Two Stage Membrane Biofilm Reactors for Nitrification and Hydrogenotrophic Denitrification

by

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Acknowledgements

TABLE OF CONTENTS

Table of Figures

Table of Tables

ABBREVIATION

BAF	Biological aerated filters
CSTR	Continuous stirred tank reactor
DO	Dissolved Oxygen
F/M	food to microorganisms
HRT	Hydraulic retention time
ICP-OES	Inductively coupled plasma – Optical emission spectrometry
IFAS	Integrated fixed film activated sludge
LOT	Limit of Treatment
MBBR	Moving bed biofilm reactors
MBfR	Membrane biofilm reactor
PMP	Polymethylpentene
PP	Polypropylene
PVA	Polyvinyl alcohol
SDNR	Specific denitrification rate (g $N/m^2 d$)
SNR	Specific nitrification rate (g $N/m^2 d$)
SS	Suspended solids
TN	Total nitrogen
TP	Total phosphorous
VSS	Volatile suspended solids

EXECUTIVE SUMMARY

Membrane biofilm reactors (MBfR) utilize membrane fibers for bubble-less transfer of gas by diffusion and provide a surface for biofilm development. Nitrogen removal was attempted using MBfR in various configurations - nitrification, denitrification and consecutive nitrification and denitrification.

Effects of loading rate and dissolved oxygen on nitrification performance were primarily investigated in a stand-alone nitrifying MBfR. Specific nitrification rate increased linearly with specific loading rate, up to the load of 3.5 g N/m^{2}d. Beyond that load, substrate diffusion limitation inhibited further increase of specific nitrification rate. 100% oxygen utilization was achievable under limited oxygen supply condition.

Effects of mineral precipitation, dissolved oxygen and temperature on hydrogenotrophic denitrification were investigated in a stand-alone denitrifying MBfR. Mineral precipitation, caused by intended pH control, caused the deterioration of denitrification performance by inhibiting the diffusion of hydrogen and nitrate. Operating reactor in various dissolved oxygen conditions showed that the denitrification performance was not affected by dissolved oxygen in MBfR. Optimum temperature of the hydrogenotrophic denitrification system was around 28 °C.

Total nitrogen removal in a two-step MBfR system incorporating sequential nitrification and hydrogen-driven autotrophic denitrification was investigated in order to achieve nitrogen removal by autotrophic bacteria alone. Long-term stable operation, which proved difficult in previous studies due to excessive biofilm accumulation in autotrophic denitrification systems, was attempted by biofilm control. Average

Jong Hyuk Hwang, Ph.D. Thesis

specific nitrification rate of 1.87 g N/m^{2}d was achieved and the performance was very stable throughout the experimental periods over 200 days. Performance of autotrophic denitrification was maintained stably throughout the experimental periods, however biofilm control by nitrogen sparging was required for process stability. The average specific denitrification rate increased from 1.50 g N/m^{2}d to 1.92 g N/m^{2}d with nitrogen sparging, over 190 days thus demonstrating the feasibility of stable long-term operation. Biofilm thickness was also stably maintained at an average of 270 µm by the gas sparging biofilm control. Maximum achievable nitrogen removal rate in consecutive operation turned out to be 6 g N/m^{2}d.

According to the cost analysis of denitrifying MBfR, hydrogenotrophic denitrification can be an economical tertiary treatment option compared to conventional denitrifying filter although its economic feasibility highly depends on the cost of hydrogen gas.

Although this study was conducted in a lab-scale, the findings from this study can be a valuable stepping stone for larger scale application and open the door for system modifications in future.

Jong Hyuk Hwang, Ph.D. Thesis

1. INTRODUCTION

1.1 Importance of nitrogen removal

The importance of removing nutrients from the effluent of wastewater treatment plants is continually emphasized by researchers and regulators, as increasingly severe problems such as eutrophication or hypoxia of lakes, reservoirs, estuaries and the near-shore ocean are forcing environmental regulators to impose more stringent effluent requirements on total nitrogen (TN) and total phosphorus (TP) (Toet *et al*., 2005; Jeong *et al*., 2006; Jung *et al*., 2006; Zhang *et al*., 2008).

In North America, the limit of treatment technology (LOT) concept has been defined as the lowest economically achievable effluent quality, which for TN is < 1.5 - 3 mg/L and TP is < 0.07 mg/L. These concentrations are becoming the targets in fragile eco-regions such as Gulf Dead Zone, Chesapeake Bay, and Las Vegas in North America. There is a drive towards combining existing biological nutrient removal process trains with additional new biological or chemical unit operations to meet these effluent limits (Oleszkiewicz and Barnard, 2006).

The most common forms of nitrogen in wastewater are ammonia (NH_3), ammonium ion (NH_4-N), nitrogen gas (N_2), nitrite (NO_2-N), nitrate (NO_3-N), and organic nitrogen. Municipal wastewater primarily contains ammonium (60%) and organic nitrogen (40%). Nitrogen can be removed by physical, chemical or biological means in wastewater treatment streams. However, biological removal of nitrogen is

Jong Hyuk Hwang, Ph.D. Thesis

considered as the most economical and applicable way. Nitrogen removal can be either an integral part of the biological treatment system or an add-on process to an existing treatment plant (Metcalf and Eddy, 2003). This study is intended to remove nitrogen by the means of an add-on process.

1.2 Nitrification and hydrogenotrophic denitrification

Biological nitrogen removal usually occurs in two steps. The first step is nitrification, in which ammonium ion (NH_4-N) is oxidized to nitrate (NO_3-N) or nitrite (NO_2-N). This is followed by denitrification, a process which converts nitrate (NO_3-N) or nitrite (NO_2-N) to nitrogen gas (Metcalf and Eddy, 2003).

1.2.1 Nitrification

Nitrification is a two-step process involving two groups of aerobic autotrophic bacteria. In the first stage (Equation 1-1), NH_4-N is oxidized to NO_2-N by autotrophic bacteria, including *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus* and *Nitrosorobrio* (Painter, 1970). In the second stage (Equation 1-2), NO_2-N is oxidized to NO_3-N by autotrophic bacteria, including *Nitrobacter, Nitrococcus, Nitrospira, Nitrospina* and *Nitroeystis* (Painter, 1970). Abundance and activity of specific

Jong Hyuk Hwang, Ph.D. Thesis

autotrophic bacteria shown above differ from the environmental situation such as DO, pH and temperature (Daebel, 2007).

The following stoichiometric relationships have been proposed for autotrophic nitrification.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \qquad \text{(Equation 1-1)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \qquad \text{(Equation 1-2)}$$

$$NH_4^+ + 1.863O_2 + 0.098CO_2$$

$$\rightarrow 0.0196C_5H_7NO_2 + 0.98NO_3^- + 0.941\ H_2O + 1.98H^+$$
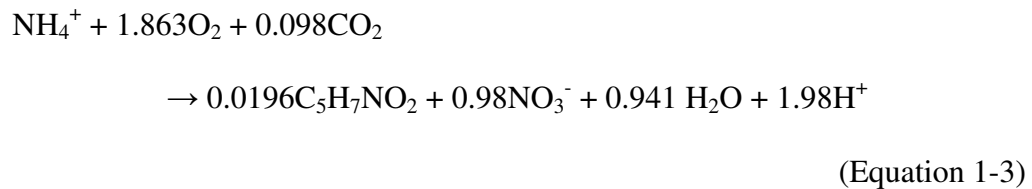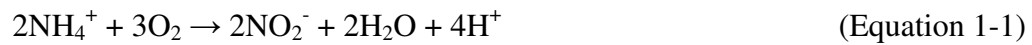
$$\text{(Equation 1-3)}$$

According to the above stoichiometric equations, for each g of ammonia nitrogen (as N) converted, 4.25 g of O_2 are utilized, 0.16 g of new cells are formed, 7.07 g of alkalinity as $CaCO_3$ is removed (only when oxidizing ammonia to nitrite), and 0.08 g of inorganic carbon is utilized in the formation of new cells.

1.2.2 Hydrogenotrophic denitrification

Biological denitrification is a microbial process that converts nitrate or nitrite to nitrogen gas. Denitrifiers can be divided into two types, heterotrophs and autotrophs, based on type of their carbon source needed for growth.

Jong Hyuk Hwang, Ph.D. Thesis

Heterotrophic denitrification requires organic substrates for biomass growth and development. There are a wide range of heterotrophic denitrifiers, which include: *Achromobacter, Acinetobacter, Agrobacterium, Alcaligenes, Bacillus* and *Chromobacterium*. The process has been applied in conventional wastewater treatment for decades (WEF and ASCE, 2006). In heterotrophic denitrification, bacteria use nitrate as the terminal electron acceptor and an organic carbon as the electron donor in their respiratory processes in the absence of oxygen or under limited DO concentrations. Usually, an external carbon source such as methanol, ethanol or starch is being used to enhance denitrification due to lack of sufficient carbon source in wastewater. Denitrification stoichiometry with methanol, the most common carbon source used as electron donor, is shown in Equation 1-4 (Metcalf and Eddy, 2003).

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \qquad \text{(Equation 1-4)}$$
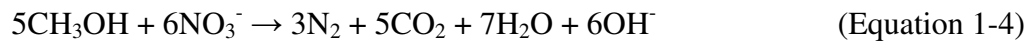
Major disadvantages of heterotrophic denitrification include the cost of adding external carbon source (which might contribute significantly to the overall operating cost of the plant), high biomass yield, and possible toxicity to final effluent, requiring further treatment (MacAdam and Judd, 2006). Alternative carbon sources such as industrial wastewater could help to alleviate the cost impact; however the toxicity of residual carbon or the costs associated with higher biomass yield, (compared to autotrophic growth) remain to be resolved (Mansell and Schroeder, 2002).

Jong Hyuk Hwang, Ph.D. Thesis

Autotrophic denitrification on the other hand requires inorganic carbon such as carbon dioxide or bicarbonate as a carbon source, and inorganic compounds, such as hydrogen (Kurt *et al*., 1987), thiosulfate (Claus and Kutzner, 1985), sulfide (Kleerebezem and Mendeza, 2002) and sulfur stone (Flere and Zhang, 1999) as electron donors. One of the major advantages of autotrophic over heterotrophic denitrification is low biomass production. Among autotrophic denitrification processes, hydrogenotrophic denitrification refers to the process that utilizes hydrogen as an electron donor, which would be better than sulfur which yields sulphuric acid into the effluent stream.

Advantages of using hydrogen over other electron donors for autotrophic denitrification include (1) its low solubility in water (1.6 mg/L at 20 °C and 1 atm); (2) the residual hydrogen that remains in water is harmless and does not interfere with subsequent treatment (Rittman and Huck, 1989); (3) only inorganic carbon is used and, therefore, there are no organic residues for further treatment; (4) reaction by-products are harmless (Water Environment Research Federation, 2003). Therefore, the need for further treatment, required for other options, could be eliminated. However, the low solubility of H_2 in water can be a drawback as well, hindering the access to hydrogen by autotrophic denitrifiers. The explosive nature of hydrogen during use, transportation and storage should be considered as well. Therefore, an efficient and safe way of delivering hydrogen (minimizing the wastage of hydrogen) needs to be established to make this alternative competitive.

Jong Hyuk Hwang, Ph.D. Thesis

The following stoichiometric relationship has been proposed for hydrogenotrophic denitrification, which reduces nitrate to nitrite and subsequently to nitrogen gas (Kurt *et al.*, 1987). Equation 1-7 shows the overall reaction including cell synthesis.

$$2NO_3^- + 2H_2 \rightarrow 2NO_2^- + 2H_2O \qquad\qquad \text{(Equation 1-5)}$$

$$2NO_2^- + 3H_2 \rightarrow N_2 + 2H_2O + 2OH^- \qquad\qquad \text{(Equation 1-6)}$$

$$NO_3^- + H^+ + 2.86H_2 + 0.15CO_2 \rightarrow 0.0286C_5H_7NO_2 + 0.49N_2 + 3.14H_2O$$
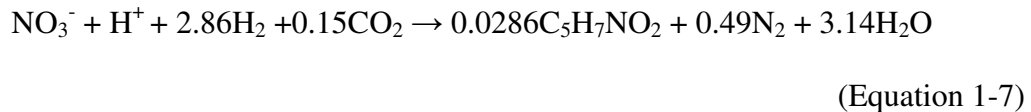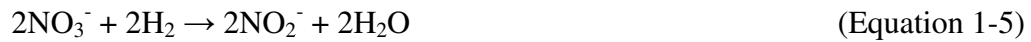
$$\text{(Equation 1-7)}$$

According to above equations, 0.41 g of hydrogen gas is required to reduce 1 g of nitrate to nitrogen gas, while the reduction of nitrite to nitrogen gas generates 3.57 g of alkalinity as $CaCO_3$ per 1 g NO_3-N reduced. The subsequent increase in pH can cause inorganic precipitation and affect the denitrification performance, which will be discussed in Chapter 6.1.

1.3 Membrane biofilm reactor

Membrane bioreactor (MBR) technology, involving membranes for solid-liquid separation, has experienced several breakthroughs over the past 30 years to become one of the major technologies for wastewater treatment. Beside these most well

Jong Hyuk Hwang, Ph.D. Thesis

known applications of membrane in wastewater treatment, there are technologies that are intended to utilize membranes as biofilm supports, rather than solid-liquid separators.

Different mechanisms of pollutants removal can be achieved in membrane-attached biofilm systems, which can be classified below and shown in Figure 1.1 with concentration profiles.

- Membrane biofilm reactors (MBfR) refer to the systems with biofilm growing on top of the membrane fiber, where the pressurized gas diffuses through the membrane lumen, in order to provide the bacteria with the components needed to oxidize or reduce the soluble constituents present outside the membrane lumen. Air, oxygen, hydrogen or methane, have been used as the process gas depending on the treatment objectives.

- Extractive membrane bioreactors refer to systems in which the contaminated liquid flows inside of the membrane fiber and only selective contaminants are transported through the membrane for biodegradation on the membrane surface or in bulk solution (Brookes & Livingston (1994) and Freitas dos Santos *et al.* (1997)).

- Membrane biofilters refer to systems in which a waste gas diffuses through the membrane lumen and is biologically removed by the biofilm growing on the membrane surface (Parvatiyar *et al*. (1996) and Reij *et al*. (1996)).

In this study a membrane-attached biofilm system (MBfR) will be implemented for nitrogen removal in tertiary wastewater treatment.
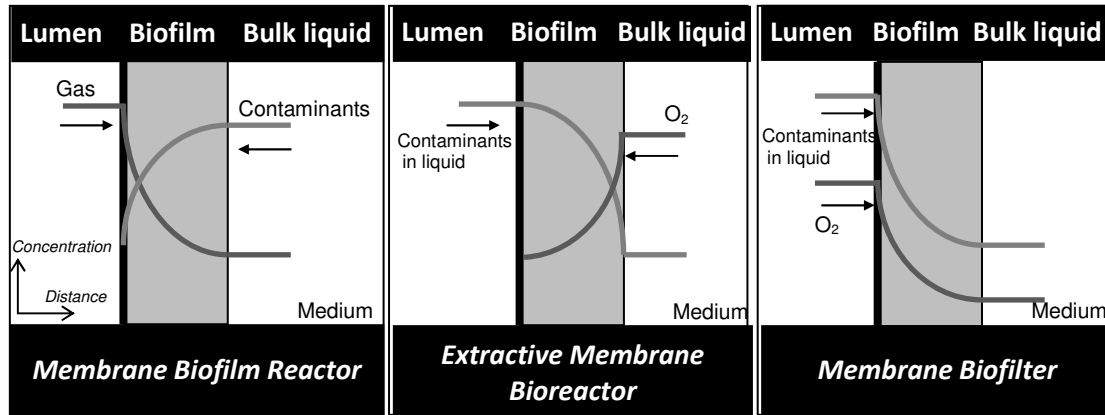
Figure 1.1 Comparison of pollutants removal mechanisms in membrane-attached biofilm system

The concept of using membranes for gas diffusion has started during the late-1980s and early-1990s (Essila *et al.,* 2000). Membranes can be used as a means of just transferring gas or they can be used as means of both supplying gas and supporting biological growth. By transferring gas through membrane fibers, the biomass attached to the membrane surface can effectively access the process of gas. Hollow fiber membranes are particularly attractive because they can provide a large specific surface area for bacterial attachments.

MBfR became the focus of research as they (1) provide efficient, bubble-less gas delivery, (2) provide extensive surface area for biofilm attachment and growth, (3) allow for extended solids retention times and high biomass concentration within the biofilm, (4) enable high volumetric removal rates, and (5) require a relatively small reactor footprint.

Jong Hyuk Hwang, Ph.D. Thesis

MBfR has an advantage of reducing the overall reactor volume. Usually, 50-60% of reactor volume is occupied by the support medium in conventional biofilm reactors, such as trickling filters, rotating biological contactors, biological aerated filters and submerged aerated filters (Goncalves *et al.*, 1996, Fdz-Polanco *et al.*, 1994). Meanwhile, MBfR occupy only 1-2% of this volume for treating similar substrate loadings (Brindle *et al.*, 1998).

In a conventional biofilm application, both substrate and gas are abundant at the side of the bulk liquid; therefore anoxic/anaerobic conditions may be induced deep inside of biofilm. However, in MBfRs, the gas concentration will be highest and substrate concentration will be lowest at the surface of the membrane. Figure 1.2 shows the difference of substrate and gas profiles in conventional biofilm systems as compared to MBfR.
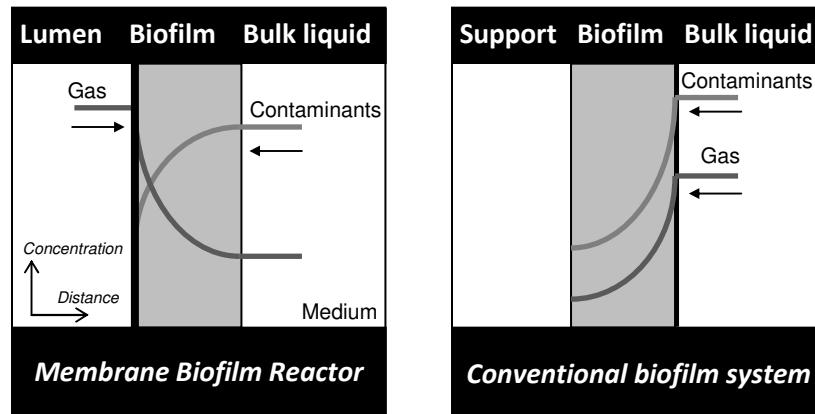
Figure 1.2 Comparison between MBfR and conventional biofilm system

Jong Hyuk Hwang, Ph.D. Thesis

2. LITERATURE REVIEW

This chapter includes the literature review regarding MBfRs and its application for nitrogen removal.

A database of a 102 research papers published in peer-reviewed international journals between 1984 and 2008 was analyzed. The literature database was partially based on the references of a recent review paper (Syron and Casey, 2008), and also supported by search results from online databases including Web of Science, ScienceDirect, and PubMed. Extensive literature review regarding MBfR applications for nitrogen removal was done in terms of reactor configuration and performance.

2.1 Chronology and geographical distribution of MBfR studies

Figure 2.1 shows the chronological distribution of the number of peer-reviewed journal articles involving studies on MBfR. A linear increase of research papers over the years can be observed, even though there were only a small number of papers in 1997 and 1998, mainly due to a limited pool of researchers. The overall number of papers in early periods tends to fluctuate depending on the activity of individual researchers, becoming more consistent after 2000, when more research groups started to study MBfR.
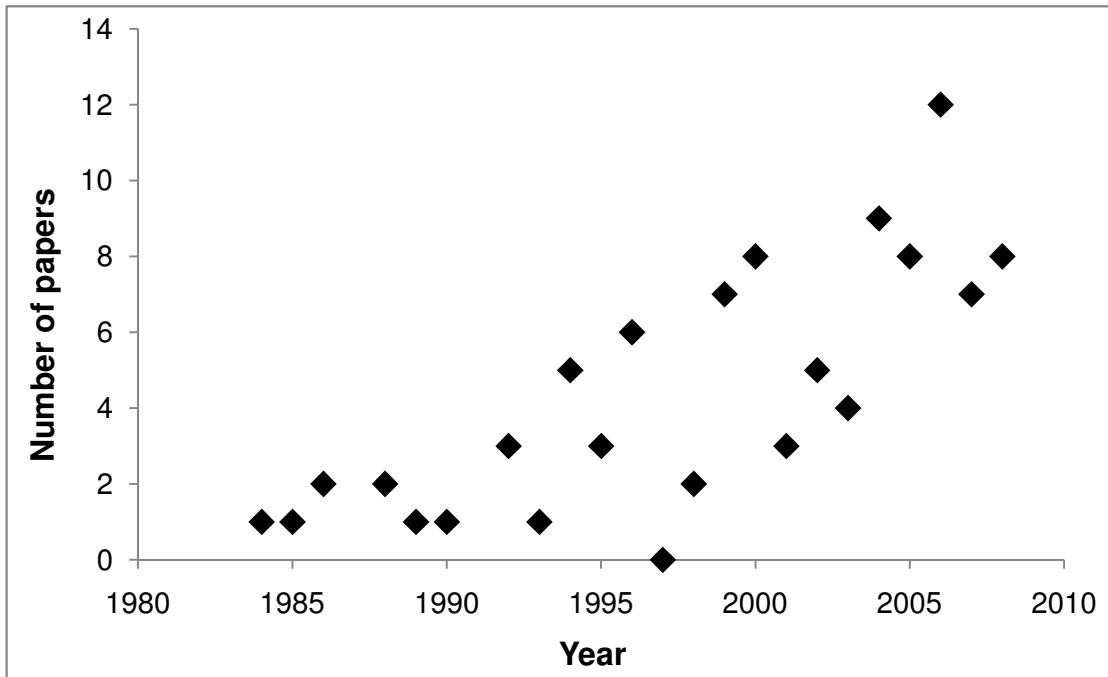
Figure 2.1 Chronological distribution of peer-reviewed journal articles

Geographically, research studies were conducted in 14 countries: Australia, Canada, China, Denmark, France, Germany, Japan, Korea, Sweden, Switzerland, Taiwan, Thailand, UK and USA; i.e. there was at least one peer-reviewed journal paper from each country. More than three quarters of research projects were conducted in the UK, USA and Japan.

Figure 2.2 shows the regional and historical distributions of published papers. Researchers from Asia, Europe and North America account for 24, 29, and 49 peer-reviewed journal articles, respectively. Most papers before 2000 originated from Europe, as it was where this technology was initiated. Although countries like USA, UK and Japan keep publishing papers consistently, more countries, especially in Asia, (particularly China and Korea) started to conduct MBfR studies after 2000, and this is

Jong Hyuk Hwang, Ph.D. Thesis

why the number of papers from Asia has increased substantially after 2000. The trend of regional distribution from Figure 2-2 is interestingly similar to the trend that can be found in membrane bioreactors (MBRs) (Yang *et al.*, 2006).
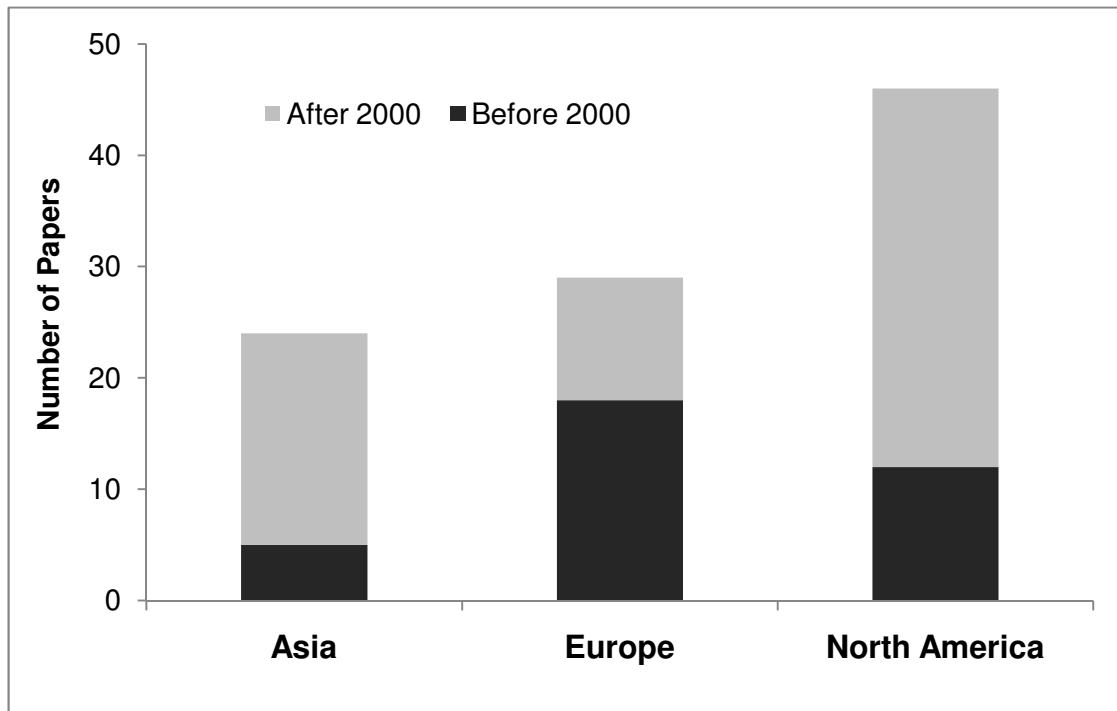
Figure 2.2 Regional distribution of peer-reviewed journal articles

2.2 Classification by research areas

All 102 journal articles were categorized into four main research areas as shown in Figure 2.3.

