Investigation of microfiltration performance of denitrifying granular sludge developed in sequencing batch reactors

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**INVESTIGATION OF MICROFILTRATION
PERFORMANCE OF DENITRIFYING GRANULAR
SLUDGE DEVELOPED IN SEQUENCING BATCH
REACTORS**

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**Investigation of Microfiltration Performance of
Denitrifying Granular sludge developed in
Sequencing Batch Reactors**

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ABSTRACT

Nitrogen-containing compounds released into the environment can create serious problems, such as water eutrophication, deterioration of water quality and potential hazard to human health. Biological nitrogen removal through two-stage nitrification and denitrification has thus been practiced for decades. Bulking sludge in denitrification has often been reported. In order to overcome such problem, denitrifying membrane bioreactor (MBR) has drawn more and more attention. However, biofouling due to microbial attachment onto membrane surfaces remains a big challenge that in turn hinders further application of denitrifying MBR technology.

The aims of this thesis were to look into denitrifying granular sludge MBR for effective control of biofouling, and further investigate the biofouling mechanism. For these purposes, the study was carried out in two parts: (i) development of denitrifying granular sludge at different cycle times of 4, 6 and 8 h and feed calcium concentrations of 0, 50 and 100 mg Ca²⁺/L, and (ii) investigation of fouling mechanisms of denitrifying granular sludge. It was found that a short cycle time could favor denitrifying granulation and helped improve the granules properties, such as size, settleability, strength and bioactivity, whereas a high calcium concentration could facilitate the formation of big size and dense denitrifying granules.

To investigate the fouling mechanisms of denitrifying granular sludge, the microfiltration tests were conducted using nitrocellulose membranes with respective pore sizes of 0.45 and 0.22 µm, and the permeate fluxes and membrane resistances were compared for the suspension and supernatant of denitrifying granular sludge as well as conventional bioflocs sludge. Results showed that the permeability of denitrifying granular sludge was greatly improved in comparison with conventional suspended flocs, leading to a higher permeate flux and lower membrane fouling. The permeate flux of granules suspension with 100 mg/L calcium supplement was 2-fold higher than that of granules suspension without calcium addition. It was further revealed that the fine particles with size of 1.0 to 2.7 µm appeared to be the

major contributors to the observed membrane fouling. The presence of denitrifying granules in the suspension could contribute to 50-60% of the observed reduction in the total filtration resistance due to the fact that the faster-settling denitrifying granules would form a cake layer on the membrane surface, which eventually served as a prefilter to prevent the fine particles, colloidal particles and soluble extracellular polymeric substances (sEPS) from depositing on the membrane surface. In addition, sEPS was found to be the main foulants for the membrane pore blocking.

This study demonstrated the feasibility of denitrifying MBR for high-efficiency denitrification with the lower fouling tendency.

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LIST OF SYMBOLS

A	Volume of PVSK added into the sample (mL),
A_{eff}	Effective membrane surface area (m^2)
ABR	Anaerobic baffled reactor
AFR	Anaerobic fluidized bed reactor
AGMBR	Aerobic granular membrane bioreactor
Anammox	Anaerobic ammonium oxidization
AnSBR	Anaerobic sequencing batch reactor
AOB	Ammonium-oxidizing bacteria
B	Volume of PVSK added into the blank (mL)
BSA	Bovine serum albumin
Canon	Completely autotrophic nitrogen removal over nitrite
C_m	Initial methanol concentration (mg TOC /L)
COD	Chemical oxygen demand (mg/L)
CP	Concentration polarization
C/N	Ratio of organic carbon to nitrate-nitrogen (mg TOC/mg NO_3^- -N)
DCB	Bivalence cation binding
DNR	Specific denitrification rate (mg N/g VSS·d)
DO	Dissolved oxygen (mg/L)
DO_0	Initial dissolved oxygen (mg/L)
EBCT	Empty bed column time (h)
EGSB	Expanded granular sludge bed
EMLSS	Mixed liquor suspended solids in effluent (mg/L)
EPS	Extracellular polymeric substances (mg/g VSS)
FA	Free ammonia
HRT	Hydraulic retention time (h)
ICAR	Internal circulation anaerobic reactor
J	Permeate flux ($\text{L}/\text{m}^2\cdot\text{h}$)
J_0	Initial permeate flux ($\text{L}/\text{m}^2\cdot\text{h}$)
K	Constant depending on the fouling mechanism involved

K_a	Blocked membrane surface per unit of the total volume permeated through the membrane (1/m).
K_c	Areas of the cake per unit of total permeate volume (1/m).
K_d	Decay rate of nitrifying bacteria (1/d)
K_n	NH_4^+ -N based Monod constant (mg/L)
$K_{\text{NO}_3^-}$	Constants (mg/L)
K_o	Constants (mg/L)
K_s	Hermia's parameter, which is the decrease in the cross section area of the pores per unit of permeates volume (1/m).
LSCFB	Liquid-solid circulating fluidized bed bioreactor
MBR	Membrane bioreactor
MF	Microfiltration
MFI	Modified fouling index (s/L^2)
MLI	Microbial load index
MLSS	Mixed liquor suspended solids (mg SS/L)
MLVSS	Mixed liquor volatile suspended solids (mg VSS/L)
n	Compressibility index ($0 \sim 1$)
n	Blocking index (0, 1, 1.5 or 2)
N	Normality of PVSK standard (0.0005 N)
NFABR	Normal feed anaerobic baffled reactor
$[\text{NH}_4^+ \text{-N}]$	Ammonium concentration (mg/L)
$[\text{NO}_2^- \text{-N}]_o$	Initial nitrite-nitrogen concentration (mg NO_2^- -N/L)
$[\text{NO}_3^- \text{-N}]_o$	Initial nitrate-nitrogen concentration (mg NO_3^- -N/L)
NOB	Nitrite oxidizing bacteria
Oland	Oxygen-limited autotrophic nitrification-denitrification
OLR	Organic loading rate (g COD/L d)
ORP	Redox potential
$P_{0.22}$	Permeate of granules supernatant filtrated through membrane with pore size of 0.22
$P_{0.45}$	Permeate of granules supernatant filtrated through membrane with pore size of 0.45
$P_{1.0}$	Permeate of granules supernatant filtrated through membrane with

	pore size of 1.0
P _{2.7}	Permeate of granules supernatant filtrated through membrane with pore size of 2.7
PN	Polymeric protein (mg/g VSS)
PS	Polysaccharides (mg/g VSS)
PVA	Polyvinyl alcohol
PVDF	Polyvinylidene difluoride
PVSK	Polyvinyl sulfate potassium salt
q _n	Specific ammonium utilization rate (g NH ₄ ⁺ -N/g VSS d)
q _{n,max}	Maximum specific ammonium utilization rate (g NH ₄ ⁺ -N/g VSS d)
q _{NO₃}	Specific growth rate of denitrifiers (1/d)
q _{NO₃⁻, max}	Maximum specific growth rate of denitrifiers (1/d)
RH	Relative hydrophobicity
R _c	Cake resistance (1/m)
R _m	Membrane resistance (1/m)
R _p	Pore block resistance (1/m)
R _r	R _r is the ratio of the cake resistance to the clean membrane resistance (dimensionless).
R _t	Total resistance (1/m)
SBR	Sequencing batch reactor
sEPS	Soluble extracellular polymeric substances (mg/L)
SFABR	Split feed anaerobic baffled reactor
SLAD	Sulfur–limestone autotrophic denitrification
SMA	Specific methanogenic activity (g methane-COD/ g VSS d)
SND	Simultaneous nitrification-denitrification
sPN	Soluble polymeric protein (mg/L)
sPS	Soluble polysaccharides (mg/L)
S _c	Organic carbon concentration (mg TOC/L)
S _{NO₃⁻}	Nitrate concentration (mg NO ₃ ⁻ -N/L);
SMP	Soluble microbiological products
SVI	Sludge volume index (mL/g SS)
TMP, ΔP	Trans-membrane pressure (kPa)

TOC	Total organic carbon (mg TOC/L)
$t_{1/2}$	Filtration time when permeate flux declined to half of the initial permeate flux (min)
UASB	Upper anaerobic sludge blanket reactor
UF	Ultrafiltration
V	Volume of sample in charge determination (mL)
VFAs	Volatile fatty acids
VLR	Volumetric loading rate
Y_n	Observed growth yield (g VSS/g NH_4^+ -N).
α	Specific cake resistance (m/kg)
η_{NO_3}	Nitrate removal efficiency (%)
η_{TOC}	TOC removal efficiency (%)
μ	Viscosity of the filtrate (Pa s)
μ_n	Specific growth rate (1/d)
$\mu_{n,\text{max}}$	Maximum specific growth rate (1/d)
μ'_n	Observed ammonium biomass growth rate (1/d)
ρ	Density of the filtrate (g/L)
θ	Temperature coefficient
ΔM	Change of filtrate mass during filtration time (g)
Δt	Time duration of mass change in microfiltration (h)

LIST OF PUBLICATIONS

Journal Papers:

Liu Y.J., Sun D.D. (2012) Membrane fouling mechanism in dead-end microfiltration of denitrifying granular sludge mixed liquors developed in SBRs at different calcium concentrations. *Journal of Membrane Science*, 396: 74-82.

Liu Y.J., Sun D.D. (2012) Particle size-associated membrane fouling in microfiltration of denitrifying granules supernatant. *Chemical Engineering Journal*, 181-182: 494-500.

Liu Y.J., Sun D.D. (2011) Calcium argumentation for enhanced denitrifying granulation in sequencing batch reactors. *Process Biochemistry*, 46: 987-992.

Liu Y.J., Sun D.D. (2011) Development of denitrifying granules in sequencing batch reactors. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substance & Environmental Engineering*, 46(5): 518-525.

Liu Y.J., Sun D.D. (2010) Comparison of membrane fouling in dead-end microfiltration of denitrifying granular sludge suspension and its supernatant. *Journal of Membrane Science*, 352(1-2): 100-106.

Liu Y., **Liu Y.J.** (2008) Biosorption isotherms, kinetics and thermodynamics. *Separation and Purification Technology*, 61(3): 229-242.

Khor S.L., Sun D.D., **Liu Y.J.**, Leckie J.O. (2007). Biofouling development and rejection enhancement in long SRT MF membrane bioreactor. *Process Biochemistry*, 42(12): 1641-1648.

Book Chapter:

Liu Y.J., Sun D.D., Liu Y. (2011). Denitrification process for wastewater treatment. In: Denitrification: Processes, Regulation and Ecological Significance. Nova Science Publishers, New York.

CHAPTER 1

INTRODUCTION

1.1. Background

The intensive exploitation of farms through a profligate application of fertilizers coupled with increasing industrial and landfill leachate has increased the nitrogen loading in receiving waterways (Vitousek et al. 1997; Visvanathan et al. 2008; Wu et al. 2009). Ground water distributed in rural and suburban areas is an essential drinking-water source common to both humans and other organisms. Nitrogen-containing compounds released into water body can create serious problems, such as eutrophication and deteriorated water quality. These problems have potentially adverse effects on human health and can be responsible for the development of methemoglobinemia, a medical condition occurring when nitrate concentration in drinking water is in excess of 10 mg/L (Foglar and Briski 2003; Dybas 2005; Chen et al. 2010). In recent decades, the nitrification-denitrification process has been successfully applied to remove nitrogen from municipal and industrial wastewaters (Chuang et al. 1996; Dincer and Kargi 1999; Bernat and Wojnowska-Baryla 2007). In this process, nitrifying bacteria nitrify ammonia to form nitrate which is subsequently reduced to nitrogen gas by denitrifying bacteria.

Some operational problems have been encountered when using the conventional denitrification unit. Conventional denitrification has often been operated in suspended culture in which high biomass concentration cannot be easily achieved due to the poor settleability of denitrifying sludge. In order to achieve the high nitrate removal efficiency, sufficient denitrifying biomass should be accumulated in the reactor. In this case, a large reactor is usually required due to the long sludge retention time (SRT). In some cases, a separate settling tank is required to further settle the sludge which is recycled to the reactor. One solution to this problem is to develop suspended denitrifying sludge into granular sludge with excellent settleability. Anaerobic granulation has been studied for high-strength organic wastewater treatment since 1980 (Lettinga et al. 1980), while development of denitrifying granules has drawn strong interest (Eiroa et al. 2004; Franco et al.

2006; Qin and Liu 2006). Microbial and hydraulic selection pressures would facilitate the formation of compact and dense granules with excellent settleability. Such a granulation process would be influenced by substrate composition, organic loading rate, hydraulic retention time (HRT), up-flow velocity, shear force, reactors configuration etc. (Grotenhuis et al. 1991; Omil et al. 1996; Picioreanu et al. 2004; Pol et al. 2004; Muda et al. 2010).

Membrane bioreactor (MBR) technology for wastewater reclamation enjoys a global popularity owing to its modular design and high effluent quality. MBR successfully combines the merits of both biological and membrane separation processes. In practice, however, the MBR process continues to be affected by severe membrane fouling that represents a significant cost and energy burden for the system. Suspended microorganisms readily attach onto the membrane surface during process operation, resulting in cake layer formation which decline in permeate flux and reduced effluent quality (Magara and Itoh 1991; Le-Clech et al. 2006). It has been shown that membrane fouling is related to membrane's physical and chemical properties, such as material, pore size, surface hydrophobicity and charge (Chang et al. 2002; Hwang et al. 2008), whereas it is also affected by the characteristics of biomass, such as mixed liquor suspended solids (MLSS) concentration, particle size, surface charge, amount and distribution of extracellular polymer substances (EPS) (Paul and Hartung 2008; Yu et al. 2009). In this, granular sludge would have advantages over suspended microorganisms in alleviating membrane fouling in a MBR.

A denitrifying granular sludge MBR would appear to be an ideal process for high-efficiency denitrification and production of high-quality effluent with controllable membrane fouling. Therefore, this study looked into development of denitrifying granules under various conditions, and filterability and fouling mechanisms of denitrifying granular sludge.

1.2. Objectives and Scope

The objectives and scope of study include (i) development of denitrifying granules at different cycle times which serve as a hydraulic pressure in sequencing

batch reactors (SBRs); (ii) investigation of the impact of calcium concentration on denitrifying granulation in SBRs; (iii) microfiltration performance of denitrifying granular sludge suspension and its supernatant, and comparison was made with denitrifying suspended sludge; (iv) mechanisms of membrane fouling by denitrifying granules, fine particles, colloids, solutes and sEPS; (v) influence of the calcium concentration, granules concentration and fine particles concentration on microfiltration of granules suspension. Details are illustrated in Fig. 1.1.

Figure 1.1 Illustration of research scope.

1.3. Organization of the Thesis

This thesis consists of 8 chapters with the following organization. Chapter 1 introduced the background information, objectives and scope of this study. Chapter 2 presented a detail literature review about denitrification, anaerobic granulation and membrane bioreactor. The results and discussion of all this study were grouped into chapters 3 to 7. Chapter 3 discussed the development of denitrifying granules in SBRs and their physicochemical characteristics at different cycle times. Chapter 4 compared microfiltration of denitrifying granular sludge suspension and its supernatant with the suspended denitrifying sludge. Chapter 5 investigated the influence of calcium concentration on denitrifying granulation, physicochemical characteristics and the bioactivity of denitrifying granules. Chapter 6 focused on the membrane fouling mechanisms of denitrifying granular sludge mixed liquor. The impact of the composition of granules mixed liquor on membrane fouling was studied in this chapter. The impact of calcium concentration, granules concentration and fine particles concentration on the microfiltration of denitrifying granules mixed liquor were also systematically investigated and discussed. Chapter 7 looked into the influence of the fine particles size distribution and composition of the supernatant on microfiltration behaviors and membrane fouling. The effects of calcium concentration on the microfiltration of supernatants were thoroughly interpreted. The conclusions derived from this study were summarized in Chapter 8 with recommendations for the future study.

CHAPTER 2

LITERATURE REVIEW

2.1. Problems Caused by Nitrogen

Nitrogen-containing compounds released into the environment can create serious problems, such as eutrophication and deterioration of water quality which poses potential health hazards to humans. Regulators continue to implement ever stringent laws and enforcement protocols effort to minimize nitrogenous pollution. In the conventional wastewater treatment processes, ammonia is oxidized by ammonia oxidizers to nitrite that is subsequently oxidized by nitrite oxidizers to nitrate under aerobic condition. Nitrate produced is reduced to nitrogen gas under anoxic condition. Over the last decade, new nitrogen removal pathways have also been reported. These include anaerobic ammonium oxidization (Anammox), simultaneous nitrification and denitrification (SND) etc., all of which represent potential candidates for development into new high-efficiency and low-energy biological nitrogen removal technologies (Zhang et al. 2004; Liu et al. 2008; Zhang et al. 2009; Uemoto and Morita 2010; Wang et al. 2010).

2.2. Nitrification

Nitrification is a chemolithoautotrophic process, in which ammonium is oxidized to nitrate using inorganic carbon as a carbon source under strict aerobic condition, and involves two consecutive oxidative steps: ammonium oxidation to nitrite by ammonia oxidizers (e.g. *Nitrosomonas*, *Nitrosococcus*, *Nitrosopira*, *Nitrosovibrio* and *Nitrosolobu*), and nitrite oxidation to nitrate by nitrite oxidizer (e.g. *Nitrobacter*, *Nitrospira*, *Nitrospina*, *Nitrococcus* and *Nitrocystis*) (Foley et al. 2010; Yu et al. 2010)

2.2.1. Nitrification stoichiometry and kinetics

Eq. 2.1 shows the overall biosynthetic reaction of nitrifying bacteria (USEPA 1993):

The salient points derived from Eq. 2.1 include: (i) nitrification is an obligatorily aerobic reaction, e.g. about 4.18 g O₂ is required to nitrify 1 g NH₄⁺-N; (ii) the overall growth yield of nitrifying bacteria is about 0.17 g VSS/g NH₄⁺-N, which is much lower than that of heterotrophic bacteria; (iii) the oxidation of NH₄⁺-N produces hydrogen ions, as a result, approximately 7 g alkalinity as CaCO₃ per g of NH₄⁺-N oxidized is required to buffer the system against the hydrogen ions released.

The growth of nitrifying bacteria is often described by a Monod-type equation:

$$\mu_n = \mu_{n,\max} \frac{[\text{NH}_4^+ - \text{N}]}{K_n + [\text{NH}_4^+ - \text{N}]} \quad (2.2)$$

where μ_n and $\mu_{n,\max}$ are the respective specific growth and maximum specific growth rates (1/d), $[\text{NH}_4^+ - \text{N}]$ is ammonium concentration (mg/L) and K_n is NH₄⁺-N based Monod constant (mg/L). Meanwhile, the specific NH₄⁺-N removal rate (q_n , g NH₄⁺-N/g VSS d) is related to μ_n in a way such that:

$$q_n = \frac{\mu_n}{Y_n} = q_{n,\max} \frac{[\text{NH}_4^+ - \text{N}]}{K_n + [\text{NH}_4^+ - \text{N}]} \quad (2.3)$$

where $q_{n,\max}$ is the maximum specific ammonium utilization rate (g NH₄⁺-N/g VSS d), and Y_n is the observed growth yield (g VSS/g NH₄⁺-N).

The growth of microorganisms can also be expressed in terms of their generation time. The generation times of nitrifying bacteria are much longer than those of heterotrophic bacteria, indicating that a long solids retention time or sludge

age (SRT) is required to retain an adequate population and amount of nitrifying bacteria in the nitrification process. At steady state, the solids leaving the system are equal to the solids produced, thus the growth rate and SRT of organisms are related by:

$$\frac{1}{\text{SRT}} = \mu_n - K_d \approx \mu'_n \quad (2.4)$$

where K_d is decay rate of nitrifying bacteria (1/d), and μ'_n is the observed nitrifying bacteria growth rate (1/d).

2.2.2. Factors affecting nitrification

Nitrifying bacteria are sensitive to changes in the culture environment. Evidence shows that many factors, including free ammonia, temperature, pH, DO concentration and presence of inhibitors, can affect nitrifying growth and subsequently nitrification efficiency (Fdz-Polanco et al. 1996; Park et al. 2010; Qiu et al. 2010).

Existing evidence shows that free ammonia (FA) can inhibit nitrifying bacteria at relatively high concentration levels, while nitrite oxidizer is much more sensitive to FA than ammonium oxidizer. According to Ford et al. (1983), FA concentration (mg/L) can be estimated as follows:

$$\text{FA} = \frac{17}{14} \frac{[\text{NH}_4^+ - \text{N}] \times 10^{\text{pH}}}{\exp[6334/(273 + T)] + 10^{\text{pH}}} \quad (2.5)$$

Eq. 2.5 shows that the FA concentration is strongly dependent upon the culture pH. The respective inhibition threshold of FA has been reported to be 10 to 150 mg/L for ammonium oxidizer, and 0.1 to 4.0 mg/L for nitrite oxidizer (Anthonisen et al. 1976; Fdz-Polanco et al., 1996).

Hydrogen ions produced during nitrification (Eq. 2.1) would lower water pH. Evidence shows that the optimal pH for nitrification falls into a very narrow range of 7.8 to 8.0, and the culture pH higher than the optimal values would inhibit the activity of nitrifying bacteria due to the pH-dependent production of FA (Eq. 2.5).

At lower pH, the nitrification rate declines for both unacclimated and acclimated cultures. For example, nitrification would be completely blocked at pH values below 5.0. Consequently, for performance stability, it is best to maintain a pH at 6.5-8.0.

Nitrifying bacteria can survive over a wide range of temperature from 4 to 45°C (Laudelout and van Techelen 1960; Qiu et al., 2010; Tokutomi et al. 2010). The maximum growth rate and the half-saturation constant of nitrifying bacteria are temperature-sensitive, and some Arrhenius-type expressions for the effect of temperature on the maximum growth rate and half-saturation constant of ammonium oxidizer over a temperature range of 5-30°C was proposed for design of a suspended growth process (USEPA 1975):

$$\mu_{n, \max} = 0.18e^{0.116 (T - 15)} \quad (2.6)$$

$$K_n = 0.405 e^{0.118 (T - 15)} \quad (2.7)$$

Nitrification is a strict aerobic reaction, i.e. molecular oxygen serves as the final electron acceptor. For pure cultures of ammonium and nitrite oxidizers, the critical dissolved oxygen concentration below which nitrification does not occur is around 0.2 mg/L (Dowing et al. 1964; Park et al., 2010). However, in full-scale wastewater treatment process, dissolved oxygen should not be less than 2.0 mg/L (Strankewich 1974; Park et al., 2010; Zapata et al. 2010). In real wastewater treatment systems, the effects of ammonia, temperature, pH, and dissolved oxygen on the nitrification process are interrelated. It has been proposed that the combined effect of these factors on biological nitrification can be described in the form of the Monod-type expression (Chen 1970; USEPA 1975):

$$\mu_n = \mu_{n, \max} \times \frac{[\text{NH}_4^+ - \text{N}]}{K_n + [\text{NH}_4^+ - \text{N}]} \times \frac{\text{DO}}{K_o + \text{DO}} \times [1 - 0.833 (7.2 - \text{pH})] \quad (2.8)$$

Nitrifying bacteria are susceptible to inhibitory and toxic compounds. Ammonium oxidizer has been found to be more susceptible than nitrite oxidizer. Most heavy metals can inhibit the growth of nitrifying bacteria. For example, Paolo

et al. (1999) reported that the inhibition of heavy metals to nitrifying sludge was in the order of $\text{Cd} > \text{Cu} > \text{Zn}$ and $\text{Pb} > \text{Cr}$.

2.3. Denitrification

Denitrification is a heterotrophic bioconversion of nitrite or nitrate to nitrogen gas under anoxic conditions. Denitrification involves the transfer of electrons from electron donor (organic carbon) to the electron acceptor (nitrate or nitrite). The electron donor can be organics in the raw wastewater or external organic carbon (e.g. methanol, ethanol etc).

2.3.1. Denitrification reaction and kinetics

According to Metcalf & Eddy (2003), the overall denitrifying reaction can be written as follows with methanol as the electron donator:

It appears that 2.47 g methanol is required to denitrify one gram of nitrate-nitrogen. In practice, wastewater often contains nitrite and dissolved oxygen, i.e. consumption of organic carbon is also related to the nitrite reduction and oxygen utilization. As suggested by McCarty and Bremner (1992), the concentration of initial organic carbon required for denitrification can be calculated as follows:

$$C_m = 2.47 [\text{NO}_3^- - \text{N}]_0 + 1.53 [\text{NO}_2^- - \text{N}]_0 + 0.87 \text{DO}_0 \quad (2.10)$$

where C_m is initial methanol concentration (mg TOC/L); $[\text{NO}_3^- - \text{N}]_0$ is initial nitrate-nitrogen concentration (mg $\text{NO}_3^- - \text{N}/\text{L}$); $[\text{NO}_2^- - \text{N}]_0$ is initial nitrite-nitrogen concentration (mg $\text{NO}_2^- - \text{N}/\text{L}$); and DO_0 is initial dissolved oxygen (mg/L).

In a denitrification process, the growth rate of denitrifiers is not only determined by the nitrate concentration, but also strongly depends on the organic carbon and oxygen concentrations. The Monod-type equation is often used to describe the denitrification kinetics (Batchelor 1982):

$$q_{\text{NO}_3^-} = q_{\text{NO}_3^-, \text{max}} \times \frac{S_{\text{NO}_3^-}}{K_{\text{NO}_3^-} + S_{\text{NO}_3^-}} \times \frac{S_C}{K_C + S_C} \times \frac{1}{K_O + \text{DO}} \quad (2.11)$$

where $q_{\text{NO}_3^-}$ is the specific growth rate of denitrifiers (1/d); $q_{\text{NO}_3^-, \text{max}}$ is the maximum specific growth rate of denitrifiers (1/d); $S_{\text{NO}_3^-}$ is nitrate concentration (mg NO_3^- -N/L); S_C is organic carbon concentration (mg TOC/L); DO is dissolved oxygen concentration (mg/L); $K_{\text{NO}_3^-}$, K_C and K_O are constants (mg/L).

