Effects of Nutrients Conditions and Solids Retention Time (SRT) on Performance and Membrane Fouling of Aerobic Membrane Bioreactors (MBRs)

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Abstract

This thesis investigated the effect of chemical oxygen demand (COD) to nitrogen ratio (COD:N) in feed on the biological performance of aerobic membrane bioreactor (MBR). Meanwhile, the effects of nutrients condition (COD:N ratios) and solids retention time (SRT) (7, 12 and 20 days) on sludge properties and their role in membrane fouling were systematically studied using well-controlled aerobic membrane bioreactor receiving a synthetic high strength industrial wastewater containing glucose. The results showed an increased COD:N ratio from 100:5 to 100:2.5 and 100:1.8 had limited impact on COD removal efficiency and further led to a significant improvement in membrane performance, a reduced sludge yield, and improved effluent quality in terms of residual nutrients. The results suggest that an increased COD:N ratio will benefit the industrial wastewater treatment using membrane bioreactors by reducing membrane fouling and sludge yield, saving chemical costs, and reducing secondary hand pollution by nutrients.

Membrane performance was improved with an increase in the COD:N ratio (e.g. reduced N dosage). Surface analysis of sludge by X-ray photoelectron spectroscopy (XPS) suggests that significant differences in the surface concentrations of elements C, O and N were observed under different COD:N ratios, implying significant differences in extracellular polymeric substances (EPS) composition. A unique characteristic peak at 1735 cm $^{-1}$ was observed under nitrogen limitation conditions by using Fourier transform-infrared spectroscopy (FTIR). Total EPS, proteins and the ratio of proteins to carbohydrates in EPS decreased with an increase in COD:N ratio, while carbohydrates in EPS increased with an increase in COD:N ratio. There were no significant differences in the total soluble microbial products (SMPs) but the ratio of proteins to carbohydrates in SMPs decreased with an increase in COD:N ratios. Sludge cake formation was the dominant mechanism of membrane fouling.

Membrane performance was improved with an increase in SRT. Surface analysis of sludge by X-ray photoelectron spectroscopy (XPS) suggests that significant differences in the surface concentration of element C and N were observed under different SRTs, implying significant differences in EPS composition. A larger amount of total EPS was found at the lowest SRT (7 days) tested but the ratio of proteins to carbohydrates in EPS increased with an increase in SRT. Similarly, the quantity of SMPs decreased with an increase in SRT but the ratio of proteins to carbohydrates in SMPs increased with an increase in SRT. The quantity of total EPS, total SMPs, and proteins to carbohydrates ratios positively correlated to membrane fouling rates. Sludge cake formation is the dominant mechanisms of membrane fouling.

Keywords: Nutrient requirements; COD:N; industrial wastewater treatment; sludge yield; membrane bioreactor; membrane fouling; surface properties; solids retention time; Extracellular polymeric substances; soluble microbial products

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List of Nomenclature and Abbreviations

AFM	Atomic Force Microscopy
AMBR	Aerobic Membrane Bioreator
BAP	Biomass Associated Products
BOD	Biochemical Oxygen Demand
BSA	Bovine Serum Albumin
CER	Cation Exchange Resin
CSLM	Confocal Scanning Laser Microscopy
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
ED	Electrodialysis
EDX	Energy-dispersive X-ray spectroscopy
EEM	Excitation-emission Matrix
EPS	Extracellular Polymeric Substances
F	Full scale
F/M	Food/Microorganisms
FTIR	Fourier Transform Infrared
HRT	Hydraulic Retention Time
ICP	Inductively Coupled Plasma
L	Laboratory/bench scale
LB-EPS	Loosely Bound- Extracellular Polymeric Substances
LPM	Liters Per Minute
MBRs	Membrane Bioreactors

MF	Microfiltration
MLSS	Mixed Liquor Suspended Solids
MW	Molecular Weight
MWCO	Molecular Weight Cut off
NF	Nano filtration
NMR	Nuclear Magnetic Resonance
OLR	Organic Loading Rate
P	Pilot scale
Powered Activated Carbon	Powered Activated Carbon
PE	Polyethylene
PN	Protein
PN/CH	Protein/ Carbohydrates
PSD	Particle Size Distribution
PVC	Polyvinyl Chloride
PVDF	Polyvinylidene Fluoride
RO	Reverse Osmosis
Rt	Total Resistance
Rc	Cake Resisitance
R_f	Resistance due to pore blocking and adsorption
R_m	Intrinsic membrane resistance
SAMBR	Submerged Aerobic Membrane Bioreator
SEM	Scanning Electron Microscopy
SMP	Soluble Microbial Products
SRT	Solid Retention Time

SS	Suspended Solids
TB-EPS	Tightly Bound-Extracellular Polymeric Substances
TMP	Transmembrane Pressure
TSS	Total Suspended Solids
UAP	Utilization Associated Products
UF	Ultrafiltration
UV	Ultraviolet VFAs Volatile fatty acids
XPS	X-ray photoelectron spectroscopy

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Chapter 1 Introduction

In this chapter conventional technologies and promising methods for industrial wastewater treatment are presented. In addition, the factors affecting the performance of biomass properties and MBR technologies are briefly discussed. The chapter concludes with the presentation of the research objectives.

1.1 Industrial wastewater and conventional remediation technologies

With competing demands on limited water resources, the awareness of water pollution has been increased for both developing and developed countries. Industrial effluent is one of the major sources of wastewater. Over 70% of industrial wastewater treated inadequately before discharging into rivers or lakes in developing countries, thus lead to over half of the river and lake are unsafe to contact directly (WWAP, 2009). Lacking of capital investment, increasing industrial waste discharges, coupled with limited wastewater treatment capacity might be the reasons for improperly disposal, which threatens environmental health and safety. According to a report released by World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), around 780 million people do not have access to clean drinking water (WHO/UNICEF, 2008). Therefore, it is urgent and necessary to develop proper and sustainable methods for industrial wastewater treatment.

The source of industrial effluents include food processing, chemical, pulp and paper, pharmaceutical, mining, textile industry as well as other manufacturing industries. The characteristics of industrial wastewaters is generally described as high organic strength (>1000 mg COD/L) and extreme physicochemical nature, such as pH, temperature, salinity (Lin et al., 2012). Industrial wastewater may also contain toxic compounds, such as, heavy metals, phenols, chlorinated and biocides (Sipma et al., 2010) that may lead to inhibition of biodegradation. More importantly, industrial effluents present nutrients limitation, like nitrogen and phosphorus (N, P), which are essential for bacteria growth. A balanced chemical oxygen demand to nutrients ratio (COD: N) is required for healthy growth of microbial communities for biodegradation (Henze et al., 1997; Tchobanoglous and Burton, 1991). Thus, costly nutrients (N and P) are generally added to the feed for biodegradation in industrial wastewater treatment.

The conventional treatment of industrial wastewater involves physicochemical methods (e.g. adsorption, coagulation and flocculation, sedimentation, flotation, filtration, extraction and air stripping etc.), chemical oxidation methods as well as conventional biological process (Tchobanoglous et al., 2003). However, all these methods have some limitations when treating industrial wastewater. For instance, adsorption and filtration are insufficient to achieve the discharge limits and simply transfer the pollutant to another form (by adsorption) or concentrate it; coagulation and flotation yield a large amount of sludge; chemical oxidation processes, such as advanced oxidation processes that use ozone, H_2O_2, Fenton's reagent, or UV, are high in cost (Mantzavinos and Psillakis, 2004). Even though the biological treatment has been successfully used in industrial wastewater treatment, it still suffers problems with solid-liquid separation, poor effluent quality as well as large aeration and sedimentation tanks (Marrot et al., 2004). Under these circumstances, more effective, lower-cost and feasible technologies are required for wastewater treatment and water reuse to meet the human demand.

1.2 Promising Methods for Industrial Wastewater Treatment

Membrane technology integrated biological degradation (e. g. membrane bioreactor (MBR)) has been successfully applied in the municipal and industrial wastewater treatment to provide a direct solid-liquid separation by membrane filtration. Compared to the conventional activated sludge process, MBRs offer a number of advantages, such as complete biomass retention, a reduced sludge yield, enhanced high quality effluent, and a more compact treatment facility (Brindle and Stephenson, 1996; Marrot et al., 2004; Rosenberger and Kraume, 2003; Visvanathan et al., 2000). Additionally, the MBR technology appears to be a promising technology for nutrients management in industrial wastewater treatment, as it can overcome the problems of an increased COD/N or COD/P ratio encountered in conventional activated sludge processes, and reduce chemical costs and secondary pollution by nutrients addition. However, membrane fouling is still a major problem that hinders their more widespread and large-scale application. This is because membrane fouling reduces productivity, shortens membrane lifespan and increases operation costs. In general, membrane fouling can be caused by many factors, such as influent characteristics, solid retention time (SRT), hydraulic retention time (HRT), organic loading rate (ORL), and dissolved oxygen level. Among these parameters, the influent nutrient conditions (COD/N) and SRT remain the most important factors. This is largely because COD/N ratio can influence the physiological properties of microorganisms and chemical compositions of biomass in MBRs (Choi et al., 2001; Gao et al., 2004; Nagaoka, 1999). The COD/N ratio also can influence the amount of extracellular polymeric substances (EPS) and the composition of protein and carbohydrate in EPS which can affect membrane performance. In terms of SRT, although it has no direct impact on membrane filterability, it does influence the biomass properties including the particle size, mixed liquor suspended solids (MLSS) concentration, extracellular polymeric substances (EPS) and soluble microbial products (SMP), all of which could foul the membrane (Le-Clech et al., 2006).

There are few reports on the effect of COD/N ratio on biomass properties and membrane fouling in submerged membrane bioreactors and, more importantly, focused on the range of COD/N ratios for excess N removal in municipal wastewater treatment; On the other hand, an optimal nutrient dose or reduced nutrients doses (N and P) are important to the feed to maintain the biomass biodegradation as well as save chemical cost in industrial wastewater treatment.

Previous studies on the effect of SRT on the membrane performance are controversial. This is probably due to the fact that the fouling behaviors were investigated at various MLSS concentrations under tested SRTs.

1.3 Rationale and Scope of This Thesis

Considering the disadvantages and problems associated with MBR technology mentioned above, the factors affecting the membrane fouling and sludge properties are needed for further investigation. In this context, the main objectives of this study are:

(1) To investigate the feasibility of reduced nutrient (N) usage for high strength industrial wastewater treatment and the relationship between the COD/N ratios of the feed and the biological and membrane performance of aerobic membrane bioreactor.

(2) To explore the effect of nutrients ratio COD/N on sludge properties and their role in membrane fouling of MBRs for high strength industrial wastewater treatment.

(3) To clarify the effect of SRT on biological performance and membrane fouling at the same biomass concentration by regulating the feed COD.

This thesis is composed of six chapters. Chapter 1 presents the research background and the study objectives. A comprehensive literature review is presented in Chapter 2. Chapter 3 and 4 present a systematic study of nutrients requirements and nutrients conditions on biological performance, surface properties of sludge and their role in membrane fouling of aerobic membrane bioreactor. Chapter 5 discusses the effect of solid retention time on sludge properties and its role in membrane fouling. The final chapter, chapter 6, provides a summary of the results and conclusions.

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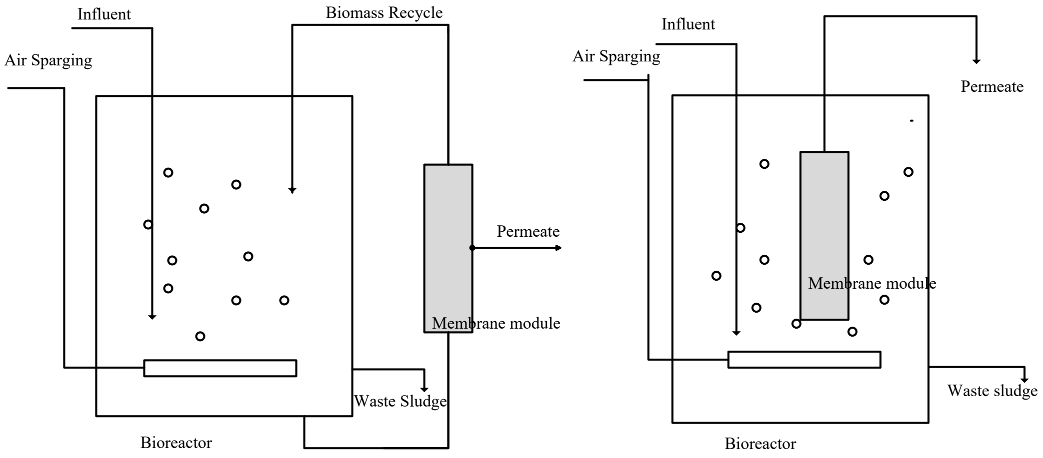
Chapter 2 Literature Review

2.1 Membrane Bioreactor Technologies

Membrane bioreactor (MBR) technology has been successfully incorporated into the activated sludge process to provide a direct solid-liquid separation by membrane filtration. In MBR systems, the bioreactor is responsible for the biodegradation of the organic pollutant and the membrane unit for the physical separation of suspended solid from treated water. MBR technology has been widely used for municipal and industrial wastewater treatment since early 1990s (Skouteris et al., 2012). The application of the micro- or ultra-filtration membrane in MBRs leads to significant improvements and numerous advantages compared to conventional activated sludge processes: (1) MBR eliminates the biomass separation problems and thus provides high quality effluent by its membrane filtration (Ferraris et al., 2009); (2) MBR allows complete suspended solids retention and consequently achieves higher biomass concentrations than conventional activated sludge process (Ferraris et al., 2009); (3) membrane module replaces the traditional large clarifying basins to settle out the biomass and enables the system to be more compact, thus lead to less footprint (Lapara and Alleman, 1999). Submerged MBR takes only half the land area of the conventional activated sludge process, and produces approximately half sludge(Mayhew and Stephenson, 1997). Thus, MBR offers superior effluent quality, smaller footprint and less sludge production. Due to these advantages, this technology has received considerable attention by both researchers and industrialists (Le-Clech, 2010).

There are two main types of configuration of MBRs: external/side-stream configuration and submerged/immersed configuration. Fig.2.1 shows the membrane bioreactor with different configurations (Lin et al., 2012). External configuration features the recirculation of the mixed liquor through a membrane module that is outside the bioreactor. This configuration simplifies the membrane replacement and helps control the membrane fouling but requires high energy (Le-Clech et al., 2006). In order to reduce energy consumption (Judd, 2004; Le-Clech et al., 2006; Liao et al., 2004), submerged configuration where the membrane modules are directly placed in the mixed liquor has been developed. The effluent is drawn by the pump, which impose negative pressure on the permeate side. The aeration employed in this configuration is to scour and clean the exterior of the membrane to reduce the cake layer formation. For immersed configuration, the operating conditions are much milder than in externalMBR systems due to the lower tangential velocities. To date, both configurations have been extensively employed for municipal and industrial wastewater treatments.



(a) (b)

Fig. 2.1 Membrane bioreactor with different configurations: (a) external configuration, (b) submerged configuration

The types of membrane can be classified into microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO), electrodialysis (ED), dialysis and pervaporation (PV). Coarse ultrafiltration (UF) and fine microfiltration range (MF) are commonly used in MBRs

(Stuckey, 2012). Various membrane modules including capillary, hollow fiber, tubular, plate and frame modules made from polymeric and ceramic membranes were tested for biomass retention in both suspended and attached growth anaerobic bioreactors (Liao et al., 2006). In general, the membranes for the current commercial submerged MBR processes are MF and UF membranes in the form of flat sheet or vertical or horizontal hollow fibres.

The MBR technology has been increasingly used in municipal wastewater and industrial effluent treatment with high-quality discharge. For municipal wastewater treatment, MBR technology becomes attractive for many reasons. For example, more compact facility is required in urban areas due to the limited land areas, stringent effluent discharge standards as well as increasing demand for water re-use. Single operation process of MBR can solve all the issues mentioned above and therefore contributes to its wide application in municipal waste water treatment (Ben Aim and Semmens, 2003; Howell, 2004; Xing et al., 2000). MBR technology also has wide applications in purification of industrial wastewaters include mechanical newsprint mill whitewater (Ragona and Hall, 1998), paper mill effluent (Lopetegui and Sancho, 2003) synthetic wastewater (LaPara et al., 2001), kraft pulp mill foul condensates (Dias et al., 2005), high strength oily pet food wastewater (Kurian et al., 2005; Kurian et al., 2006), and landfill leachate (Visvanathan et al., 2007). By 2006, over 100 municipal MBR plants with a capacity larger than 500 person equivalent and around 300 industrial large-scale plants (> 20 m^3/d) were in operation in Europe (Lesjean and Huisjes, 2008). To date, thousands MBRs have been installed worldwide and some of them with a wastewater treatment capacity up to 100 MLD. Yang et al., (Yang et al., 2006) provides a detailed literature review of MBRs and theirs applications in industrial wastewater treatment.

2.1.1 Anaerobic Bioreactors

An anaerobic membrane bioreactor (AnMBR) is a biological treatment process operated without oxygen to degrade the complex organic matter to methane (CH_4) and carbon dioxide (CO_2) and separate solid from liquid using a membrane (Chan et al., 2009). Anaerobic processes have been successfully used to treat high strength industrial effluents (Chan et al., 2009), food processing wastewaters (Perez et al., 1997), agricultural wastewaters (Diamantis et al., 2003), sludges from municipal wastewater treatment plants (Kim et al., 2004) and animal manures (Masse et al., 2005). Compared to conventional aerobic processes, no oxygen is required for anaerobic processes so there is no need of energy to dissolve oxygen into water (Tanaka and Hvitved-Jacobsen, 1998). Methane produced from the anaerobic digestion can also be used to power the plant. Less sludge production, low nutritional requirements, and the ability to deal with high organic loads are the merits of the anaerobic process (Chan et al., 2009). Removal efficiencies ranging from 63.8% to 86.3% for COD, 65.5% to 87.0% for BOD and 79.2% to 89.1% for TSS were achieved for the treatment of palm oil mill effluent (POME) by anaerobic process (Chan et al., 2011). The COD, BOD and TSS removal efficiencies initially increase with MLSS and OLR up to a maximum level, and then decline with the further increase of either parameter (Chan et al., 2011). Proper selection of the MLSS concentration and OLR is important for efficient anaerobic process (Chan et al., 2011). Meanwhile, anaerobic process suffers from the slow growth rates of the methanogenic organisms and the microbial community of the systems. Thus, it usually requires longer time to start up (Stuckey, 2012). Commercial high-rate anaerobic reactors retain biomass either by the formation of granular sludge or by attachment to a fixed or mobile support material (Liao et al., 2006).

2.1.2 Aerobic Membrane Bioreactors

Aerobic membrane bioreactors (AMBRs) have gained considerable popularity in the past decade for the treatment of both high and low strength wastewater (Abeynayaka and Visvanathan, 2011). Compared to anaerobic systems, aerobic systems can achieve higher COD removal and better flocculated biomass, resulting in lower effluent suspended solids concentration. As a result, the effluent quality from an aerobic system is generally higher than those from anaerobic system (Chan et al., 2009). Combination of biological treatment and membrane makes it possible to eliminate both dissolved detrimental substances (COD, BOD_5, calcium) and solids. It has high efficiency to degrade the organic matters and is the dominant technology for treating municipal wastewater which is characterized by low organic strength (250 - 800 mg COD/L) and low suspended solids concentrations (120 - 400 mg/L). MBR technology has been widely applied in industrial effluent treatment at mesophilic or room temperatures (Bouhabila et al., 2001; Scott and Smith, 1997). For example, the submerged MBR treatment managed to remove 98.13% of anionic surfactant (AS) and 83.73% of chemical oxygen demand (COD) for cosmetic industry wastewater treatment (Friha et al.). Zhidong et al. (Zhidong et al., 2009) reported that achieved chemical oxygen demand (COD) and 5-day biochemical oxygen demand (BOD5) were around 92% for Oil refinery wastewater treatment by MBR technology. A detailed literature review on industrial wastewater treatment by MBRs process was conducted by Marrot et al. (Marrot et al., 2004). They stated that the MBR systems have good removal efficiency and a potential for water reuse in manufacturing industry.

Additionally, in many industrial processes, the wastewaters are produced at a high temperature and contain relative high concentrations of organic compounds. For economic consideration, treatment at thermophlic temperatures can reduce operation and capital cost. This is because it can eliminate the cooling facility prior to biological treatment (Chan et al., 2010) and allow the treated water to be reused in the processes without heating. Thermophilic aerobic treatment has made it a preferential treatment option for effluents from pulp and paper industry, food industry and landfill (Prado et al., 2007; Visvanathan et al., 2007).

2.2 Membrane fouling and control

2.2.1 Mechanisms of membrane fouling

Despite these advantages and potential, membrane fouling remains the most critical issues that hinder the wider application of MBRs (Le-Clech et al., 2006). Pore blocking/narrowing and cake layer formation usually accompany by a reduction of permeate flux or an increase in transmembrane pressure (TMP) which lead to added energy consumption and frequent membrane cleaning or replacement. Membrane fouling can directly result in the reduced productivity and increased operating costs (Gander et al., 2000). Hence, it is important to understand the mechanisms and characteristics of membrane fouling to develop effective anti-fouling strategies in MBR applications.

As shown in 2.2, fouling mechanisms can be described as adsorption, accumulation and deposition of undesirable particulate and colloids onto or into the membrane under complex physical and chemical interactions between various components of sludge suspension and the membrane surface. Lee et al. (Lee et al., 2001) reported that sludge cake layer resistance is the major contributor to the total resistance.

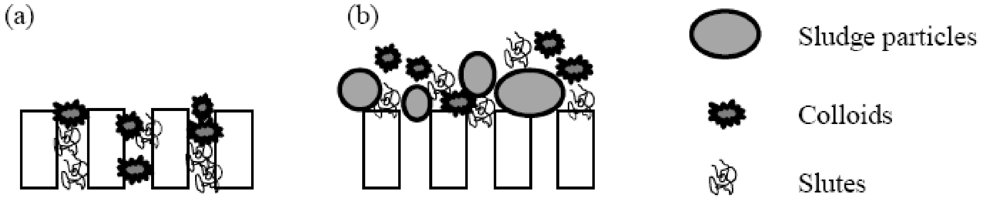


Fig. 2.2 Membrane fouling process in MBRs: (a) pore blocking; (b) cake layer

Membrane fouling can be classified as removable, irremovable or irreversible based on the removability of foulants. The removable fouling implies that the foulants can be eliminated by application of physical cleaning (e.g., backwashing) because it is loosely attached to the surface. Irremovable fouling refers to fouling needed to be removed by chemical approaches and it is caused by the pore blocking and strongly attached foulants. Fouling that cannot be eliminated by physical or chemical cleaning is defined as irreversible fouling (or permanent fouling) (Meng et al., 2009). When irreversible fouling occurs, the membrane should be conducted extensive chemical washing or be replaced.