2.2.1 Literature and critical reviews – Category I

Jong Hyuk Hwang, Ph.D. Thesis

There were 5 literature and critical reviews. Research progress of aerated MBfR, where oxygen or air was supplied through the membrane fiber was reviewed in three papers (Brindle and Stephenson, 1996; Casey *et al.*, 1999; Syron and Casey, 2008). MBfR with hydrogen addition were reviewed relatively recently (Rittmann *et al.*, 2004; 2006).

2.2.2 Fundamental aspects including modeling – Category II

There were 38 papers on the fundamental aspects of MBfR. Initially, oxygen transfer through membrane lumen without biofilm attachment was studied. Research at that time focused on the feasibility of supplying oxygen and the measurement of oxygen transfer rate through membrane fibers. Rapid increase of oxygen concentration in a sequencing batch reactor (SBR) system could be achieved by using silicone tubing where pure oxygen was pressurized (Wilderer *et al.*, 1985). Oxygen mass transfer in silicone rubber hollow fibers (Cote *et al.*, 1989) and individually-sealed hollow fiber membranes (Ahmed *et al.*, 1992) was also investigated. Once the biofilm growth on the membrane fiber was observed, studies began to evaluate its potential and its applicability to various cases (See Category III). Wilderer (1995) suggested that membrane oxygenation and biofilm SBR technology can be favourably combined to treat wastewater which contain volatile organics, organics in low concentration or organics which are degraded only by selected, slow growing microorganisms.
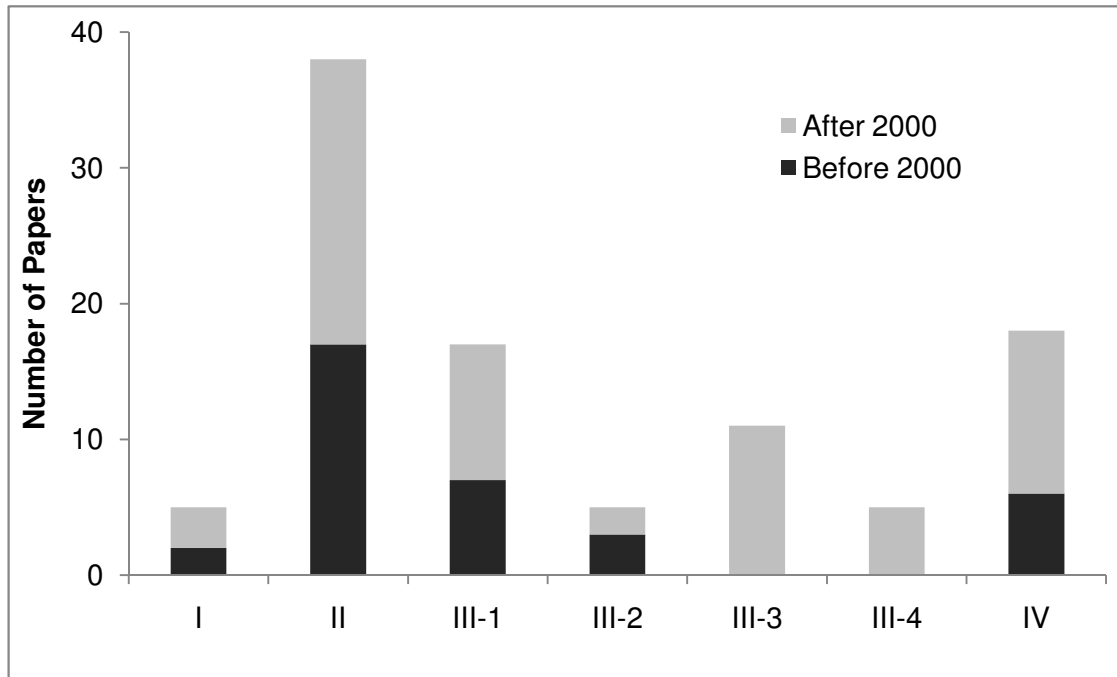
Figure 2.3 Distribution of research topics (Category I: literature and critical reviews; Category II : fundamental aspects including modeling; Category III-1: organic removal and simultaneous nitrification (including heterotrophic denitrification); Category III-2: nitrification only; Category III-3: autotrophic denitrification only; Category III-4 : total nitrogen removal with autotrophic bacteria only; Category IV: other applications)

Many papers focused on the investigation of diffusion characteristics of gas or substrate and biofilm stratification by experiments or by modeling. It was shown by Casey *et al.* (1999), that greater thicknesses of active biomass can be maintained in MBfR compared to conventional biofilm reactors. However, some modeling results showed that even though the performance of MBfR was superior to that of conventional biofilm systems, when the biofilm thickness was in the range of 450 µm - 1400 µm, the efficiency deteriorated with a further increase in biofilm thickness

Jong Hyuk Hwang, Ph.D. Thesis

(Terada *et al.*, 2007). A modeling study also showed that there were substantial differences in substrate and oxygen concentration and activity profiles between MBfR and conventional biofilm systems (Essila *et al.* 2000). Models predicted that the oxygen flux can drop to zero resulting in anaerobic conditions at the external regions of the biofilm, which may facilitate simultaneous nitrification and denitrification (Semmens and Essila, 2001).

Another modeling study by Matsumoto et al. (2005) showed that even nitrifiers would be stratified in MBfR, suggesting that ammonia oxidizing bacteria (AOB) and specific nitrite oxidizing bacteria (NOB) dominated the oxic part of biofilm (at the surface of membrane fiber), while NOB were abundant at the oxic-anoxic interface. Cole *et al.* (2004) analyzed changes in biomass density, respiratory activity, and bacterial community structure as functions of biofilm depth. Biomass density was generally highest near the membrane surface where the gas is supplied and declined with distance from the membrane, while respiratory activity was highest in the middle of the biofilm.

Community analysis demonstrated substantial stratification of the community structure across the biofilm. Surface-modified hollow fiber membranes to enhance the bacterial adhesivity for partial nitrification was tested and showed that the amount of attached nitrifiers on surface-modified fiber was 3 times greater than those on unmodified fiber surfaces (Tarada *et al.*, 2004). FISH analysis proved that ammonia-oxidizing bacteria were located near the surface of membrane, whereas other bacteria were located throughout the biofilm thickness (Terada et al., 2006). The thickness of

Jong Hyuk Hwang, Ph.D. Thesis

biofilm attached to the membrane surface was reported to be 1600 μm; however oxygen was capable of penetrating only about 300 to 700 μm from the surface of membrane, according to measurements using a microelectrode. A study with flat-sheet membranes showed the difference of oxygen transfer in upstream and downstream sections of the membrane due to biofilm growth, suggesting that reactor configuration may have a significant influence on oxygen transfer rates (Shanahan and Semmens, 2006).

2.2.3 Applications for nitrogen removal – Category III

Category III includes research articles mainly focusing on applications and case studies using MBfR. Since many papers reviewed here fall into this category, it was divided into 4 sub categories, as shown in Figure 2-3, (3-1) organic removal and nitrification simultaneously (including heterotrophic denitrification); (3-2) only nitrification; (3-3) only autotrophic denitrification using hydrogen; (3-4) total nitrogen removal with only autotrophic bacteria. Overall, there were 38 papers (16, 6, 11 and 5 for each sub-category, respectively).

1) Category III-1: organic removal and simultaneous nitrification (including heterotrophic denitrification

The papers in this sub-category dealt with the heterotrophic activity for organic removal and denitrification, and/or simultaneous nitrification.   Combined

Jong Hyuk Hwang, Ph.D. Thesis

heterotrophic oxidation of organics, denitrification and nitrification in MBfR could be successfully achieved in several studies (Timberlake *et al.*, 1988; Satoh *et al.*, 2004).

A gas-permeable flat sheet membrane with a surface area of 585cm^2 for the biofilm support was tested for combined aerobic heterotrophic oxidation, nitrification and denitrification (Timberlake *et al.*, 1988). Pure oxygen was supplied through the membrane and the outer solution was sparged with nitrogen gas to keep the bulk solution anoxic. The efficiency of organic and nitrogen removal turned out to be 47 % and 21 % respectively, and only 50-75 % of nitrification was achieved. Even though the performance of this study was inferior to subsequent studies, it showed that combined aerobic heterotrophic oxidation, nitrification and denitrification can occur in MBfR. The potential of microporous hollow fiber membrane for oxygen transfer was investigated (Ahmed *et al.*, 1992), suggesting that bubble-less transfer of oxygen and 100% transfer efficiency could be achieved.

7.6 kg/m^{3}d of COD removal could be achieved with a hydraulic retention time as low as 36 min, (Pankhania *et al.*, 1994) with 280 μm diameter polypropylene based, dead-end hollow fibers, although a daily backwash was necessary to prevent channelling. Although some simultaneous nitrification and denitrification (SND) was detected, backwashing also resulted in the deterioration of nitrification by reducing the biomass retention time and denitrification by removing thick biomass which was potentially anoxic.

1.9 g/m^{2}d of specific nitrification rate could be achieved during simultaneous organic carbon removal and nitrification (Yamigawa *et al.*, 1998) with attached biofilm in a fibrous support woven around hollow membrane fibers. Denitrification

Jong Hyuk Hwang, Ph.D. Thesis

was minimal in this study since the concentration of dissolved oxygen was kept above 2 mg/L. Nitrification and denitrification in a single reactor by adding two membrane modules, supplying oxygen for nitrification and methanol for heterotrophic denitrification was attempted, showing effective coexistence of nitrifier and denitrifier cultures in a single tank (Chang *et al.*, 1999). Semmens *et al.* (2003) tried to control biofilm growth by an internal gas recycle; however, the performance could not be sustained due to the excess biofilm accumulation, emphasizing the importance of biofilm thickness control.

Recently a hybrid system utilizing biofilm on the membrane fibers for nitrification and suspended biomass for organic removal and denitrification was tested (Downing and Nerenberg, 2007). Since nitrifiers existed in the biofilm, the system could be operated with low SRT (5 days). Maximum 75 % of total nitrogen removal, 80 % of nitrification with a specific nitrification rate of 0.85 g/m^2d and 99 % of BOD removal could be achieved. Biological phosphorus removal was also attempted using nitrifying biofilm in MBfR and denitrifying polyphosphate-accumulating organisms (DNPAOs) in a bulk solution (Terada *et al.*, 2006). A sequencing batch reactor was operated including an anaerobic period for phosphorous release and a subsequent membrane aeration period for nitrification and phosphorous uptake, resulting in 96% of total nitrogen removal and 90% of total phosphorus removal.

2) Category III-2 : nitrification only

The papers in this sub-category dealt with the application of MBfR for only nitrification. Relatively small number of studies was conducted for nitrification only

Jong Hyuk Hwang, Ph.D. Thesis

(Brindle *et al.*, 1988; Suzuki *et al.*, 2000), because many researchers intended to achieve simultaneous removal of organics or denitrification. Brindle *et al.* reported that complete nitrification could be achieved throughout the experiment with average 98 % of nitrification efficiency, while backwashing was not conducted as excessive biomass growth was not observed (Brindle *et al.*, 1998). A maximum nitrification rate of 0.98 kg NH_4-N/m^3d was achieved, which corresponded to a specific nitrification rate of 5.4 g NH_4-N/m^2d. Up to 100 % of oxygen uptake rates could be achieved as the oxygen supply balanced out with the oxygen amount used by the nitrifiers. Cell immobilization techniques were also tested for nitrification purposes in an MBfR system (Hsieh *et al.*, 2002).

Table 2.1 summarizes the operating parameters and results from previous nitrification only or both organic removal and nitrification studies in MBfR. Systems operated with pure oxygen tended to have higher specific nitrification rates (average SNR of 3.7 g N/m^2d) than those with air (average SNR of 1.3 g N/m^2d), although only small number of data was compared. Table 2.1 also includes the nitrification results from consecutive nitrification and denitrification, which will be discussed later.

3) Category III-3 : autotrophic denitrification only

The papers in this sub-category deal with the application of MBfR in autotrophic denitrification using hydrogen. The ability to efficiently deliver hydrogen through membrane fibers, combined with low sludge yield of autotrophic growth, makes autotrophic denitrification in MBfR an interesting alternative over conventional heterotrophic denitrification.

Jong Hyuk Hwang, Ph.D. Thesis

Table 2.1 Comparison of MBfR operated for ammonia and organic carbon removal

	Timberlake et al. (1988)	Pankhania et al. (1994)	Brindle et al. (1998)	Yamagiwa et al. (1994)	Semmens et al. (2003)	Terada et al. (2003)	Shin et al. (2005)	Cowman et al. (2005)	Downing et al. (2006)
Target	C + N	C + N	N	C + N	C + N	C + N	N	N	C + N
Influent type	Actual	Synthetic	Synthetic	Synthetic	Synthetic	synthetic	Synthetic	Synthetic	Synthetic
Influent COD or TOC (mg/L)	90	290	-	200	400	4500	-	-	120
Influent nitrogen (mg/L)	50	50	50	41	90	4000	100	50	20
Organic removal (%)	47	60	-	over 95	over 95	96	-	-	99
Nitrogen removal (%)	21	76	over 95	over 90	over 90	83	over 95	over 90	75
Oxygen source	Pure oxygen	Pure oxygen	Pure oxygen	Air	Air	Air	Air	Pure oxygen	Air
Membrane type	Flat Sheet	Hollow Fiber	Hollow Fiber	Fiber + support	Hollow Fiber	Hollow Fiber	Hollow Fiber	Hollow Fiber	Hollow Fiber
Surface area (m^2)	0.0585	0.687	0.078	0.056	2.82	0.0075	0.13	-	0.13
HRT (h)	25	2.13	4	12	6	360	6	1.5	6
Reactor volume (L)	3.15	1.345	0.43	1.63	6.68	0.15	0.075	0.01	3.25
Specific removal rate for organics (g COD/m^2d)	4.28	3.84	-	6.3	3.6	5.76	-	-	-
Specific Nitrification Rate for nitrogen (g N/m^2d)	1.92	0.84	5.4	2	0.767	4.48	1.43	-	0.85
Experimental period (days)	42	150	180	90	190	350	260	7	100

Jong Hyuk Hwang, Ph.D. Thesis

Studies regarding this sub-category started to appear after the year 2000. Application to nitrate removal from contaminated drinking water showed promise over conventional denitrification (Lee and Rittmann, 2000). Up to 2.2 g N/m^{2}d of specific denitrification rate could be obtained, however stable operation of reactor could not be achieved due to excessive biomass growth (Ergas *et al.*, 2001).

Carbon dioxide could be added to neutralize the alkalinity generated in denitrification in order to inhibit nitrite accumulation (Ho *et al.*, 2001). However, the effect of pH on autotrophic denitrification is still unclear (Lee and Rittmann, 2003, Rezania *et al.*, 2005). High rate of denitrification (5.38 g N/m^{2}d) could be achieved; however the duration of reactor operation (6 days) was not long enough to judge the system performance.

Performance deterioration by mineral precipitation is another operational concern and biofilm control is required to maintain reactor performance (Celmer *et al.*, 2008). Celmer *et al.* (2006) found that biofilm density rather than thickness was the determining factor in substrate diffusion and biofilm sloughing in an MBfR system operated under limited hydrogen supply condition. Celmer *et al.* (2008) also evaluated the impact of different shearing strategies, including mixing and nitrogen sparging to scour off the excessive biomass in MBfR. As high as 0.93 g N/m^{2}d of specific denitrification rate could be achieved using nitrogen sparging reducing to maintain the biofilm thickness below 500 μm.

Terada *et al.* reported that immobilized hydrogenotrophic bacteria in fibrous slag could achieve higher denitrification rate than other studies when hydrogen was supplied in excess (Terada *et al.*, 2006). Over supply of hydrogen also allowed

Jong Hyuk Hwang, Ph.D. Thesis

autotrophic sulfate reducing bacteria to grow. Therefore, the significance of hydrogen pressure control for suppressing the occurrence of sulfate reducing bacteria was emphasized.

Table 2.2 summarizes the previous studies for hydrogenotrophic denitrification in MBfR. Table 2.2 also includes the nitrification results from consecutive nitrification and denitrification, which will be discussed later.

Table 2.2 Summary of hydrogenotrophic in MBfR

	Influent NO_x-N (mg/L)	Surface area (m^2)	HRT (h)	Volumetric Denitrification Rate (kg/m^3d)	Specific Nitrification Rate (g/m^2d)
Ergas *et al.* (2001)	100	0.37	4	0.36	1.17
Ho *et al.* (2001)	120	0.059	8.3	0.21	5.38
Lee *et al.* (2002)	15	0.075	0.7	0.478	2.68
Shin *et al.* (2005)	200	0.13	2	1.43	0.84
Shin *et al.* (2008)	50	0.814	5	0.175	1.4
Celmer *et al.* (2008)	20	0.375	4	0.11	0.93

4) Category III-4 : total nitrogen removal with autotrophic bacteria only

The papers in this sub-category deal with the application of MBfR for total nitrogen removal with only autotrophic denitrification. Many MBfR studies have showed successful nitrification or denitrification performance so far when operated separately. However, only a few research studies (Shin *et al.*, 2005, 2008, Cowman *et al.*, 2005) have attempted to perform nitrification and sequential hydrogenotrophic denitrification in MBfR systems.

Shin *et al.* (2005) reported up to 2.48 kg/m^3d and 1.43 g N/m^2d of volumetric and specific nitrification rate in an MBfR with consecutive nitrification and denitrification.

Jong Hyuk Hwang, Ph.D. Thesis

Nitrification could be accomplished almost completely even in low DO conditions of 0.6 mg/L. Slight denitrification in nitrification reactor, which might be induced by the heterotrophic activity outside of the biofilm, was also reported in the nitrification reactor. Although up to 1.33 g N/m^2d of specific denitrification rate could be achieved, denitrification efficiency was reduced at pH higher than 8.3. They reported even higher specific nitrification and denitrification rate up to 2.06 g N/m^2d and 1.72 g N/m^2d, respectively, in their CSTR (continuous stirred tank reactor) (Shin *et al.*, 2008). However, excessive biofilm growth in the denitrification reactor was observed, leading to manual cleaning at a frequency of 3 times during their 300 days of operation.

Cowman *et al.* tested several different O_2 pressures in order to determine the optimal gas pressure in nitrifying and denitrifying MBfRs (Cowman *et al.*, 2005). Nitrification was greatly inhibited at low O_2 pressure, due to the limited O_2 supply for nitrification, causing carry-over of un-oxidized NH_4-N to the following denitrification reactor and final effluent. Meanwhile, nitrate concentration in the final effluent increased when the O_2 pressure was raised, which resulted in the carry-over of excess dissolved oxygen to the denitrification reactor, emphasizing the need of proper control of O_2 pressure in this type of configuration.

Although some studies showed the potential of consecutive nitrification and hydrogenotrophic denitrification in MBfR, the accomplishments so far have been focused on mainly reactor performance with little in-depth information. The impact of the nitrification reactor on the following denitrification reactor did not get much attention so far, even though there were controversial results about the role of

Jong Hyuk Hwang, Ph.D. Thesis

dissolved oxygen in autotrophic denitrification (Ho *et al.*, 2002; Cowman *et al.*, 2005). Carried-over components from the nitrification reactor such as NH_4-N or washed-out biomass would also affect the performance of the subsequent autotrophic denitrification reactor, which differentiates this option from stand-alone reactor operations.

2.2.4 Other applications – Category IV

19 papers focused on the applications of removing contaminants other than nitrogen. Oxidation of contaminants such as xylene (Debus *et al.*, 1994) and trichloroethylene (Clapp *et al.*, 1999; Edstrom *et al.*, 2005), was investigated in MBfR. Limited or partial aeration with MBfR could be applied to other biological system such as anaerobic bioreactors (Kappel *et al.*, 2005), and anaerobic ammonium (Annamox) oxidation (Gong *et al.*, 2008).

Hydrogen-based MBfR is capable of reducing many oxidized pollutants such as perchlorate (Nerenberg *et al.*, 2002), arsenate (Chung and Rittmann, 2006), selenate (Chung *et al.*, 2006), and hexavalent chromium (Chung *et al.*, 2006), which are difficult to remove in conventional biological processes.

Jong Hyuk Hwang, Ph.D. Thesis

2.3 Research needs

2.3.1 Biofilm control

Many researchers identified biofilm control as the most challenging aspect of operating an MBfR (Pankhania *et al.*, 1994, Semmens *et al.*, 2003; Celmer *et al.*, 2006). Excessive biofilm growth will not only cause non-uniform flow distribution and channelling, but also the inhibition of substrate or gas diffusion, eventually deteriorating the system performance. Some past studies suffered from biofilm overgrowth, which prevented long-term steady operation. Excessive biofilm growth tends to be more often found in the application for organic removal, which requires heterotrophic growth. Higher biomass yields of heterotrophic bacteria, combined with high performance of MBfR, contribute the excessive biofilm growth (Semmens *et al.*, 2003).

To ensure stable operation, the detachment or sloughing of biomass needs to be balanced with biomass accumulation. Daily backwashing, which consists of compressed air scouring and the complete replacement of the bulk liquid to remove the detached biomass, was required to prevent channelling and remove excessive biomass in MBfR, at a COD loading of 8.94 kg/m^{3}d (Pankhania *et al.*, 1994). The absence of backwashing for 6 days resulted in extensive biofilm growth and performance deterioration. Air scouring, coupled with bulk liquid replacement was beneficial, causing the increase of biofilm density and its adherence to the membrane

Jong Hyuk Hwang, Ph.D. Thesis

surface (Pankhania *et al.*, 1999, Brindle *et al.*, 1999). MBfR system with 5.1 kg COD/m^{3}d loading (lower than 8.94 kg COD/m^{3}d from above, Semmens *et al.*, 2003) and generous gas recycle (2 L/min) to mix the reactor and control biofilm, was able to maintain its performance for 3 months of operation. However, eventually the excessive biofilm growth inhibited the oxygen transfer and resulted in the development of anaerobic biomass. Increase in biofilm thickness also reduced the effective hydraulic retention time (HRT), resulting in the deterioration of reactor performance.

Excessive biofilm growth was also found in MBfR for nitrogen removal without organic oxidation, mainly due to overgrowth of heterotrophic denitrifiers, growing at the outer side of biofilm (Suzuki *et al.*, 2000). Interestingly, no excessive biofilm growth was reported in only nitrifying MBfR.

Hydrogen-based MBfR is vulnerable to mineral accumulation due to high local pH generated by high denitrification activity, as well as biomass overgrowth due to higher cell yield than nitrification. Calcium ions present in water and wastewater could precipitate with some anions, such as phosphate or carbonate, both of which are commonly used during the operation of MBfR for pH control or inorganic carbon supply (Lee and Rittmann, 2003).

Precipitated minerals associated with the biofilm could create a VS/TS ratio of biofilm lower than 0.25, indicating that the biofilm is carrying unnecessary solids (Celmer *et al.*, 2006). Increased shear force including faster mixing and nitrogen sparging could reduce the biofilm thickness, and then ensure higher performance

Jong Hyuk Hwang, Ph.D. Thesis

(Celmer *et al.*, 2008a). Ultrasound was also proven to be effective for biofilm control without damaging the membrane itself (Celmer *et al.*, 2008b). Excessive biofilm growth was also found during perchlorate removal using hydrogen (Padhye *et al.*, 2007).

2.3.2 Pilot- or full- scale operations

This sub-chapter addresses the general research needs for MBfR. Pilot- or full-scale operation of MBfRs was beyond the objectives of this thesis. Unfortunately, none of the journal papers reported the successful application of MBfR at demonstration or full-scale so far. Reactor sizes of lab-scale studies vary from 0.2 – 7 L, and most researchers used only one membrane module, while a large number of membrane modules would be implemented at demonstration or full-scale. Considering the short history of MBfR, it would be premature to expect full-scale MBfR applications. However, a few simulation studies have been conducted to foresee whether MBfR can be a promising alternative to previous technologies.

Thanks to higher biomass concentrations and efficient gas delivery, preliminary economic analysis reported that MBfR with pure oxygen would require less electricity for oxidation of designated amount of organics than conventional activated sludge systems or high purity oxygen activated sludge system (Syron and Casey, 2008).