2.3.2. Factors affecting denitrification

2.3.2.1. Temperature, pH and dissolved oxygen

Temperature is one of the most important factors that affect the denitrification process. The temperature-effect on denitrifying activity can be described by an Arrhenius-type equation (Orhon et al. 2000):

$$\text{DNR}_{T_1} = \text{DNR}_{T_2} \cdot \theta^{(T_1 - T_2)} \quad (2.12)$$

where DNR_{T_1} and DNR_{T_2} are the specific denitrification rates at temperature T_1 and T_2 (mg N/g VSS·d), respectively and θ is temperature coefficient. It can be seen that a faster denitrification would be expected at a higher temperature in the temperature range of 2-35°C (Barlindhaug and Odegaard 1996). For example, DNR was increased from 140 mg N/g VSS d to 830 mg N/g VSS d when experimental temperature was raised from 8 to 30°C (Obaja et al. 2003).

The specific denitrification rate is related to water pH (Timmermans and Van Haute 1983; Shen et al. 2009). Optimal pH for denitrification is usually within neutral range. The nitrite accumulation has been observed due to the reduced denitrifying activity at the pH lower than 6.8 (Glass and Silverstein 1998; Foglar and Briski 2003). Denitrification occurs at dissolved oxygen (DO) concentration below 1 mg/L, whereas denitrifying activity would be greatly inhibited when DO concentration is higher than 2 mg/L (Alefounder et al. 1981).

2.3.2.2. External organic carbon

Methanol, glucose, glycerol, acetic-acid, lactic-acid and broth, etc continue to be widely used as external organic carbon source for denitrification (Akunna et al. 1993; Christensson et al. 1994; Shen et al., 2009; Adav et al. 2010). The growth of denitrifying bacteria, DNR and nitrate and TOC removal efficiency are all influenced significantly by the organic carbon source (Blaszczyk 1993; Lee and Welander 1996; Bilanovic et al. 1999; Mokhayeri et al. 2008; Wu et al. 2010; Zhao et al. 2010). The ratio of organic carbon to nitrate-nitrogen (C/N) influences denitrification. According to the denitrification reaction stoichiometry with ethanol, 0.72 mg ethanol-TOC is needed to denitrify 1 mg of nitrate-nitrogen. Foglar et al. (2004) reported that at the methanol to nitrate-nitrogen ratios of 0.75 mg TOC/mg NO_3^- -N and 0.56 mg TOC/mg NO_3^- -N, the nitrate removal efficiency in 7 h reaction was only about 42% and 38%, respectively. However, when the C/N ratio was increased to 1.35 mg TOC/mg NO_3^- -N, 700 mg NO_3^- -N/L in the influent was removed completely within 7 h (Fernandez-Nava et al. 2008). The similar phenomenon was also observed in denitrification with ethanol as external carbon source (Gomez et al. 2003; McAdam and Judd 2007).

2.3.2.3. Nitrite accumulation

In biological nitrogen removal process, nitrite accumulation can inhibit denitrification (Glass and Silverstein 1998). Nitrite accumulation is believed to related to increase of pH of the mixed liquor (Lee and Rittmann 2003; Sun et al. 2009). The optimum pH for heterotrophic denitrification is in the range of 7 to 8 (Hiscock et al. 1991; Kapoor and Viraraghavan 1997; Li et al. 2008; Shen et al., 2009). A pH outside the optimal range may cause accumulation of intermediates, such as nitrite. For example, nitrite tended to accumulate at alkaline pH, whereas the nitrate-nitrogen removal efficiency was found to decline when pH was increased from 8.4 to 9.5 (Lee and Rittmann 2003; Wang et al. 2009). Evidence shows that inhibition of nitrite to denitrification is due to the formation of free nitric acid (HNO_2), and the inhibitory threshold of HNO_2 to denitrification was reported to be in the range of 0.13 to 0.2 mg/L (Abeling and Seyfried 1992; Ma et al. 2010).

2.4. Novel Nitrogen Removal Pathways

Biological nitrogen removal highly efficient and cost-effective with broad applied in wastewater treatment. Various novel biological nitrogen removal processes, such as stimulation nitrification and denitrification (SND), anaerobic ammonium oxidation (Anammox), completely autotrophic nitrogen removal over nitrite (Canon) process and oxygen-limited autotrophic nitrification-denitrification (Oland) process, have been developed.

2.4.1. Aerobic denitrification

Denitrification can occur under aerobic conditions (Bernat and Wojnowska-Baryla 2007), which is called aerobic denitrification. Some bacteria, such as *Microvirgula aerodenitrificans* (Patureau et al. 1998; Bouchez et al. 2009), *Thiosphaera pantotropha*, *Alcaligenes faecalis* (Otte et al. 1996; Kim et al. 2008; Taylor et al. 2009; Miyahara et al. 2010; Zhao et al. 2010), *Pseudomonas nautical* (Bonin and Gilewicz 1991; Miyahara et al., 2010), known as aerobic denitrifiers, have ability to tolerate oxygen at the levels of 5.0-6.0 mg/L and undertake denitrification under oxic conditions. Most aerobic denitrifiers are heterotrophic organisms and widespread in the environment. These denitrifiers can reduce nitrate rapidly to nitrogen gas without nitrite accumulation under aerobic condition. The simultaneous consumption of oxygen and nitrate by aerobic denitrifiers has been known as co-respiration, which allows nitrifiers and aerobic denitrifiers to co-exist in a single aerated reactor (Patureau et al., 1998; Zhao et al., 2010).

2.4.2. Autotrophic denitrification

Heterotrophic denitrifiers utilize simple organic substances (e.g. methanol and ethanol) as electron donor, while some denitrifying bacteria, such as *Thiobacillus denitrificans* and *Thiomicrospira denitrificans*, are chemolithoautotrophic and can use reduced sulfur compounds, e.g. elemental sulfur (S^0), sulfide (S^{2-}), thiosulfate or sulfite as electron donors (Zumft 1992; Byun et al.

2008; Shao et al. 2009; Zhang et al. 2009; Moon et al. 2010). Chemolithotrophic denitrification with elemental sulfur as the electron donor is known as the sulfur–limestone autotrophic denitrification (SLAD) process. Chemolithotrophic denitrification has attracted increasing interest due to the following major advantages compared over heterotrophic denitrification: (i) inorganic substances are utilized as electron donors, eliminating potential problems associated with residual organics in the effluent; (ii) no external organic carbon is needed, leading to reduced operation cost and (iii) lower cell yield. Since no organic carbon source is needed in the SLAD process, and autotrophic denitrifiers exist widely in natural sediments or soil. As an alternative, the SLAD process may be considered as a replacement for heterotrophic denitrification in constructed wetlands or stabilization ponds (Zhao et al. 2004; Ruan et al. 2009; Zhang et al., 2009).

2.4.3. Simultaneous nitrification-denitrification

Simultaneous nitrification-denitrification (SND) allows the nitrogen removal in a single reactor without separation of the two processes in time or space. In the traditional biological nitrogen removal process, ammonium is oxidized to nitrate in the nitrification process under aerobic condition, and nitrate is reduced to nitrogen gas in the denitrification process by heterotrophic denitrifiers under anoxic or anaerobic condition. In the SND process, ammonium is only oxidized to nitrite by selective growth of ammonium-oxidizing bacteria (AOB) under aerobic condition, and nitrite is subsequently reduced to nitrogen gas by denitrifying bacteria under anoxic condition (Blackburne et al. 2008). SND process via nitrite pathway is illustrated in Fig. 2.1. Compared to the traditional nitrification and denitrification via nitrate, the main advantages of SND include: (i) 25% lower oxygen consumption in the aerobic stage, implying a 60% energy savings; (ii) in the anoxic stage the electron donor requirement is lowered by up to 40%; (iii) nitrite denitrification rate is 1.5 to 2 times higher than that of nitrate; (iv) reduced CO₂ emission by 20%; (v) 33-35% lower sludge production in nitrification process and 55% in denitrification process. (Andrade do Canto et al. 2008; Li et al. 2008): A liquid-solid circulating fluidized bed bioreactor (LSCFB) with anoxic and aerobic

columns and lava rock as the biofilm carrier has been reported to be very efficient for SND with more than 90% nitrification and 70% of overall nitrogen removal at an empty bed column time (EBCT) of 0.44 h with the ratio of organic loading rate (OLR) to nitrogen loading rate of 10 kg COD/kg N (Chowdhury et al. 2008).

Figure 2.1 Simultaneous nitrification-denitrification via nitrite pathway (Peng and Zhu 2006).

2.5. Anaerobic Granulation

Anaerobic degradation process has been well developed and widely applied to remove the nutrients and biodegradable organics from the industrial and domestic wastewaters (Chuang et al., 1996; Choi et al. 2008). Anaerobic biomass flocs are commonly used in the conventional biodegradation process. In order to achieve high removal efficiency, a large quantity of biomass flocs should be accumulated in the reactor, leading to a bigger volume of reactor due to the longer sludge retention time (SRT). Moreover, the poor settleability of anaerobic biomass would cause the poor effluent quality with high biomass concentration. Therefore, a separated sludge retention tank is often needed to reduce the biomass concentration in the effluent. These in turn lead to increased capital and operation cost for biomass recycling (Lettinga et al. 1980).

In order to solve the problems of poor settleability of anaerobic sludge, anaerobic granular sludge has been developed in upflow anaerobic sludge blanket (UASB) reactor (Fig. 2.2), and applied to treat various types of industrial and municipal wastewaters (Lettinga et al., 1980; Chang et al. 1995; Baloch and Akunna 2003). Anaerobic granules are dense and spherical aggregates of the

biomass. The settleability of anaerobic granules in term of SVI was reported to be as low as 7.8 mL/g SS (Grotenhuis et al., 1991), which was much lower than anaerobic floc biomass. As compared to suspended anaerobic sludge, anaerobic granules have excellent settleability that in turn ensures a much easy and fast solid-effluent separation, higher biomass concentration and greater ability to withstand shock loadings (Liu et al. 2009). The size of anaerobic granules may vary in a wide range from 0.3 to 3.0 mm (Peng et al. 1999; Lin and Chen 2001; Chang and Lin 2004; Muda et al., 2010). It has been believed that the upflow velocity in UASB would provide a unique driving force to separate the poorly settling biomass from the well settling biomass (Alphenaar et al. 1993; Shayegan et al. 2005; Subramanyam and Mishra 2008; Basu and Gupta 2010). Nowadays, anaerobic granular sludge process has been regarded as one of the most successful and promising anaerobic technologies for wastewater treatment.

Figure 2.2 Schematic UASB reactor (Franco et al., 2006).

2.5.1. Factors affecting anaerobic granulation

Anaerobic granulation is self-aggregation of anaerobic bacteria under certain conditions. It is understood that many factors may affect the formation of anaerobic

granules. These factors include solid and hydraulic retention times, liquid upflow velocity, separation methods of the aggregated biomass from the dispersed biomass, organic loading rate and wastewater composition, etc. (Bhatti et al. 2001; Angenent et al. 2004; Peveré et al. 2007; Muda et al., 2010).

2.5.1.1. Temperature and pH

Full-scale anaerobic granular sludge plants have been generally operated in the mesophilic (20-45°C) or thermophilic (45-65°C) temperature range (She et al. 2006; van Hullebusch et al. 2007; Zhou et al. 2007; Sahinkaya and Yucesoy 2010). Operational temperature apparently affects the granulation process and biological activity of methanogenesis more than the acidogenesis (Chou et al. 2004). O'Reilly et al. (2009) reported that both granulation ratio and COD removal efficiency of methanogenic granules in an expanded granular sludge bed (EGSB) operated at $37\pm1^\circ\text{C}$ was higher than that at $15\pm1^\circ\text{C}$. It was found that the compositions of the granules altered when the operation temperature varied. Temporal shifts in methanogenic community towards the dominance of hydrogenotrophic methanogens, particularly methanomicrobiales and methanobacteriales were observed in mesophilic granular biofilms incubated at low temperatures (Connaughton et al. 2006). So far, a number of successful low-temperature anaerobic digestion trials, treating various types of synthetic and real wastewaters, have demonstrated robust and stable operation with comparable performance to mesophilic systems. Treating wastewaters at their discharge temperature below 18°C could reduce the operation cost by removing a heating process and increasing net biogas yield (McCarty 2001; Connaughton et al., 2006). Some sudden temperature changes resulting in the reactor failure were also reported. Therefore, the temperature of the reactors should be controlled carefully in order to maintain long-term stability and activity of the granules.

A suitable pH value is necessary for stable operation of anaerobic granules bioreactors. The optimum pH range may vary for different microorganisms. For example, the hydrogen producing microorganisms could produce hydrogen gas efficiently at pH value above 4.0 (Lay 2000), whereas the best pH range for

acidogenes and methanogenes growth fall in to 5.0-6.5 and 6.5-7.5, respectively. In addition, acidogenes and methanogenes would compete with each other in growth at the pH of 6.2 to 6.5 (He 1998; Ji et al. 2009). However, methanogenes are more sensitive to pH changes than acidogenes. e.g. acetoclastic methanogenic activity of UASB was found to be reduced by 61% when pH declined from 7 to 5 (Ahn et al. 2002). It should be noted that organic acids can be accumulated in anaerobic process due to the different growth rates of fermentative bacteria. The build-up of organic acids in turn could suppress the methanogenic activity of UASB. Therefore, the alkalinity buffer should be provided to mitigate the effect of acids (Kanat and Saral 2009; Sakar et al. 2009). Evidence shows that the control of pH at an optimum level is important for the successful operation of an anaerobic granules bioreactor (Turkdogan-Aydinol and Yetilmezsoy 2010).

2.5.1.2. Effect of organic loading

Organic loading rate (OLR) is one of the most important design and operation parameters of anaerobic granulation processes, and it is determined by both influent chemical oxygen demand (COD) concentration and flow rate. This suggests that the OLR describes the food availability to microorganisms in a biological system. At a low OLR, the microorganisms might not have sufficient food sustain their growth, whereas a high OLR would encourage a fast microbial growth. It had been widely reported that gradual increase in OLR during the start-up would favour anaerobic granulation (Najafpour et al. 2006; Zhou et al., 2007; Zhang et al. 2008). It appears that a reasonably high OLR would be essential for rapid granulation and subsequent process stability. It was observed that granulation in an upflow anaerobic sludge-fixed film (UASFF) reactor was increased from 2 to 4 months to 20 d when OLR was increased from 2.63 to 23.15 g COD/L d (Najafpour et al., 2006). A practical way for rapid anaerobic granulation in UASB systems is to increase the OLR based on an 96% reduction of biodegradable COD with close monitoring washout of suspended solid (Zhang et al. 2009). In addition, it appears from previous study that the UASB start-up could be guided by a microbial load index (MLI), which is defined as the ratio of applied OLR to specific

methanogenic activity (SMA) in terms of g methane-COD produced per g VSS per day (Tay and Yan 1996). According to Tay and Yan (1996), a MLI value of around 0.8 would be appropriate for rapid anaerobic granulation in UASB systems. Bhanja and Ghangrekar (2008) observed that proper granulation could not be achieved and COD removal efficiency was drastically reduced when OLR was less than 1.0 kg COD/m³ d, in both the reactors inoculated with and without polymer additive.

It should be pointed out that anaerobic bacteria would grow faster at a high OLR, and this in turn may result in a reduced strength of granules, leading to granule disintegration (Quarmby and Forster 1995). According to the Monod equation, a high microbial growth rate would be expected at an increased OLR, while high microbial growth rate would reduce the strength of three-dimensional structure of anaerobic granules (Morvai et al. 1992). Moreover, more biogas would be eventually produced at high applied OLR. If the applied OLR is too high in the period of UASB start-up, increased biogas production rate would cause serious hydrodynamic turbulence and further leads to the washout of seed sludge from the reactor, which sometimes is responsible for unsuccessful UASB start-up (Liu et al 2003). It was indicated that too high loading rate should be avoided because it could cause the unrecoverable decay of methanogenic activity and the serious unbalance between the feed stuff and biological requirement (Zhou et al., 2007).

2.5.1.3. Effect of hydraulic retention time and liquid up-flow velocity

Strong evidence shows that a high liquid upflow velocity would favour anaerobic granulation, unsuccessful granulation was observed at a weak hydrodynamic shear force which is also associated with the liquid upflow velocity in UASB systems (Alphenaar et al., 1993; Bhunia and Ghangrekar 2008). It has been reported that rapid anaerobic granulation could be achieved in UASB reactor by increasing high liquid upflow velocity, and shortening hydraulic retention time (HRT) (Alphenaar et al., 1993; Zhou et al., 2007). For example, Alphenaar et al. (1993) found that the mean size of anaerobic granules developed in UASB was increased from 0.6 mm to 1.3 mm after 140 d reaction when the up-flow velocity was increased from 0.05 m/h to 0.65 m/h. Recent studies on aerobic granulation

clearly suggested that biogranulation would be driven by various selection pressures (Liu et al. 2005), e.g. environmental conditions (e.g. temperature), process operation conditions (e.g. HRT, upflow velocity, wastewater type, organic loading rate and pH), type of reactor and seed sludge. According to Hulshoff Pol et al. (1988), a short HRT combined with a high liquid upflow velocity could cause washout of light and dispersed bioflocs from the system, and in turn would promote sludge granulation. This indicates that anaerobic granulation could be manipulated through controlling the hydrodynamic conditions in UASB reactors. Experiments by Noyola and Moreno (1994) provided strong support to such view, i.e. rapid conversion of suspended anaerobic flocs to anaerobic granular sludge was achieved by simply shortening HRT to less than 8 h, whereas the settleability of anaerobic granules developed was improved markedly with increasing the liquid upflow velocity. As the result, the sludge washout was reduced from 46 to 2%. A high upflow velocity combined with a short HRT appears to be a feasible strategy towards rapid anaerobic granulation.

2.5.1.4. Effect of cations

Addition of poly-valence cations, such as Al^{3+} , Ca^{2+} and Mg^{2+} , was a promising alternative for enhancing anaerobic granulation (Pevero et al., 2007; Ismail et al. 2010). Calcium is a common cation that has been used for speeding-up anaerobic granulation process. Addition of calcium has the following benefits on anaerobic granulation: (i) enhanced physicochemical interaction among the anaerobic biomass leading to strengthened granule structure and bigger granules. This is because Ca^{2+} can reduce the cell surface negative charge density and acts as a bridge to interconnect extracellular polysaccharide molecules; (ii) reduced the viscosity of the anaerobic granules suspension; (iii) improved aggregation/flocculation of fine particles, leading to good-quality effluent and (iv) increased size of bioparticles which help to prevent their wash-out from anaerobic reactors. It should be noted that calcium addition would eventually result in calcification of anaerobic granules. These would in turn negatively impact the bioactivity of the granules (Van Langerak et al. 2000; Bhunia and Ghangrekar 2008). According to the bivalence cation binding (DCB) theory, bivalence cations

could combine with the negative charged biomass to form granules with the strong structure. It has been believed that EPS secreted during the anaerobic granulation can help the biomass to attach each other, whereas calcium ion could combine with the EPS to form EPS- Ca^{2+} -EPS complexes, and further reduce the porosity inside the granules, and therefore increase the structural stability of anaerobic granules.

A high Ca^{2+} concentration of greater 150 mg/L has been reported to be detrimental to anaerobic granulation (Yu et al. 2001; Chang and Lin 2006; Ismail et al., 2010). This can be explained by the fact that microorganisms might completely change their surface charge properties from negative to positive by binding with Ca^{2+} at extremely high Ca^{2+} concentration. This in turn implies that over-dosed Ca^{2+} would recreate repulsive force between positively charged microorganisms that would slow down or even prevent microbial granulation. In addition, high-concentration Ca^{2+} would also pose some operational problems, such as precipitation of Ca^{2+} on the surface of granules or accumulation of Ca^{2+} inside anaerobic granules leading to decreased microbial activity etc.

2.5.1.5. Heavy metals

Various metal ions are often needed for the maintenance of microbial metabolism and growth in bioreactors (Beyenal et al. 1997). However, excessive amounts of heavy metals present in influent wastewater would be inhibitory, or even toxic for biochemical reactions, leading to the failure of the wastewater treatment process (Yilmaz 2003; Li and Fang 2007; Altas 2009). High-concentration heavy metals, such as zinc (II), chromium (VI), nickel (II), cadmium (II), copper (II) and lead (II), may have a negative impact on anaerobic granular sludge. The heavy metal concentrations for the prohibition of methane production from a glucose-containing wastewater were found in the following order: Ni (44.82 mg/L)>Cd (28.73 mg/L)>Cr (15.52 mg/L)>Zn (0.65 mg/L) (Altas 2009). The relative toxicity of some heavy metals to the H_2 -producing activity of granules in a packed-bed upflow reactor treating sucrose-containing wastewater was found in the following order: Cu>>Ni~Zn>Cr>Cd>Pb (Li and Fang 2007).

The inhibition or toxicity of heavy metals to microorganisms is mostly due to (i) the strong sorption of metal ions by granules through precipitation, coprecipitation, adsorption and binding by EPS and bacterial interfaces (Guibaud et al. 2008); (ii) formation of metal crystals due to the precipitation with sulfur, carbonate or phosphate (Van der Veen et al. 2007); (iii) inhibition of membrane-transport processes; and (iv) electron siphoning (Harrison et al. 2007). It should be noted that different inhibitory concentrations of heavy metals for granules bioactivity have been reported in the literature. In fact, the toxicity of heavy metals to anaerobic granules also depend on the structure and composition of granules: (i) EPS concentration and distribution in granular sludge (Li and Fang 2007); (ii) size structure (e.g. loose or compact) of granules (Gonzalez-Gil et al. 2001); and (iii) precipitation and adsorption of soluble metals (Artola et al. 2000). In addition, Lin and Chen (1999) found that toxicity-resistance of granules to heavy metals was also HRT-dependent.

2.5.2. Reactor configuration

2.5.2.1. Upflow anaerobic sludge blanket (UASB) and its modifications

The formation of anaerobic granules in UASB is largely dependent on upflow velocity, organic loading rate and gas production, etc (Liu et al. 2006; Zhou et al., 2007; Bhunia and Ghangrekar 2008). At the top of UASB, treated water is separated from sludge solids and gas by a three-phase separator known as the gas-liquid-solids separator (Fig. 2.2). The upflow velocity applied in most laboratory and industrial scale UASB is often kept at < 2 m/h (Kalogo et al. 2001; Vivanco et al. 2006). This allows an extreme uncoupling of the SRT of over 200 d from the HRT of 6 h, making possible the use of compact and economical wastewater treatment plants. UASB had advantages of simple design, low sludge production, low energy requirement and high methane production, etc (Turkdogan-Aydinol and Yetilmezsoy 2010). While, long start-up period due to the slow growth rate of anaerobic bacteria and the stability of the reactor are still the shortages of UASB.

The expanded granular sludge bed (EGSB) reactor (Fig. 2.3) is a modification of the traditional UASB reactor (Chen et al. 2008). A much higher

superficial velocity in EGSB (2-10 m/h) can be achieved due to a high height to diameter ratio and a high recirculation rate (Vivanco et al., 2006; Abreu et al. 2007). These in turn improve the mixing and the contact between the wastewater and the sludge in the EGSB reactor (Hulshoff Pol et al. 2004). As a result, a higher COD removal efficiency and methane production were achieved in EGSB than UASB with OLR of 1.9 g COD/L d (Puyol et al. 2009). It was also reported that EGSB was more efficient and stable in treating 2,4-dichlorophenol (2,4-DCP) and the methanogenic granular biomass from the EGSB reactor was more tolerant to 2,4-DCP (Puyol et al., 2009).

Figure 2.3 The schematic of EGSB (Chen et al., 2008).

Internal circulation anaerobic reactor (ICAR) is another modification of UASB (Fig. 2.4). ICAR contains two layers of UASB, dividing the reactor into two reaction stages (the 1st stage and 2nd stage). The hydraulic circulation between the two gas-liquid-solids separators results in an increased hydraulic rate (Habets et al. 1997). High superficial liquid velocity of 10-20 m/h in the 1st stage could enhance the mass transfer between granules and wastewater while relatively low liquid

velocity of the 2nd stage (2 m/h) avoids strong disturbance to the sedimentation (Wu et al. 2009). The two stage design of ICAR allows 3-6 times higher loading rate than UASB. And the experimental results showed that methanogenic granules developed in ICAR were larger than those in UASB when treating the similar wastewater (Pereboom and Vereijken 1994).

Figure 2.4 The schematic diagram of ICAR (Wu et al. 2006).

2.5.2.2. Anaerobic baffled reactor (ABR)

Anaerobic baffled reactor (ABR) has been developed since 1980s and has shown the potential to produce granular sludge (Barber and Stuckey 1999; Sallis and Uyanik 2003). A typical ABR contained one or more compartments separated by a series of vertical baffles and the influence could be fed into an ABR via one (Fig. 2.5a) or more inlets (Fig. 2.5b). The wastewater containing organic pollutants flows under and over the baffles when it passes from the inlet to the outlet. The development of granules in ABR could be enhanced by the flow characteristics and gas production (Uyanik et al. 2002; She et al., 2006).

Compared with other anaerobic granules bioreactors, ABR has the capability of separating acidogenesis and methanogenesis longitudinally down the reactor and allowing the reactor to behave as a two-phase system without the associated control problems and high cost (Baloch and Akunna 2003). In the investigation of the bacteria distribution and concentration of volatile fatty acid in four compartments, it was conducted that the entire system of ABR could be operated into separated phases. Acidogenesis were dominated in the first compartment, while methanogenesis were main retained in the subsequent compartments near the outlet (Zhu et al. 2008). Such operation of ABR could overcome the accumulation of intermediate acid products which inhibited slow growing methanogenesis and increase the stability of reactor to organic and hydraulic shock loading (She et al., 2006).