Membrane fouling in MBRs also can be classified as internal or external fouling according to the molecular weight (MW) distributions (Zhu et al., 2011). It has been found that the external foulants have wider MW range than internal foulants. Foulants adsorbed into the membrane pores are considered as internal fouling and is irreversible. The external foulants refer to the foulants deposited on the membrane surface which are easier to remove (Lee et al., 2001; Ramesh et al., 2007).

Due to the fouling periods, membrane fouling can be divided into short-term and long-term fouling. Previous studies found that short-term and long-term fouling were formed due to distinct different fouling mechanisms(Geng and Hall, 2007). Ye et al. (Ye et al., 2005) noticed that the short-term fouling is caused by foulant deposition and pore narrowing and is mostly reversible. Long-term fouling including reversible and irreversible fouling results from the long contact time of deposited compounds into the membrane pores.

According to fouling components, membrane fouling also can be subdivided into three major categories: biofouling, organic and inorganic fouling (Liao et al., 2006). Biofouling is defined as the deposition, growth and metabolism of particulate materials or cell debris on the membranes, as well as undesirable accumulation of extracellular polymeric substances (EPS) and soluble microbial products (SMP) (Liao et al., 2006; Liao et al., 2004; Meng et al., 2009; Ramesh et al., 2007). Organic fouling refers to the accumulation and deposition of biomass aggregates (i.e. proteins and polysaccharides) on membranes. Soluble microbial products (SMP) are considered as the major component of organic fouling which are derived from the biological treatment processes due to decomposition of organic compounds (Drewes and Fox, 1999). Inorganic compounds also can cause fouling when chemical and biological precipitation occurs on the membrane. This is due to the high concentration of inorganic substances (i.e., calcium, magnesium, carbonate, sulfate, silica and iron), anions and ionisable groups of biopolymers (i.e., CO_3^{2-}, SO_4^{2-}, PO_4^{3-} , OH^-, COO^-) (Meng et al., 2009). All three fouling mechanisms are usually observed simultaneously. However, the membrane fouling is mainly governed by biofouling and organic fouling rather than inorganic fouling (Tang et al., 2010), and their relative contribution of each mechanism to membrane fouling depends on membrane characteristics, sludge characteristics, environmental conditions, reactor design, and the operating strategy (Liao et al., 2006).

2.2.2 Factors affecting membrane fouling

A number of studies have been conducted to illustrate the effect of various factors on membrane fouling. These factors can be classified into three groups (Le-Clech et al., 2006): membrane properties, biomass characteristics, and operating /environmental conditions.

2.2.2.1 Membrane properties

Membrane properties (such as membrane material, pore size, porosity, roughness, surface charge, hydrophilicity/hydrophobicity, and module structure, etc.) have direct impacts on membrane fouling. Membrane materials include ceramic (i.e., aluminum, zirconium, and titanium oxide etc.) and polymeric membranes (polyolefin, polyethylene (PE), polyvinylidene fluoride (PVDF) and polyvinyl chloride (PVC) Ceramic membranes offer advantages with higher fluxes since they have higher porosity and more hydrophilic surface compared to organic membranes. However, polymeric membranes are more commonly used due to its lower cost. Polyvinylidene fluoride (PVDF) membrane, for example, has been widely used in MBRs due to its high mechanical strength, high thermal stability, and high chemical resistance (Chae et al., 2009; Liu et al., 2011). Based on the pore size (or molecular weight cut-off (MWCO)), membranes can be classified into microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) membranes. As for the pore size of membrane, a narrow pore size is preferred to control the membrane fouling of the pore blocking in membrane filtration process. Stephenson et al. (Stephenson et al., 2000) pointed that the suitable membrane pore sizes used in wastewater treatments range from 0.02 to 0.5 µm. Several studies also have revealed the importance of hydrophobicity of membrane materials. Hydrophobic membranes are easily susceptible to fouling. However, in contrast to hydrophilic surfaces, fouling on hydrophobic membrane is often reversible (Le-Clech et al., 2006).

2.2.2.2 Biomass characteristics

The interactions between the MBR membrane and the biological suspension (biomass) contribute significantly to the fouling in the MBR. Biomass can be ideally fractionated into three components including suspended solids, colloids and solutes to account for their relative contribution of each biomass fraction on MBR fouling (Le-Clech et al., 2006). Soluble and colloidal materials are assumed to be responsible for the membrane pore blockage, while suspended solids account mainly for the cake layer formation (Jeison and van Lier, 2006). Various methodologies have been applied to appropriately separate the biomass fractions but no standard method exists. However, this approach neglects the synergistic effects occurring among the different components of the biomass. The sludge properties including floc size, mixed liquor suspended solids (MLSS) concentration, sludge hydrophobicity, surface charge, soluble and bound exopolymeric substances (EPS) can greatly influence the membrane fouling (Ng and Hermanowicz, 2005; Rosenberger and Kraume, 2002). It is well known that particle size distribution has great impact on membrane fouling (Meng and Yang, 2007; Van den Broeck et al., 2010). Smaller floc particles have higher tendency depositing on membrane which attribute to larger hydraulic resistance, and thus worsen the filtration performance (Altmann and Ripperger, 1997). Lim and Bai (Lim and Bai, 2003) also concluded that smaller particles tend to cause much severer fouling than larger particles since a preferential deposition of smaller particles on membranes and form a compact and dense cake layer.

Mixed liquor suspended solids (MLSS) concentration is a key operational parameter for membrane filtration performance. It has been reported that high MLSS level (eg., > 15 g/L) has a negative impact on membrane permeability (Rosenberger et al., 2005; Trussell et al., 2007). This can be theoretically explained as the increased MLSS lead to higher bulk viscosity which may weaken the hydrodynamic conditions to scour the solids away from the membrane, thus the fouling layers will build up faster on the membrane (Pritchard et al., 1995). However, Rosenberger et al. (Rosenberger et al., 2005) pointed out that the MLSS (8-12 g/L) exhibited little influence on membrane fouling. Hong et al. (Hong et al., 2002) also revealed that almost no effect of sludge on the transmembrane pressure is observed when MLSS concentration ranges from 3.6-8.4 g/L. Lee et al. [36] even claimed that an improvement of membrane permeability was obtained at higher MLSS concentration.

It has been acknowledged that bound extracellular polymeric substances (EPS) and soluble microbial products (SMP) are controlling factors on membrane fouling (Cho et al., 2005; Yamato et al., 2006). Bound EPS can be described as complex mixture of polymers consisting of polysaccharides, proteins, acid polysaccharides, lipids, DNA, and humic acid compounds which have been found at or outside the cell surface and in the intercellular space of microbial aggregates (Andreadakis, 1993; Liao et al., 2001). Bound EPS are further subdivided into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). Both of them have some influence on the characteristics of microorganism aggregates (Li and Yang, 2007; Yu et al., 2008). To date, a large number of peer-reviewed articles have been published regarding the correlations of EPS with membrane fouling (Nuengjamnong et al., 2005; Sweity et al., 2011; Tansel et al., 2006; Wang et al., 2009). Chang and Lee (Chang and Lee, 1998) observed higher EPS levels resulted in flux decline in the membrane-coupled activated sludge process. Cho et al. (Cho et al., 2005) showed experimentally that the increased amount of bound EPS would lead to higher specific cake resistance. Several studies, however, reported that no clear correlation between bound EPS and membrane fouling when bound EPS concentration was lower than 10 mg/g SS (Yamato et al., 2006). Rosenberger and Kraume (Rosenberger and Kraume, 2003) observed no impact of bound EPS on the filterability.

Soluble EPS, namely SMP, are biodegradable organic compounds excreted from substrate metabolism during biomass growth and decay (Barker and Stuckey, 1999). The main chemical components of SMP consist of proteins, polysaccharides, and organic colloids (Rosenberger et al., 2006). SMP can be classified into two categories (Laspidou and Rittmann, 2002): utilization associate products (UAP) which originated from substrate metabolism and biomass growth, and non-growth related biomass associated products (BAP) derived from cell lysis during biomass decay. It has been reported that SMP exhibit a broad range of molecular weight. The high molecular weight compounds play an important role in creating high resistance of the membrane leading to a reduction of permeate flux (Jarusutthirak and Amy, 2007). The soluble EPS is easily accumulated to the membrane surface to clog membrane pores or form the gel layer on membrane resulting in the poor filterability. Soluble microbial products (SMP) are found to be the majority soluble organic matter in biological wastewater treatment effluent. SMP accounting for the majority membrane fouling for microfiltration or ultrafiltration membranes in MBRs has been studied by (Bouhabila et al., 2001; Wisniewski and Grasmick, 1998). It also has been well recognized that the proteins are more hydrophobic and polysaccharide tends to be more hydrophilic. Thus, the polysaccharide (PS) fraction of the SMP contribute to higher fouling tendency (Judd, 2004; Rosenberger et al., 2006). A linear correlation between the concentration and composition of SMP and fouling propensity was presented (Rosenberger et al., 2006).

2.2.2.3 Operating and environmental conditions

The biomass properties and membrane performance can be greatly influenced by its operational and environmental parameters such as MLSS, OLR, dissolved oxygen (DO) concentrations, SRT, HRT, pH and temperature.

Solid retention time (SRT), for instance, can affect the biomass characteristics and contribute to changes in the microorganism physiological state. A positive impact on membrane performance by extended SRT is due to the low concentration of SMP and bound EPS concentrations (Cho et al., 2005; Han et al., 2005; Masse et al., 2006). An extended SRT is also conducive to biomass concentration and activated sludge bioflocculation and therefore lower fouling rates. While other investigators observed an opposite trend. Lee et al. [11] reported fouling resistance increase due to the prolonged SRT (20 to 40 and 60 days). Han et al. [12] also reported that the membrane foulants and sludge viscosity increased as SRT prolonged (30, 50, 70, and 100 d), which lead to intensive fouling.

HRT and organic loading rate (OLR) are main operating parameters affecting the biomass growth rate and membrane fouling rate. Dufresne et al. (Dufresne et al., 1996) found that shorter HRT or higher F/M provides more nutrients to the biomass result in an increased MLSS concentration of the bioreactor. Several investigators observed the shorter HRT or higher OLR results in increased fouling rates. Meng et al. (Meng et al., 2007) revealed that the concentration of extracellular polymeric substances (EPS) and sludge viscosity increased significantly by decreasing HRT. However, Visvanathan et al. (Visvanathan et al., 1997) concluded that an higher HRT helps reduce the membrane fouling rate.

Aeration used in aerobic MBR systems not only provides oxygen to the biomass and maintains the activated sludge dispersed but also minimize fouling by constant scouring of the membrane surface (Gander et al., 2000). Studies show that the membrane foulants grow faster at low level of dissolved oxygen since the preferential deposition of small particles on the membrane thus leading to reduced filterability of membrane (Jin et al., 2006). Ji et al. (Ji and Zhou, 2006) found that the bubbling rate directly governs the quantity and composition of the polymeric compounds (EPS) in the biological flocs and thus affects the ratio of protein/carbohydrate deposited on the membrane surface. Strong aeration intensity may also destroy the floc structure to smaller floc and release EPS into the bioreactor (Ji and Zhou, 2006; Park et al., 2005).

Substrate characteristics associated with nitrogen and phosphorus contents play an important role in membrane performance as they are closely correlated with the production of EPS and colloid formation. Nutrients (COD/N) are necessary elements for the growth of bacteria and they are also responsible for the production of biopolymers. Many attentions have been given on effect of nutrients (COD/N/P) on activated sludge properties like flocculation (Chao and Keinath, 1979), settleability (Forster, 1985), filterability (Wu et al., 1982), and dewatering (Pere et al., 1993). Others studied revealed that variation of carbon-to-nitrogen ratio (COD/N) and nutrient level of feed results in the changes of bound EPS level and composition. Liu and Fang (Liu and Fang, 2003) reported that biomass fed with low COD/ N ratio tends to produce EPS with a high proteins/carbohydrates. However, Miqueleto (Miqueleto et al., 2010) suggested that high COD/ N ratio favors EPS production in the ASBBR used for wastewater treatment. On the other hand, there are very limited studies on the effect of nutrients conditions on the performance and membrane fouling in MBRs.

2.3 Membrane foulants characterization

To better understand the characteristics of fouling, a series of methods has been established. Scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM) are common tools for physical characterization. SEM can provide high resolution images with nano/micrometer scale. It is commonly used to observe the development of biofouling and the formation of cake layer on the membrane (Chu and Li, 2005; Lee et al., 2001). However, the main limitation of SEM is that the specimens need to be pretreated such as dehydration and pre-coating, thus the fouling layer may be destroyed. Another useful tool is the AFM which could identify sludge cake morphology and roughness of the membrane surface (Lee et al., 2006). Among the various optical microscopic techniques, CSLM could distinguish and visualize the structural features and the spatial distribution of EPS, polysaccharides, proteins as well as bacterial cells (Chen et al., 2006; Jin et al., 2006; Lawrence et al., 2004). CLSM makes it possible to visualize and demonstrate bacterial cells and correlated substance accumulation at different depths of the membrane when combined with the fluorescent probes (Ferrando et al., 2005; Yun et al., 2006).

Additionally, chemical components of membrane foulants have been systematically examined by energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and three-dimensional excitation-emission matrix (EEM). Fluorescence spectroscopy FTIR spectrometer has been utilized to identify the functional groups of biopolymers adsorbed on membrane (Loh et al., 2009; Nataraj et al., 2008; Wang et al., 2009). EEM fluorescence spectroscopy is a sensitive technique and has been proven as the proper and effective approach to identify the EPS from various origins in wastewater treatment systems (Sheng and Yu, 2006). Structural and morphological changes on the membrane surface can be determined by X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) (Yu et al., 2005). Results from EDX and XRF showed that Mg, Al, Ca, Si, and Fe were the major inorganic elements in fouling cake.

2.4 Fouling Control Strategies

Operating conditions optimizations, activated carbon/coagulant/flocculent addition and membrane cleaning are common practices to minimize the fouling in MBRs. Process parameter optimization plays an important role for fouling mitigation in MBR systems. It includes the selection of optimal solid retention time (SRT), hydraulic retention time (HRT), organic loading rates (OLR), substrate type, temperature and intermittent suction intervals(Le-Clech et al., 2006). Among these parameters, SRT is probably the most important factor. It has been suggested that either too short SRT (Ng et al., 2006) or too long SRT (Han et al., 2005) has negative effect on membrane performance. (Meng et al., 2009) concluded that proper SRT for mitigating fouling should be controlled at 20- 50 days depending on HRT and feed water quality. Proper aeration also can be utilized to provide certain surface shear to prevent the particles deposition on membrane surface. The implement of air scouring with periodic backwashing are preferred for almost all aerobic MBRs (Chaize and Huyard, 1991; Choo and Stensel, 2000; Scott et al., 1998). Besides all the measures mentioned above, the management of flux might be a solution to control fouling. Critical flux is closely related to membrane filtration and fouling (Field et al., 1995). It was defined as operation below which the decline of permeability or the increase of trans-membrane pressure (TMP) does not occur. Adoption of sub-critical operation has been proven as an effective approach to avoid serious fouling and lengthen the operational period of MBR.

The use of additives, such as adsorbents or coagulants and other chemical agents, is expected to lower fouling propensity and improve flux. Powdered activated carbon (PAC) has been widely used as “flux enhancers” in MBRs. It can not only absorb soluble organics in the sludge suspension but also helps bioflocs to form strong biologically activated carbon (BAC) which improves flocculation ability and prevent particles accumulation on membranes (Hu and Stuckey, 2007; Ng et al., 2006). Coagulants including alum, chitosan, filter acids, polymeric aluminum chloride and polymeric ferric sulphate have been proven to have positive effects on membrane performance (Iversen et al., 2008; Ji et al., 2008; Song et al., 2008; Tian et al., 2008; Zhang et al., 2008) .

Fouled membrane also can be remedied through physical cleaning and chemical cleaning. In general, a physical cleaning is performed by relaxation and periodic backwashing with tap water which can successfully remove most of colloidal particles and loosely attached sludge cake from the membrane surface (Le-Clech et al., 2006). When physical cleaning method is not effective enough to recover the permeate flux to an acceptable level, chemical cleaning should be applied. Chemical cleaning has been extensively used for both restoring membrane permeability and reducing the frequency of recovery cleaning. In chemical cleaning, acids, bases, oxidants and surfactants are commonly used agents for MBR systems (Liao et al., 2004). For example, sodium hypochlorite (NaOCl) has been applied to remove microorganisms and organic-based compounds (Al-Amoudi and Lovitt, 2007). Citric acid is considered as an effective way to remove inorganic foulants. Biological fouling is normally treated by alkaline cleaning agent NaOH (Liao et al., 2006). In many cases, multi-step chemical cleaning are employed in order to get enhanced cleaning efficiency. Fouled membrane could be implemented by sodium hypochlorite (NaOCl) and followed by acid for further cleaning.

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Chapter 3 Nutrients requirements in aerobic membrane bioreactor

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Abstract:

Effect of chemical oxygen demand (COD) to nitrogen ratio (COD/N) in feed on the performance of aerobic membrane bioreactor (MBR) for a synthetic high strength industrial wastewater treatment was studied for over 370 days. The results showed that the thumb rule of nutrients ratio (COD:N=100:5) is not necessary for aerobically biological industrial wastewater treatment. An increased COD: N ratio from 100:5 to 100:2.5 and 100:1.8 had limited impact on COD removal efficiency and further led to a significant improvement in membrane performance, a reduced sludge yield, and improved effluent quality in terms of residual nutrients. An increased COD:N ratio will benefit the industrial wastewater treatment using membrane bioreactors by reducing membrane fouling and sludge yield, saving chemical costs, and reducing secondary hand pollution by nutrients. Optimization of nutrients usage should be conducted for specific industrial wastewater streams.

Key words: Nutrient requirements; COD/N; industrial wastewater treatment; sludge yield; membrane bioreactor; membrane fouling

3.1 Introduction

Activated sludge processes have been widely used for industrial wastewater treatment, including pulp and paper effluent, food processing wastewater, petrochemical wastewater treatment (Orhon et al., 2002). In these wastewaters, the streams are rich in organic carbons but short of other nutrients, like nitrogen and phosphorus (N, P), which are essential for bacteria growth. A balanced chemical oxygen demand to nutrients ratio (COD: N) is required for healthy growth of microbial communities for biodegradation (Henze et al., 1997; Tchobanoglous and Burton, 1991). Thus, the nutrients (N and P), which are costly, are generally added to the feed for biodegradation in industrial wastewater treatment. It is generally believed that a COD: N ratio of 100:5 is recommended for aerobic biological wastewater treatment, based on the rule of thumb (Tchobanoglous and Burton, 1991). In recent years, studies have been conducted to reduce nutrients cost by increasing the COD: N ratios. For example, (Ammary, 2004) found that a reduced COD: N ratio could be used for olive mill and pulp and paper mill effluent treatment and achieved a 75-80% COD removal. Furthermore, effects of nutrients conditions (COD:N) on conventional biological processes of municipal wastewater treatment were studied on sludge properties including settleability (Durmaz and Sanin, 2003), filterability (Wu et al., 1982), and flocculation and dewatering (Sanin et al., 2006). Moreover, other studies focused on the COD: N ratios on nitrification and denitrification processes as well as nutrients removal efficiency in municipal wastewater treatment (Hwang et al., 2009; McAdam and Judd, 2007; Meng et al., 2008). It is generally believed that unfavorable nutrients conditions will cause biomass separation problems, like sludge bulking and foaming (Jenkins et al., 2004). Therefore, the application of a reduced COD: N ratio is limited by the COD removal efficiency and biomass separation problems in conventional activated sludge processes for industrial wastewater treatment.

On the other hand, membrane bioreactor (MBR) is a relatively new technology which combines activated sludge treatment with a membrane filtration process and thus completely eliminates biomass separation problems with superior quality of treated effluent. Compared to the conventional activated sludge process, MBR offers several advantages like smaller footprint, superior effluent quality as well as low space requirement (Brindle and Stephenson, 1996; Marrot et al., 2004; Rosenberger and Kraume, 2003; Visvanathan et al., 2000). The MBR technology appears to be a promising technology for nutrients management in industrial wastewater treatment, as it can overcome the problems of a reduced COD: N or COD: P ratio encountered in conventional activated sludge processes, and reduce chemical costs and secondary pollution by nutrients addition. However, the nutrients conditions (COD: N) of the influent, one of the crucial parameters of microbial growth, can greatly affect the biological and membrane performance of MBR. Until now, there are only limited studies reported the nitrogen removal rate under various COD:N ratios in municipal wastewater treatments using MBRs (Feng et al., 2012; Fu et al., 2009). The potential of MBR for industrial wastewater treatment under reduced nutrients addition has not been explored.

Therefore, further studies are necessary for a better understanding the influence of reduced nutrients levels on the biological and membrane performance of MBRs in industrial wastewater treatment. The objective of this study was to investigate the feasibility of reduced nutrient (N) usage for high strength industrial wastewater treatment and the relationship between the COD: N ratios of the feed and the biological and membrane performance of aerobic membrane bioreactor.

3.2 Methods and materials

3.2.1 Experimental set-up

Fig. 3.1 shows the schematic diagram of the MBR experimental system. A 6 L (effective volume) submerged MBR equipped with a flat sheet microfiltration membrane module (0.03 m^2) was used for a synthetic high strength industrial wastewater treatment in this study. The membranes (SINAP Membrane Science & Technology Co. Ltd., Shanghai, China) were made of polyvinylidene fluoride (PVDF) materials with a pore size of 0.3 μm and a molecular weight cut off (MWCO) of 70,000 Da. The immersed air bubble diffusers (one coarse bubble 1.9 LPM and one fine bubble diffuser 1.3 LPM on each side of the membrane module) were installed under the membrane module to provide oxygen as well as air souring to limit fouling. Dissolved oxygen (DO) level was maintained at larger than 2 ppm. A magnetic stirrer (Thermolyne Cimarec, Model S47030) was located at the bottom of the reactor for mixing. The temperature of the bioreactor was maintained constant at a mesophilic temperature of 35 ± 1 °C by means of water jacket. The pH was monitored by a pH electrode (Thermo Scientific, Beverly, MA), and automatically adjusted to 7.0 ± 0.2 by a pH regulation pump using 0.25M NaOH solution.

The system was continuously fed on a synthetic high strength wastewater containing glucose, stored at 4 °C. The feed was pumped automatically by a peristaltic pump (Masterflex Model 7520-50, Barnant Co., USA), which was controlled by a liquid level sensor (Madison Co., USA) as well as a controller (Flowline, USA). The effluent was obtained by means of a suction pump connected to the membrane modules. The operational cycles were applied with 4 min suction followed by 1 min relaxation. The trans-membrane pressure (TMP) was monitored by a pressure gauge which connected between the membranes and suction pump. An instant membrane flux of 10 $L/m^2 \cdot h$ was applied in this study.