Very recently, pilot-scale results started to appear backed by promising lab-scale application results. A 120 L active volume, pilot scale hybrid MBfR for nitrification

Jong Hyuk Hwang, Ph.D. Thesis

in the biofilm, and denitrification in the bulk liquid, was reported to achieve comparable performance as other full scale, attached growth nitrifying systems, such as IFAS (Integrated fixed film activated sludge). However, the reported nitrification rates were lower (0.35 g N/m^{2}d) in the pilot-scale system than those from bench scale study (up to 2 g N/m^{2}d), possibly due to challenges in biofilm control (Downing *et al.*, 2008). Biofilm control, which was widely reported as the main barrier to sustainable operation in laboratory scale experiments, would be expected to be more difficult in pilot- or full-scale systems, where more membrane modules are needed and higher packing densities can be expected. Although this study focused on lab-scale experiments, further studies should be conducted to assess whether MBfR can become a technically and commercially viable alternative to existing technologies. Pilot scale test by Applied Process Technology, Inc. also reported the problems related to biofilm overgrowth and mineral precipitation, as well as initial capital cost (Rittmann, 2007). Studies of reactor design considering biofilm control, membrane packing ratio and membrane replacement are an essential prerequisite for implementing MBfR in larger scale.

2.4 Objective of this research

Nitrification and autotrophic denitrification with hydrogen in MBfR were investigated in this study. Although heterotrophic denitrification can be performed simultaneously with nitrification in MBfR, depending on the DO and organic supply,

Jong Hyuk Hwang, Ph.D. Thesis

autotrophic denitrification was chosen due to its advantages explained previously. The drawbacks of hydrogenotrophic denitrification, previously mentioned, can be overcome in MBfR.

Objectives of this research were 1) to demonstrate feasibility of a stable and long-term operation of MBfRs for consecutive nitrification and autotrophic denitrification, 2) to investigate the effect of operational parameters such as DO, loading rate, temperature and mineral precipitation in separate operation or in a consecutive operation, 3) discuss the engineering challenges and perspectives of such a process.

Jong Hyuk Hwang, Ph.D. Thesis

3. GENERAL OBJECTIVES OF RESEARCH

The research comprised experimental preparation, operation of nitrification reactor, operation of denitrification reactor and operation of consecutive nitrification and denitrification reactors. Detailed timeframe of the studies undertaken is shown in Figure 3.1.1. Preparation in Figure 3.1.1 indicates the period when membranes were tested to decide their feasibility or applicability for nitrification. Feasibility for the transfer of hydrogen gas for autotrophic denitrification had been confirmed before by Celmer (2009). Two experimental objectives, the effect of loading rate and dissolved oxygen, were investigated during the operation of a stand-alone nitrifying MBfR after proper acclimation of biofilm. During the operation of the stand-alone denitrifying MBfR, the effect of mineral precipitation and temperature was investigated. Acclimation of denitrifying biofilm was frequently required to provide the working biofilm for consecutive nitrification and denitrification reactor, as the denitrification reactor stopped working due to improper control of biofilm overgrowth. During the operation of nitrification and subsequent denitrification reactor, which is the major part of this research, three research objectives were investigated including the effect of dissolved oxygen, loading rate and biofilm control. Since many previous research studies on MBfR have failed due to lack of appropriate biofilm control, this study pursued the proper biofilm control to achieve long-term stable operation of an MBfR system for total nitrogen removal by only autotrophic bacteria. Detailed research objectives, results and discussions during 27 months of experiments will be provided in following chapters.

Jong Hyuk Hwang, Ph.D. Thesis

Figure 3.1 Timeframe of whole experiments

Year	2007												2008												2009				
Month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5
PREPARATION			PP test				PMP test																						
NITRIFICATION												Accli.		Loading						DO									
DENITRIFICATION	A		Precipitation				pH						Acclimation								DO				Temp.				
NITRIFICATION - DENITRIFICATION																Loading			DO			Biofilm Control							

Accli., A and Acclimation mean the biofilm acclimation for subsequent research objectives.
Accli. : two nitrification reactors were acclimated for separate and consecutive nit-den. operations.
Acclimation : three denitrification reactors were prepared one by one for separate and consecutive nit-den. operation.

Jong Hyuk Hwang, Ph.D. Thesis

4. MATERIALS AND METHODS

This chapter describes the reactor configurations, membrane specification, influent characteristics and general outline of whole experiments performed for this study. Detailed materials and methods will be provided later in corresponding chapters about experimental results and discussions.

4.1 Reactor configurations

Two types of reactors were used, one modified 0.5 L graduate cylinder and the other custom cylindrical reactor, made by the technicians in Department of Biosystems, University of Manitoba.

Figure 4.1 shows the dimensions of 0.5 L modified polypropylene graduated cylinder. Total volume of the reactor is 580 mL and reactor volume to effluent outlet port is 555 mL. The true working volume when the membrane is submerged in the reactor is 500 mL (over 55 mL is occupied by membrane fibers and membrane module). Since there is not enough space for a magnetic bar at the bottom of the cylinder, internal recirculation was employed to provide reactor mixing. The liquid was taken from the bottom of the reactor and then recycled with the rate of, at least, 5 times of influent flow rate. The bottom end of the membrane module just is placed on the reactor bottom and the upper-end is fixed by a rubber cap. This type of reactor was used for nitrification since December, 2007.

Jong Hyuk Hwang, Ph.D. Thesis
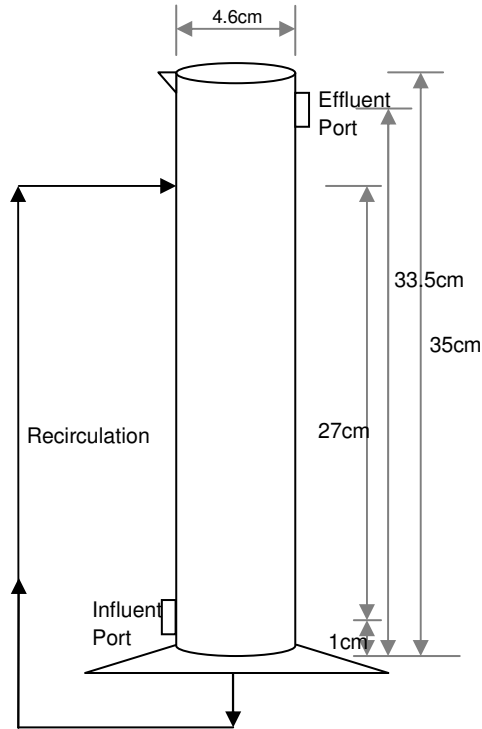
Figure 4.1 Dimensions for 0.5 L modified polypropylene graduated cylinder

Custom cylindrical reactors were manufactured in January, 2007 by the technicians in Department of Biosystems, University of Manitoba. Four reactors were made with acrylic glass and their detailed dimensions are shown in Figure 4.2. Total volume of the reactors was 2.01 L and reactor volume to effluent outlet port was 1.65 L. True working volume when the membrane was submerged in the reactor was 1.595L.

There were three holes on the top of the reactors for gas input to the membrane module and the other two holes for pH or DO probe. Spacing between two membrane supports (28 cm) is slightly shorter than membrane fiber length (30 cm) to provide free movement of membrane fibers. A thin glass plate was placed on the bottom of the inside of reactor to prevent the plastic surface from wearing out by magnetic bar.

Jong Hyuk Hwang, Ph.D. Thesis

Mechanical mixing by magnetic bar was provided for mixing. This type of reactor was used for nitrification and denitrification studies from March, 2007.
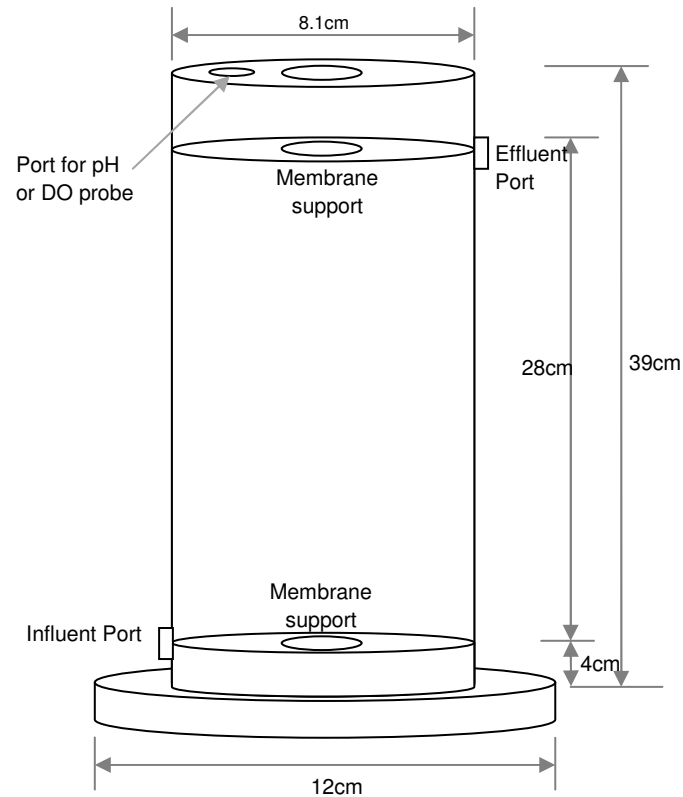
Figure 4.2 Dimensions for 1.6 L custom made reactor

4.2 Membrane characteristics

Membranes were provided by GE Water & Process Technologies (former ZENON Membrane Solutions) for research purpose. Two types of membrane, PP (Polypropylene) and PMP (Polymethylpentene), were used for the experiments. Gas transfer would occur only by diffusion due to non-porous characteristics of the membrane. Physical characteristics of membrane materials were shown in Table 4.1.

Jong Hyuk Hwang, Ph.D. Thesis

PP membrane had robustness but low diffusivity of gas, while the PMP membrane was weak but was able to deliver much more gas through the membrane fibers.

Table 4.1 Physical characteristics of two types of membranes used

		PP	PMP
Tensile Strength (MPa)		220	25.5
Elongation at break (%)		50	15
Diffusivity (mol/m·s·KPa)	H_2	13.48	Not available
	O_2	0.76	8.93
	N_2	0.13	2.24
	CO_2	2.70	31.52

The PP membrane modules were initially tested for nitrification and denitrification. The nitrification study using PP membrane modules was performed from February, 2007 to June 2007, but was not successful due to its inability to deliver oxygen (experimental results were shown in Chapter 5.1). Therefore, only PMP membrane modules were used for nitrification studies after July, 2007 when PMP membrane was first provided from GE.

The PP membrane was used for denitrification at the beginning of the experiment with the objective of investigating the effect of inorganic precipitation on hydrogenotrophic denitrification in MBfR. Although the experimental objective could be achieved, the specific denitrification rate was not comparable to other previous studies, due to low H_2 delivery rates. PP membrane modules were discarded in

Jong Hyuk Hwang, Ph.D. Thesis

December, 2007 and only PMP membrane modules were used for denitrification studies. Table 4.2 provides the details of membranes used for experiments.

Table 4.2 Membrane details

	PP	PMP
Outer diameter (μm)	60	80
Wall thickness (μm)	12	12
Fiber length (cm)	34.7	30
Number of fiber per Tow	48	48
Number of Tow	42	42
Surface area (m^2)	0.114	0.152
Specific surface area (m^2/m^3)	67000	50000

4.3 Influent characteristics

Different types of synthetic wastewater were used for nitrification and denitrification, as they need different form of nitrogen as a substrate. Synthetic wastewater representing non-nitrified secondary effluent from municipal wastewater treatment plants was fed into the bottom of the nitrification reactors or consecutive nitrification and denitrification reactors, and its composition throughout the whole experiments is shown in Table 4.3. Any changes from this composition for specific tests were addressed in respective chapters describing experimental results. Nitrogen concentration for nitrification was 34 mg N/L with sufficient alkalinity provided for complete nitrification. The concentration of each constituent was decided based on the literature and modified considering the influent nitrogen concentration (Head *et al.* 2004, Shin *et al.* 2005, Lee *et al.* 2003).

Jong Hyuk Hwang, Ph.D. Thesis

Table 4.3 Influent composition for the nitrification reactor

Chemical Constituent	Concentration (mg/L)
NH_4Cl	130 (34 as N)
$NaHCO_3$	600
Na_2CO_3	100
$MgSO_4 \cdot 7H_2O$	12.5
$ZnSO_4 \cdot 7H_2O$	1.25
$CaCl_2 \cdot 2H_2O$	4
$MnSO_4 \cdot H_2O$	5
$CoCl_2 \cdot 6H_2O$	0.3
$FeSO_4 \cdot 7H_2O$	2
$CuSO_4$	0.1
$Na_2MoO_4 \cdot 2H_2O$	0.35
KCl	7
K_2HPO_4	7.4

Table 4.4 Influent composition for the denitrification reactor

Chemical Constituent	Concentration (mg/L)
$NaNO_3$	200 (33 as N)
$NaHCO_3$	300
$MgSO_4 \cdot 7H_2O$	12.5
$ZnSO_4 \cdot 7H_2O$	1.25
$CaCl_2 \cdot 2H_2O$	4
$MnSO_4 \cdot H_2O$	5
$CoCl_2 \cdot 6H_2O$	0.3
$FeSO_4 \cdot 7H_2O$	2
$CuSO_4$	0.1
$Na_2MoO_4 \cdot 2H_2O$	0.35
KCl	7
K_2HPO_4	7.4

For stand-alone denitrification reactor operation, synthetic wastewater (See Table 4.4 for its composition), representing nitrified secondary effluent from municipal wastewater treatment plants or groundwater, was used as feed. Since tap water was used to prepare the feed, the actual concentration of minerals might be higher than

Jong Hyuk Hwang, Ph.D. Thesis

shown in Table 4.3 and 4.4. Stand-alone denitrification reactors were operated in order to investigate the effect of inorganic mineral precipitation and of the temperature on the performance of hydrogenotrophic denitrification in MBfR, as well as to prepare denitrifying biofilm for consecutive nitrification and denitrification.

All influent and effluent samples were filtered through a 0.45 µm glass-fiber filter before daily water quality analysis. Alkalinity (Standard Methods, APHA, 1998) and pH were measured. NH_4-N, NO_3-N and NO_2-N were also analyzed daily by flow injection analysis (LACHAT Quickchem 8500). DO inside the reactor was measured using a DO selective electrode (Hach SensION378). Suspended solids (SS) and volatile suspended solids (VSS) in the effluent were also measured twice a week (Standard Methods, APHA, 1998). Reactors were operated at room temperature, thus temperature inside of reactors was maintained at 20±1°C throughout the experimental period unless specific tests on temperature effects where conducted in environmental chambers.

4.4 Reactor operations

As addressed in Chapter 4.1, two types of reactors were used for this study. Hollow fiber membrane modules were submerged in the center of the reactors. Influent was fed into the bottom part of the reactor and the effluent was overflowed from the top part of the reactor. Necessary gas (Air or O_2 or H_2) was introduced to the top of the dead-end configuration membrane module. Air was used as an oxygen

Jong Hyuk Hwang, Ph.D. Thesis

source for the nitrification reactor in the early stages, but just pure oxygen was used

for nitrification due to oxygen transfer issues. H_2 was fed into the lumen of the

membrane fibers for denitrification. The configurations of reactor operation for two

types of reactors were shown in Figure 4.3. Applied gas pressure was up to 5 psi in

PP membrane and 2.5 psi in PMP membrane. Any modification to the reactor

configurations for specific experiments will be discussed later in respective chapters.

Investigation of specific goals, which will be addressed later, was conducted after

steady state operation with separate reactors was achieved to ensure the acclimation of

biofilm.
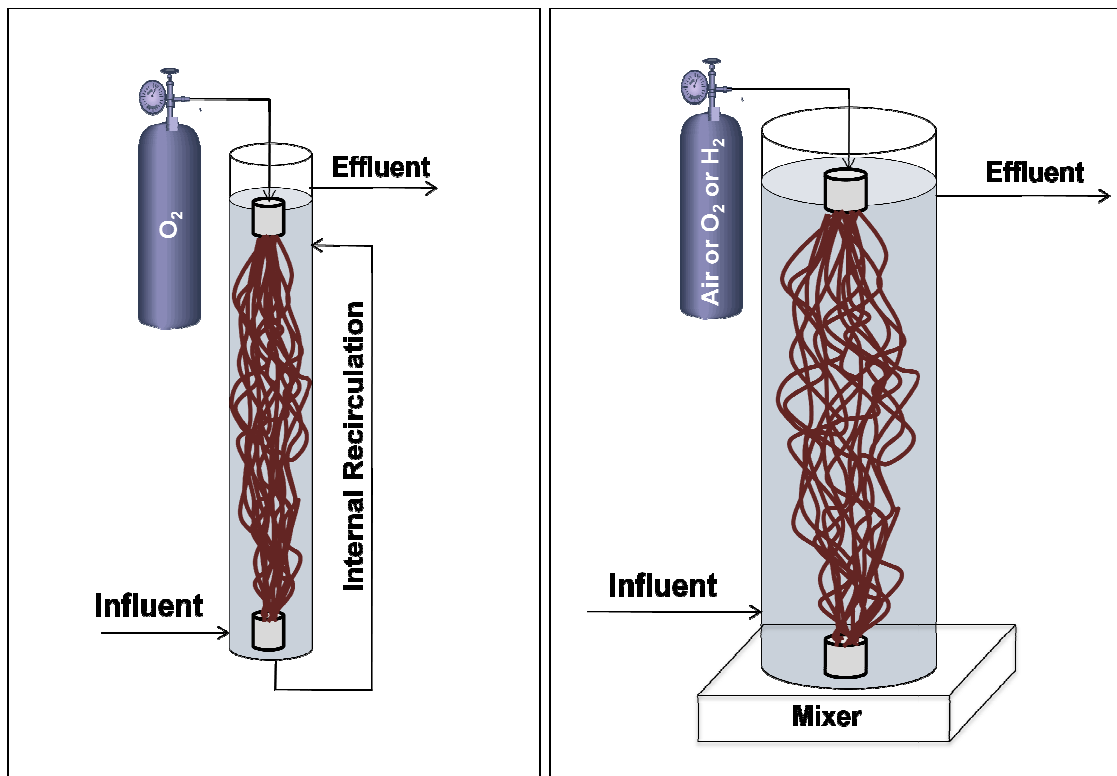
Figure 4.3 Reactor configurations for two types of reactors

Jong Hyuk Hwang, Ph.D. Thesis

5. STAND-ALONE NITRIFICATION REACTOR

This chapter includes all the experimental results and discussion from the operation of stand-alone nitrification reactors. It also includes the membrane tests for the applicability to nitrification.

5.1 Membrane feasibility test for nitrification

Membranes, provided by GE Water & Process Technologies, required testing for their feasibility for nitrification, as they had never been tested for nitrification before. The PP membrane module, which was initially provided for denitrification, was tested for nitrification starting February, 2007. Figure 5.1 shows the pictures of these reactors before seeding and after seeding.

Since feasibility of nitrification was not confirmed in PP membrane, lower loadings were attempted at the beginning. Two reactors with HRT of 7 hr and 12 hr were operated for approximately 4 months. NH_4-N concentration of incoming synthetic wastewater ranged from 10 mg N/L to 20 mg N/L. Initially, air was supplied as an oxygen source for nitrification. Gas pressure was 5 psi and DO concentration in the bulk was around 0.5 mg/L. Figure 5.2 shows the specific nitrification rates (SNR) from two reactors. SNR was calculated based on the NH_4-N concentration difference between influent and effluent, flowrates and provided surface area of membrane fibers for biofilm development.

Jong Hyuk Hwang, Ph.D. Thesis

$$\text{SNR} = \frac{((NH_4 - N_{inf}) - (NH_4 - N_{eff})) \times \text{flowrate}}{\text{surface area}}$$

Equation (5.1)

Average SNRs were 0.12 g N/m^2d from HRT 7 hr reactor and 0.123 g N/m^2d from HRT 12 hr reactor, showing no difference. Over 0.3 g N/m^2d at Day 30 was possible due to the leak of air. Moreover, SNRs from these two reactor operations were far below those reported in previous similar studies (Brindle *et al.*, 1998; Shin *et al.*, 2005; Terada *et al.*, 2006).
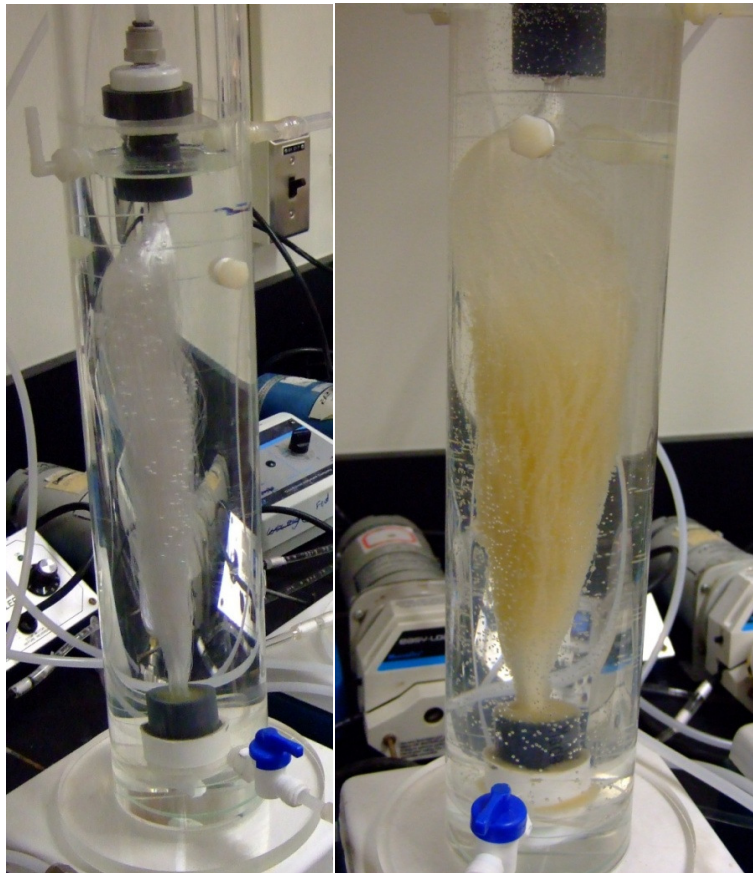
Figure 5.1 Pictures of the nitrification reactor before seeding (left) and after seeding (right)

Jong Hyuk Hwang, Ph.D. Thesis

Various attempts were made to find out the reason for the low nitrification performance, such as providing extra alkalinity (200 mg $CaCO_3$/L to 600 mg $CaCO_3$/L) although the sufficient alkalinity was provided, change of influent pH (from 7.5 to 8.7) favourable for nitrification, supplements of micro-minerals such as Cu, Mo and Co, the occasional seeding of new nitrifiers, and adding small amount of organics to facilitate initial attachment of nitrifiers to the membrane fibers. Without significant improvements in rates following the above attempts, it was concluded that the PP membrane fibers were not able to deliver sufficient oxygen to the biofilm.
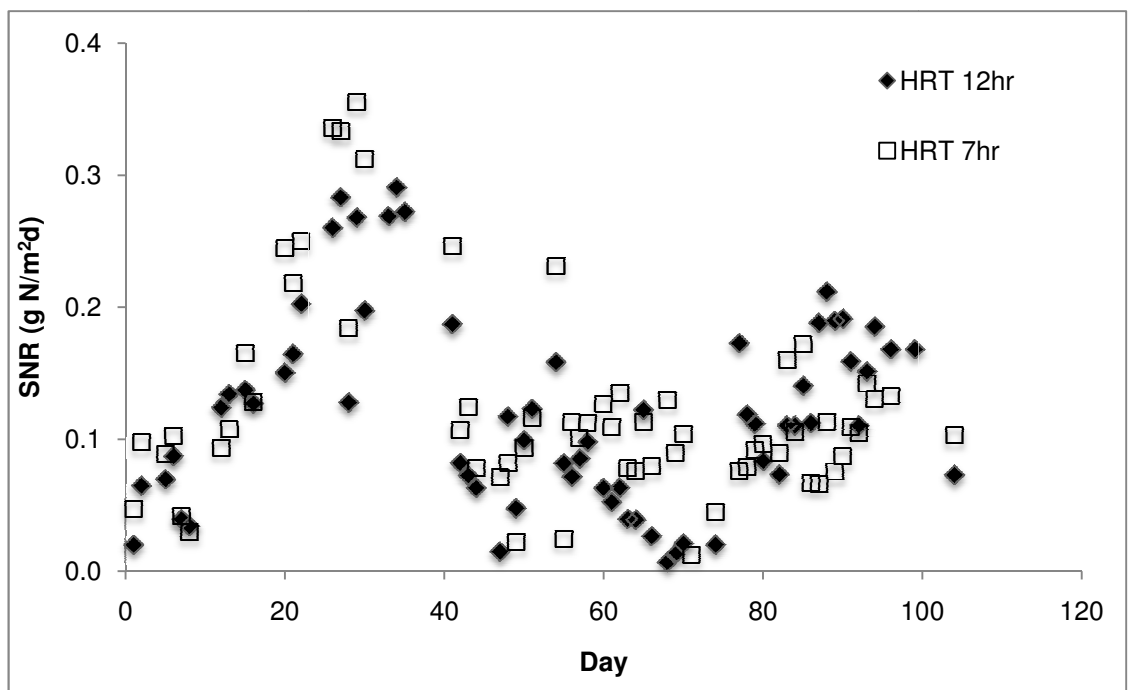
Figure 5.2 Specific nitrification rates during PP membrane test for nitrification

Jong Hyuk Hwang, Ph.D. Thesis

After communicating with GE Water & Process Technologies regarding the problems with nitrification, it was decided to test a new membrane module made with PMP fibers for nitrification. The nitrification tests using newly manufactured PMP membranes began after July, 2007. Figure 5.3 shows SNR from the PMP membrane test.