Figure 2.5 Schematic diagram of (a) normal feed anaerobic baffled reactor (NFABR) and (b) split-feed anaerobic baffled reactor (SFABR) (Sallis and Uyanik 2003)

2.5.2.3. Anaerobic fluidized bed reactor (AFB)

Anaerobic granulation in UASB, EGBS and ABR is due to the auto-aggregation of anaerobic biomass without the addition of carrier particles. AFB was a fixed-film reactor in which the granules were developed because of the attachment of biomass on the biocarrier particles (Hulshoff Pol et al., 2004; Kumar et al. 2007). Activated carbon, sand, clay and polyvinyl alcohol (PVA) gel beads were often considered as effective biocarrier particles which were suitable for bacteria retention (Zhang et al., 2009). Due to the high specific gravity of the carriers used in AFB, the settling velocity of the anaerobic granules formed in AFB was very high (200 m/h), which enabled to apply higher upflow velocity (10-30 m/h) in the reactor. As known that high upflow velocity could increase the formation of better settleability granules and thus high biomass up to 610 g VSS/L could be accumulated in AFB (Zhang et al., 2009). Compared with other high rate anaerobic reactors, AFB had the potentially high purification capacity and seldom problem of sludge retention (Verma et al. 2005).

2.5.2.4. Anaerobic sequencing batch reactor (AnSBR)

Anaerobic granules was reported to be formed in anaerobic sequencing batch reactor (AnSBR) when selectively preserving the heavier aggregates and washing out the poorly settling flocs by imposing selection pressure during the reaction (Ong et al. 2002). Strong selection pressure could be achieved in AnSBR by shortening settling time, decreasing HRT and increasing volume exchange ratio of effluent to enhance the rapid anaerobic granulation process (Sung and Dague 1995; Wu et al. 2009). Hydrogen production rate was increased by 3 times with the decrease of HRT from 24 h to 8 h (Wu et al., 2009). Liang et al. (2005) indicated that hydrogen-producing granules were formed within the first 10 d of the AnSBR operation at pH 5.5. While hydrogen-producing granules with diameter of 0.4 to 0.5 mm were appeared in an UASB after 140 d operation (Mu and Yu 2006).

Anaerobic sequencing batch reactor (AnSBR) has been extensively studied due to its advantages: (i) no short circuit; (ii) high efficiency for both COD removal and gas production; (iii) no primary and secondary settles; (iv) flexibly control, etc.

AnSBR has been successfully applied in laboratory and pilot scales for treatment of high strength wastewaters, such as landfill leachate, slaughterhouse wastewater, municipal sludge, dairy effluent and brewery wastewater, etc (Zhang et al. 1996; Kennedy and Lentz 2000; Sarti et al. 2007; Shao et al. 2008)

2.6. Membrane Bioreactor

Membrane bioreactor (MBR) is a combination of biological reactor and a membrane filtration module. MBR systems are increasingly adopted for the treatment of various types of wastewaters (Chiemchaisri and Yamamoto 1994; Lee et al. 2001; Lee et al. 2003; Liang et al. 2010). Compared with conventional biological processes, MBR has the advantages of compact design with small footprint, separated hydraulic retention time (HRT) and suspended solids retention time (SRT), high-quality effluent (Anderson et al. 1986; Yamamoto et al. 1989; Langlais et al. 1992). It has been demonstrated that there is great potential in substituting conventional biological process with MBR.

2.6.1. Operation modes of MBR

Membrane filtration is used to separate solids from fluid in a suspension, where the fluid can be a liquid or a gas. During membrane filtration, solids bigger than membrane pore size in the suspension are rejected; while particles smaller than pore size and fluid can go through the membrane. The filtrate collected from the process is called permeate. Membrane filtration can also be used for the separation of materials from different chemical composition in chemistry in order to purify compounds, such as the extraction of soluble antibiotics from fermentation liquors. So far, MBR have been operated in two modes, i.e. dead-end filtration and cross-flow filtration as illustrated in Fig. 2.6.

Figure 2.6 Schematic of membrane filtration process: (a): dead-end filtration; (b): cross-flow filtration (Wakeman and Tarleton 1999)

2.6.1.1. Dead-end filtration

Dead-end filtration operates with the feed flowing in the same direction with the permeate flow (Fig. 2.6a.). In dead-end filtration, the feed is passed through a membrane under a downward permeate drag force (e.g. vacuum or pressure). The solids are accumulated on the surface of the membrane or trapped in the pore of the membrane and the filtrate is released from the other side of the membrane. In dead-end filtration, the thickness of the cake formed on the membrane is usually the major contributor to filtration resistance. Generally, the increase in thickness is approximately proportional to the total amount of filtrate collected over time (Fig. 2.7a). Other process parameters, like solids concentration and flux can also influence the cake deposition layer which would inadvertently require periodic cleaning.

(a) Dead-end filtration

(b) Cross-flow filtration

Figure 2.7 Comparison of flux rate and particle boundary layer thickness. (a) dead-end filtration and (b) cross-flow filtration.

2.6.1.2. Cross-flow filtration

In cross-flow filtration (Fig. 2.6b), the majority of the feed flow travels tangentially across the surface of the membrane (Koros et al. 1996). Different from dead-end filtration, cross-flow filtration is a continuous process. The formed cake layer can be substantially washed away during the cross-flow filtration, thus the membrane filtration process can last long operation time. Cross-flow filtration is typically selected for feeds containing a high proportion of small particle solids because solid material can quickly block the membrane surface in the dead-end filtration process. In cross-flow filtration, the feed water passes through a passage across the membrane and only a fraction that flows out in the perpendicular direction is recovered as permeate. Therefore, the feed flow needs to be recirculated to maintain a high velocity of flow parallel, or cross-flow, to the membrane surface. During the cross-flow filtration, the particles will also accumulate on the membrane surface; however, the cross-flow can serve to offer the particle deposit another degree of movement along the horizontal direction (Fig 2.6b). In cross-flow filtration, deposition of foulants will occur constantly until the adhesive forces that induce the cake to attach onto the membrane are equalized by the scouring forces of the liquid passing through the membrane. A cross-flow filtration process would usually reach steady-state conditions determined by concentration polarisation (CP).

Stabilised conditions, also known as pseudo-steady-state conditions, can be achieved only in practice where deposition or adsorption of foulants is unavoidable. In fact, the shear force generated by cross-flow can minimize the particle build-up; thereby maintain certain permeate flux after some time of filtration. Fig 2.7b shows the relationship between particle boundary layer thickness and permeate flux for cross-flow filtration.

2.6.2. Configurations of MBR

There are two main configurations of MBR applied for wastewater treatment, namely submerged MBR and side-stream MBR.

2.6.2.1. Submerged MBR

In submerged MBR, MF or UF membranes are immersed in the mixed liquor and used for solid–liquid separation (Fig. 2.8). In addition to the removal of biodegradable organics, suspended solids and inorganic nutrients, the submerged MBR also retains particles and slow-growing organisms and removes a very high percentage of pathogens (DiGiano et al. 2004). Compared to the conventional biological processes, the submerged MBR has the advantages of high biomass concentration, small footprint and excellent effluent quality (You et al. 2006; Arabi and Nakhla 2008).

Submerged MBR is often operated under aerobic conditions, which means that the membrane filtration process is combined with the aerobic bioreactor, such as activated sludge and aerobic granules. In aerobic submerged MBR, air is diffused into the reactor through the diffuser located at the bottom of the reactor (Fig. 2.8). Aeration can provide dissolved oxygen required for microbial growth, while helps to prevent the biomass accumulation on the membrane surface. As a result, the cake layer formation would be reduced (Yamamoto et al., 1989; Liang et al., 2010).

Figure 2.8 Schematic diagram of submerged MBR (Liang et al., 2010).

2.6.2.2. Side-stream MBR

The distinction between a submerged MBR and a side-stream MBR is the latter's placement of the membrane module in a separated reactor (Fig. 2.9). The influent enters the bioreactor, where it is brought into contact with the biomass. The mixture is then pumped from the bioreactor to the membrane unit, and further filtered through the membrane under pressure. Permeate is discharged from the system and the entire biomass is returned to the bioreactor. Excess sludge is pumped out and membrane is regularly cleaned by backwashing or chemical cleaning (Sun et al. 2010). Side-stream MBR has higher fouling tendency than submerged MBR. This is because side-stream MBR has high flux operations and thus results in lower permeability. Furthermore, the liquid pumping can induce higher shear stress on the flocs and cause them to break-up (Wisniewski and Grasmick 1998). As a result, particle size is reduced and foulant materials bounded within the flocs are released.

Despite of the high energy consumption of side-stream MBRs, they possess a number of advantages, like declined fouling with increasing cross-flow velocity, in-situ chemical cleaning of membranes where biomass is not exposed to chemical risk, low maintenance and plant downtime costs, manageable precipitation of

sparingly soluble inorganic solids and organic matter, possible operations at high MLSS levels and lastly, aeration can be optimised for oxygen transfer and mixing.

Figure 2.9 Configuration of side-stream MBR (Sun et al., 2010).

2.6.3. Challenge in MBR: membrane biofouling

MBR systems enable the complete physical retention of biomass flocs and all suspended solids within MBR. However, the main drawback hindering the practical application and further improvement of the MBR systems is membrane fouling which decreases the membrane permeability and the quantity of the treated water, leading to increased costs due largely to clean and replacement of the clogged membranes (Magara and Itoh 1991). Membrane fouling occurs when suspended solids attach on the membrane surface and form a layer deposition or when the pores of the membrane are completely or partially blocked by the soluble substances (Belfort et al. 1994).

Membrane fouling can be classified into two groups: reversible and irreversible fouling (Geng et al. 2007). Reversible fouling is primarily formed on the membrane surface in the form of cake layer due to adsorption and deposition of the particles, and it can be removed by physical methods, such as washing and scrubbing (Ognier et al. 2002). The cake layer can cause the cake resistance (R_c) and further reduce the net driving force of filtration and the permeate flux.

Difference from reversible fouling, irreversible fouling is referred to the completely or partially internal pore blocking of the membrane due to the adsorption or attachment of the soluble organic materials, such as organic or inorganic molecules, extracellular polymeric substances (EPS), soluble microbiological products (SMPs) etc. (Peng et al. 2004; Nuengjamnong et al. 2005; Wang et al. 2009). Irreversible fouling is often called as pore blocking (R_p) leading to substantial reduction in the permeate flux. Usually, irreversible fouling can be reduced by chemical cleaning to prolong the usage of the membrane, while it cannot be removed completely.

2.6.4. Mathematical description of fouling phenomenon in MBR

2.6.4.1. Resistance-in-series model

The key elements of membrane process are related to the overall permeate flux, which is mainly influenced by the following parameters: (i) the membrane resistance; (ii) the operational driving force; (iii) the hydrodynamic conditions at the membrane-liquid interface; (iv) the fouling and subsequent cleaning of the membrane surface.

The permeate can be described by the following equation (Cheryan 1986):

$$J = \frac{\Delta P}{\mu R_t} \quad (2.13)$$

where ΔP is the trans-membrane pressure (Pa); R_t is total resistance (1/m), μ is the filtrate viscosity sensitive to temperature (Pa s); J is the permeate flux calculated from the following expression based on Darcy's law ($L/m^2 h$).

$$J = \frac{\Delta M / \rho}{A_{\text{eff}} \times \Delta t} \quad (2.14)$$

where ΔM is the mass of filtrate collected over Δt (g); ρ is density of filtrate (g/L); A_{eff} is the effective membrane surface area (m^2); and Δt is the filtration time (h).

According to the resistance-in-series model for filtration, R_t is the sum of three main components of resistance, i.e. membrane resistance (R_m) which is related

to the membrane properties; cake resistance (R_c) caused by the formation of the cake layer on the membrane surface; internal pore blocking resistance (R_p):

$$R_t = R_m + R_c + R_p \quad (2.15)$$

Inserting Eq. 2.15 into Eq. 2.13 yields:

$$J = \frac{\Delta P}{\mu(R_m + R_c + R_p)} \quad (2.16)$$

2.6.4.2. Membrane blocking model

It is considered in the membrane blocking model that flux decline usually depends on the ratio of the particle size to the pore diameter, and there are four different pore blocking mechanisms, i.e. complete blocking, standard blocking, intermediate blocking and cake filtration (Fig. 2.10).

Figure 2.10 Fouling mechanisms: (a) complete blocking; (b) standard blocking; (c) intermediate blocking; and (d) cake filtration (Bowen et al. 1995).

According to the complete blocking model (Fig. 2.10a), each particle arriving to the membrane participates in blocking some pore or pores with no superposition of particles. Standard blocking model shows the direct adsorption (Fig. 2.10b), i.e. each particle arriving to the membrane is deposited onto the internal pore walls, leading to a decrease in the pore volume (Hermia 1982; Bowen et al., 1995). In the intermediate blocking model (Fig. 2.10c), it is assumed that each particle can settle on other particle previously arrived and already blocking some pores or it can also directly block some membrane area. Intermediate blocking refers to a long term adsorption of particles in membrane pores. As to cake filtration model (Fig. 2.10d), it is considered that each particle locates on others already arrived and already blocking some pores and there is no room for directly obstructing some membrane area.

2.6.5. Factors affecting membrane fouling

As mentioned earlier, the major obstacle for the wide application of MBRs is the rapid decline of permeate flux as a result of membrane fouling (Le-Clech et al., 2006). Evidence shows that membrane fouling is related to the physical and chemical properties of membrane, such as material, pore size, hydrophobicity and charge (Chang et al., 2002), whereas it is also controlled by the characteristics of feed solutions, mixed liquor suspended solids (MLSS) concentration, particle size, surface charge, extracellular polymer substances (EPS) (Lesjean et al. 2005; Paul and Hartung 2008; Thanh et al. 2008; Yu et al., 2009). In addition, the MBR operation conditions may also influence the fouling tendency of MBR.

2.6.5.1. Characteristics of membrane

It had been reported that the overall performance of the membrane is crucially determined by the configuration of the membrane (Ho and Zydney 1999; Hwang et al., 2008). An ideal membrane configuration might have the following criteria: (i) a high ratio of membrane area to module bulk volume, (ii) a high degree of turbulence for mass transfer promotion on the feed side, (iii) a low energy expenditure per unit product water volume, (iv) a low cost per unit membrane area,

(v) a design that facilitates cleaning, and (vi) a modular design. In membrane design, the following characteristics of membrane need to be considered: (i) membrane geometry; (ii) surface characteristics, such as pore size and material, surface charge, hydrophobicity, and porosity; and (iii) inter-membrane separation.

The impact of membrane pore size on critical flux (J_c) was observed at low pore size and/or low MLSS levels (Le-Clech et al. 2003). In the study of the effect of membrane pore size on the particle fouling in dead-end microfiltration, Hwang et al. (2008) found that the blocking index of polymethyl methacrylate (PMMA) particles with mean size of 0.15 μm for membrane with 0.4 μm pore size was always larger than that for membrane with 0.2 μm pore size under the same filtration pressure due to more severe membrane blocking. Natural organic matter (NOM) is a major membrane foulant in drinking water treatment with membrane technology. In the study of the behavior of NOM components in low-pressure membrane fouling, Lee et al. (2004) observed more significant flux decline in microfiltration (MF) than in ultrafiltration (UF) membrane filtration. It was also found that MF membrane fouling may be caused by pore blockage associated with large (macromolecular) hydrophobic molecules and/or organic colloids, whereas, the flux decline in NF would be caused by sequential or simultaneous processes of surface (gel layer) coverage during filtration.

Hydrophobic interactions occur between solutes, microbial cells of EPS and the membrane material, thus resulting in more severe membrane fouling in hydrophobic than hydrophilic membranes. According to Chang et al. (2001a), the solute rejection is a result of adsorption onto or sieving by the cake layer on the membrane which eventually leads to direct adsorption into the membrane pores and the at the membrane surface. It has also been suggested by Fang and Shi (2005) that membranes with higher hydrophilicity are more exposed to deposition of hydrophilic foulants. Hence, hydrophilic membranes tend to be more porous and are more susceptible to fouling problems. It is necessary to modify the base material to produce a hydrophilic surface. This can be achieved using chemical oxidation and organic chemical reaction. Due to its high level of reliability, MBR membranes are often made from the polymer, polyvinylidene difluoride (PVDF) using this modification process (Gander et al., 2000).

2.6.5.2. Characteristics of feed wastewater

In the membrane filtration process, development of membrane fouling is also dependent on the properties of the substrate fed into the MBR, such as mixed liquor suspended solids (MLSS) concentration, sludge particle size distribution (PSD), extracellular polymeric substances (EPS), soluble microbial products (SMP), dynamic viscosity, relative hydrophobicity (RH), and zeta potential, etc (Yamamoto et al., 1989; Le-Clech et al., 2003; Fuchs et al. 2005; Schrader et al. 2005; Shen et al. 2009; Wang et al., 2009).

2.6.5.2.1. Characteristics of biomass

In submerged MBRs, membrane modules are immersed in the bioreactors. Therefore, the membrane fouling mechanisms are very complicated due to the complex characteristics of mixed liquors. High biomass concentration in MBR has often been reported due to the long SRT (Rosenberger and Kraume 2002). Therefore, biomass concentration in MBR is a main parameter affecting membrane fouling, e.g. the fouling in MBR was found to increase with the sludge concentration (Le-Clech et al., 2003; Katayon et al. 2004; Farizoglu and Keskinler 2006). The different phenomenon was also observed and reported by Lee et al. (2001). Moreover, Wu et al. (2007) concluded that there was no significant correlation between MLSS concentration in the range of 4.2 to 25.0 g/L and membrane fouling in a pilot submerge MBR.

The physiological characteristics of the biomass had been found to play an important role in development of membrane fouling. In comparison of the filtration characteristics of attached and suspended growth biomass in submerged MBRs, the suspended growth biomass had 7 times higher permeate flux than attached growth biomass because suspended sludge could deposit on the surface membrane to form the rougher cake layer which could be removed from the membrane surface by air-liquid flow easily (Lee et al., 2001). In the discussion of biomass size, experimental results revealed that large size biomass with nearly spherical shape and monosize distribution could effectively eliminate the membrane fouling since the size of the

particles in MBRs would effect the interactions of the cake particles with the apertures in the filter medium (Wakeman 2007). It was also noted that the final permeate flux in short-term microfiltration of aerobic granules was 2 times higher than that of the activated sludge (Jun et al. 2007). The higher fouling of activated sludge indicated that the sludge floc had a stronger tendency to deposit on the membrane surface and less porous in the formed cake layer than granules sludge.

Some research also revealed that highly hydrophobic flocs are partial causes of membrane fouling, in which relative hydrophobicity of floc is directly related to the bacterial adhesion or partition (Jang et al., 2005b). Hydrophobicity measurement of sludge and EPS by Jang et al. (2005a,b) showed that EPS level and filamentous index can directly influence biomass flocs hydrophobicity and zeta potential. Filamentous index is a parameter indicating the relative presence of filamentous bacterial in sludge. Excess filamentous growth will result in higher EPS level, lower zeta potentials, more irregular flocs shape and higher hydrophobicity (Meng et al. 2006)

2.6.5.2.2. Characteristics of supernatant

Supernatant, the liquid phase of the mixed liquor, consists of colloidal particles and soluble EPS macromolecules (Trussell et al. 2007). The relative contribution of the floc biomass and the supernatant to the membrane fouling has been evaluated (Bouhabila et al. 2001; Iritani et al. 2007). Some studies showed that the supernatant would play an important role in controlling the membrane fouling (Nuengjamnong et al., 2005; Iritani et al., 2007). In study of the performance of a MBR using a hollow fibre membrane with pore size of 0.1 μm , Bouhabila et al. (2001) reported that the supernatant contributed about 76% to the total resistance at a MLSS concentration of 20.7 g/L and SRT of 20 d, whereas Iritani et al. (2007) also found that the relative contribution of supernatant to the membrane fouling of the activated sludge was nearly 100%. However, it should be pointed out that some different results were reported in the literature. For example, Lee et al. (2003) found that the relative contribution of supernatant to the membrane fouling of the sludge was as low as 29 to 37%, while Defrance et al. (2000) reported that the supernatant

would contribute 35% to the total fouling resistance in microfiltration of activated sludge at the concentration of 4500 mg MLSS/L through a ceramic membrane with pore size of 0.1 μm .

Soluble EPS (sEPS) in the supernatant were observed to play a critical role in membrane fouling in MBRs and have been widely recognized as one of the main membrane foulants (Lee et al., 2003). EPS are of biological origin, participate in the formation of microbial aggregates and consist of insoluble materials (sheaths, capsular polymers, condensed gel, loosely bound polymers, and attached organic material); whereas sEPS or SMP are soluble cellular components (Mayer 1999; Laspidou and Rittmann 2002). It has been reported that sEPS had more potential influence on the membrane fouling than bound EPS (Wang et al., 2009). Furthermore, not only sEPS composition, but also EPS distribution and properties (solubility and bindability) would all be involved in the development of membrane fouling (Li et al. 2008). Thanh et al. (2008) observed that the soluble polysaccharides (sPS) in the supernatant of the granular sludge MBR accounted for 84 % of the total soluble extracellular polymer substances (sEPS), and the membrane fouling rate was proportionally related to the sPS concentration in the supernatant of MBR.

2.6.5.3. Operation conditions of the MBR

Some important operation parameters, such as solids residence time (SRT), shear force, aeration all influence the development of membrane fouling in a MBR. As a MBR has double functions of biodegradation and solid-liquid separation, a long SRT allows the system to keep a sufficient amount of slow-growing microbes for degrading refractory compounds (Stamper et al. 2003). A proper SRT can help to improve water quality, while prevent serious membrane fouling by minimizing the accumulation of large amount of EPS in MBR system (Fan et al. 2005; Le-Clech et al., 2006; Djamila et al. 2008).

Shear force can be applied along the membrane surface to control biofouling caused by the cake layer formation due to the deposition of the biomass. However, shear force may also affects the physicochemical and biological properties of MBR

biomass and the production of sEPS (Kim et al. 2001; Meng et al. 2008). It was reported by Menniti et al. (2009) that the production of sEPS would be reduced at high shear force, while due to the lower concentration of floc-associated EPS and the production of stickier floc-associated EPS that is more erosion resistant in the high shear reactor. As higher concentration of sEPS would result in increased membrane fouling potential, increased shear could increase membrane fouling.

2.6.6. New development of granular sludge MBR

As discussed earlier, membrane fouling is related to several factors including the types of membrane, the sludge characteristics, the feed water, mixed liquor components and the operating conditions (Peng et al., 2004; Paul and Hartung 2008). In the past a few years, a new granular sludge MBR has been developed in an attempt to retard membrane fouling as well as to maintain long-term stable operation (Chu et al. 2005; Li et al. 2005; Chu et al. 2006; Li et al. 2007; Wang et al. 2008; Krause et al. 2010; Wang et al. 2010).

Compared with the conventional suspended sludge MBR, the introduction of granular sludge into the reactor offers some advantages in controlling membrane fouling. For example, the membrane performance could be improved and maintained at a low fouling rate below 0.1 kPa/d at the MLSS concentration greater than 18 g/L (Tu et al. 2010). An aerobic granular membrane bioreactor (AGMBR) system was able to effectively remove organics and turbidity throughout the operation period. During the granulation process, the reduced SVI led to a decrease in the supernatant TOC concentration that corresponded to a slower rise in TMP and less membrane fouling (Tu et al., 2010). The protein to carbohydrate ratio of both EPS and SMP increased after the formation of aerobic granules. This led to an increase in the relative hydrophobicity and zeta potential of the sludge, as well as the filterability of mixed liquor (Jun et al., 2007; Thanh et al., 2008). Big granular sludge can be easily retained and form removable cake layer on the surface of the membrane. Furthermore, it was found that chemical cleaning could remove most of foulants on the membrane surface and within the pores with the recovery of near 100% (Li et al., 2005).

It appears from previous study that the mixed liquor in aerobic granular sludge MBR had a much better filterability. The substitute of floc sludge by aerobic granules in the MBR system is beneficial for controlling flux decline and membrane fouling with good treatment performance (Zhou et al. 2007). The loss of the overall membrane permeability of the aerobic granular sludge was 1.68-fold lower than that of aerobic flocs (Tay et al. 2007), whereas the membrane permeability of aerobic granular sludge MBR was reported to be more 50% higher than that of conventional MBR (Li et al., 2005). An expanded granular sludge bed (EGSB) reactor coupled with hollow fibre membrane filtration has been proven to be an attractive technology to treat low strength wastewater (Chu et al., 2005). The membrane-coupled EGSB reactor can achieve a high quality effluent due to the effective removal of soluble pollutants by EGSB and the capability of the membrane module in retaining all suspended solids, pathogenic bacteria and even macromolecules within the reactor (Seghezzo et al. 1998). In addition, the fine disturbance caused by a high upflow velocity is advantageous in reducing membrane fouling.

2.7. Summary

Biological nitrogen removal process has been widely practiced for wastewater treatment, whereas MBR has been identified as an effective way for enhancing the quality of treated water. In order to mitigate membrane fouling, the novel granular sludge MBR has been developed for reduction of membrane fouling. In operation of MBR, potential contribution the suspended mixed liquor to membrane fouling is a very complex phenomenon. Little information is currently available regarding the effect of the biomass properties on membrane fouling and subsequent flux decline. It also appears that the mechanisms of membrane fouling caused by suspended or granular sludge remain unclear. Therefore, more comprehensive study is needed to provide deeper understanding of membrane fouling mechanisms and associated phenomena.

CHAPTER 3

DEVELOPMENT OF DENITRIFYING GRANULES AT DIFFERENT CYCLE TIMES

3.1. Introduction

With increasing demands on water reuse and recycle, advanced and cost effective techniques for nitrogen removal from wastewater are highly desirable. So far, biological nitrogen removal typically through nitrification-denitrification has been practiced worldwide (Metcalf & Eddy 2003), whereas almost all denitrification processes are based on suspended culture in which it is still difficult to achieve high biomass concentration, effective retention and separation of denitrifying sludge. These would eventually lead to a large volume of denitrification tank and potential high-energy demand on mixed liquor recirculation.