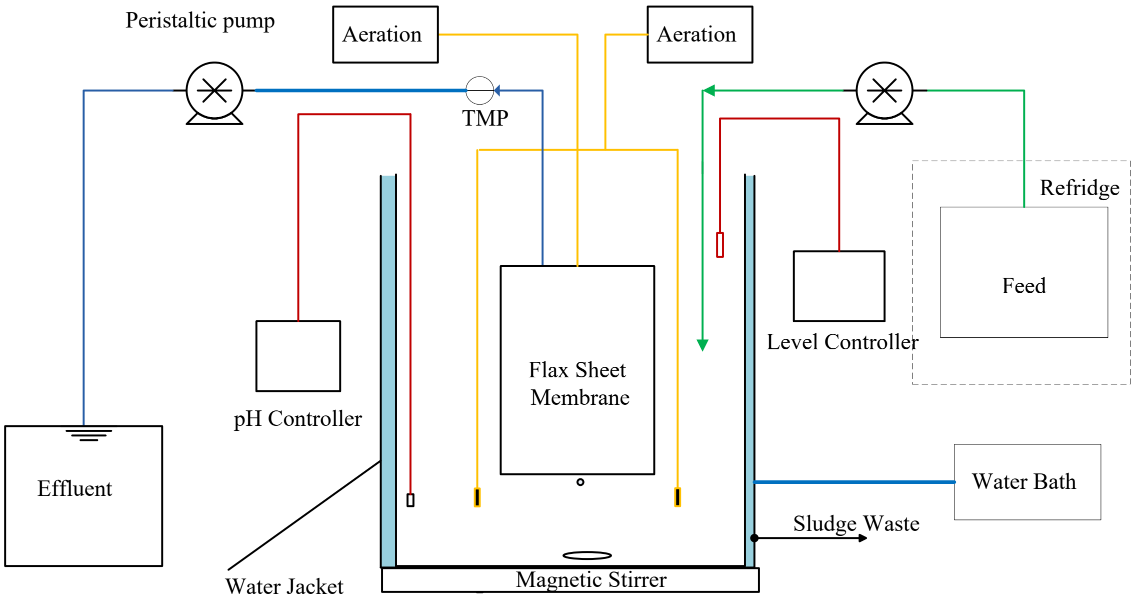

Fig. 3.1 Schematic of the submerged aerobic membrane bioreactor set-up

3.2.2 Synthetic wastewater and operating conditions

The synthetic influent was composed of the following components: glucose ($C_6H_{12}O_6$), nitrogen (NH_4Cl) and phosphorus (KH_2PO4) as major nutrients. In addition, trace nutrients derived from $MgSO_4 \cdot 7H_2O$, 5.07 mg/l; $FeSO_4 \cdot 7H_2O$, 2.49 mg/l; $Na_2MoO_4 \cdot 2H_2O$, 1.26 mg/l; $MnSO_4 \cdot 4H_2O$, 0.31 mg/l; $CuSO_4$, 0.25 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.44 mg/l; NaCl, 0.25 mg/l; $CoCl_2 \cdot 6H_2O$, 0.43 mg/l; $CoCl_2 \cdot 6H_2O$, 0.41 mg/l were used in the system. The influent COD concentration was approximately 2500 mg/L. This synthetic industrial wastewater showed the similarity with the food industrial wastewater (Lin et al., 2012).

During the operation of the bioreactor, a volume of 400 mL of sludge was wasted daily to maintain a solids retention time (SRT) of 15 days. Reactor was started at an HRT of approximately 1day and an organic loading rate (OLR) of 2.5 g COD L^{-1} day^{-1}. The operation of the reactor system included three phases: Phase 1 (1 – 106 day) is characterized as a nutrients ratio (COD:N:P) of 100:5:1 period; Phase 2 (107 –240 day) is the nutrients ratio of 100:2.5:1 period; Phase 3 (241- 370 day) is the nutrients ratio of 100:1.8:1 period.

3.2.3 Analytical Methods

The influent synthetic industrial wastewater, permeate and mixed liquor were sampled periodically from the system. The COD and mixed liquor suspended solids (MLSS) were analyzed according to Standard Methods (APHA, 2005). Supernatant COD was determined after centrifuging the mixed liquor for 20 min at 18700 x *g*. Permeate ammonia cation was measured by ion chromatography using a Dionex 120 equipped with a CG16 guard column and CS16 analytical column. Nitrate and nitrite anions were also examined by ion chromatography using the Dionex 120 equipped with an AG14 guard column and AS14 analytical column. The particle size distribution (PSD) of the mixed liquor was determined by using a Malvern Mastersizer 2000 instrument (Malvern Instruments Ltd., Worcestershire, UK) with a detection range of 0.02 – 2000 μm. Scattered light is detected by a detector converting the signal to a size distribution based on volume.

3.2.4 Statistical analysis

Statistical analysis was conducted by using the Statistical Package for the Social Science (SPSS) 16.0. An analysis of variance (ANOVA) was employed to identify whether there is significant difference between treatment means when studying permeate and supernatant COD, COD removal efficiency, MLSS, and sludge yield under different nutrients conditions. The difference was considered statistically significant at a 95% confidence interval ($p<0.05$).

3.3 Results and Discussion

3.3.1 COD removal efficiency under different COD: N ratios

Fig. 3.2 shows the feed, supernatant and effluent COD values during the whole operation period. The influent COD loading was maintained at approximately 2500 mg COD/L, while the influent COD: N ratio (100:5; 100:2.5; 100:1.8) was shifted by changing nitrogen loading rate supplied to the system. The steady-state permeate COD concentration was 10.6 ± 5.9, 16.5 ± 12.7, 39.6 ± 18.2 mg/L (ANOVA, $p<0.05$), which corresponded to a removal efficiency of 99.6 ± 0.3%, 99.3 ± 0.5% and 98.4 ± 0.7% (ANOVA, $P<0.05$), at the different COD:N ratio of 100:5, 100:2.5, and 100:1.8, respectively. Overall, the total COD removal efficiency of the aerobic MBR process could be kept over 98.4% regardless of nitrogen loading rate. An upset stage (day 117-129) occurred, this was probably due to the nutrients transition (the COD:N ratio shifted from 100:5 to 100:2.5) and broken air sparging with oxygen stress. A low DO concentration could cause the supernatant COD increase (Jin et al., 2006; Kang et al., 2003). Although the concentration of supernatant COD appears greater than permeate COD, the permeate COD stabilized at low levels. This is largely due to the membrane was responsible for complete retention of all particulate COD and macromolecular COD components. It was remarkable that most of the COD was removed by biological degradation and the MBR system can achieve high quality of effluent. These findings indicate that the COD removal efficiency of the MBR was not obviously affected by the significantly reduced nitrogen level. Thus, saving of chemical cost is feasible by reducing nutrients dosage in industrial wastewater treatment in MBRs.

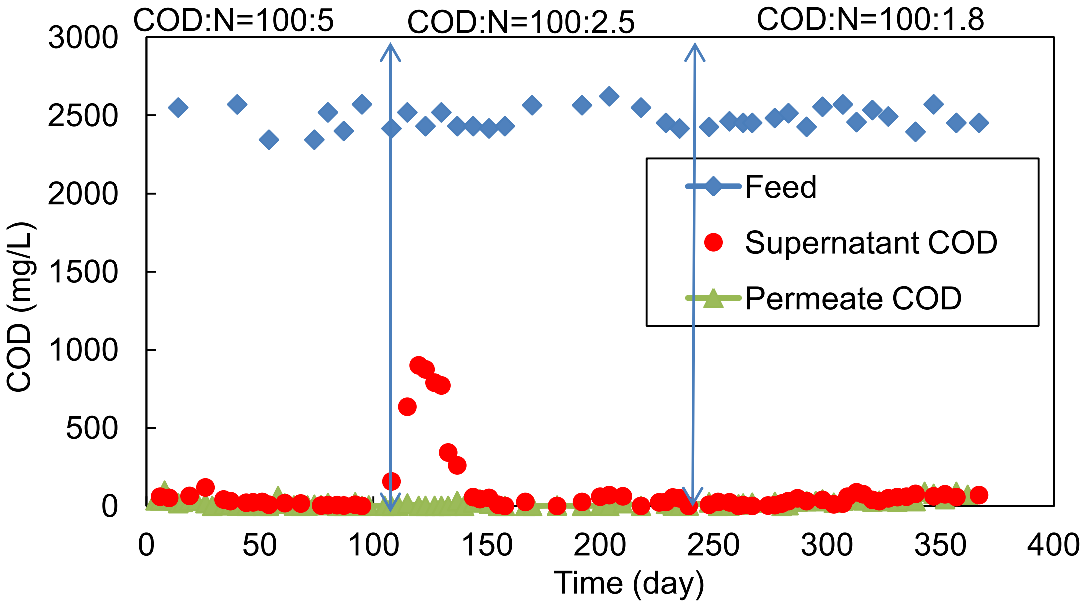

Fig. 3.2 Variations of the influent, supernatant and permeate COD with experimental time

3.3.2 Sludge yields and fate of influent COD

Fig. 3.3 shows the variations in sludge yield under different COD: N ratios. A sludge yield of 0.26 ± 0.03, 0.20 ± 0.01 and 0.18 ± 0.01 mg sludge/mg COD removal, which corresponded to a state-state mixed liquor suspended solids (MLSS) concentration of 8631 ± 376, 7268 ± 289 and 6363 ± 181 mg/L, was found under a COD:N ratio of 100:5, 100:2.5, 100:1.8, respectively. There were significant difference among the three sludge yield values (ANOVA, $P<0.05$). An increase in the COD: N ratio led to a significant decrease in sludge yield. A 31% reduction in sludge production was achieved when the COD:N ratio was changed from 100:5 to 100:1.8. The sludge yield values are lower than the sludge yield (0.4 g MLSS/ g COD) of conventional activated sludge process (Tchobanoglous et al., 2003) but similar to that from other studies (Ammary, 2004; Langevin and Liao, 2010). The results suggest that the nitrogen loading had significant effect on biomass growth and the biomass growth rate exhibited pronounced decrease when cutting the nitrogen dosage to the COD: N ratio of 100:1.8 (ANOVA, $p < 0.05$). It accounts for a 31% reduction in sludge yield comparing with stage one (100:5). In general, the sludge yield for stage two and three were found to be lower than stage one and this finding was consistence with previous studies conducted by (Miqueleto et al., 2010).

After the biological degradation of influent COD, the fate of influent COD includes three paths: 1.) converted into new biomass (sludge production); 2.) converted into CO_2; and 3.) left as residual COD. The distribution of the COD in the three paths is showed in Fig 3.4, based on COD mass balance calculations. Clearly, 25.7 - 33.1% of the influent COD was converted into biomass, and 66.6 - 72.7% of the influent was converted into CO_2; and the residual COD in final effluents only accounts for 0.4 - 1.7% of the influent COD. Furthermore, the COD: N ratio had a significant impact on the distribution of the influent COD. Much less influent COD was converted into biomass when the influent COD: N ratio was increased from 100:5 to 100:1.8 (ANOVA, $P<0.05$). Slightly increase in residual COD (from 10.6 ± 5.9 to 39.6 ± 18.2 mg/L) was observed when the COD: N ratio was increased from 100:5 to 100:1.8.

Excess sludge reduction was one of the two important problems (sludge reduction and nutrients removal) associated with biological wastewater treatment plants (Banu et al., 2009). By increasing COD:N ratio, it is possible to achieve a significant reduction in sludge production while maintaining excellent COD removal. This result suggests that operation of activated sludge process at a COD:N ratio of 100:5 is not necessary for industrial wastewater treatment. The optimal doses of N and P to maintain an excellent COD removal and to reduce sludge production should be experimentally determined for specific type of industrial wastewater, in addition to saving chemical costs and reducing secondary hand pollution by nutrients addition.

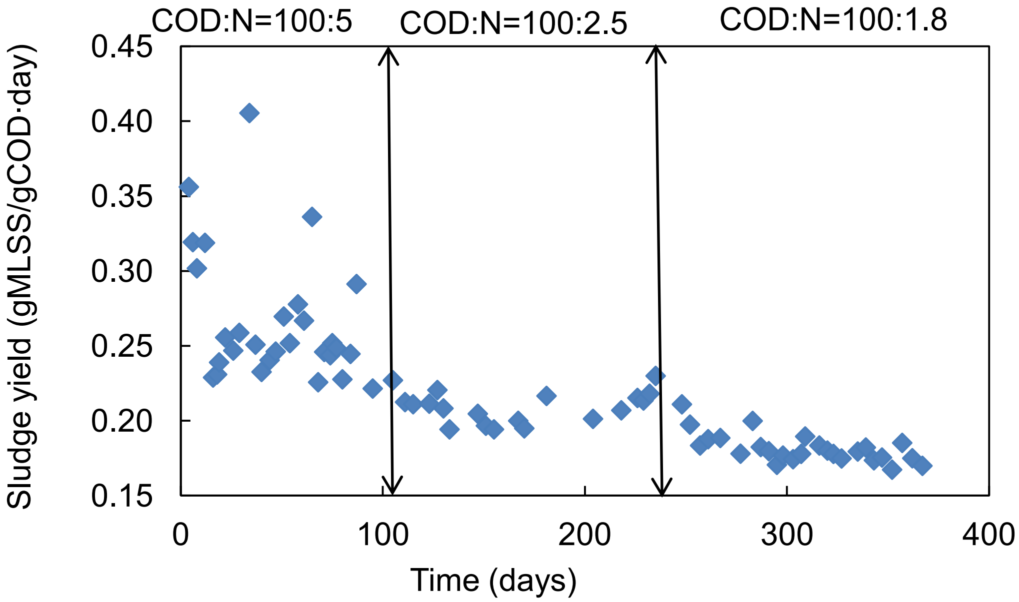

Fig. 3.3 Variation of sludge yield under different COD: N conditions

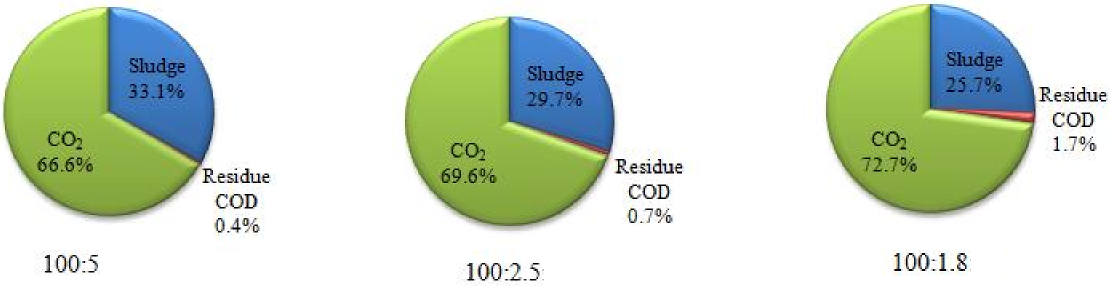

Fig. 3.4 The distribution of the COD in the three paths under three COD: N conditions

3.3.3 Residual N

Table 3.1 summarizes the total inorganic nitrogen, in terms of ammonia, nitrate and nitrite, concentration in treated effluent under different COD: N ratios. The results suggest significant difference in residual nitrogen in the treated effluents (ANOVA, $P<0.05$). There were some residual ammonia, nitrate and nitrite left in treated effluent under a COD:N ratio of 100:5 and 100:2.5, while all the added nitrogen was taken up by microorganisms with residual nitrogen concentrations below detection limitations in treated effluent at a COD:N of 100:1.8. In most cases, the ammonia, nitrate and nitrite concentrations are regulated at a concentration less than 1 mg/L for each type of nitrogen in pulp and paper effluent treatment (Marshall, 2008). Thus, the residual ammonia concentration (1.3-2.2 mg/L) was over target under a COD:N ratio of 100:5 and 100:2.5. Therefore, a COD:N of 100:1.8 should be applied to achieve residual nitrogen target.

The challenge for nutrient management in industrial wastewater treatment is to achieve a sufficient degree of effluent treatment (COD removal) while at the same time minimizing nutrient residuals. If nutrient levels are too low, COD removal efficiency drops resulting in higher effluent total suspended solids (TSS) and BOD in conventional activated sludge processes. However, this can also result in higher nitrogen and phosphorus residuals because of the nitrogen and phosphorus contained in the sludge being lost to the final effluent, due to biomass separation problems associated with nutrient deficiency. On the other hand, the use of MBR technology completely eliminate biomass separation problems associated with low nutrients levels and achieve zero solids in final effluent. Thus, the nutrients levels can go as low as possible, as long as the COD removal efficiency is acceptable.

Table 3.1 Effluent concentration of residue N at different COD:N ratio

	Nutrients condition of COD:N ratio		
	100:5	100:2.5	100:1.8
Effluent NH_4^+(mg/L)	2.176±0.267	1.256±0.125	< 0.010
Effluent NO_3^-(mg/L)	0.292±0.093	0.185±0.092	< 0.009
Effluent NO_2^-(mg/L)	0.574±0.132	< 0.009	< 0.009

*Sample mean ± standard deviation, number of measurements: n=4 for each test.

3.3.4 Membrane performance

Although MBR has the advantages of completely eliminating biomass separation problems associated with nutrients deficiency and achieve zero solids in final effluent, one of the major challenges in MBRs is membrane fouling, which is an obstacle for wide applications of MBRs. Therefore, it is interesting to know how nutrients loading affect membrane performance and membrane fouling. Fig. 3.5 shows changes in trans-membrane pressure (TMP) under different COD:N ratios. An increase in TMP indicates membrane fouling. The fouling profiles of two stages of TMP changes show the similar behavior under different COD:N ratios: a relatively stable and low of TMP for the first stage and followed by a rapid increase in TMP, also known as the TMP jump in second stage. This finding is consistent with the findings of previous studies in which two stages of TMP changes were observed (Brookes et al., 2006; Hwang et al., 2008). The sudden jump in TMP was probably caused by the local flux effect as well as the sudden changes to the biofilm or cake layer structure (Zhang et al., 2006). The trans-membrane pressure (TMP) observed in the aerobic MBR exhibited a more rapid fouling at COD:N = 100: 5, but the fouling rate declined (e. g. a longer operation time before rapid TMP jump) with decreasing nitrogen loading rate at COD: N = 100: 2.5 and 100: 1.8. The rate of increase in TMP is an important factor that reflecting the membrane permeability in a submerged MBR system since it is correlated to the extent of membrane fouling. An acclimation stage (day 1-14), the system was in start-up stage and the TMP was unstable. During 115-129 days was upset stage, excessive fouling occurred due to the high concentration of supernatant. Physical cleaning was applied everyday by using tap water and sponge during this period of time. All these findings indicate that the reduced nitrogen dosage significantly improved membrane filterability. The improvement in membrane performance at a reduced nitrogen dose could be explained by the increase in floc sizes of bulk sludge (Fig. 3.6). It is well known that smaller floc sizes have a higher tendency to attach to membrane surfaces and cause membrane fouling (Meng et al., 2006).

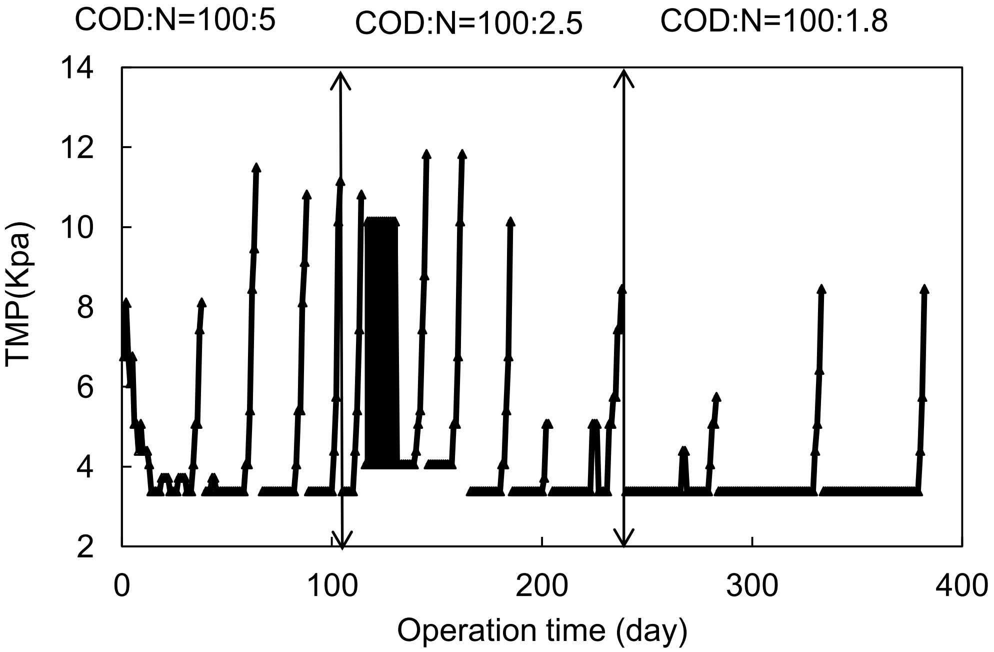

Fig. 3.5 TMP profile under different COD: N ratios

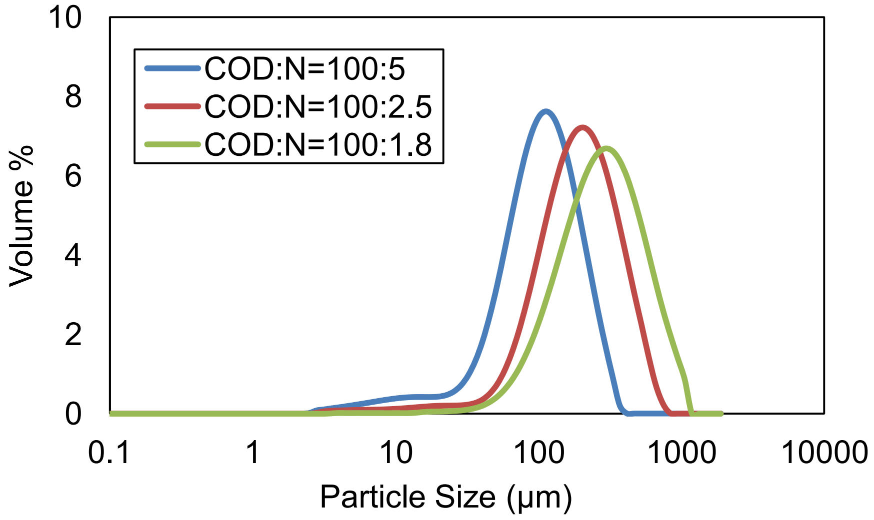

Fig. 3.6 Particle size distribution of bulk sludge under different COD: N ratios

3.3.5 Economic analysis

Assuming the industrial wastewater treatment plant with a capacity of 2000 m^3/d and the COD level of the wastewater around 2000 mg COD/L, the yearly required addition amount of NH_4Cl is about 278.4 ton at a COD: N of 100:5. When the COD: N ratio is increased to 100:1.8, the required NH_4Cl amount is reduced to approximately 100.23 tons/year. Assuming a price of NH_4Cl is \$3000/ton, the saving for NH_4Cl cost is approximately \$533,310/year. It is obvious that the decreased nitrogen loading rate has significant economic benefit in the industrial wastewater treatment plant.