SNR of 0.2 g N/m^{2}d, which was higher than SNR by PP membrane before, was easily achieved in PMP membrane within 20 days of operation. Further attempt to improve nitrification performance by increasing the air supply failed due to the leak of air from the top of the membrane module. Therefore, the oxygen source was changed from air to pure oxygen starting day 26, as shown in Figure 5.3.
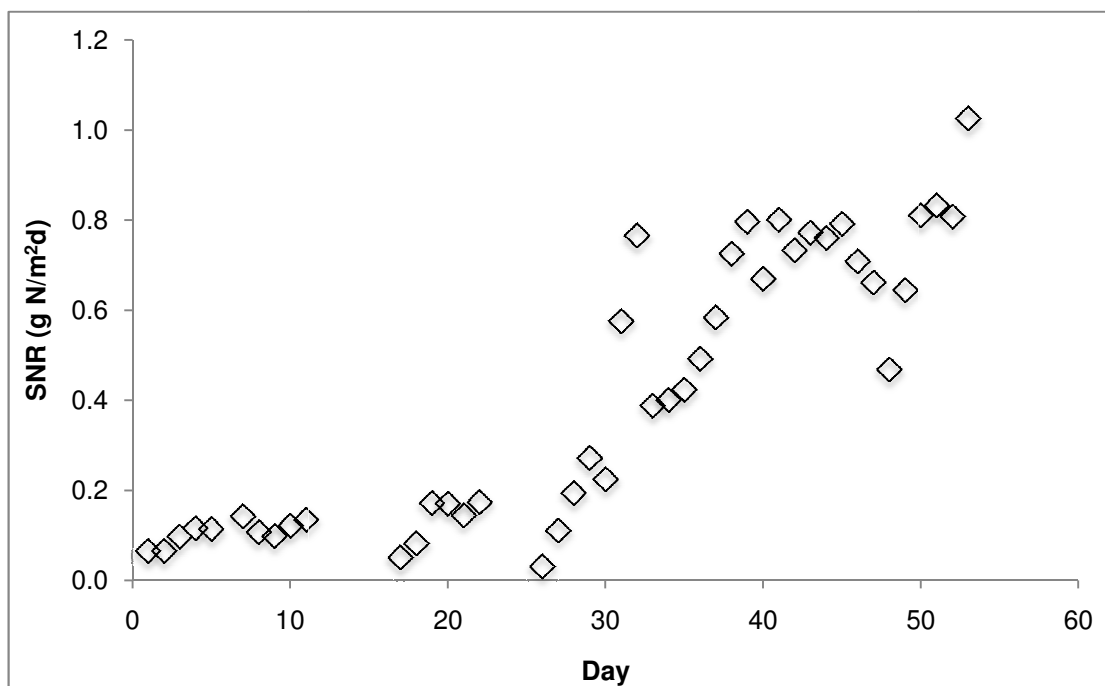
Figure 5.3 Specific nitrification rate during PMP membrane test for nitrification

Jong Hyuk Hwang, Ph.D. Thesis

Following the introduction of the pure oxygen, SNR increased up to over 0.8 g N/m^2d during an additional 30 days of operation. Although up to 1 g N/m^2d of SNR could be achieved, the membrane had to be replaced due to a leak, which was assumed to be caused by the testing of higher flowrate with air. Although SNR with PP membrane was not measured with pure oxygen supply, it would be expected to go up to 0.6 g N/m^2d, as the average was 0.12 g N/m^2d with air. And PMP membrane was able to achieve higher than 0.6 g N/m^2d with pure oxygen supply.

PMP membrane tests confirmed that low nitrification rate from PP membrane was caused by membrane material rather than experimental problems, and PMP membranes would be appropriate for the nitrification studies.

5.2 Acclimation of nitrification for future use

After preparing additional PMP membrane module for testing, one nitrifying reactor (0.5 L reactor volume) was prepared for future use as the first part of consecutive operation. This reactor was operated at an HRT of 1.4 hr for 4 months from December, 2007 to March, 2008, prior to the connection to a denitrification reactor. Shorter HRT than Chapter 5.1 was chosen because higher performance was confirmed with PMP membrane and pure oxygen supply. Figure 5.4 shows the picture of nitrification reactor with PMP membrane.

Figure 5.5 shows NH_4-N concentration of influent and effluent, and SNR. Influent NH_4-N concentration was varied from 27 mg N/L to 50 mg N/L in early 30 days to

Jong Hyuk Hwang, Ph.D. Thesis

find out optimal influent concentration for experimental purposes and was set to 32 mg N/L after 35 days of operation. Seeding of biomass was performed only once at the beginning of the experiment.

As shown in Figure 5.5, it took 40 days for biofilm development and system stabilization, and the reactor was stably working afterwards. Deterioration of performance due to excessive biomass growth or major detachment of biomass was not observed.

Figure 5.4 Pictures of the nitrification reactor (PMP)

Jong Hyuk Hwang, Ph.D. Thesis

Average SNR for the final 80 days was 1.62 g N/m^{2}d, volumetric nitrification rate was 0.492 kg N/m^{3}d, and the maximum SNR was 1.92 g N/m^{2}d. Since stable and high nitrification rate could be maintained, this reactor was considered as acclimated biofilm and then used as the first part for consecutive operation with the denitrification reactor. Detailed results and discussion from consecutive operation will be presented later.
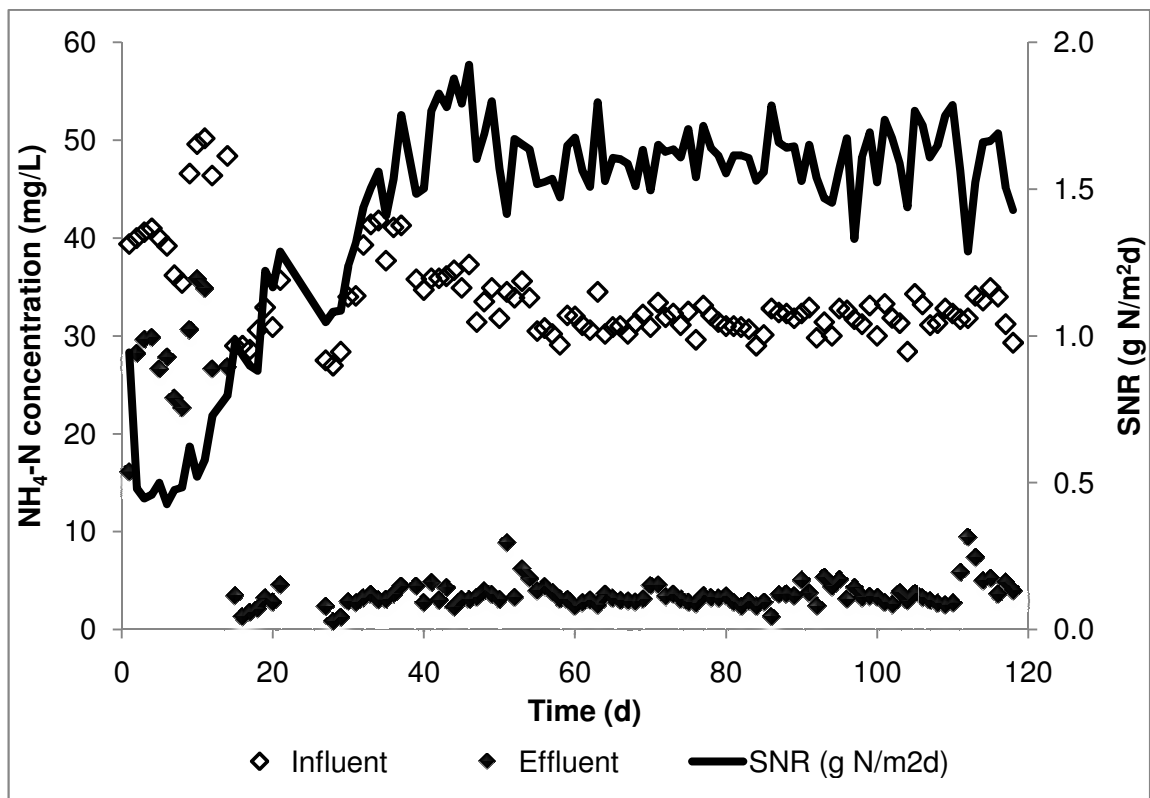
Figure 5.5 NH$_4$-N concentrations and SNR from acclimation stage

Jong Hyuk Hwang, Ph.D. Thesis

5.3 Effect of ammonia loading rate on nitrification

5.3.1 Objectives

In this study, the effect of ammonia loading rate on the nitrification efficiency in MBfR was investigated under oxygen-excess conditions, in order to select the optimum loading rate.

5.3.2 Materials and methods

The working volume, excluding the head space and volume occupied by the membrane and module, was 1.595 L. Detailed reactor configuration was provided in Chapter 3.1. The reactor was operated under five different loading conditions by adjusting the HRT, ranging from 1.4 hours to 3.9 hours. Each test lasted 3 weeks. The reactor was operated for over 100 days with different ammonia loading conditions. Synthetic wastewater was prepared, simulating non-nitrifying secondary wastewater treatment effluent. Gas pressure was 2.5 psi. Detailed composition was shown in Chapter 3.3. The reactor temperature was maintained at 21±1°C throughout the experimental period.

Jong Hyuk Hwang, Ph.D. Thesis

5.3.3 Results and discussion

Figure 5.6 shows the ammonia concentration of influent and effluent, and SNR with changing HRTs. Table 5.1 summarizes the operational conditions, SNRs and their standard deviations during the study.
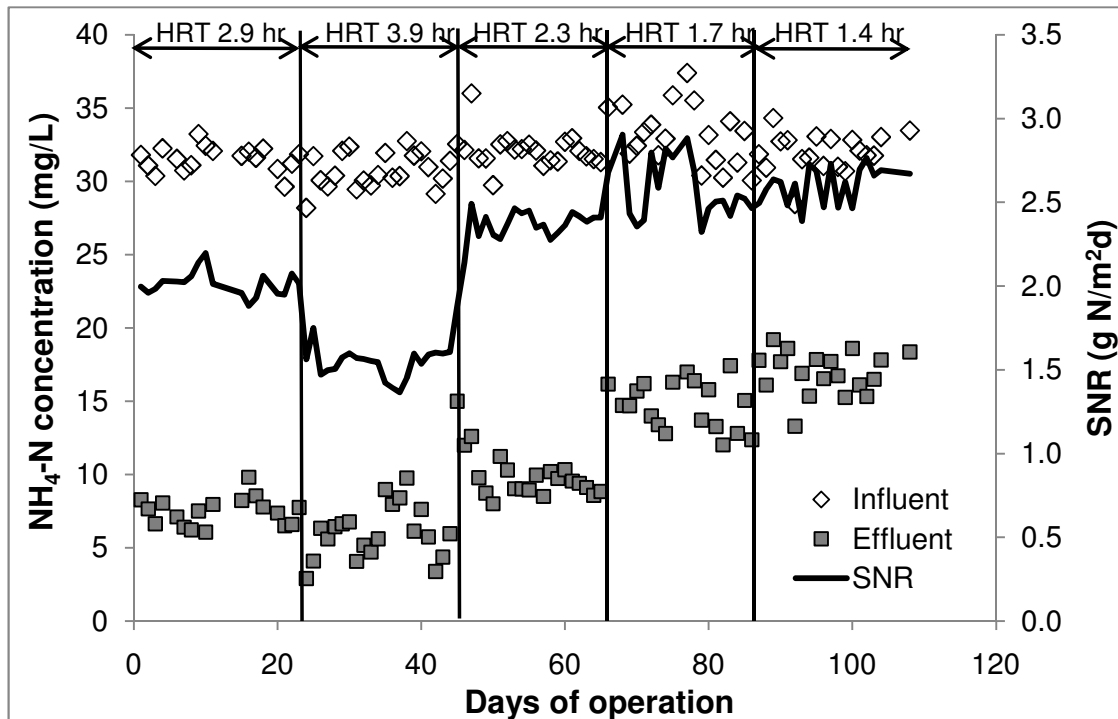
Figure 5.6 Nitrogen concentration and SNR in nitrifying MBfR

To investigate the relationship between specific NH_4-N loading rate and specific nitrification rate, Figure 5.7 was plotted showing the ratio between these two rates. All the experimental data used in this chapter were collected under oxygen excess conditions, as the bulk DO concentration was higher than 2 mg/L at any given time.

Jong Hyuk Hwang, Ph.D. Thesis

Table 5.1 Summary of operational conditions

Averages	Days	HRT	Influent NH$_4$-N (mg/L) and standard deviation	Effluent NH$_4$-N (mg/L) and standard deviation	SNR (g N/m^{2}d) and standard deviation
Days (1-23)	23	2.9	31.5 (0.8)	7.5 (1.0)	2.01 (0.08)
Days (24-44)	21	3.9	30.7 (1.2)	6.0 (1.8)	1.54 (0.09)
Days (45-65)	21	2.3	32.1 (1.2)	9.9 (1.6)	2.35 (0.13)
Days (66-86)	21	1.7	33.1 (2.1)	14.7 (1.7)	2.58 (0.18)
Days (87-108)	22	1.4	32.0 (1.3)	16.9 (1.5)	2.60 (0.11)
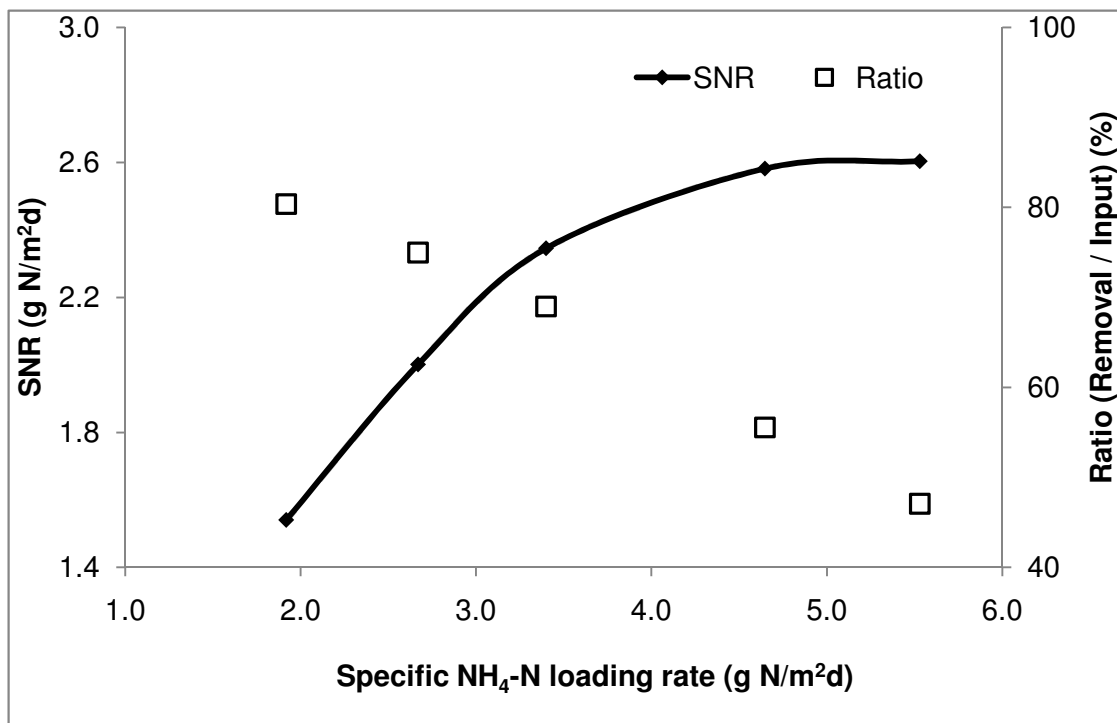
Figure 5.7 Relationship between loading rate and nitrification rate

As shown in Figure 5.7, SNR increased linearly with specific loading rate, up to around 3.5 g N/m^{2}d. Substrate diffusion limitation seemed to occur at loads above 3.5

Jong Hyuk Hwang, Ph.D. Thesis

g N/m^{2}d, due to the low HRT of 1.4 hours. Despite that, over 2 g NH$_4$-N/m^{2}d were nitrified, which is comparable or higher than reported in previous studies (Brindle *et al.*, 1998, Shin *et al.*, 2005, Cowman *et al.*, 2005).

Figure 5.8 illustrates the role of bulk NH$_4$-N concentration on the nitrification efficiency in MBfR. The trend in Figure 5.8 was almost identical to that in Figure 5.7, indicating that bulk NH$_4$-N concentration has a linear relationship with SNR when it was lower than 10 mg N/L.

$$SNR = \frac{4.01 \times (NH_4 - N)}{8.32 + (NH_4 - N)}$$

$$R^2 = 0.93$$
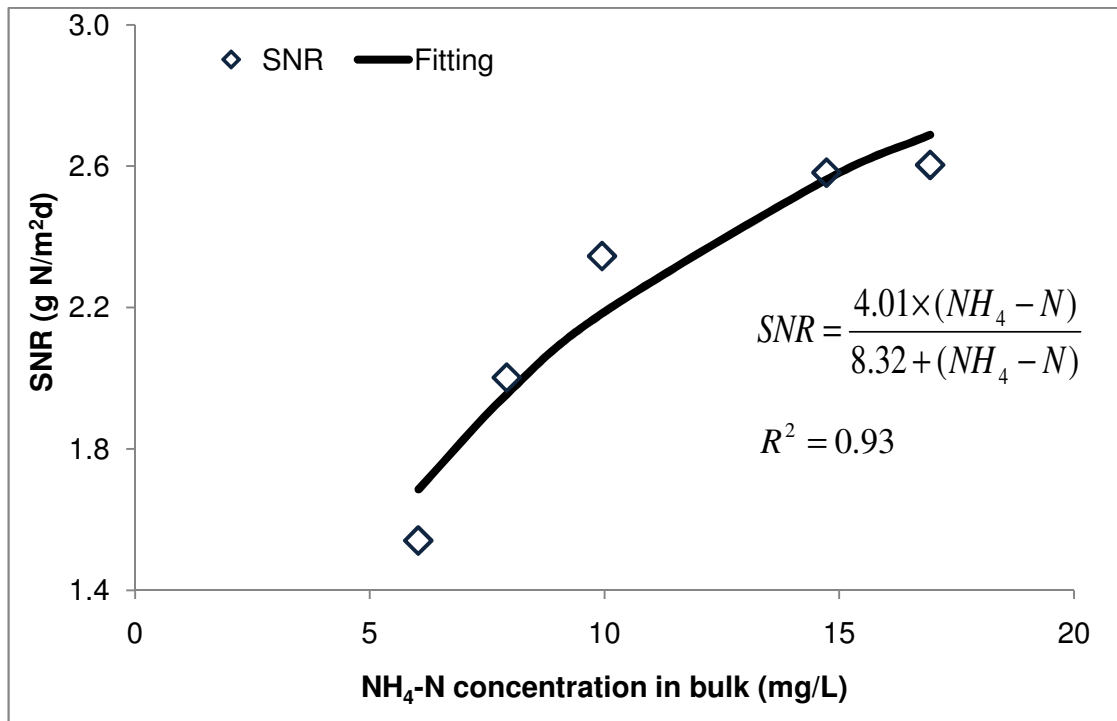
Figure 5.8 Relationship between bulk NH$_4$-N and SNR

Figure 5.8 demonstrates that in biofilm systems, the rate of mass transfer is linearly related to the substrate concentration gradient, and the rate of substrate utilization can be expressed by a saturation-type reaction (Equation (5.2), Metcalf & Eddy, 2003).

Jong Hyuk Hwang, Ph.D. Thesis

$$SNR = \frac{kX \times S_{NH4-N}}{K_S + S_{NH4-N}}$$
Equation (5.2)

The constants from the regression, based on the least-square method, were 4.01 for kX (maximum specific substrate utilization rate × biomass concentration) and 8.32 for Ks (half-velocity utilization coefficient). Figure 5.8 does not provide a clear fit with the experimental data which could be attributed to the changing biofilm thickness or density throughout the experiments.

5.3.4 Conclusions

Nitrifying MBfR was operated over 110 days. Effect of loading rate on the nitrification efficiency was investigated. Five different loading conditions were used by adjusting the HRT, ranging from 1.4 hours to 3.9 hours, which resulted in specific nitrification rate (SNR), ranging from 1.54 to 2.60 g N/m^{2}d. SNR increased linearly with the specific loading rate, up to approximately 3.5 g N/m^{2}d, as the rate of mass transfer is linearly related to the bulk ammonia concentration. However, beyond that, the substrate diffusion limitation inhibited further increase of SNR.

Jong Hyuk Hwang, Ph.D. Thesis

5.4 Effect of dissolved oxygen on nitrification

5.4.1 Introduction and specific objectives

Nitrification rates are affected by DO concentration in activated sludge systems. For nitrification in suspended biomass, DO concentration has little impact when exceeding 2 mg/L, but at low DO (<0.5 mg/L) inhibition effect has been shown to be greater for *Nitrobacter* than for *Nitrosomonas*, which can cause increased NO_2-N concentration in the effluent (MetCalf and Eddy, 2003).

In conventional biofilm processes, the limitation of DO is one of the most critical factors affecting nitrification performance (Zhang & Bishop, 1996; Lazarova *et al.*, 1998). This is more crucial when organic and ammonia oxidiation are simultaneously attempted, as heterotrophs would outperform autotrophs where DO is mostly present, and excessive oxygen supply might be required to deliver oxygen to the nitrifiers. It was reported that liquid DO above 5 mg/L should be maintained for stable nitrification efficiency in biofilm systems simultaneously oxidizing organic pollutants and ammonia nitrogen (Park *et al.*, 2008).

Substrate or oxygen transfer mechanisms in MBfR differ from those in conventional biofilm systems. One of the advantages of nitrifying MBfR is that nitrifers can easily utilize oxygen, with less competition from heterotrophic bacteria at the membrane surface, where oxygen is provided. The fraction of heterotrophs would still be higher even at the membrane surface, however the activity of nitrifiers would

Jong Hyuk Hwang, Ph.D. Thesis

be much higher than that of heterotrophs in the system removing organics and ammonia simultaneously (Shanahan & Semmens, 2004).

In this chapter, the effect of DO concentration, focusing on very low DO levels, was investigated. Almost 100% of oxygen utilization was possible without sacrificing nitrification efficiency in MBfR for nitrification (Brindle *et al.*, 1998). Nitrification efficiency was unaffected in a low bulk DO condition, at levels as low as 0.6 mg/L (Shin *et al.*, 2005), and Cowman *et al.* (2005) reported that nitrification was greatly inhibited by low O_2 pressure of 8.6 KPa (1.25 psi), due to the limited O_2 supply in the nitrification reactor.

The effect of oxygen supply was specifically investigated in this research in order to find out whether the nitrification would be possible under low DO conditions, as the effluent from the nitrification reactor was intended to be used as feed in the subsequent denitrification reactor.

5.4.2 Materials and methods

Experimental conditions were the same as in the previous Chapter 5.3 on the effect of loading rate. Reactor was operated over 80 days with 2.9 hours HRT in various bulk DO conditions. DO was measured using a DO selective electrode (Hach SensION378).

5.4.3 Results and discussion

Jong Hyuk Hwang, Ph.D. Thesis

Only limited amount of oxygen was supplied during the first 6 weeks of the experiment in order to keep DO concentration in the bulk liquid below 0.2 mg/L. Average SNR during this period was 1.08 g N/m^2d, which was lower than previous similar studies (Brindle *et al.*, 1998, Shin *et al.*, 2005, Cowman *et al.*, 2005). Low DO concentration indicates oxygen-limited conditions for nitrifiers at the outer (bulk liquid portion) of the biofilm, which caused low specific nitrification efficiency. As shown in Figure 5.9, a correlation between DO and nitrification efficiency can be observed at very low DO, ranging from 0.01 to 0.8 mg/L (oxygen-limiting conditions), while no noticeable difference of nitrification rate in DO ranging from 0.6 to 7.1 mg/L was observed (Shin *et al.*, 2005).

Low DO (< 0.5 mg/L) usually results in accumulation of nitrite by inhibiting the activity of nitrite oxidizing bacteria rather than ammonia oxidizing bacteria in suspended growth biomass (Metcalf and Eddy, 2003). The NO_2-N build-up was not observed in this study due to the different oxygen delivery mechanisms of MBfR. It is quite difficult to accurately determine whether the system is oxygen-limited by solely monitoring bulk DO. Having oxygen supplied from the membrane surface, DO concentration at the surface of membrane fiber could be higher than 3 mg/L, even though DO was depleted at the bulk liquid (Satoh *et al.*, 2004).