Anaerobic granules have been successfully developed in upflow anaerobic sludge blanket (UASB) reactor for treating a wide spectrum of industrial wastewaters, whereas aerobic granulation has been reported in sequencing batch reactors (Lettinga et al. 1984; Schmidt and Ahring 1996; Liu and Tay 2004; Oliveira and von Sperling 2009). Compared to conventional anaerobic sludge with a poor settleability, anaerobic granules have regular, dense microbial structure, excellent settleability and high biomass retention. Such features of anaerobic granules allow development of highly compact anaerobic granular sludge bioreactor, such as UASB. However, one major drawback of UASB system is the long start-up period required for the development of anaerobic granules. In cases where the inoculation is done with municipal digester sludge, it usually takes 3 to 4 months or a longer period before the process can be put into operation. In view of the longer start-up period, enhanced anaerobic granulation is highly desirable for reducing space-time requirement. It appears from the literature that most of studies of denitrifying granulation have been conducted in upflow sludge blanket reactor in which it is difficult to effectively control hydraulic selection pressure (Bhatti et al., 2001; Franco et al., 2006; Ting and Huang 2006). Compared to conventional UASB

reactor, sequencing batch reactor (SBR) provides high flexibility in operation, e.g. hydraulic selection pressure in SBR can be easily controlled. So far, little information is available about the characteristics of denitrifying granules developed in SBR operated at different cycle times which may serve as a selection pressure on biosolids. Therefore, this study aimed to investigate the physicochemical characteristics of denitrifying granules developed in SBRs at different cycle times.

3.2. Materials and Methods

3.2.1. Experimental set-up and operation conditions

Three identical columns with 60 cm height and 8 cm internal diameter were used as sequencing batch reactors (SBRs), namely as R1, R2 and R3, for development of denitrifying granular sludge. Fig. 3.1 showed the schematic diagram of one SBR set-up. Each SBR had the same geometrical configuration. The effective working volume of each reactor was 2.5 L. Substrate for denitrifying granules development was pumped into each reactor from the upper port of the reactor. The suspension in each reactor was mixed completely with an electronic mixer at the low speed of 100 rpm. The effluent with volume a half as much as the effective working volume was discharged from the middle port of the reactor after each cycle's reaction. All reactors were operated under the same anaerobic conditions. The experiments were conducted in a temperature control room of 25°C. R1 to R3 were operated at respective cycle times of 8, 6 and 4 h, each cycle comprising 15 min feeding, 30 min settling, 5 min effluent withdrawal and the rest time for reaction. The cycle operation of three reactors was controlled by using a logic program (Zelio-Logic Smart Relay, Schneider Electric). The experimental set-up was shown in Fig. 3.2.

Figure 3.1 Schematic diagram of a laboratory scale SBR.

Figure 3.2 Photo of the experimental set-up of SBRs.

3.2.2. Seed sludge

The seed sludge taken from Ulu Pandan Water Reclamation Plant was enriched with the synthetic wastewater for two weeks. One litre of acclimated denitrifying sludge was seeded to each reactor. The seed sludge had a sludge volume index (SVI) of 152 mL/g SS after 30 min settling, initial particle size of 125 μm , and mixed liquor suspended solids (MLSS) concentration of 3000 mg/L.

3.2.3. Synthetic wastewater

The synthetic wastewater consisting of methanol as sole carbon source, nitrate and other necessary nutrients was used for acclimation of sludge and development of denitrifying granules. The methanol and nitrate concentrations were fixed at 173 mg TOC/L and 140 mg N/L, respectively in R1, R2 and R3. Details of the composition of the synthetic wastewater were presented in Table 3.1.

Table 3.1 Synthetic wastewater used (adopted from Modin et al. (2008)).

Component	Concentration (mg/L)
KNO_3	1000
CH_3OH	460
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	100
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	40
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	6.25
Phosphate buffer*	2 mL/L
Trace element solution**	1 mL/L

*: 1 L of phosphate buffer contained 24.4 g KH_2PO_4 and 12.2 g NaHPO_4

**: Trace element solution (pH = 7.5) was composed with 500 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 50 mg ZnCl_2 , 100 mg $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, 50 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 26 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 50 mg H_3BO_3 , 300 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

3.2.4. Extraction and examination of extracellular polymeric substances

Bound extracellular polymeric substance (bEPS) in the sludge and granules were extracted using formaldehyde and sodium hydroxide method described by (Liu and Fang 2002). The details of the procedure were described as following:

- (1) 10 mL of sludge collected from SBRs was washed twice with deionized water;
- (2) Washed sludge was separated from supernatant by centrifugation at 4000 rpm for 20 min and was pulverized;
- (3) The pulverized sludge was re-suspended in 20 mL of 0.22% formaldehyde and kept at 4°C for 1 h;
- (4) 4 mL of 2 M NaOH was then added into the suspension and mixed completed at 4°C for 3 h;
- (5) The liquid part was separated from the suspension by centrifugation at 10000rpm for 30 min and then filtrated through 0.45 μ m membrane;
- (6) Extracted EPS was collected and kept at -20°C before measurement.

The soluble EPS (sEPS) was collected directly by filtrating the suspension through membrane with pore size of 0.45 μ m.

The bEPS and sEPS were analyzed for proteins and polysaccharides. Proteins were determined by Folin-Ciocalteau phenol method with BSA (Bovine Serum Albumin) as standard (Lowry et al. 1951), whereas polysaccharides were determined by Phenol-Sulfuric method with glucose as standard (Dubois et al. 1956).

3.2.5. Surface charge determination

The colloid titration technique was used to determine the surface charge of the denitrifying granular sludge in this experiment (Kawamura and Tanaka 1966; Morgan et al. 1990; Jia et al. 1996). Polybrene (Sigma) and polyvinyl sulfate potassium salt (PVAK) (Aldrich) were used as positive and negative standards,

respectively. The details of the procedure of the surface charge determination was stated below:

- (1) 2 mL sample was added in 100 mL deionized water to make suspension.
- (2) 5 mL of 0.001 N polybrene was added into the suspension and react with the sludge.
- (3) 0.0005 N PVSK was then used to back-titrate the excess amount of polybrene using 0.1 mL of 0.1% toluidine blue as end-point indicator.
- (4) Titration was terminated when electrical neutrality was reached where the color changed from blue to pink.
- (5) An equal volume of polybrene in deionized water was used as a blank for each series of titration.

The surface charge was calculated using Eq. 3.1 and the result was expressed as milliequivalent per liter. For sludge sample, it was calculated using Eq. 3.2 and preferable to express the surface charge as milliequivalent per gram of dried sludge solids.

$$\text{Charge (meq/L)} = \frac{(A - B) \times N}{V} \times 1000 \quad (3.1)$$

$$\text{Charge (meq/g SS)} = \frac{(A - B) \times N}{V \times X} \times 1000 \quad (3.2)$$

where A is the volume of PVSK added into the flask (mL); B is the volume of PVSK added into the blank (mL); V is the volume of the sample (mL); X is the biomass concentration (g/L) and N is normality of PVSK standard (0.0005 N)

3.2.6. Granule strength determination

Granule strength was determined by the sonication technique described by Morgan and Forster (1992). The measurement procedure was stated below:

- (1) 10 g denitrifying granular sludge suspended in 200 mL quarter-strength Ringer's solution was used to sonication.

- (2) The sonication energy was kept at a constant power level of 60 w.
- (3) 1 mL of sample was taken at different sonication time and diluted 100 times in quarter-strength Ringer's solution.
- (4) The turbidity of the diluted solution was measured using a turbidity meter (Hach, 2100A).

3.2.7. Analytical methods

Nitrate and nitrite were determined by using an ionic chromatograph system (DIONEX ICS-1000). TOC concentration was analyzed by a Shimadzu TOC analyzer (TOC-500 model).

Denitrification rate (DNR) expressed as mg N/g VSS·d was calculated as follows:

$$\text{DNR (mg N/g VSS} \cdot \text{d)} = \frac{[\text{NO}_3^- - \text{N}]_b - [\text{NO}_3^- - \text{N}]_e}{t \times \text{MLVSS}} \quad (3.3)$$

where $[\text{NO}_3^- - \text{N}]_b$ is $\text{NO}_3^- - \text{N}$ concentration at the beginning of denitrification in each cycle (mg N/L); $[\text{NO}_3^- - \text{N}]_e$ is $\text{NO}_3^- - \text{N}$ concentration at the end of denitrification (mg N/L); t is reaction time (d); MLVSS is the mixed liquor volatile suspension sludge (g VSS/L).

Measurement of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI) were preceded according with the standard methods (APHA 1998). The mean size of the flocs and granular sludge was measured by a laser particle size analyzer (Malvern Mastersizer Series 2600, Malvern). The morphologies of microbial aggregates were visualized using an imaging analysis system (Olympus SZX9).

3.3. Results and Discussion

3.3.1. Morphology of denitrifying granules

Fig. 3.3 demonstrates that the mean size of denitrifying sludge gradually increased and stabilized at 0.19 mm in R1, 0.21 mm in R2 and 0.22 mm in R3 (Fig. 3.3), indicating that relatively big denitrifying granules would be developed at the short cycle time. Fig. 3.3 also reflects the appearance of denitrifying granules in all three reactors after 20 days operation. Lin et al. (2001) reported when the hydraulic retention time (HRT) was decreased from 10 to 1.5 days, the diameter of the anaerobic granules developed in a UASB reactor increased from 0.56 mm to 0.89 mm. In fact, the size of denitrifying granules was found to be comparable with that of nitrifying granules cultivated in SBR (Tay et al. 2002). As compared to the seed sludge with a mean size of 0.12 mm, denitrifying granules had a regular outer shape and compact and dense physical structure (Fig. 3.4). It should be realized that small denitrifying granules as shown in Fig. 3.4 would have advantages over big ones in the sense of substances diffusion, while bigger granules may help improve the settleability of biosolids. According to Li and Liu (2005), diffusion limitation would be negligible for a granule smaller than 0.5 mm.

Figure 3.3 Mean size evolution of denitrifying granulation in R1, R2 and R3.

Figure 3.4 Morphology of biomass. bar = 200 μm ; (a) seed sludge; (b) denitrifying granules in R1; (c) denitrifying granules in R2; (d) denitrifying granules in R3.

Granules of R1 to R3 were sampled at the 45th day during the granulation.

3.3.2. Biomass settleability

In the field of biological wastewater treatment, sludge volume index (SVI) is a key indicator of microbial sludge settleability. Fig. 3.5 shows that SVI of biomass in R1 to R3 all tended to decrease in the course of operation, whereas the fastest decline in SVI and the lowest SVI of about 20 mL/g SS were observed in R3 operated at the shortest cycle time. These results in turn suggest that the shorter cycle time would be more favorable for improving the biomass settleability. In addition, the SVI values of the mature denitrifying granules were found to be comparable with those reported for the UASB granules and aerobic granules (Tay et al., 2002). Fig. 3.6 further reveals a linear relationship between the SVI and mean size of denitrifying granules, i.e. a bigger denitrifying granule would result in a lower SVI. It is obvious that the settleability of denitrifying sludge can be improved

significantly through granulation, eventually leading to increased biomass retention in SBRs.

Figure 3.5 SVI evolution of denitrifying granulation in R1, R2 and R3.

Figure 3.6 Correlation of mean size and SVI of denitrifying aggregates.

3.3.3. Denitrifying granulation rate

The initial denitrifying granulation rates in terms of change in mean size and SVI were determined at different cycle times. The higher initial denitrifying granulation rate was obtained at a shorter cycle time (Fig. 3.7). It should be pointed out that in study of nitrifying granulation at different cycle times in SBR, Tay et al. (2002) only focused on the effect of cycle time on the mean size of mature nitrifying granules, but no attention was given to how cycle time would affect the kinetics of nitrifying granulation. The cycle time of SBR represents the frequency of biosolids discharge through effluent withdrawal, and is also related to hydraulic retention time (HRT) for a given volume exchange ratio, indicating that cycle time of SBR indeed represents a type of hydraulic selection pressure exerted on bioparticles. For microbial granulation, light and dispersed biosolids should be washed out, whereas denser bioparticles would be selected and retained in the system (Liu and Tay 2004). In the operation of SBR, a short cycle time would help to suppress the growth of suspended biosolids due to the more frequent washout of the light suspended biosolids (Qin et al. 2004). Consequently, a faster denitrifying granulation can be expected at a shorter cycle time, i.e. a speedy denitrifying granulation is achievable through manipulation of selection pressure (Fig. 3.7). Ting and Huang (2006) also reported that biological denitrification in the continuous upflow sludge bed reactor could be improved through the formation of denitrifying granules induced by sludge wasting from the bioreactor. On the contrary, anaerobic granulation failed when the hydraulic selection pressure was too weak in UASB reactor (Alphenaar et al., 1993; Oflaherty et al. 1997).

Figure 3.7 Initial change rate of denitrifying granulation versus cycle time.

3.3.4. Extracellular polymeric substances

Extracellular polymeric substances (EPS) have been believed to mediate microbial aggregation and play an essential role in maintaining the structural integrity of microbial granules and biofilms (Flemming and Wingender 2000; Sutherland 2001; Liu and Tay 2004; Zhou et al. 2006; Vlaeminck et al. 2010). Figs. 3.8 and 3.9 show changes in the contents of proteins (PN) and extracellular polysaccharides (PS) of biomass in the course of operation of R1 to R3. It was found that the PS content of biomass fluctuated in a narrow range in all three SBRs operated at different cycle times of 4 to 8 h, whereas the PN content of biomass tended to increase over the operation time. In addition, the PN content of biomass was significantly higher than that of PS in all three SBRs. Fig. 3.10 further indicates that the ratio of PN to PS of biomass in three reactors increased from 0.3 to 2.6 along with denitrifying granulation. Compared to Fig. 3.3, the observed increase in the PN content of biomass was positively correlated to the increased size of denitrifying sludge, implying the importance of extracellular proteins in the formation of denitrifying granules. In fact, Franco et al. (2006) found that denitrifying granular sludge treating soft groundwater in a continuous UASB reactor exhibited a higher ratio of proteins to polysaccharides, while McSwain et al.

(2005) also reported that the formation and stability of aerobic granules were related to extracellular proteins instead of polysaccharides. According to Vlaeminck et al. (2010), EPS would take about 50% of the autotrophic space of aerobic ammonium-oxidizing and anoxic ammonium-oxidizing granules developed under the oxygen-limited autotrophic nitrification and denitrification conditions, while no attention was given to specific role of PN over PS.

Figure 3.8 Changes of proteins during denitrifying granulation versus time.

Figure 3.9 Changes of extracellular polysaccharides during denitrifying granulation versus time.

Figure 3.10 Ratio of PN to PS during denitrifying granulation.

In study of microbial attachment to a solid surface, Flint et al. (1997) reported that treatment of cells with sodium metaperiodate, lysozyme or tricholoacetic acid to remove cell surface polysaccharides has no significant effect on microbial adhesion, however a 100-fold reduction in the number of attached bacteria was achieved when cells were treated with trypsin to disrupt cell surface proteins. In another investigation of microbial adhesion to glass and polystyrene by Dufrene et al. (1996), a correlation between protein concentration at the cell surface or at the support surface and adhesion density was observed under different experimental conditions, which provided a direct demonstration of the involvement of extracellular PN in microbial adhesion onto inert surfaces. Furthermore, Allison and Sutherland (1987) also reported that a non-polysaccharide-producing mutant as well as the polysaccharide-producing wild type adhered to glass surface. These seem to indicate that specific contribution of extracellular PN and PS to microbial granulation is subject to further study. It should be pointed out that the role of extracellular PS, to some extent, would be over-highlighted against extracellular PN for microbial granulation as recently noted by Jiang and Liu (2010).

3.3.5. Mechanical strength of denitrifying granules

To date scant information is available describing the effect of SBR cycle time on the stability of denitrifying granules. To look into the stability of mature denitrifying granules developed at different cycle times, mature denitrifying granules harvested from R1 to R3 were subject to various sonication strengths, and the resultant changes in turbidity were measured (Fig. 3.11). Some salient points from Fig. 3.11 are: (i) the turbidity of the supernatant for the samples collected from R1 to R3 all tended to increase with the increase in the sonication strength until a plateau was reached, e.g. 28 NTU for R1 denitrifying granules, 22 NTU for R2 and 16 NTU for R3; (ii) the change rate of turbidity was inversely related to the SBR cycle time, i.e. a quicker increase in turbidity was observed for denitrifying granules cultivated at a longer cycle time. These results clearly show that denitrifying granules developed at the shorter cycle time would have the higher mechanical strength. Consequently, the SBR cycle time as a hydraulic selection pressure would not only determine the formation rate of denitrifying granules (Fig. 3.7), but also their mechanical strength (Fig. 3.11).

Figure 3.11 Changes in turbidity of denitrifying granules versus sonication strength.

In order to better understand the effect of the SBR cycle time on the strength of mature denitrifying granules, changes in surface charges of mature denitrifying granules subjected to various sonication strengths were also determined (Fig. 3.12). It can be seen that the mature granules developed in R1, R2 and R3 all carried negative charges and the surface charge density of denitrifying granules tended to decrease with the increase in the SBR cycle time, e.g. the negative surface charge density of denitrifying granules cultivated at the cycle time of 8 h was nearly 3-fold higher than that developed at the cycle time of 4 h. Furthermore, Fig. 3.13 shows that the mechanical strength of denitrifying granules was inversely related to the surface charge density, i.e. high surface charge density would weaken the structure of denitrifying granules. In a study of the structure of thermophilic and mesophilic anaerobic granules developed in UASB reactor, Quarmby and Forster (1995) also reported that the increased cell surface negative charge density would weaken the strength of anaerobic granules. In fact, similar phenomenon has also been observed in biofilm and bioflocculation processes (Dickson and Koohmaraie 1989; Liao et al. 2001).

Figure 3.12 Change in surface charge of denitrifying granules versus sonication strength.

Figure 3.13 Correlation of specific cell surface charge and turbidity.

3.3.6. Denitrification efficiency

The respective cycle denitrification efficiency in R1, R2 and R3 was examined on day 50 when the denitrifying granules were mature. Figs. 3.14 to 3.16 show the typical concentration profiles of TOC, nitrite and nitrate observed in R1, R2 and R3. The initial nitrate concentrations in R1 to R3 were fixed at 140 mg N/L. The nitrate was removed to the minimum levels in 240 min, 150 min and 60 min for denitrifying granules in R1, R2 and R3, whereas the fastest specific denitrification rate (DNR) of 630 mg N/g VSS·d was observed in R3 run at the shortest cycle time of 4 h (Fig. 3.17), meaning that DNR of R3 was 3 times and 2.75 times higher than that of R1 and R2, respectively. These seem to imply that a shorter cycle time of SBR could favor denitrification reaction. The increased granules activity shorting the cycle time was also reported in aerobic granulation fed with acetate, e.g. Liu et al. (2007) observed that nitrification ability in term of ammonium consumption rate was increased when the cycle time was shortened from 4 h to 2 h.

Figure 3.14 Cycle profiles of nitrite-N, nitrate-N and TOC in R1 with 8 h cycle time on day 50.

Figure 3.15 Cycle profiles of nitrite-N, nitrate-N and TOC in R2 with 6 h cycle time on day 50.

Figure 3.16 Cycle profiles of nitrite-N, nitrate-N and TOC in R3 with 4 h cycle time on day 50.

Figure 3.17 Comparison of specific denitrification rate of denitrifying granules developed under different cycle time.

Denitrification has been stoichiometrically described by the following reaction:

This equation shows that 0.93 mg TOC is theoretically required for removing 1 mg NO_3^- -N using methanol as the organic carbon source. As shown in Figs. 3.14 to 3.16, 1.00 mg, 1.03 mg and 1.05 mg TOC were needed to denitrify 1 mg NO_3^- -N in R1, R2 and R3, respectively, which are very close to the theoretical requirement. In the suspended denitrifying sludge processes, sludge flotation and subsequent washout have been often observed, especially at high nitrogen loading (Franco et al., 2006). However, it can be expected that such problems may be overcome in denitrifying granular sludge reactor due to the excellent settleability, compact and dense structure of denitrifying granules.

3.4. Summary

The denitrifying granules were successfully developed in SBRs operated at different cycle time of 4, 6 and 8 h, respectively. Some remarks can be drawn from this chapter:

- (1) The denitrifying granules developed in R3 operated at 4 h cycle time had the largest size of 220 μm and the lowest SVI of 20 mL/g SS. The mean size and settleability of denitrifying granules were inversely related to the SBR cycle time, and increased size of denitrifying granules would result in a lower SVI.
- (2) A shorter cycle time would promote denitrifying granulation in terms of initial denitrifying granulation rate. For example, the initial denitrifying granulation rate in terms of mean size at the cycle time of 4 h was 1.2 and 2.2 times higher than those developed at the cycle times of 6 h and 8 h. It was found that extracellular proteins would contribute more to denitrifying granulation than extracellular polysaccharides.

- (3) The higher mechanical strength of denitrifying granules was observed at the shorter cycle time of 4 h, whereas high cell surface negative charge density would weaken the granule strength regardless of the applied cycle times.
- (4) It appears that denitrifying granular sludge bioreactor would be a robust system suitable for higher-efficiency nitrogen removal from wastewater.

CHAPTER 4

COMPARISON OF MEMBRANE FOULING IN DEAD-END MICROFILTRATION OF DENITRIFYING GRANULAR SLUDGE SUSPENSION AND ITS SUPERNATANT

4.1. Introduction

With increasing demands on the reuse and recycle of municipal wastewater, advanced and cost-effective techniques for nitrogen removal from wastewater are highly desirable. So far, biological nitrogen removal typically through nitrification-denitrification has been practiced worldwide (Metcalf & Eddy 2003), and almost all denitrification processes are based on suspended culture in which it is still difficult to achieve high biomass concentration, effective retention and separation of denitrifying sludge. These would eventually lead to a large volume of denitrification tank and potential high-energy demand on mixed liquor recirculation. Anaerobic granules have been successfully developed in upflow anaerobic sludge blanket (UASB) reactor for treating a wide spectrum of high-strength organic wastewaters (Lettinga et al., 1984; Schmidt and Ahring 1996; Oliveira and von Sperling 2009). Most of studies on denitrifying granulation have been conducted in UASB reactor in which it is difficult to effectively control hydraulic selection pressure for rapid granulation (Bhatti et al., 2001; Franco et al., 2006; Ting and Huang 2006). As presented in Chapter 4, denitrifying granules developed in SBR appear to be a promising nitrogen removal technology.

Membrane bioreactor (MBR) has attracted more attention and is increasingly used in the wastewater reclamation due to its compact design and high quality product water as compared with the conventional activated sludge processes (Chiemchaisri and Yamamoto 1994; Lee et al., 2001; Lee et al., 2003). However, the main drawback hindering the practical application and further improvement of the MBR systems is membrane fouling leading to decreased membrane permeability and quantity of the treated water, while increased operation cost incurs due to frequent cleaning and replacement of the clogged membranes (Magara and

Itoh 1991). Membrane fouling occurs when the suspended solids attach on the membrane surface and form a layer of cake or when the pores of the membrane are completely or partially blocked by the soluble substances (Belfort et al., 1994).

So far, extensive research has been pursued to investigate the possible ways to prevent or reduce biofouling in MBR. All the methods can be divided into two main types: (i) modification of the membrane surface to improve anti-fouling property leading to reduced membrane fouling (Yu et al. 2005), and (ii) change of biomass types and characteristics by increasing the particle size or biomass coagulant ability (Lee et al., 2003; Yu et al., 2009). For example, one of the effective ways to reduce membrane fouling is to treat the wastewater using MBR combined with aerobic granular sludge (Li et al., 2005; Tay et al., 2007). However, little has been known about the possible combination of denitrifying granular sludge with membrane filtration. Therefore, this study aims to investigate microfiltration of the suspension and supernatant of denitrifying granular sludge developed in sequencing batch reactors (SBRs) operated at different cycle times (Chapter 3), while the possible fouling mechanisms were also discussed.

4.2. Materials and Methods

4.2.1. Microfiltration experimental set-up

In order to evaluate and compare the filterability of mature denitrifying granular sludge cultivated at different cycle times and the seed sludge, the microfiltration experiments were carried out using a dead-end filtration module (Fig. 4.1). The flat-sheet nitrocellulose membrane (Millipore, Ireland) with a pore size of 0.45 μm and an effective membrane area was 11.94 cm^2 was used for study of microfiltration. The applied pressure was kept at 80 kPa provided by compressed nitrogen gas.

Figure 4.1 Schematic diagram of a dead-end microfiltration set-up.

4.2.2. Feed solutions in microfiltration experiment

Suspension and supernatant of mature denitrifying granular sludge and seed sludge were used as feed solutions in the microfiltration experiment. Suspension of granules was taken from three SBRs, namely R1, R2 and R3) which were operated at the respective cycle time of 8 h, 6 h and 4 h for denitrifying granules development as described in Chapter 3 and Figs. 3.1 and 3.2. Mixed liquor suspension solids (MLSS) of three denitrifying granules suspensions and seed sludge were maintained at 5000 mg/L. The mean size of the mature denitrifying granules of R1 to R3 was 195 to 220 μm . Sludge volume index (SVI) of granules of R1, R2 and R3 was 33 mL/g SS, 23 mL/g SS and 20 mL/g SS, respectively, while SVI of seed sludge was 290 mL/g SS. Prior to the experiment, the supernatant of mature denitrifying granules and seed sludge was separated from the sludge suspension by centrifugation at 10000 rpm for 30 min (KUBOTA, 5922) to remove the big particles.

4.2.3. Determination of permeate flux

In order to describe the performance of mature denitrifying granular sludge, the permeation flux and filtration resistance were calculated and compared. According to Darcy's law, permeation flux (J) was determined by using Eq. 4.1:

$$J = \text{Flux (L/m}^2 \cdot \text{h)} = \frac{\Delta M / \rho}{A_{\text{eff}} \times \Delta t} \quad (4.1)$$

Where, ΔM is change of filtrate mass during filtration time Δt (g); ρ is the density of the filtrate (g/L); A_{eff} is effective membrane surface area (m²); Δt is time duration of mass change (h).

4.2.4. Determination of membrane resistance

Membrane resistance was determined using the following equation based on the resistance-in-series model (Cheryan 1986):

$$R = \frac{\Delta P}{\mu \Delta J} \quad (4.2)$$

where R is the filtration resistance (1/m); ΔP is the trans-membrane pressure (N/m²); μ is the viscosity of the filtrate (Ns/m²); ΔJ is the permeate flux (L/m²h).

The details of the membrane resistance can be calculated using Eqs. 4.3 and 4.4:

$$R_t = R_m + R_c + R_p \quad (4.3)$$

$$R_f = R_m + R_p \quad (4.4)$$

where R_t is the total resistance (1/m), R_m is membrane resistance (1/m), R_c is the cake layer resistance (1/m), R_p is the pore blocking resistance (1/m), R_f is the membrane fouling resistance (1/m).