3.4 Conclusions

Effects of COD:N ratios on the performance of aerobic MBR for high strength industrial wastewater treatment were studied for over 370 days. The conclusions are summarized as below.

* An increase in COD: N ratio from 100:5 to 100:1.8 led to only slightly decrease in the COD removal efficiency from 99.6% to 98.4%.

* An increase in COD: N ratio from 100:5 to 100:1.8 resulted in a significant decrease in sludge yield (31% reduction in sludge production)

* An increase in COD: N ratio from 100:5 to 100:1.8 significantly improved membrane performance and the length of operation time, before membrane fouling occurs, was significantly increased.

* An increase in COD: N ratio from 100:5 to 100:1.8 significantly improved effluent quality in terms of residual nitrogen. No residual nitrogen (NH_4^+, NO_3^-, NO_2^-) was detected in effluent under COD: N ratio of 100:1.8.

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Chapter 4 Influence of nutrient conditions on surface properties of sludge and their role in membrane fouling of aerobic membrane bioreactor

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Abstract

Effect of nutrients condition (COD:N ratios) on sludge properties and their role in membrane fouling was studied using a well-controlled aerobic membrane bioreactor receiving a synthetic high strength industrial wastewater containing glucose. Membrane performance was improved with an increase in the COD:N ratio (e.g. reduced N dosage). Surface analysis of sludge by X-ray photoelectron spectroscopy (XPS) suggests that significant differences in surface concentrations of elements C, O and N were observed under different COD:N ratios, implying significant differences in extracellular polymeric substances (EPS) composition. A unique characteristic peak at 1735 cm $^{-1}$ was observed under nitrogen limitation conditions by using Fourier transform-infrared spectroscopy (FTIR). Total EPS, proteins and the ratio of proteins to carbohydrates in EPS decreased with an increase in COD:N ratio, while carbohydrates in EPS increased with an increase in COD:N ratio. There were no significant differences in the total soluble microbial products (SMPs) but the ratio of proteins to carbohydrates in SMPs decreased with an increase in COD:N ratios. Sludge cake formation was the dominant mechanism of membrane fouling.

Key words: Nutrient conditions; membrane bioreactor; membrane fouling; industrial wastewater; extracellular polymeric substances; surface properties

4.1 Introduction

Membrane technology integrated biological degradation has been successfully applied in the municipal and industrial wastewater treatment to provide a direct solid-liquid separation by membrane filtration. Compared to the conventional activated sludge process, MBR offers several advantages like smaller footprint, superior effluent quality as well as low space requirement (Brindle and Stephenson, 1996; Marrot et al., 2004; Rosenberger and Kraume, 2003; Visvanathan et al., 2000). However, membrane fouling which leads to poor membrane performance and high operational costs (Kraume and Drews, 2010), is still a major challenge that limits the further development and widespread application of MBRs. In general, membrane fouling is caused by many factors, such as influent characteristics, solid retention time (SRT), hydraulic retention time (HRT), organic loading rate (ORL), and dissolved oxygen level etc. Among these parameters, the influent characteristics like COD:N ratios remain one of the most important factors. This is largely because COD:N ratio can influence the physiological properties of microorganisms and chemical compositions of biomass in MBRs. The COD:N ratio also can influence the amount of extracellular polymeric substances (EPS) and the composition of protein and carbohydrate in EPS which can affect membrane performance.

Many attentions have been given to the effects of nutrients conditions (COD:N) in conventional biological processes on sludge properties including settleability (Durmaz and Sanin, 2003), filterability (Wu et al., 1982), and flocculation and dewatering (Sanin et al., 2006). Other studies focused on the COD: N ratios on nitrification and denitrification processes as well as nutrients removal efficiency in municipal wastewater treatment (Hwang et al., 2009; McAdam and Judd, 2007; Meng et al., 2008). Moreover, some studies also focused on the effect of COD:N ratio on the changes of EPS and microbial community structure of the bioreactor. Durmaz and

Sanin (Durmaz and Sanin, 2001) found that EPS increased with the increase of COD:N ratio. Miqueleto (Miqueleto et al., 2010) suggested that high COD:N ratio favored EPS production in the ASBBR used for wastewater treatment.

A careful review of the literature finds that the limited studies on effect of COD:N ratio were conducted with conventional activated sludge processes and focused on N removal in municipal wastewater treatment. There were only few reports on the effect of COD:N ratio on biomass properties and membrane fouling in submerged membrane bioreactors and, more importantly, focused on the range of COD:N ratios for excess N removal in municipal wastewater treatment. On the other hand, a number of industrial wastewaters are short of nutrients (N and P) and thus nutrients have to be added to promote microorganisms growth and biodegradation (Henze et al., 1997; Tchobanoglous and Burton, 1991). Finding the optimal nutrients doses or reduced nutrients doses not only improve process efficiency but also saves chemical cost and reduces second-hand pollution of treated effluent with added nutrients and thus has significant industrial importance. The objective of this research was to explore the effect of nutrients ratio (COD:N) on sludge properties and their role in membrane fouling of MBRs for high strength industrial wastewater treatment. In this study, an aerobic MBR was fed at different COD:N ratios (100:5,100:2.5, and 100:1.8). The membrane filtration resistance, surface concentrations of elements (C, N, and O) on sludge surfaces, extracellular polymeric substances (EPS) and soluble microbial products (SMP) were monitored. XPS and Fourier transform infrared (FTIR) spectroscopy were employed to characterize the sludge samples.

4.2 Materials and method

4.2.1 Experimental set-up and operating conditions

The laboratory-scale submerged MBR system was comprised of a 6 L working volume with flat sheet microfiltration membranes (SINAP Membrane Science & Technology Co. Ltd., Shanghai, China). The flat sheet membrane were made of polyvinylidene fluoride (PVDF) materials with a pore size of 0.3 μm and a molecular weight cut off (MWCO) of 70,000 Da. The immersed coarse air bubble diffusers (3.8 L/min (LPM)) were installed under the membrane module in order to provide oxygen for biodegradation as well as air scouring to limit membrane fouling. Finer air aeration (2.6 LPM) was also used to maintain satisfying dissolved oxygen (DO) level large than 2 mg/L during the aerobic period. A magnetic stirrer (Thermolyne Cimarec, Model S47030) was located at the bottom of the reactor to help sludge mixing. The temperature of the bioreactor was maintained constant at mesophilic temperature of 35 ± 1^{o}C by means of water jacket. The pH was monitored by a pH electrode (Thermo Scientific, Beverly, MA), and automatically adjusted to 7.0 ± 0.2 by a pH regulation pump using 0.25 M NaOH solution.

The system was continuously fed with a synthetic high strength industrial wastewater containing glucose, stored at 4 ◦C. The feed was pumped automatically by a peristaltic pump (Masterflex Model 7520-50, Barnant Co., USA), which was controlled by a liquid level sensor (Madison Co., USA) as well as a controller (Flowline, USA). The effluent was obtained by means of a suction pump connected to the membrane module. The operational cycles were applied with 4 min suction followed by 1 min relaxation. The trans-membrane pressure (TMP) was monitored by a pressure gauge connected between the membrane module and the suction pump. An instant membrane flux of 10 $L/m^2 \cdot h$ was applied in this study.

The major composition of influent includes glucose, nitrogen (NH_4Cl) and phosphorus (KH_2PO_4) in a proportion of chemical oxygen demand (COD): N: P= 100:5:1, 100:2.5:1 and 100:1.8:1 and other trace metals were summarized as shown in Table 4.1. During the operation of the bioreactor, a volume of 400 mL of sludge was discharged daily from the MBR for bulk sludge characterization and maintaining the SRT at 15 days. Reactor was started at an HRT of approximately 1 day and an organic loading rate (OLR) of 2.5 g COD L^{-1} day^{-1}. The operation of the reactor system included three phases. Phase 1 (0 - 107day), the MBR was fed at a COD: N: P ratio of 100:5:1. In phase 2 (108 - 240 day), the reactor was fed with COD: N: P ratio of 100:2.5:1; In Phase 3 (240- 380 day) the MBR was operated at nutrients ratio (COD: N: P) of 100:1.8:1.

Table 4.1 Composition and concentration of micronutrients in the feed

Components	Concentration (mg/L)
$MgSO_4 \cdot 7H_2O$ (mg/L)	5.07
$FeSO_4 \cdot 7H_2O$(mg/L)	2.49
$Na_2MoO_4 \cdot 2H_2O$ (mg/L)	1.26
$MnSO_4 \cdot 4H_2O$ (mg/L)	0.31
$CuSO_4$ (mg/L)	0.25
$ZnSO_4 \cdot 7H_2O$(mg/L)	0.44
NaCl(mg/L)	0.25
$CaSO_4 \cdot 2H_2O$ (mg/L)	0.43
$CoCl_2 \cdot 6H_2O$ (mg/L)	0.41

4.2.2 Analytical methods

4.2.2.1 Water quality measurement

The influent synthetic industrial wastewater, permeate and mixed liquor were sampled periodically from the system. The COD and mixed liquor suspended solids (MLSS) were analyzed according to Standard Methods (APHA, 2005) (APHA, 2005). Supernatant COD was determined after centrifuging the mixed liquor for 20 min at 18700 × g.

4.2.2.2 Extracellular polymeric substances (EPS) extraction and measurement

The bound EPS from sludge suspensions samples was extracted according to cations exchange resin (CER) (Dowex Marathon C, Na^+ form, Sigma-Aldrich, Bellefonte, PA) method (Frolund et al., 1996). 100 mL sample of the sludge suspension was taken and centrifuged (IEC MultiRF, Thermo IEC, Needham Heights, MA, USA) at 18,700×g for 20 min at 4°C. The sludge pellets were re-suspended to their original volume using a buffer consisting of 2mM Na_3PO4, 4mM NaH_2PO_4, 9mM NaCl and 1mM KCl at pH 7. The sludge was then transferred to an extraction beaker filled with buffer and the CER (80 g/g-MLSS). The extraction was lasted for 2 hours at 4°C. The EPS was determined as the sum of proteins and carbohydrates and was measured colorimetrically by the methods of Lowery et al. (Lowery et al., 1951) and DuBois et al. (DuBois et al., 1956), respectively. The total bound EPS was represented by adding the concentrations of bound polysaccharides and proteins.

4.2.2.3 Soluble microbial products (SMP) measurement

The extracted supernatant by centrifugation was filtered by 0.45 µm membrane filters (Millipore) for SMP analysis. The proteins and carbohydrates of SMP were measured using the methods of Lowery et al. (Lowery et al., 1951) and DuBois et al. (DuBois et al., 1956), respectively. The total SMP was the sum of the concentrations of soluble carbohydrates and soluble proteins.

4.2.2.4 Surface composition of sludge by X-ray photoelectron spectroscopy (XPS)

XPS method was used to examine the surface concentrations of elements, such as C, O, and N, etc. The wet sludge samples were freeze-dried at −35 °C for one week. The freeze-dried sludge samples were ground to powders before being analyzed by ThermoFisher Scientific K-Alpha XPS Spectrometer equipped with monochromatic AlKα X-ray source with a spot source of 400μm. Charge compensation was also provided. The position of the energy scale was adjusted to place the main C 1s feature (C−C) at 285.0 eV except for those samples where the C−O peak was more dominant. A survey spectrum was taken at low resolution (PE −150 eV). High resolution spectra were taken of C1s regions (PE−25 eV). All data processing was performed using the software (Advantage) provided with the instrument.

4.2.2.5 Molecular composition of sludge by Fourier transform-infrared spectroscopy (FTIR)

A Bruker Ten 37 FTIR Spectrometer (Bruker Co., Ltd.) was employed to determine the major functional groups of organic matters and to predict the chemical composition of the bulk sludge. The FTIR analysis was performed using absorbance mode. The sludge samples were freeze-dried at −35 °C for one week before FTIR measurement.

4.2.2.6 Membrane Resistance

The extent of fouling rate can be evaluated by the derivative of the filtration resistance:

$$R_t = \frac{\Delta P}{J \times \eta_T} = R_m + R_f + R_c$$

$$\eta_T = \eta_T^{20^{\circ C}\cdot e^{-0.0239(T-20)}}$$

Where R_t is the total filtration resistance (m^{-1}), J represents the permeate flux (m^3/m^2h), ΔP is the trans-membrane pressure difference (Pa), and η_T is the permeate dynamic viscosity (Pa·s). T is the permeate temperature in ^{o}C. R_t was calculated with the temperature corrected to 20 °C to compensate for the dependence of viscosity on temperature. The experimental procedure to determine each resistance value is as follows: R_t is total membrane resistance (m^{-1}) and calculated from the filtration data at the end of operation. R_m is intrinsic membrane resistance and evaluated by the water flux of tap water. Rc is fouling layer resistance (m^{-1}). R_f is fouling resistance due to irreversible adsorption and pore blocking (m^{-1}). When fouling occurred, membrane surfaces was conducted by wiping away the fouling layer with a sponge and tap water, and then the membrane was submerged in tap water for flux and TMP measurement. New membranes were used in each membrane operation cycle, in order to maintain the same membrane conditions for comparison and reproducibility of results in repeated membrane operation cycles at each nutrient (COD:N) ratio.

4.2.3 Statistical Analysis

Statistical analysis was conducted by using the Statistical Package for the Social Science (SPSS) 16.0. An analysis of variance (ANOVA) was employed to identify whether there is significant difference between treatment means when evaluating membrane fouling and EPS concentration under different COD:N ratios. The difference was considered statistically significant at a 95% confidence interval ($p < 0.05$). The student t-test also was applied to analyze the content of surface chemical composition of bulk sludge. The paired p values were calculated for the differences between COD: N ratios of 100:5 and 100:2.5; 100:2.5 and 100:1.8; and 100:5 and 100:1.8. Data sets were considered statistically different at a 95% confidence interval ($p < 0.05$).

4.3 Results and discussion

The MBR was operated at three different COD: N ratios from 100:5 to 100:2.5 and to 100:1.8. The COD removal efficiency of the MBR was only slightly affected at a reduced N usage (98.4% to 99.5%) (Hao and Liao, 2014). Furthermore, the sludge yield was reduced and effluent quality was improved in terms of nutrients residual (Hao and Liao, 2014).The results suggest that it is feasible to operate the aerobic MBR for high strength industrial wastewater treatment at a reduced nutrients addition and achieve excellent biological performance (Hao and Liao, 2014). In this manuscript, effect of nutrients conditions (COD:N) on sludge properties and their role in membrane fouling will be presented and discussed.

4.3.1 Membrane performance

The increase in filtration resistance in MBRs is an important indicator of membrane fouling since it directly reflects the membrane permeability. Fig. 4.1 shows the changes of filtration resistance under different COD: N ratios. A relatively stable and low filtration resistance for the first stage and followed by a sudden increase in filtration resistance in the second stage. The sudden increase in filtration resistance can lead to failure in membrane performance. Aimed to recover the membrane performance, physical cleaning or chemical cleaning would be employed at the end of the filtration cycles. As shown in Fig. 4.1, a more rapid membrane fouling rate (shorter operation time before filtration resistance jump) was observed at COD: N = 100: 5, and the membrane fouling rate gradually declined (longer operation time before filtration resistance jump) with an increase in COD:N ratio (100: 2.5 and 100: 1.8). During acclimation stage (day 1-14), the TMP was unstable. Excessive membrane fouling occurred at days 115-129 (transition and upset stage) due to the high concentration of supernatant. Physical cleaning with water and sponge was applied to recover membrane performance. The filtration resistance data obtained from the current investigation suggests that the reduced nitrogen dosage improved membrane performance.

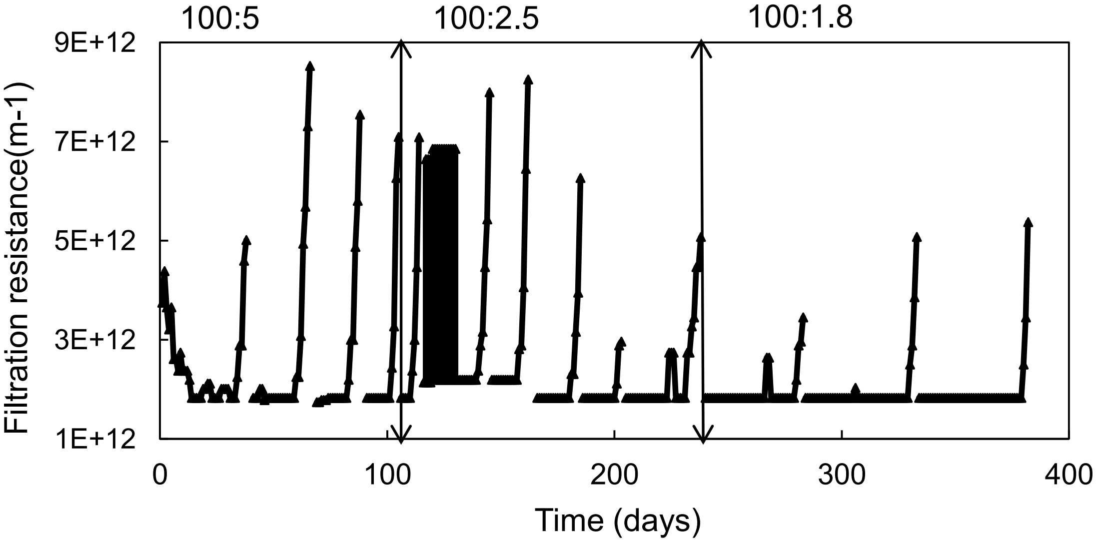


Fig. 4.1 Membrane filtration resistance over time respect to different COD: N ratios.

4.3.2 Fouling resistance

Membrane filtration resistances were evaluated at the end of an operation cycle. The filtration resistances distributions under different COD: N ratios are listed in Table 4.2. It was noted that the total fouling resistance (R_t) decreased with the declining of nitrogen level from

COD: N of 100:5, 100:2.5, 100:1.8, respectively. The value of $R_c/(R_c + R_f)$ (100%) indicates the cake layer formation was the dominant mechanism of membrane fouling in the MBRs. More importantly, R_c under each condition was equivalent to almost 100% of the total fouling resistance (R_t), suggesting no irreversible membrane fouling by adsorption and/or pore blocking. The variation of fouling resistance directly shows the degree of membrane fouling. Therefore, over one year operation period, the greater fouling resistance was observed at nutrition condition of COD: N of 100:5.

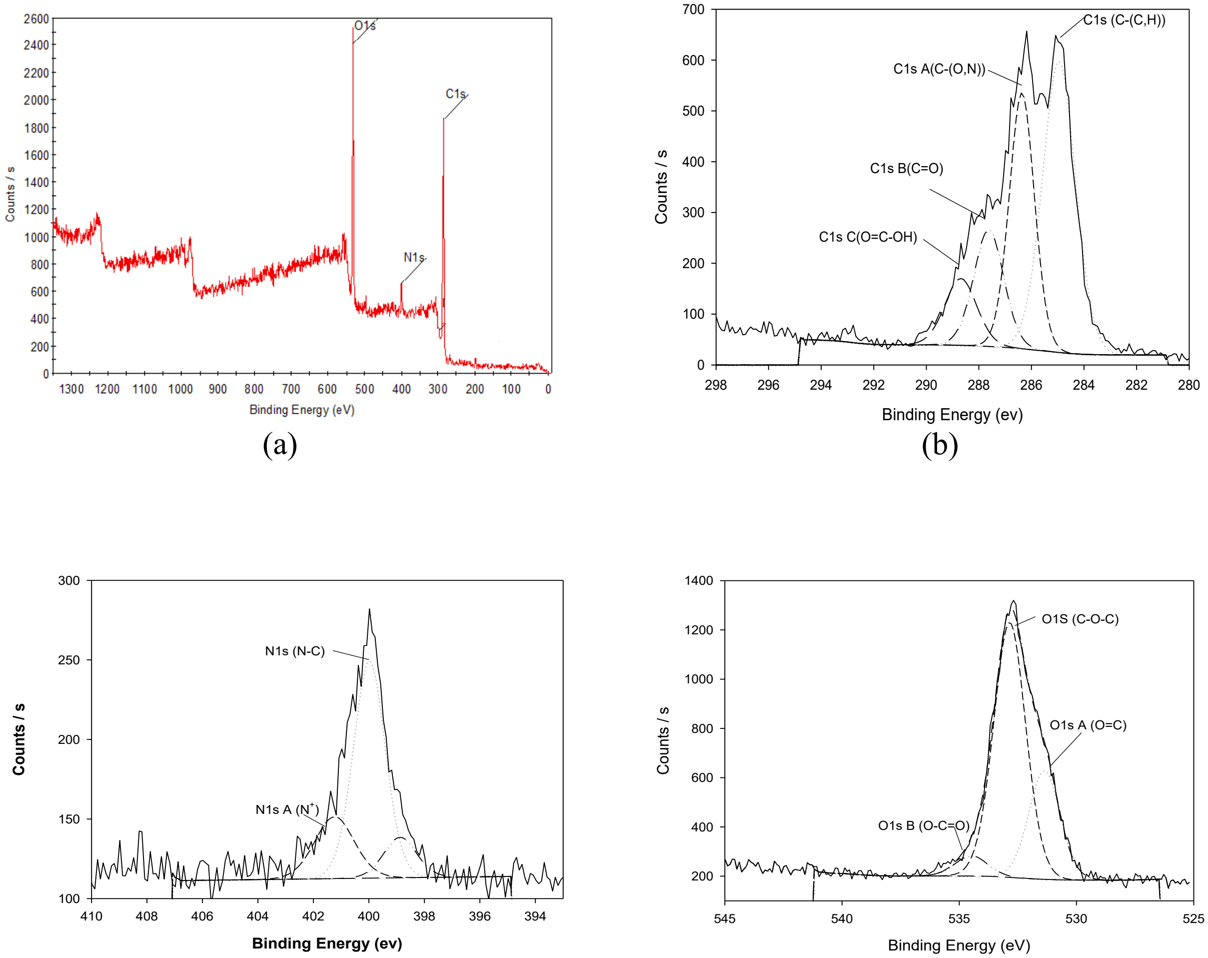
Table 4.2 A series of resistances at the end of the study point of membrane filtration.