Jong Hyuk Hwang, Ph.D. Thesis
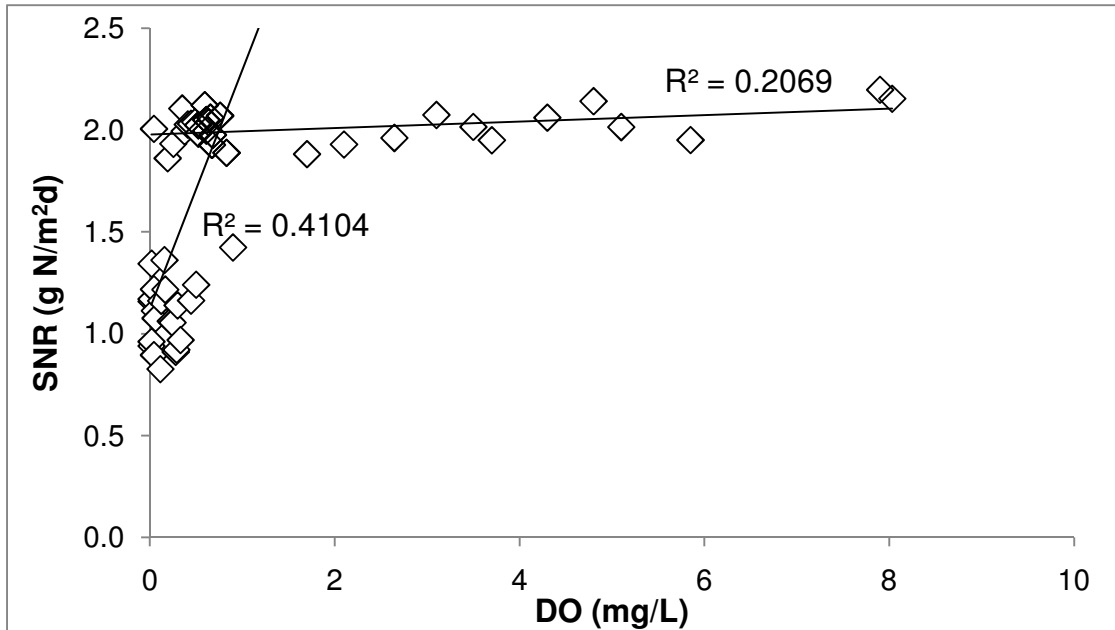
Figure 5.9 Relationship between bulk DO and SNR

During 43 to 84 days of operation, the oxygen supply was increased to achieve higher nitrification rates, and it was still possible to maintain DO concentration at less than 0.2 mg/L with improved SNR, which means that the oxygen uptake efficiency was 100% up to this point, as shown in Figure 5.9. Oxygen supply was increased further to obtain higher efficiency, however it only caused the increase of oxygen concentration in the bulk liquid, which generated large DO distribution at the SNR around 2 g NH_4-N/m^2d, as shown in Figure 5.9. High DO concentration means that oxygen was not a limiting factor during this period, so additional oxygen supply would not result in significantly higher nitrification efficiency. However, higher DO concentration in the bulk liquid would promote the growth of nitrifiers at the outer side of biofilm, where nitrifiers could not grow due to oxygen diffusion limitations under low DO conditions. Figure 5.9 shows the slight increase of nitrification rate

Jong Hyuk Hwang, Ph.D. Thesis

from 1.9 to 2.2 g N/m^2d in higher bulk DO conditions, which might indicate the presence of nitrifiers at the outer side of biofilm.

5.4.4 Conclusions

Effect of DO under limited-oxygen-supply conditions was investigated. Operating the system with 100% oxygen utilization was achievable, however the higher oxygen supply allowed for higher SNR due to the growth of nitrifiers at the outer side of biofilm.

5.5 Batch nitrification tests with effluent from the nitrification reactor

5.5.1 Introduction and specific objectives

Nitrification batch tests were performed in order to confirm the growth of nitrifiers at the outer side of biofilm when the diffusion of oxygen was limiting or sufficient. The solids escaping into the effluent originated and detached from the outer side of biofilm, therefore the measurement of nitrifiers from the edge of biofilm for these batch tests can be facilitated. This batch test was intended to provide evidence to why nitrification would be possible in the denitrification reactor when consecutive reactors were operated.

Jong Hyuk Hwang, Ph.D. Thesis

5.5.2 Materials and methods

The fractions of nitrifiers was estimated by the high F/M (food to microorganisms) protocol (WERF, 2003), which is usually used for the determination of maximum specific growth rate of nitrifiers. This test is to be performed with a relatively low concentration of nitrifying mixed liquor with spiked ammonia, which should be high enough to provide a high F/M condition for the maximum and exponential growth of nitrifiers. Ammonia nitrogen and alkalinity ($NaHCO_3$) adequate for full nitrification were added to 7 L of reactor effluent (which was collected overnight), mixed and aerated sufficiently until full oxidation of ammonia nitrogen was measured. pH was controlled to prevent pH drop and a nitrogen balance was checked by the analysis of each form of nitrogen components.

Initial NH_4-N concentration in the batch test was high enough (relative to the half-saturation coefficient) to ensure that the nitrification rate is at the maximum, as expressed in Equation (5.2).

$$\mu = \mu_{AUT} \frac{S_{NH}}{K_{NH}+S_{NH}} \approx \mu_{AUT} \qquad \text{Equation (5.2)}$$

Since the effluent suspended solids were as low as 1 mg/L and the concentration of initial ammonia nitrogen was at least 18 mg/L, the initial experimental conditions were similar to the experimental condition, proposed by high F/M method.

In the beginning of the test, the nitrite/nitrate production rate would be:

Jong Hyuk Hwang, Ph.D. Thesis

$$\frac{dS_{NO}}{dt} = \frac{\mu_{AUT}}{Y_{AUT}} \cdot X_{AUT} \qquad \text{Equation (5.3)}$$

And the change of biomass concentration, X_{AUT}, is the result of growth and decay of nitrifiers:

$$\frac{dX_{AUT}}{dt} = (\mu_{AUT} - b_{AUT}) \cdot dt \qquad \text{Equation (5.4)}$$

$$X_{AUT,t} = X_{AUT,0} \cdot e^{(\mu_{AUT} - b_{AUT})t} \qquad \text{Equation (5.5)}$$

Combining and integrating Equation (5.3) and (5.5) would yield:

$$S_{NOx,t} = S_{NOx,0} + \frac{\mu_{AUT} \times X_{AUT,0}}{Y_{AUT} \times (\mu_{AUT} - b_{AUT})} \times \left(e^{(\mu_{AUT} - b_{AUT})} - 1\right) \qquad \text{Equation (5.6)}$$

Exponential increase of nitrite/nitrate concentration in Equation (5.6) can be estimated by using initial nitrifier concentration ($X_{AUT,0}$) and nitrifiers' growth rates (μ_{AUT} and b_{AUT}).

Definitions and used values of each parameter in Equation (5.6) were shown in Table 5.2. $X_{AUT,0}$, the initial nitrifier concentration, was estimated using $S_{NOx,t}$ and $S_{NOx,0}$, measured from the batch tests, and Y_{AUT}, b_{AUT}, and μ_{AUT}, assumed from the simulation model, BIOWIN 3 (Envirosim Associates Ltd., 2008).

Jong Hyuk Hwang, Ph.D. Thesis

Table 5.2. Definitions and values in Equation (5.6)

Item	Definition	Value
$S_{NOx,t}$	oxidized nitrogen concentration at time t (mg/L)	From water quality analysis
$S_{NOx,0}$	oxidized nitrogen concentration at time zero (mg/L)	From water quality analysis
Y_{AUT}	nitrifier yield coefficient (unitless)	0.15
$X_{AUT,0}$	nitrifier concentration at time zero (mg/L)	Values to be estimated
b_{AUT}	nitrifier decay rate (/d)	0.17 @ 20°C
μ_{AUT}	maximum specific nitrifier growth rate (/d)	0.9 @ 20°C

Since no seeding of microorganisms was conducted, the growth of nitrifiers in the batch test was assumed to originate from the effluent solids, detached from the biofilm.

Tests were performed twice when the reactor was working on different DO conditions, less than 0.1 mg/L and higher than 7 mg/L.

5.5.3 Results and discussions

Figure 5.10 (a) and 5.10 (b) show the change of nitrogen components during the batch tests, performed using the effluents from nitrification reactor when operated under less than 0.1 mg O_2/L and higher than 7 mg O_2/L, respectively.

Two graphs show similar NO_x-N increase trends, except the initial NO_x-N, due to the different SNR when the effluents were taken. More than 3 days were required to achieve full nitrification due to low initial concentration of nitrifiers. The fitted curves, plotted in Figure 5.10 (a) and (b), were found using the initial concentration of

Jong Hyuk Hwang, Ph.D. Thesis

nitrifiers, calculated by the least-square method. Table 5.3 shows the results from both batch tests.

Effluent SS concentration was almost the same in both cases, however twice the amount of nitrifiers was found in the high DO case. Low bulk DO in the MBfR indicates that most of the nitrifiers exist on the surface of the membrane where oxygen is supplied, with decreasing population at the outer side of the biofilm. High bulk DO suggests that nitrifiers can exist at the outer side of biofilm, where both oxygen and substrate are abundant. Therefore, these results could explain the slightly higher SNR with increasing DO even at O_2 excess conditions as shown in Figure 5.9.

Table 5.3 Results from nitrification batch tests

DO concentration in nitrification reactor (mg/L)	0.1	7
Temperature during the test (°C)	23	24
SS in nitrification reactor effluent (mg/L)	1.7	1.72
VSS in nitrification reactor effluent (mg/L)	1.4	1.33
Nitrifier amount (mg/L)	0.094	0.19
Nitrifier fraction / SS (%)	5.5	11.0
Nitrifier fraction / VSS (%)	6.7	14.2

Even though there are no incoming organics for heterotrophic growth in the nitrification reactor, the results indicate the existence of heterotrophic bacteria in the effluent. Earlier studies have clearly shown that the existence of heterotrophs, growing on nitrifier decay products in autotrophic nitrifying biofilm (Kindaichi *et al.,* 2004; Okabe *et al*., 2005; Lackner *et al*., 2008).

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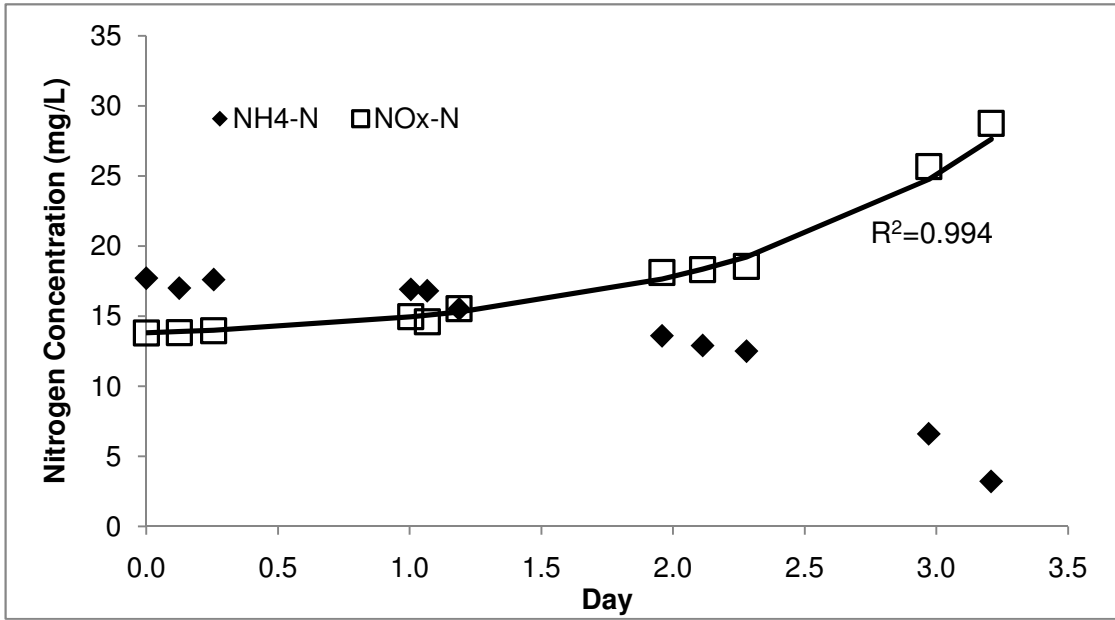
Figure 5.10 (a) Change of nitrogen components in batch test with low DO effluent
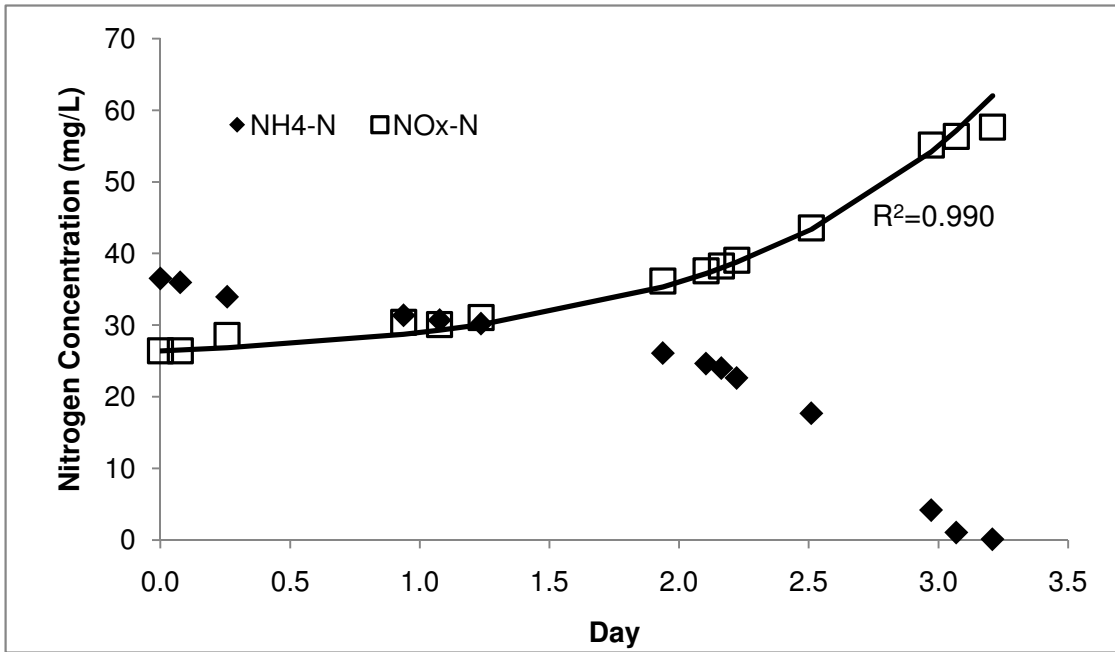
Figure 5.10 (b) Change of nitrogen components in batch test with high DO effluent

Jong Hyuk Hwang, Ph.D. Thesis

They have shown that up to 50% of the biomass can be heterotrophic in autotrophic biofilms without any external carbon sources through experimental and modeling studies. Autotrophic (Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria) fraction from autotrophic biofilm varies from 5% (out of VSS, Lackner *et al.*, 2008), 50% (out of active cell, Kindaichi *et al.* 2004), and 70% (out of active cell, Okabe *et al.*, 2005). The nitrifying bacteria fractions were found in this study ranging from 6.7% to 14.2%. The fraction was based on volatile solids rather than on active biomass, which was used in other studies. The results from other studies were from the biomass throughout the biofilm, while the nitrifying bacteria fractions in this study were measured with the effluent from the nitrification reactor, rather than the inside of the biofilm. In membrane-aerated nitrification systems, the nitrifying bacteria are most abundant on the surface of the membrane where the oxygen is supplied, and its fraction decreases with biofilm depth away from the membrane surface (Shanahan *et al.*, 2004).

5.5.4 Conclusions

The nitrifiers' fraction was measured to assess the existence of nitrifiers in the bulk liquid or outer portion of the biofilm. Fraction of nitrifiers, detached from the edge of biofilm and washed with the effluent, was twice as high when oxygen was over-supplied, compared to oxygen-limited condition.

Jong Hyuk Hwang, Ph.D. Thesis

5.6 Summary of nitrification reactor operation

Nitrification in MBfR has been tested since February, 2007. Initial unsuccessful nitrification performance due to inadequate selection of membrane material (polyprophylene, PP) for the oxygen delivery led to a change of membrane material (polymethylpentene, PMP), which eventually ensured adequate oxygen delivery. Specific nitrification rate, comparable or higher than in other previous studies, could be finally achieved by the implementation of PMP membranes.

Specific research objectives, investigated during the operation of nitrification reactor, included the effect of loading rate and DO, and assessing nitrifiers' presence in the effluent of the reactor.

Effect of loading rate on the nitrification efficiency was investigated under five different loading conditions by adjusting the HRT, ranging from 1.4 hours to 3.9 hours. SNR increased linearly with specific loading rate, up to approximately 3.5 g N/m^{2}d, as the rate of mass transfer is linearly related to the bulk ammonia concentration. However, beyond that, substrate diffusion limitation inhibited further increase of SNR. Effect of DO under limited-oxygen-supply conditions was also investigated. Operating the system with 100% oxygen utilization was achievable, however the higher oxygen supply allowed for higher SNR due to the growth of nitrifiers at the outer side of biofilm. Fraction of nitrifiers, detached from the edge of biofilm and washed with the effluent, was twice as high when oxygen was over-supplied, compared to oxygen-limited condition.

Jong Hyuk Hwang, Ph.D. Thesis

6. STAND-ALONE DENITRIFICATION

This chapter includes all the experimental results and discussion from the operation of stand-alone denitrification reactors. Unlike the operation of nitrification reactor, the membrane test was not required as its diffusivity of hydrogen was proven previously. Hence, the research was initiated after one month of biofilm acclimation. According to the timeframe in Figure 4.1.1, almost 10 months in 2008 were spent for biofilm acclimation due to the preparation of consecutive nitrification and denitrification reactor operations, and stand-alone denitrification operation for investigating the effect of DO and temperature. Since one of denitrification reactor failed during the consecutive operation, the reason of which was addressed in Chapter 7.2.3, longer acclimation periods were required than planned.

6.1 Effect of mineral precipitation

6.1.1 Introduction and objectives

Membrane fouling mechanisms in MBfR are different from those in conventional membrane bioreactors. In an MBfR, fouling is usually caused by the precipitation of minerals at the membrane surface due to local changes of pH or redox potential. Accumulation of mineral solids on the membrane surface can result in the decrease of gas and substrate transfer rates, leading to a reduction in overall system performance

Jong Hyuk Hwang, Ph.D. Thesis

(Semmens, 2007). For a system treating chlorinated solvents in groundwater using a hydrogen-driven MBfR, iron sulfide was reported to precipitate on the surface of the membrane, decreasing the gas transfer coefficient, even though the system had not failed under a given experimental condition (Roggy *et al.*, 2002).

To prevent prohibitively high pH and maintain the operating pH at an appropriate working range, carbon dioxide or phosphate buffer is commonly added. However, these buffers could induce the precipitation of calcium carbonate or calcium phosphate in the hydrogen-driven MBfR. Here are some of the relevant reactions responsible for the formation of these precipitants (Snoeyink and Jenkins, 1980).

$$CaCO_{3(s)} + 2H^+ \leftrightarrow Ca^{2+} + H_2CO_3 \qquad K_{SP}=2.2\times10^{-8} \qquad \text{Equation (6.1)}$$

$$Ca_3(PO_4)_2 \leftrightarrow 3Ca^{2+} + 2PO_4^{3-} \qquad K_{SP}=1\times10^{-27} \qquad \text{Equation (6.2)}$$

$$Ca_5(PO_4)_3OH_{(s)} \leftrightarrow 5Ca^{2+} + 3PO_4^{3-} +OH^- \qquad K_{SP}=8\times10^{-55} \qquad \text{Equation (6.3)}$$

Carbonate is known to compete and co-precipitate with phosphate to form calcium precipitants (Ferguson and McCarty, 1971). This indicates that the precipitation would be unavoidable, regardless of the type of buffer used in an MBfR for denitrification. Generally, phosphate precipitation would be more likely than carbonate precipitation, as the solubility constants of common phosphate precipitants are much smaller than those of carbonate precipitants. When both buffers were used for pH control in MBfR for H_2-driven denitrification, the inorganic contents stabilized at levels higher than 60% of total solids, although it decreased when CO_2 was used (Celmer *et al*, 2007). Chemical control of foulants (in order to dissolve precipitants)

Jong Hyuk Hwang, Ph.D. Thesis

using HCl or EDTA can be an option to prevent precipitation. Long-term operation of a denitrification reactor, dosed with 10 mg/L of EDTA to the influent, did not result in serious precipitation problem (Shin *et al.*, 2005). However, the use of chemical anti-scaling agents should be limited since usually it is poorly degradable in conventional wastewater treatment processes, and MBfR are usually considered as an effluent polishing or final treatment step option.

The effect of pH on the denitrification performance has not been clarified so far. Some studies have reported the optimum pH for hydrogenotrophic denitrification would be 8.4 (Lee and Rittmann, 2003) and 9.5 (Rezania *et al.*, 2006). Conversely, one study reported that denitrification was inhibited above pH 7.8 and that complete denitrification could only be achieved below pH 7.6 (Shin *et al.*, 2005).

The objective of this chapter was to investigate the effect of HRT and pH on the performance of an H_2-driven denitrifying MBfR, and address the extent of inorganic precipitation. The solids, scoured off from the membrane surface were quantified and identified, and possible mitigation strategies for reducing the inorganic content of the biofilm were investigated.

6.1.2 Materials and methods

Two denitrifying MBfR were operated with different loading conditions. The first reactor was operated at an HRT of 7 h for 170 days. The second reactor was operated at an HRT of 18 h for 90 days and then an HRT of 13.5 h for 60 days. Reactor configuration and operation were depicted in Chapter 3.1 and 3.4 – 1.6 L reactor. PP

Jong Hyuk Hwang, Ph.D. Thesis

membrane was used for this part of the study. Figure 6.1 shows the reactor and membrane with developed biofilm.

Figure 6.1 Picture of denitrification reactor with developed biofilm

Synthetic wastewater, simulating nitrified secondary effluent (Table 6.1), which was different from the composition shown in Table 3.4 due to the use of KH_2PO_4 and K_2HPO_4 as a pH buffer, was fed during the operation of the MBfR.

Jong Hyuk Hwang, Ph.D. Thesis

Accumulation of solids on the membrane surface due to precipitation of inorganic matter and growth of biofilm was expected during operation. Once the deterioration of denitrification performance was detected, the membranes were scoured to remove solids, loosely attached to the fibers, on a regular basis by mechanical vibration. The sloughed off solids were collected and analyzed for inorganic and organic content and particular inorganic precipitants were identified using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Varian Liberty 200) analysis.

Scouring was performed at Day 37, 51, 71, 87, 106, 126, 142, 155, 170 (HRT 7 h), and Day 19, 39, 55, 68, 83 (HRT 13.5 h) and Day 37, 51, 71, 87 (HRT 18 h).