The experimental determination of each resistance was described by the following procedure:

- (1) R_m can be calculated by measuring the flux of deionized water before the filtration experiment;

- (2) R_t can be estimated by the final flux of the microfiltration;
- (3) R_f was calculated by measuring the flux of deionized water again, after cleaning the cake layer and washing the membrane surface;
- (4) R_p was calculated by deducting R_m from Eq. 4.4 and R_c was calculated from the difference between Eqs. 4.3 and 4.4.

4.2.5. Extracellular polymeric substances

Bound extracellular polymeric substances (bEPS) of denitrifying granules were extracted using formaldehyde and sodium hydroxide method (Liu and Fang 2002). The extracted EPS was kept at -20°C before use. The soluble extracellular polymeric substances (sEPS) were harvested by direct filtration of the suspension using a membrane with the pore size of $0.45\text{ }\mu\text{m}$. The extracted bound and soluble EPS were analyzed for proteins and polysaccharides, respectively. Proteins were determined by modified Folin-Ciocalteu phenol method with BSA (Bovine Serum Albumin) as standard (Lowry et al., 1951; Frølund et al. 1995). Polysaccharides were determined by phenol-sulfuric method using glucose as standard (Dubois et al., 1956). The detailed procedure of EPS extraction and measurement are given in Section 3.2.4, Chapter 3.

4.2.6. Surface charge

The colloid titration technique was employed to determine the surface charge of denitrifying granular sludge. This method has been widely used in the literature (Kawamura and Tanaka 1966; Morgan et al., 1990; Jia et al., 1996). Details can be found Section 3.2.5, Chapter 3.

4.2.7. Analytical methods

Analytical methods employed in this chapter were described in Section 3.2.7, Chapter 3.

4.3. Results and Discussion

4.3.1. Suspension microfiltration of denitrifying granular sludge

In order to investigate the filterability of the denitrifying granular sludge developed at different cycle times, the suspension microfiltration experiments were conducted using a dead-end microfiltration module (Fig. 4.1). 200 mL of denitrifying granular sludge suspension with the biomass concentration of 5000 mg/L taken from each reactor was used for the microfiltration experiment. Fig. 4.2 shows that the granular sludge developed at the shorter cycle time of 4 h had a faster filtration velocity and a higher permeate flux than the granules developed at the longer cycle times of 6 and 8 h. For instance, in the first 500 seconds, 190 g of permeate was collected from the membrane filtration of the R3 suspension, while 112 g and 90 g from the R2 and R1 suspensions respectively, but only 27 g from the seed sludge filtration. The observed initial decline in the permeate flux could be due to the development of pore blocking and cake layer on the membrane surface. It can be seen in Fig. 4.2 that the steady-state permeate fluxes of the denitrifying granular sludge suspensions declined with the increase in the cycle time, e.g. the permeate flux of the R3 suspension was about 1.7 times and 4 times higher than those of the R2 and R1 suspensions, and 9.4 times higher than the seed sludge.

Figure 4.2 Microfiltration flux of denitrifying granular sludge suspension (Pore size = $0.45\ \mu\text{m}$, TMP = 80 kPa, and MLSS = 5000 mg/L).

High initial fluxes observed in Fig. 4.2 have often been reported in the dead-end microfiltration filtration experiments (Nuengjamnong et al., 2005; Zhou et al. 2007). For example, Zhou et al. (2007) reported an initial flux of $200\ \text{L/m}^2\ \text{h}$ in a dead-end microfiltration ($0.1\ \mu\text{m}$) experiment. It should be pointed out that the microfiltration membrane used in this study had a pore size of $0.45\ \mu\text{m}$. The experimental measurement of the initial flux indeed depends on preset sampling time interval (Δt). In this study, the time interval for sampling was fixed at 10 seconds for the suspension microfiltration (Fig. 4.2), i.e. the initial flux points in Fig. 4.2 indeed represent the fluxes at the end of the first 10 seconds. As the resistance development is strongly related to the properties of mixed liquors to be filtered, this may explain the different initial fluxes obtained in the microfiltration of the R1 to R3 suspensions and the seed sludge. The filtration of supernatant is much faster than that of its corresponding sludge suspension, thus in the microfiltration experiments of supernatants (Fig. 4.3), the sampling time interval was fixed at 1 second, i.e. the initial fluxes in Fig. 4.3 are the fluxes obtained at the end of 1 second. As discussed above, this would be the main reason why the initial

fluxes of the supernatants were found to be much higher than those of the suspensions.

Figure 4.3 Microfiltration flux of denitrifying granular sludge supernatant (Pore size = $0.45\ \mu\text{m}$, TMP = 80 kPa).

4.3.2. Resistance analysis of microfiltration of suspension granules and seed sludge

Table 4.1 shows the respective filtration resistances of the denitrifying sludge suspensions from R1 to R3 and the seed sludge. It appears that the total and cake resistances for the microfiltration of the R3 denitrifying granular sludge suspension were lower than those for R2, R1 and the seed sludge. For example, the total filtration resistance of the R3 denitrifying granular sludge suspension was 1.69-fold and 2.35-fold lower than that of R2 and R1, respectively. The fraction of the cake resistance in the total filtration resistance of the denitrifying granular sludge suspensions varied from 82.4% to 94.5% in R3 to R1, indicating that the cake resistance would be the main contributor to the total filtration resistance as compared to the pore blocking in the microfiltration of the denitrifying granular sludge suspensions. In fact, in study of filterability of aerobic granules, Zhou et al.

(2007) also found that the cake resistance of aerobic granular sludge contributed to 34.7% of the total resistance, while 73.68% for the activated sludge flocs.

The specific cake resistance (α , m/kg) was further calculated from the slope of the plot of t/V versus V (Suarez and Veza 2000; Ognier et al. 2002):

$$\frac{t}{V} = \frac{\mu C \alpha}{2 \Delta P A_{\text{eff}}^2} V + \frac{\mu R_{\text{initial}}}{\Delta P A_{\text{eff}}} \quad (4.5)$$

where t is filtration time (s), V is cumulative permeate volume (m^3), μ is viscosity of permeate (Ns/m^2), C is total suspended solid concentration (kg/m^3), and R_{initial} is initial membrane resistance ($1/\text{m}$).

Table 4.1 shows that the specific cake resistances of the R1 to R3 denitrifying granular sludge suspensions were much lower than that of the seed sludge, e.g. the specific cake resistance of the R3 denitrifying granular sludge suspension is only 2.1% of that of the seed sludge. In this study, the biomass concentrations used in the membrane filtration experiments were all kept at the same level. As the mean sizes of denitrifying granules in R1 to R3 were bigger than that of the seed sludge and the granules were sphere, the potential cake resistance of denitrifying granular sludge suspension would be significantly lower than that of the seed sludge. The cake resistance of suspension of R1, R2 and R3 were 17.67 %, 12.55 % and 6.56 % of the seed sludge.

Table 4.1 Resistance distribution in microfiltration of suspension

	$R_m (\times 10^{11} \text{ 1/m})$	$R_m/R_t (\%)$	$R_p (\times 10^{11} \text{ 1/m})$	$R_p/R_t (\%)$	$R_c (\times 10^{11} \text{ 1/m})$	$R_c/R_t (\%)$	$R_t (\times 10^{11} \text{ 1/m})$	$\alpha (\times 10^{11} \text{ m/kg})$
R3	0.22	4.13	0.73	13.52	4.45	82.35	5.40	6.06
R2	0.22	2.41	0.38	4.21	8.52	93.39	9.13	16.50
R1	0.22	1.73	0.48	3.78	12.0	94.49	12.70	27.60
seed sludge	0.19	0.28	0.38	0.56	67.9	99.20	68.40	286

R_m : membrane resistance; R_p : pore blocking resistance; R_c : cake layer resistance; R_t : total resistance; α : specific resistance.

4.3.3. Supernatant microfiltration of denitrifying granular sludge

In the experiment of supernatant microfiltration, 400 mL supernatant was harvested from R1 to R3 by filtering the corresponding suspension from each reactor. The experiments were carried out under the same experimental conditions as described for the suspensions microfiltration. It was found that time required for collecting 200 g of permeates was 200 seconds for the R3 supernatant, 326 seconds for the R2 supernatant, 600 seconds for the R1 supernatant and 891 seconds for the seed sludge supernatant. These suggest that the membrane filtration of the supernatant of R3 operated at the shortest cycle time of 4 h is much faster than those from R1, R2 and the seed sludge. In fact, Fig. 4.3 clearly shows that the R3 supernatant exhibited the highest permeate flux as compared with those obtained from R1 and R2 run at the cycle times of 8 and 6 h as well as the seed sludge. The steady state permeate flux of the R3 supernatant was found to be 1.8-fold and 2.69-fold higher than those from R2 and R1, and 4.2-fold of that from seed sludge, respectively.

4.3.4. Resistance analysis of microfiltration of supernatant of granules and seed sludge

Table 4.2 shows that the total resistances of the R1 to R3 supernatants were significantly smaller than those obtained in the microfiltration of the denitrifying granular sludge suspensions. The supernatant of the denitrifying granular sludge suspension from R3 run at the shortest cycle time of 4 h exhibited the lowest total resistance as compared to those found in R1, R2 and the seed sludge. Moreover, the fraction of the cake resistance of the supernatant in the total resistance was reduced to 47-50%, which was much smaller than that observed in the suspension microfiltration (Table 4.1). It was found in R3 that the fraction of the pore blocking resistance in the total resistance for the supernatant was increased from 13.5% for the suspension to about 47%. In fact, the pore blocking resistance of the supernatant from an aerobic granular sludge suspension was reported to be as high as 59% of the total resistance (Thanh et al., 2008).

Table 4.2 Resistance distribution in microfiltration of denitrifying granules supernatant.

	$R_m (\times 10^{11} \text{ 1/m})$	$R_m/R_t (\%)$	$R_p (\times 10^{11} \text{ 1/m})$	$R_p/R_t (\%)$	$R_c (\times 10^{11} \text{ 1/m})$	$R_c/R_t (\%)$	$R_t (\times 10^{11} \text{ 1/m})$
R3	0.22	5.91	1.75	47.05	1.75	47.03	3.72
R2	0.26	3.41	3.53	46.26	3.85	50.40	7.63
R1	0.24	2.09	5.06	44.00	6.20	53.91	11.5
seed sludge	0.23	1.24	6.65	35.75	11.7	63.04	18.6

R_m : membrane resistance; R_p : pore blocking resistance; R_c : cake layer resistance; R_t : total resistance.

4.3.5. Factors affecting membrane fouling

4.3.5.1. Effect of cycle time on membrane fouling

In order to better understand the influence of the cycle time of SBRs on the filtration performance of the suspension and supernatant, the concept of half-life ($t_{1/2}$) was introduced, which represents time required for initial permeate flux (J_0) to decline to $0.5J_0$. The smaller $t_{1/2}$ is, the faster flux decline would be. Fig. 4.6 shows that $t_{1/2}$ values obtained in the suspension and supernatant microfiltration of denitrifying granules and seed sludge. It was found that the $t_{1/2}$ values of suspensions of granules and seed sludge were smaller than their corresponding supernatants. The permeate flux decline of suspension of granules and seed sludge was faster than the corresponding supernatant (Figs 4.2 and 4.3). It was also found that $t_{1/2}$ was related to the cycle time of SBR. Granulation has been proved to enhance biomass filtration and to reduce the membrane resistance (Li et al., 2007). Shorter cycle time would increase the formation of granules and separation of the solids phase from the liquid phase. Fig. 4.6 shows that $t_{1/2}$ of both granules suspension and supernatant of R3 operated at the shorter cycle time of 4 h was greater than that of R1, R2 and seed sludge, meaning that the flux decline of R3 granules suspension and supernatant is much slower than those observed in R1, R2 and seed sludge. These in turn suggest that the granules suspension and supernatant of R3 had a higher permeate flux and lower resistance compared with the corresponding suspension and supernatant of R1, R2 and seed sludge (Figs. 4.2 and 4.3; Tables 4.1 and 4.2).

Figure 4.4 Comparison of $t_{1/2}$ in the suspension and supernatant microfiltration of denitrifying granules and the seed sludge.

4.3.5.2. Effect of granule size on membrane fouling

The performance of microfiltration would strongly depend on the size of denitrifying granule. According to the cake filtration theory (Eq. 4.5), the slope of the linear portion in the t/V - V curve can be defined as modified fouling index (MFI) and used to evaluate the fouling potential. MFI can be expressed as follows:

$$\text{MFI} = \frac{\mu C \alpha}{2 \Delta P A_{\text{eff}}^2} = k \alpha \quad (4.6)$$

At a given ΔP , the size-effect of mature denitrifying granules on MFI was shown in Fig. 4.5. It can be seen that MFI was inversely correlated to the square of the mean diameter of mature denitrifying granules developed in R1 to R3. In fact, such an observation can be explained by the well-known Carmen-Kozeny equation showing that the specific cake resistance is proportional to the inverse of the square of the mean particle size. These in turn provide theoretical basis for better understanding the filtration performance and resistance data presented in Fig. 4.2 and Table 4.1.

Figure 4.5 Plot of MFI versus granule size.

4.3.5.3. Effect of granule strength on membrane fouling

The total resistance of the denitrifying granular sludge suspension developed at the shorter cycle time was lower than those cultivated at the longer cycle times (Table 4.1). In addition to the bigger size, the denitrifying granular sludge developed at the shorter cycle time also had higher mechanical strength (Fig 3.11). Obviously, the granules with higher mechanical strength could easily resist to the shape deformation during filtration. As the result, it can be expected that the porosity of the cake layer formed by the high-strength R3 denitrifying granules would be high vis-à-vis denitrifying granules from R1 and R2 with low mechanical strength. This was supported by the data presented in Table 4.1 showing that the cake resistance of the R3 granular sludge suspension had relatively small portion in the total resistant compared with the R1 and R2 granular sludge suspensions. Obviously, the cake layer with high porosity would be less effective in retaining soluble compounds, such as sEPS and colloidal particles, which would eventually cause the pore blocking of membrane. This view is supported by the fact that the cake resistance of the R3 denitrifying granular sludge suspension had relatively small portion in the total resistance, but its pore blocking resistance largely contributed to the total resistance as compared to R1 and R2.

In addition, the data presented in Tables 4.1 and 4.2 suggest that the dominant fouling mechanism for the microfiltration of denitrifying granular sludge and the seed sludge suspensions can be attributed to the resistance associated with the cake layer, whereas the pore blocking seems to be dominant over the cake layer resistance during the microfiltration of the supernatants of the denitrifying granular sludge and the seed sludge suspensions. These results indicate that the cake layer developed during the microfiltration of sludge suspension can serve as a barrier to further remove the pore blocking-causing substances (e.g. sEPS or colloidal particles) through filtration or adsorption.

4.3.5.4. Effect of soluble EPS on the development of the pore blocking

Soluble EPS (sEPS) have often been thought to be one of the main causes responsible for filtration resistance development in MBRs (Thanh et al., 2008). Fig. 4.6 shows the concentration of soluble polysaccharides (sPS) and soluble proteins (sPN) in the R1 to R3 supernatants. The sPS concentration in the supernatant was found to be proportionally related to the cycle time, i.e. high sPS concentration was observed at the longer cycle time, whereas the sPN concentration nearly remained unchanged. The correlation between the soluble PS and the resistances due to cake layer and pore blocking for the R1 to R3 supernatants is further shown in Fig. 4.7. It appears that sPS would contribute to both cake resistance and pore blocking. In study of aerobic granular sludge MBR, Thanh et al. (2008) also found that the sPS would have more profound influence on the membrane fouling than the total sEPS. In addition, Nuengjamnong et al. (2005) reported that the permeate flux of the MBR with a sludge retention time (SRT) of 80 days was higher than that of the MBRs with a SRT of 8 or 20 days due to lower concentration of the sEPS in the former MBR (Wang et al., 2009).

Figure 4.6 Soluble extracellular polysaccharides (sPS) and soluble proteins (sPN) in supernatant of denitrifying granular sludge.

Figure 4.7 Effect of sPS on membrane resistances in microfiltration of supernatant of denitrifying granules.

4.3.5.5. Effect of surface charge on the membrane fouling

Fig. 4.8 shows that the R1 to R3 denitrifying granules all carried the negative surface charge, and the lowest surface charge density was observed in denitrifying granules developed at the shortest cycle time of 4 h. Obviously, lowered negative surface charge density would help biomass to form the denser and stronger granules, and further prevent the development of membrane fouling as reported widely in the literature (Quarmby and Forster 1995; Sallis and Uyanik 2003).

Figure 4.8 Cell surface charge of denitrifying granular sludge suspension and its corresponding supernatant.

It appears from Fig. 4.8 that the denitrifying granular sludge suspension carried much higher negative surface charge than its supernatant. High negative surface charge of microbial sludge in MBR has been reported to cause serious membrane fouling (Lee et al., 2003). The correlation of the surface charge density to the cake resistance and pore blocking resistance of the suspension and the supernatant of the denitrifying granular sludge were presented in Figs. 4.9 and 4.10, respectively. The cake resistance of the suspension and supernatant tended to increase with the increase in the negative charges, but the surface charge had a more

significant influence on the microfiltration of the granular sludge supernatant than its suspension.

Figure 4.9 Resistances versus surface charge of the suspension of denitrifying granular sludge.

Figure 4.10 Resistances versus surface charge of the supernatant of denitrifying granular sludge.

4.4. Summary

The denitrifying granules were successfully developed in SBRs operated at different cycle time of 4, 6 and 8 hours, respectively, and microfiltration studies were carried with the suspension and supernatant of the denitrifying granular sludge cultivated. The following concluding remarks can be drawn from the present study:

- (1) As compared to the seed sludge, denitrifying granules exhibited a superior permeate flux, indicating a great potential of denitrifying granular sludge MBR for high-efficiency nitrogen removal from wastewater.
- (2) The microfiltration performances of denitrifying granular sludge developed at different cycle times were closely related to their characteristics. For example, denitrifying granular sludge developed at the cycle time of 4 h had higher permeate flux and lower fouling due to bigger size and less negative surface charge of granules. Such observation was further evidenced by the lower MFI obtained at the cycle time of 4 h, e.g. MFI of mature denitrifying granules decreased from 59300 s/L² to 12100 s/L² when the size of granules was increased from 190 µm to 220 µm.
- (3) Soluble polysaccharides were more responsible for the observed membrane fouling than soluble proteins. Consequently, this study clearly showed that combination of granular sludge with membrane filtration would be an alternative for effectively mitigating membrane fouling in MBR.

CHAPTER 5

CALCIUM AUGMENTATION FOR ENHANCED DENITRIFYING GRANULATION IN SEQUENCING BATCH REACTORS

5.1. Introduction

With increasing demands on water reuse and recycle, advanced and cost-effective technologies for nitrogen removal from wastewater are highly desirable. In this regard, biological nitrogen removal through nitrification-denitrification has been practiced worldwide for decades (Metcalf & Eddy 2003; Wu et al., 2009). However, it should be realized that almost all denitrification processes are based on suspended culture in which it is difficult to achieve high biomass concentration, effective retention and separation of denitrifying sludge due to frequent sludge bulking. These would eventually lead to a large volume of denitrification tank and potential high-energy demand on mixed liquor recirculation.

Anaerobic granules are self-immobilized bacterial aggregates and have been cultured in various types of reactors, such as up-flow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB), upflow bed and filter (UBF), anaerobic baffled reactor (ABR) and anaerobic migrating blanket reactor (AMBR), etc (Lettinga et al., 1980; Uyanik et al. 2002; Angenent et al., 2004; Lant and Hartley 2007; Puyol et al. 2009). Although extensive research has been conducted, the mechanisms of anaerobic granulation still remain unclear and debatable as reviewed by Liu et al. (2003). Anaerobic granulation has been known to be a very slow process that may require for 2 to 4 months of careful operation. Divalent cations, such as calcium, have been recognized to play an important role in microbial self-immobilization by reducing cell surface charges and/or bridging extracellular polymeric substances (Flemming and Wingender 2010). It has been reported that removal of calcium by ethylene glycol-bis (beta-aminoethylether)-N, N-tetraacetic acid (EGTA) eventually led to break-up of methanogenic granular sludge (Grotenhuis et al. 1991). So far, little information has been known about

calcium augmentation for enhancement of denitrifying granules in sequencing batch reactors (SBRs). Therefore, the purpose of this study was to systematically evaluate if calcium augmentation would lead to accelerated and strengthened denitrifying granulation with the production of granules having superior physical and biological characteristics in SBRs.

5.2. Materials and Methods

5.2.1. Experimental set-up and synthetic wastewater

Three identical columns (60 cm height and 8 cm in internal diameter) with a working volume of 2.5 L were used as sequencing batch reactors (SBRs), namely R1 to R3 (Figs. 3.1 and 3.2). A mechanical mixer at 100 rpm was employed to provide the mixing power in each reactor. R1 to R3 were operated at a fixed cycle time of 4 h with a volume exchange ratio of 50% under anaerobic conditions. Each cycle consisted of 15 min feeding, 205 min reaction, 15 min settling and 5 min effluent discharge. The experiments were conducted in a temperature control room of 25°C.

Synthetic wastewater used in this study was made up of potassium nitrate and methanol as the main nitrogen and organic carbon sources (Table 5.1). Nitrate and TOC concentrations in the synthetic wastewater were kept constant at 120 mg N/L and 120 mg TOC/L, respectively. Calcium chloride was used as the main source of calcium, and calcium concentrations in the synthetic wastewaters to R1, R2 and R3 were kept at the levels of 0, 50 and 100 mg Ca^{2+} /L, respectively. The synthetic wastewater was prepared in deionized water (DI water).

Table 5.1 Composition of the synthetic wastewater

Component	Concentration (mg/L)
KNO ₃	865.7
CH ₃ OH	320
CaCl ₂ •2H ₂ O	0 (for R1)
	183.75 (for R2)
	367.5 (for R3)
MgSO ₄ •7H ₂ O	40
FeSO ₄ •7H ₂ O	6.25
Phosphate buffer [*]	2 mL/L
Trace element solution ^{**}	1 mL/L

*: 1 L of phosphate buffer contained 24.4 g KH₂PO₄ and 12.2 g NaHPO₄

**: 1 L trace element solution (pH = 7.5) was composed with 500 mg MnCl₂•4H₂O, 50 mg ZnCl₂, 100 mg NiSO₄•6H₂O, 50 mg CoCl₂•6H₂O, 26 mg Na₂MoO₄•2H₂O, 50 mg H₃BO₄ and 300 mg CuSO₄•5H₂O.

5.2.2. Seed sludge

Activated sludge taken from a local wastewater reclamation plant was enriched with the synthetic wastewater under anaerobic condition for two weeks. 6.75 g of enriched sludge was seeded into R1 to R3, respectively, equivalent to an initial mixed liquor suspended solids (MLSS) concentration of 2700 mg SS/L in all the three reactors, whereas the sludge volume index after 15 min settling (SVI₁₅) of the seed sludge was about 104.65 mL/g SS.

5.2.3. Extracellular polymeric substances

Extracellular polymeric substances (EPS) were extracted from denitrifying biomass using formaldehyde and sodium hydroxide method (Liu and Fang 2002). The extracted EPS were kept at -20°C before analysis. Extracellular proteins (PN) in extracted EPS were determined by Lowry method with Bovine Serum Albumin

(BSA) as standard (Lowry et al., 1951), whereas extracellular polysaccharides (PS) were determined using phenol-sulfuric acid method with glucose as standard (Dubois et al., 1956). The detailed procedures of EPS extraction and analysis were all presented in Section 3.2.4, Chapter 3.

5.2.4. Cell surface charge

The surface charge densities of microbial sludge and granules were determined according to colloid titration method used by Jia et al. (1996) and Morgan et al. (1990). The details were presented in Section 3.2.5, Chapter 3.

5.2.5. Mineral contents in denitrifying granules

100 mg of mature denitrifying granules and seed sludge were dried at 105°C until constant weight. According to method described by Sandroni and Smith (2002), dried biomass was grinded and digested with 6 mL of 1N HNO₃ solution for 50 min in a microwave reaction system (Multiwave 3000, Anton Paar). The digested biomass was further diluted to 25 ml with DI water, and the diluted samples were analyzed for Ca, Mg, Fe and K etc by an inductively coupled plasma emission spectrometer (Optima, 2000DV).

5.2.6. Analytical methods

Other analytical methods were presented earlier in Section 3.2.7, Chapter 3.

5.3. Results and Discussion

5.3.1. Development of denitrifying granules

R1 to R3 were supplemented with the respective calcium concentration of 0, 50 and 100 mg Ca²⁺/L, and operated for 96 days under anaerobic condition. Fig. 5.1 shows changes in the biomass size over time. It was found that addition of calcium ion could accelerate denitrifying granulation process, e.g. the mean size of denitrifying aggregates was increased at the growth rate of 1.22 µm/d, 2.32 µm/d

and 4.46 $\mu\text{m}/\text{d}$, whereas the mean size of biomass gradually stabilized at 140 μm , 260 μm and 420 μm in R1, R2 and R3, respectively. As shown in Fig. 5.2, R1 denitrifying granules without the addition of calcium had smaller size and much looser structure than those developed in R2 and R3 supplemented with 50 and 100 $\text{mg Ca}^{2+}/\text{L}$.

Figure 5.1 Evolution of mean size of microbial aggregates in R1, R2 and R3.

Figure 5.2 Morphology of mature denitrifying granules on day 92 and seed sludge.

(a) seed sludge; (b) R1; (c) R2; and (d) R3. Bar = 500 μm .

The size distribution of mature denitrifying granules in R1 to R3 on day 92 was analyzed and compared with that of the seed sludge (Fig. 5.3). It was found that over 90% of mature granules in R3 had a size bigger than 200 μm , while most R1 granules were smaller than 200 μm , i.e. addition of calcium could favor the formation of the big denitrifying granules. In study of anaerobic granulation in a UASB reactor, Yu et al. (2001) also found that Ca^{2+} at concentrations of 150 to 300 mg/L enhanced anaerobic granulation, whereas addition of calcium would induce the formation of bigger particles and thus helped to prevent the wash-out of biosludge from anaerobic bioreactors (Pevere et al., 2007).

