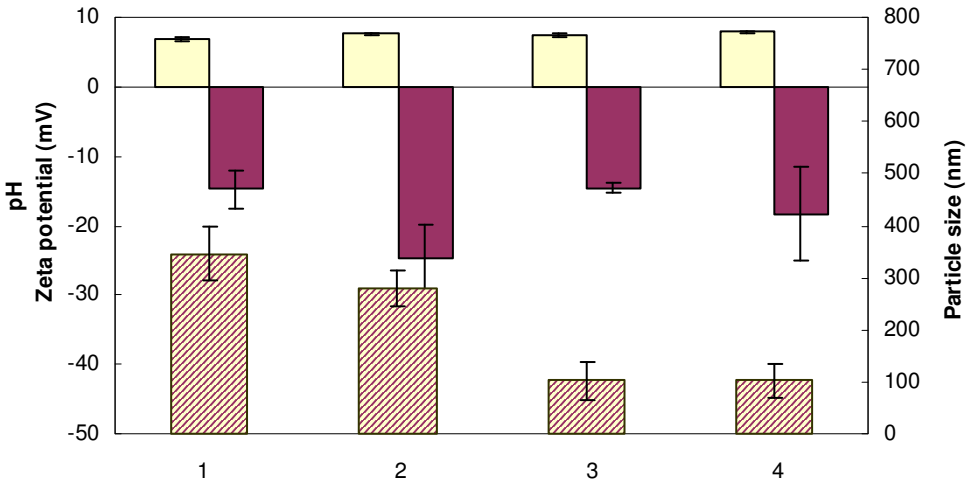
Figure 5.7 Contributions of suspended solids and dissolved solids to membrane fouling in the Bedok MBR system with regard to EPS loading (Light bar) Dissolved solids without stirring (Dark bar) Suspended solids without stirring (Striped bar) Activated sludge without stirring

5.3.2 Study of the membrane fouling mechanisms caused by dissolved solids (including colloids)

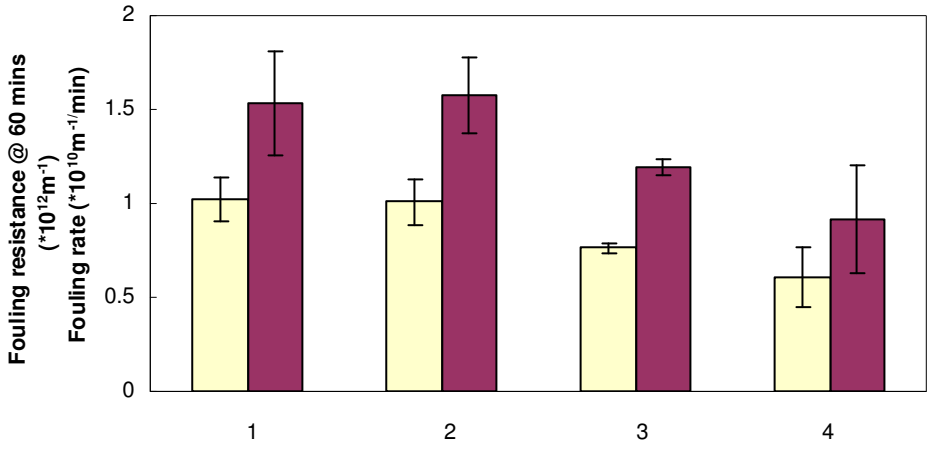
Results above (in Section 5.3.1) indicated that dissolved solids performed a key function in membrane fouling although they held less EPS amount than the suspended solids. This is correlated with the observation that the dissolved solids can be the main factor in flux decline and induce irreversible membrane fouling in

the initial phase of filtration (Bae and Tak, 2005; He et al., 2005). Nevertheless, the contribution ratios of dissolved solids from the lab-scale MBR and the pilot MBRs were different, which suggests that different organic macromolecules in the dissolved solids have different potentials for binding together and affecting membrane fouling. Variations in composition (polysaccharides and proteins) or removal of some specific components, therefore, may lead to changes in the characteristics of dissolved solids as well as their membrane fouling tendencies.

Figure 5.8 summarizes the changes in pH, zeta potential and particle size of dissolved solids before and after removing colloids or/and cations (85-90% cations removed based on methods described in the Figure 5.1). The 'raw' sample had a pH value of 6.8 and there was a slight increase to 7.5~7.9 after removing colloids or/and cations. After removing the cations, the apparent zeta potential decreased dramatically from -14.8 mV to -24.7 mV, but it seemed to be unaffected by the removal of colloids. The surfaces of EPS species tend to display negative charges, leading to the presence of large concentrations of counter-ions within the dissolved solids. After removing the cations, the zeta potential decreased dramatically (more negative) because some negative charged groups would be exposed due to the lack



1. Dissolved solids samples 2. Samples with cations removed
3. Samples with colloids removed 4. Samples with cations and colloids removed
Figure 5.8 Changes of characteristics of dissolved solids before and after removing cations or/and colloids
(Light bar) pH (Dark bar) Zeta potential (Striped bar) Particle size



1. Dissolved solids samples 2. Samples with cations removed
3. Samples with colloids removed 4. Samples with cations and colloids removed
Figure 5.9 Changes of membrane fouling resistances of dissolved solids before and after removing cations and colloids
(Ligh bar) Fouling resistance @ 60 mins (Dark bar) Fouling rate

of positive charges. After removing the colloids, the total amount of positive and negative charges decreased together, so that the zeta potential value was not affected significantly. It was found that the zeta potential of dissolved solids was -18.4 mV after removing colloids and cations, which was higher than that after removing cations. The mean particle size dropped by about 25%, 80% and 80% after removing cations, colloids and cations and colloids from the dissolved solids compared with the raw sample, respectively. Thus, the effect of the colloids was more significant on the mean particle size than the cations. Figure 5.9 reveals that the membrane fouling rates and final fouling resistances of the dissolved solids after removing colloids, cations plus colloids were significantly less than those of the dissolved solids. There was not a clear difference after only removing cations. Compared with the 'raw' dissolved solids, the removal of colloids and colloids plus cations reduced the fouling characteristics by 22% and 41%, respectively.

Since the fine colloids and macrosolids in the supernatant importantly influenced membrane fouling tendency, the distribution of molecular weights was examined to investigate the fouling mechanisms caused by these species. The samples were derived from phase 4.1 and 4.3 (Section 4.1 and 4.3 in Chapter 4). Previous results

showed that higher substrate loading and longer SRT had higher membrane fouling tendency (Figures 4.1.12 and 4.3.11 in Chapter 4). This trend coincides with the distribution of molecular weights ranging from 10K to 50K Dalton, whose range covered the majority of the dissolved solids (Figures 5.10 and 5.11). Since the pore size of the membrane employed for the membrane filtration test was 50K Dalton, this shows that fine colloids and macrosolids with a similar molecular weight to the membrane pore size play major roles, presumably due to pore blocking.

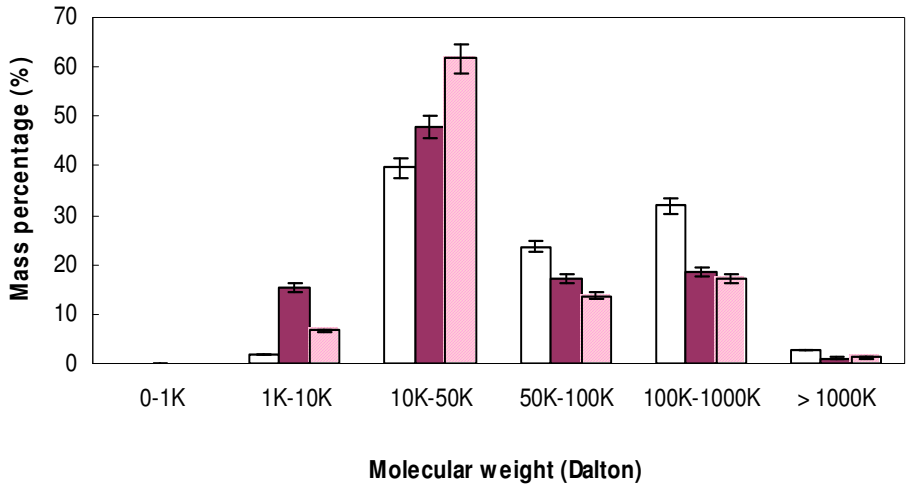


Figure 5.10 Molecular weight distributions of dissolved solids in the MBR under different substrate loadings1
(Light bar) TOC 106 mg/L (Dark bar) TOC 212 mg/L (Striped bar) TOC 424 mg/L

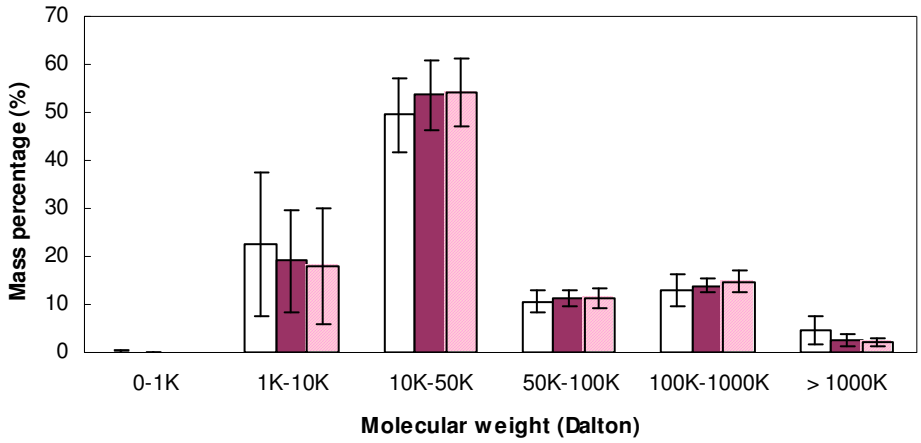


Figure 5.11 Molecular weight distributions of dissolved solids in the MBR under different SRTs
(Light bar) SRT 10 days (Dark bar) SRT 30 days (Striped bar) SRT Infinity

5.4 Discussion

5.4.1 The contribution of suspended solids and dissolved solids to membrane fouling

This study focused on the contributions of suspended solids and dissolved solids to the membrane fouling tendency. The suspended solids are considered as the bacterial flocs, which are described as the microorganisms embedded in a matrix of EPS. The supernatant (dissolved solids, including 'fine' colloids) contain a variety of soluble organics including residual degradable and non- or slowly-biodegradable substrates in the influent, intermediate substrates, the end products as well as excreted and lysed substances by microorganisms. The presence of dissolved solids is a matter of great interest concerning not only current discharge standards, but also the membrane filtration performance in MBRs.

The results indicate that the dissolved solids dominated in the membrane foulants of the lab-scale MBR system, but suspended solids performed the major functions to membrane fouling in the Bedok MBR system using a fouling assay. Possibly, the different concentrations and mean particle sizes of the suspended solids in the two systems can explain this apparent conflict. The activated sludge had a much higher

mean particle size in the lab-scale MBR compared with that in the Bedok MBR systems. Normally, the cake layer resistance is related to the shape and size of the activated sludge flocs and the porosity of the cake layer accumulated on the membrane surface (Chang et al., 1999). If the particles are relatively large compared to the size of the membrane pore, the deposit layer of the particles is not dense enough create a significant resistance (Kwon and Vigneswaran, 1998). This suggests that larger particles depositing on the membrane surface do not increase TMP up to a significant value. In addition, the biomass amounts in the Bedok MBR system were ten times those in the lab-scale MBR system when the same volume of sample was employed, which would enhance the thickness of the cake layer. Hence, significant membrane fouling in the fouling assay tests in the Bedok MBR system is attributed to a thick and dense cake layer caused by suspended solids.

Since this incompatible phenomenon appears to be related to the activated sludge characteristics, which are ascribed to different scale MBR systems with different operating conditions, a direct comparison may not provide much useful and complete information on the contributions of suspended solids and dissolved solids. One of the difficulties is that in practice MBRs are operated at a flux which aims to limit deposition of floc cake layers. Fouling tends to be very slow under these conditions. It is thus difficult to apply short-term assays to compare very different MBR systems (lab vs plant-scale). However our evidence is that the short-term tests can be useful to compare the effects of operating conditions (substrate load, composition etc.) for parallel lab-scale MBRs.

Previous studies have shown that EPS influences the characteristics of activated sludge and is also a key foulant in membrane fouling in MBRs. Thus specific resistance of EPS was employed to compare the contributions of suspended solids and dissolved solids by eliminating the effects of activated sludge characteristics, such as biomass concentration and particle size. The consensus is arrived at that the dissolved solids play an important role in the membrane fouling in both MBR systems after normalizing with their own EPS loading. It is evident that membrane fouling, as an increase in resistance, is relatively sensitive to the EPS characteristics.

This confirms the conclusions drawn by Cho and Fane (2002) that even at low values of EPS the resistance increases steadily, possibly due to pore plugging and closure.

5.4.2 Study on the membrane fouling mechanisms caused by dissolved solids (including colloids)

Dissolved solids (including colloids) have been shown to be a large contributor to membrane fouling although their contributions may vary depending on the source of the activated sludge samples. In MBRs, dissolved solids mainly consist of organic compounds that result from substrate metabolism and biomass decay during the complete mineralization of simple substrates (Park et al., 2005). Based on this study, cations and colloids combined are significant (40~50%) components of the dissolved solids performing important functions in membrane fouling. The presence of multivalent cations is essential for the formation of ionic bridges and the maintenance of ordered structures since they are involved in electrostatic interactions with surface groups of protein and polysccharides, such as carboxylates, hydroxyls (Wingender et al., 1999; Flemming et al 2000b; Park et al., 2005). Hence the surface charges of soluble species and colloids have significant effects on membrane fouling behavior and cleaning efficiency (He et al., 2005). Colloids normally have a particle size ranging from several nanometers to several millimeters, however based on the protocol the colloids upper limit would be about 0.45 μm. Therefore, they would easily cause membrane pore blocking and closure. In this study the characteristics and filtration properties of the dissolved solids before and after removing cations or/and colloids were measured in order to clarify their influencing mechanism.

After removing the cations or/and colloids, the surface charge and particle size in the dissolved solids was significantly influenced, and this caused changes of membrane fouling resistances. Surface charge can influence interaction of organic and inorganic colloidal substances with membrane surfaces in the suspension (Kwon and Vigneswaran, 1998). It was reported that the surface charge of a polyethersulfone membrane at pH 7.0 was about -15 mV (Burns and Zydney, 2000). The dissolved solids with lower cations have more negative surface charges, which

provides less permeate flux decline due to a repulsion force created by the similar charge between the membrane and dissolved solids. This explanation is confirmed by He et al. (2005). In addition, presumably, the decrease of the zeta potential (greater negative value) provides a loose deposit layer with higher porosity, which also reduces the membrane fouling tendency. The lack of response to cations removal from the 'raw' dissolved organics (sample 2 in Figure 5.9), may be due to an interplay between the various foulants (fine colloids and macrosolutes) etc.

After removal of colloids alone (sample 3 in Figure 5.9) the fouling tendency dropped by 22%, indicating that the colloid species were a significant fouling component of the dissolved solids. The fact that removal of both colloids and cations (sample 4 in Figure 5.9) further reduced fouling tendency suggests that the cations were interacting with the non-colloid organics. One possibility is the ability of divalent cations to 'cross-link' and bind with carboxylic groups. This could reduce the permeability of the macromolecular deposits on the membrane.

In order to clarify the roles of the fine colloids and macrosolids on the membrane fouling, the distributions of molecular weights in the dissolved solids were monitored and a relationship between the molecular weight distribution and membrane fouling tendency was established. Normally, EPS with its large molecules contributes to a "sorptive sponge" structure, in which microorganisms are embedded. This property benefits from microbial metabolism of organic matter by binding and concentrating available substrates. Different bacteria possess specific EPS production and composition characteristics, which display different binding affinities for dissolved organic matter and ions (Wingender et al., 1999). In addition, due to the effect of chemical factors (protease, ionic strength and salinity) and physical factors (pH, temperature, UV irradiation and shear force), degradation or detachment of these fragile structures occur. Thus, the dissolved solids (mainly EPS molecules) display a broad spectrum of molecular weight distribution. The results have indicated the distribution of molecular weight has a significant influence on membrane filtration tendency. Since the pore size of the polyethersulfone (PES) membrane used in this study was 50,000 Dalton, it can not

retain small molecular weights species as low as several thousands Dalton. But the species whose molecular weights are close to the membrane pore size are more easily deposited into the pores to foul the membrane. Once most of the membrane pores are occupied by the dissolved solids, the membrane performance is compromised. In addition, higher molecular weight substances are retained in the solution due to the membrane separation. It is assumed that a portion of these particles are transported to the membrane surface where they are absorbed to form a thin cake layer.

In summary, a same trend is observed in the lab-scale MBR and the Bedok MBRs that the dissolved solids cause more serious membrane fouling when specific resistance of EPS is considered as the comparison factor. The membrane fouling tendency of dissolved solids after removing colloids plus cations decreases by amount of about 40~50%. Further the species whose molecular weights are close to membrane pore size appear to be more important in influencing membrane fouling.

CHAPTER 6

STUDY OF THE FUNCTIONS OF ISOLATED BACTERIA FROM THE MBR SYSTEM

6.1 Introduction

In Chapter 4, it was shown that operating parameters (substrate composition, substrate loading, SRT) in the MBR system could influence the bacterial community structure. The change of microbial diversity would further appear to have an effect on EPS production, which finally causes different membrane fouling tendencies in the MBR system. Although a comparison of the microbial diversity of activated sludge may give important information about the functions of bacterial consortia, how individual bacterial strains function in MBRs is still a question. Answering this question could be an important step towards understanding and controlling membrane biofouling in MBRs.