Table 6.1 Influent composition for denitrification reactor

Chemical Constituent	Concentration (mg/L)
$NaNO_3$	185 (30 as N)
KH_2PO_4	325
K_2HPO_4	225
$NaHCO_3$	300
$MgSO_4 \cdot 7H_2O$	12.5
$ZnSO_4 \cdot 7H_2O$	1.25
$CaCl_2 \cdot 2H_2O$	4
$MnSO_4 \cdot H_2O$	5
$CoCl_2 \cdot 6H_2O$	0.3
$FeSO_4 \cdot 7H_2O$	2
$CuSO_4$	0.1
$Na_2MoO_4 \cdot 2H_2O$	0.35
KCl	7

Jong Hyuk Hwang, Ph.D. Thesis

6.1.3 Results and discussions

1) Effect of HRT

Figure 6.2 shows the average specific denitrification rate (SDNR) from three different HRT periods. SDNR was calculated based on the concentration change between the influent and effluent nitrogen, and the associated membrane surface area for biofilm growth (Equation (6.4).

$$SDNR = \frac{((NO_X-N_{inf})-(NO_X-N_{eff}))\times flowrate}{surface\ area} \qquad Equation\ (6.4)$$

Figure 6.2 Effect of HRT

Jong Hyuk Hwang, Ph.D. Thesis
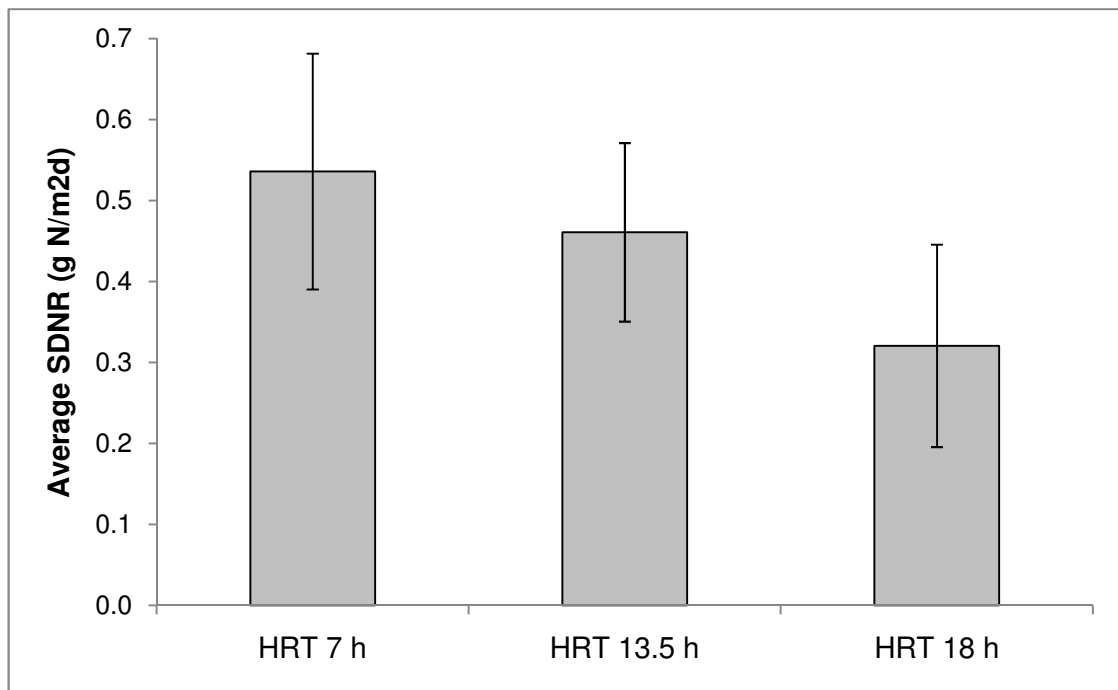
Average SDNR was 0.54, 0.46 and 0.32 g N/m^{2}d at HRT of 7 h (170 days of operation), 13.5 h (60 days of operation) and 18 h (90 days of operation), respectively. Maximum SDNRs, observed from each HRT case, were 0.84, 0.71 and 0.61 g N/m^{2}d at HRT of 7 h, 13.5 h and 18 h, respectively. High standard deviation values were obtained due to highly fluctuating denitrification performance.   Denitrification performance from this experiment was lower than reported earlier (Terada *et al.*, 2006, Ergas and Reuss, 2001), possibly due to the effect of mineral precipitation.

Average SDNR increased with decreasing HRT, as shown in Figure 6.2. As mentioned earlier, diffusion of substrate would be the major driving force of pollutants removal in MBfR. High NO_3-N concentration in bulk with lower HRT would promote more diffusion into biofilm, thus resulting in higher removal rate. Additionally, added introduction of buffer with influent in the case of lower HRT would suppress pH increase, which is favorable to mineral precipitation. Average pH in bulk solution was 7.5, 7.8 and 7.95, at HRT of 7 h, 13.5 h and 18 h, respectively, however the pH within the biofilm is expected to be higher than that in the bulk solution.

Despite the increasing SDNR with decreasing HRT, due to extensive accumulation of solids within the biofilm (which was clearly visible during the experiments), SDNR values were highly scattered in every HRT case, as can be seen from the standard deviations in Figure 6.2.

2) Effect of pH

Jong Hyuk Hwang, Ph.D. Thesis

Results shown in Figure 6.2 would suggest that higher pH would be unfavourable in terms of system performance due to mineral precipitation, and higher NO_2-N accumulation was observed under high pH conditions, as shown in Figure 6.3.

These results led to the investigation of the effect of pH on the reactor performance by controlling pH through directly adding phosphate buffer at designated pH levels to the reactor.
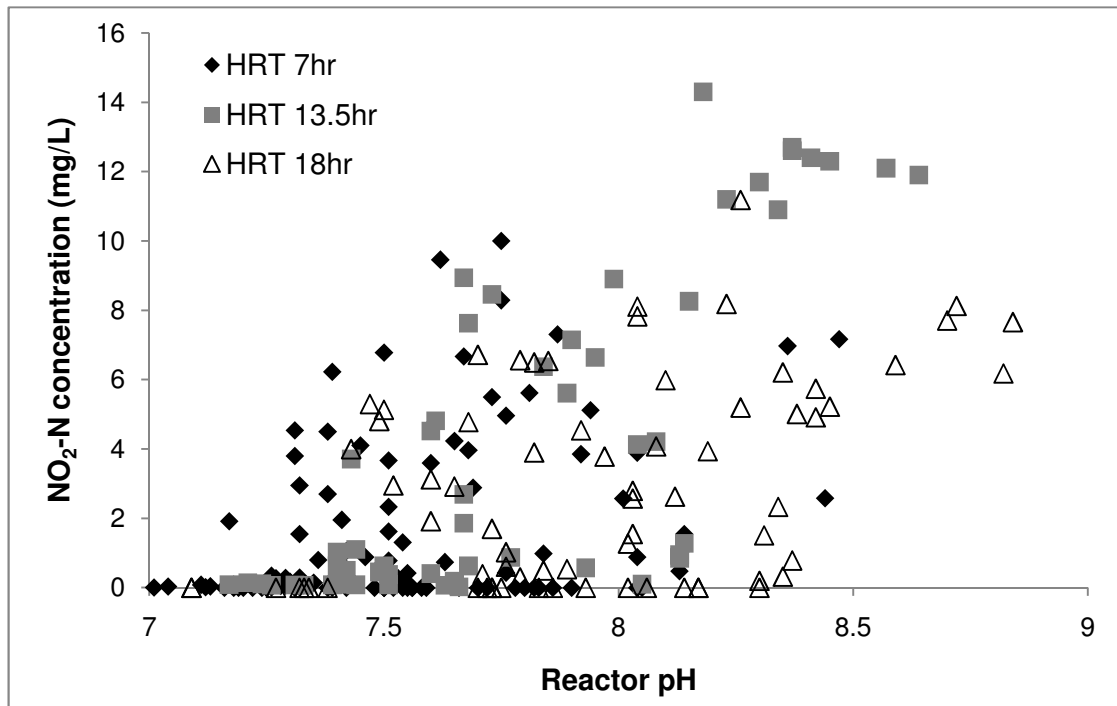
Figure 6.3 Effluent NO_2-N concentration vs. reactor pH

Two pH conditions were tested in two reactors with different HRT. pH was maintained at 7.5 for the first 20 days and then maintained at pH 8 for the next 20 days. Shorter HRT (2.7 h and 5.4 h) were tested in order to maximize the effect of phosphate buffer addition. pH was controlled by adding phosphate buffer and buffer

Jong Hyuk Hwang, Ph.D. Thesis

consumption was monitored. The tests with the pH control could not be maintained for extended periods due to the deteriorating performance over time caused by extensive accumulation of solids. Figure 6.4 shows SDNRs obtained from the two reactors and their phosphate buffer consumption to maintain the designated pH levels.

As shown in Figure 6.4, the buffer consumption was dependent upon SDNR, as pH increase would be caused by denitrification activity. Performance worsened over time in both cases as extensive introduction of phosphate buffer for pH adjustment accelerated the precipitation of calcium phosphate.
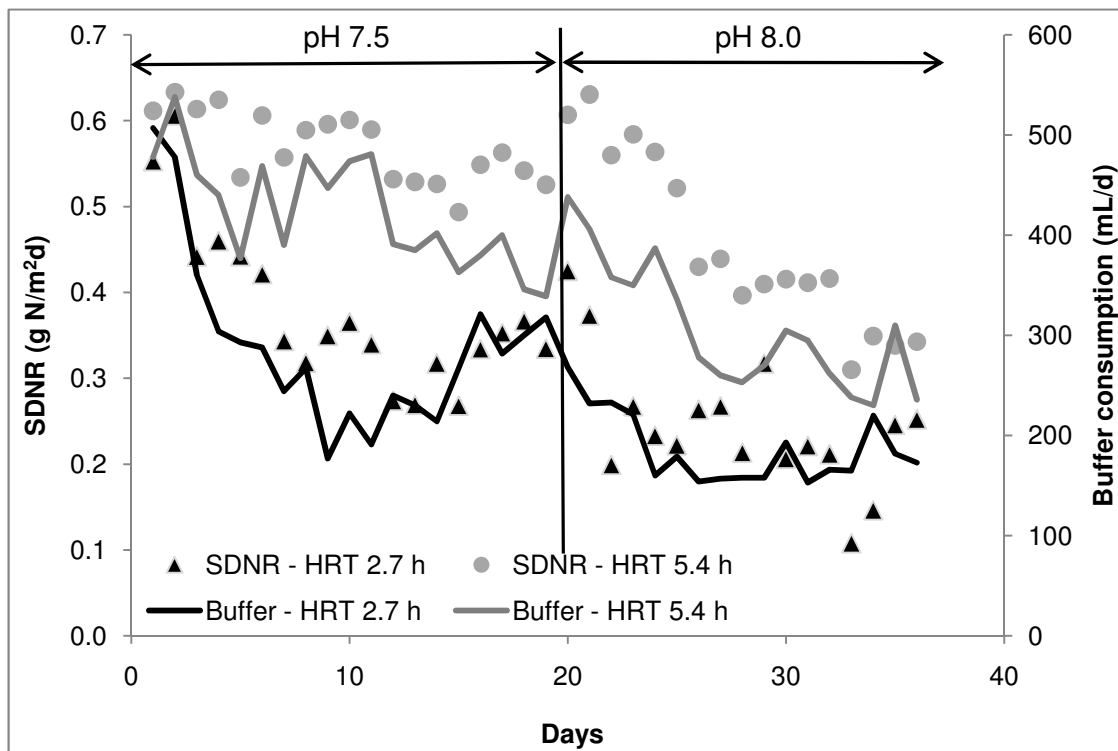
Figure 6.4 SDNR and buffer consumption

3) Membrane scouring

Jong Hyuk Hwang, Ph.D. Thesis

Since solids accumulation was assumed to be the main reason for the deterioration of performance, solids attached to the membrane fibers were collected and tested. The frequency of the mechanical scouring of the membrane surface was initially based on the drop in SDNR and implemented regularly thereafter. Response to the scouring was instantaneous, as SDNR jumped up to 220%, compared to that before scouring. Figure 6.5 shows the typical response of denitrification performance to membrane scouring in the reactor operated at HRT of 13.5 h. Removing excessive solids from the biofilm resulted in an immediate increase of SDNR, however continuing solids accumulation resulted in abrupt decreases of SDNR, resulting in high fluctuations of performance. These abrupt drops of SDNR were attributed to the precipitated minerals, which limit substrate and gas transfer efficiencies.
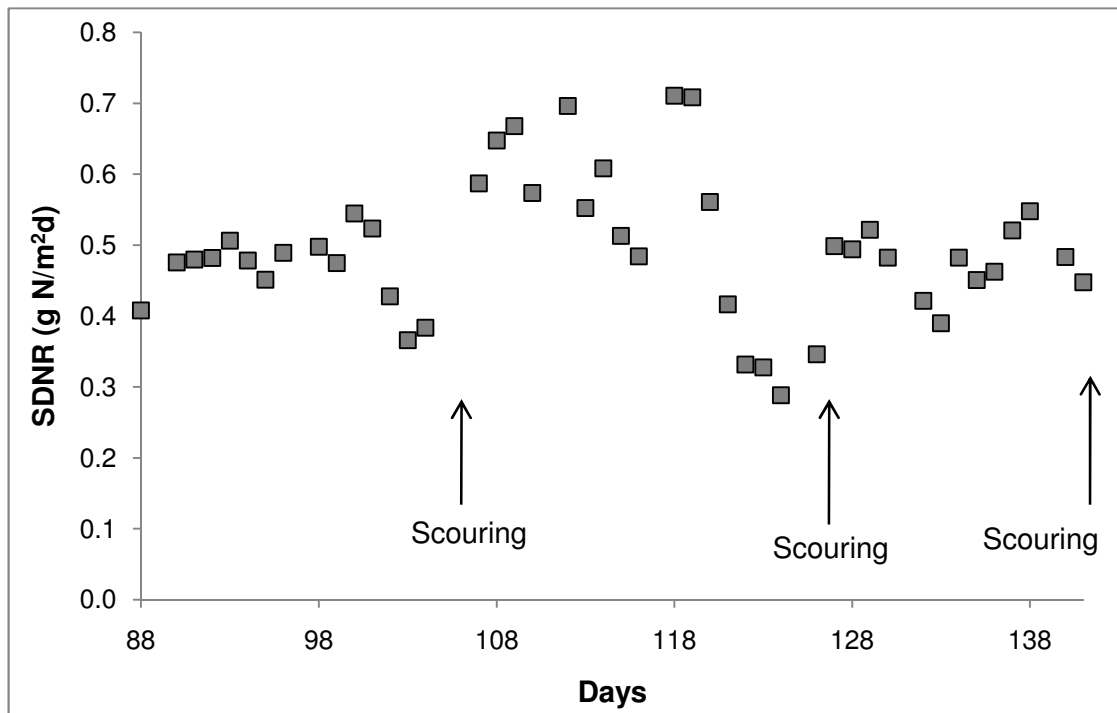
Figure 6.5 Increase of SDNR after each scouring

Jong Hyuk Hwang, Ph.D. Thesis
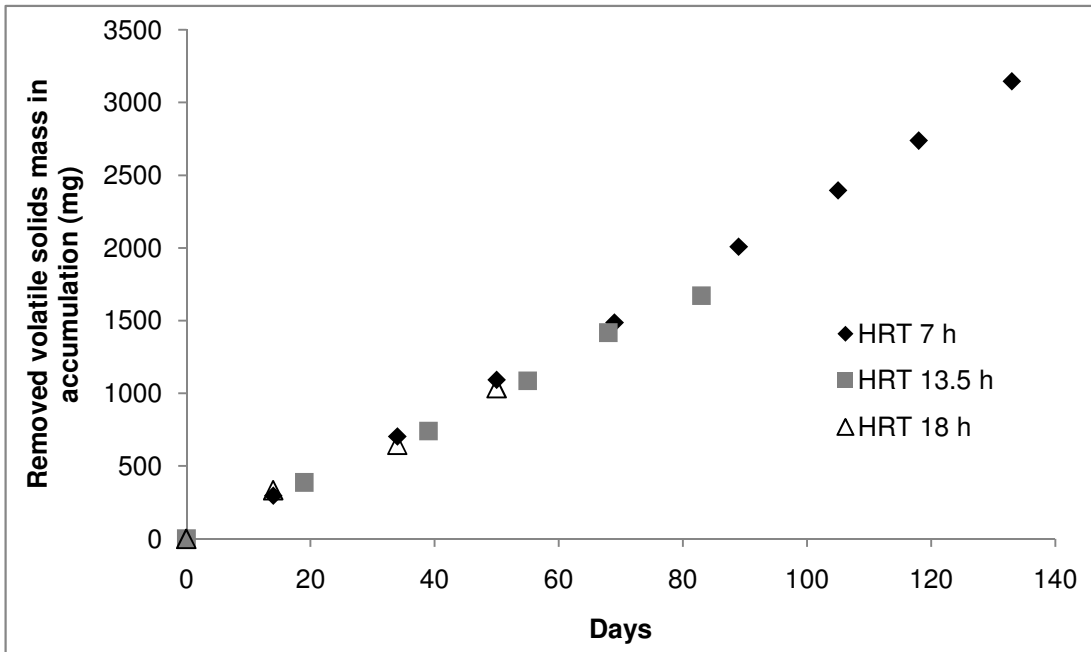
(a) Cumulative volatile solids mass
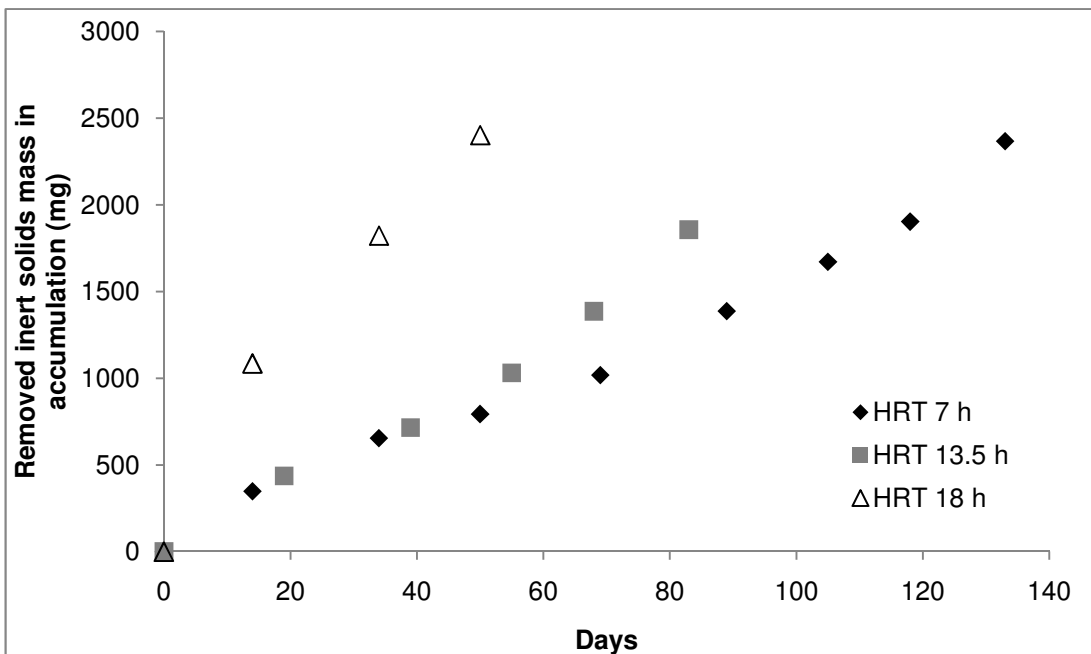
(b) Cumulative inert solids mass

Figure 6.6 Accumulated solids mass from membrane scouring

Jong Hyuk Hwang, Ph.D. Thesis

The sloughed off solids from the scouring operation were collected and analyzed for volatile and inert components. Since the solids concentrations in the influent and effluent were below detectable levels, the sloughed-off solids from the membrane surface were considered to be generated during the long-term operation between scouring. Figure 6.6 shows net volatile and inert solids generations for each tested HRT.

The accumulation of volatile solids at the examined HRTs showed a linear trend, indicating the exerted strength of membrane cleaning was fairly consistent although it was performed manually. Rates of volatile solids accumulation were 23.2 mg/d (HRT 7 h), 20.8 mg/d (HRT 13.5 h) and 20.7 mg/d (HRT 18h).

The theoretical yield coefficient for autotrophic denitrification can be calculated as 0.23 g cell/g N from the stoichiometric relationship shown in Equation (6.5). Similar true yield of 0.27 mg active cell/mg N has been reported (Rezania *et al.*, 2006).

$$NO_3^- + H^+ + 2.86H_2 + 0.15CO_2 \rightarrow 0.0286C_5H_7NO_2 + 0.49N_2 + 3.14H_2O$$
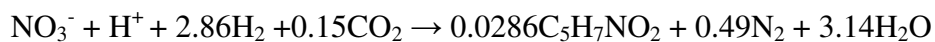
<div align="right">Equation (6.5)</div>

Observed yield, which can be calculated from the VSS generated and nitrogen reduced, should be higher than the theoretical yield as volatile suspended solids include active biomass, cell debris, extracellular polymeric substances (EPS) and possible trapped organics in the microbial aggregates. Reported observed yields for

Jong Hyuk Hwang, Ph.D. Thesis

autotrophic denitrification were 1.2 g VSS/g N (Benedict, 1996) and 0.78 g VSS/ g N (Pierkiel, 2002). Even though the daily volatile solids generation rate was higher at lower HRT, the observed yields, based on solids accumulation and denitrification rate from this study were 0.38 g VSS/g N (HRT 7 h), 0.40 g VSS/g N (HRT 13.5 h) and 0.57 g VSS/g N (HRT 18h).

If the fraction of cell synthesis is governed by the theoretical yield coefficient and the fraction of cell debris is linearly related with cell mass, solids at higher HRTs (and lower SDNR) can be expected have higher EPS contents. As the membrane surface area is constant, higher EPS (considered a foulant) in the biofilm attached to the membrane would result in performance deterioration normalized to the surface area.

As for the inorganic accumulation, at higher HRTs (especially HRT of 18 hrs), consistently more inorganic solids were generated than at lower HRTs, which was attributed to higher pH conditions. Mineral precipitation would increase at higher pH, as the driving force of hydroxyapatite ($Ca_5(OH)(PO_4)_3$) precipitation, which is the most common form of calcium phosphate precipitation, and its co-precipitation product with carbonate ($Ca_{10}(PO_4)_6(CO_3)_{0.5}(OH)$), would also increase (Lu and Leng, 2005). Added phosphate buffer at high pH due to denitrification activity would result in extensive mineral precipitation, thus the use of phosphate buffer for pH control in the autotrophic denitrification system should be avoided.

4) Effect of accumulation of inert matter on SDNR

Since hydrogen gas diffuses from the membrane surface in an MBfR, any deposit other than active biomass on the membrane surface would limit the gas and substrate

Jong Hyuk Hwang, Ph.D. Thesis

transfer rates. As the inert solids fraction from removed solids by membrane scouring ranges from 26% to 76%, the relationship between SDNR and inert solids fraction can be established as shown in Figure 6.7. SDNR values used in this graph were the average values between each membrane scouring. The error bars in Figure 6.7 represent the standard deviations in the measured data.
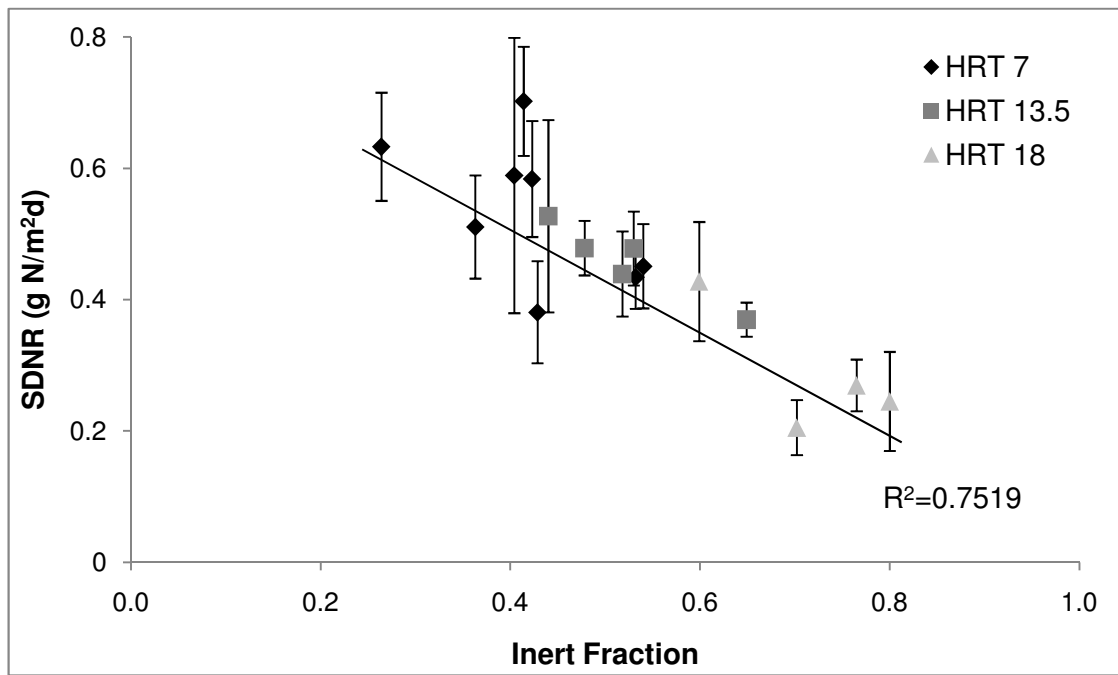
Figure 6.7 Relationship between inert fraction and SDNR

Even though many parameters may inter-act with each other and affect the system performance of an MBfR, SDNR appears to be significantly affected by inorganic precipitation. This indicates that inert precipitation is a major reason for inhibiting the transfer of gas or substrate in MBfR operation. Figure 6.8 shows the micro-photograph of inorganic precipitation, which covers the membrane surface and then

Jong Hyuk Hwang, Ph.D. Thesis

reduces the active surface area for hydrogen delivery. Measures, such as adjusting the operational parameters or controlling biofilm structure should therefore be taken to avoid or reduce the generation of inorganic precipitants in order to maintain a consistent system performance.
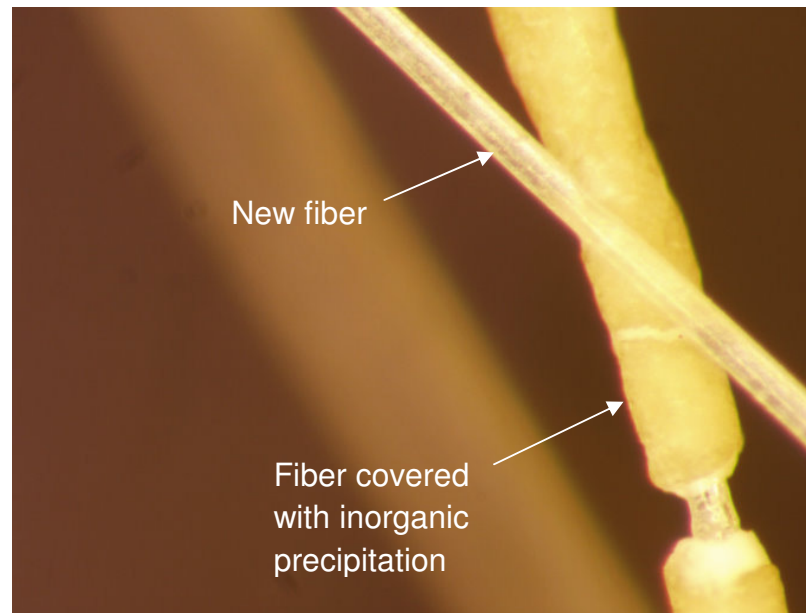
Figure 6.8 Microscopic picture of new and covered membrane fiber with inorganic precipitants.