Figure 5.3 Size distribution of the seed sludge and the denitrifying granules in R1, R2 and R3 on day 92.

5.3.2. Biomass concentration

Figs. 5.4 to 5.6 show changes in MLVSS, MLSS and MLVSS/MLSS ratio in R1 to R3. The MLVSS concentrations in R1 to R3 all tended to increase, but at different rates, e.g. 100 mg VSS/L·d in R1, 130 mg VSS/L·d in R2 and 165 mg VSS/L·d in R3 during denitrifying granulation (Fig. 5.4). The MLSS concentration in the steady-state R3 fed with 100 mg Ca^{2+} /L was 2.98-fold and 1.29-fold higher than those in R1 and R2, respectively (Fig. 5.5). The lowest MLVSS/MLSS ratio of 0.37 was observed in R3 (Fig. 5.6). However, even at such low MLVSS/MLSS ratio, the MLVSS concentration in R3 was still higher than those in R2 and R3. These implied that addition of calcium in the range studied would not affect the accumulation of MLVSS. In fact, high mineral content in R3 granules would result from the accumulation of calcium (Table 5.2).

Figure 5.4 Changes of MLVSS in the course of operation of R1 to R3.

Figure 5.5 Changes of MLSS in the course of operation of R1 to R3.

Figure 5.6 Changes of MLVSS/MLSS ratios in R1 to R3.

5.3.3. Mineral contents of denitrifying granules

Table 5.2 shows the mineral contents of the seed sludge and mature denitrifying granules developed in R1 to R3. More calcium was accumulated in denitrifying granules fed with higher influent calcium concentration, and calcium was found to be the main component against other ions in R2 and R3 denitrifying granules. The calcium content of R1 granules was found to be lower than that of the seed sludge due to the fact that no calcium ion was supplemented to R1, thus release of calcium from R1 granules would eventually occur. Titration of denitrifying granules by 1 M HCl showed that carbon dioxide produced against accumulated calcium was nearly in a molar ratio of 1 to 1, suggesting that calcium in R1 to R3 granules would primarily exist in the form of calcium carbonate.

Table 5.2 Mineral contents in the mature denitrifying granules on day 90 and the seed sludge

Mineral content (mg/g SS)	Seed sludge	R1	R2	R3
Calcium	37.18	5.63	154.61	177.63
Magnesium	1.93	9.08	6.85	1.15
Iron	7.28	6.85	1.61	1.98
Copper	0.83	1.15	0.46	0.49
Zinc	0.33	0.60	0.19	0.15
Potassium	15.89	9.08	2.31	2.19
Sodium	3.63	1.48	0.44	0.37
Total	67.07	33.89	166.47	183.96
CaCO ₃	92.95	14.08	386.53	444.08

The estimated fraction of calcium carbonate in biomass (Table 5.2) was consistent with the MLVSS/MLSS ratio presented in Fig. 5.6. As discussed previously, the addition of calcium would favor the development of big denitrifying granules (Fig. 5.2). The formation of calcium carbonate is responsible for this observation because it serves as a binding force as noted by Chang and Lin (Chang and Lin 2006). Previous study also showed that calcium carbonate (CaCO₃) was an important constituent of anaerobic granules cultivated in UASB reactors (Van Langerak et al., 2000; Yu et al., 2001; Ren et al. 2008).

5.3.4. Settleability of denitrifying granules

In this study, the influence of feed calcium concentration on the settleability of denitrifying granules was evaluated using 15 min SVI (SVI₁₅). As shown in Fig. 5.7, the lowest SVI₁₅ of 14.36 mL/g SS was observed in R3 supplemented with 100 mg Ca²⁺/L. It should be noted that the SVI₁₅ values of denitrifying granules were comparable with those of aerobic and anaerobic granules (Tsuneda et al. 2003; Angenent et al., 2004; Qin and Liu 2006; Liu et al., 2007).

Figure 5.7 SVI₁₅ profiles of denitrifying granules in the course of operation of R1 to R3.

The settleability of mature denitrifying granules developed at the different calcium concentrations was correlated to the MLVSS/MLSS ratio (Fig. 5.8). As discussed above, the biomass MLVSS/MLSS ratio is largely dependent on the fraction of calcium carbonate in denitrifying granules (Table 5.2). Such an observation is consistent with that reported for UASB granules (El-Mamouni et al. 1995). As noted by Fang et al. (2008), settleability of anaerobic granules would be more closely related to their ash content and structure than their size.

Figure 5.8 Correlation between SVI₁₅ and MLVSS/MLSS ratio.

5.3.5. Extracellular polymeric substances

Extracellular polymeric substances (EPS) are believed to play an important role in microbial granulation (Zhou et al., 2006), and extracellular proteins (PN) and polysaccharides (PS) are the main components of EPS. Figs. 5.9 to 5.11 show that the PN contents in R1 to R3 denitrifying sludge all gradually increase to their respective stable values, whereas the PS contents do not change significantly during denitrifying granulation. These results would suggest that PN would play a more important role than PS in denitrifying granulation. In addition, the steady-state PN contents tended to increase from 113.24 mg/g VSS in R1 to 157.35 mg/g VSS in R3, i.e. calcium ion, to some extent, would stimulate the production of PN instead of PS.

Figure 5.9 The PS and PN contents versus operation time observed in R1.

Figure 5.10 The PS and PN contents versus operation time observed in R2.

Figure 5.11 The PS and PN contents versus operation time observed in R3.

Recently, Jiang and Liu (2010) reported that the reduced PN content caused by chemical uncoupler to the failure of aerobic granulation. In study of microbial attachment to stainless steel, treatment with the bacterial surface polysaccharide disruptor, sodium metaperiodate, did not affect the attachment ability of the bacteria. In contrast, treatment of bacteria with trypsin or sodium dodecyl sulfate to remove cell surface proteins resulted in a 100-fold reduction in the number of attached bacteria onto the surface of stainless steel (Flint et al., 1997). Moreover, Dufrene et al. (1996) also observed the correlation between protein concentration at the cell surface and adhesion density under various conditions. More interestingly, Allison and Sutherland (1987) found that both the nonpolysaccharide-producing mutant and the polysaccharide-producing wild type could adhere to glass surface. It appears from this and previous study that specific contribution of extracellular PN and PS to microbial attachment and granulation deserves further study. As noted by Jiang and Liu (2010), the role of extracellular PS, to some extent, is over highlighted against extracellular PN for microbial granulation.

In this study, the calcium content in extracted EPS was also determined. It was found that calcium in EPS accounted for about 2.5-2.9% of total calcium in R1

to R3 denitrifying granules. In fact, high calcium content in granules would favor formation of EPS- Ca^{2+} -EPS bridge as illustrated in Fig. 5.12 (Flemming and Wingender 2010). Obviously, such EPS- Ca^{2+} -EPS complex in turn would further strengthen the three-dimensional structure and stability of microbial community including biofilms and biogranules. In fact, the high affinity between EPS and calcium has been reported in both aerobic and anaerobic processes (Forster and Lewin 1972; Wang et al. 2006; Ren et al., 2008).

Figure 5.12 The classes of weak physicochemical interactions and the entanglement of biopolymers that dominate the stability of the EPS matrix (Mayer 1999; Flemming and Wingender 2010).

5.3.6. Cell surface charge

Calcium as a typical divalent ion would alter cell surface charge property that in turn affects bacterium to bacterium interaction. At the normal water pH, microorganisms often carry negative charges that prevent close contact of bacteria.

Fig. 5.13 shows changes in bacterial surface charge density in R1 to R3. Compared to the seed sludge, the surface charge density of R1 to R3 denitrifying granules on day 92 was reduced by 51.3%, 68.7% and 82.4%, respectively. These indicate a clear charge neutralization effect of calcium. Reduced negative surface charge density would facilitate microbial interaction leading to faster and stronger microbial aggregation (Fig. 5.1). Consequently, it is reasonable to consider that addition of calcium would help to enhance denitrifying granulation and further improve the strength and stability of denitrifying granules through surface charge neutralization and the formation of EPS- Ca^{2+} -EPS binding as discussed earlier.

Figure 5.13 Changes in surface charge of biomass along with denitrifying granulation.

5.3.7. Performance of denitrifying granules

Figs. 5.14 and 5.15 show the respective cyclic concentration profiles of NO_3^- -N and TOC in R1 to R3 on different days. It can be seen that the influent NO_3^- -N and TOC were nearly 100% removed after 40 days of operation. The activities of denitrifying granules in R1 to R3 tended to increase over the operation time,

while the faster increase in the activity in terms of TOC and nitrate removal was observed in the reactor fed with calcium. This is probably due to calcium-enhanced denitrifying granulation, leading to high biomass retention in R2 and R3 as shown in Fig. 5.5. It should also be noted that no significant NO_2^- accumulation was observed in R1 to R3 over 96 days of operation. The amount of TOC required to denitrify 1 mg NO_3^- -N was computed from Figs. 5.14 and 5.15. It was found in Fig. 5.16 that 1.0 mg NO_3^- -N removed requires 0.996 mg TOC, which is very close to the theoretical TOC to NO_3^- -N ratio of 0.93 derived from the stoichiometry of denitrification reaction using methanol as carbon source (Eq. 5.1).

Figure 5.14 Cycle concentration profiles of NO_3^- -N in R1 (a), R2 (b) and R3 (c) on different operation days.

Figure 5.15 Cycle concentration profiles of TOC in R1 (a), R2 (b) and R3 (c) on different operation days.

Figure 5.16 TOC consumption versus NO₃⁻-N utilization in R1 to R3.

The specific substrate utilization rate has been widely used to describe microbial activity under various conditions. In this study, the specific denitrification rate defined by Eq. 5.2 was calculated from the data presented in Fig. 5.14.

$$\text{DNR (mg N/g VSS} \cdot \text{d)} = \frac{[\text{NO}_3^- - \text{N}]_i - [\text{NO}_3^- - \text{N}]_e}{t \times \text{MLVSS}} \quad (5.2)$$

where $[\text{NO}_3^- - \text{N}]_b$ is NO₃⁻-N concentration at the beginning of denitrification in each cycle (mg N/L); $[\text{NO}_3^- - \text{N}]_e$ is NO₃⁻-N concentration at the end of denitrification (mg N/L) and t is reaction time (d). As can be seen in Fig. 5.17, denitrifying granules from R1 without addition of calcium had the lowest DNR compared to R2 and R3 granules with supplement of calcium. The highest DNR of 1040 mg N/g VSS d was recorded with R2 denitrifying granules supplemented with 50 mg Ca²⁺/L. According to Fig. 5.17, excessive dosage of Ca²⁺ (e.g. in the case of 100 mg Ca²⁺/L in R3) would not favor microbial activity of denitrifying granules, on the other

hand, insufficient supply of Ca^{2+} would inhibit microbial activity of denitrifying granules in the term of DNR (e.g. in the case of 0 mg Ca^{2+} /L in R1). In fact, a significant loss of biological activity of anaerobic granules at high calcium concentration had been reported in UASB systems (El-Mamouni et al., 1995; Van Langerak et al., 2000). In study of the removal of nitrate from rinse wastewater generated in the stainless steel manufacturing process by denitrification in a SBR, Fernandez-Nava et al. (2008) found that a high calcium concentration led to a decrease in both the biomass growth rate and denitrification rate. In fact, the DNR obtained in this study was higher than those previously reported, but except for the pure cultures as shown in Table 5.3.

Figure 5.17 Changes of specific denitrification rates in the course of R1, R2 and R3 operation.

Table 5.3 Comparison of the maximum specific denitrification rate using methanol as organic carbon source

Sludge form	Reactor	DNR (mg N/g VSS d)	References
Suspended sludge	Continuous	14-37	(Argaman 1986)
Suspended sludge	Batch	648	(Akunna et al., 1993)
Suspended sludge	SBR	768	(Hallin et al. 1996)
Suspended sludge	SBR	696	(Lee and Welander 1996)
Suspended sludge	SBR	19	(Louzeiro et al. 2002)
Suspended sludge	SBR	19	(Louzeiro et al., 2002)
Suspended sludge	Continuous	170	(Carrera et al. 2003)
Suspended sludge	SBR	220.8	(Mokhayeri et al., 2008)
Granules	SBR	729.6	(Fernandez-Nava et al., 2008)
Granules	SBR	28.8	(Adav et al., 2010)
Granules	USB	319.2	(Pagacova et al. 2010)
Pure suspended culture	SBR	1440	(Ginige et al. 2004)
Pure denitrifying culture	Batch	2200	(Christensson et al., 1994)
Denitrifying granules	SBR	600-1040	This study

5.3.8. Effluent quality

In suspended denitrifying process, effluent biomass concentration is often high due to poor settleability of denitrifying sludge. Fig. 5.18 shows the respective MLSS concentrations in effluents (EMLSS) from the steady-state R1 to R3. Although the biomass concentrations in the steady-state R2 and R3 were about 2.27-fold and 2.98-fold higher than that in R1 free of Ca^{2+} addition, the EMLSS concentrations from R2 and R3 were much lower than that from R1. Moreover, the EMLSS/MLSS ratio in R3 supplemented with 100 mg/L Ca^{2+} was found to be 26.1% and 75.4% smaller than in R2 and R1, respectively (Fig. 5.18).

Figure 5.18 Comparison of mixed liquor suspended solids in the effluent from and EMLSS/MLSS ratio in R1 to R3.

It should be noted that high EMLSS concentration has often been reported in the conventional activated sludge processes due to the poor settleability of activated sludge (Liao et al., 2001). Calcium added to R2 and R3 appeared to have double functions: (i) accelerating denitrifying granulation and strengthening structure of denitrifying granules, which is beneficial for having a high effluent quality as demonstrated in Fig. 5.19; (ii) coagulation of colloidal particles, soluble EPS and dispersed microorganisms in the suspension. These in turn would result in high

quality of the effluent even at the very high MLSS concentration of 13,000 mg/L as observed in R3 fed with 100 mg Ca^{2+} /L.

Figure 5.19 Correlation between EMLSS/MLSS ratio and SVI₁₅ of the denitrifying granules in R1, R2 and R3.

5.4. Summary

This study showed that Ca^{2+} augmentation would be an alternative for rapid denitrifying granulation, and strengthen the structure and stability of denitrifying granules. It was found that calcium could significantly reduce the negative surface charge density of biomass, while facilitated formation of EPS- Ca^{2+} -EPS complex, leading to high-strength structure and excellent settleability of denitrifying granules. The results also revealed that extracellular proteins would play a more important role than extracellular polysaccharides in denitrifying granulation regardless of addition of calcium. Excellent effluent quality Furthermore, a high-quality effluent was achievable with the addition of calcium even at the MLSS concentration as high as 13 g/L. The maximum specific denitrification rate of 1040 mg N/g VSS d was obtained with denitrifying granules developed at 50 mg/L calcium, however further increased calcium concentration would hinder the activity of denitrifying granules. This study offers in-depth insights into the mechanisms of denitrifying granulation and its potential application.

CHAPTER 6

MEMBRANE FOULING MECHANISMS IN MICROFILTRATION OF DENITRIFYING GRANULAR SLUDGE SUSPENSION

6.1. Introduction

Membrane bioreactors (MBRs) have been applied to treat municipal and industrial wastewater for water recycle and reuse (Choo and Lee 1996; Li et al., 2005; Jeison et al. 2009; Lew et al. 2009). However, membrane fouling is the major problem that causes increased operation cost and energy consumption due to frequent cleaning and replacement of clogged membrane (Magara and Itoh 1991; Chang et al., 2002). It has been reported that the nature and extent of membrane fouling during the operation of MBRs would be mainly determined by the biomass characteristics, operation conditions and membrane surface properties (Chang et al., 2002). For example, biomass concentration and sludge morphology would impact on the membrane fouling due to the formation of sludge cake on the membrane surface (Lee et al., 2001; Thanh et al., 2008), whereas reduced membrane fouling was observed at lowered biomass concentration (Chang and Kim 2005; Farizoglu and Keskinler 2006; Li and Wang 2006). Nowadays, the major challenge is how to effectively control membrane fouling in MBR.

As presented in the precedent chapter, denitrifying granules were successfully cultivated at different calcium concentrations. Although it has been reported that aerobic granular sludge would help alleviate membrane fouling (Zhou et al. 2007), little has been known about the fouling mechanisms of granular sludge. Therefore, this chapter aimed to investigate the fouling mechanisms during the microfiltration of denitrifying granular sludge mixed liquors.

6.2. Materials and Methods

6.2.1. Microfiltration set-up

A standard dead-end microfiltration module was employed in this study, which was connected with a mini-reservoir as shown in Fig. 4.1. The nitrocellulose membrane with a pore size of 0.22 μm and an effective surface area of 45.3 cm^2 was used (GSPW, Millipore, Ireland). Trans-membrane pressure (TMP) was provided by compressed nitrogen gas and kept at 5 kPa for all the experiments.

6.2.2. Feed solutions used in microfiltration experiments

As presented in Chapter 5, denitrifying granules were successfully developed in R1 to R3 supplemented with the calcium concentrations of 0, 50 and 100 $\text{mg Ca}^{2+}/\text{L}$, respectively. In this study, the granular sludge mixed liquors collected from R1 to R3 were used to prepare three kinds of feed solutions for the microfiltration experiments, i.e. (i) mixed liquor, (ii) granules solution and (iii) supernatant.

(i) Preparation of mixed liquor: Mixed liquors were directly taken from the steady state R1 to R3. The concentrations of denitrifying granules in R1 to R3 mixed liquors were adjusted to 5000 mg SS/L using the corresponding supernatants. (ii) Preparation of granules solution: Denitrifying granules were separated and further collected after 15 min settling of R1 to R3 mixed liquors. The harvested granules were re-suspended in DI water with a granules concentration of 5000 mg SS/L . (iii) Preparation of supernatant: After 15 min settling of R1 to R3 mixed liquors, the upper liquid was collected as the supernatant.

6.2.3. Determination of permeate flux and membrane resistance

Permeate flux was determined by Darcy's law (Eq. 4.1, Chapter 4), whereas total resistance (R_t), cake resistance (R_c), pore blocking resistance (R_p) and membrane resistance (R_m) were determined using resistance-in-series model (Cheryan 1986). Details can be found in Sections 4.2.3 and 4.2.4, Chapter 4.

6.2.4. Determination of specific cake resistance

According to cake filtration model, the specific cake resistance (α) can be calculated from the slope of the plot of t/V versus V :

$$\frac{t}{V} = \frac{\mu C \alpha}{2 \Delta P A_{\text{eff}}^2} V + \frac{\mu R_{\text{initial}}}{\Delta P A_{\text{eff}}} \quad (6.1)$$

where t is filtration time (s), V is cumulative permeate volume (m^3), μ is viscosity of permeate ($\text{Pa}\cdot\text{s}$), C is total suspended solid concentration (kg/m^3), ΔP is trans-membrane pressure (Pa), A_{eff} is effective membrane surface area (m^2), and R_{initial} is initial membrane resistance ($1/\text{m}$).

6.2.5. Determination of compressibility of cake

Compressibility index n was used to describe the cake compressibility formed on the membrane surface by granules during microfiltration. The cake layer is considered incompressible when n is null. The higher n is, the more compressible the cake would be. n can be determined by Eq. 6.2 (Bennett et al. 1983; Chudacek and Fane 1984; Nagaoka et al. 1996):

$$\alpha = k \cdot \text{TMP}^n \quad (6.2)$$

where k is an empirical constant equal to α when TMP is 1 Pa (m/kg). Eq. 6.2 can be rearranged to a linear form of $\log \alpha$ versus $\log \text{TMP}$:

$$\log \alpha = n \cdot \log \text{TMP} + \log k \quad (6.3)$$

For determination of n by Eq. 6.3, microfiltration experiments were conducted at different TMP of 2 to 200 kPa, respectively.

6.2.6. Analytical methods

Extracellular polymeric substances (EPS) in granules were extracted using formaldehyde and sodium hydroxide method (Liu and Fang 2002). As the main components of EPS, extracellular proteins (PN) and polysaccharides (PS) were measured using Lowry method with BSA (Bovine Serum Albumin) as standard (Lowry et al., 1951) and phenol-sulfate method with glucose as standard (Dubois et al., 1956), respectively. Details can be found in Section 3.2.7, Chapter 3. Mixed liquor suspension solids (MLSS), mixed liquor volatile suspension solids (MLVSS), sludge volume index (SVI) and turbidity were all determined according to standard methods (APHA 1998).

6.3. Results and Discussion

6.3.1. Characteristics of mature denitrifying granules

As presented in Chapter 5, denitrifying granules were successfully developed in R1 to R3 supplemented with the calcium concentration of 0, 50 and 100 mg Ca^{2+}/L , respectively. Some characteristics of R1 to R3 denitrifying granules were recalled in Table 6.1.

Table 6.1 Characteristics of mature denitrifying granules in R1 to R3

Parameter	R1	R2	R3
Substrate calcium concentration (mg Ca^{2+}/L)	0	50	100
MLSS concentration in reactors (mg SS/L)	4400	10150	13000
MLVSS/MLSS (%)	88	43	37
Mean size (μm)	140	260	420
SVI (mL/g SS)	39.41	18.75	14.36
Calcium content (mg/g SS)	5.63	154.61	177.63
PN content (mg/g VSS)	100.37	124.69	173.34
PS content (mg/g VSS)	11.03	8.39	8.06

6.3.2. Flux comparison of mixed liquor, granules solution and supernatant in microfiltration

The changes in fluxes of R1 to R3 mixed liquors, granules solutions and supernatants are presented in Figs. 6.1 to 6.3, respectively. For R1 without calcium addition, a greater decline rate in initial flux is observed for supernatant than for mixed liquor and granules solution (Fig. 6.1). Moreover, the ratio of permeate flux at steady state to initial flux (J/J_0) of R1 supernatant is only 0.049, which is much lower than that of R1 granules solution (0.071) and mixed liquor (0.068), respectively. However, there is no significant difference in J/J_0 for R1 granules solution and mixed liquor in the course of the microfiltration.

Figure 6.1 Normalized fluxes in microfiltration of granules solution, mixed liquor and supernatant prepared from R1 without calcium addition.

For R2 supplemented with 50 mg Ca^{2+}/L , the initial decline rate of flux was found in the order of R2 supernatant > R2 mixed liquor > R2 granules solution, whereas the lowest steady state flux was observed during the microfiltration of R2 supernatant, and followed by R2 mixed liquor and granule solution (Fig. 6.2). For

example, the J/J_0 ratio of R2 granules solution at the end of the filtration was 1.65-fold and 2.51-fold of R2 mixed liquor and supernatant, respectively (Fig. 6.2). It should be noted that the similar phenomena are also observed for R3 (Fig. 6.3).

Figure 6.2 Normalized fluxes in microfiltration of granules solution, mixed liquor and supernatant prepared from R2 supplemented with 50 mg Ca^{2+}/L .

Figure 6.3 Normalized fluxes in microfiltration of granules solution, mixed liquor and supernatant prepared from R3 supplemented with 100 mg Ca^{2+}/L .

In this study, supernatant was mainly made up of fine particles including colloids and soluble EPS (sEPS), whereas mixed liquor indeed was the mixture of granules and corresponding supernatant. Although R1 to R3 mixed liquors contained high granules concentration of 5000 mg SS/L, their permeate fluxes were all higher than those of R1 to R3 supernatants (Figs. 6.1 to 6.3). These results indicate that fine particles including colloidal particles and sEPS in supernatant seems to have a more significant negative impact on permeate flux, whereas the presence of granules in the mixed liquor would help to improve permeate flux.

In order to further evaluate the permeability of granules solutions, mixed liquors and supernatants of R1 to R3, the concept of half-life ($t_{1/2}$) was introduced in this study. $t_{1/2}$ is defined as the microfiltration time at which initial flux is reduced by 50%, i.e. smaller $t_{1/2}$ value means a faster flux decline and more severe membrane fouling. Fig. 6.4 shows the comparisons of $t_{1/2}$ values obtained in the microfiltration of mixed liquors, granules solutions and supernatants of R1 to R3. It appears that $t_{1/2}$ for the microfiltration of the R1 to R3 supernatants had smaller values as compared to those of the corresponding mixed liquors and granules solutions. These in turn imply that membrane fouling would develop faster during microfiltration of R1 to R3 supernatants than their mixed liquors and granules solutions, respectively. It seems that granules in the mixed liquors would play a positive role in mitigating membrane fouling. This phenomenon will be further elaborated in the following sections.

Figure 6.4 Comparison of $t_{1/2}$ for microfiltration of mixed liquors, granules solutions and supernatants from R1 to R3.

6.3.3. Comparison of filtration resistances of mixed liquor, granules solution and supernatant

The permeate flux decline observed in Figs. 6.1 to 6.3 can be attributed to the two main phenomenon: formation of cake layer on the membrane surface and membrane pore blocking. To examine the possible fouling mechanism of microfiltration of mixed liquors, granule solutions and supernatants from R1 to R3, filtration resistances were calculated using the resistance-in-series model, and results are presented in Table 6.2. It can be seen that R1 to R3 supernatants exhibited the highest total resistances as compared to their corresponding mixed liquors and granules solutions. For example, for R3 supplemented with 100 mg Ca^{2+}/L , the total resistance of R3 supernatant was $6.96 \times 10^{11}/\text{m}$, which was about 1.41-fold of the R3 mixed liquor and 3.82-fold of granule solution. For R2 with addition of 50 mg Ca^{2+}/L , total resistance of supernatant was found to be 1.47-fold and 2.43-fold of the mixed liquor and granules solution. Similarly, for R1 without calcium addition, total resistance of supernatant was 1.47-fold and 1.84-fold of mixed liquor and granules solution. These results clearly indicate that supernatant

would be the main contributor to the observed total resistance regardless of calcium addition.