Molecular phylogenetic approaches, such as Denaturing Gradient Gel Electrophoresis (DGGE), and culture-dependent methods like isolation have been used for the screening of isolates that have dominant phylogentic signatures of original ecosystems (Muyzer et al., 1993; Miambi et al., 2003). Laboratory pure-culture experiments are essential for detailed analyses of the physiology of microorganisms together with their functions in membrane fouling. As the ecological behavior of a microorganism is determined by interactions with other microorganisms based on its multiple physiological traits, study of relationships of microorganisms will be an important approach for understanding the membrane fouling mechanisms. It is possible that such information, obtained from identified microorganisms, could be used for manipulating the important 'membrane-fouling' bacteria or 'membrane-friendly' bacteria to optimize the operation of MBRs.

Therefore, in this study, the 16S rRNA DGGE technique was employed to obtain evidence of temporal and spatial variations in the bacterial community structure in order to compare bacterial diversities on the membrane surface and in the mixed liquor. In addition, pure cultures were obtained by traditional isolation methods and the functions of isolated bacteria were evaluated by investigating EPS characteristics and membrane fouling tendency. The characteristics of pure cultures influencing membrane fouling are discussed.

6.2 Materials and methods

6.2.1 Sampling

The types of membrane used in this study were hollow-fiber membrane modules made of Polyacrlonitrile (Blue Star Company, China) with a pore size of 500 K Dalton, membrane area of $2.64*10^{-3}$ m^2 (refer to section 3.2.1 in Chapter 3). Three bioreactors were used operating at different SRTs; Reactor 1 had SRT 10 days, R2 had 30 days and R3 had SRT infinity (no wastage). Before placing into each reactor, the membrane modules were cleaned with 5~10% sodium hypochlorite. The membrane modules were soaked in the mixed liquor when the reactors were operating under the initial phase and stable phase (Operating parameters were the same as the description in Phase 4.3, see Section 4.3 in Chapter 4) and constant flux filtration (30 L/m^2*hr) was carried out. Membrane fibres were cut from the membrane modules after working for 48 hours in the three reactors. The fibres were then gently washed with 0.85% NaCl solution in order to remove any floc loosely attached on the membrane surface. The fibres were put into sterile tubes filled with 50 ml sterile 0.85% NaCl solution. Then the suspended bacterial solutions were prepared from the fibres by ultrasonication for 30 seconds. Meanwhile, a mixed liquor sample was obtained from the reactor and put into a sterile tube, which was ultrasonicated for 30 seconds to disperse the floc.

Samples were also obtained from a flat-sheet MBR system operating in the same laboratory. This MBR system was operated under HRT of 6 hours, SRT of 30 days, aeration rate of 20 L/min and feed organic loading rate at 0.6 kg COD/m^3.day. The

membrane module was made of Polyethylene (Kubota Pte Ltd) with 0.2 μm pore size. When the MBR was operated under stable conditions (after 150 days), the membrane was placed into the reactor. The samples were obtained from the membrane surface (before and after washing with distilled water for 10 minutes) and mixed liquor after this module had operated for 6 days.

6.2.2 Analytical methods

6.2.2.1 SEM observation

The mixed liquor samples were centrifuged at 4,000 rpm for 10 minutes to remove the water. Then the samples were fixed by adding 2% glutaraldehyde. After 2 hours, the samples were washed in 0.10 M sodium cacodylate buffer for 20 minutes three times. Then the samples were dehydrated in series of 10 minute washes in 50%, 70%, 85% and 95% ethanol. After filtering with a 0.2 μm membrane, the samples were dried with freeze-drier equipment (Christ, Germany). The samples were gold-coated using a sputter gun (SPI supplies, USA). Photographs of the sample structure were observed and taken with a scanning electron microscopy (Jeol, UK).

6.2.2.2 Isolation

Serial dilutions of the solutions from the membrane surface and mixed liquor within the range of 10^{-4}-10^{-7} were spread on to petri dishes containing R2A agar (Difco, USA) and synthetic wastewater agar (the compositions of the synthetic wastewater were described in Section 3.2.1.2 in Chapter 3), and incubated at 25ºC for 10 days. Colonies were counted on the plates every two days, until no new colonies were observed.

6.2.2.3 Direct lysis PCR conditions

The genomic DNA of the pure culture was extracted by the direct lysis method. Several colonies were picked from the agar plates into a PCR tube and 87 μl lysis buffer was added to the tube. The lysis buffer consisted of 10 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$ and 0.05% Nonidet P40 (NP40). Then the tube was put in a thermal cycler (Mastercycler, Eppendorf, Germany) for 30 minutes at 98ºC. A 5.3 μl mixture including 0.8 μl 200 μM of deoxynucleoside triphosphate (including deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxythymidine

triphosphate, deoxycytidine triphosphate), 2 µl 25 pmol forward primer Eubac27F (5'-AGAGTTTGATCCTGGCTCAG-3'), 2 µl 25 pmol reverse primer Universal 1492R1 (5'-GGTTACCTTGTTACCTT-3') and 0.5 µl 5 U/µl *Taq* DNA polymers was added into the tube. PCR was run in the thermal cycler under the following conditions: 30 cycles consisting of 1 minute and 30 seconds at 94°C, 1 minute and 30 seconds at 62°C, 2 minutes at 72°C (Maszenan et al., 2000). 1.5% (wt/vol) agarose gel in 1×TAE buffer was used to run electophoresis with ethidium bromide staining to confirm the proper size of the PCR products. These DNA products were purified with Qangen DNA clean kit (Qangen, Germany). Then the 16S rRNA gene was amplified from genomic DNA by the PCR method described in Section 4.1.2 (Chapter 4).

6.2.2.4 Culture

Cells were removed from the R2A agar plates and inoculated in a screwcapped test tube with sterile R2A broth. The composition of R2A was as follows: yeast extract 0.5 g/L, proteose peptone 0.5 g/L, casein hydrolysate 0.5 g/L, glucose 0.5 g/L, soluble starch 0.5 g/L, sodium pyruvate 0.3 g/L, dipotassium hydrogenphate 0.3 g/L, magnesium sulphate 0.05 g/L. The tubes were gently shaken at approximately 150 rpm at room temperature of about 25ºC.

6.2.2.5 Gram staining

In order to determine the Gram staining behavior of the isolated strains, the staining kit (DIFCO, BD Inc, USA) was used as recommended by the manufacturer. The cell morphology was observed by an optical microscope (Olympus, Japan).

6.2.2.6 Growth curve observation

The pure culture was taken from the culture tube with the same initial concentration and put into a 250 ml glass flask with 100 ml sterile synthetic wastewater whose TOC was 286 mg/L. The bacterial concentration was monitored over a period of time by UV-VIS spectrometer (Jasco V-550, Japan). The samples for EPS and filtration analysis were obtained when the bacterial growth was under the stationary phase.

6.2.2.7 EPS measurement

Bacterial cells were harvested by centrifugation at 4,000 rpm for 10 minutes. The resulting pellet was separated from the supernatant and resuspended in distilled water. 1.2 µl formaldehyde (37%) was added into the floc solution. After 1 hour, 80 µl NaOH (1 N) was added into the floc solution and kept for 3 hours at 4°C. Then, the supernatant solution and floc solution were centrifuged (13,200 rpm, 20 minutes, 4°C) before chemical component analysis as described in Section 3.2.2.2 (Chapter 3).

6.2.2.8 Dead-end filtration test and cross-flow filtration test

Dead-end filtration tests and cross-flow filtration tests were performed as described in section 4.1.2 (Chapter 4) and section 5.2.4 (Chapter 5). After 60 minutes filtration, the membrane was removed and washed with distilled water for several minutes to remove the cake layer depositing on the membrane surface. 20 ml distilled water was added to the filtration cell and the irreversible membrane fouling was measured. For the cross-flow filtration test, the sterile synthetic wastewater was fed into the filtration cell continuously from the reservoir. The whole system was sterilized with 10% NaOCl followed by UV exposure before the experiments.

6.2.2.9 Contact angle measurement

The hydrophobicity of each bacterial strain was evaluated by water contact angle measurement on a prepared strain cake. This measurement was carried out with a modified axis symmetric drop shape analysis method using a contact angle goniometry (Dataphysics, Germany). The strain sample was filtered onto a cellulose acetate membrane (Millipore, 0.22 µm) held on a glass filter with a vacuum pump. The membrane with the deposited strain cake was then carefully transferred into an oven at 105 °C to remove the moisture content of the cake. Then the dried strain cake was analyzed by the contact angle goniometry. A drop of distilled water was placed on the strain cake by a micrometer syringe equipped with a stainless steel needle. The sessile drop shape was captured with no further shrinking of the water drop after several seconds by an image system (Duncan-Hewitt et al., 1989; Sharma and Rao et al., 2002).

6.2.2.10 Surface charge and particle size determination

The surface charge and particle size of the strain solution and supernatant were determined by a ZetaPALS (Brookhaven, USA).

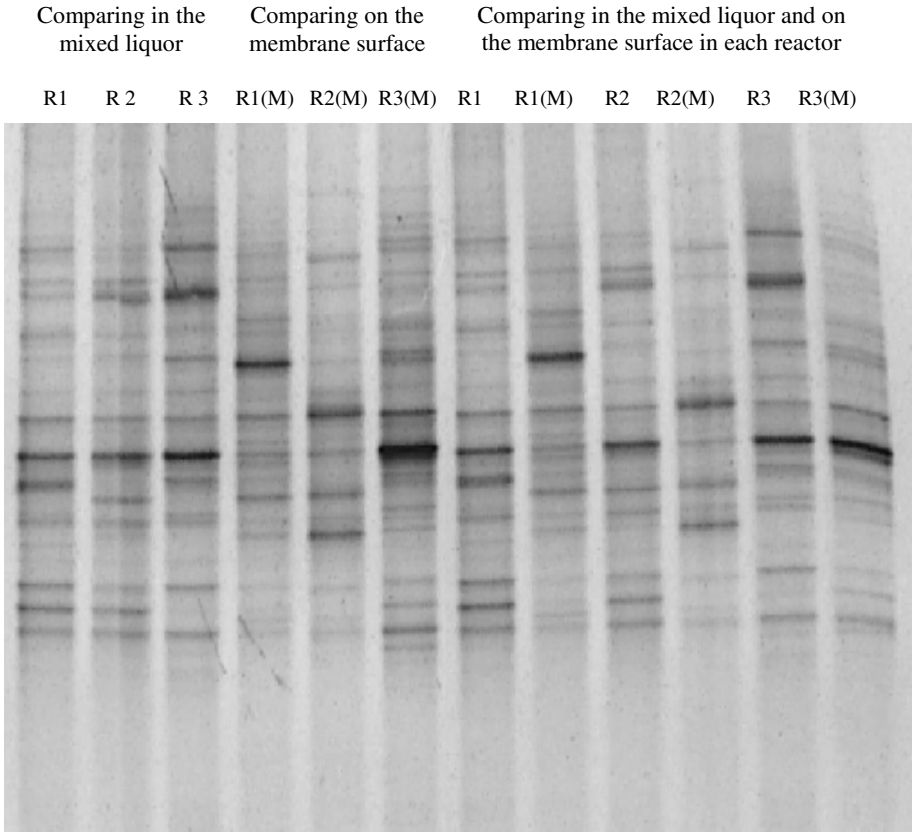
6.3 Results

6.3.1 Comparisons of bacterial community structures in the mixed liquor and on the membrane surface

The DGGE profiles of the bacterial community structures attached on the membrane surface and suspended in the mixed liquor were compared for the three hollow fibre MBRs (R1 (SRT 10 days), R2 (SRT 30 days), R3 (SRT infinity)). A highly diverse bacterial community with a limited number of strongly dominant bands was found in the DGGE profiles. In the initial period of operation (Figure 6.1), bacterial community structures in the mixed liquor of the three reactors showed high similarity (Figure 6.2), which suggests that the bacterial diversities are not significantly different for the three reactors. A probable reason is that the same seed was fed into the reactors and during this short period, the bacterial communities mainly focused on regulation of metabolisms in order to acclimatize to the new environment. Also, although DGGE profiles contained some similar bands, the dominant bacteria in the mixed liquor and on the membrane surface were not similar for each reactor. However, during the stable conditions of the reactors (Figure 6.3) the bacterial PCR-DGGE fingerprints demonstrated that there were distinctly different bacterial community structures in the mixed liquors for the three MBRs due to the different SRTs being employed (see also discussion in Section 4.3.4). Comparing the DGGE profiles of the bacterial consortia from the mixed liquor and the membrane surface in the each reactor, numerous common bands were observed, which are demonstrated by the similarity indexes (Figure 6.4).

Figure 6.5 presents the bacterial community structures from the mixed liquor and membrane surface in the flat sheet MBR system during the stable phase. The formation of a thick gel layer was observed on the flat sheet membrane surface after 6 days' filtration. High similarity indexes were shared by the bacterial community structures from different positions on the membrane surface before washing. This

confirms that distribution of bacteria on the fouled membrane is consistent in the well mixed MBR system. Comparing the DGGE profiles of microbial communities from the membrane surface and the mixed liquor, it was found that most of the dominant bands are similar.



R1 (SRT 10 days), R2 (SRT 30 days), R3 (SRT infinity)

Figure 6.1 DGGE of PCR-amplified 16S rRNA from the bacterial communities during the initial phase (Day 3)

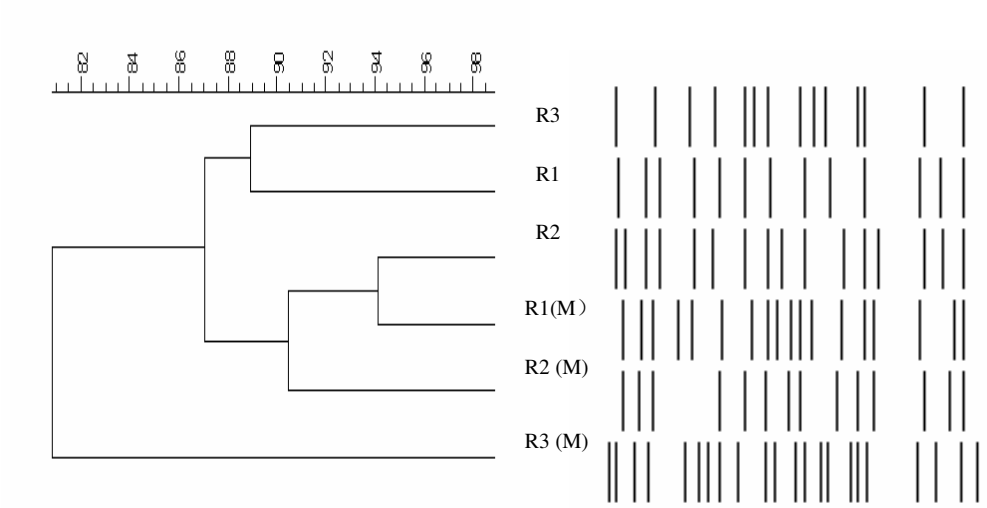
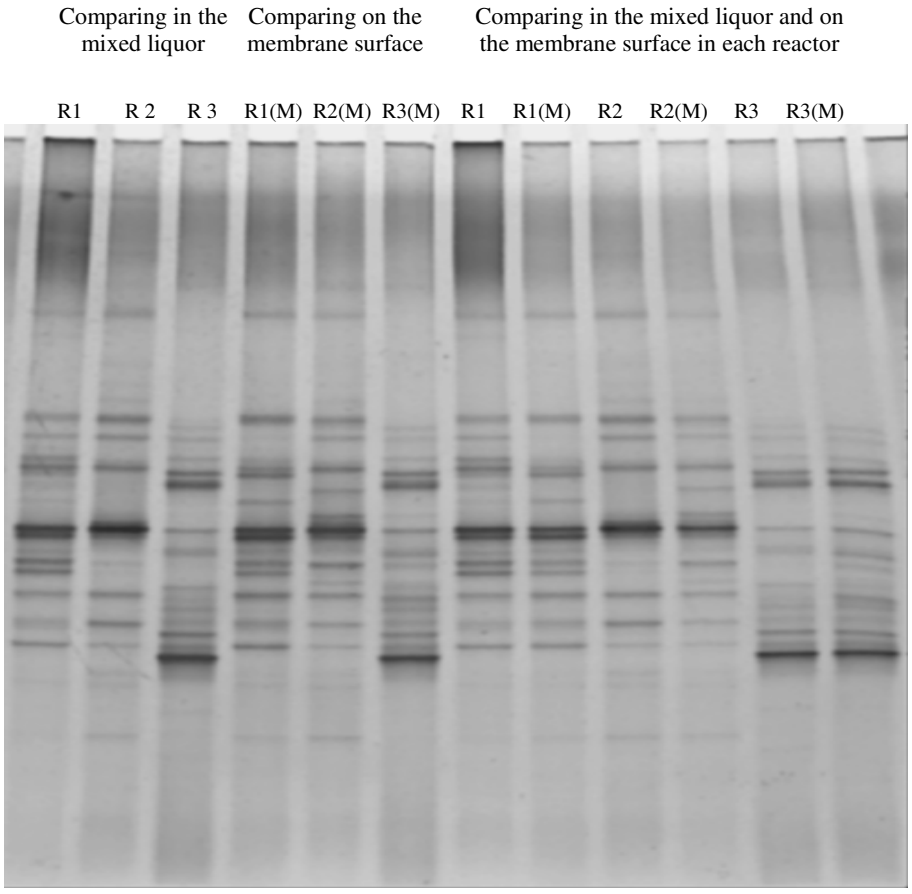