5) Identification of sloughed-off solids

The sloughed-off biofilm solids were analyzed by ICP-OES to identify the precipitants. The relationship between weight fractions of calcium and phosphorus, and the inert fraction of solids is shown in Figure 6.9.

The abundance of Ca and P, compared to Mg, Fe and S (insignificant, data not shown) indicates that the calcium-based precipitants are dominant in the reactors.

Jong Hyuk Hwang, Ph.D. Thesis

Weight fraction of phosphorous ranged from 6% to 13% of removed solids mass. Considering 1% organic phosphorous in average biomass by weight, it can be suggested that most of P in the sloughed-off solids are in an inorganic form.
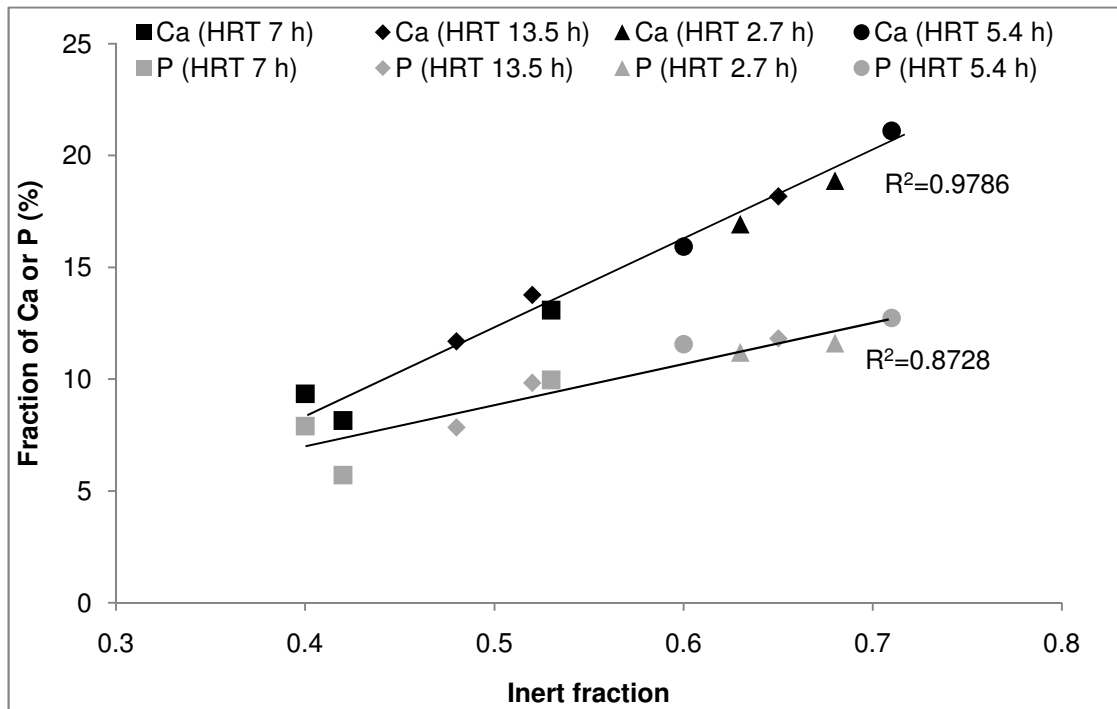
Figure 6.9 Inert fraction vs. Ca and P from sloughed-off solids

Weight fraction of calcium and phosphorous in sloughed-off solids were linearly related with the inert fraction from sloughed-off solids, suggesting that the calcium phosphate component was the major precipitant. Sum of weight fraction of calcium and phosphate comprised around 50% of the inert fraction. The statistical fit between calcium and inert fraction was better than that between P and the inert fraction as calcium results include possible calcium carbonate precipitants. Lower P/Ca ratio in

Jong Hyuk Hwang, Ph.D. Thesis

higher inert fraction also indicated that more calcium-based precipitants would be probable in higher inert fraction.

The form of calcium phosphate could be estimated based on the slopes of calcium and phosphate, shown in Figure 6.9, as the increase of inert fraction indicates the increase of certain forms of calcium phosphate. Among the three common forms of calcium phosphate ($Ca_5(OH)(PO_4)_3$, $Ca_3(PO_4)_2$, $CaPO_4$, $CaHPO_4$, CaH_2PO_4), the form removed in this study, would be $Ca_5(OH)(PO_4)_3$ (Hydroxyapatite), the most common form of calcium phosphate as P/Ca ratio (0.477) from the slopes of Figure 6.9 was very close to that (0.462) of hydroxyapatite.

6.1.4 Conclusions

The effect of inorganic precipitation on the performance of H_2-driven MBfR for autotrophic denitrification was investigated. Operating reactors at different HRTs revealed that shorter HRT led to higher denitrification rates due to better pH maintenance at higher flow rates. The performance of reactors with pH control using phosphate buffer did not improve due to extensive precipitation of calcium phosphate. The membrane was cleaned periodically to investigate the solids contents generated in the system. The reactor with long HRT generated more inert solids than the reactor with short HRT and had exhibited a lower specific denitrification rates (SDNR), even though the generation of volatile solids was almost identical in both cases. Inorganic precipitation was strongly and linearly related with specific denitrification rate, which indicates that inert precipitation is a major cause for inhibiting the transfer of gas or

Jong Hyuk Hwang, Ph.D. Thesis

substrate in an MBfR. Characterization of sloughed-off solids revealed an abundance of calcium phosphate, which showed good relationship with inert solids generation.

6.2 Effect of dissolved oxygen

6.2.1 Introduction and specific objectives

Dissolved oxygen (DO) is one of the major operating parameters for biological nitrogen removal processes. It could be an especially important operating parameter for hydrogenotrophic denitrification because it is usually applied to groundwater treatment, which contains a significant amount of oxygen, or to wastewater treatment trains after nitrification where oxygen is sufficiently supplied.

Even though some studies reported denitrification under aerobic conditions with specific microorganisms such as *Alcaligenes faecalis* (Joo *et al.*, 2005), *Thiosphaera pantotropha* (Dalsgaard *et al.*, 1995; Robertson *et al.*, 1995), most of the reported denitrification studies were conducted by facultative anaerobes in the absence of oxygen, because organisms capable of performing aerobic denitrification are out-competed in natural environments. Inhibition of heterotrophic denitrification with the presence of oxygen is extensively reported (Tiedje, 1988; Henze *et al.*, 1995; Oh and Silverstein, 1999).

Jong Hyuk Hwang, Ph.D. Thesis

So far, the studies regarding the effect of DO on hydrogenotrophic denitrification reported controversial and conflicting results. Over-supply of hydrogen was required to remove DO that adversely affected hydrogenotrophic denitrification (Kurt *et al.*, 1987; Gross *et al.* 1986). Ho *et al.* (2002) reported reduced denitrification performance in saturated DO condition from their denitrification batch tests under various DO conditions using PVA (Polyvinyl alcohol)-immobilized biofilms on a silicone tube. Cowman *et al.* (2005) operated consecutive nitrification and hydrogenotrophic denitrification in MBfR by changing O_2 and H_2 pressures and observed the increase of nitrate concentration in the final effluent due to the carry-over of excess oxygen, caused by higher O_2 pressure.

However, Schnobrich *et al.* (2007) conducted a field-scale column study for treating groundwater containing high DO and nitrate. Even though DO concentration in their feed was over 5.5 mg/L, complete denitrification could be achieved. Sahu *et al.* (2007) reported that the presence of DO in the influent did not adversely affect the denitrification rate in a bench-scale MBfR.

Despite conflicting reports on the effect of oxygen in literature, a shared observation was that hydrogenotrophic denitrification was not completely inhibited by the presence of oxygen as was observed in heterotrophic denitrification. Since past studies were mostly intended for investigating the performance of hydrogenotrophic denitrification in various situations rather than focusing on the effect of oxygen, the effect of oxygen on hydrogenotrophic denitrification has not been fully elucidated.

Jong Hyuk Hwang, Ph.D. Thesis

The objective of this chapter was to investigate the effect of oxygen on hydrogenotrophic denitrification in MBfR. Two separate MBfR systems for consecutive nitrification-denitrification and separate denitrification were operated under various DO conditions. This chapter describes the results and discussion from the operation of a stand-alone denitrification reactor.

6.2.2 Materials and methods

Denitrification reactor was inoculated with 200 mL of removed biomass from a lab-scale MBfR for separate hydrogenotrophic denitrification study. Reactor was acclimated over 90 days for biofilm development.

Working volume of reactor was 1.6 L (HRT 3.81 h) and its configuration was shown in Figure 6.10, slightly different from Figure 3.4. PP membrane was no longer used for denitrification due to its low diffusivity, and PMP membrane was used, because it was proven that PMP membrane had the ability to deliver more gas. The composition of synthetic wastewater is shown in Table 3.4.

During 100 days of operation, various DO conditions were tested. From day 1 to day 14, the DO in the influent and reactor was not controlled. From day 15 to day 54, air was introduced via a diffuser inside the reactor in order to manually increase the DO level of the reactor (as shown in Figure 6.10), however the flow rate of air was carefully controlled not to scour off existing biofilm.

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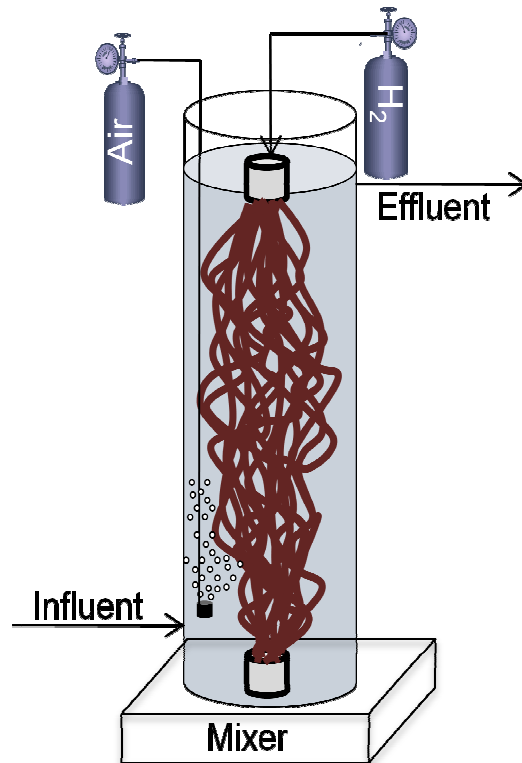
Figure 6.10 Reactor configurations

From day 55 to day 81, influent was sparged with nitrogen gas to remove DO from the influent. From day 82 to day 95, DO was not controlled again. From day 96 to day 100, air was introduced to the reactor once more to increase the DO concentration.

6.2.3 Results and discussions

Table 6.2 shows the DO entering and DO inside the reactor and the SDNR corresponding to the applied DO control measures.

Jong Hyuk Hwang, Ph.D. Thesis

Table 6.2 DO entering and DO inside the reactor and SDNR

Days	Feed DO (mg/L)	Reactor DO (mg/L)	SDNR (g N/m²d)	DO control
Day 1-14	8.6	0.4	0.82	None
Day 15-54	6.2	4.9	0.87	Reactor aeration
Day 55-81	0.7	0.0	0.86	Feed N_2 addition
Day 82-95	6.5	0.8	0.87	None
Day 96-100	4.6	1.6	0.87	Reactor aeration

An average reactor DO concentration of 4.9 mg/L could be maintained by reactor aeration, and all of the DO entering the reactor could be removed by feed N_2 addition. Identical DO control strategies to the first two stages were tested during Day 82-95 and Day 96-100, respectively, to evaluate the reproducibility of denitrification performance under changing DO conditions. Figure 6.11 shows the relationship between SDNR and incoming DO(a), reactor DO(b).

The change of DO neither favourably nor adversely affected the hydrogenotrophic denitrification as can be seen in Table 6.2 and Figure 6.11. During day 82-100 of operation, when the previous DO condition was replicated, no considerable differences were observed. Therefore, it can be concluded that the effect of DO on hydrogenotrophic denitrification in stand-alone operation was negligible. Detailed discussion regarding the effect of DO on hydrogenotrophic denitrification will be addressed in the subsequent Chapter 7.3 with results from the operation of consecutive nitrification and denitrification reactors.

Jong Hyuk Hwang, Ph.D. Thesis
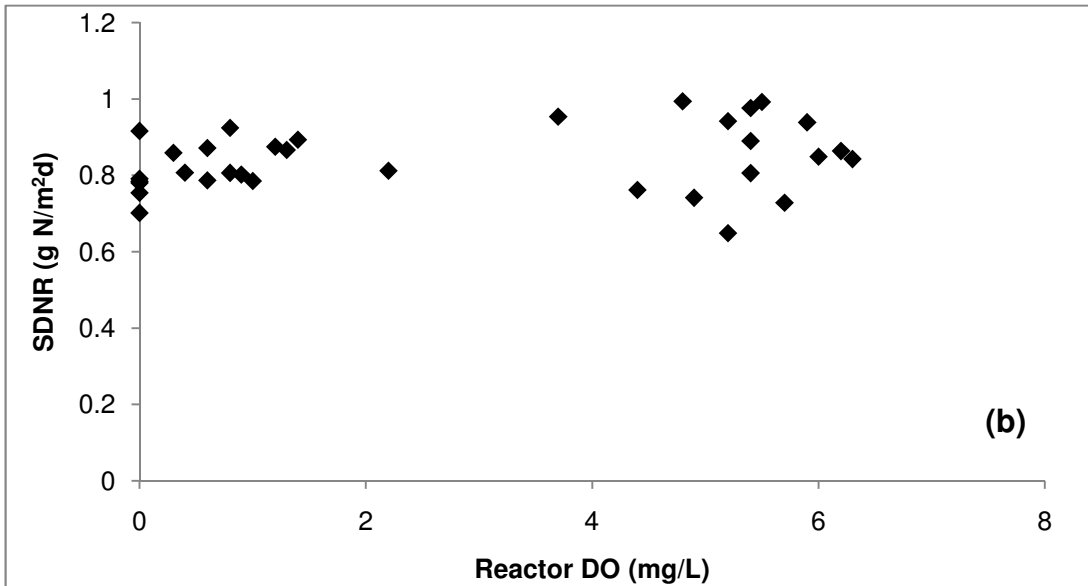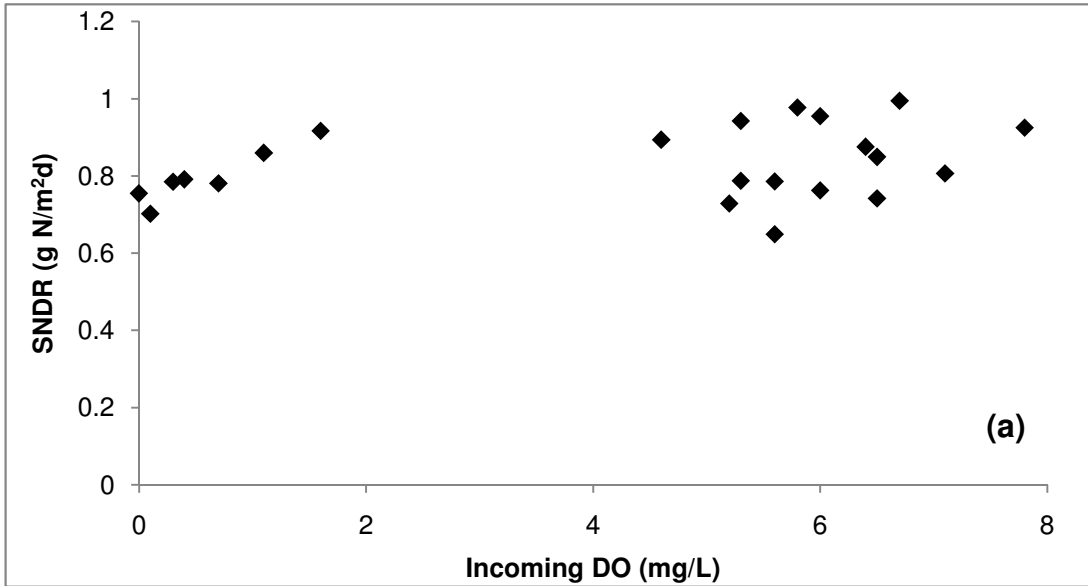
Figure 6.11 Relationship between SDNR and incoming DO (a), reactor DO (b)

6.2.4 Conclusions

Jong Hyuk Hwang, Ph.D. Thesis

The effect of oxygen on hydrogenotrophic denitrification in MBfR was investigated. One stand-alone denitrification reactor, representing the system treating non-nitrified secondary effluent from domestic wastewater treatment plants, and groundwater or nitrified secondary effluent from domestic wastewater treatment plants, was operated under various DO conditions.

A wide range of DO was tested in the stand-alone denitrification reactor. Aeration was used to increase the level of DO while nitrogen scouring was used to remove any oxygen from the influent. From the results of 100 days of operation under a variety of DO conditions (ranging from 0 to 4.9 mg O_2/L in the reactor), no noticeable difference of specific denitrification rate between various DO conditions was found. It was concluded that the hydrogenotrophic denitrification was not affected by the presence of DO in our MBfR system.

6.3 Effect of temperature

6.3.1 Introduction and specific objectives

Temperature is one of the major influencing factors in biological wastewater treatment. The effect of temperature on the reaction rate of biological processes can be expressed by the Arrhenius equation – shown below – with each type of organism, e.g. carbon oxidizing heterotrophs, nitrifiers, or denitrifiers having their own

Jong Hyuk Hwang, Ph.D. Thesis

temperature correction factor (Tchobanoglous, Burton, and Stensel, 2003; Oleszkiewicz and Berquist, 1988).

$$\mu_T = \mu_{\max,20^0 C} \times \theta^{T-20}$$
<div align="right">Equation (6.5)</div>

Nitrification is known as the most temperature-sensitive step among the biological processes in wastewater treatment. The temperature correction factor for nitrifiers' maximum growth rate ranging from 1.072 to 1.127 have been found historically, however 1.072 is recently accepted for designing wastewater treatment plants (WERF, 2003; Painter and Loveless, 1983; Downing and Hopwood, 1964; Oleszkiewicz and Berquist, 1988; EnviroSim and Associates Ltd., 2006).

The effect of temperature on hydrogenotrophic denitrification has not been reported so far. Therefore, the objective of this chapter is to investigate the denitrification performance in stand-alone denitrification reactor with changing temperature.

6.3.2 Materials and methods

After finishing the investigation of the effect of DO in Chapter 6.2, the denitrification reactor was moved to a temperature-controlled incubator to investigate the effect of temperature on hydrogenotrophic denitrification. Influent feed, feeding pump and effluent reservoir were also moved to the incubator. Since NO_3-N was the

Jong Hyuk Hwang, Ph.D. Thesis

major component of feed, no significant change of feed composition was expected inside the incubator.

The reactor was operated for 62 days under various temperature conditions, ranging from 8 °C to 34 °C. Since the denitrification performance was very responsive and stabilized quickly following the temperature change, the temperature was switched every week. Detailed temperature profile is shown in Table 6.3 with operating duration and corresponding SDNRs.

Influent pump setting was unchanged, therefore the expected HRT was 3.81 hrs as shown in Chapter 6.2. However, the actual flowrate fluctuated slightly with temperature as the performance of the feed pump, placed inside incubator, was affected by the temperature change. The actual HRT ranged from 3.47 hr to 3.87 hr. Although the loading rate was slightly different as HRT changed around 10%, the effect of loading rate was not considered for data analysis from this phase. The composition of synthetic wastewater was shown in Table 3.4.

6.3.3 Results and discussions

Table 6.3 shows operational days, HRT, temperature and SDNR. The reactor was operated at each temperature for about 1 week. Reproducibility of SDNR was confirmed by operating a reactor in same temperatures couple times (twice (13 °C, 28 °C) and three times (22 °C)). Figure 6.12 also shows the change of SDNR with operating temperature.

Jong Hyuk Hwang, Ph.D. Thesis

Table 6.3 Effect of temperature on hydrogenotrophic denitrification

Days of operation	HRT (hr)	Temperature (℃)	SDNR (g N/m^{2}d)
6	3.71	22.3	1.003
7	3.80	13.0	0.737
7	3.62	8.2	0.316
7	3.48	13.0	0.738
7	3.47	21.7	1.044
8	3.83	28.1	1.088
7	3.74	34.2	1.100
7	3.84	28.4	1.100
6	3.87	23.0	1.049
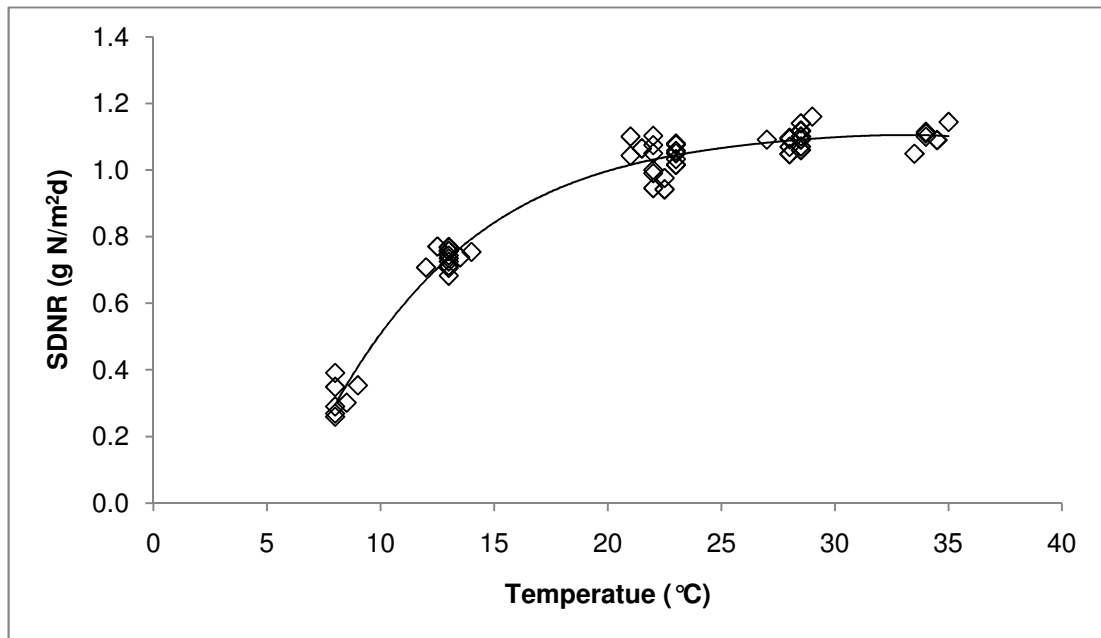
Figure 6.12 SDNR with temperature change

According to Figure 6.12, the optimum temperature for hydrogenotrophic denitrification appears to be between 25 °C and 30 °C, which is lower than the

Jong Hyuk Hwang, Ph.D. Thesis

optimum temperature for autotrophic nitrification (35 °C) (Tchobanoglous, Burton, and Stensel, 2003). The result from this operation indicates that the performance of hydrogenotrophic denitrification can be maximized without temperature control.

Regarding temperature sensitivity, however, the temperature sensitivity below 20 °C was much higher than conventional heterotrophic bacteria and similar to that for autotrophic nitrifiers (Tchobanoglous, Burton, and Stensel, 2003) – SDNR dropped by 50% from 1 g N/m^{2}d to 0.5 g N/m^{2}d when the temperature dropped from 20 °C to 10 °C.

6.3.4 Conclusions

Effect of temperature on hydrogenotrophic denitrification was investigated in a stand-alone denitrification reactor with changing temperature.

The optimum temperature for hydrogenotrophic denitrifiers appears to be between 20 °C and 25 °C. No improvement of denitrification performance was observed above 25 °C. Temperature sensitivity below 20 °C was much higher than conventional heterotrophic bacteria and similar to that for autotrophic nitrifiers.

Jong Hyuk Hwang, Ph.D. Thesis

6.4 Summary of the denitrification reactor operation

The denitrification reactor was tested starting in March 2007.