Nutrients COD:N	Resistances				Cake resistance ratio (%)
	R_m	R_c	R_f	R_t	$R_c/(R_c+R_f)$
100:5	$(1.82 \pm 0.07)\times10^{12}$	$(6.32\pm 0.10)\times10^{12}$	0	$(8.15 \pm 0.08)\times10^{12}$	100
100:2.5	$(1.80 \pm 0.02)\times10^{12}$	$(4.53 \pm 0.01)\times10^{12}$	0	$(6.33\pm 0.01)\times10^{12}$	100
100:1.8	$(1.81\pm 0.07)\times10^{12}$	$(2.71 \pm 0.18)\times10^{12}$	0	$(4.52\pm 0.11)\times10^{12}$	100
* Sample average ± relative error, number of measurements: n = 2 for each COD:N conditions					

4.3.4 Surface composition of sludge measured by XPS

The surface chemical composition of bulk sludge at different COD: N ratios were analysed by XPS. XPS has been used to study the surface functional groups of materials, including bacteria, and each peak corresponds to electrons with a characteristic binding energy from a particular element (Badireddy et al., 2008; Dengis and Rouxhet, 1996; Dufrene et al., 1997). As shown in Fig. 4.2, the C peaks (C1s, C1sA, C1sB, C1sC) were decomposed into four different bonds: C bound only to C and H, C−(C,H) at a binding energy of 284.8 eV; C singly bound to O or N, C−(O, N), including ether, alcohol, amine, and amide, at a binding energy of 286.3 eV; C bound to O making two single bonds or one double bond, C−O or O−C−O, including amide, carbonyl, carboxylate, ester, acetal, and hemiacetal, at a binding energy of 288.0 eV. The O peaks (O1s, O1sA, and O1sB) could be attributed into three bonds: O−C bond, including hydroxide (C−OH), acetal, and hemiacetal (C−O−C), at a binding energy of 532.7 eV, and O−C in carboxylic acid, carboxylate, ester, carbonyl and amide at a binding energy of 531.4 eV. The N peaks (N1s and N1sA) were attributed to the two different bonds: N−C bond in amide or amine at a binding energy of 400.12 eV and N−H bonds in ammonia or protonated amine at a binding energy of 402.10 eV.

Surface concentrations of element C, N and O on sludge surfaces under different nutrients conditions (COD: N) were summarized in Table 4.3. Although there was no significant difference in total C (Student t-test, $P>0.05$), significant differences in the quantity of C-(C, H), C-(N, N) and C=O were observed between COD:N of 100:5 and 100:2.5, 100:5 and 100:1.8, 100:2.5 and 100:1.8 (95% confidence level, Student t-test). Furthermore, the total O on sludge surface increased with an increase in the COD:N ratio (reduced N dosage) (Student t-test, $P<0.05$). Moreover, the general tendency of total N on sludge surface decreased with an increase in the COD:N ratio, although no statistically significance was observed (ANOVA, $P>0.05$). But student t-test suggested significance in total N on sludge surface existed between the COD: N ratio of 100:5 and 100:1.8 ($P<0.05$). This can be attribute to the decreasing nitrogen level (increasing COD:N ratio) in the system. The XPS results strongly suggest that significant difference in EPS composition on sludge surface under different COD: N ratios. This may not be surprising that microorganisms responding to the stress of nutrient (N) deficient will produce different biopolymers (Omoike and Chorover, 2004)(Omoike and Chorover, 2004).



(a) (b)

(c) (d)

Fig. 4.2 XPS spectra of mesophilic sludge, (a) whole spectra, (b) C1s spectra, (c) N1s spectra, and (d) O1s spectra.

It is well known that the bound EPS consist of proteins, polysaccharides, nucleic acids, lipids, humic acids, etc, which are located at or outside the cell surface. Proteins in the activated sludge EPS are the primary source for the elemental nitrogen which is represented as N. Earlier XPS studies (Badireddy et al., 2008; Dengis and Rouxhet, 1996; Dufrene et al., 1997) have reported that the C-(C, H) bonds might originate from lipids or from amino acid side chains. Polysaccharides contain hydroxide and acetal or hemiacetal building blocks. The higher content of C-(C, H), and N and lower concentration of O in COD: N of 100:5 might correlate to a higher surface concentration of lipids and proteins and a lower surface concentration of carbohydrates in the sludge. This is supported with the EPS measurement results as discussed in later section.

Table 4.3 Surface composition of the sludge under different COD:N ratios which determined by XPS: average atom fraction (%)

	COD:N ratio			Significant difference[a] of COD:N ratio		
Element component	100:5	100:2.5	100:1.8	100:5 and 100:2.5	100:2.5 and 100:1.8	100:5 and 100:1.8
Total C	64.89± 1.49	64.41± 0.85	62.99± 1.42	N(0.328)	N(0.100)	N(0.090)
C-(C,H)	33.09± 1.82	28.51± 0.49	26.03± 1.52	Y(0.006)	Y(0.027)	Y(0.003)
C-(O,N)	19.35± 1.54	24.74± 0.59	25.15± 0.73	Y(0.002)	N(0.113)	Y(0.001)
C=O	9.03±0.80	6.28± 0.61	7.33± 0.53	Y(0.005)	Y(0.044)	Y(0.019)
O=C-OH	3.41±0.90	4.88± 0.96	4.09± 0.30	N(0.063)	N(0.125)	N(0.141)
Total O	24.66±0.31	26.46±0.98	29.42±0.88	Y(0.039)	Y(0.017)	Y(0.000)
Total N	7.00±1.59	6.29±1.09	4.53±1.34	N(0.289)	N(0.067)	Y(0.044)

a sig. value shown in parentheses. Sample number n=3 for each conditions.

4.3.4 EPS production and components

In order to study the role of EPS in membrane fouling, the content of proteins and carbohydrates of EPS were measured. The bound EPS contents at each COD: N ratios are shown in Fig. 4.3. Statistical analysis using ANOVA also confirmed that differences in total EPS, proteins (PN), carbohydrates (CH) were all statistically significant (ANOVA, $p < 0.05$) at different COD:N ratios studied. The total EPS were 35.5(± 3.08), 19.12 (± 2.04) and 15.21 (±

1.22) mg/g MLSS for COD: N ratios of 100:5, 100:2.5 and 100:1.8, respectively. At the lowest COD: N ratio of 100:5, the microorganisms are more likely to produce more EPS with the highest PN/CH ratio. This observation is consistent with previous findings (Durmaz and Sanin, 2001; Liu and Fang, 2003). After reducing the nitrogen content in the feed (increased COD: N ratio), apparently, the proteins contents presented a rapid decrease. This is mainly because the nitrogen in the system is utilized for synthesis of proteins and insufficient nitrogen is supplied for protein synthesis when COD: N ratio increased. At the lowest COD: N ratio of 100:5, the majority of carbon is used in biomass synthesis instead of carbohydrate production. However, with an increase in the COD: N ratio, the carbohydrate concentration in the EPS increased. These results are parallel to the results by Durmaz et al. (Durmaz and Sanin, 2001).

Another important result observed from Fig. 4.3. is the change in proteins to carbohydrates (PN/CH) ratio in EPS as the COD:N ratio changes. The PN/CH values (ANOVA, $p < 0.05$) decreased with an increase in the COD: N ratios. These results are consistent with the results of XPS as discussed above in that significant difference in EPS composition and concentration exist under different COD:N ratios. In this study, PN/CH ratio shows great positive correlation to cake resistance. Higher PN/CH ratio in bound EPS favors more cake layer formation thus corresponding with greater filtration resistance. This observation is in consistent with previous studies (Lin et al., 2011). It is also believed that total amount of EPS is well correlated to the membrane fouling as the increase in EPS content could deteriorate membrane fouling (Wang et al., 2009). Furthermore, Lee et al. (Lee et al., 2001) suggested that proteins are more hydrophobic and have more tendencies to adhere on membrane surface and inducing membrane fouling. Current investigation suggested that the decrease of the nitrogen content can reduce the production of EPS and protein content and may be the reason of membrane fouling mitigation.

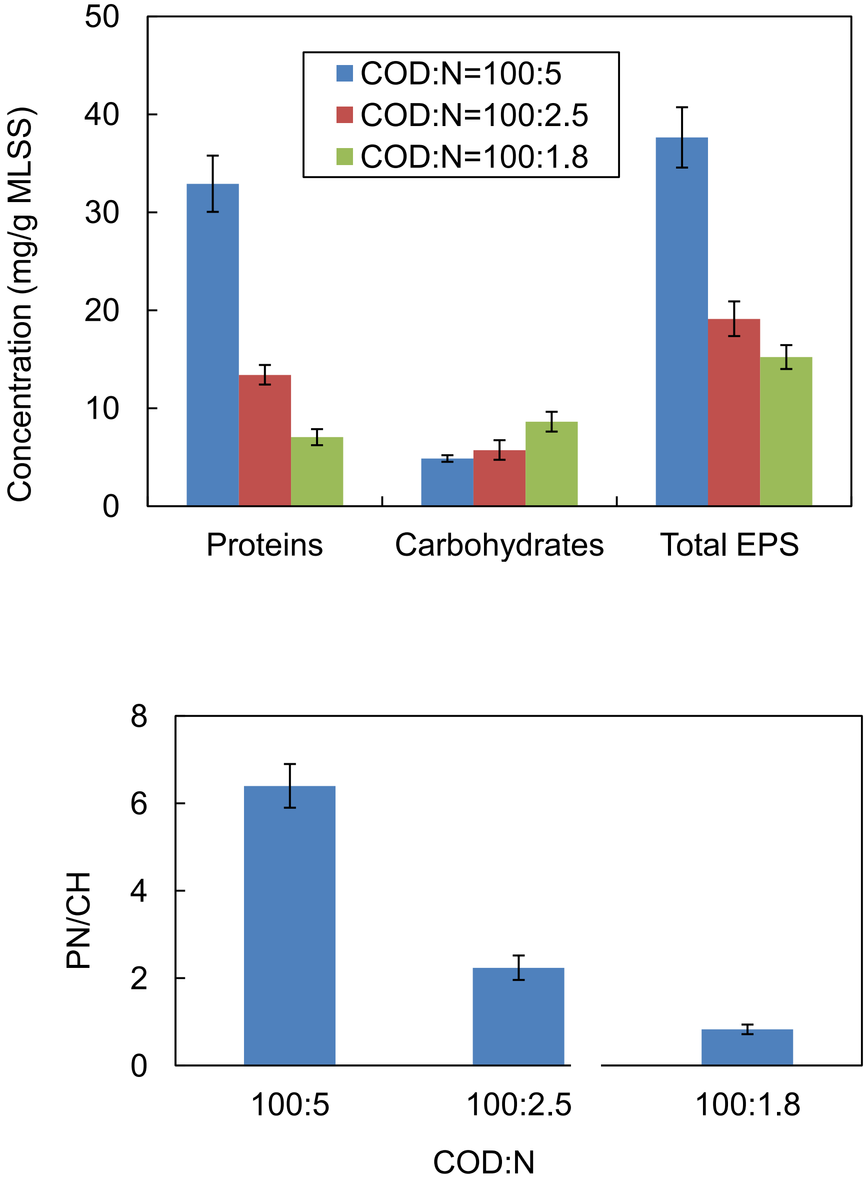


Fig. 4.3 Comparison of bound EPS of bulk sludge at different COD: N ratios (ANOVA, $p<0.05$, number of measurements: n=5 for each condition).

4.3.5 SMP

The total SMP and soluble proteins and carbohydrates concentrations at three COD: N ratios are shown in Fig. 4.4. The amount of SMP (ANOVA, $p < 0.05$) reached to a peak of 32.6 (± 3.10) mg/L at the COD: N ratio of 100:5. After decreasing nitrogen content in the feed, the SMP production presented a decrease trend for other two conditions. Obviously, the protein content decreased with a decrease in the nitrogen content in feed, while the biomass produced similar carbohydrate level in SMPs at COD: N of 100:5 and 100:2.5. At the COD: N ratio of 100:1.8, the carbohydrate concentration in SMPs was almost twice of carbohydrates concentrations produced at the other two COD: N ratios. The PN/CH values (ANOVA, $p < 0.05$) showed the similar trend as shown in EPS in that PN/CH ratio decreased with an increase in the COD:N ratio. Carbohydrate were found as the predominant fraction and accounted for more than half of the SMP concentration at COD: N of 100:1.8.

The total SMP content could not explain the improvement of membrane performance at an increase in the COD: N ratio but an increase in the PN/CH ratio is positively correlated to the membrane fouling rate (e. g. a decrease in the length of membrane operation before TMP jump). This may suggest that it is the composition and PN/CH ratio but not the total SMP content that controls membrane fouling rate. It was reported that proteins have a hydrophobic nature and thus have a higher affinity to membrane surface, as compared to carbohydrates (Meng et al., 2006).

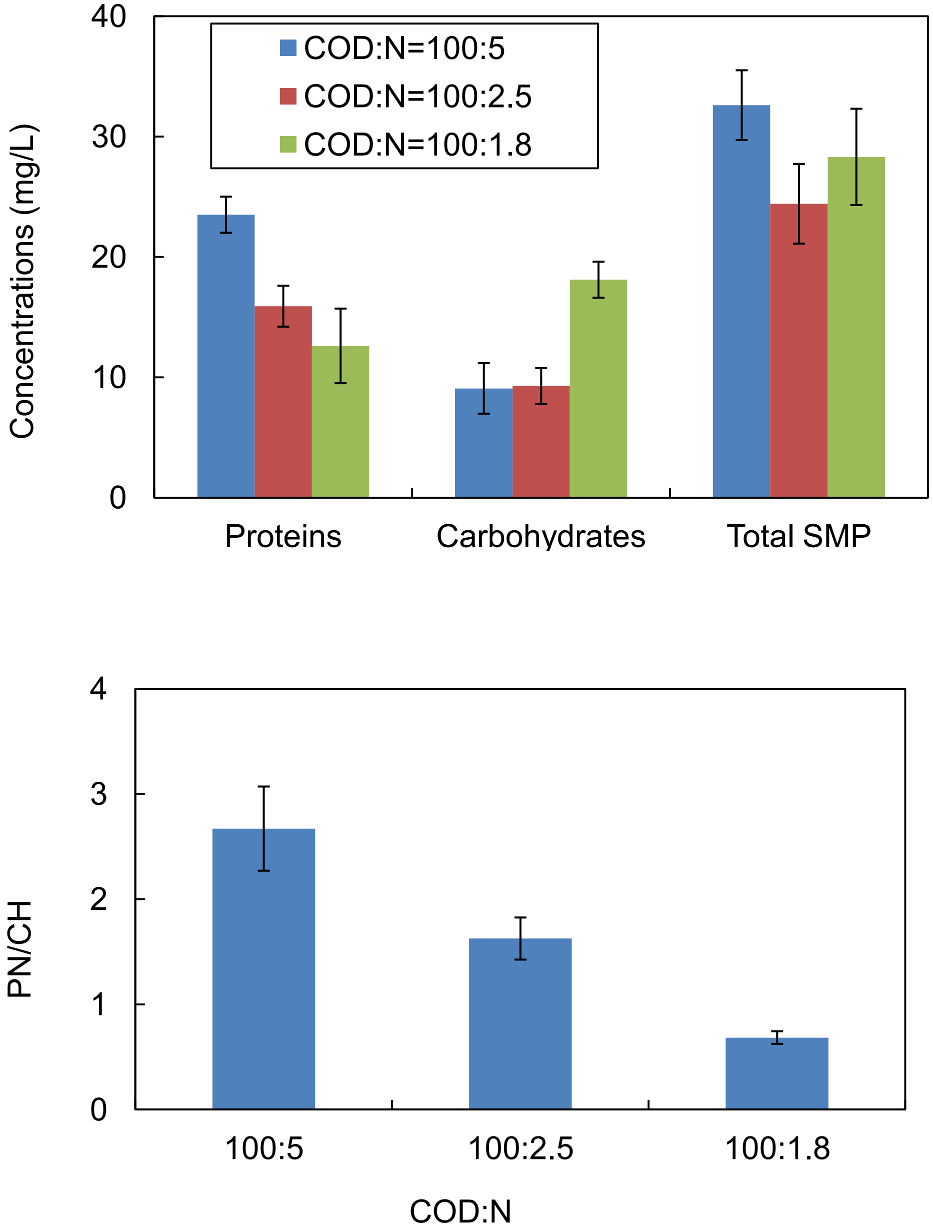


Fig. 4.4 SMP concentrations under different COD: N ratios (ANOVA, $p<0.05$, number of measurements: n=5 for each condition).

4.3.6 FTIR analysis

The FT-IR spectra under different nutrients conditions are shown in Fig. 4.5. All three samples shown peaks at 3300 cm^{-1} (overlapping of bands from the stretching vibrations of N–H and O–H) (Kumar et al., 2006). Peaks, at wave numbers of 2926, 2847 and 1445 cm^{-1}, are reflecting C-H bonds in the alkenes class (Kim and Jang, 2006). The doublets at 2370 and 2343 cm^{-1} attribute to carbon dioxide from atmosphere adsorbed on the sample surface. The asymmetrical stretching peak observed at 1735 cm^{-1} was representing humic acids (Jarusutthirak et al., 2002), which associated with nitrogen shortage of COD:N=100:2.5 and COD:N=100:1.8. This results indicated that the large amount of fat can be produced when biomass grown under the stress of nutrients limitation (Hung et al., 1996). Additionally, the relative intensity of the peak at 1735 cm^{-1} was stronger under COD:N=100:1.8 than COD:N=100:2.5. A stronger intensity of the unique characteristic peak at 1735 cm^{-1} correlated to a better membrane performance (e. g. a lower membrane fouling rate or a longer operation time bore TMP jump). The peaks located at 1660 (stretching vibration of C=O and C-N amide I) and 1540 (N-H deformation and C=N stretching amide II) and 1245 cm^{-1} are important as they indicate the presence of proteins (Croue et al., 2003). The peaks of 1384 and 1244 cm−1 suggest the presence of amide III (Jun et al., 2007). A peak near 1100 cm^{-1} (due to C-O stretching) was due to the functional group of carbohydrate (Croue et al., 2003). According to the FTIR spectra, it can be concluded that the sludge under the nitrogen limitation conditions can generate great amount of humic acids. The characteristic peak at 1735 cm^{-1} under nitrogen limitations (COD: N of 100:2.5 and 100:1.8) may be used as an indicator of nutrients conditions in feed. FTIR may be used as a tool to indirectly monitor the nutrients condition in the MBRs.

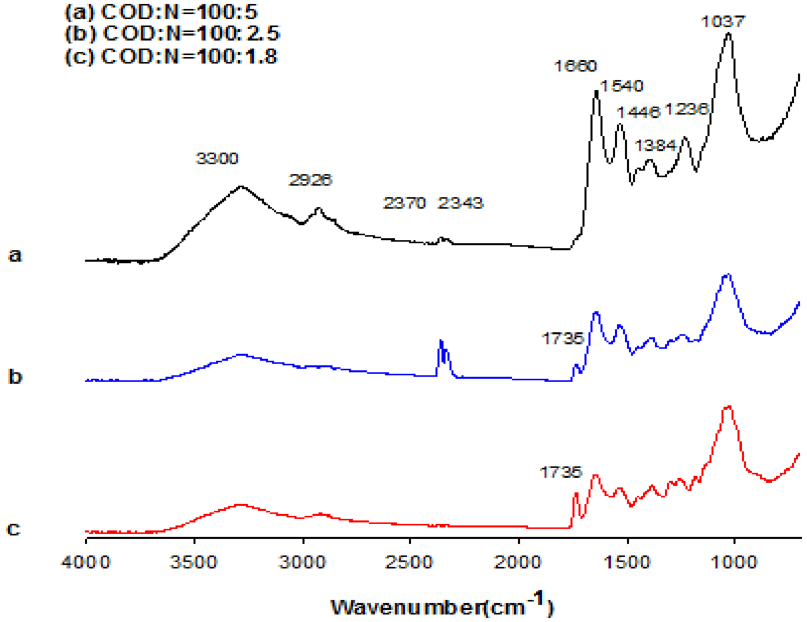


Fig. 4.5 FTIR spectrum of bulk sludge under different COD: N ratios.

4.4 Conclusions

Effects of nutrients conditions on sludge properties and their role in membrane fouling were studied using well-controlled aerobic MBR fed with a synthetic high strength industrial wastewater containing glucose. The main conclusions are summarized below.

* An increase in COD/N ratio from 100:5 to 100:1.8 led to an improved membrane performance and the longer operation time of membrane before membrane cleaning.

* The XPS results demonstrated significant differences in surface concentrations of elements C, O and N under different COD/ N ratios, which suggest significant difference in EPS composition.

* A decrease in total EPS content and PN/CH ratio in EPS was observed with an increase in COD: N ratio. Statistical analyses shows the total bound EPS and PN/CH ratio positively

correlated to membrane fouling rates, indicating the importance of EPS content and composition in governing membrane fouling.

* A decrease in PN/CH ratio in SMP was observed with an increase in COD/N ratio but no general tendency was observed with the total SMPs. The results might suggest that the SMP composition is more important than the total SMPs in governing membrane fouling.

*A unique characteristic peak at 1735 cm^{-1} was observed by FTIR under nitrogen limitations (a higher COD/ N) conditions. The relative intensity of the characteristic peak at 1735 cm^{-1} increased with an increase in COD/N ratios and thus positively correlated to membrane fouling rate. The results suggest FT-IR may be used as nutrient management tool to indirectly monitor nutrient conditions and sludge properties in MBR and predict membrane filterability.

Acknowledgement

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Chapter 5 Effect of Solids Retention Time (SRT) on Sludge Properties and Their Role in Membrane Fouling in Membrane Bioreactors (MBRs)

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Abstract:

Effect of solids retention time (SRT) (7, 12 and 20 days) on sludge properties and their roles in membrane fouling was studied under a well-controlled aerobic membrane bioreactor at the same biomass concentration for over 390 days using a synthetic high strength wastewater containing glucose. Membrane performance was improved with an increase in SRT. Surface analysis of sludge by X-ray photoelectron spectroscopy (XPS) suggests that significant differences in the surface concentration of element C and N were observed under different SRTs, implying significant differences in EPS composition. A larger amount of total EPS was found at the lowest SRT (7 days) tested but the ratio of proteins to carbohydrates in EPS increased with an increase in SRT. Similarly, the quantity of SMPs decreased with an increase in SRT but the ratio of proteins to carbohydrates in SMPs increased with an increase in SRT. The quantity of total EPS, total SMPs, and proteins to carbohydrates ratios in EPS and SMPs positively correlated to membrane fouling rates. Sludge cake formation is the dominant mechanisms of membrane fouling.