Figure 6.2 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns during the initial phase (Day 3)



R1 (SRT 10 days), R2 (SRT 30 days), R3 (SRT infinity)

Figure 6.3 DGGE of PCR-amplified 16S rRNA from the bacterial communities during the stable phase (Day 120)

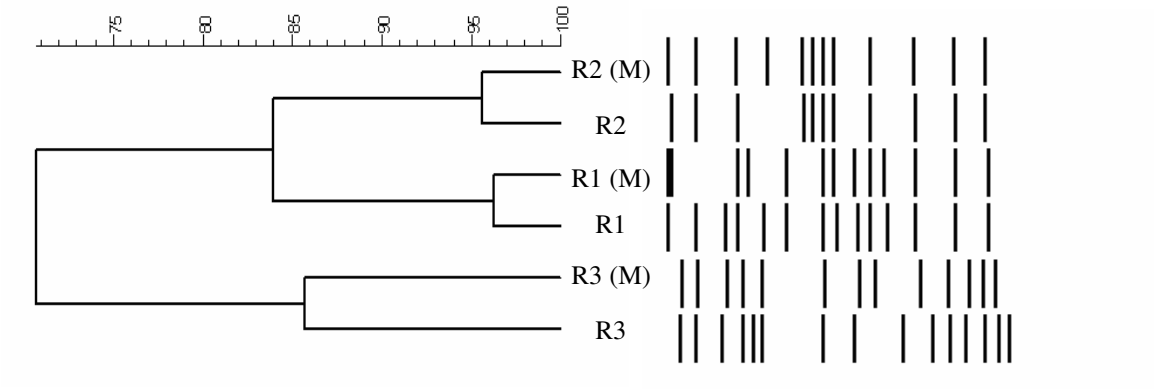
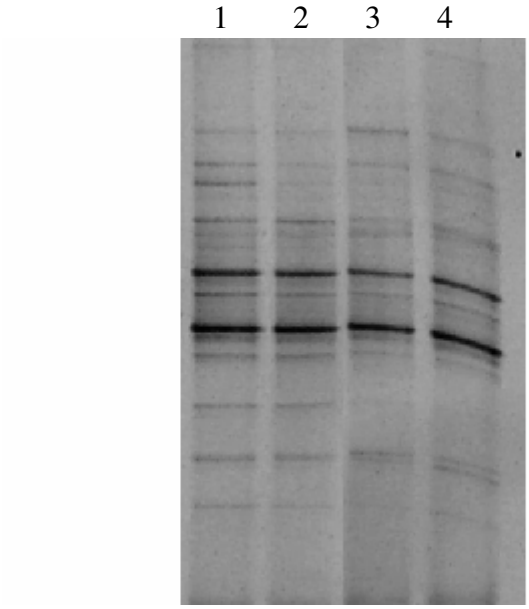


Figure 6.4 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns during the stable phase (Day 120)



1: before washing, from the upper part of the flat sheet membrane module
2: before washing, from the lower part of the flat sheet membrane module
3: after washing, from a random position 4: from the mixed liquor
Figure 6.5 DGGE of PCR-amplified 16S rRNA from the bacterial communities during the stable phase in the flat sheet MBR system

Figure 6.6 UPGMA dendrogram of bacterial community DGGE fingerprints with schematics of banding patterns during the stable phase in the flat sheet MBR system

6.3.2 Isolation and identification of dominant bacteria

The traditional isolation method was utilized to analyze microbial diversities from the membrane surface and the mixed liquor. Considering that an SRT of 30 days is commonly used in the operation of MBRs, the samples were picked up from R2 during the stable stage (Operating parameters were the same as described in Section 4.2, Chapter 4). After 10 days' incubation on R2A agar and synthetic wastewater agar media, a higher number of colonies on the R2A agar were observed. Therefore, R2A medium was used for further analysis of the total microflora from the membrane surface and the mixed liquor in the MBR system.

Among the colonies growing on R2A agar, about 50 strains were picked up based on the different morphologies observed by eye. Further observations by microscope and Gram-staining of these isolated bacteria were carried out and about 35 strains were chosen. 20 strains were identified by direct lysis-PCR-DGGE analysis. The characteristics of the isolated bacteria are listed in Table 6.1. It was revealed that most strains were Gram-negative with hydrated surfaces. Among these 20 strains, 9 strains were isolated from the mixed liquor and 14 strains isolated from the membrane surface. Of those, 3 strains (ME12 and LQ1; ME13 and LQ2; ME14 and LQ3) existed on the membrane surface as well as in the mixed liquor. Assessing microbial assemblages requires quantitative information about community members. Hence, the initial concentrations of strains in the mixed liquor and membrane surface were calculated based on the liquor volume and membrane surface area,

Table 6.1 Characteristics of isolated strains from the membrane surface and the mixed liquor

Strain	Colony			Cell	
	Shape	Color	Size (mm)	Shape	Gram stain
ME1	circular,circle,smooth,hydrate	light yellow	1.5~2	rod	-
ME2	circular,rough	yellow	3	rod	-
ME3	circular,smooth, dry	orange red	2	short rod	-
ME4	circular,smooth,hydrate	orange	0.7	rod	+
ME5	circular,smooth,hydrate	light orange	1	short rod	-
ME6	circular, smooth, hydrate	white	5	rod	-
ME7	circular,circle,smooth,hydrate	white	1	rod	+
ME8	unnormal bound,smooth,hydrate	light yellow	1~2	rod	-
ME9	translucent, smooth, brown center	white	2	coccus	-
ME10	unnormal bound,smooth,hydrate,yellow center	white	5	rod	-
ME11	circular,translucent,smooth,hydrate,brown center	red	3	short rod	-
ME12, LQ1	circular, unsmooth, hydrate	yellow	2	rod	-
ME13,LQ2	circular,smooth, hydrate	yellow	1	short rod	-
ME14,LQ3	translucent, smooth,hydrate	white	0.5	short rod	-
LQ4	circular,rough	light yellow	2	short rod	-
LQ5	circular,smooth,hydrate,translucent	light yellow	1	coccus	+
LQ6	dry,curled margin,rough	white	1~2	coccus	+
LQ7	circular, translucent, growth into medium	white	1	short rod	-
LQ8	circular, smooth, hydrate	white	2	short rod	+
LQ9	circular, growth into medium	white	2	rod	-

* “ME” represents the strain isolated from the membrane surface and “LQ” represents the strain isolated from the mixed liquor.

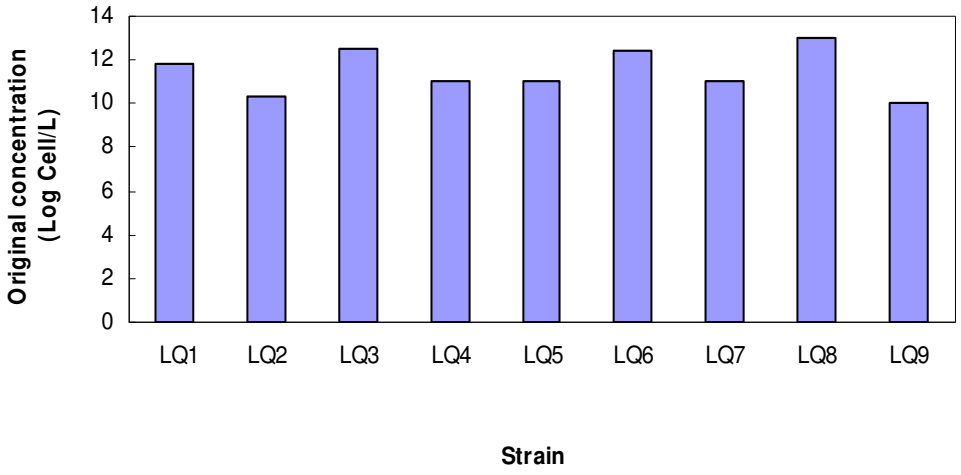


Figure 6.7 Original concentrations of the strains isolated from the mixed liquor

respectively (Figure 6.7 and Figure 6.8). LQ8 and ME12 were the representative strains with the greatest amounts growing on R2A agar from the mixed liquor and membrane surface, respectively. Nevertheless, it appears that the amounts of the strains were not significantly different in each group.

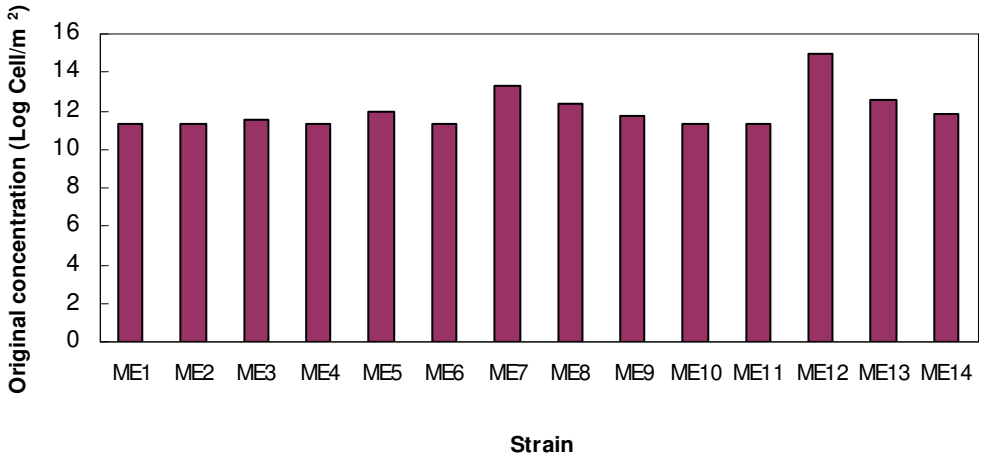


Figure 6.8 Original concentrations of the strains isolated from the membrane surface

6.3.3 Comparisons of EPS production and membrane fouling tendency of isolated bacteria

Previous researchers have found that EPS production and membrane fouling tendency of bacteria were related with their growth phase (Chang and Lee, 1998; Mukai et al., 1999). In this study, the pure culture samples were removed from the shaking flasks when their growths approached the stationary phase. The times for the pure cultures to reach the stationary stage were recorded (Figure 6.9). It was observed that strain ME9 and strain LQ4 were fast-growing bacteria under these culture conditions. Also strains LQ6, LQ7, LQ8, ME10 and ME14/LQ3 have achieved comparatively higher MLSS concentration under the stationary phase.

Each strain had its own EPS characteristics as shown as Figure 6.10. The differences in EPS concentrations (mg/L) among these strains were less significant than that of EPS production (mg/gMLSS). Obviously, strains ME5, ME8, ME13/LQ2, LQ4 and LQ9 had higher EPS production due to their lower MLSS concentration during the stationary phase. In order to compare the EPS productions of the strains from the membrane surface and the mixed liquor, the two groups were classified and the averaged EPS production values of these strains in the two groups were calculated. The averaged EPS production of the strains from the membrane surface was about 1522.8 mg/g MLSS, including 74.3 mg/g MLSS from the suspended strains (pellet) and 1448.5 mg/g MLSS from the soluble solids (supernatant). The averaged EPS production of the strains from the mixed liquor was about 1512.4 mg/g MLSS, containing 67.3 mg/g MLSS from the suspended strains (pellet) and 1445.1 mg/g MLSS from the soluble solids (supernatant). A slightly higher EPS production by the strains from the membrane surface was estimated compared with that from the mixed liquor. These values can not be differentiated within experimental error.

Protein and polysaccharide production are shown in Figure 6.11. Strain ME3, ME8, ME9, ME13/LQ2, ME14/ LQ3, LQ8 and LQ9 produced comparatively little protein under this culture condition. But ME5 and LQ4 not only produce higher amounts of protein but also polysaccharides. The average protein production and concentration

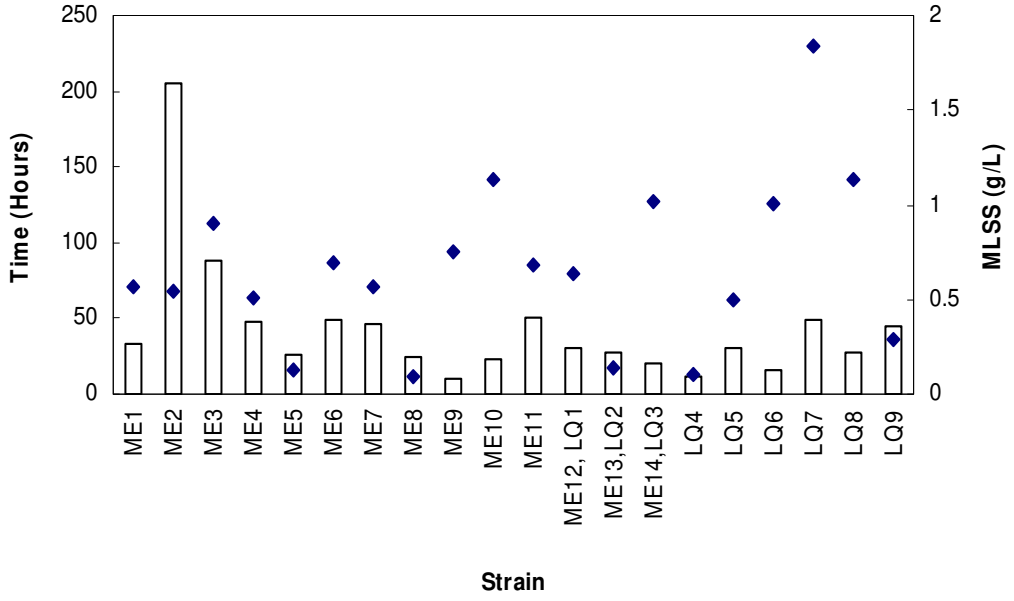


Figure 6.9 The times to reach the stationary phase and MLSS concentrations under the stationary phase of each strain (Light bar) Time (◆) MLSS

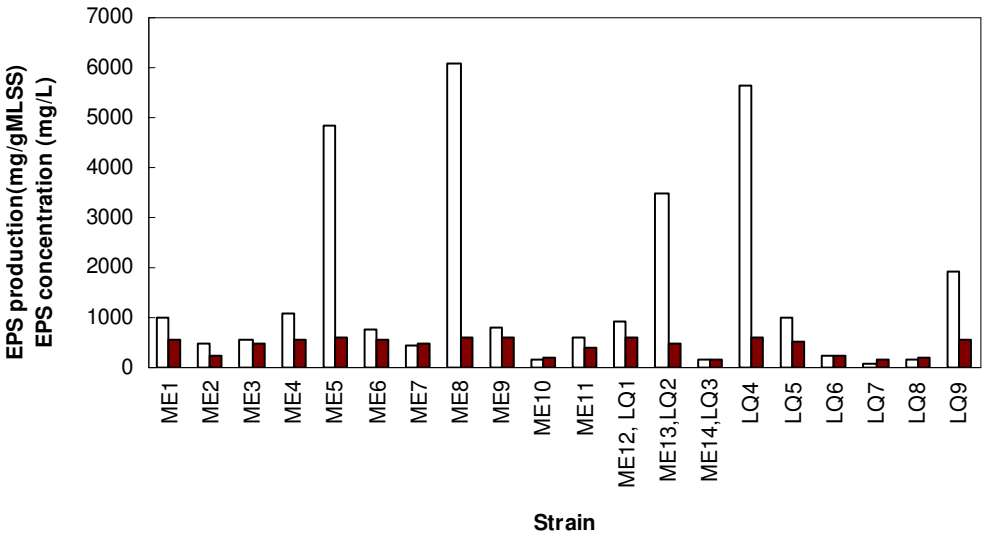


Figure 6.10 EPS production and EPS concentration of each strain (Light bar) EPS production (Dark bar) EPS concentration

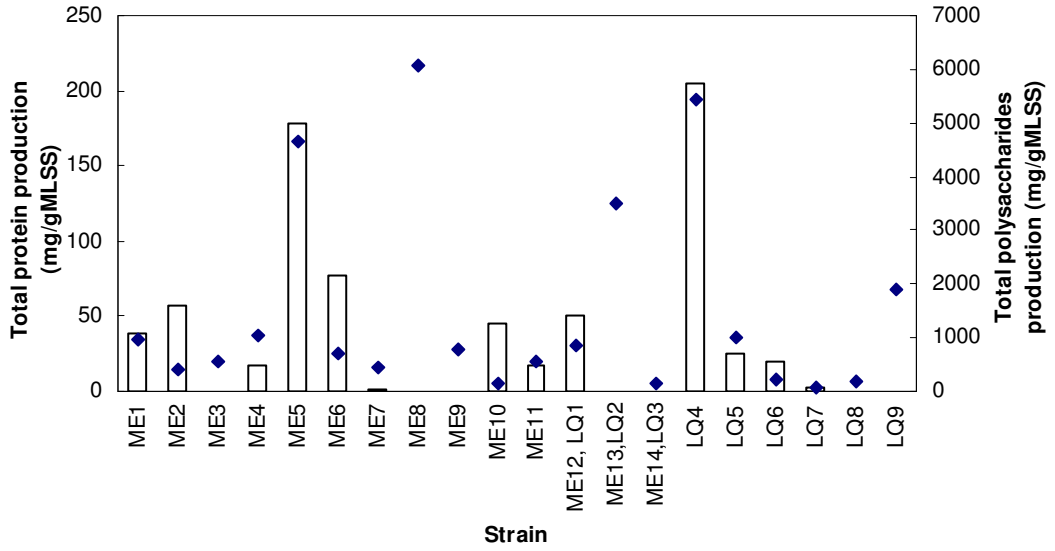


Figure 6.11 EPS composition of each strain
(Light bar) Protein production (◆) Polysaccharides production