It also appears from Table 6.2 that the respective ratios of cake resistance to total resistance (R_c/R_t) of mixed liquor, granules solution and supernatant fell into the range of 64.45 % to 93.39 %, indicating that cake resistance would be dominant over pore blocking in microfiltration of R1 to R3 mixed liquors, granules solutions and supernatants. Furthermore, the cake layers formed by R1 to R3 supernatants exhibited the higher specific cake resistances than those formed by R1 to R3 mixed liquors and granules solutions. For example, specific cake resistances of R1 to R3 supernatants were found to be 290-fold to 1943-fold higher than the R1 to R3 granules solutions, and 222-fold to 288-fold of the R1 to R3 mixed liquors. These further reveal that R1 to R3 supernatants would be the main contributor of the observed membrane fouling.

Although the granules concentrations were the same in R1 to R3 mixed liquors and granules solutions, it should be noted that total resistance, cake resistance and specific cake resistance of R1 to R3 granules solutions were much lower than those of R1 to R3 mixed liquors. Such difference would be attributed to fine particles present in mixed liquors. Consequently, the presence of fine particles eventually including colloids and sEPS, would have a stronger negative impact on the membrane fouling than granules.

Table 6.2 Resistances analysis of microfiltration of R1 to R3 mixed liquors, granules solutions and supernatants ^a

Feed solutions	R_m (1/m)	R_m/R_t (%)	R_p (1/m)	R_p/R_t (%)	R_c (1/m)	R_c/R_t (%)	R_t (1/m)	α (m/kg)
R1								
Granules solution	3.44E+10	5.97	1.52E+10	2.64	5.26E+11	91.39	5.76E+11	1.43E+12
Mixed liquor	3.58E+10	4.96	2.66E+10	3.69	6.59E+11	91.35	7.22E+11	1.87E+12
Supernatant	3.47E+10	3.29	3.52E+10	3.32	9.90E+11	93.39	1.06E+12	4.15E+14
R2								
Granules solution	3.45E+10	10.74	1.88E+10	5.84	2.68E+11	83.42	3.22E+11	3.13E+11
Mixed liquor	3.36E+10	6.26	2.33E+10	4.35	4.79E+11	89.34	5.36E+11	1.23E+12
Supernatant	3.53E+10	4.51	3.21E+10	4.10	7.16E+11	91.39	7.83E+11	3.84E+14
R3								
Granules solution	3.44E+10	18.93	3.02E+10	16.62	1.17E+11	64.45	1.82E+11	1.41E+11
Mixed liquor	3.60E+10	7.28	2.24E+10	4.53	4.36E+11	88.19	4.94E+11	9.50E+11
Supernatant	3.57E+10	5.13	3.20E+10	4.60	6.35E+11	91.27	6.96E+11	2.74E+14

R_m is inherent membrane resistance; R_p is pore blocking resistance; R_c is cake resistance; R_t is total resistance; α is specific cake resistance.

^a Granule concentration in mixed liquors and granule solutions are 5000 mg SS/L. Membrane pore size is 0.22 μ m and TMP is 5 kPa.

6.3.4. Cake layer as a prefilter for mitigating membrane fouling

As presented earlier, mixed liquor consists of both denitrifying granules and supernatant. However, it appears from Table 6.2 that the total filtration resistances of R1 to R3 mixed liquors are much smaller than the sum of the total resistance of granules solution and supernatant. These suggest that there should be some unrevealed function of granules cake during microfiltration of mixed liquors. In this case, the resistance reduction of R1 to R3 mixed liquors against R1 to R3 supernatants was calculated using Eqs. 6.4 to 6.6 for the cake, total and specific cake resistance (Table 6.3).

$$\text{Reduction of } R_c (\%) = \frac{R_{c,SP} - R_{c,ML}}{R_{c,SP}} \times 100 \quad (6.4)$$

where, $R_{c,SP}$ is cake resistance of supernatant (1/m); $R_{c,ML}$ is corresponding cake resistance of mixed liquor (1/m).

$$\text{Reduction of } R_t (\%) = \frac{R_{t,SP} - R_{t,ML}}{R_{t,SP}} \times 100 \quad (6.5)$$

where, $R_{t,SP}$ is total resistance of supernatant (1/m); $R_{t,ML}$ is corresponding total resistance of mixed liquor (1/m).

$$\text{Reduction of } \alpha (\%) = \frac{\alpha_{SP} - \alpha_{ML}}{\alpha_{SP}} \times 100 \quad (6.6)$$

where, α_{SP} is specific cake resistance of supernatant (m/kg); α_{ML} is corresponding specific cake resistance of mixed liquor (m/kg).

Table 6.3 Resistance reductions of mixed liquors versus supernatants

	Reduction of R_c (%)	Reduction of R_t (%)	Reduction of α (%)
R1	33.41	31.92	99.55
R2	33.10	31.55	99.68
R3	31.34	29.02	99.65

These results provide strong support for the idea of that the granules cake layer formed on the membrane surface during microfiltration of mixed liquors would favor the removal of fine particles including colloids and sEPS through prefiltration by granules cake, leading to a lower fouling intensity as reflected in Table 6.3. In study of microfiltration performance of bovine serum albumin (BSA) with and without the presence of yeast, Guella et al. (1999) found that the presence of yeast prevented the membrane fouling by BSA since the yeast layer on the membrane surface could capture BSA, and thus prevent BSA from clogging the internal structure of the membrane, whereas Iritani et al (2007) observed that the presence of the activated sludge in microfiltration of the supernatant would not cause further increase in membrane fouling. Although it has been reported that supernatant would be a major contributor to membrane fouling (Lee et al., 2003; Nuengjamnong et al., 2005; Iritani et al., 2007), little is currently known about the role of granular sludge in fouling development during microfiltration. As discussed above, granules in mixed liquors would help alleviate membrane fouling caused by supernatants from R1 to R3. To properly explain the observed phenomenon, a hypothesis is thus put forward in this study, as elaborated below.

Based on the experimental results, it is reasonable to consider that the cake layer formed by granules can serve as a prefilter that will retain fine particles present in supernatants, and subsequently help to alleviate membrane fouling. Such a prefiltration mechanism by granules cake layer is schematically illustrated in Fig. 6.5 for microfiltration of mixed liquors, granules solutions and supernatants from R1 to R3.

Figure 6.5 Fouling development in microfiltration of (a) granules solution; (b) supernatant; and (c) mixed liquor.

(a) Microfiltration of granules solution

During microfiltration of granule solution, fast-settling denitrifying granules can quickly form a porous granules cake layer on the membrane surface as illustrated in Fig. 6.5a. In this study, granules solution was made up of denitrifying granules and DI water, i.e. there would not be significant fouling due to fine

particles, colloids or sEPS at the initial filtration stage. As the result, high permeate flux of granule solution is observed at the initial stage in R1 to R3 (Figs. 6.1 to 6.3), and this is also supported by the $t_{1/2}$ values presented in Fig. 6.4. However, in the course of microfiltration driven by an external pressure, denitrifying granules in the cake layer eventually undergo deformation in their shape, which would be caused by applied pressure. Moreover, under such pressure, part of bound EPS and fine particles attached to granules would be gradually released out, which in turn would be responsible for the observed flux decline (Figs. 6.1 to 6.3) due to pore blocking and reduced porosity of the granules cake layer.

The deformation of denitrifying granules would largely depend on their physicochemical characteristics and applied pressure. It can be seen in Table 6.1 that R3 denitrifying granules supplemented with 100 mg Ca^{2+} /L are much stronger and denser than those developed in R2 and R1. In fact, addition of Ca^{2+} has been adopted for enhancing the strength of UASB granules (Yu et al., 2001). As discussed in Chapter 5, Ca^{2+} could complex with EPS to form EPS- Ca^{2+} -EPS binding, which would strengthen spatial structure and stability of denitrifying granules. Fig. 6.6 further illustrates the relationship between granules cake compressibility and calcium content in denitrifying granules. As expected, the compressibility of the granules cake layer is inversely related to the calcium content in denitrifying granules, e.g. the cake compressibility of R1 to R3 granules was decreased from 0.98 to 0.71 with the increase in the calcium content of denitrifying granules.

Figure 6.6 Effect of calcium content in denitrifying granules on compressibility of granule cake layer.

Fig. 6.7 further shows the effect of the cake compressibility on both specific cake resistance and $t_{1/2}$. When the cake is more compressible, the specific cake resistance increase and $t_{1/2}$ becomes smaller. These observations provide a plausible explanation for the recorded difference in microfiltration resistances of R1 to R3 granules solutions (Table 6.2). As discussed in Chapter 4, size-effect on cake resistance would not be ignored. The specific cake resistances determined from microfiltration of R1 to R3 granules solutions are plotted against $1/d^2$ (Fig. 6.8). With the increase in granule size, smaller specific cake resistance was observed as predicted by the well-known Carmen-Kozeney equation.

Figure 6.7 Effect of compressibility of granule cake layer on specific cake resistance and $t_{1/2}$.

Figure 6.8 Effect of granules size on specific cake resistances.

(b) Microfiltration of supernatant

Supernatants from R1 to R3 contained fine particles including colloids and sEPS except denitrifying granules. As shown in Figs. 6.1 to 6.3, much membrane fouling was observed during microfiltration of R1 to R3 supernatants as compared R1 to R3 mixed liquors and granule solutions. Furthermore, it appears from Table 6.2 that cake resistance would be the main contributor to the total resistance in microfiltration of supernatants, e.g. the R_c/R_t ratios were 93.39%, 91.39% and 91.27% for R1 to R3 supernatants, respectively. Moreover, it should be noted that the total microfiltration resistances of R1 to R3 supernatant are much greater than their corresponding mixed liquors and granule solutions. These seem to indicate that fine particles with a size bigger than the membrane pore size (0.22 μm) would deposit on the membrane surface and form a thin, but substantially dense and compact cake layer. This layer would cause extremely high cake resistance as illustrated in Fig. 6.5b.

Table 6.2 also reveals that R3 supernatant exhibited the lowest total and cake resistances compared to those from R2 and R1. This can be reasonably explained by the calcium-assisted coagulation effect. Addition of positive ions, such as calcium, has been believed to facilitate particle-to-particle coagulation leading to increased size of fine particles (Wang et al. 2008).

(c) Microfiltration of mixed liquor

In this study, mixed liquor contains both denitrifying granules and supernatant. As described in Fig. 6.5c, at the initial filtration stage, denitrifying granules present in mixed liquor would settle much faster than those fine particles, colloids and sEPS, and quickly develop into a granule cake layer on the membrane surface. As illustrated in Fig. 6.5c, this granule cake layer serves as a prefilter that will retain slow-settling fine particles. This is supported by the results presented in Table 6.2, i.e. the cake and total resistances of the R1 to R3 mixed liquors were much smaller than those of R1 to R3 supernatants, but greater than R1 to R3 granules solutions. Theoretically, rejection of fine particles in R1 to R3 mixed liquors by granule cake layer is related to the size of granules. Fig. 6.9 shows that

the rejection rate of fine particles decreases from 86.57% to 49.76% when the size of granule in the granule cake layer increases from 140 μm to 420 μm . Lousada-Ferreira et al. (2010) also found that activated sludge at the concentration higher than 10000 mg SS/L could entrap particles smaller than 20 μm in the activated sludge bulk, leading to reduced membrane resistance. Furthermore, it appears from Table 6.2 that the specific cake resistances of R1 to R3 mixed liquors were only about 0.45% to 0.35% of those of R1 to R3 supernatants, respectively.

As discussed above, it is a reasonable consideration that the filtration performance can be improved by the formation of a more porous and less compressible cake layer (Pirbazari et al. 1996; Lesage et al. 2005). For example, Benoit et al. (2011) reported that the addition of melamine and polystyrene latex in activated sludge supernatant could induce non-compressible fouling at a certain TMP and lead to better filtration performances. The specific cake resistance in dead-end microfiltration of suspensions of melamine and supernatant were only 12.15% to 36.26% of that in the filtration of supernatant alone when TMP was varied from 50 to 100 kPa.

Figure 6.9 Effect of granules size on fine particle rejection rate by granules cake pefilter.

6.3.5. Effect of granules concentration on microfiltration

In order to further understand the prefiltration function of granule cake layer during microfiltration of mixed liquors as described above, a series of mixed liquors were artificially prepared with different granule concentrations of 0 to 13000 mg SS/L, while the fine particles concentration was fixed at 30 mg/L. These mixed liquors were prepared by mixing 300 mL of R3 supernatant with different quantity of R3 granules.

6.3.5.1. Effect of granules concentration on permeate flux

Fig. 6.10 shows the flux profiles obtained in microfiltration of the mixed liquors with different granules concentrations. The highest stable permeate flux was observed in microfiltration of the mixed liquor having the highest granule concentration of 13000 mg SS/L. These results seem to imply that denitrifying granules are able to help to mitigate membrane fouling. Fig. 6.11 shows the effect of granule concentrations in the prepared mixed liquors at different filtration times. Some salient points can be drawn from Fig. 6.11: (i) at the initial filtration stage (e.g. 5 min), higher flux was recorded as compared those measured after 10 and 30 min, indicating that granule cake layer was formed firstly on the membrane surface; (ii) the J/J_0 ratio tended to increase with the increase in the granule concentration in the range of 0 to 5000 mg SS/L regardless of filtration time. This supports the prefiltration function of the granule cake layer; (iii) at the granules concentration above 5000 mg SS/L, the J/J_0 ratio remained nearly constant. Furthermore, Fig. 6.12 reveals that decline in permeate flux in terms of $t_{1/2}$ was slower at higher granules concentrations. These provide additional experimental evidence to the prefiltration mechanism of granule cake layer as illustrated in Fig. 6.5.

Figure 6.10 Flux profiles in microfiltration of R3 mixed liquors with different granules concentrations.

Figure 6.11 Effect of granules concentration on J/J_0 ratio at different filtration times.

Figure 6.12 Effect of granules concentration on $t_{1/2}$.

6.3.5.2. Effect of granules concentration on membrane resistance

As discussed above, the granule cake layer can function as a prefilter that helps to retain fine particles, leading to a reduced filtration resistance. Figs. 6.13 and 6.14 show the correlation between granules concentration and total and specific cake resistances, respectively. It was found that the total and cake resistances were reduced by 37.63% and 39.86%, respectively, when granules concentration was increased from 0 to 5000 mg SS/L (Fig. 6.13), while nearly stable total resistance was observed at the granule concentration greater than 5000 mg SS/L. Moreover, it can also be seen in Fig. 6.14 that the specific cake resistance decreased exponentially with the increase of granules concentration. Once again, these results clearly support the prefiltration function of the granules cake layer as illustrated in Fig. 6.5, meanwhile also provide strong experimental evidence showing that the main foulant would be fine particles instead of denitrifying granules.

Figure 6.13 Effect of granules concentration on cake and total resistances.

Figure 6.14 Effect of granule concentration on specific cake resistance.

6.3.6. Effect of fine particle concentration on membrane resistance

It appears from the above discussion that fine particles (including colloids and sEPS) present in mixed liquors would be the main foulant in the microfiltration

of R1 to R3 mixed liquors. To investigate the effect of fine particle concentration on membrane fouling, a series of mixed liquors were prepared with the fixed granules concentration of 5000 mg SS/L and various concentrations of fine particles. For this purpose, R3 supernatant was first diluted with DI water at dilution factors of 8, 4, 1.6 and 1, giving diluted supernatants with different fine particles concentration of 3.75 mg/L, 7.5 mg/L, 18.75 mg/L and 30 mg/L, respectively. Dead-end microfiltration experiments were then carried out with the prepared supernatants (Fig. 6.15). It is obvious that a faster flux decline was observed at a higher concentration of fine particles, whereas the stable flux was inversely related to the fine particles concentration. In these experiments, the denitrifying granule concentration was kept at the same level, thus the observed changes in flux would be mainly due to the presence of fine particles at different concentrations.

Figure 6.15 Flux profiles in microfiltration of R3 granules mixed liquors with different particles concentrations

Figs. 6.16 and 6.17 show the effects of different concentrations of fine particles on filtration resistances. It can be seen from Fig. 6.17 that specific cake resistance was increased exponentially with increased concentration of fine

particles, whereas total and specific cake resistance were increased by 2.72-fold and 8.7-fold when fine particles concentration was increased from 0 to 30 mg/L, respectively (Figs. 6.16 and 6.17). As granule concentrations were kept at the same level in this study, Figs. 6.16 and 6.17 further confirm that fine particles present in mixed liquors would be the major contributor of membrane fouling as compared to denitrifying granular biomass.

Figure 6.16 Effect of fine particles concentration on cake and total resistances.

Figure 6.17 Effect of fine particles concentration on specific cake resistance.

6.4. Summary

This study investigated the microfiltration behaviors of denitrifying granular sludge mixed liquors, granule solutions and supernatants prepared from R1 to R3 supplemented with different calcium concentrations. The following conclusions can be drawn:

- 1) As compared to denitrifying granules, supernatants were mainly responsible for the observed membrane fouling in microfiltration of granular sludge mixed liquors. Specific cake resistances of supernatant prepared from R1 to R3 were 290 to 1943 times of their corresponding granule solutions.
- 2) In microfiltration of granular mixed liquor, cake layer first formed by fast-settling denitrifying granules would serve as a prefilter for preventing slow-settling fine particles, colloids and sEPS from depositing on the membrane surface, which indeed helped to mitigate membrane fouling intensity. The prefiltration phenomenon of granule cake layer was observed in all three cases regardless of calcium addition.
- 3) The reduction of resistance due to the granule cake layer prefilter was increased with the granule concentrations ranging from 0 to 5000 mg SS/L in microfiltration of mixed liquors. While no further improvement was observed when granule concentrations increased from 5000 mg SS/L to 13000 mg SS/L.
- 4) For mixed liquors with same granule concentration, increasing of the concentration of fine particles, colloids and sEPS could increase the membrane fouling.

CHAPTER 7

ANALYSIS OF PARTICLE SIZE-ASSOCIATED MEMBRANE FOULING IN MICROFILTRATION OF DENITRIFYING GRANULE SUPERNATANT

7.1. Introduction

Fine particles, colloids, soluble extracellular polymeric substances (sEPS) are main components of supernatant. The molecular weight distribution and hydrophobicity of supernatant has been correlated to fouling development in microfiltration (Hodgson et al. 1993; Defrance et al., 2000; Bouhabila et al., 2001; Jefferson et al. 2004; Kim and Jang 2006), whereas model EPS solutions (e.g. pure protein solution and polysaccharide solutions) were used to simulate the EPS-associated membrane fouling (Ho and Zydney 1999; Ghosh 2002; Ye et al. 2005; Duclos-Orsello et al. 2006). Nuengjamnong et al. (2005) reported that the supernatant contributed to approximately 50% of total specific cake resistance, while Iritani et al. (2007) found that supernatant was fully responsible for the resistance in microfiltration of the activated sludge. However, little is currently known about the particle size-associated membrane fouling in microfiltration of supernatants prepared from real biomass liquor.

It has been shown in Chapter 6 that supernatant would play an important role in development of membrane fouling, e.g. as compared to granules solution and mixed liquor, granule supernatant was found to contribute more to the observed membrane fouling due to the formation of the thin, compact and dense particle cake layer on membrane surface. Therefore, this chapter further looked into particle size-associated membrane fouling mechanisms in microfiltration of supernatants from R1 to R3 fed with different calcium concentrations of 0 to 100 mg/L. Meanwhile, both resistances-in-series and blocking models were applied to analyze fouling mechanisms of microfiltration of supernatants with different-size particles.

7.2. Materials and Methods

7.2.1. Microfiltration experimental set-up

Microfiltration experiments were carried out in a dead-end microfiltration module as described in Chapter 4. Flat-sheet nitrocellulose membranes with pore size of 0.22 μm and effective membrane area of 45.3 cm^2 (Millipore, Ireland) were used in this study. Trans-membrane pressure (TMP) was kept constant at 5 kPa by compressed nitrogen gas.

7.2.2. Preparation of feed solutions for microfiltration experiments

Three identical SBRs named as R1, R2 and R3 were used for development of denitrifying granules at the different calcium concentrations of 0, 50 and 100 $\text{mg Ca}^{2+}/\text{L}$, respectively. Details can be found in Section 5.2, Chapter 5. Prior to microfiltration experiment, granules supernatants taken from the steady-state R1 to R3 were filtrated through a series of membranes with various pore sizes of 2.7 μm , 1.0 μm , 0.45 μm and 0.22 μm , respectively. The corresponding permeates are designated as $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$, and their characteristics are presented in Table 7.1.

Table 7.1 Characteristics of feed solutions for microfiltration experiments

Feed solutions	Particle size (μm)	Main compositions
$P_{2.7}$	<2.7	Fine particles, colloids and sEPS
$P_{1.0}$	<1.0	Colloids and sEPS
$P_{0.45}$	<0.45	Colloids smaller than 0.45 μm and sEPS
$P_{0.22}$	<0.22	Colloids smaller than 0.22 μm and sEPS

7.2.3. Determination of permeate flux and membrane resistance

Permeate flux was determined using Darcy's law (Eq. 4.1, Chapter 4). Total resistance (R_t), cake resistance (R_c), pore blocking resistance (R_p) and membrane

resistance (R_m) were calculated by resistance-in-series model (Cheryan 1986). More details can be found in Sections 4.2.3 and 4.2.4, Chapter 4.

7.2.4. Analytical methods

Soluble proteins (sPN) and soluble polysaccharides (sPS) are the two major components of sEPS. sPN were measured using Folin-Ciocalteu phenol method with Bovine Serum Album (BSA) as standard (Lowry et al., 1951), and sPS were determined using phenol-sulfate method with glucose as standard (Dubois et al., 1956). The detailed procedures were presented in Section 3.2.4, Chapter 3. The turbidity of water sample was measured using a turbidity meter (Hach 2000), whereas size distributions in $P_{2.7}$ -R1 to $P_{2.7}$ -R3 were measured by a laser particle size analyzer (Mastersizer Series 2600, Malvern).

7.3. Results and Discussion

7.3.1. Characteristics of feed solutions for microfiltration experiments

R1 to R3 were used to develop denitrifying granules with the addition of 0, 50 and 100 mg/L calcium, respectively (Chapter 5). As described above, $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of steady-state R1 to R3 were prepared for investigating mechanisms of membrane fouling by R1 to R3 supernatants. For this purpose, turbidity, sPN, sPS and sEPS in $P_{2.7}$ to $P_{0.22}$ of R1 to R3 were measured (Table 7.2). The salient points from Table 7.2 are: (i) the turbidity, sPS and sPN are in the order of $P_{2.7} > P_{1.0} > P_{0.45} > P_{0.22}$ for R1 to R3; (ii) the turbidity, sPS and sPN of $P_{2.7}$ -R3 < $P_{2.7}$ -R2 < $P_{2.7}$ -R1; and the same phenomenon is observed for $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$. These results suggest that addition of Ca^{2+} ion could help to reduce the turbidity and sEPS through coagulation and bridging of particles and EPS molecules to form large particles. In fact, the coagulation and bridging of negatively charged functional groups in EPS by calcium had been reported (Kim and Jang 2006).

Table 7.2 Characteristics of P_{2.7}, P_{1.0}, P_{0.45} and P_{0.22}, of R1 to R3

Feed solutions	Turbidity (NTU)	sPN (mg/L)	sPS (mg/L)	sEPS (mg/L)
R1				
P _{2.7} -R1	7.57	16.22	9.00	25.22
P _{1.0} -R1	1.93	12.00	8.65	20.65
P _{0.45} -R1	0.78	9.08	5.41	14.49
P _{0.22} -R1	0.19	5.84	3.14	9.98
R2				
P _{2.7} -R2	4.55	12.98	4.52	17.50
P _{1.0} -R2	1.29	10.05	3.39	13.44
P _{0.45} -R2	0.58	8.09	3.17	11.26
P _{0.22} -R2	0.17	3.89	1.27	5.16
R3				
P _{2.7} -R3	3.85	12.32	1.59	13.91
P _{1.0} -R3	0.64	8.11	1.35	9.46
P _{0.45} -R3	0.18	5.51	1.23	6.74
P _{0.22} -R3	0.16	2.92	0.92	3.84

Fig. 7.1 further shows the effect of calcium on the size distribution of particles in P_{2.7} of R1 to R3 fed with 0, 50 and 100 mg/L calcium. It was found that calcium ion can effectively reduce the quantity of particles with size smaller than 1.0 μm in P_{2.7}-R3 and P_{2.7}-R2 as compared to P_{2.7}-R1. These results suggest that coagulation-flocculation of colloidal particles promoted by calcium ion would favor growth of particles to a size bigger than 1.0 μm . This is also supported by the observation of that P_{2.7} prepared from R1 without calcium addition had the highest quantity of smaller particles (Fig. 7.1).

Figure 7.1 Size distributions of particles in $P_{2.7}$ -R1 to $P_{2.7}$ -R3.

7.3.2. Flux profiles of $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ in microfiltration

Figs. 7.2 to 7.4 show the respective flux profiles of $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 to R3 observed in microfiltration. It is clearly seen that permeate fluxes of $P_{2.7}$ -R1 to $P_{2.7}$ -R3 tended to decline rapidly at the initial filtration stage, and relatively low permeate fluxes at the stable filtration stage were observed as compared with those of $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 to R3. For example, for R1, J/J_0 of $P_{2.7}$ at the stable filtration stage was 1.99-fold, 5.15-fold and 9.70-fold lower than those of $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$, respectively (Fig. 7.2). Similar phenomenon was also observed in R2 and R3 (Figs. 7.3 and 7.4). The rapid decline of J/J_0 at initial filtration stage of $P_{2.7}$ can be attributed the formation of cake layer by fine particles as discussed in Chapter 6.

Figure 7.2 Permeate fluxes of $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 without calcium addition as a function of filtration time (Membrane pore size = $0.22\ \mu\text{m}$, TMP = 5 kPa).

Figure 7.3 Permeate fluxes of $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R2 fed with $50\ \text{mg}\ \text{Ca}^{2+}/\text{L}$ as a function of filtration time (Membrane pore size = $0.22\ \mu\text{m}$, TMP = 5 kPa).

Figure 7.4 Permeate fluxes of $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R3 fed with 100 mg Ca^{2+}/L as a function of filtration time (Membrane pore size = 0.22 μm , TMP = 5 kPa).