Key words: membrane bioreactor; membrane fouling; surface properties, solids retention time. Extracellular polymeric substances, soluble microbial products;

5.1 Introduction

Membrane bioreactors (MBRs) as an efficient technology have been widely used in municipal and industrial wastewater treatment and reclamation. MBRs technology combines biodegradation processes with membrane filtration for solid/liquids separation and provides high quality discharge. Thus, it replaces the solids/liquid separation of the secondary clarifier in conventional activated sludge processes. However, membrane fouling which reduces productivity and, hence, increases operational costs is still the main challenge limiting the wide application of MBRs (Le-Clech et al., 2006; Meng et al., 2009; Wang and Wu, 2009). A number of factors, including hydrodynamic conditions, membrane properties and module design, process and environmental conditions, affect the rate of membrane fouling (Huang et al., 2011; Le-Clech et al., 2006). Among these factors, solids retention time (SRT) has been widely acknowledged to be one of the key factors. Although the SRT itself has no direct impact on membrane filterability, it does influence the biomass properties including the particle size, mixed liquor suspended solids (MLSS) concentration, extracellular polymeric substances (EPS) and soluble microbial products (SMP), all of which could foul the membrane (Le-Clech et al., 2006).

Previous studies on the effect of SRT on membrane performance in MBRs are controversial. Some studies observed improved membrane permeability at longer SRTs of 2–10 days (Trussell et al., 2006)), 8–80 days (Nuengjamnong et al., 2005) and 10–40 days (Liang and Song, 2007)). On the other hand, some other investigators found exactly the reverse trend. Lee et al. (Lee et al., 2003) reported that the prolonged SRT ranging from 20 days to 40 days and 60 days could increase the membrane fouling resistance. Han et al. (Han et al., 2005) also reported that the membrane fouling and sludge viscosity increased with lengthening SRT from 30 to 100 days. Furthermore, Annop et al. (Annop et al., 2014) found that an increase in SRT (15, 30 and

60 days) led to a poorer membrane performance in an anaerobic membrane bioreactor. The above mentioned opposite observations are probably due to the fact that the fouling behavior were investigated at various MLSS concentrations under tested SRTs. It is well known that MLSS is an important factor affecting the membrane fouling as microbial floc and supernatant containing colloids and solutes are the two main fractions of biomass. Each fraction has its own physicochemical and biological properties and affects membrane fouling. Different membrane fouling behaviors are anticipated at different MLSS concentrations even with the same sludge (Rosenberger et al., 2005). Therefore, it is desirable to eliminate the influence of MLSS on membrane performance while studying the effect of SRT and focusing the physiological state (cell age or growth rate) only. Furthermore, in some cases, the MLSS is preferred to be maintained at an optimal level in commercial pilot-plant or full-scale MBRs. When unexpected disturbances in feed conditions, such as flow rate and COD concentration, occurred, waste of biomass is adjusted accordingly to maintain the same biomass concentration, which results in changes of SRTs (Wu et al., 2013). However, there are few studies which focus on the effect of SRT on MBR performance at the same biomass concentration. The objective of this research was to clarify the effect of SRT on biological performance and membrane fouling at the same biomass concentration by regulating the feed COD. At the same MLSS, membrane fouling rate will be affected only by the different physiological sates (cell age) of sludge but not the MLSS under different SRTs. Therefore, a better controlled study was designed to investigate the effect of physiologic states (e. g. cell age or SRT) on membrane fouling in this study. The effects of three different SRTs (7, 12, and 20 days) on the membrane fouling behaviours were evaluated at the same biomass concentration by regulating feed COD. The relationship between the evolution of membrane fouling and sludge properties at different SRTs were also established. The

membrane flux, fouling resistance, surface composition of sludge, extracellular polymeric substances (EPS), soluble microbial products (SMP) and particle size (PSD) were monitored. XPS and Fourier transform infrared (FTIR) spectroscopy were employed to characterize surface properties of sludge samples.

5.2 Materials and methods

5.2.1 Experimental set up and operating conditions

The laboratory-scale submerged MBR system was comprised of a 6 L working volume bioreactor with flat sheet microfiltration membranes (SINAP Membrane Science & Technology Co. Ltd., Shanghai, China). The flat sheet membrane were made of polyvinylidene fluoride (PVDF) materials with a pore size of 0.3 μm and a molecular weight cut off (MWCO) of 70,000 Da. The immersed coarse air bubble diffusers (2.6 LPM on each side of the membrane module) were installed under the membrane module in order to provide air scouring for membrane fouling control and oxygen for biodegradation. Finer aeration (1.2 LPM on each side of the membrane module) was also used to maintain satisfying dissolved oxygen (DO) level approximately 4 mg/L. A magnetic stirrer (Thermolyne Cimarec, Model S47030) was located at the bottom of the reactor to help sludge mixing. The temperature of the MBR was maintained constant at mesophilic temperature of $35 \pm 1^{o}C$ by means of water jacket. The pH was monitored by a pH electrode (Thermo Scientific, Beverly, MA), and automatically adjusted to 7.0 ± 0.2 by a pH regulation pump using 0.5 M NaOH solution.

The feed was automatically pumped into the bioreactor by a peristaltic pump (Masterflex Model 7520-50, Barnant Co., USA), which was controlled by a liquid level sensor (Madison Co., USA) as well as a controller (Flowline, USA). The major composition of influent includes glucose, nitrogen (NH_4Cl) and phosphorus (KH_2PO_4) in a proportion of chemical oxygen demand (COD): N: P= 100:5:1 and other trace metals ($MgSO_4 \cdot 7H_2O$, 5.07 mg/L; $FeSO_4 \cdot 7H_2O$, 2.49 mg/L; $Na_2MoO_4 \cdot 2H_2O$, 1.26 mg/L; $MnSO_4 \cdot 4H_2O$, 0.31 mg/L; $CuSO_4$, 0.25 mg/L; $ZnSO_4 \cdot 7H_2O$, 0.44 mg/L; NaCl, 0.25 mg/L; $CoCl_2 \cdot 6H_2O$, 0.43 mg/L; $CoCl_2 \cdot 6H_2O$, 0.41 mg/L). The system was continuously fed on prepared synthetic high strength wastewater, stored at 4 °C. The membrane operational cycles with 4 min of operation and 1 min of relaxation were carried out to mitigate membrane fouling in the continuous operation. The vacuum gauges were installed between the membranes and suction pump in order to monitor the variation of the trans-membrane pressure (TMP). The instant membrane flux was maintained at 7.0 $L/m^2 \cdot h$. The main operating conditions are summarized in Table 5.1. New membranes were used for each operation cycle, in order to maintain the same conditions for comparison along different SRTs.

Table 5.1 Main operational conditions of the aerobic MBR

	Day 1-117	Day 118-269	Day 270-390
Three solid retention time (days)	7	12	20
Influent COD concentration (g COD/L)	5.07 ± 0.16	3.52 ± 0.10	2.46 ± 0.10
Hydraulic retention time (hour)	36	36	36
Food to microorganisms ratio (F/M) (kg COD/kg MLSS·d)	0.42 ± 0.01	0.31±0.01	0.21±0.01
Operation pH	6.9 ± 0.1	6.9 ± 0.1	6.9 ± 0.1
Operation temperature (°C)	35	35	35

5.2.2 Analytical methods

5.2.2.1 Water quality measurement

The influent synthetic industrial wastewater, permeate and mixed liquor were sampled periodically from the system. The COD and mixed liquor suspended solids (MLSS) were analyzed according to Standard Methods (APHA, 2005) (APHA, 2005). Supernatant COD was determined after centrifuging the mixed liquor for 20 min at 18700 × *g*.

5.2.2.2 Extracellular polymeric substances (EPS) extraction and measurement

The bound EPS from sludge suspensions samples was extracted according to cations exchange resin (CER) (Dowex Marathon C, Na^+ form, Sigma–Aldrich, Bellefonte, PA) method (Frolund et al., 1996). 100 mL sample of the sludge suspension was taken and centrifuged (IEC MultiRF, Thermo IEC, Needham Heights, MA, USA) at 18,700×g for 20 min at 4°C. The sludge pellets were re-suspended to their original volume using a buffer consisting of 2mM Na_3PO_4, 4mM NaH_2PO_4, 9mM NaCl and 1mM KCl at pH 7. The sludge was then transferred to an extraction beaker filled with buffer and the CER (80 g/g-MLSS). The extraction was lasted for 2 hours at 4°C. The EPS was determined as the sum of protein and carbohydrate and were measured colorimetrically by the methods of Lowery et al. (Lowery et al., 1951) and DuBois et al. (DuBois et al., 1956), respectively. The total bound EPS was represented by adding the concentrations of bound carbohydrates and proteins.

5.2.2.3 Soluble microbial products (SMP) measurement

The extracted supernatant was filtered by 0.45 µm membrane filters (Millipore) for SMP analysis. The proteins and carbohydrates of SMP were measured using the methods of Lowery et al. (Lowery et al., 1951) and DuBois et al. (DuBois et al., 1956), respectively. The total SMP was the sum of the concentrations of soluble carbohydrates and soluble proteins.

5.2.2.4 Particle size distribution measurement and analysis

The particle size distribution (PSD) measurements of bulk sludge were routinely conducted. The PSD was determined by a Malvern Mastersizer 2000 instrument (Worcestershire, UK) with a detection range of 0.02–2000 μm. The scattered light is detected by means of detectors that convert the signal to a size distribution based on volume or number. Each sample was measured three times with a standard deviation of 0.1–4.5%.

5.2.2.5 Fourier transform-infrared spectroscopy (FTIR)

A Bruker Ten 37 FTIR Spectrometer (Bruker Co., Ltd.) was employed to determine the major functional groups of organic matters and to predict the chemical composition of the bulk sludge. The FTIR analysis was performed using absorbance mode. The sludge samples were freeze-dried at −35 ◦C for one week before FTIR measurement.

5.2.2.6 Membrane Resistance

The extent of fouling rate can be evaluated by the derivative of the filtration resistance:

$$R_t = \frac{\Delta P}{J \times \eta_T} = R_m + R_f + R_c$$

$$\eta_T = \eta_T^{20^{\circ}C \cdot e^{-0.0239(T-20)}}$$

Where R_t is the total filtration resistance (m^{-1}), J represents the permeate flux (m^3/m^2h), ΔP is the trans-membrane pressure difference (Pa), and η_T is the permeate dynamic viscosity (Pa·s). T is the permeate temperature in °C.

R_t was calculated with the temperature corrected to 20 °C to compensate for the dependence of viscosity on temperature. The experimental procedure to determine each resistance value is as follows: R_t is total membrane resistance (m^{-1}) and calculated from the filtration data at the end of operation, R_m intrinsic membrane resistance and evaluated by the water flux of tap water, Rc fouling layer Resistance (m^{-1}). R_f fouling resistance due to irreversible adsorption and pore blocking (m^{-1}). When fouling occurred, membrane surfaces was conducted by wiping away the fouling layer with a sponge and tap water, and then the membrane was submerged in tap water for flux and TMP measurement.

5.2.3 Statistical Analysis

Statistical analysis was conducted by using the Statistical Package for the Social Science (SPSS) 16.0. An analysis of variance (ANOVA) was employed to identify whether there is significant difference between treatment means when evaluating membrane fouling and EPS concentration under different SRTs. The difference was considered statistically significant at a 95% confidence interval ($p < 0.05$). The student t-test also was applied to analyze the content of surface chemical composition of bulk sludge. The paired p values were calculated for the differences between SRT of 7 and 12 day; 12 day and 20 day; and 7 day and 20 day. Data sets were considered statistically different if the p values were less than 0.05.

5.3 Results and discussion

5.3.1 Overall COD removal efficiency

As shown in Fig. 5.1, operating periods can be divided into 3 stages: SRT of 7, 12 and 20 days stages, respectively. In order to avoid the influence of various MLSS on biological and membrane performance, the MLSS values for SRTs of 7, 12, and 20 days were maintained at approximately 7580 ± 289 mg/L by regulating the feed COD concentration. The influent COD values were varying from 5070 ±165 mg COD/L to 3517± 103 mg COD/L and then to 2460 ± 100 mg COD/L for SRT of 7 days, 12 days and 20 days stages, respectively. During Day 1- 44, membrane leaking occurred and the COD removal efficiency was only 81%, due to the leaking of supernatant to effluent. At steady-state, the permeate COD values remained between 0 mg/L and 69 mg/L corresponding to the removal efficiency of 100% and 98%, respectively. A steady-state permeate COD of 39.9 ± 15.5 mg/L, 43.7 ± 18.7, 24.2 ± 7.1 mg/L was achieved for SRT of 7, 12 and 20 days, respectively. Similarly, a supernatant COD of 326.9 ± 60.8, 85.2 ± 44.3, 53.9 ± 20.1 mg/L was found for an SRT of 7, 12 and 20 days, respectively. It is clear that the removal efficiency of COD increased and supernatant COD decreased with an increase in SRT. Meanwhile, the supernatant COD values were higher than the permeate COD indicating the presence of a large quantity of colloidal particles and soluble microbial products (SMPs) in the sludge suspension that retained by the membrane. This can be the explanation of the higher supernatant COD at SRT= 7 days stage that leads to much faster fouling. Membrane leaking occurred on day 200 and 250, which gave rise of deterioration in COD degradation: both permeate and supernatant COD jumped to high values and the COD removal efficiency dropped. All of the findings indicate that the SRT has limited influence on COD removal efficiency.

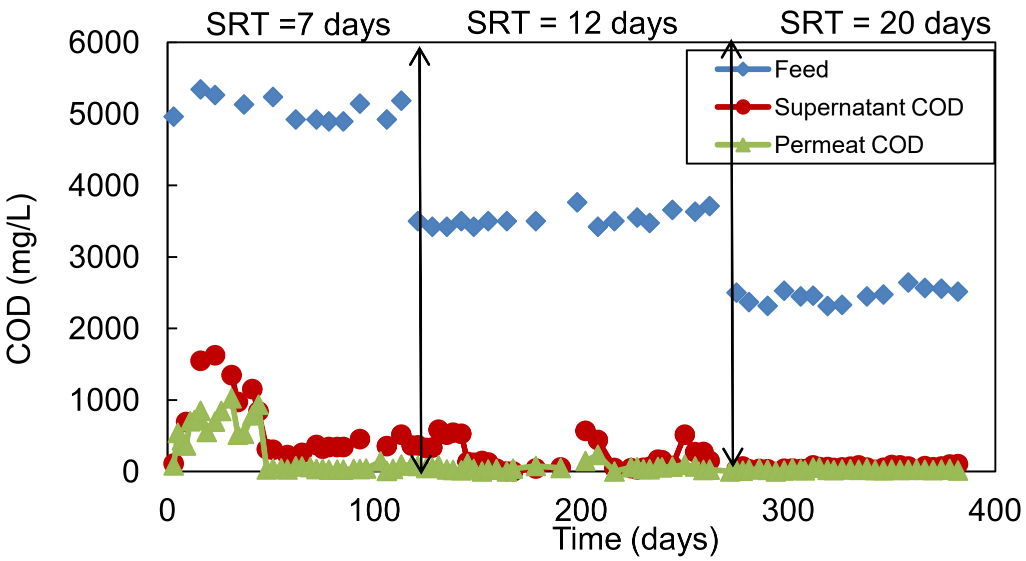


Fig. 5.1 Variation of the influent, supernatant and permeate COD

5.3.2 Trans-membrane pressure (TMP)

Fig. 5.2 shows the change of trans-membrane pressure (TMP) of the MBRs with time at different SRTs. The first period of operation at an SRT of 7 days (day 1 to 117) had severe membrane fouling and rapidly increased TMP. When trans-membrane pressure (TMP) jumped, physical cleaning with wet sponge was carried out daily for flux recovery. During the second period of operation at an SRT 12 days, the first 21 days (day118 to 139) was the transition stage and followed by a steady-state stage operation (day 140 to 269). A subtle fluctuation of TMP was observed at the beginning (transition period). After the transition period, a relatively low and stable TMP was achieved for 21 to 24 days before TMP jumps. For the SRT of 20 days (day 270 to 390), an extended stable and low TMP period of operation (29 to 41 days) was achieved in each membrane operation cycle. A three stage TMP profile was observed for SRT of 12 and 20 days: a rapid increase in TMP in the first few hours followed by a flat and low and stable TMP period (weeks) and eventually by a TMP jump. The TMP profile exhibited the order of membrane fouling rate from high to low: SRT 7 days > SRT 12 days > SRT 20days. This suggested that lengthened SRT tends to result in lower membrane fouling potential. This result was obtained at the same MLSS and thus eliminated the influence of MLSS concentration. Thus, the change of membrane fouling rate can be correlated to changes in sludge properties caused by physiological age but not MLSS, which will be discussed in later sections. The change in membrane fouling rate under different SRTs might be explained by the fact that the membrane fouling can be caused by the high concentration of soluble organic matter and bound extracellular polymeric substances (EPS). Soluble microbial products (SMP) have been found as the major foulants in the operation of MBRs for wastewater treatment (Drews et al., 2008; Paul and Hartung, 2008; Rosenberger et al., 2006). A higher concentration of soluble EPS may attribute to faster membrane fouling rate (Kimura et al., 2005).

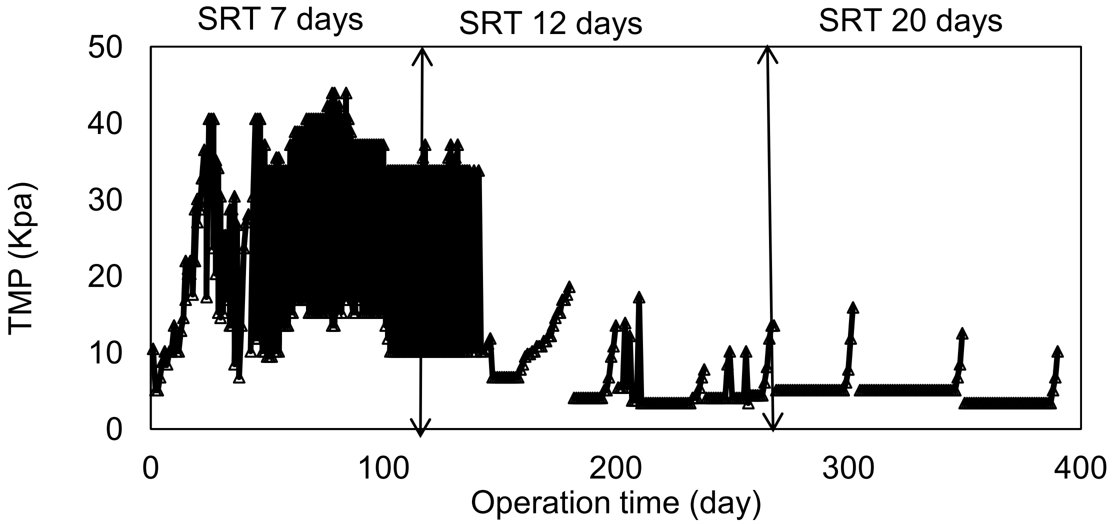


Fig. 5.2 TMP profile at different SRTs.

5.3.3 Fouling resistance

In order to clarify membrane fouling, the operational cycle for each operation condition was usually defined as the period between two physical and chemical cleaning procedures and resistance analysis was conducted at the end of each run. The total filtration resistance (R_t), the cake layer resistance (R_c), and resistance caused by pore blocking and adsorption (R_f) are shown in Table 5.2. In our study, a greater fouling occurred at SRT 7 days which resulted in the rapid rise of TMP. The total fouling resistance (R_t) decreased with an increase of SRT from 7 to 20 days, which correlated well with the variations of TMP (as shown in Fig. 5.2). The high percentage (94-99%) of cake lay resistance suggests that cake layer formation was the dominant mechanisms of membrane fouling under tested SRT range. Thus, physical cleaning of cake layer using wet sponge could reinstall membrane performance and no chemical cleaning was needed.

Table 5.2 A series of resistances for the filtration of activated sludge under different SRTs.

SRT	Resistances				Cake resistance ratio (%)
	R_m	R_c	R_f	R_t	$R_c/(R_c+R_f)$
7 days	$(1.88 \pm 0.24)\times10^{12}$	$(3.29 \pm 0.06)\times10^{13}$	$(19.1 \pm 7.3)\times10^{11}$	$(3.75 \pm 0.14)\times10^{13}$	94
12 days	$(1.88 \pm 0.18)\times10^{12}$	$(1.73 \pm 0.53)\times10^{13}$	$(7.41 \pm 1.69)\times10^{11}$	$(1.85 \pm 0.73)\times10^{13}$	96
20 days	$(2.42 \pm 0.52)\times10^{12}$	$(1.22 \pm 0.26)\times10^{13}$	$(3.93\pm 0.20)\times10^{11}$	$(1.49 \pm 0.31)\times10^{13}$	99
* Sample mean ± relative error, number of measurements: n = 2 for SRT 7 days; n = 3 for SRT 12 days; n= 2 for SRT 20 days.					

More importantly, R_c at SRT 20d represented over 99% of the total fouling resistance (R_t) and it was higher than Rc values observed at SRT 7 and 12 days. This result suggests that less irreversible fouling at prolonged SRT. The variation of fouling resistance directly shows the degree of membrane fouling. Therefore, over one year operation period, the greater fouling resistance was observed at shorter SRTs.

5.3.4 Particle size distributions (PSD) measurement

The particle size distributions of bulk sludge at different SRTs were routinely measured and shown in Fig 5.3. It is clear that a significantly larger portion of smaller particles existed in the shorter SRT of 7 days. The particle size of bulk sludge increased as the SRT extended. The mean size of particles at SRT of 7 days shifted from a much smaller range while those at SRT 12 and 20 days move to larger range. This result is consistent with the findings of previous studies (Ahmed et al., 2007) in that floc sizes increased with an increase in SRT.