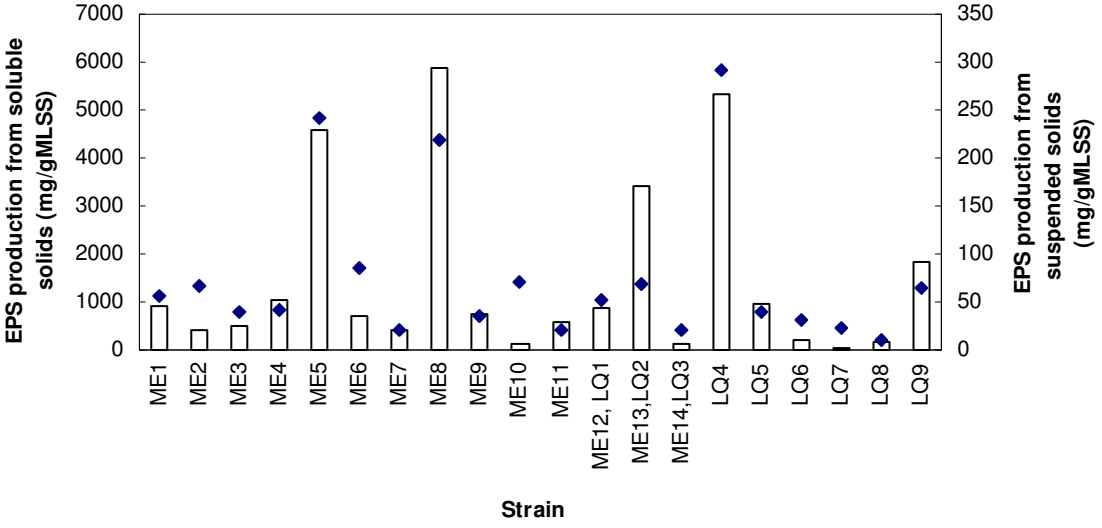


Figure 6.12 EPS distribution of each strain
(Light bar) Dissolved solids (◆) Suspended solids

(34.6 mg/gMLSS, 16.5 mg/L) of the strains on the membrane surface was slightly higher than that from the mixed liquor (33.7 mg/gMLSS, 10.1 mg/L). The average polysaccharides production of the strains on the membrane surface was 1488.2

mg/gMLSS, a little higher than that from the mixed liquor (1478.7 mg/gMLSS). The average polysaccharide concentration of the strains on the membrane surface (449.7 mg/L) was also higher than that from the mixed liquor (377.0 mg/L). Figure 6.12 shows the distribution of EPS production in the suspended solids (pellet) and soluble solids (supernatant). Obviously, EPS mainly came from soluble solids (supernatant), which were 1.5~28.5 times greater than that from the suspended solids (pellet). This may be attributed to a significant amount of residual nondegraded substrate in the flask under the batch mode, which would be measured as a part of soluble TOC and reported as EPS. However this could only apply to the polysaccharides as there were no proteins in the substrate.

Dead-end filtration assays were used to compare the membrane fouling tendencies of these strains. 20 ml samples were taken from the flask when the growth of the strain was at the stationary phase and added to the filtration cell. After 60 minutes' filtration through a PES membrane, it was apparent that strain ME11, ME14/LQ3, LQ6, LQ7 and LQ8 had more significant membrane fouling rates and final fouling

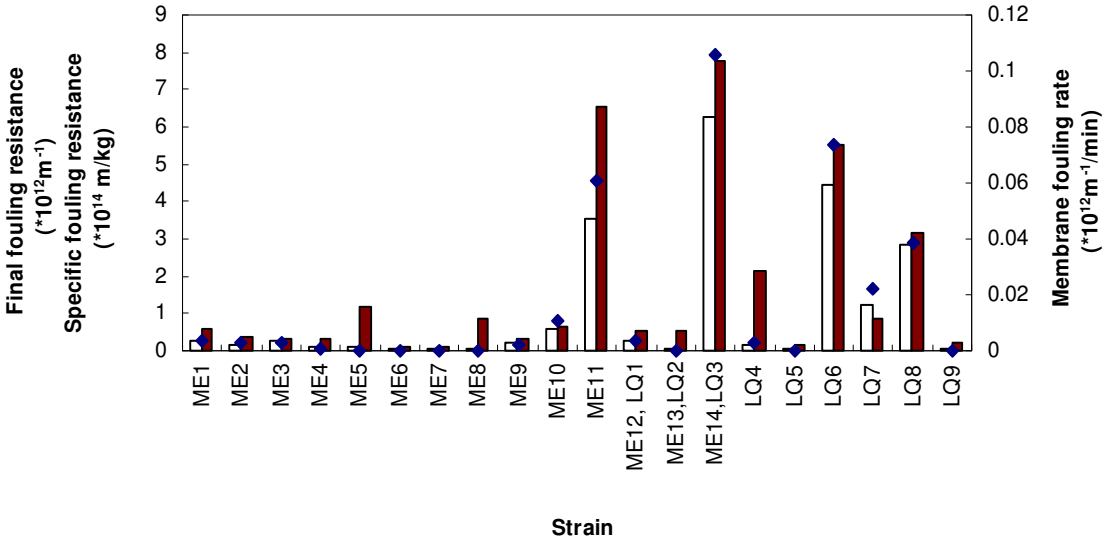


Figure 6.13 Membrane fouling tendency of each strain
(Light bar) Final fouling resistance (Dark bar) Specific fouling resistance
(◆) Membrane fouling rate

resistances compared with other strains (Figure 6.13). Considering the differences of biomass concentrations of strains, specific fouling resistances were calculated based on the measured final fouling resistance divided by the amount of material filtered onto the membrane surface during the tests. It was observed that the specific fouling resistances of the five strains followed the same trend as the final fouling resistance and fouling rate although they also had higher biomass concentrations. Surprisingly, the EPS concentrations of these five strains were not significantly predominant compared with the other strains. This phenomenon is not consistent with the previous conclusions from the activated sludge in MBRs that EPS concentration is major factor causing membrane fouling. The relationship appears to be more complex and may be some feature of the batch-fed cultures compared with continuous cultures. Alternatively there may be a difference between a mixed system compared with a pure culture system.

6.3.4 Comparisons of EPS production and membrane fouling tendency of mixtures from the mixed liquor and the membrane surface

In order to evaluate the effect of EPS characteristics on the membrane fouling tendency of the mixed strains, the nine strains from the mixed liquor (Group I) and the thirteen strains from the membrane surface (Group II) were mixed together, respectively. The two groups of mixtures which had similar initial strain concentrations were inoculated with 100 ml sterile synthetic wastewater (TOC: 286 mg/L) in the flasks and cultured for 36 hours.

MLSS concentrations of Group I and II reached 1.0 and 1.1 g/L after 36 hours, respectively. The total protein production in Group I was about 26.5 mg/gMLSS, which was almost half the amount of Group II (Figure 6.14). However, the total polysaccharides and EPS productions observed in Group I were about 133.0 and 159.5 mg/gMLSS, which were significantly higher than those in Group II (49.0 and 104.4 mg/gMLSS, respectively). Figure 6.15 illustrates that EPS concentrations followed a similar trend to EPS production due to similar MLSS concentration.

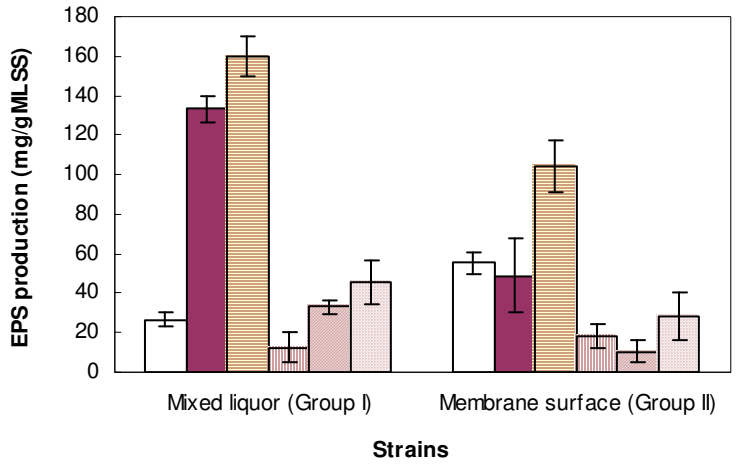


Figure 6.14 EPS production of the mixture strains from the membrane surface and the mixed liquor (Light bar) Total protein production (Dark bar) Total polysaccharides production (Horizontal striped bar) Total EPS production (Vertical striped bar) Protein production from suspended solids (Diagonal striped bar) Polysaccharides production from suspended solids (Point bar) EPS production from suspended solids

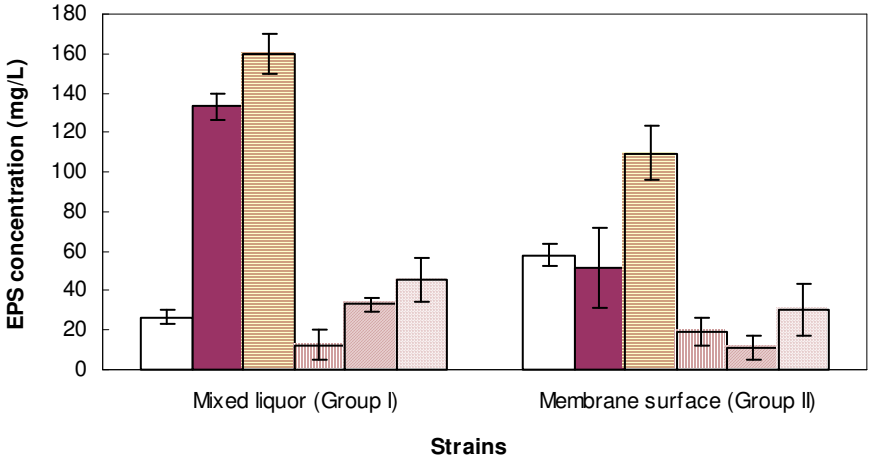


Figure 6.15 EPS concentration of the mixture strains from the membrane surface and the mixed liquor (Light bar) Total protein concentration (Dark bar) Total polysaccharides concentration (Horizontal striped bar) Total EPS concentration (Vertical striped bar) Protein concentration from suspended solids (Diagonal striped bar) Polysaccharides concentration from suspended solids (Point bar) EPS concentration from suspended solids

Two kinds of ultrafiltration membrane (PES 50K Dalton and PAN 40K Dalton) and one kind of microfiltration membrane (PVDF 0.22 μm) were chosen to compare the

membrane fouling tendencies of the two groups. The results (Figure 6.16, 6.17 and 6.18) reveal that Group I exhibited higher membrane fouling rate and final fouling resistance compared with Group II filtered with these three kinds of membranes. Correspondingly, the irreversible fouling resistance in Group I was also higher than that in Group II. These results positively correlated with EPS concentration which

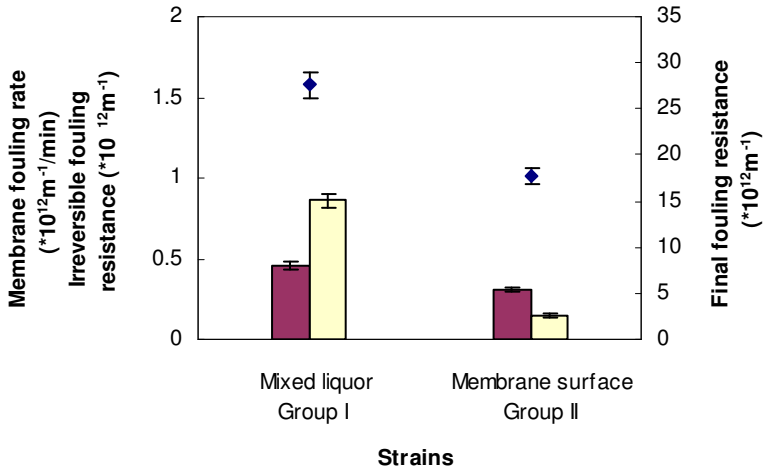


Figure 6.16 Membrane fouling tendency of the mixture strains from the membrane surface and the mixed liquor (PES, UF membrane)
(Dark bar) Membrane fouling rate (Light bar) Irreversible fouling resistance
(◆) Final fouling resistance

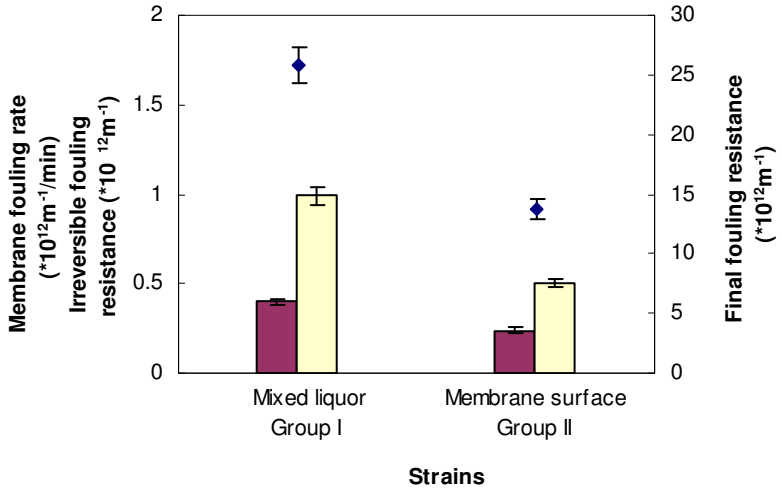


Figure 6.17 Membrane fouling tendency of the mixture strains from the membrane surface and the mixed liquor (PAN, UF membrane)
(Dark bar) Membrane fouling rate (Light bar) Irreversible fouling resistance
(◆) Final fouling resistance

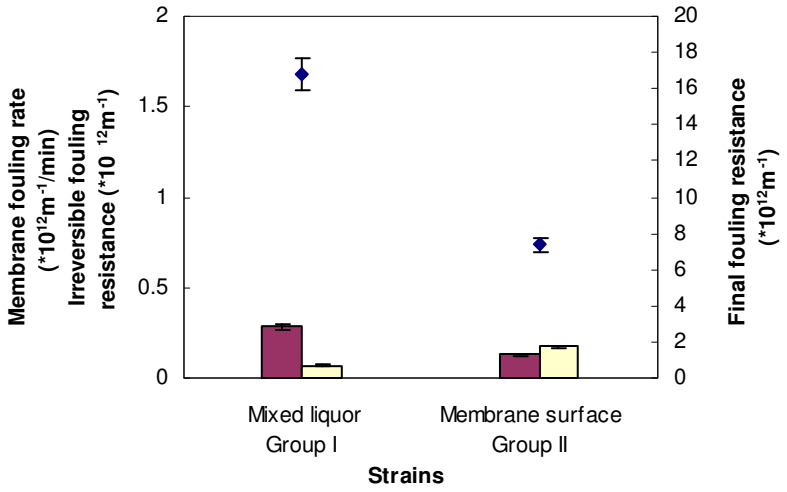


Figure 6.18 Membrane fouling tendency of the mixture strains from the membrane surface and the mixed liquor (PVDF, MF membrane) (Dark bar) Membrane fouling rate (Light bar) Irreversible fouling resistance (◆) Final fouling resistance

confirms that EPS concentration is a crucial factor causing membrane fouling for the mixed system. However, using the fouling protocol described, the contribution of irreversible fouling resistance was not significant, being only 0.4-4% of the total fouling resistance. This indicates that the cake layer, which was formed by the strain mixtures depositing on the membrane surface rather than pore blocking, plays a major role in membrane fouling. This is correlated with the previous results (Section 5.3.1 in Chapter 5) that the colloids and macrosolutes in the supernatant could be present in the cake layer mixed with the suspended solids and not form a separate layer. Meanwhile, the results illustrate when the two mixtures were filtered with ultrafiltration membranes, higher membrane fouling tendencies were achieved compared with the microfiltration membrane.

6.3.5 Comparison of the 'membrane-fouling' bacteria and 'membrane-friendly' bacteria

In order to study the fouling mechanisms of the pure culture, which appears to behave differently in terms of the relationship between EPS characteristics and membrane fouling from the mixtures, two distinctly different bacterial strains (ME 14/LQ3 and LQ 9) were chosen and named as 'membrane-fouling' bacteria and 'membrane-friendly' bacteria respectively, based on their membrane fouling

tendencies (Figure 6.10 and 6.13). Suspended strains (pellet) were harvested at the stationary phase by centrifuging (4000 rpm, 10 minutes, 4°C) the strain solution (suspended solids and supernatant) and then resuspended in distilled water. The two strain samples were regulated to a similar MLSS concentration (about 0.3 g/L) for the filtration test.

It was found that the 'membrane-fouling' bacteria had more significant membrane fouling tendency compared with 'membrane-friendly' bacteria with regard to membrane fouling rate, final fouling resistance and irreversible fouling resistance under the dead-end filtration mode (Table 6.2). The contributions of the suspended strains (pellet) to the total membrane fouling resistances (caused by pellet and supernatant) for the two strains were different. For 'membrane-fouling' bacteria, 16% of the total fouling resistance was derived from suspended strain, but for the 'membrane-friendly' bacteria, the suspended strain contributed 55% to the total fouling resistance. In addition, the irreversible fouling resistances were below 7% based on the final membrane fouling resistance for the two strains. This shows that the TMP increase is attributed to a cake layer formation, which is easily removed, rather than pore blocking or narrowing. It is proposed that in the process of cake layer formation, dissolved solids occupy the void space between the suspended solids to form a more dense cake layer that results in a higher membrane fouling tendency for the 'membrane-fouling' bacteria compared with 'membrane-friendly' bacteria. This suggests that the dissolved solids are the more important substances, which play their role in membrane fouling by infiltrating cake layer of suspended solids.