As presented in Chapter 4, $t_{1/2}$ is defined as filtration time at which permeate flux (J) is reduced to half of the initial permeate flux (J_0). In this study, $t_{1/2}$ was used to evaluate the filtration performances of $P_{2.7}$ to $P_{0.22}$ of R1 to R3. The slower the flux declines, the greater $t_{1/2}$ value is. As can be seen in Fig. 7.5, $t_{1/2}$ values for different feed solutions were in order of $P_{0.22} > P_{0.45} > P_{1.0} > P_{2.7}$ for R1 to R3, indicating that cake formed by fine particles was the primarily contributor to the observed flux decline. Moreover, it appears from Fig. 7.5 that addition of calcium could significantly help to alleviate membrane fouling, i.e. longest $t_{1/2}$ values were obtained in the microfiltration of feed solutions prepared from R3 supplemented with 100 mg/L calcium. Such observation is in good agreement with Fig. 7.1, i.e. calcium-associated size distribution of particles.

Figure 7.5 $t_{1/2}$ values of P_{2.7} to P_{0.22} of R1 to R3.

7.3.3. Mechanistic analysis of membrane fouling

To analyze the possible mechanisms of membrane fouling by different-size particles present in R1 to R3 supernatants, four filtration models, namely complete blocking model, standard blocking model, intermediate blocking model and cake filtration model, were employed in this study. According to Hermia (1982) and Bowen et al. (1995), these four models can be unified to the following expression:

$$\frac{dJ}{dt} = -KJ(A_{\text{eff}}J)^{2-n} \quad (7.1)$$

where J is permeate flux at filtration time t ($\text{L}/\text{m}^2\cdot\text{h}$); t is filtration time (h), A_{eff} is effective membrane surface area (m^2); K is a membrane-associated constant; and n is the so-called blocking index. Eq. 7.1 reduces to the complete blocking model, standard blocking model, intermediate blocking model and cake filtration model when n takes the respective values of 2, 1.5, 1 or 0 (Table 7.3). Standard blocking model assumes that adsorption of macromolecules onto the internal walls of membrane pores would cause reduced effective pores radius. Complete blocking model assumes that each biomass molecule or its aggregate clogs one pore. As assumed by intermediate blocking model, attachment of biomass molecules or aggregates on the nonporous surface of the membrane and pore blocking happens at

the same time during filtration. Cake filtration model is suitably applied when the cake layer formed on the membrane surface.

In this study, flux data (Figs. 7.2 to 7.4) were fitted to Eqs. 7.2 to 7.5, respectively, and statistical analyses of curve fitting by these models in the term of square of correlation coefficient (R^2) are presented in Table 7.4. It appears that the cake filtration model (Eq. 7.5) can provide the best description of flux data collected from microfiltration of P_{2.7}-R1 to P_{2.7}-R3, whereas flux data of P_{1.0}, P_{0.45} and P_{0.22} of R1 to R3 can be best fitted to intermediate blocking model (Eq. 7.4). These suggest that for particles bigger than 1.0 μm , the main fouling mechanism would be the formation of cake layer, while a combined fouling mechanism of cake and pore blocking appears to be dominant when particles are smaller than 1.0 μm .

In this study, the supernatants from R1 to R3 fed with different calcium concentrations were fractionated into the four size categories for better understanding of the membrane fouling mechanisms (Table 7.2). Study on such detail fractionation of supernatant is very limited in the literature. Yang et al. (2007) reported that high turbidity in supernatant could result in severe membrane fouling due to the accumulation of colloids and soluble matter on the membrane surface, but without clear differentiation in particle size of supernatant. In fact, to evaluate the mechanism of membrane fouling by sludge supernatant (e.g. colloid particles and sEPS), bovine serum albumin (BSA) and sodium alginate had been used as model protein and polysaccharide, respectively (Bowen et al., 1995; Velasco et al. 2003; Duclos-Orsello et al., 2006). In study of the flux decline in microfiltration of 1 g BSA/L solution, Herrero et al. (1997) found that the initial filtration step was fitted well to the standard model, while the final filtration step was assumed to obey an intermediate blocking model. However, it should be noted that the concentration of model polysaccharide or protein used in this kind of studies appeared to be too high, while such high concentration (e.g. 1 g BAS/L) has never been found in the real supernatants from MBRs. For example, the EPS and colloids concentrations in the P_{1.0}, P_{0.45} and P_{0.22} fractions of the R1 to R3 supernatants were much lower than the model EPS concentrations used in the literature. This in turn implies that the fouling mechanisms at high concentration of model EPS may not be applicable in real situation.

Table 7.3 Mathematical expressions of the fouling models

Model	n	Permeate flux decline	Constants	Eq.
Complete blocking	2	$\frac{J}{J_o} = e^{-Kt}$	$K = K_a \cdot J_o$ K_a is the blocked membrane surface per unit of the total volume permeated through the membrane (1/m).	(7.2)
Standard blocking	1.5	$\frac{J}{J_o} = \left(1 + 2K_s A_{eff}^2 J_o^{0.5} t\right)^{-2}$	K_s is Hermia's parameter, which is the decrease in the cross section area of the pores per unit of permeate volume (1/m).	(7.3)
Intermediate blocking	1	$\frac{J}{J_o} = \left(1 + K A_{eff} J_o t\right)^{-1}$	$K = K_a / A_{eff}$ K_a is the blocked membrane surface per unit of the total volume permeated through the membrane (1/m).	(7.4)
Cake filtration	0	$\frac{J}{J_o} = \left(1 + 2K_c A_{eff}^2 J_o^2 t\right)^{-0.5}$ $= \left(1 + 2K_c R_r J_o t\right)^{-0.5}$	$K = K_c R_r / A_{eff}^2 J_o$ K_c is area of the cake per unit of total permeate volume (1/m). R_r is the ratio of the cake resistance to the clean membrane resistance (dimensionless).	(7.5)

Table 7.4 Statistic analysis of curve fitting of fluxes data obtained in microfiltration of $P_{2.7}$ to $P_{0.22}$ of R1 to R3

Feed solutions	R^2				Remark
	Complete blocking (Eq.7.2)	Standard Blocking (Eq.7.3)	Intermediate blocking (Eq.7.4)	Cake Filtration (Eq.7.5)	
$P_{2.7}$					
$P_{2.7}$ -R1	NA	0.0036	0.7589	0.9990	Best fitted by Eq. 7.5
$P_{2.7}$ -R2	NA	NA	0.8696	0.9923	
$P_{2.7}$ -R3	NA	0.2289	0.8051	0.9971	
$P_{1.0}$					
$P_{1.0}$ -R1	0.8543	0.9962	0.9811	0.7885	Best fitted by Eq. 7.4
$P_{1.0}$ -R2	0.8292	0.9780	0.9951	0.9290	
$P_{1.0}$ -R3	0.9145	0.9681	0.9849	0.8448	
$P_{0.45}$					
$P_{0.45}$ -R1	0.8787	0.9837	0.9992	0.9549	Best fitted by Eq. 7.4
$P_{0.45}$ -R2	0.8997	0.9875	0.9991	0.9417	
$P_{0.45}$ -R3	0.9332	0.8822	0.9993	9.8497	
$P_{0.22}$					
$P_{0.22}$ -R1	0.9830	0.9879	0.9998	0.9760	Best fitted by Eq. 7.4
$P_{0.22}$ -R2	0.9811	0.9794	0.9991	0.9593	
$P_{0.22}$ -R3	0.9919	0.8713	0.9993	0.5571	

NA: Not Applicable.

7.3.3.1. Fouling mechanism of $P_{2.7}$ of R1 to R3 in microfiltration

Table 7.4 indicates that among the models listed, cake filtration model best explains the experimental data of $P_{2.7}$. This indicated by a correlation coefficient as high as 0.99 (Fig. 7.6). This is conclusive evidence that cake formation was the major fouling mechanism in microfiltration of $P_{2.7}$ -R1 to $P_{2.7}$ -R3. This observation occurs when particles size is much bigger than the membrane pore size and is consistent with the results presented in Chapter 6.

Figure 7.6 Time evolution of $(J/J_0)^{-2}$ for $P_{2.7}$ -R1 to $P_{2.7}$ -R3 predicted by Eq.7.5. The straight line correspond to the fitting of fouling to cake filtration model (Membrane pore size = $0.22 \mu\text{m}$ and TMP = 5 kPa).

When using resistance-in-series model to estimate the cake and total resistances, it was found that the contribution of cake resistance to total resistance (R_c/R_t) in microfiltration of $P_{2.7}$ -R1 to $P_{0.22}$ -R1 decreased from 93.52% to 24.75%. The similar phenomena were also observed in microfiltration of $P_{2.7}$ -R2 to $P_{0.22}$ -R2 and $P_{2.7}$ -R3 to $P_{0.22}$ -R3 with the decrease of R_c/R_t from 92.42% to 8.02% and 89.58% to 14.82%, respectively. In order to further investigate the influence of particles size on fouling behaviors, the relative contributions of different-size

particles to cake resistances (RC) was estimated from Eqs. 7.6 to 7.9 and results were presented in Table 7.5.

$$RC_{1.0-2.7} (\%) = \frac{R_{c-P2.7} - R_{c-P1.0}}{R_{c-P2.7}} \times 100\% \quad (7.6)$$

$$RC_{0.45-1.0} (\%) = \frac{R_{c-P1.0} - R_{c-P0.45}}{R_{c-P2.7}} \times 100\% \quad (7.7)$$

$$RC_{0.22-0.45} (\%) = \frac{R_{c-P0.45} - R_{c-P0.22}}{R_{c-P2.7}} \times 100\% \quad (7.8)$$

$$RC_{\leq 0.22} (\%) = \frac{R_{c-P0.22}}{R_{c-P2.7}} \times 100\% \quad (7.9)$$

where $R_{c-P2.7}$, $R_{c-P1.0}$, $R_{c-P0.45}$ and $R_{c-P0.22}$ are cake resistances of $P_{2.7}$, $P_{1.0}$, $P_{0.45}$, estimated from resistance-in-series model, respectively. The data in Table 7.5 suggests that particles in the range of 1.0 to 2.7 μm were the major contributors to the cake resistance compared with particles smaller than 1.0 μm . This validates further the application of cake filtration model for interpreting flux declines of $P_{2.7}$ -R1 to $P_{2.7}$ -R3. In study of membrane fouling in a MBR, Fan and Zhou (2007) also noted that colloidal particles with size of around 1.0 μm might play a critical role in fouling development.

Table 7.5 Relative contributions of different-size particles to cake resistances

Size of particles (μm)	RC (%)		
	R1	R2	R3
1.0 ~ 2.7	86.82 $\pm$ 9.94	93.13 $\pm$ 4.56	94.34 $\pm$ 4.33
0.45 ~ 1.0	4.36 $\pm$ 0.47	5.70 $\pm$ 0.42	4.53 $\pm$ 0.31
0.22 ~ 0.45	7.60 $\pm$ 0.50	0.76 $\pm$ 0.25	0.37 $\pm$ 0.02
0 ~ 0.22	1.22 $\pm$ 0.34	0.41 $\pm$ 0.05	0.76 $\pm$ 0.09
Total	100	100	100

It has been reported that supernatant would contributed 75-100% of the total resistance in microfiltration of mixed liquors (Bouhabila et al., 2001; Iritani et al., 2007). However, Defrance et al. (2000) found that the contribution of colloids and dissolved molecules to filtration resistance was only about 35% in microfiltration of activated sludge suspension. It should be pointed out that in the literature, “supernatant” was prepare by mean of centrifuging mixed liquor at a specific speed. The size and quantity of particles remaining in the supernatant was therefore largely dependent on applied centrifugation speed and duration. The varied centrifugal speeds possibly contributed to inconsistent compositions of supernatant form sample to sample. This is a possible explanation for the inconsistent conclusions regarding the contribution of fine particles to resistance in microfiltration that is often reported in the literature (Defrance et al., 2000; Bouhabila et al., 2001; Ognier et al., 2002; Lee et al., 2003; Yu et al. 2006; Meng and Yang 2007). In this study, $P_{2.7}$, $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 to R3 were prepared from the corresponding supernatant filtrated through membrane with different pore size. Therefore, the particles size and distribution can be examined accurately. As discussed in Chapter 6, it was found that granules supernatants of R1 to R3 were the major contributor of the cake and total resistances. It was also noted that the cake and total resistances of $P_{2.7}$ -R1 to $P_{2.7}$ -R3 were higher than the corresponding granules supernatants of R1 to R3 (Table 6.2). These phenomena could be explained by the prefiltration model developed in Chapter 6. The cake layer formed by big particles in granules supernatants of R1 to R3 could serve as a prefilter to capture smaller particles and further help alleviate pore blocking.

According to Eq. 7.5, value of $K_c R_f$ is an important indicator of the cake layer formation during microfiltration and can be calculated from the gradient of the plot of $(J/J_0)^{-2}$ versus filtration time. It appears that $P_{2.7}$ -R1 had the higher $K_c R_f$ value than $P_{2.7}$ -R2 and $P_{2.7}$ -R3 (Fig. 7.7). As discussed in Chapter 5, R1, R2 and R3 were fed with 0, 50 and 100 mg Ca^{2+} /L, respectively. Addition of Ca^{2+} helped to reduce turbidity and sEPS as well as the percentage of smaller particles in $P_{2.7}$ -R1 to $P_{2.7}$ -R3 (Table 7.2 and Fig. 7.1) due to Ca^{2+} -enhanced coagulation and aggregation of colloidal particles and sEPS. These suggest that addition of calcium favors mitigation of membrane fouling. It is observed in Figs 7.8 and 7.9 that $K_c R_f$ was

positively related to the turbidity and sEPS of P_{2.7}-R1 to P_{2.7}-R3. In practical applications of MF and UF for wastewater reclamation, addition of polyvalent ions (e.g. Al³⁺, Ca²⁺ and Mg²⁺) would effectively improve the permeability via control of size and structure of aggregated colloids and sEPS (Waite et al. 1999; Arabi and Nakhla 2008; Wang et al., 2009). For example, Waite et al. (1999) reported that a very low resistance was achieved during UF of hematite suspension at calcium concentrations greater than 40 mM.

Figure 7.7 Values of $K_c K_r$ obtained from microfiltration of P_{2.7}-R1 to P_{2.7}-R3.

Figure 7.8 Effect of turbidity of $P_{2.7}$ -R1 to $P_{2.7}$ -R3 on $K_c K_r$.

Figure 7.9 Effect of sEPS in $P_{2.7}$ -1 to $P_{2.7}$ -3 on $K_c K_r$.

7.3.3.2. Fouling mechanism of $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 to R3 in microfiltration

Blocking models have often been applied to study the fouling mechanisms in microfiltration or ultrafiltration of colloids, proteins and polysaccharides (Bowen et al., 1995; Ghosh 2002). Table 7.4 shows that intermediate blocking model (Eq.

7.4) can provide the best description for the permeate fluxes obtained during microfiltration of $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$. The curve fittings by Eq. 7.4 for $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 to R3 are presented in Fig. 7.10. It appears that with the decrease of particles size from 2.7 to below 1.0, smaller particles (e.g. sEPS and colloids) might enter pores of membrane, and subsequently cause partial pore blocking in microfiltration of the colloids and sEPS when the prefilter formed by bigger particles is lack. Two key points can be seen from Fig. 7.10: (i) $P_{1.0}$ -R1 to $P_{0.22}$ -R1 exhibited the steepest slopes as compared to the others; (ii) $P_{1.0}$ -R1 to $P_{1.0}$ -R3 had the greatest slope in comparison with $P_{0.45}$ -R1 to $P_{0.45}$ -R3 and $P_{0.22}$ -R1 to $P_{0.22}$ -R3, respectively. As discussed earlier, these phenomena can be explained by both particle size and distribution in the R1 to R3 supernatants.

Figure 7.10 Time evolution of $(J/J_0)^{-1}$ of $P_{1.0}$ to $P_{0.22}$ (a-c) and estimated K_a values (d). The straight lines in a-c represent prediction by Eq. 7.4. (Membrane pore size = $0.22 \mu\text{m}$ and TMP = 5 kPa)

K_a in intermediate blocking model reflects the degree of pore blocking during microfiltration, and can be calculated from the slope of Fig. 10a to 10c. The results presented in Fig. 7.10d demonstrated that the largest K_a values were observed in R1 without addition of calcium ion. According to intermediate blocking model, both attachment of particles onto membrane surface and direct pore clogging by particles would occur simultaneously during filtration. The relative contribution of cake resistance to pore blocking resistance of $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 to R3 were estimated by resistance-in-series model and presented in Table 7.6. It appears that relative contribution of pore blocking resistance against cake resistance varied in the range of 13.3% to 83.67%, suggesting that intermediate blocking model was suitable for description of microfiltration behaviors of $P_{1.0}$, $P_{0.45}$ and $P_{0.22}$ of R1 to R3.

Table 7.6 Relative contributions of pore blocking and cake to the resistances

Feed solutions	Contributions (%)					
	R1		R2		R3	
	Cake	Pore blocking	Cake	Pore blocking	Cake	Pore blocking
$P_{1.0}$	53.41	46.59	68.81	31.19	62.31	37.69
$P_{0.45}$	85.74	14.26	16.33	83.67	50.03	49.97
$P_{0.22}$	86.70	13.30	38.18	61.82	54.97	45.03

7.4. Summary

This study assessed the fouling mechanisms of supernatants from three denitrifying granular sludge SBRs (R1 to R3) fed with different calcium concentrations. The following observations can be drawn from the present study.

- (1) Compared with the results presented in Chapter 6, it was found that particles smaller than $2.7\ \mu\text{m}$ in R1 to R3 supernatants would be the main contributor to the observed membrane fouling.
- (2) The resistance of microfiltration of $P_{2.7}$ of R1 to R3 mainly resulted from the formation of cake layer on membrane surface, which in turn helped to remove smaller colloidal particles and sEPS. The statistical analysis also showed that the flux profiles of $P_{2.7}$ of R1 to R3 in microfiltration can be best described by cake filtration model, indicating a cake-dominant fouling mechanism during microfiltration of $P_{2.7}$ of R1 to R3.
- (3) For microfiltration of $P_{1.0}$ to $P_{0.22}$ of R1 to R3, both cake formation and pore blocking were identified as the main fouling mechanisms responsible for the observed flux decline. This was supported by the fact that intermediate pore blocking model could provide the best description for the experimental data. However, it was shown that relative contribution of pore blocking and cake resistances varied and would be related to calcium concentrations fed to R1 to R3.
- (4) Addition of calcium ion would improve the permeate fluxes of microfiltration of $P_{2.7}$ to $P_{0.22}$ of R1 to R3. It was demonstrated that calcium could alter size distribution of particles present in R1 to R3 supernatants through coagulation and bridging with colloids and sEPS, and further led to improved quality of supernatant in terms of turbidity.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

This study investigated denitrifying granulation under various conditions and the prevalent fouling mechanisms influencing microfiltration of denitrifying granular sludge suspensions. The microfiltration performance of denitrifying granular sludge was correlated to the physicochemical and biological characteristics of denitrifying granules.

The SBR cycle time is recognized as an important selection pressure for aerobic granulation, while little has been known about its effect on denitrifying granulation under anoxic condition. In this study, denitrifying granules were developed at different cycle times of 4, 6 and 8 h, respectively. The mean size and settleability of denitrifying granules were inversely correlated to the SBR cycle time, such that, an increased denitrifying granules size would result in a lower SVI at a shorter cycle time. It was found that extracellular proteins play a more significant role in denitrifying granulation than extracellular polysaccharides. The ratios of proteins to polysaccharides in R1 to R3 were increased from 0.3 to 2.6 along the granulation. A higher mechanical strength of denitrifying granules was obtained at the shorter cycle time. These contrasts with the diminished granular strength observed in longer cycle times which are responsible for a higher cell surface negative charge density of denitrifying granules. These results demonstrated that a shorter cycle time promotes denitrifying granulation. As a consequence, a more robust and stable denitrifying granular sludge bioreactor without sludge bulking would be developed to provide more efficiency nitrogen removal from wastewater.

Calcium ion is believed to play an important functional role in the neutralization of cell surface negative charges. In this part of study, the effect of calcium ion on denitrifying granulation was thoroughly investigated. The results showed that addition of 50 and 100 mg/L calcium could promote rapid denitrifying

granulation, and denser denitrifying granules with excellent settleability were developed at the higher calcium concentration. For example, denitrifying granules formed at 100 mg/L calcium had a lowest SVI of 14 mL/g SS. It was further revealed that the presence of calcium ion in the culture media might not be essential for denitrifying granulation, but significantly influenced the characteristics of denitrifying granules. The structure of denitrifying granules developed without calcium addition had looser structure and lower mechanical strength than those developed at 50 or 100 mg Ca^{2+} /L. In fact, the settleability and structure of denitrifying granules were positively related to the calcium content in denitrifying granules instead of their size. More importantly, addition of calcium could significantly reduce the suspended solids concentration in the effluent even at the biomass concentration as high as 13000 mg SS/L. The ratio of EMLSS/MLSS in the effluent from the SBR run at 100 mg Ca^{2+} /L was as low as 3 mg ESS/g SS. The accumulation of calcium in denitrifying granules would facilitate the formation of the EPS- Ca^{2+} -EPS complex, which in turn would improve the strength and reduce the negative surface charge of denitrifying granules. It was confirmed again that denitrifying granulation was more related to extracellular proteins content in biomass than polysaccharides as found in study of cycle time. The highest specific denitrification rate of 1040 mg N/g VSS d was observed at the calcium concentration of 50 mg/L.

The combination of denitrifying granules with microfiltration represents strong interest in developing a novel granular sludge MBR for high-efficiency denitrification. However, filterability and fouling mechanisms of denitrifying granular sludge should be investigated before the development of such a MBR system. For these purposes, microfiltration experiments of denitrifying granular sludges developed at respective cycle times of 4, 6, and 8 h and calcium concentrations of 0, 50 and 100 mg Ca^{2+} /L were conducted. As compared to the seed suspended sludge, denitrifying granules exhibited a superior permeate flux (e.g. 2.3 to 9.4-times higher than that of seed sludge), indicating a great potential of denitrifying granular sludge MBR for high-efficiency nitrogen removal from wastewater. Denitrifying granules with big size, strong structure and less negatively charged surface would result in higher microfiltration permeate flux, longer

filtration time for half-flux decline and lower fouling potentials. It was found that denitrifying granules with the high calcium content was resistant to granule deformation at high filtration pressure, and this would help the membrane fouling during the microfiltration of denitrifying granular sludge.

In order to primarily distinguish contributions of various components in denitrifying granular sludge suspension, the microfiltration experiments with denitrifying granules, supernatant, colloidal solution and solutes were carried out. The results indicated denitrifying granules themselves only marginally contributed to the total filtration resistance. Fine particles with sizes ranging from 1.0 to 2.7 μm were found to contribute most to the observed membrane fouling responsible for the recorded flux decline. The theoretical analysis by blocking model revealed that colloids and soluble extracellular polymeric substances were also responsible for the observed membrane fouling. It appears that fine particles in the sludge suspension play a far more important role than denitrifying granules on the development of membrane fouling. The specific cake resistances of supernatants separated from granular sludge mixed liquors by sedimentation were 290 to 1943-fold higher than their corresponding granule solutions. Therefore, microfiltration experiments with denitrifying granular sludge suspensions which had different concentrations of fine particles, but the same concentration of denitrifying granules (i.e. 5000 mg SS/L) were conducted. It was found that membrane fouling in terms of specific cake resistance tended to increase exponentially from 1.41×10^{11} to 9.5×10^{11} m/kg when the concentration of fine particles, colloids and sEPS was increased from 0 to 30 mg/L. Addition of calcium would reduce the concentration of fine particles, colloids and sEPS in suspensions, thus less membrane fouling was observed at high calcium concentration.

It was also observed that the cake layer formed on the membrane surface by fast-settling denitrifying granules could serve as a prefilter during the microfiltration of denitrifying granular sludge suspension. This phenomenon greatly reduces the membrane fouling caused by the retention of fine particles and colloids. To further investigate the concentration-effect of denitrifying granules, sludge suspensions with different granule concentrations ranging from 0 to 13000 mg SS/L, but having the same concentration of fine particle, colloids and sEPS were

prepared for microfiltration. It was shown that the total resistance of granular sludge suspensions was greatly reduced by 37.63% when the concentration of denitrifying granules was increased from 0 to 5000 mg SS/L, and almost remained constant when the concentration of denitrifying granules was further increased from 5000 to 13000 mg SS/L. These results provide further experimental evidence supporting the pre-filtration effect of the cake layer of denitrifying granules on the membrane surface.

8.2 Recommendations

Future work will need to look into the following aspects: (i) characteristics of cake layer formed by denitrifying granules with various biomass concentrations in terms of porosity, deformability and their relation to the retention of colloidal particles and EPS; (ii) strategy to reduce the concentration of fine particles in sludge suspension as it was clearly showed in this study that fine particles with the size smaller than 2.7 μm would be the main cause of the observed membrane fouling; and (iii) integration of denitrifying granular sludge with microfiltration membrane unit into one set-up.

In study of the characteristics of cake layer formed by denitrifying granules, the major components of cake layer can be systematically examined by scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), X-ray fluorescence (XRF), energy-dispersive X-ray analyzer (EDX), and Fourier transform infrared (FTIR) spectroscopy. According to these analyses, spatial and temporal distribution of microbial species, EPS and multi-valent cations (e.g. Ca^{2+} , Mg^{2+} , Al^{3+} etc) in cake layer will be linked to membrane fouling and subsequent membrane performance. Furthermore, based on the characteristics of cake layer, tailored high-efficiency backwashing strategy can be developed.

The present study showed that addition of calcium ion would greatly help to reduce fine particle concentration in granules sludge suspension. Thus, the future work will also look into selection and optimal dosage of multi-valent cations (such as Al^{3+} , Ca^{2+} , Mg^{2+} and Fe^{3+}), and their effects on the formation of the denitrifying

granules and reduction of fine particles in granules suspension will be investigated as well.

The present study clearly demonstrated that denitrifying granular sludge would help to mitigate membrane fouling and further improve membrane performance. Biological nitrogen removal basically requires a two-step reaction, i.e. nitrification and denitrification. In the future study, a combined nitrifying-denitrifying granules-membrane bioreactor will be developed and optimized for high-efficiency and low-cost nitrogen removal from municipal wastewater.

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