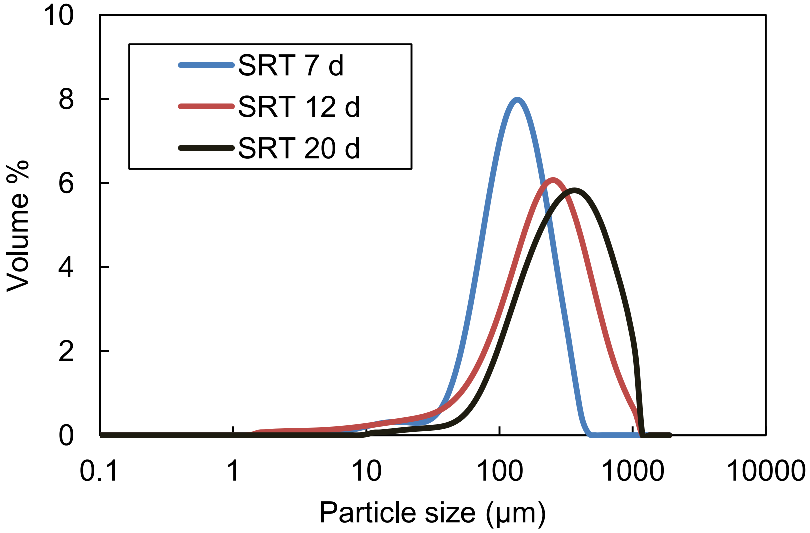
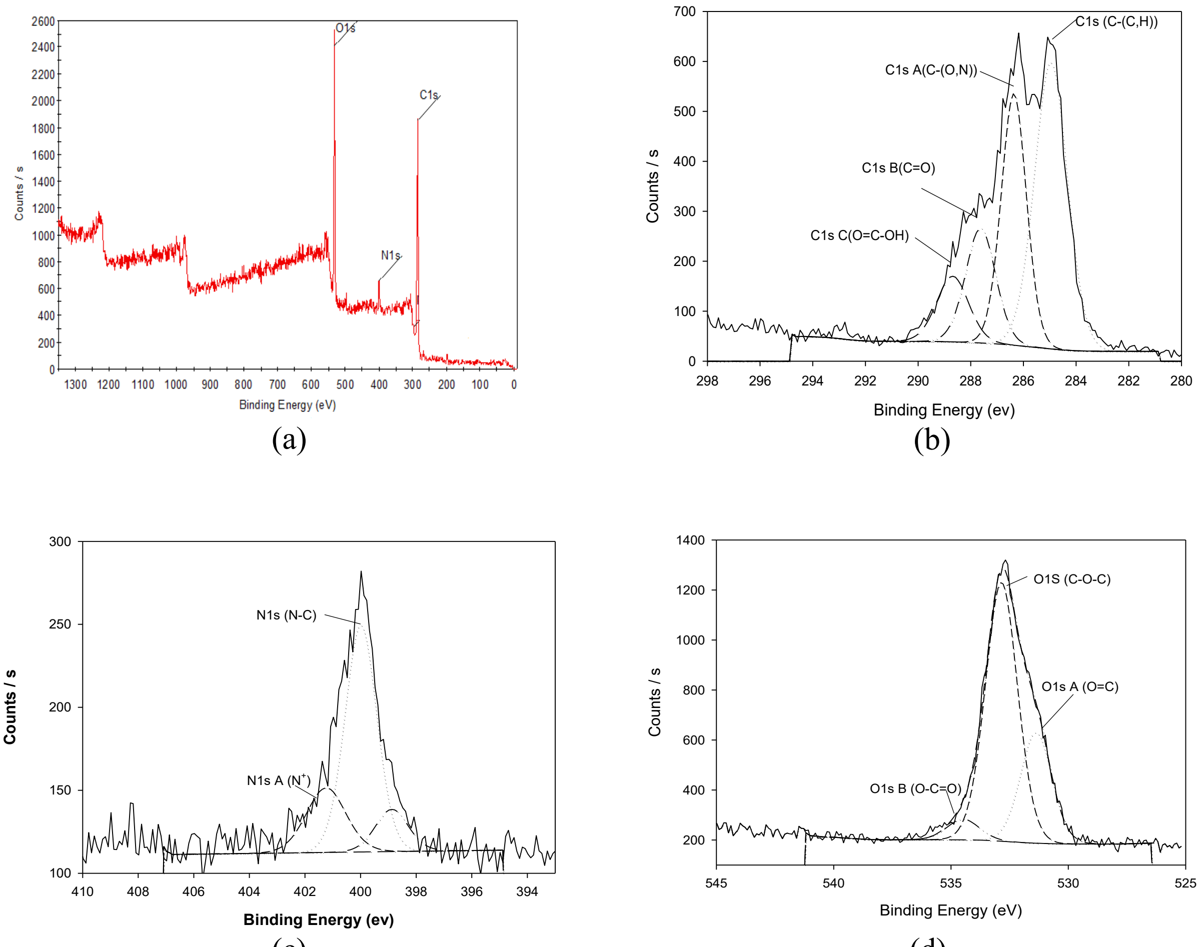


Fig. 5.3 Particle size distribution of bulk sludge at different SRTs.

The small particles have stronger tendency to deposit on the membrane surface to form a compact and dense cake layer which induces poorer filtration (Lim and Bai, 2003). The change in floc size distribution provides an explanation on the changes in membrane fouling rate under different SRTs. Additionally, higher cake layer resistance was observed in this study with smaller particle size. which is in great agreement with previous studies (Defrance et al., 2000).

5.3.5 Surface composition of sludge by XPS analysis

The surface chemical composition of bulk sludge at different SRTs were analysed by XPS. XPS has been used to study the surface functional groups of materials, including bacteria, and each peak corresponds to electrons with a characteristic binding energy from a particular element (Badireddy et al., 2008; Dengis and Rouxhet, 1996; Dufrene et al., 1997). As shown in Fig. 5.4. The C peaks (C1s, C1sA, C1sB, C1sC) was resolved into four component peaks: C bound only to C and H, C−(C,H) at a binding energy of 284.8 eV; C singly bound to O or N, C−(O, N), including ether, alcohol, amine, and amide, at a binding energy of 286.3 eV; C bound to O making two single bonds or one double bond, C−O or O−C−O, including amide, carbonyl, carboxylate, ester, acetal, and hemiacetal, at a binding energy of 288.0 eV. The O peaks (O1s, O1sA, and O1sB) could be attributed into three bonds: O−C bond, including hydroxide (C−OH), acetal, and hemiacetal (C−O−C), at a binding energy of 532.7 eV, and O−C in carboxylic acid, carboxylate, ester, carbonyl and amide at a binding energy of 531.4 eV. The N peaks (N1s and N1sA) were attributed to the two different bonds: N−C bond in amide or amine at a binding energy of 400.12 eV and N−H bonds in ammonia or protonated amine at a binding energy of 402.10 eV.



(a) (b)

(c) (d)

Fig. 5.4 XPS spectra of activated sludge, (a) whole spectra, (b) C1s spectra, (c) N1s spectra, and (d) O1s spectra

Concentrations of surface composition C, O, N under different SRT conditions were summarized in Table 5.3. Significant differences in the surface concentration of element N and C on sludge surface were observed between SRT of 7, 12 and 20 days (95% confidence level, Student t-test). These results suggest that significant difference in EPS composition existed at different SRTs. No significant difference (Student t-test, P>0.05) in the quantity of O was observed between SRT conditions. It is well known that the bound EPS consist of proteins, polysaccharides, nucleic acids, lipids, humic acids, etc. which are located at or outside the cell surface. Proteins in the activated sludge EPS are the primary source for the elemental N. Previous studies (Badireddy et al., 2008; Dengis and Rouxhet, 1996; Dufrene et al., 1997) revealed that the C-(C, H) bonds might originate from lipids or from amino acid side chains. Polysaccharides contain hydroxide and acetal or hemiacetal building blocks. Proteins and uronic acids in polysaccharides might contain carboxylate and carboxyl function groups. The amide may represent peptidic bonds in proteins. The ammonium might be a counter-ion of surface negative sites and the protonated amine could be due to basic amino acids (Dengis and Rouxhet, 1996).

Table 5.1 Surface composition of the sludge at different SRT which determined by XPS: average atom fraction (%)

Element component				Significant difference[a]		
	SRT 7d	SRT 12d	SRT 20d	SRT 7d and 12d	SRT 12d and 20d	SRT 7d and 20d
Total C	66.29±0.14	63.78± 1.09	65.35± 0.51	Y(0.008)	Y(0.043)	Y(0.018)
C-(C,H)	27.22±2.44	26.09± 5.67	27.70± 1.28	N(0.309)	N(0.339)	N(0.386)
C-(O,N)	23.37±0.39	20.57± 2.81	23.05± 3.18	N(0.115)	N(0.184)	N(0.440)
C=O	10.09±1.11	9.83± 2.87	9.10± 1.38	N(0.445)	N(0.359)	N(0.194)
O=C-OH	5.61±0.13	7.27± 1.70	5.57± 0.07	N(0.132)	N(0.124)	N(0.459)
Total O	25.08±1.64	27.55±1.26	25.30±0.34	N(0.053)	N(0.096)	N(0.413)
C-OH	6.27±0.93	6.59±1.16	6.93±0.18	N(0.384)	N(0.218)	N(0.111)
O=C	16.84±1.40	16.43±1.82	16.90±0.73	N(0.386)	N(0.349)	N(0.465)
O-C=O	3.14±0.81	3.71±1.90	1.17±0.29	N(0.391)	N(0.070)	N(0.170)
Total N	5.65±0.11	4.71±0.58	6.25±0.10	Y(0.025)	Y(0.005)	Y(0.001)
N-C	3.49±0.78	2.90±0.55	4.51±0.43	N(0.172)	N(0.123)	N(0.059)
N+	1.40±0.13	1.08±0.25	1.18±0.19	N(0.190)	N(0.332)	N(0.181)

[a] sig. value shown in parentheses. Sample number n=3 for each conditions.

5.3.6 Extracellular polymeric substances (EPS)

Proteins and carbohydrates are considered as dominant components representing the total amount of EPS. Fig. 5.5 (a) presents the comparison of bound EPS content in the bulk sludge. Statistical analysis using ANOVA confirmed that differences in proteins, carbohydrates and total EPS were all statistically significant (ANOVA, $p < 0.05$) among different SRTs. Proteins, carbohydrates and total EPS concentration decreased with an increase in SRT. Proteins were observed as the predominant compounds in EPS compared to carbohydrates. On average, the total EPS were 61.01(± 5.16), 30.32 (± 3.45) and 26.07 (± 1.73) mg/g MLSS for SRTs of 7, 12 and 20 days, respectively. It is well known that the EPS is growth-related and is produced in direct proportion to substrate utilization (Laspidou and Rittmann, 2002). At shorter SRTs, the great amount of EPS were produced which probably due to the fact that the biomass at lower SRTs did not consume all the carbon sources available for growth. The excess carbon substrates have more tendency to be converted to EPS and more proteins were produced and thus resulted in more excretion of intracellular polymers (Lee et al., 2003). Meng et al. (Meng et al., 2007) also reported that the increased EPS were induced by a higher organic loading or F/M ratio. In contrast, the biomass at a higher SRT exhibits a lower metabolism rate to consume less food and generate less microbial products. Therefore, a shorter SRT with a higher organic loading rate or F/M ratio led to a larger amount of EPS. Previous investigations revealed that the EPS are controlling factor for membrane fouling in MBRs (Laspidou and Rittmann, 2002; Lee et al., 2003; Meng et al., 2007). Therefore, the findings from this study were in agreement with studies that greater EPS content led to faster fouling rates.

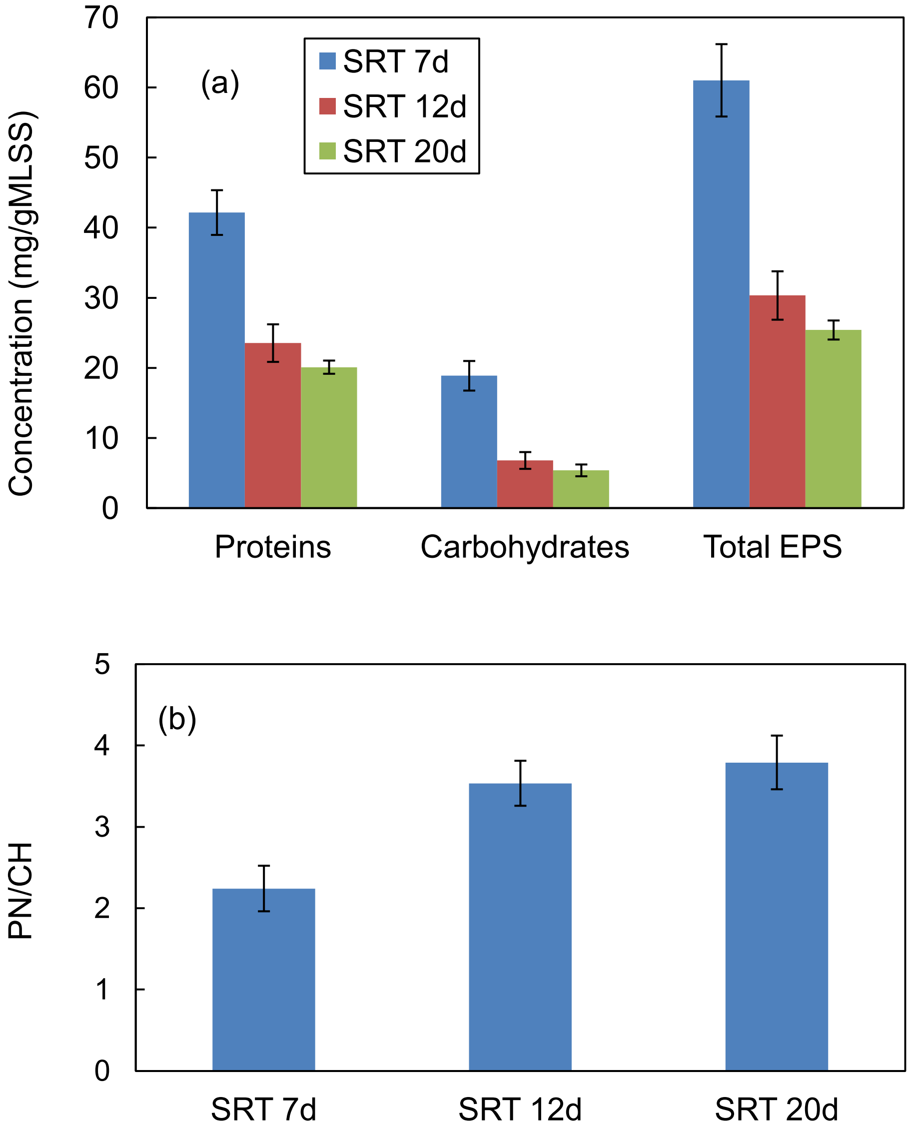


Fig. 5.5 Comparison of bound EPS of bulk sludge at different SRTs (ANOVA, $p<0.05$, number of measurements: $n=6$ for each SRT).

The change in the ratio of proteins to carbohydrates (PN/CH) in EPS with respect to SRT is shown in Fig. 5.5 (b). An increase in SRT led to an increase in PN/CH ratio. The change in the ratio of PN/CH indicates the difference in EPS composition and concentration at different SRTs and is consistent with the finding of XPS results (differences in element C and N surface concentration at different SRTs) from this study. Similar trends in the ratio of PN/CH in EPS has been reported by Liao et al. (Liao et al., 2001). Meng et al. (Meng et al., 2006) found that proteins are more hydrophobic and have more tendencies to adhere on membrane surface and induce membrane fouling. The larger amount of proteins in bound EPS might explain the higher fouling rate at the SRT of 7 days. Furthermore, a negative relationship exists between the PN/CH ratio and membrane fouling rate. This can be explained by the fact that the lower PN/CH ratio in EPS led to a poorer bioflocculation and thus smaller floc sizes, which is responsible for the higher propensity of membrane fouling (Liao et al., 2001).

5.3.7 Fouling potential of SMP

SMP, namely soluble EPS, which is released from the microbial aggregates into the water phase (Flemming and Wingender, 2001; Rosenberger and Kraume, 2002). As shown in Fig. 5.6, carbohydrates were found as the predominant fraction and accounted for more than half of the SMP concentration for SRT 7 days. However, their fraction was changed when SRT increased to 20 days. The ANOVA results indicated that SRT had a significant impact on the SMP production (ANOVA, $p < 0.05$) (Fig. 5.6). The total SMP, soluble proteins and carbohydrates contents in SMPs at three SRTs exhibited the similar trend which typically dropped with an increase in SRT. SMP had significant correlation to fouling potential. Higher SMP concentration derived from shorter SRT would enhance membrane fouling and induce a faster TMP increase. The proteins to carbohydrates (PN/CH) ratios in SMPs (ANOVA, $p < 0.05$) were also compared to provide more information on characteristics of SMP. The lower PN/CH ratio at a shorter SRT might probably be explained by the fact that the carbohydrates were originated from the original substrates and metabolism products. The higher feed COD at the short SRT would result in a higher residual carbohydrate (glucose) in supernatant.

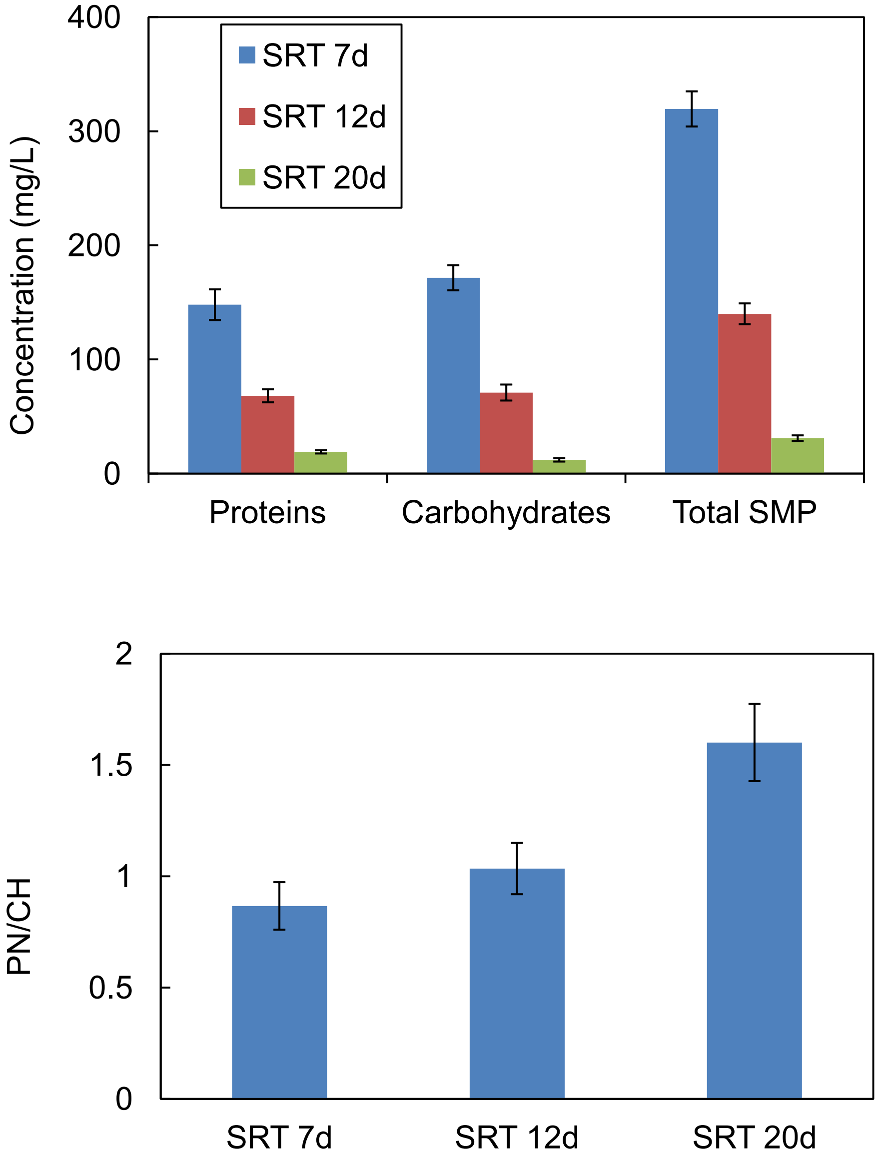


Fig. 5.6 SMP concentrations at different SRTs (ANOVA, $p<0.05$, number of measurements: n=4 for SRT 7d, n=4 for SRT 12d, n=6 for SRT 20d).

On the other hand, the fouling potentials of SMP in effluents were monitored to determine the changes of SMP composition after filtration. It appears that the concentration of SMP (ANOVA, $p<0.05$) as well as proteins and carbohydrate of effluents were significantly lower than those of supernatants. This indicates that the SMP from sludge suspension are more prone to foul the membrane. As show in Fig. 5.7, concentrations of both carbohydrates and proteins of effluent SMP decreased as SRT lengthened, which corresponded well to the variation of total SMP concentration. Therefore, the fouling control strategies can be conducted by operating MBR at longer SRT which greatly minimizes the content of proteins and carbohydrate of SMP.

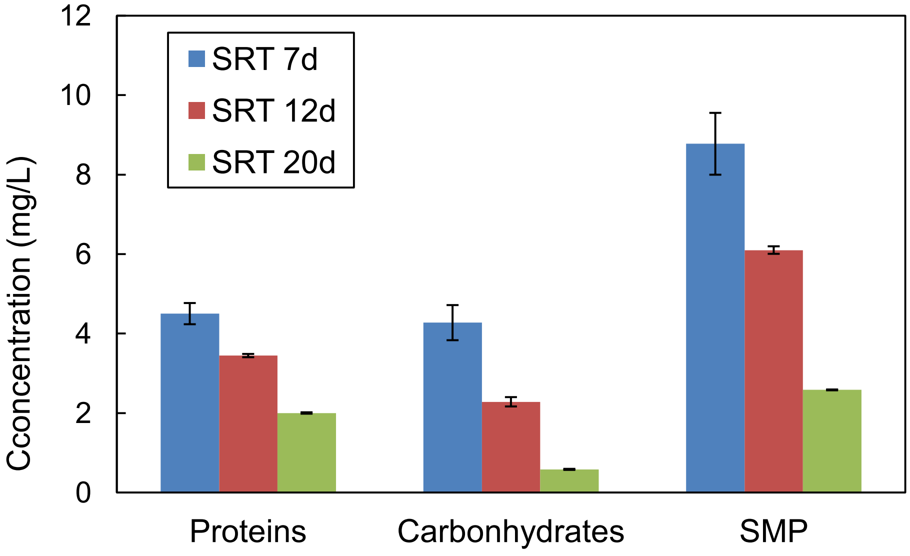


Fig. 5.7 SMP concentrations in effluent at different SRTs (ANOVA, $p<0.05$, number of measurements: n=4 for each SRTs).

5.3.8 Fourier transform infrared (FTIR) spectroscopy

The FTIR spectrums of bulk sludge taking from three SRT of 7, 12 and 20 days are illustrated in Fig. 5.8. The sludge samples exhibited similar peaks for all tested SRTs. All three samples shown peaks at 3400cm^{-1} (overlapping of bands from the stretching vibrations of N-H and O-H) (Kumar et al., 2006) . Peaks at wave numbers of 2908, 2847 and 1445 cm^{-1}, are reflecting C-H bonds in the alkenes class (Kim and Jang, 2006). Peaks near 1647 cm^{-1} (stretching vibration of C-O and C-N amide I) and 1540 cm^{-1} (N-H deformation and C-N stretching amide II) indicate the presence of proteins.