Figure 6.19 presents the membrane fouling tendencies of the two strains (Pellet + supernatant) in the cross-flow filtration mode (See section 6.2.2.8). At a constant flux of 30 L/m^2*hr, a period of relatively low fouling resistance was observed followed by a sudden transition to a rapid resistance rise for the two strains. For the 'membrane-fouling bacteria', the TMP exhibited a slow increase between 700 and 1000 minutes and jumped to a peak at about 1300 minutes. The 'membrane-friendly' bacteria had no marked increase of TMP until about 1900 minutes.

Table 6.2 Membrane fouling tendencies of the 'membrane-fouling' bacteria and 'membrane-friendly' bacteria under dead-end filtration mode

	'Membrane fouling' bacteria	'Membrane friendly' bacteria
Membrane fouling rate ($*10^{12}m^{-1}$/min) (pellet +supernatant)	0.0264	0.0033
Final fouling resistance ($*10^{12}m^{-1}$) (pellet +supernatant)	1.5339	0.156
Irreversible fouling resistance ($*10^{12}m^{-1}$) (pellet +supernatant)	0.077 (5%)	0.006 (4%)
Final fouling resistance ($*10^{12}m^{-1}$) (pellet)	0.2527 (16%)	0.0854 (55%)

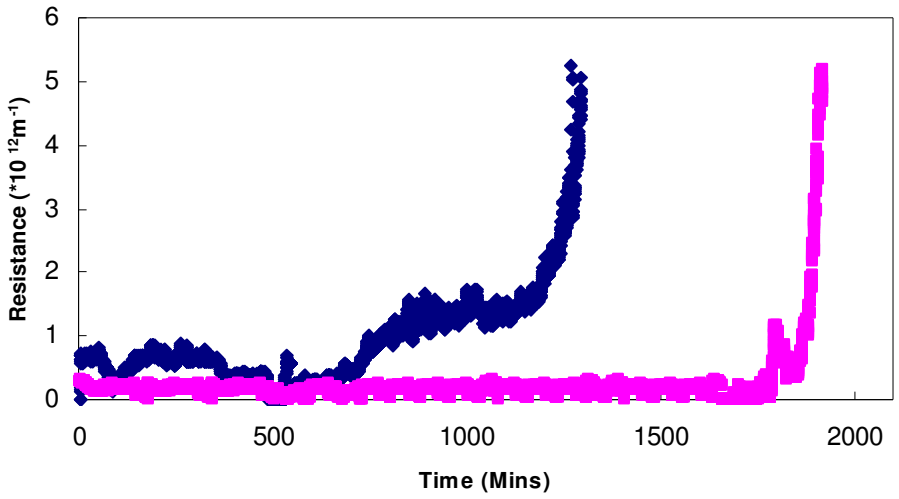


Figure 6.19 Membrane fouling tendency of the 'membrane-fouling' bacteria and 'membrane-friendly' bacteria under the cross-flow filtration mode (◆) 'Membrane-fouling' bacteria (■) 'Membrane-friendly' bacteria

Possibly, the trace foulant slowly accumulates on the membrane surface and this loose cake layer can be more easily limited by the shear force which alleviates the initial TMP increase. The sudden rise in TMP is believed to be caused by local flux in some regions of the membrane increasing under the fixed permeate flow rate and exceeding the critical flux of the strain solution (Cho and Fane, 2002). This result is

qualitatively consistent with the phenomenon under dead-end filtration mode, which identifies considerable fouling for 'membrane-fouling' bacteria.

6.4 Discussion

Complex bacterial communities are directly responsible for the effectiveness and success of organic/inorganic reduction, nitrogen and phosphorus removal as well as major foulants causing membrane fouling in MBRs. Hence, bacterial community structure and function have direct effects not only on the quality of the final effluent but also on the performance of the membrane filtration process. In this study, PCR-DGGE technology as well as bacterial isolation have been employed to compare the bacterial community structures and their functions. The combination of the two techniques is expected to provide more information on the key functional microbial groups that show different membrane fouling tendencies in MBRs.

In light of recent molecular studies, 16S rRNA-PCR-DGGE information has been exploited to give an estimate of the number and relative abundance of numerically dominant bacteria and allow a comparison of different microbial communities. It is considered that in the filtration process, the predominant bacteria in the reactor will easily attach initially on the membrane surface due to their higher concentration combined with the hydrodynamic effects. Some non-dominant bacteria in the reactor which prefer to live as "attached mode" also affix on the membrane surface. Due to limited amounts of the non-dominant bacteria, it is not easy to detect them by the DGGE technique. Under the non-stable condition of MBRs, previous studies (Section 4.3 in Chapter 4) indicated that the bacterial community structures changed significantly with operating time. So, during the two days filtration with the membrane module, the bacterial diversities that initially accumulated on the membrane surface may have differed from those in the mixed liquor due to obvious bacterial community shifts occuring in the reactor. However, during the stable phase, steady bacterial community structures in the mixed liquor could be achieved with the stable reactor performance. Hence, after two days filtration by the membrane module, the microbial diversity on the membrane surface became similar to that in the mixed liquor due to the more stable bacterial strains existing in the

reactor. This phenomenon has also been observed in the flat-sheet MBR system, which indicates there is no marked difference in the bacterial community structures in DGGE profiles between the membrane surface and in the mixed liquor during the stable phase of MBRs. Apparently these bacteria are very limited and therefore, it is suggested that the bacterial community structure on the membrane surface is associated with the dominant bacteria in the mixed liquor when the system approaches the stable stage by DGGE analysis.

Although the DGGE results provide evidence that the bacterial community structures on the membrane surface and in the mixed liquor share high similarity under the steady conditions, the strains obtained by the isolation approach from the membrane surface and mixed liquor showed more variety. Fourteen strains were obtained from the membrane surface and nine strains were obtained from the mixed liquor. Only three of these strains exist both on the membrane surface and in the mixed liquor. This suggests that culture-independent (PCR-DGGE) and culture-dependent (Isolation) techniques yield different characteristics of microbial community structures in the MBR system. Both techniques have their limitations. It has been confirmed that the most abundant species masked the presence of less abundant in DGGE profiles (Boon et al., 2002; Muyzer et al. 1993). Hence, in DGGE analysis, some non-dominant but culturable bacteria may not be distinguished due to their relatively low abundances. Also different species containing similar GC amounts may halt at identical positions in the DGGE patterns, which are then too close to be distinguished by the eye. On the other hand, for activated sludge the percentage of culturable bacteria in comparison with total cell counts is estimated to be in the range from 1 to 15% (Boon et al., 2002). Hence some living, dominant but not readily culturable bacteria may not be obtained by isolation. Another possibility is that some dominant bacteria are overgrown by more rapid growers, or their full nutritional requirements are not provided by the chosen medium (Kämpfer, 1997). Nevertheless, these isolates do provide important information on the functions of bacteria in MBRs.

In order to investigate the contributions of various bacterial strains to membrane fouling, the analysis of characteristics of strains, such as growth phase, EPS properties as well as membrane fouling tendency were carried out. Although the same initial substrate concentration and bacterial inoculum concentration were employed, the strains isolated from the membrane surface and mixed liquor exhibited different growth kinetics and EPS productions. Since the initial F/M ratios were the same, this suggests that if the available energy is over the bacterial maintenance energy requirement, the surplus energy is utilized for bacterial growth or for bacterial energy storage inside or outside of cells. Due to the individual bacterial strains in pure cultures using artificial growth media, it can be speculated that the energy-requiring process of EPS production does not confer any selective advantage to cells grown in the pure culture systems in contrast to the competitive multispecies environments in the mixed culture systems, where EPS functions seem to be essential for survival of bacterial populations (Wingender et al., 1999). Also, some strains produced less protein, which suggests that possibly the bonds between inert or higher molecular weight substances and EPS prevent the excess production

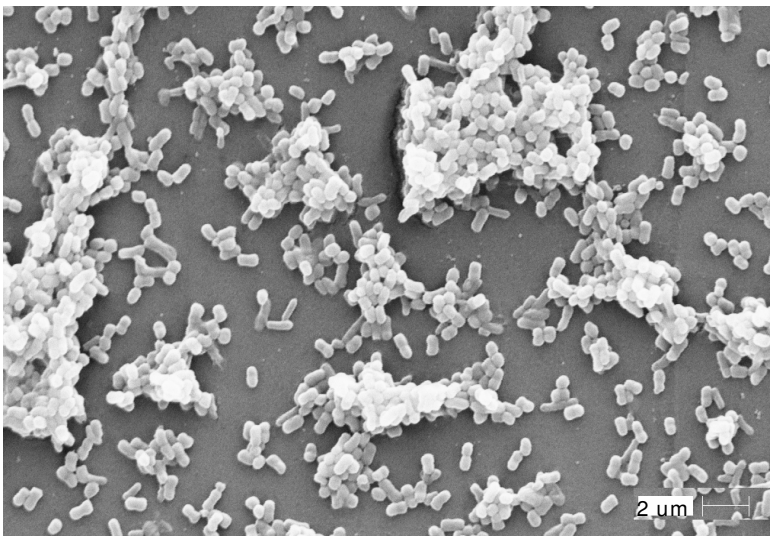


Figure 6.20 SEM of mixture of the isolated bacteria from the membrane surface (×3000)

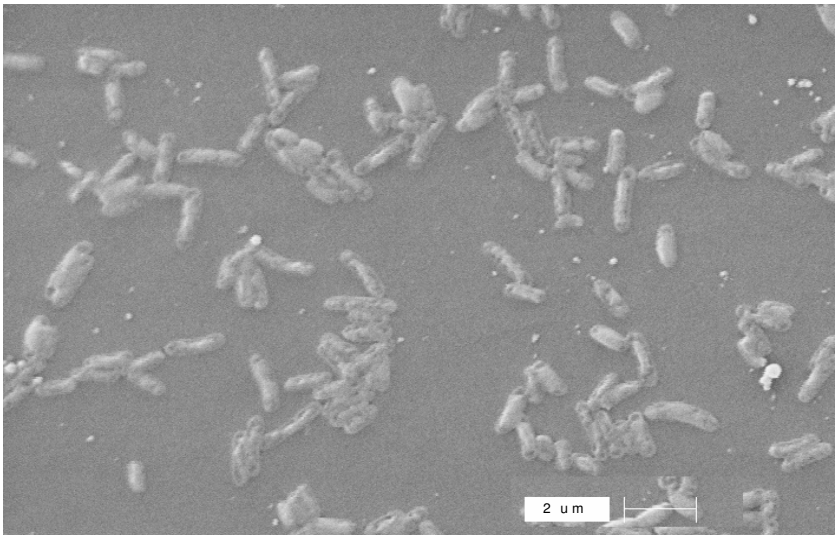


Figure 6.21 SEM of individual strain from the membrane surface (×3000)

of extracellular enzymes based on protein (Sponza, 2003). Further, different strains displayed different membrane fouling tendencies during the stationary phase. However, it was difficult to establish a relationship between EPS properties, such as EPS concentration, production or composition, and membrane fouling tendency for the pure culture systems. On the other hand, the results of the filtration characteristics of the strain mixtures from the membrane surface and the mixed liquor revealed that membrane fouling tendency was a function of EPS concentration for different membrane materials and membrane pore sizes. Previously in this thesis, it has been strongly suggested that EPS concentration and composition are major factors influencing membrane fouling tendency because EPS is advantageous for microorganisms to physically associate with solid surfaces. In the mixed strains system (Figure 6.20), it is inevitable that cells gathered together to form fairly stable, contiguous, multicellular associations due to protein ligands and carbohydrate receptors or protein receptors and carbohydrate ligands (Calleja, 1984). Hence, EPS production seems to be an important feature of survival because most bacteria occur in microbial aggregates such as flocs and their structural and functional integrity is based essentially on the presence of an EPS matrix. However, laboratory pure cultures are in a different situation (Figure 6.21), where EPS may

not be so essential for bacterial structures, since loss of EPS does not impair growth and viability of the cells (Wingender et al., 1999). Therefore, it can be concluded in both complex mixture systems (activated sludge systems) and simple mixture systems (pure culture mixed systems) EPS is an important factor affecting membrane fouling tendency with the exception of pure culture systems (single cell systems).

In order to evaluate the membrane fouling mechanism by the individual strain, 'membrane-fouling' bacteria and 'membrane-friendly' bacteria were selected from the strains based on their membrane fouling tendency and EPS characteristics. Although 'membrane-friendly' bacteria had a higher EPS concentration compared with 'membrane-fouling' bacteria, it showed lower membrane fouling tendency, possibly related to its cell properties, such as surface charge, hydrophobicity and particle size (Table 6.3).

Table 6.3 Cell characteristics of the 'membrane-fouling' bacteria and 'membrane-friendly' bacteria

Sample	Zeta potential (mV)	Contact angle (°)	Particle size (d_{50}) (nm)
'Membrane-fouling' bacteria	-20.3±1.61	66.0±4.0	161.0±10.1
'Membrane-friendly' bacteria	-15.7±1.57	30.8±1.0	215.9±12.9

The surface charge is related to the ionizable groups present on the surface, it increases the polar interactions of EPS with water molecules (Liao et al. 2000). In the mixed system, activated sludge containing more EPS has more negative surface charges (Sponza, 2003). However, in the pure culture system, the 'membrane-fouling' bacteria had more negatively charged surfaces with less EPS concentration compared to the 'membrane-friendly' bacteria. Possibly, this phenomenon is related to the detailed composition of protein and polysaccharides in EPS. It has been reported that the surface charge of polyethersulfone membrane at pH 7.0 was about -15 mV (Burns and Zydney, 2000). Thus, the decrease of zeta potential represented increasing negative charges on the surface of strain, which increases concentration polarization thickness between the particles along the membrane surface due to

electrostatic repulsion (Kwon and Vigneswaran, 1998; Park et al., 2005). This would lead to loose deposit layer with high porosity, which ameliorated the membrane fouling tendency. However, this beneficial property of the 'membrane-fouling' bacteria was negated by other characteristics.

The results also showed that the contact angle of the 'membrane-fouling' bacteria was about 66°, but for the 'membrane-friendly' bacteria, the water drop shrunk little by little from the initial shape (30.8°) and wetted the strain surface, which indicates this strain is more hydrophilic. Both strains were Gram-negative which confirms the idea that the general organization of the cell wall does not allow us to make an a priori decision as to the hydrophobic quality of the bacterial surfaces (Jorand et al., 1995). Previous studies have pointed out that increases in bacterial hydrophobicity are probably linked to surface proteins due to the contribution of amino acids with hydrophobic sites (Overmann and Pfennig, 1992; Jorand et al. 1998). However, in this case, the difference in hydrophobicity may not be derived from protein as the two strains produced little extractable protein. It is likely that a better understanding of the hydrophobicity requires more fundamental information about the physical nature of EPS molecules. Since hydrophobic interactions happen between the membrane surface and the strain surface, this indicates that the higher hydrophobicity of the strains could render a stronger adherence of floc particles with one another as well as onto the membrane surface. As a result the filtration resistance would be augmented. Some studies have suggested that the higher resistance of activated sludges can be attributed to the hydrophobic and waxy nature of the sludge surface (Chang and Lee, 1998; Chang et al., 1999). In addition, some researchers have found a positive correlation between the hydrophobicity of the activated sludge flocs or bacteria isolated from activated sludge and their flocculation performance (Singh and Vincent, 1987; Jorand et al., 1995). Hence, the hydrophobicity is expected to be associated with the mean particle size of the aggregates of pure cultures. The 'membrane-fouling' bacteria had greater hydrophobicity and it appears that the hydrophobic interactions promoted the aggregation of cells to form smaller and denser particles, which result in serious membrane fouling. Whereas for the 'membrane friendly' bacteria, the lower

hydrophobic interactions may have promoted formation of looser and bigger particles.

Apparently, lower zeta potential, less hydrophobicity and bigger particle size of the strain play an important role in reducing membrane fouling. Therefore, the combined effects of surface charge, hydrophobicity and particle size of the strain on membrane fouling are important for pure culture systems.

In summary, the bacterial community structure on the membrane surface was associated with the dominant bacteria in the reactor when the system approached the stable stage as analyzed by DGGE. The contributions of the individual strains isolated from the membrane surface and the mixed liquor to membrane fouling were independent of EPS concentration, production or composition. However, for the mixtures of strains from the membrane surface and the mixed liquor, the membrane fouling tendency was proportional to EPS concentration. The selected ‘membrane-fouling’ bacteria that caused more serious membrane fouling had greater hydrophobicity and smaller particle size compared with ‘membrane-friendly’ bacteria.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

7.1.1 Dynamic EPS production under different operating parameters

A fundamental study of the dynamics of EPS production was performed in order to clarify the changes of EPS production with the shifts in operating parameters (substrate loading, SRT, aeration rate, HRT), and the resultant effect on reactor performance and membrane fouling tendency in MBRs. When the MBR operating parameters changed, a significant and rapid increase in EPS production and concentration occurred at the initial stage of operation. This phenomenon correlated well with variations in protein production. In addition, EPS distribution patterns (attached to floc or in supernatant) were independent of the shifts in operating parameters, and showed that attached EPS contributed much more to total EPS than soluble EPS under the four phases of operation examined.

Obviously, periodic and sequential changes of operating parameters in a MBR system could influence EPS production and composition. Results in this study indicated that increasing F/M ratios, decreasing SRTs as well as shortening HRTs would result in higher average EPS production. However, increase in aeration rate insignificantly influenced EPS production.