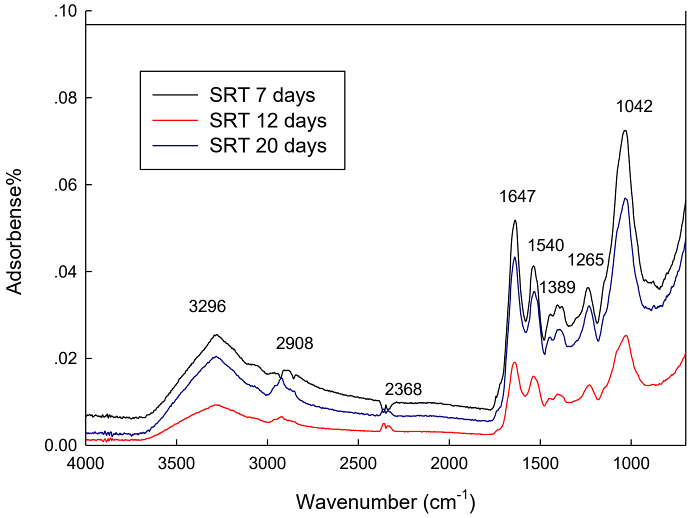


Fig. 5.8 FTIR spectrum of bulk sludge at different SRTs.

In all three spectra, a broad peak due to C-O bonds near 1100 cm^{-1} was attributed to carbohydrates or carbohydrate-like substances (Croue et al., 2003). The peaks of 1389 and 1265 cm^{-1} suggest the presence of amide III (Jun et al., 2007). It is evident from above observations that proteins and carbohydrates were presented in the sludge, suggesting a significant organic fouling derived from EPS. The FTIR results are consistent with EPS results that both proteins and carbohydrates are present in sludge.

5.4 Conclusions

This study focused on clarification of the effect of physiological state (e. g. SRT) on sludge properties and their role in membrane fouling by designing an experiment at the same MLSS, which emphasized the effect of growth rate and eliminated the effect of MLSS. The following conclusions were obtained from this study.

- At the same MLSS, membrane performance was improved with an increase in SRT.
- Surface analysis by XPS indicates significant difference in surface concentration of element C and N on sludge surface at different SRTs, which suggest different compositions in EPS at different SRTs.
- The total EPS and total SMP quantity decreased but the ratio of PN/CH increased with an increase in SRT. The change in PN/CH ratio in EPS is consistent with the difference in surface element N of sludge detected with XPS.
- Floc sizes increased with an increase in SRT.
- Statistical analyses shows total bound EPS, bound carbohydrate, bound proteins, soluble total SMP, soluble carbohydrate, and soluble proteins in supernatant positively correlated to the fouling resistance, indicating that bound EPS, SMP have significant effect on sludge properties and membrane fouling.

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Chapter 6 Conclusion and recommendations for future work

6.1 Conclusions

An increased COD:N ratio from 100:5 to 100:2.5 and 100:1.8 had limited impact on COD removal efficiency and further led to a significant improvement in membrane performance, a reduced sludge yield, and improved effluent quality in terms of residual nutrients. The results suggest that an increased COD:N ratio will benefit the industrial wastewater treatment using membrane bioreactors by reducing membrane fouling and sludge yield, saving chemical costs, and reducing secondary hand pollution by nutrients.

The membrane performance was improved by increasing in COD:N ratio from 100:5 to 100:1.8. The XPS results demonstrated significant differences in surface concentrations of elements C, O and N under different COD:N ratios, which suggest significant difference in EPS composition. A unique characteristic peak at 1735 cm^{-1} was observed by FTIR under nitrogen limitations (a higher COD:N) conditions. The relative intensity of the characteristic peak at 1735 cm^{-1} increased with an increase in COD:N ratios and thus positively correlated to membrane fouling rate. The results suggest FT-IR may be used as nutrient management tool to indirectly monitor nutrient conditions and sludge properties in MBR and predict membrane filterability. A decrease in total EPS content and PN/CH ratio in EPS was observed with an increase in COD:N ratio. Statistical analyses shows the total bound EPS and PN/CH ratio positively correlated to membrane fouling rates, indicating the importance of EPS content and composition in governing membrane fouling. A decrease in PN/CH ratio in SMP was observed with an increase in COD:N ratio but no general tendency was observed with the total SMPs. The results might suggest that the SMP composition is more important than the total SMPs in governing membrane fouling.

At the same MLSS, membrane performance was improved with an increase in SRT. Surface analysis by XPS indicates significant difference in surface concentration of element N on sludge surface at different SRTs, which suggest different compositions in EPS at different SRTs. The total EPS and total SMP quantity decreased but the ratio of PN/CH increased with an increase in SRT. The change in PN/CH ratio in EPS is consistent with the difference in surface element N of sludge detected with XPS. Statistical analyses shows total bound EPS, bound carbohydrate, bound proteins, soluble total SMP, soluble carbohydrate, and soluble proteins in supernatant positively correlated to the fouling resistance, indicating that bound EPS, SMP have significant effect on sludge properties and membrane fouling.

6.2 Recommendations for Future Work

The following topics are suggested for further studies to improve the membrane bioreactor performance and biomass properties.

1. To continue the study on the influence of nutrient conditions on surface properties of sludge and their role in membrane fouling though increase the COD: N ratios.
2. To investigate the effect of nutrient conditions of COD: P conditions on sludge properties and membrane performance.
3. Characterize the microbial community under nutrients limitation conditions (increased COD: N ratios).

Appendix

Appendix A: Chapter 3 Variations of the influent, supernatant and permeate COD with experimental time under different COD/N

Date	Days	Influent	Supernatant COD (mg/L)	Permeate COD (mg/L)	MLSS (g/L)
2013/6/5	4	1733		36.0	8.7
2013/6/7	6	1733	59.1	36.0	7.8
2013/6/9	8	1733		97.7	7.5
2013/6/11	10	1733	51.4	48.8	
2013/6/13	12	1733		33.4	7.8
2013/6/15	14	2550		18.0	
2013/6/17	16	2550		25.7	8.3
2013/6/20	19	2550	64.3	33.4	8.7
2013/6/23	22	2550		33.4	8.8
2013/6/27	26	2550	118.3	18.0	9.0
2013/6/30	29	2550		0.0	9.5
2013/7/5	34	1598	41.1	5.1	9.3
2013/7/8	37	2570	30.8	5.1	8.8
2013/7/11	40	2570		2.6	8.6
2013/7/15	44	2570	20.6	0.0	8.9
2013/7/18	47	2570	23.1	2.6	8.3
2013/7/22	51	2344	25.7	0.0	9.1
2013/7/25	54	2344	7.7	0.0	8.5
2013/7/29	58	2344		61.7	
2013/8/1	61	2344	18.0	15.4	8.0
2013/8/5	65	2344		2.6	
2013/8/8	68	2344	15.4	0.0	8.0
2013/8/12	71	2344		2.6	
2013/8/15	74	2344		7.7	8.2
2013/8/19	77	2519	2.6	5.1	8.0
2013/8/22	80	2519	5.1	18.0	8.0
2013/8/26	84	2300	5.1	0.0	7.9
2013/8/29	87	2300	2.6	0.0	8.2
2013/9/3	92	2571	7.7	23.1	
2013/9/6	95	2571	0.0	0.0	8.2
2013/9/9	98	2571		2.7	
2013/9/16	105	2416		0.0	7.9

2013/9/19	108	2416	156.8	0.0	
2013/9/22	111	2519		2.6	7.7
2013/9/26	115	2519	635.0	15.4	7.8
2013/10/1	120	2431	899.7	0.0	8.0
2013/10/4	123	2431	874.0	0.0	7.4
2013/10/8	127	2519	789.2	0.0	7.2
2013/10/11	130	2519	771.2	0.0	6.8
2013/10/14	133	2431	341.9	0.0	6.4
2013/10/18	137	2431	259.6	30.8	6.0
2013/10/21	140	2431		0.0	6.4
2013/10/25	144	2431	56.6	23.1	6.6
2013/10/28	147	2416	43.7	41.1	7.0
2013/10/31	151	2416	51.4	15.4	6.8
2013/11/4	155	2431	7.7	0.0	6.8
2013/11/7	158	2431	0.0	0.0	6.5
2013/11/11	162	2431			5.5
2013/11/16	167	2431	25.7	0.0	5.5
2013/11/19	170	2565		0.0	5.8
2013/11/30	181	2565	0.0		7.6
2013/12/11	192	2565	25.7	0.0	8.0
2013/12/19	200	2565	59.1	2.6	8.0
2013/12/23	204	2622	69.4	0.0	7.8
2013/12/29	210	2622	61.7	18.0	8.0
2014/1/6	218	2550	0.0	0.0	7.8
2014/1/14	226	2452	23.1		7.6
2014/1/17	229	2452	25.7	12.9	7.5
2014/1/20	232	2416	54.0	2.6	7.2
2014/1/23	235	2416	48.8	0.0	7.2
2014/1/27	239	2426		2.6	8.0
2014/2/5	248	2426	7.7	23.1	8.2
2014/2/9	252	2462	25.7	0.0	7.0
2014/2/14	257	2463	23.1	2.6	6.5
2014/2/18	261	2452	0.0	10.3	6.6
2014/2/21	263	2452	5.1	15.4	6.9
2014/2/24	267	2452	0.0	20.6	6.6
2014/3/3	274	2483	2.6		
2014/3/6	277	2483	5.1	25.7	6.3
2014/3/9	280	2514	15.4	0.0	
2014/3/12	283	2514	30.8	5.1	6.5
2014/3/17	287	1426	48.8	28.3	

2014/3/21	291	2427	30.8	30.8	6.2
2014/3/25	295	2555		30.8	6.2
2014/3/28	298	2555	38.6	41.1	6.4
2014/4/2	303	2570	10.3	20.6	6.4
2014/4/6	307	2571	15.4	33.4	6.5
2014/4/8	309	2457	59.1	38.6	6.6
2014/4/12	313	2458	87.4	46.3	6.8
2014/4/15	316	2534	74.6	36.0	6.6
2014/4/19	320	2535	38.6	28.3	6.5
2014/4/22	323	2493	30.8	33.4	6.3
2014/4/26	327	2494	48.8	30.8	6.2
2014/4/30	331	2394	56.6	28.3	6.4
2014/5/4	335	2394	59.1	33.4	6.1
2014/5/8	339	2395	77.1	30.8	6.2
2014/5/12	343	2570		90.0	6.4
2014/5/16	347	2571	61.7	77.1	6.3
2014/5/22	352	2658	74.6	43.7	6.3
2014/5/28	357	2452	56.6	90.0	6.3
2014/6/3	362	2452		70.7	6.0
2014/6/9	367	2452	69.4		6.0

Appendix B: Chapter 5 Variation of the influent, supernatant and permeate COD and MLSS under different SRT

Date	Days	Influent (mg/L)	Supernatant COD (mg/L)	Permeate COD (mg/L)	MLSS (g/L)
2013/6/7	3	4961	110.5	84.8	6.6
2013/6/9	5	4961		539.8	
2013/6/11	7	4961		437.0	5.9
2013/6/13	9	4961	691.5	362.5	
2013/6/15	11	5500		701.8	6.7
2013/6/17	13	5342		717.2	
2013/6/20	16	5342	1547.6	848.3	6.7
2013/6/23	19	5342		552.7	7.1
2013/6/27	23	5263	1624.7	696.7	8
2013/6/30	26	4631		845.8	8.2
2013/7/5	31	4631	1347.0	1023.1	8.5
2013/7/8	34	5131	971.7	514.1	8.8
2013/7/11	37	5131		527.0	8.3
2013/7/15	41	5131	1151.7	784.1	8.6
2013/7/18	44	5131	840.6	933.2	8.4
2013/7/22	48	5237	311.1	28.3	8.2
2013/7/25	51	5237	303.3	41.1	8
2013/7/29	55	5237		41.1	7.9
2013/8/1	58	5237	228.8	28.3	7.8
2013/8/5	62	4921		72.0	
2013/8/8	65	4921	259.6	69.4	8
2013/8/12	69	4921		61.7	
2013/8/15	72	4921	370.2	33.4	8
2013/8/19	75	4895	318.8	36.0	8
2013/8/22	78	4895	341.9	25.7	8.2
2013/8/26	82	4895	341.9	25.7	
2013/8/29	85	4895	341.9	25.7	8.2
2013/9/3	90	5142		36.0	
2013/9/6	93	5142	452.4	33.4	8
2013/9/9	96	5142		41.1	
2013/9/16	103	4921		141.4	7.8
2013/9/19	106	4921	354.8	7.7	
2013/9/23	109	5184		36.0	7.8
2013/9/26	113	5184	514.1	97.7	8
2013/10/1	118	3500	362.5	82.3	8

2013/10/4	121	3500	365.0	77.1	7.6
2013/10/8	125	3421	329.0	48.8	
2013/10/11	128	3421	334.2	51.4	7.4
2013/10/14	131	3421	583.5	84.8	7.4
2013/10/18	135	3421	514.1	28.3	7.6
2013/10/21	138	3500	542.4	20.6	7.6
2013/10/25	142	3500	527.0	15.4	7.8
2013/10/28	145	3421	133.7	108.0	7.6
2013/10/31	148	3421	120.8	33.4	7.8
2013/11/4	152	3500	146.5	0.0	7.8
2013/11/7	155	3500	126.0	25.7	7.7
2013/11/11	159	3500	38.6	33.4	7.5
2013/11/16	164	3500		0.0	7.4
2013/11/19	167	3500		38.6	7.4
2013/11/30	178	3500	41.1	77.1	
2013/12/11	190	3763	56.6	48.8	7.2
2013/12/19	198	3763			7.5
2013/12/23	202	3421	568.1	146.5	
2013/12/29	208	3421	439.6	231.4	7
2014/1/6	216	3500	48.8	0.0	7.4
2014/1/14	224	3500	46.3	59.1	7
2014/1/17	227	3500		59.1	
2014/1/20	230	3474	51.4	30.8	
2014/1/23	233	3474	64.3	30.8	
2014/1/27	237	3657		61.7	7.4
2014/1/29	239	3657	156.8	56.6	7.5
2014/2/5	244	3657	77.1	66.8	7.6
2014/2/9	250	3632	514.1		7.2
2014/2/14	255	3632	269.9	69.4	7.2
2014/2/18	259	3711	272.5	20.6	7
2014/2/21	262	3711	149.1	25.7	7
2014/3/6	275	2500	30.8	15.4	7.2
2014/3/9	278	2368	69.4	10.3	7.4
2014/3/12	281	2368	30.8	28.3	7.4
2014/3/17	286	2315	36.0	38.6	7.4
2014/3/21	290	2316	30.8	23.1	7.5
2014/3/25	294	2526		0.0	
2014/3/28	298	2526	38.6	12.9	7.6
2014/4/2	302	2447	41.1	46.3	7.7
2014/4/6	306	2447	33.4	28.3	7.5

2014/4/8	308	2458	30.8	15.4	
2014/4/12	312	2458	84.8	54.0	7.5
2014/4/15	315	2313	61.7	54.0	7.4
2014/4/19	319	2314	61.7	23.1	7.3
2014/4/22	322	2329	48.8	18.0	7.5
2014/4/26	326	2329	56.6	28.3	7.3
2014/4/30	330	2447	66.8	30.8	
2014/5/4	334	2447	84.8	23.1	7.4
2014/5/8	338	2447	54.0	41.1	7.3
2014/5/12	342	2474	23.1	18.0	
2014/5/16	346	2474	51.4	15.4	7.6
2014/5/20	350	2642	90.0	25.7	
2014/5/24	354	2642	84.8	20.6	7.4
2014/5/28	358	2642	69.4	23.1	7.5
2014/6/1	362	2566		30.8	7.5
2014/6/5	366	2566	74.6	23.1	7.5
2014/6/9	370	2555	59.1	18.0	7.7
2014/6/13	374	2555	69.4	25.7	7.6
2014/6/17	378	2514	97.7	33.4	7.6

Appendix C: EPS analysis

Table C1 for Chapter 4 Comparison of bound EPS of bulk sludge under different COD/N

Proteins (COD:N)			Carbohydrates(COD:N)			Total EPS(COD:N)		
100:5	100:2.5	100:1.8	100:5	100:2.5	100:1.8	100:5	100:2.5	100:1.8
33.16	15.07	6.53	5.08	7.22	8.85	38.23	21.34	15.38
34.63	13.33	7.89	4.51	4.78	7.41	39.14	17.28	15.30
30.66	12.53	6.11	4.56	5.16	6.63	35.22	16.91	12.73
29.75	12.80	7.89	4.20	5.73	6.61	33.95	17.73	14.51
36.79	13.07	6.74	4.86	5.71	6.39	41.64	18.70	13.13

Table C2 for Chapter 5 Comparison of bound EPS of bulk sludge at different SRT

Protein			Carbohydrate			Total EPS		
SRT 7d	SRT12d	SRT 20d	SRT7d	SRT 12d	SRT 20d	SRT7d	SRT 12d	SRT 20d
44.51	22.31	19.92	21.01	5.01	4.20	65.52	27.32	24.82
42.13	27.20	21.02	17.80	7.12	5.37	59.92	34.32	26.38
47.01	21.23	18.33	21.11	7.66	4.85	68.12	28.89	24.58
41.05	25.60	22.21	19.03	7.87	5.90	60.08	33.47	27.49
38.09	24.53	19.02	15.45	7.50	6.14	53.54	32.04	27.12
40.10	20.31	20.10	18.80	5.58	5.83	58.89	25.89	26.08

Appendix D: SMP analysis

Table D1 for Chapter 4 SMP analysis under different COD:N

Proteins (COD:N)			Carbohydrates(COD:N)			Total SMP(COD:N)		
100:5	100:2.5	100:1.8	100:5	100:2.5	100:1.8	100:5	100:2.5	100:1.8
24.06	16.00	8.42	10.17	8.83	14.95	34.23	24.83	23.37
22.81	14.00	9.21	8.68	7.94	15.09	31.49	21.94	24.30
21.25	17.67	15.26	6.09	8.06	18.06	27.34	25.72	26.48
25.00	16.00	15.00	10.26	9.61	19.58	35.26	25.61	31.95
24.52	12.00	15.26	10.17	11.83	22.96	34.69	23.83	35.59

Table D2 for Chapter 5 SMP analysis under different SRT

Proteins			Carbohydrates			SMP total		
SRT 7d	SRT 12d	SRT 20d	SRT 7d	SRT 12d	SRT 20d	SRT 7d	SRT 12d	SRT 20d
149.06	75.91	21.25	155.48	76.72	14.05	304.54	152.63	35.30
164.38	69.27	19.75	172.62	68.28	11.70	336.99	137.54	31.45
131.56	67.13	17.50	177.14	63.71	13.64	308.71	130.84	31.14
146.88	62.65	17.50	180.95	78.79	10.42	327.83	141.44	27.92
		19.25			11.21			30.46
		19.00			11.02			30.02

Appendix E: XPS analysis

Table E 1 for Chapter 4 XPS analysis for bulk sludge under different COD:N ratios

Peaks (P)\Peaks	C	N	O
COD:N ratios	At%	At%	At%
100:5 sample 1	66.39	5.81943	24.66
100:5 sample 2	64.89	6.39439	24.35
100:5 sample 3	63.41	8.83446	24.97
100:2.5 sample 1	63.50	7.55893	25.86
100:2.5 sample 2	65.19	4.88236	27.60
100:2.5 sample 3	64.56	6.42386	25.93
100:1.8 sample 1	62.58	3.92968	30.44
100:1.8 sample 2	61.82	5.24881	28.91
100:1.8 sample 3	64.57	4.41826	28.92

Table E2. XPS analysis results for C under different COD:N ratio

	Name	Peak BE	At. %	At. %	At. %	After calculation		
COD:N= 100:5	C1s	284.98	50.83	53.89	48.29	32.98616	34.97195	31.33783
	C1s A	286.49	30.85	27.1	31.51	20.02165	17.5879	20.44999
	C1s B	287.83	12.81	15.25	13.69	8.31369	9.89725	8.88481
	C1s C	288.75	5.51	3.76	6.51	3.57599	2.44024	4.22499
COD:N= 100:2.5	C1s	284.98	43.65	44	45.11	28.11933	28.3448	29.05986
	C1s A	286.49	38.48	39.29	37.46	24.78882	25.31062	24.13173
	C1s B	287.83	9.28	10.86	9.14	5.978176	6.996012	5.887988
	C1s C	288.75	8.59	5.85	8.3	5.533678	3.76857	5.34686
COD:N= 100:1.8	C1s	284.98	43.65	44	45.11	28.11933	28.3448	29.05986
	C1s A	286.49	38.48	39.29	37.46	24.78882	25.31062	24.13173
	C1s B	287.83	9.28	10.86	9.14	5.978176	6.996012	5.887988
	C1s C	288.75	8.59	5.85	8.3	5.533678	3.76857	5.34686

Table E 3 for Chapter 5 XPS analysis for bulk sludge under different SRT

Components	C	N	O
Position	At%	At%	At%
SRT-7day-Sample-1	66.26	5.56	23.35
SRT-7day-Sample-2	66.17	5.62	26.63
SRT-7day-Sample-3	66.44	5.78	25.26
SRT-12day-Sample-1	62.53	4.09	26.32
SRT-12day-Sample-2	64.53	5.24	27.5
SRT-12day-Sample-3	64.29	4.81	28.84
SRT-20day-Sample-1	65.42	6.13	25.30
SRT-20day-Sample-2	65.82	6.34	24.97
SRT-20day-Sample-3	64.81	6.29	25.65

Table E4 for Chapter 5 XPS analysis for C under different SRT

	Peak BE	Name	At. %	At. %	At. %	After calculation		
SRT 7d	284.98	C1s	42.14	44.05	36.92	27.93461	29.20075	24.47427
	286.49	C1s A	34.57	35.54	35.66	22.91645	23.55947	23.63901
	287.83	C1s B	15.91	13.32	16.46	10.54674	8.829828	10.91133
	288.75	C1s C	7.37	7.08	10.96	4.885573	4.693332	7.265384
SRT 12 d	284.98	C1s	37.55	34.2	51.01	23.94939	21.81276	32.53418
	286.49	C1s A	29.68	37.36	29.74	18.9299	23.82821	18.96817
	287.83	C1s B	19.92	15.4	10.92	12.70498	9.82212	6.964776
	288.75	C1s C	12.86	13.04	8.33	8.202108	8.316912	5.312874
SRT 20 d	284.98	C1s	42.52	40.37	44.3	27.78682	26.3818	28.95005
	286.49	C1s A	38.25	37.94	29.66	24.99638	24.79379	19.38281
	287.83	C1s B	13.61	11.99	16.2	8.894135	7.835465	10.5867
	288.75	C1s C	5.62	9.69	9.85	3.67267	6.332415	6.436975