7.1.2 MBR Performance and bacterial community structures under different operating conditions in parallel MBR systems

7.1.2.1 MBR performance under different operating conditions

This chapter focused on the effect of different substrate loadings (Phase 4.1), substrate compositions (Phase 4.2) and SRTs (Phase 4.3) on EPS production and

membrane fouling tendency in three parallel MBR systems. Excellent organic removal efficiencies were achieved consistently in the MBRs during the three phases.

In phase 4.1, three parallel MBR systems were operated with substrate volumetric loadings of 0.57, 1.14 and 2.28 g COD L^{-1} day^{-1} with a fixed SRT of 30 days, HRT of 12 hours, and aeration rate of 3 L/min. With substrate loading increased four times from 0.57 to 2.28 g COD L^{-1} day^{-1}, the biomass concentration correspondingly increased by almost four times from 2.2 to 8.4 g/L at steady state. However, similar F/M ratios were achieved in the three reactors. The mean particle sizes in R1 and R2 changed from 120 μm initially to 148 μm and 400 μm at stable conditions, respectively. However the mean particle size in R3 increased before day 45 and dramatically dropped to 190 μm during the stable stage. Under stable conditions, the dissolved oxygen was about 6.9, 5.4 and 0.6 mg/L for R1, R2 and R3. In phase 4.2, nitrogen-rich and phosphorus-rich MBRs were operated at a fixed SRT of 30 days, HRT of 12 hours and aeration rate of 2 L/min. R1 in Phase 4.1 was regarded as the control reactor. The nitrogen-rich MBR had a higher F/M ratio of 0.26 day^{-1} and larger mean particle size of 252 μm compared with the phosphorus-rich MBR, whose F/M ratio was 0.22 day^{-1} and particle size was 207 μm at steady state. In phase 4.3, three parallel MBR systems were operated at SRT of 10 days, 30 days and infinity with the same substrate loading, HRT, and aeration rate. As the SRT increased from 10 days, to 30 days to infinity, the F/M correspondingly dropped from 0.44, to 0.27 to 0.17 day^{-1}, the mean particle size of the activated sludge decreased from 246, to 235 to 181 μm and the dissolved oxygen concentration declined from 6.2, to 3.8 to 1.8 mg/L at steady state.

7.1.2.2 EPS characteristics under different operating conditions

Analysis of EPS production (mg/g MLSS) and composition (protein and polysaccharides) indicated that at the beginning of operation, a distinct increase in EPS production, mainly proteins, happened in the MBRs. With increasing substrate loadings from 0.57 (R1), to 1.14 (R2), to 2.28 g COD L^{-1} day^{-1} (R3), the EPS production decreased from 102, to 66, to 58 mg/gMLSS at steady state. It proposed

that the limited dissolved oxygen concentration and inefficient oxygen transfer caused the reduced EPS production. Increasing the nitrogen concentration in the substrate reduced protein production, the ratio of protein to polysaccharide and EPS production compared with the control reactor. Increasing phosphorus amount promoted protein production and the ratio of protein to polysaccharide significantly. However the effect of the phosphorus concentration on the EPS production relative to the control was negligible. By extending SRT from 10 days, to 30 days to infinity, EPS production decreased from 130, to 111, to 80 mg/ gMLSS under steady state conditions, which indicates that the EPS production as a characteristic of microorganisms is dependent on the living conditions of the microorganism, such as F/M and dissolved oxygen.

7.1.2.3 Bacterial community structures under different operating conditions

During the three phases of tests, DGGE analysis of PCR-amplified 16S rRNA genes from nucleic acids extracted from the activated sludge showed that significant population shifts appeared during the start-up period and that a stable microbial community was achieved at steady state conditions in each reactor. Increasing substrate loading or extending SRTs caused the dominant bacterial consortia to vary markedly although the same seed was employed in the initial period. However, similar dominant bacteria were evident in the nitrogen-rich and phosphorus-rich MBR, which illustrates that changes in substrate composition may insignificantly influence dominant bacteria.

7.1.2.4 Relationship between EPS production and bacterial community structure under different operating conditions

Similarity index analysis of the bacterial community structures showed that the EPS production was related to the bacterial function and population shifts. At the beginning of operation, the higher bacterial community similarity values and increasing trends in EPS production suggest that the bacteria were acclimatizing to the new living environment and experiencing metabolic stress causing the production of more enzymes, which are the main protein components of EPS. Subsequently, some acclimatized bacterial populations became dominant in the

reactor and were restored to normal metabolism with gradual adaptation to the environment, and this would have lead to a slow decrease in EPS production. At steady state, constant bacterial diversity and EPS production occurred, which suggests that EPS production was associated with bacterial community structures.

7.1.2.5 Membrane fouling tendencies under different operating conditions

Under lower substrate loading or shorter SRTs, lower membrane fouling rates and specific fouling resistances were obtained. A crucial reason is that lower substrate loading or shorter SRT resulted in higher F/M ratio as well as higher DO values. These changes of reactor performance leaded to shifts of bacterial community structures and functions of microorganisms that produced higher EPS in the MBRs. However, EPS concentration (mg/L, equals to EPS production (mg/g MLSS)× MLSS concentration (g/L)) achieved lower values due to lower MLSS, which finally caused lower membrane fouling tendency of the activated sludge. The nitrogen-rich MBR showed a lower membrane fouling rate and specific fouling resistance than the phosphorus-rich MBR although there were no significant differences in F/M, DO, as well as bacterial community structure. However, nitrogen-rich MBR had lower EPS concentration, possibly due to the effect of bacterial metabolism function, which obtained lower membrane fouling tendency.

Therefore, substrate loading, substrate composition and SRT are important operational parameters that appear to have a significant effect on EPS characteristics by influencing reactor performance and bacterial community structure or their metabolism function. Further, the changes of EPS concentration would affect membrane fouling tendency of the activated sludge. In this study, it is concluded that membrane fouling tendency, such as membrane fouling rate and specific fouling resistance, was positively associated with EPS concentration (mg/L) in the reactor. This agrees with previous studies that showed EPS to be a major foulant in MBRs. The findings derived from this study are expected to explain the membrane fouling mechanisms in submerged membrane bioreactors with different bioreactor parameters (SRT, substrate loading, substrate composition).

7.1.3 Membrane fouling mechanisms caused by EPS

In this series of experiments, the results indicated that the dissolved solids predominated in the membrane fouling in the lab-scale MBR system, but suspended solids performed an important role in membrane fouling in the Bedok MBR system. However, the results showed that the dissolved solids played an important role in membrane fouling in the both MBR systems after normalizing by EPS loading, which indicates that EPS characteristics are a key factor influencing membrane fouling. The effects of cations and colloids in the dissolved solids on membrane fouling were also investigated. After removing cations from the dissolved solids, the membrane fouling resistance was not significantly changed due to the counteractive effects of surface charge and particle size. However, the membrane fouling tendency of the dissolved solids after removing colloids decreased noticeably. When both colloids and cations were removed, the fouling tendency decreased by almost 50%. By analyzing molecular weight distribution of dissolved solids, it was found that species whose molecular weights (and sizes) were close to membrane pore size were more likely to influence membrane fouling due to their ability to accumulate in the pores. Higher molecular weight substances were retained in the solution due to membrane separation and smaller molecular weight substances passed through the membrane pores with the permeate.

7.1.4 Study of the functions of isolated bacteria from the MBR system

During the start-up period of the operation, the bacterial community structures on the membrane surface and in the mixed liquor were significantly different due to the unstable bacterial diversity in the initial non-stable phase. However, when the system achieved steady state conditions, high similarity indices (from DGGE analysis) were observed for the membrane surface and the mixed liquor, which suggests that bacterial community structure on the membrane surface was associated with the dominant bacteria in the reactor when the system approached the stable stage. Twenty pure cultures were isolated from the membrane surface as well as the mixed liquor at steady state conditions in the MBR by the traditional series dilution method. However, using this method, only three strains co-existed on

the membrane surface and in the mixed liquor which did not support the findings from the DGGE analysis because of the limitations of the two methods.

Further, the contributions of these bacterial strains to membrane fouling were evaluated by analyzing the characteristics of the strains, such as growth kinetics, EPS properties as well as membrane fouling tendency. Surprisingly, the results showed that membrane fouling tendency of the isolates was independent of the EPS properties, such as EPS concentration, production as well as composition. However, when the strains from the membrane surface and mixed liquor were mixed, respectively, the membrane fouling tendency was proportional to EPS concentration. These results show that EPS is an important factor to influence membrane fouling tendency in a mixture system, but that this influence may not be clearly evident in the pure culture system.

The fouling mechanisms of 'membrane-fouling' bacteria and 'membrane-friendly' bacteria were analyzed by comparing their surface charge, hydrophobicity and particle size. The results revealed that under dead-end and crossflow filtration modes, the 'membrane-fouling' bacteria caused serious membrane fouling and that coincided with their more negative surface charge, greater hydrophobicity and smaller particle size.

7.2 Limitations of this research and recommendations for future work

In this study, the membrane fouling characteristics of activated sludge was measured ex-situ in a dead-end filtration apparatus. Although these results show clear relationships between EPS properties and membrane fouling tendency, further research needs to be conducted to observe the magnitude of change of membrane resistance in a real MBR in order to confirm these observations. The findings derived from this study are expected to explain the membrane fouling mechanisms in submerged membrane bioreactors with different types of membrane modules. However further investigation at large scale is required to confirm this. In addition, statistical approach is a powerful tool that allows quick and cheap evaluation of data,

leading to optimal solutions, which is not obviously foreseen. By employing different statistical approaches, correlation between operating conditions and reactor performances in MBRs can be determined.

Although the DGGE technology and pure culture isolation method provided detailed information on the bacterial community structures and characteristics of single strains, they may not adequately reflect the entire pool of bacteria in MBRs due to their own limitations. Hence, other advanced microbiological techniques, such as FISH, CLMS, DNA sequencing and real time PCR are recommended to be combined with these two techniques to give deeper insight into the functional bacteria in MBRs. In the future, direct amplification and analysis of ribosomal DNA genes with specific primers could be undertaken to define the specific functional groups (e.g. 'membrane fouling' bacteria) within a complex microbial community, therefore, a more complete picture of the role of bacterial communities can be obtained. In this research, pure culture filtration experiments were carried out in a batch study. Prior to practical application to MBRs, further study should be performed in lab-scale MBR systems operated under realistic operating conditions.

Dissolved solids were the major contributors to membrane fouling and molecular weight distributions in the dissolved solids were correlated with EPS characteristics and membrane fouling tendency. Techniques to limit the concentration of these components need to be optimized to improve MBR productivity. This could be approached as a microbiological optimisation, or by means of additives (polymers, adsorbents) to uptake these key foulants.

REFERENCES

Abbassi, B., Dullstein, S. and Raebiger, N. (1999). "Mininization of Excess Sludge Production by Increase of Oxygen Concentration in Activated Sludge Flocs: Experimental and Theoretical Approach", Water Research, Vol. 34, No. 1, pp. 139-146.

Alavi Moghaddam, M. R., Guan, Y., Satoh, H. and Mino, T. (2003). "Performance and Microbial Dynamics in the Coarse Pore Filtration Activated Sludge Process at Different SRTs (solid retention time)", Water Science and Technology, Vol. 147, No. 12, pp. 73-80.

American Public Health Association (APHA). (1998). Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, D.C., USA.

Amann, R. I., Ludwig, W. and Schleifer, K. H. (1995). "Phylogenetic Identification and in situ Detection of Individual Microbial Cells without Cultivation." Microbiological Reviews, 59: 143-169.

Azeredo, J., lazarova, V. and Oliveira, R. (1999). "Methods to Extract the Exopolymeric Matrix from Biofilms: A Comparative Study", Water Science and Technology, Vol. 39, No. 7, pp. 243-250.

Bae, T. H. and Tak, T. M. (2005). "Interpretation of Fouling Characteristics of Ultrafiltration Membranes during the Filtration of Membrane Bioreactror Mixed Liquor", Journal of Membrane Science, Vol. 264, No.1-2, pp. 151-160.

Bartlett, J. M. S. and Stirling, D. (2003). PCR protocols (Second edition), Humana Press, Totowa, New Jersey, USA.

Belfort, G., Davis, R. H. and Zydney, A.L. (1994). "The Behavior of Suspensions and Macromolecular Solutions in Crossflow Microfiltration", Journal of Membrane Science, Vol. 96, pp. 1-58.

Boon, N., De Windt, W., Verstraete, W. and Top, E. M. (2002). "Evaluation of Nested PCR-DGGE (denaturing gradient gel electrophoresis) with Group-specific 16S rRNA Primers for the Analysis of Bacterial Communities from Different Wastewater Treatment Plants", FEMS Microbiology Ecology, Vol. 39, pp. 101-112.

Bouhabila, E. H., Aim, R. B. and Buisson, H. (2001). "Fouling Characterisation in Membrane Bioreactors", Separation and Purification Technology, Vol. 22-23, pp. 123-132.

Boyette, S. M., Lovett, J. M., Gaboda, W. G. and Soares, J. A. (2001). "Cell Surface and Exopolymer Characterization of Laboratory Stabilized Activated Sludge from a Beverage Bottling Plant", Water Science and Technology, Vol. 43, No. 6, pp. 175-184.

Bradford, M.M. (1976). "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-dye Binding", Analytical Biochemistry, Vol. 72, pp. 248-254.

Bura, R., Cheung, M., Liao B., Finlayson, J., Lee, B. C., Droppo, I. G., Leppard, G. G. and Liss, S. N. (1998). "Composition of Extracellular Polymeric Substances in the Activated Sludge Floc Matrix", Water Science Technology, Vol. 37, No. 4-5, pp. 325-333.

Burns, D. B. and Zydney, A. L. (2000). "Buffer Effect on the Zeta Potential of Ultrafiltration Membranes", Journal of Membrane Science, Vol. 172, pp. 39-48.

Calleja, G. B. (1984). Microbial Aggregation, CRC Press, Boca Raton, Florida, USA.

Chang, I. S. and Kim, S. N. (2005). "Water Treatment Using Membrane Filtration--Effect of Biosolids Concentration on Cake Resistance", Process Biochemisty, Vol. 40, pp. 1307-1314.

Chang, I. S. and Lee, C. H. (1998). "Membrane Filtration Characterisitcs in Membrane Coupled Activated Sludge-- the Effect of Physiological States of Activated Sludge on Membrane Fouling", Desalination, Vol. 120, pp. 221-223.

Chang, I. S., Lee, C. H. and Ahn, K. H. (1999). "Membrane Filtration Characteristics in Membrane-coupled Activated Sludge System: The Effect of Floc Structure on Membrane Fouling", Separation Science and Technology, Vol. 34, No. 9, pp. 1743-1758.

Chiemchaisri, W., Wu, J. S. and Visvanathan, C. (2001). "Methanotrophic Production of Extracellular Polysaccharide in Landfill Cover Soils", Water Science and Technology, Vol. 43, No. 6, pp. 151-159.

Chio, J. G., Bae, T. H., Kim, J. H., Tak, T. M. and Randall, A. A. (2002). "The Behavior of Membrane Fouling Initiation on the Crossflow Membrane Bioreactor System", Journal of Membrane Science, Vol. 203, No. 1-2, pp. 103-113.

Cho, S. D. and Fane, A.G. (2002) "Fouling Transients in Nominally Sub-critical Flux Operation of a Membrane Bioreactor", Journal of Membrane Science, Vol. 209, pp. 391-403.

Choi, E., Yun, Z., Park, Y., Jeong, H., Kim, K., Lee, H., Rho, K. and Gil, K. (2001). "Extracellular Polymeric Substance in Relation to Nutrient Removal from a Sequencing Batch Biofilm Reactor", Water Science and Technology, Vol. 43, No. 6, pp. 185-192.

Choi, H., Zhang, K., Dionysiou, D., D., Oerther, D. B. and Sorial, G. A. (2005). "Effect of Permeate Flux and Tangential Flow on Membrane Fouling for

Wastewater Treatment", Separation and Purification Technology, Vol. 45, pp. 68-78.

Cicek, N., Dionysiou, D., Suidan, M. T., Ginestet P. and Audic, J. M. (1999). "Performance Deterioration and Structural Changes of a Ceramic Membrane Bioreactor due to Inorganic Abrasion", Journal of Membrane Science, Vol. 163, pp. 19-28.

Cicek, N., Macomber, J., Davel, J., Suidan, M. T., Audic, J. and Genestet, P. (2001). "Effect of Solids Retention Time on the Performance and Biological Characteristics of a Membrane Bioreator", Water Science and Technology, Vol. 43, No. 11, pp. 43-50.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O. and Kroiss, H. (2005). "The Solids Retention Time----a Suitable Design Parameter to Evaluate the Capacity of Wastewater Treatment Plants to Remove Micropollutants", Water Research, Vol. 39, pp. 97-106.

Clöte, T. E. and Oosthuizen, D. J. (2001). "The Role of Extracellular Exopolymers in the Removal of Phosphorus from Activated Sludge", Water Research, Vol. 35, No. 15, pp. 3595-3598.

Curtis, T. P. and Craine, N. G. (1998). "The Comparison of the Diversity of Activated Sludge Plants", Water Science and Technology, Vol. 37, No. 4-5, pp. 71-78.

Defrance, L., Jaffrin, M. Y., Gupta, B., Paullier, P. and Geaugery V. (2000). "Contribution of Various Constituents of Activated Sludge to Membrane Bioreactor Fouling", Bioresource Technology, Vol. 73, pp. 105-112.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). "Colorimetric Method for Determination of Sugars and Related Substances", Analytical Chemistry, Vol. 28, No. 3, pp. 350-356.

Duncan-Hewitt, W. C., Policova, Z., Cheng, P., Vargha-Butler, E. I. and Neumann, A. W. (1989). "Semi-automatic Measurement of Contact Angles on Cell Layers by a Modified Axis Symmetric Drop Shape Analysis", Water Research, Vol. 42, pp. 391-402.

Ellis, R. J., Morgan, P., Weightman, A. J. and Fry, J. C. (2003). "Cultivated-dependent and -independent Approaches for Determining Bacterial Diversity in Heavy-metal-contaminated Soil", Applied and Environmental Microbiology, Vol. 69, pp. 3223-3230.

Evans, E., Brown, M. R. W. and Gilbert, P. (1994). "Iron Chelator, Exopolysaccharide and Protease Production in Staphylococcus Epidermidis: a Comparative Study of the Effects of Specific Growth Rate in Biofilm and Planktonic Culture", Microbiology, Vol. 140, pp. 153-157.

Emanuelsson, E. A. C., Arcangeli, J. P. and Livingston, A. G. (2003). "The Anoxic Extractive Membrane Bioreactor", Water Research, Vol. 37, pp. 1231-1238.

Fane, A. G. (1996). "Membranes for Water Production and Wastewater Reuse", Desalination, Vol. 106, pp. 1-9.

Fane, A. G. (2002). "Membrane Process for Water Treatment: Microfiltration and Ultrafiltration", In proceedings of Membrane Technology for Sustainable Water Seminar, Singapore.

Fang, H. H. P. and Shi, X. L. (2005). "Pore Fouling of Microfiltration Membranes by Activated Sludge", Journal of Membrane Science, Vol. 1-2, pp. 161-166.

Field, R. W., Wu, D., Howell, J. A., Gupta, B. B. (1995). "Critical flux concept for microfiltration fouling", Journal of Membrane Science, Vol. 100, pp. 259-272.

Fischer, S. G. and Lerman, L. S. (1983). "DNA Fragments Differing by Single Basepair Substitutions Are Separated in Denaturaing Gradient Gels: Correspondence with Melting Theory", Process Natl. Acad. Sci., Vol. 80, pp. 1579-1583.

Flemming, H. C., Schaule, G., McDonogh, R. and Ridgway, H. F. (1994). "Biofouling and biocorrosion in industrial water systems", in Effects and Extent of Biofilm Accumulation in Membrane Systems, Lewis publishers, USA.

Flemming, H. C. and Wingender, J. (2001a). "Relevance of Microbial Extracellularpolymeric Substances (EPSs) - Part I: Structural and Ecological Aspects", Water Science and Technology, Vol. 43, pp. 1-8.

Flemming, H.C. and Wingender, J. (2001b). "Relevance of Microbial Extracellular Polymeric Substances (EPSs) - Part II: Technical Aspects", Water Science and Technology, Vol. 43, No. 6: 9-16.

Flemming, H.C., Wingender, J., Griebe, T. and Mayer, C. (2000a). Biofilms: Recent Advances in Their Study and Control, Harwood Academic Publishers, UK.

Flemming, H. C., Wingender, J., Mayer, C., Koerstgens, V. and Borchard, W. (2000b). "Cohesiveness in Biofilm Matrix Polymers", in Community Structure and Co-operation in Biofilm, SGM symposium 59, Cambridge University Press, UK.

Frank, B. P. and Belfort, G. (2003). "Polysaccharides and Sticky Membrane Surfaces: Critical Ionic Effects", Journal of Membrane Science, Vol. 212, pp. 205-212.

Gafan, G. P. and Spratt, D. A. (2005). "Denaturing Gradient Gel Electrophoresis Gel

Expansion (DGGEGE)-An Attempt to Resolve the Limitations of Co-migration in the DGGE of Complex Polymicrobial Communities", FEMS Microbiology letters, Vol. 253, pp. 303-307.

Gander, M., Jefferson, B. and Judd, S. (2000). "Aerobic MBRs for Domestic Wastewater Treatment: A Review with Cost Considerations", Separation and Purification Technology, Vol. 18, pp. 119-130.

Gao, M. C., Yang, M., Li, H.Y., Yang, Q. X. and Zhang, Y. (2004). "Comparison between a Submerged Membrane Bioreactor and a Conventional Activated Sludge System on Treating Ammonia-bearing Inorganic Wastewater", Journal of Biotechnology, Vol. 108, pp. 265-269.

Gaudy, A. F., and Gaudy E. T. (1980). Microbiology for environmental scientists and engineers, McGraw-Hill, Inc, USA.

Gryta, M. (2002). "The Assessment of Microorganism Growth in the Membrane Distillation System", Desalination, Vol. 142, pp. 79-88.

Green, G. and Belfort, G. (1980). "Fouling of ultrafiltration membranes: lateral migration and particle trajectory model", Desalination, Vol. 35, pp. 129-147.

Günder, B. and Krauth, K. (1998). "Replacement of secondary clarification by membrane separation — Results with plate and hollow filbre modules", Water Science and Technology, Vol. 38, No. 4-5, pp. 383-393.

Günder, B. and Krauth, K. (1999). "Replacement of Secondary Clarification by Membrane Separation-Results with Tubular, Plate and Hollow Fibre Modules", Water Science and Technology, Vol. 40, No. 4-5, pp. 311-320.

Han, S. S., Bae, T. H., Jang, G. G. and Tak, T. M. (2005). "Influence of sludge retention time on membrane fouling and bioactivities in membrane bioreactor system", Process Biochemisty, Vol. 40, pp. 2393-2400.

He, Y. L., Xu, P., Li, C. J. and Zhang, B. (2005). "High-concentration Food Wastewater Treatment by an Anaerobic Membrane Bioreactor", Water Research, Vol. 17, pp. 4110-4118.

Ho, W. W. S. and Sirkar, K. K. (2001). Membrane handbook, Boston, USA, Kluwer Academic Publishers.

Hodgson, P. A., Leslie, G. L., Schneider, R. P., Fane, A. G., Fell, C. J. D. and Marshall K. C. (1993). "Cake Resistance and Solute Rejection in Bacterial Microfiltration: The Role of the Extracellular Matrix", Journal of Membrane Science, Vol. 79, pp. 35-53.

Hong, S. P., Bae, T. H., Tak, T. M., Hong, S. and Randall, A. (2002). "Fouling Control in Activated Sludge Submerged Hollow Fiber Membrane Bioreactors", Desalination, Vol. 143, pp. 219-228.

Horn, H., Neu, T.R. and Wulkow, M. (2001). "Modelling the Structure and Function of Extracellular Polymeric Substances in Biofilms with New Numerical Techniques ", Water Science and Technology, Vol. 43, No. 6, pp. 121-127.

Huang, C. T., Peretti, S. W. and Bryers, J. D. (1994). "Effects of Medium Carbon-to-Nitrogen Ratio on Biofilm Formation and Plasmid Stability", Biotechnology and Bioengineering, Vol. 44, No. 3, pp. 329-336.

Jang, A., Kim, S. M., Kim, S. Y., Lee, S. G. and Kim, I. S. (2001). "Effect of Heavy Metals (Cu, Pb and Ni) on the Compositions of EPS in Biofilm", Water Science and Technology, Vol. 43, No. 6, pp. 41-48.

Jiang, H. L. (2004). Develoment of Aerobic Granules for Enhanced Biological Wastewater Treatment (Ph.D thesis), School of Civil & Environmental Engineering, Nanyang Technology University, Singapore.

Jiang, Y. X., Dodds, J., Leclerc, D. and Lenoel, M. (1995), "A Technique for the Study of the Fouling of Microfiltration Membranes Using Two Membranes in Series", Journal of Membrane Science, Vol. 105, No. 1-2, pp. 23-30.

Jin, B., Wilén, B. M. and Lant, P. (2003). "A Comprehensive Insight into Floc Characteristics and Their Impact on Compressibility and Settleability of Activated Sludge", Chemical Engineering Journal, Vol. 95, No. 1-3, pp. 221-234

Jin, Y.-L., Lee, W.-N., Lee, C.-H., Chang, I.-S., Huang, X. and Swaminathan, T. (2006). "Effect of DO Concentration on Biofilm Structure and Membrane Filterability in Submerged Membrane Bioreactor", Water Research, Vol. 40, pp. 2829-2836.

Jorand, F., Guicherd, P., Urbain, V., Manem, J. and Block, J. C. (1995). "Hydrophobicity of Activated Sludge Flocs and Laboratory-grown Bacteria", Water Science and Technology, Vol. 30, No. 11, pp. 211-218.

Jorand, F., Boue-Bigne, F., Block, J. C. and Urbain, V. (1998). "Hydrophobicity /Hydrophilic Properties of Activated Sludge Exopolymeric Substances", Water Science and Technology, Vol. 37, No. 4-5, pp. 307-315.

Jönsson, C. and Jönsson, A. S. (1995), "Influence of the Membrane Material on the Adsorption Fouling of Ultrafiltration membranes", Journal of Membrane Science, Vol. 108, No. 1-2, pp. 79-87.

Kämpfer, P. (1997). "Detection and cultivation of filamentous bacteria from activated sludge", FEMS Microbiology Ecology, Vol. 23, pp. 169-181.

Kaiser, S. K., Guckert, J. B. and Gledhill, D. W. (1998). "Comparison of Activated Sludge Microbial Communities using Biolog Microplates", Water Science and Technology, Vol. 37, No. 4-5, pp. 57-63.

Kishino, H., Ishida, H., Iwabu, H. and Nakano, I. (1996). "Domestic Wastewater Reuse using a Submerged Membrane Bioreactor", Desalination, Vol. 106, pp. 115-119.

Klatt, C. G. and Lapara, T. M. (2003). "Aerobic biological treatment of synthetic municipal wastewater in membrane-coupled bioreactors", Biotechnology and Bioengineering, Vol. 82, No. 3, pp. 313-320.

Knoblock, M. D. (2000). "Membrane Biological Reactor of Oily Wastewater", Water Environment Research, Vol. 66, No. 2, pp. 133-139.

Kreft, J. U. and Wimpenny, J. W. T. (2001). "Effect of EPS on Biofilm Structure and Function as Revealed by an Individual-based Model of Biofilm Growth", Water Science and Technology, Vol. 43, No. 6, pp. 135-141.

Kwon, D. Y., and Vigneswaran, S. (1998). "Influence of Particle Size and Surface Charge on Critical Flux of Crossflow Microfiltration", Water Science and Technology, Vol. 38, No. 4-5, pp. 481-488.

LaPara, T. M., Klatt, C. G. and Chen, R. (2006). "Adaptations in Bacterial Catabolic Enzyme Activity and Community Structure in Membrane-coupled Bioreactors Fed Simple Synthetic Wastewater", Journal of Biotechnology, Vol. 121, pp. 368-380.

Laspidou, C. S. and Rittmann, B. E. (2002). "A Unified Theory for Extracellular Polymericsubstances Soluble Microbial Products and Active and Inert Biomass", Water Research, Vol. 36, pp. 2711-2720.

Le-Clech, P., Jefferson, B. and Judd, S. J. (2003). "Impact of Aeration, Solids Concentration and Membrane Characteristics on the Hydraulic Performance of a Membrane Bioreactor", Journal of Membrane Science, Vol. 218, No. 1-2, pp. 117-129.

Le-Clech, P., Jefferson, B. and Judd, S. J. (2005). "A comparison of submerged and sidestream tubular membrane bioreactor configurations", Desalination, Vol. 173, pp. 113-122.

Lee, W., Kang, S. and Shin, H. (2003). "Sludge Characteristics and Their Contribution to Microfiltration in Submerged Membrane Bioreactor", Journal of Membrane Science, Vol. 216, No. 1-2, pp. 217-227.

Lerman, L. S., Fischer, S. G., Hurley, I., Silverstein, K. and Lumelsky, N. (1984). "Sequence-determined DNA Separations", Annual Review of Biophysics and Bioengineering, Vol. 13, pp. 399-423.

Lesjean, B., Rosenberger, S. and Schrotter, J. C. (2004). "Membrane-aided Biological Wastewater Treatment ---- an Overview of Applied Systems", Membrane Technology, Vol. 126, No. 8, pp. 5-10.

Li, H., Yang, M., Zhang, Y., Liu, X., Gao, M. and Kamagata, Y. (2005a). "Comparison of Nitrification Performance and Microbial Community between Submerged Membrane Bioreactor and Conventional Activated Sludge System", Water Science and Technology, Vol. 51, No. 6-7, pp. 193-200.

Li, X. F., Gan F. S., Hua, Z. Z., Du, G. C. and Chen, J. (2005b). "Treatment of Synthetic Wastewater by a Novel MBR with Granular Sludge Developed for Controlling Membrane Fouling", Journal of Membrane Science, Vol. 46, No. 1-2, pp. 19-25.

Liao, B. Q., Allen, D. G., Droppo, I. G., Leppard, G. G. and Liss, S. N. (2000). "Surface Properties of Sludge and Their Role in Bioflocculation and Settleability", Water Research, Vol. 35, No. 2, pp. 339-350.

Liu, H. and Fang, H. P. (2002). "Extraction of Extracellular Polymeric Substances (EPS) of Sludges", Journal of Biotechnology, Vol. 95, pp. 249-256.

Liu, R., Huang, X., Xi, J. Y. and Qian, Y. (2005). "Microbial behaviour in a membrane bioreactor with complete sludge retention", Process Biochemistry, Vol. 40, pp. 3165-3170.

Low, E. W. and Chase, H. A. (1999). "The effect of maintenance energy requirement on biomass production during wastewater treatment", Water Research, Vol. 33, No. 3, pp. 847-853.

Lubin, M. B., Elashoff, J. D., Wang, S. J., Rotter, J. I. and Toyoda, H. (1991). "Precise gene dosage determination by polymerase chain reaction: theory, methodology, and statistical approach", Molecular and Cellular Probes, Vol. 5, pp. 307-317.

Luxmy, B. S., Nakajima, F. and Yamamoto, K. (2000). "Analysis of Bacterial Community in Membrane-separation Bioreactors by Fluorescent in situ Hybridization (FISH) and Denaturing Gradient Gel Electrophoresis (DGGE) Techniques", Water Science and Technology, Vol. 41, No. 10, pp. 259-268.

Ma, B.-C., Lee, Y.-N., Park, J.-S., Lee, C.-H., Lee, S.-H., Chang, I.-S. and Ahn, T.-S. (2006). "Correlation between Dissolved Oxygen Concentration, Microbial Community and Membrane Permeability in a Membrane Bioreactor", Process Biochemistry, Vol. 41, pp. 1165-1172.

Ma, H. M., Lakim, L. F., Bowman, C. N. and Davis, R. H. (2001). "Factors Affacting Membrane Fouling Reduction by Surface Modification and Backpulsing", Journal of Membrane Science, Vol. 189, pp. 255-270.

Ma, L., Li, X. F., Du, G. C., Chen, J. and Shen, Z. S. (2005). "Influence of the Filtration Modes on Colloids Adsorption on the Membrane in Submerged Membrane Bioreactor", Colloids and Surfaces A: Physicochemistry Engineering Aspects, Vol. 264, pp. 120-125.

Macintyre, E. A. (1989). "The Use of the Polymerase Chain Reaction in Haematology", Blood Reviews, Vol. 3, pp. 201-210.

Maszenan, A. M., Seviour, R. J. , Patel, B. K. C. , Schumann, P., Burghardt, J., Tokiwa, Y. and Stratton, H. M. (2000). "Three isolates of novel polyphosphate-accumulating Gram-positive cocci, obtained from activated sludge, belong to a new genus, Tetrasphaera gen. nov., and descriptionof two new species, Tetrasphaera japonica sp. nov. and Tetrasphaera australiensis sp. nov.", International Journal of Systematic Evolutional Microbiology, Vol. 50, pp. 593-603.

Matsuda, A., Bhatta, C. P., Kawasaki, K., Noma, Y., Machana, Y., Yamaguchi, M. and Omori, D. (2003). "Stable Operational Condition of Submerged Membrane Activated Sludge Process without Sludge Withdrawal", Journal of Chemical Engineering of Japan, Vol. 36, No. 12, pp. 1523-1527.

Matsuyama, T. and Nakagawa, Y. (1996). "Surface-active exolipids: analysis of absolute chemical structures and biological functions", Journal of Microbiological Methods, Vol. 25, No. 2, 165-175.

Metcalf & Eddy, Inc. (1991). Wastewater Engineering: Treatment, Disposal, Reuse, McGraw-Hill Inc, New York, USA.

Miambi, E., Guyot, J.-P. and Ampe, F. (2003). "Identification, Isolation and Quantification of Representative bacteria from fermented cassava dough using an integrated approach of culture-dependent and culture-independent methods", International Journal of Food Microbiology, Vol. 82, pp. 111-120.

Mikkelsen, L. H. and Nielsen, P. H. (2001). "Quantification of the Bond Energy of Bacteria Attached to Activated Sludge Floc Surfaces", Water Science and Technology, Vol. 43, No. 6, pp. 67-75.

Möckel, D., Staude, E. and Guiver, M. D. (1999). "Static Protein Adsorption, Ultrafiltration Behavior and Cleanability of Hydrophilized Polysulfone Membranes", Journal of Membrane Science, Vol. 158, pp. 63-75.

Mukai, T., Takimoto, K., Kohno, T. and Okada, M. (1999). "Ultrafiltration Behaviour of Extracellular and Metabolic Products in Activated Sludge System with UF Separation Process", Water Research, Vol. 34, No. 3, pp. 902-908.

Muller, E. B., Stouthamer, A. H., van Verseveld H. W. and Eikelboom, D. H. (1995). "Aerobic domestic waste water treatment in a pilot plant with complete sludge retention by cross-flow filtration", Water Research, Vol. 29, No. 4, pp. 1179-1189.

Muyzer, G., de Waal, E. C. and Uitterlinden, A. G., (1993). "Profiling of Complex Microbial Population by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-amplified Genes Coding for 16S rRNA", Applied and Environmental Microbiology, Vol. 59, pp. 695-700.

Nagaoka, H. (1999). "Nitrogen Removal by Submerged Membrane Separation Activated Sludge Process", Water Science and Technology, Vol. 39, No. 8, pp. 107-114.

Nagaoka, H., Ueda, S. and Miya, A. (1996). "Influence of Bacterial Extracellular Polymers on the Membrane Separation Activated Sludge Process", Water Science and Technology, Vol. 34, No. 9, pp. 165-172.

Nagaoka, H., Yamanishi, S. and Miya, A. (1998). "Modeling of biofouling by extracellular polymers in a membrane separation activated sludge system", Water Science and Technology, Vol. 38, No. 4-5, pp. 497-504.

Nielsen, P. H., FrÖlund, B., Keiding, K. (1996). "Changes in the composition of extracellular polymeric substances in activated sludge during anaerobic storage", Applied Microbiology Biotechnology, Vol. 44, pp. 823-830.

Ng, H. Y. and Hermanowicz, S. W. (2005). "Membrane Bioreactor Operation at Short Solids Retention Times: Performance and Biomass Characteristics", Water Research, Vol. 39, pp. 981-992.

Overmann, J. and Pfeng, N. (1992). "Buoyancy Regulation and Aggregate Formation in Amoebobacter Purpures from Mahoney Lake", FEMS Microbiology Ecology, Vol. 101, pp. 67-79.

Pan, S., Tay, J. H., He, Y. X. and Tay, S. T. L. (2004). "The Effect of Hydraulic Retention Time on the Stability of Aerobically Grown Microbial Granules", Letters in Applied Microbiology, Vol. 38, pp. 158-163.

Park, H., Choo, K. H. and Lee, C. H. (1999). "Flux Enhancement with Powered Activated Carbon Addition in the Membrane Anaerobic Bioreactor", Separation Science and Technology, Vol. 34, pp. 2781-2792.

Park, N., Kwon, B., Kim, I. S. and Cho, J. (2005). "Biofouling potential of various NF membranes with respect to bacteria and their soluble microbial products (SMP): Characterizations, flux decline, and transport parameters", Journal of Membrane Science, Vol. 258, pp. 43-54.

Petersen, D. G. and Dahllöf, I. (2005). "Improvements for Comparative Analysis of Changes of Microbial Communities Using Internal Standards in PCR-DGGE", FEMS Microbiology Ecology, Vol. 53, No. 3, pp. 339-348.

Pollice, A., Laera, G. and Blonda, M. (2004). "Biomass Growth and Activity in a Membrane Bioreactor with Complete Sludge Retention", Water Research, Vol. 38, pp. 1799-1808.

Pollice, A., Brookes, A., Jefferson, B. and Judd, S. (2005). "Sub-critical Flux Fouling in Membrane Bioreactors----a Review of Recent Literature", Desalination, Vol. 174, pp. 221-230.

Porter, M. C. (1972). "Concentration Polarization with Membrane Ultrafiltration", Ind. Eng. Chem. Prod. Res. Dev., Vol. 11, pp. 234.

Puñal, A., Brauchi, S., Reyes, J.G. and Chamy, R. (2003). "Dynamics of Extracellular Polymeric Substances in UASB and EGSB Reactors Treating Medium and Low Concentrated Wastewater", Water Science and Technology, Vol. 48, No. 6, pp. 41-49.

Roberts, J. A., Sutton, P. M. and Mishra, P. N. (2000). "Application of the Membrane Biological Reactor System for Combined Sanitary and Industrial Wastewater Treatment", International Biodeterioration & Biodegradation, Vol. 46, pp. 37-42.

Rosenberger, S. and Kraume M. (2002). "Filterability of Activated Sludge in Membrane Bioreactors", Desalination, Vol. 151, pp. 195-200.

Saiki, R. K., Scharf, S. J., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. and Arnheim, N. (1985). "Enzymatic Amplification of B-globin Genomic Sequences and Restriction Site Analusis for Diagnosis of Sickle Cell Anemia", Science, Vol. 230, pp. 1350-1354.

Schöberl, P., Brik, M., Bertoni, M., Braun, R. and Fuchs, W. (2005). "Optimization of Operational Parameters for a Submerged Membrane Bioreactor Treating Dyehouse Wastewater", Separation and Purification Technology, Vol. 44, pp. 61-68.

Seo, G. T., Moon, C. D., Chang, S. H. and Lee, S. H. (2004). "Long Term Operation of High Concentration Powered Activated Carbon Membrane Bioreactor for Advanced Water Treatment", Water Science and Technology, Vol. 50, No. 8, pp. 81-87.

Seviour, R. J., Mino, T. and Onuki, M. (2003). "The Microbiology of Biological Phosphorus Removal in Activated Sludge Systems", FEMS Microbiology Reviews, Vol. 27, pp. 99-127.

Sharma, P. K. and Rao, K. H. (2002). "Analysis of Different Approaches for Evaluation of Surface Energy of Microbial Cells by Contact Angle Goniometry", Advances in Colloid and Interface Science, Vol. 98, pp. 341-463.

Shin, H. S., Kang, S. T. and Nam, S. Y. (2001). "Effect of Carbohydrate and Protein in the EPS on Sludge Settling Characteristics", Water Science and Technology, Vol. 43, No. 6, pp. 193-196.

Shin, H. S. and Kang, S. T. (2003). "Characteristics and Fates of Soluble Microbial Products in Ceramic Membrane Bioreactor at Various Sludge Retention Times", Water Research, Vol. 37, pp. 121-127.

Singh, K. K. and Vincent, W. S. (1987). "Clumping Characteristics and Hydrophobic Behaviour of an Isolated Bacterial Strain from Sewage Sludge", Applied Microbiology Biotechnology, Vol. 25, pp. 396-398.

Sofia, A., Liu, W. T., Ong, S. L. and Ng, W. J. (2004). "In-situ Characterization of Microbial Community in an A/O Submerged Membrane Bioreactor with Nitrogen Removal", Water Science and Technology, Vol. 50, No. 8, pp. 41-48.

Spellman, F. R. (1997). Microbiology for Water/wastewater Operator, Technomic Publishing Company, Basel, Switzerland.

Sponza, D. T. (2002). "Extracelluar Polymer Substances and Physicochemical Properties of Flocs in Steady- and Unsteady-state Activated Sludge Systems", Process Biochemistry, Vol. 37, pp. 983-998.

Sponza, D. T. (2003). "Investigation of Extracellular Polymer Substances (EPS) and Physicochemical Properties of Different Activated Sludge Flocs under Steady-state Conditions", Enzyme and Microbial Technology, Vol. 32, pp. 375-385.

Sutherland, I. W. (2001). "Exopolysaccharides in Biofilms, Flocs and Related Structures", Water Science and Technology, Vol. 43, No. 6, pp. 77-86.

Tao, G., Kiran, K., Zhao, W., Ting, C. L., Bala, V. and Seah, H. (2005). "Membrane Bioreactors for Water Reclamation", Water Science and Technology, Vol. 51, No. 6-7, pp. 431-440.

Taverneier, P., Portais, J. C., Saucedo, J. E. N., Courtois, J., Courtois, B. and Barbotin, J. N. (1997). "Exopolysaccharides and Poly-beta-hydroxybutyrate Coproduction in Two Rhizobium Meliloti Strains", Applied and Environmental Microbiology, Vol. 63, pp. 21-26.

Tay, J. H., Zeng, J. L. H. and Sun, D. D. L. (2003). "Effects of Hydraulic Retention Time on System Performance of a Submerged Membrane Bioreactor", Separation Science and Technology, Vol. 38, No. 4, pp. 851-868.

Thomas, H., Judd, S. and Murrer, J. (2000). "Fouling Characteristics of Membrane Filtration in Membrane Bioreactors", Membrane Technology, Vol. 122, pp.10-13.

Tsuneda, S., Aikawa, H., Hayashi, H., Yuasa, A. and Hirata, A. (2003a). "Extracellular Polymeric Substances Responsible for Bacterial Adhesion onto Solid Surface", FEMS Microbiology Letters, Vol. 23, No. 2, pp. 287-292.

Tsuneda, S., Jung, J., Hayashi, H., Aikawa, H., Hirata, A. and Sasaki, H. (2003b). "Influence of Extracellular Polymers on the Electrokinetic Properties of Heterotrophic Bacterial Cells Examined by Soft Particle Electrophoresis Theory", Colloids and Surfaces B: Biointerfaces, Vol. 29, No. 2-3, pp. 181-188.

Tsuneda, S., Park, S., Hayashi, H., Jung, J. and Hirata, A. (2001). "Enhanced of Nitrifying Biofilm Formation Using Selected EPS Produced by Heterotrophic Bacteria", Water Science and Technology, Vol. 43, No. 6, pp. 197-204.

Vallaeysa, T., Topp, E., Muyzer, G., Machereta, V., Laguerrea, G., Rigauda, A. and Soulasa, G. (1997). "Evaluation of Denaturing Gradient Gel Electrophoresis in the Detection of 16S rDNA Sequence Variation in Rhizobia and Methanotrophs", FEMS Microbiology Ecology, Vol. 24, No. 3, pp. 279-285.

Van Dijk, L. and Roncken, G. C. G. (1997). "Membrane Bioreactor for Wastewater Treatment: The State of the Art and New Developments", Water Science and Technology, Vol. 35, No. 10, pp. 35-41.

Vierstraete, A. (1999). http://users.ugent.be/~avierstr/

Vocks, M., Adam, C., Lesjean, B., Gnirss, R. and Kraume, M. (2005). "Enhanced Post-Denitrification without Addition of an External Carbon Source in Membrane Bioreactors", Water Research, Vol. 39, pp. 3360-3368.

Wagner, J. and Rosenwinkel, K. H. (2000). "Sludge Production in Membrane Bioreactors under Different Conditions", Water Science and Technology, Vol. 41, No. 10-11, pp. 251-258.

Watanabe, K., Teramoto, M., Futamata, H. and Harayama, S. (1998). "Molecular Detection, Isolation, and Physiological Characterization of Functionally Dominant Phenol-degrading Bacteria in Activated Sludge", Applied and Environmental Microbiology, Vol. 64, No. 11, pp. 4396-4402.

Wilén, B. M., Jin, B. and Lant, P. (2002) "The Influence of Key Chemical Constituents in Activated Sludge on Surface and Flocculating Properties", Water Research, Vol. 37, No. 9, pp. 2127-2139.

Wilshusen, J. H., Hettiaratchi, J. P. A., De Visscher, A. and Saint-Fort, R. (2004). "Methane Oxidation and Formation of EPS in Compost: Effect of Oxygen Concentration", Environmental Pollution, Vol. 129, pp. 305-314.

Winnen, H., Suidan, M. T., Scarpino, P. V., Wrenn,, B., Cicek, N., Urban, V. and Manem, J. (1996). "Effectiveness of the Membrane Bioreactor in the Biodegradation of High Molecular-weight Compounds", Water Science and Technology, Vol. 34, No. 9, pp. 197-203.

Wingender, J., Neu, T. R. and Flemming H. C. (1999). What are bacterial extracellular polymeric substances? In: Wingender, J., Neu, T. R. and Flemming H. C, editors. Microbial Extracellular Polymeric Substances----Characterization, Structure and Function, Springer, Berlin.

Wintgens, T., Rosen, J., Brepols, C., Drensla, K. and Engelhardt, N. (2003). "Modelling of a Membrane Bioreactor System for Municipal Wastewater Treatment", Journal of Membrane Science, Vol. 216, pp. 55-65.

Wisniewski, C. and Grasmick, A. (1998). "Floc Size Distribution in a Membrane Bioreactor and Consequences for Membrane Fouling", Colloids and Surfaces, Vol. 138, pp. 403-411.

Witzig, R., Manz, W., Rosenberger, S., Krueger, U., Kraume, M. and Szewzyk, U. (2002). "Microbiological Aspects of a Bioreactor with Submerged Membranes for Aerobic Treatment of Municipal Wastewater", Water Research, Vol. 36, pp. 394-402.

Wyffels, S., Van Hulle, S. W. H., Boeckx, P., Volcke, E. I. P., Van Cleemput, O., Vanrolleghem, P. A. and Verstraete, W. (2004). "Modeling and Simulation of Oxygen-Limited Partial Nitritation in a Membrane-assisted Bioreactor (MBR)", Biotechnology and Bioengineering, Vol. 86, No. 5, pp. 531-542.

Yamamoto, K., Hiasa, H., Talat, M. and Matsuo, T. (1989). "Direct Solid Liquid Separation Using Hollow Fiber Membranes in Activated Sludge Aeration Tank", Water Science and Technology, Vol. 21, pp. 43-54.

Yamato, N., Kimura, K., Miyoshi, T. and Watanabe, Y. (2006), “Difference in Membrane Fouling in Membrane Bioreactors (MBRs) caused by Membrane Polymer Materials”, Journal of Membrane Science, Vol. 280, No. 1-2, pp. 911-919.

Yang, W., Cicek, N. and Ilg, J. (2005). "State-of-the-art of Membrane Bioreactors: Worldwide Research and Commercial Application in North America", Journal of Membrane Science, Vol. 270, No. 1-2, pp. 201-211.

Ye, Y., Le Clech, P., Chen, V. and Fane, A. G. (2005). "Evolution of Fouling during Crossflow Filtration of Model EPS Solution", Journal of Membrane Science, Vol. 264, pp. 190-199.

Yeon, K. M., Park, J. S., Lee, C. H. and Kim, S. M. (2005). "Membrane Coupled High-Performance Compact Reactor: A New MBR System for Advanced Wastewater Treatment", Water Research, Vol. 39, pp. 1954-1961.

Yi, S., Tay, J. H., Maszenan, A. M. and Tay, S. T. L. (2003). "A Culture-independent Approach for Studying Microbial Diversity in Aerobic Granules", Water Science and Technology, Vol. 47, No. 1, pp. 283-290.

Yoon, S. H. (2003). "Important Operational Parameters of Membrane Bioreactor-sludge Disintegration (MBR-SD) System for Zero Excess Sludge Production", Water Research, Vol. 37, pp. 1921-1931.

Yoon, S. H., Kim, H. S. and Yeom, I. T. (2004). "The Optimum Operational Condition of Membrane Bioreactor (MBR): Cost Estimation of Aeration and Sludge Treatment", Water Research, Vol. 38, No. 1, pp. 37-46.

Yun, M.-A., Yeon, K.-M., Park, J.-S., Lee, C.-H., Chun, J. S. and Lim, D. J. (2006). "Characterization of Biofilm Structure and Its Effect on Membrane Permeability in MBR for Dye Wastewater Treatment", Water Research, Vol. 40, pp. 45-52.

Zhang, H. M., Xiao, J. N., Cheng, Y. J., Liu, L. F. Zhang, X. W. and Yang, F. L. (2005). "Comparison between a Sequencing Batch Membrane Bioreactor and a Conventional Membrane Bioreactor", Process Biochemistry, Vol. 41, No.1, pp. 87-95.

Zhang, X. Q., Bishop, B. L. and Kinkle, B. K. (1999). "Comparison of Extraction Methods for Quantifying Extracellular Polymers in Biofilms", Water Science and Technology, Vol. 39, No. 7, pp. 211-218.

Zhang, S. Y., Houten, R. V., Eikelboom, D. H., Doddema, H., Jiang, Z. C., Fan, Y. B. and Wang, J. S. (2003). "Sewage Treatment by a Low Energy Membrane Bioreacter", Bioresource Technolgoy, Vol., 90, No. 2, pp. 185-192.

Zhuang, W. Q., Tay, J. H., Yi, S. and Tay, S. T. L. (2005). "Microbial Adaption to Biodegradation of Tert-butyl Alcohol in a Sequencing Batch Reactor", Journal of Biotechnology, Vol. 118, pp. 45-53.

PUBLICATIONS

In Journals

Yi, S., Zhuang, W.-Q., Wu, B., Tay, S. T.-L., Tay, J.-H. (2006). "Biodegradation of *p*-Nitrophenol by Aerobic Granules in a Sequencing Batch Reactor", Environ. Sci. Technol., Vol. 40, No. 7, pp. 2396-2401.

Wu, B., Tay, S.T.L., Yi, S., Ng, C.A., Zhang, J.S., Sun, D .D. L.and Fane, A.G. (2006). "MBR Performance and Bacterial Community Structure under Different Solids Retention Times", Separation Science and Technology, (Submitted).

Wu, B., Yi, S., Zhuang, W.-Q., Tay, S.T.L. and Fane, A.G (2006). Comparison of EPS "Production, Membrane Fouling and Bacterial Population Changes in Nitrogen-rich MBR and Phosphorus-rich MBR Systems", Journal of biotechnology, (Submitted).

In International Conferences

Wu, B., Tay, S.T.L. and Fane, A.G (2004). "The effect of MBR operational parameters on EPS production and membrane fouling", Proceedings of IWA Specialized Conference on Water Environment-Membrane Technology, Korea, Vol. 4, pp. 1299-1306.

Ng, C. A., Sun, D., Zhang, J. S., Chua, H. C., Wu, B., Tay, S.T.L. and Fane, A.G. (2005). "Strategies to Improve the Sustainable Operation of Membrane Bioreactors", Proceedings of International Desalination Association conference, Singapore.

Wu, B., Tay, S.T.L., Sun, D.D.L. and Fane, A.G. (2006). "Investigation on The Contribution Of Dissolved Solids To Membrane Fouling And Fouling Mechanism Caused By Dissolved Solids", Proceedings of Membrane Science and Technology symposium, Singapore.