Major findings from the operation of stand-alone denitrification reactor include the effect of mineral precipitation, DO in bulk solution and temperature. Research regarding mineral precipitation was done between March 2007 and December 2007. Research regarding DO and temperature was done between November 2008 and May 2009. There was a gap between December 2007 and November, 2008, when a couple denitrification reactors were acclimated for the consecutive operations. As one denitrification reactor failed due to excessive biofilm growth during consecutive operation, the acclimation period was longer than originally planned. Research regarding the effect of mineral precipitation on the performance of hydrogenotrophic denitrification showed that mineral precipitation, caused by intended pH control, would cause the deterioration of denitrification performance by inhibiting the diffusion of hydrogen and nitrate. A linear relationship could be achieved between the inert fraction of the collected biomass from the biofilm and SDNR. ICP confirmed that more than 50 % of the inert material was $CaPO_4$, which originated from the pH buffer used. Operating the reactor under various DO conditions showed that the denitrification performance was not affected by bulk DO in the MBfR. Optimum temperature of hydrogenotrophic denitrification found to be around 28 °C. This was much lower than that of nitrification, which is known over 30 °C. The sensitivity to temperature of denitrifier was similar to that of nitrifier when operating below 20 °C.

Jong Hyuk Hwang, Ph.D. Thesis

7. CONSECUTIVE OPERATION OF NITRIFICATION AND DENITRIFICATION

After the PMP membrane module was confirmed to have high gas delivery potential for nitrification and denitrification, the consecutive operation of nitrification and denitrification for complete nitrogen removal using only autotrophic bacteria was initiated. Consecutive operation was conducted by supplying the effluent from the nitrification reactor to the denitrification reactor. The configuration for consecutive operation is shown in Figure 7.1.

Consecutive operation was conducted from March, 2008 to May, 2009.
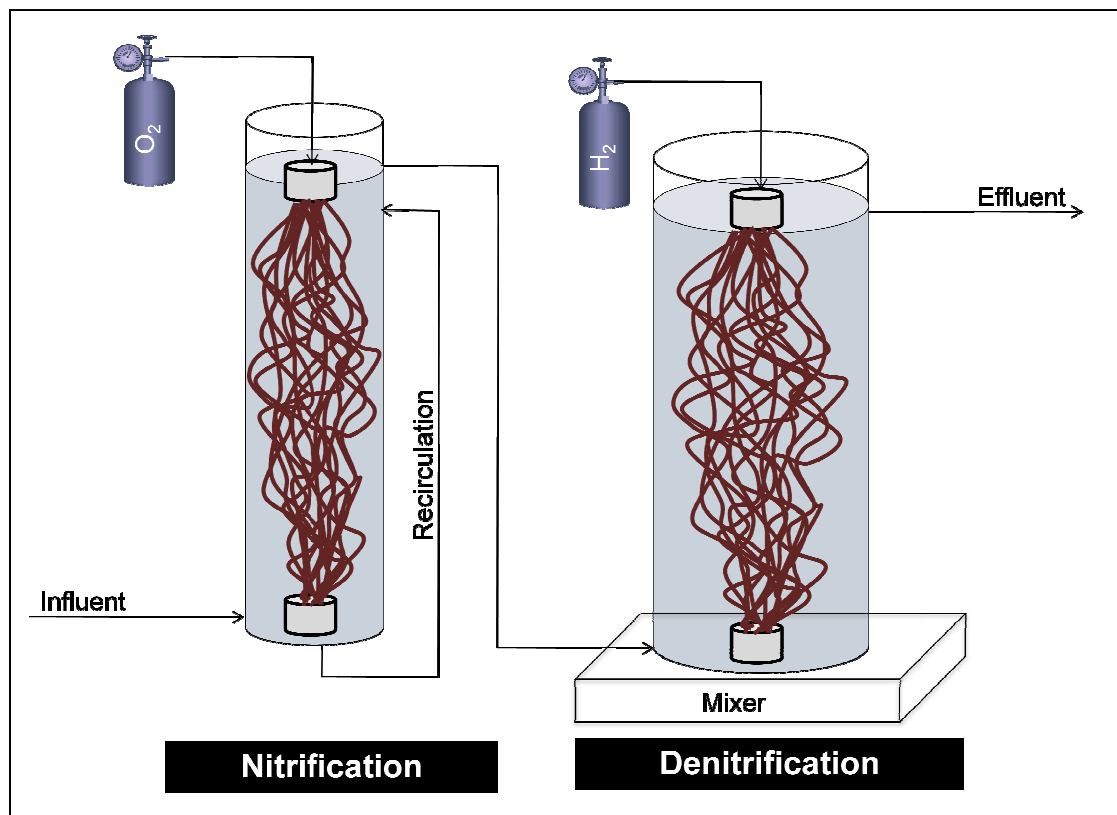
Figure 7.1 Configuration for consecutive operation

Jong Hyuk Hwang, Ph.D. Thesis

7.1 Long-term operational stability

7.1.1 Introduction and objectives

Although many studies have been reported on MBfR, many focused on the removal of organics or simultaneous removal of organics and nitrogen. Studies regarding only nitrogen removal are limited. Brindle *et al.* (1998) conducted a nitrification study with artificial wastewater without organic substrate, reporting that almost complete nitrification could be achieved throughout the experiment with average 98 % of nitrification efficiency. A maximum nitrification rate of 0.98 kg NH_4-N/m^3d was achieved, which corresponds to a specific nitrification rate of 5.4 g NH_4-N/m^2d at 30°C. Backwashing was not performed as excessive biomass growth was not observed. Nitrifying microorganisms, immobilized by polyvinyl-alcohol (PVA) and attached to the surface of a silicone membrane tube, where O_2 was supplied, were used for nitrification. The immobilized biofilm had a rubber-like elasticity and resisted shear stress over 5 months of operation (Hsieh *et al.*, 2002).

Shin *et al.* (2005) studied nitrification, as well as autotrophic denitrification with hydrogen with artificial wastewater containing nitrogen. Volumetric and specific removal rate up to 2.48 kg/m^3d and 1.43 g N/m^2d, respectively were achieved.

Ergas and Reuss (2001) operated an autotrophic denitrifying MBfR for over 4 months. Denitrification rates up to 0.77 kg N/m^3d were achieved with an influent NO_3-N concentration of 145 mg/L and HRT of 4.1 hours. Maximum specific denitrification rate of 2.2 g N/m^2d was achieved. However, denitrification rates

Jong Hyuk Hwang, Ph.D. Thesis

decreased significantly due to the build up of a thick layer of biofilm on the surface of the membranes after approximately four months of operation.

Although in these previous studies researchers were able to operate their reactors for a few months, many cases (especially denitrification reactors) experienced serious deterioration of performance due to excessive growth of biofilm and lack of proper biofilm control.

In this study, total nitrogen removal in a two-step MBfR system incorporating sequential nitrification and hydrogen-driven autotrophic denitrification was investigated. A particular goal was to achieve long-term stable operation, which was identified as a problem in previous studies with MBfR, and to investigate total nitrogen removal using purely autotrophic bacteria. Unlike the nitrification reactor, the denitrification reactor would receive the complex effluent of the nitrification reactor during consecutive operation, which would have variable pH, alkalinity, NH_4-N and especially the residual DO. The effects of these components on hydrogenotrophic denitrification have not been well documented elsewhere. Performance in this study was also compared with other similar previous studies. This operation was conducted between July 2008 and March 2009. Consecutive operation was performed starting March, 2008 however the excessive biofilm growth on the denitrification reactor caused the failure of the consecutive operation, therefore the consecutive operation had to be re-started in July, 2009, with newly acclimated denitrifying biofilm.

Jong Hyuk Hwang, Ph.D. Thesis

7.1.2 Materials and methods

Nitrifying and denitrifying MBfR (Figure 7.1) were operated sequentially over 230 days at an HRT of 1.15 h and 3.7 h, respectively. The HRT of the denitrification reactor was bigger in order to provide more void space as biofilm accumulation was expected in the denitrification reactor, according to literatures (Lee and Rittmann, 2003, Celmer *et al.*, 2008) and previous experiment regarding mineral precipitation. Identical membrane surface areas ($0.152m^2$) for gas delivery and biofilm development were provided. The composition of synthetic wastewater is shown in Table 3.3. HRT was checked daily by collecting effluent overnight.

Internal liquid recycle at approximately 5 times of influent flowrate was applied in the nitrification reactor and mechanical stirrer mixing was provided in the denitrification reactor in order to ensure homogeneous conditions. Oxygen and hydrogen were introduced to the top of the membrane module in the nitrification and denitrification reactors, respectively. Operating temperature was maintained at $20\pm1°C$ throughout the experimental period.

Nitrification batch tests were also performed in order to confirm the presence of nitrifiers escaping from the nitrification reactor to the denitrification reactor. Experimental procedures were same as described in Chapter 5.5.

7.1.3 Results and discussions

Figure 7.2 shows the NH_4-N concentration in the influent, nitrification reactor effluent, final effluent and SNR over time. Average NH_4-N concentrations in influent, nitrification reactor effluent and final effluent were 31.5, 4.0 and 0.7 mg/L, respectively. SNR was steadily maintained over time due to stable biofilm development up to 240 days of operation. In the ensuing period SNR increased slightly mainly due to slight increase in influent loading, which in turn induced diffusion of NH_4-N, which is the main driving force in MBfR. Average SNR for the entire experimental periods was 1.87 g N/m^{2}d.
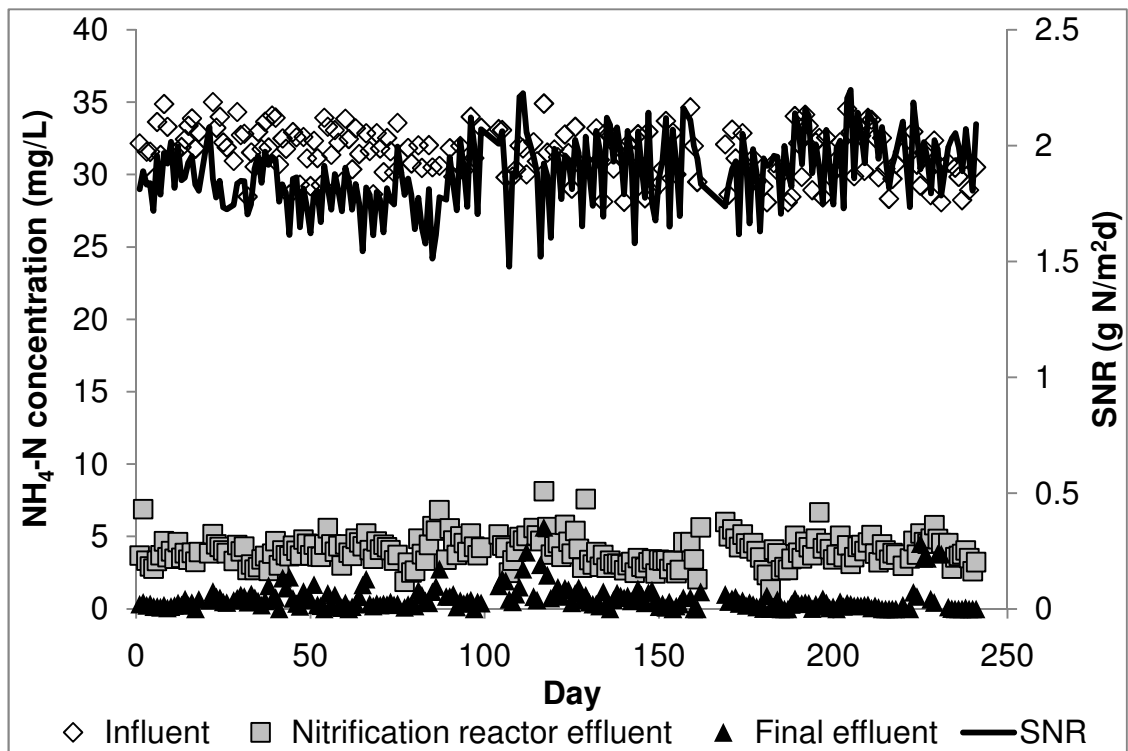
Figure 7.2 Nitrification reactor performance

Jong Hyuk Hwang, Ph.D. Thesis

Nitrification reactor was operated for over 240 days without intentional biomass removal, which meant that the only biomass removal mechanism was the natural detachment/washout by the shear force induced by the internal recirculation, from the nitrification reactor. Solids concentration from the nitrification effluent was not monitored regularly, because the SS measurement required at least 2 L of nitrification reactor effluent sample for accurate SS assessment due to its low concentration, which would have caused the stoppage of feeding to the following denitrification reactor. However, effluent solids were consistently lower than 5 mg SS/L from occasional SS measurements and no solids breakthrough were observed during this period.

There were some past studies aimed at achieving nitrification-only in MBfR (Brindle *et al*., 1998; Hsieh *et al*., 2002). Therefore, the nitrification performance from this study was compared with previous similar studies, shown in Table 7.1. Nitrification rate (both specific and volumetric) from this study was higher or comparable to those of other studies; considering it was conducted at the lowest operating temperature among the results in Table 7.1 and nitrification is one of the most temperature-sensitive biological activities.

Most of these nitrification studies did not report on the biomass accumulation, which has been a problem in MBfR studies focussing on organic removal (Pankhania *et al.*, 1994; Pankhania *et al.*, 1999; Brindle *et al.*, 1999; Semmens *et al.*, 2003). One nitrifying MBfR study reported a biomass accumulation problem when heterotrophic denitrification occurred on the outer side of the biofilm (Suzuki *et al.*, 2000).

Table 7.1 Performance comparison of previous nitrification studies with this work

Reference	Temp (℃)	SNR (g N/m²d)	Volumetric Rate (kg N/m³d)	Influent & Bulk Conc. (mg/L)	Operation periods (days)
Brindle *et al.* (1998)	30	5.4	0.98	50, 1	180
Suzuki *et al.* (2000)	30	2.2	0.04	25, 5	
Hsieh *et al.* (2002)	30	2.3	0.004	50, 5	150
Terada *et al.* (2006)	25	0.5	0.30	90, 50	120
Shin *et al.* (2005)	24	1.4	2.48	200, 5	260
This study	20	1.87	0.56	32, 5	240

   Figure 7.2 also indicates some residual nitrification activity in the denitrification reactor - equal to the difference between the nitrification reactor effluent and final effluent (after denitrification reactor). Nitrification in the denitrification reactor would be possible if the nitrification reactor effluent contains a nitrifier population, which can reattach itself in the denitrification reactor and grow in the presence of DO. NH_4-N removal in the denitrification reactor was not observed after the start of consecutive operation. It gradually started after around 10 days since the denitrification reactor started to receive the influent from the nitrification reactor effluent, suggesting the transfer of biological activity. Sufficient DO was carried over to the denitrification reactor since pure oxygen in nitrification reactor was added not to limit the nitrification activity. Cowman *et al.* (2005) reported that nitrification was greatly

Jong Hyuk Hwang, Ph.D. Thesis

inhibited by low O_2 pressure of 1.25 psi, due to the limited O_2 supply in the nitrification reactor, which left ammonium nitrogen un-oxidized.

According to nitrification stoichiometry in Equation (7.1) (Metcalf and Eddy, 2003), 1 mg of ammonia requires 4.26 mg of oxygen for nitrification.

$$NH_4^+ + 1.863O_2 + 0.098CO_2 \rightarrow 0.0196C_5H_7NO_2 + 0.98NO_3^- + 0.941\ H_2O + 1.98H^+ \qquad \text{(Equation 7.1)}$$
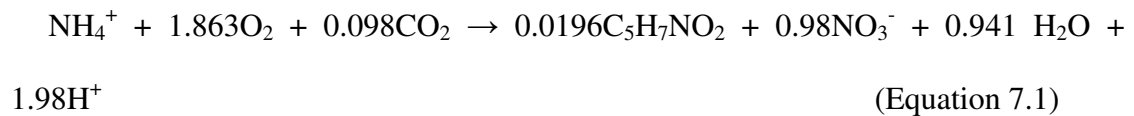
In terms of oxygen mass balance, the residual DO from the nitrification reactor effluent (10 -15 mg/L) contained enough oxygen to nitrify the carried-over NH_4-N, which is the NH_4-N concentration difference between the nitrification reactor effluent and final effluent.

As for the presence of nitrifiers, its existence from the nitrification reactor effluent was tested and confirmed by batch tests using the high F/M test (WERF, 2003).

Figure 7.3 shows the change of nitrogen components during the batch test. Three days were required to achieve full nitrification due to low initial nitrifiers' concentration. The fitted curves (using the least-square method) plotted in Figure 7.3, were used to determine the initial concentration of nitrifiers.

Out of 2.1 mg SS/L (1.5 mg VSS/L) of nitrification reactor effluent, 15.2 % of SS (21.3 % of VSS) was determined to be active nitrifiers, suggesting the possible reattachment of nitrifiers and subsequent biological nitrification in the denitrification reactor.

Jong Hyuk Hwang, Ph.D. Thesis
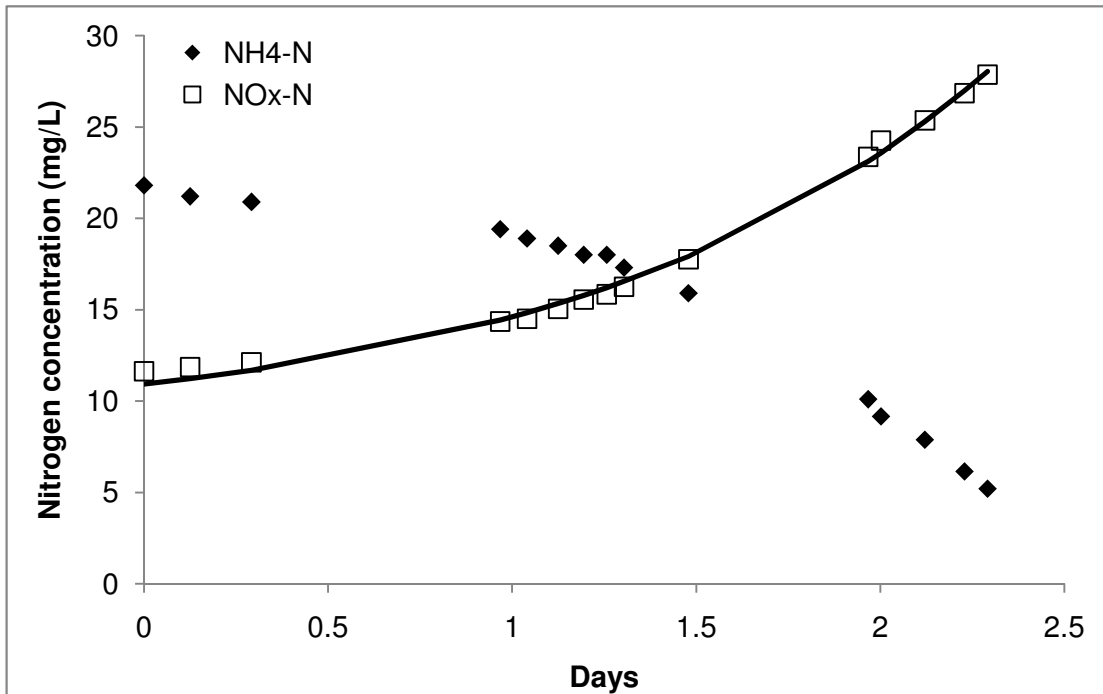
Figure 7.3 Change of NH$_4$-N and NO$_x$-N concentrations during batch test

Figure 7.4 shows the NO$_x$-N concentration of the influent and effluent of the denitrification reactor, and SDNR. Since some nitrification took place in the denitrification reactor, the influent in Figure 6.3 was defined as the sum of incoming NO$_x$-N from the nitrification reactor and the NO$_x$-N converted from NH$_4$-N (NH$_4$-N concentration difference between nitrification effluent and final effluent) in the denitrification reactor. The specific denitrification rate (SDNR) was calculated based on the NH$_4$-N, NO$_2$-N, NO$_3$-N concentration difference between the influent and effluent of the denitrification reactor, flowrate and provided surface area of membrane fibers for biofilm development as shown in Equation (7.2).

Jong Hyuk Hwang, Ph.D. Thesis

$$SDNR = \frac{(((NO_2 - N_{inf}) + (NO_3 - N_{inf})) + ((NH_4 - N_{inf}) - (NH_4 - N_{eff})) - ((NO_2 - N_{eff}) + (NO_3 - N_{eff}))) \times flowrate}{surface\_area}$$

Equation (7.2)
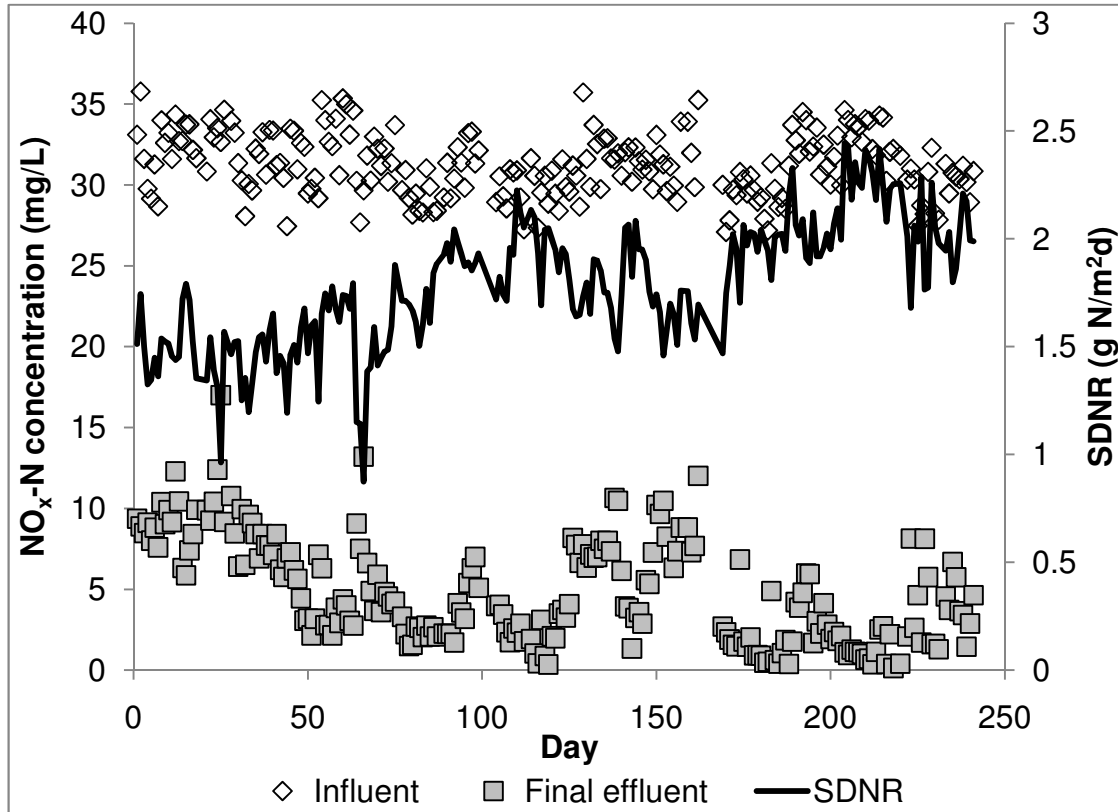
Figure 7.4 NO$_x$-N concentrations and performance of the denitrification reactor

Increasing SDNR was observed over time. Biofilm control by N$_2$ gas scouring was carried out after 70 days of operation. Nitrogen gas was introduced inside of the reactor for 2 minutes every 100 minutes at a flow rate of 15 L/min. Effluent solids concentration increased due to scoured biofilm. Average SDNRs before and after biofilm control were 1.50 and 1.91 g N/m^{2}d. Maximum specific denitrification rate was 2.4 g N/m^{2}d. Solids balance (in Figure 7.8, Chapter 7.2) indicated that the

excessive biomass accumulation can be controlled by introducing nitrogen gas for biofilm control purposes. In terms of system performance, it resulted in improved denitrification performance, as well as made long-term operation possible.

Detailed analysis and discussion regarding biofilm control were explained in Chapter 7.2.3.

Denitrification performance of this study was also compared with previous similar studies in Table 7.2. Comparing Table 7.2 with Table 7.1, operational periods of autotrophic denitrification from previous studies were relatively shorter than those of nitrification, indicating that long-term operation of hydrogen-driven denitrification may have been difficult.

Table 7.2 Performance comparison of previous denitrification studies with this work

Reference	Temp (°C)	SDNR (g/m²d)	Volumetric rate (kg N/m³d)	Operation periods (days)
Ergas *et al.* (2001)	NA	1.17	0.36	
Ho *et al.* (2001)	NA	5.38	0.21	6
Lee *et al.* (2002)	NA	2.68	0.478	0.13
Shin *et al.* (2005)	24	0.84	1.43	40
Celmer *et al.* (2008)	20	0.93	0.11	25
This study	20	1.77	0.19	240

It also should be noted that the denitrification reactor in this study was not inhibited by the carried-over oxygen from the nitrification reactor. Cowman *et al.* (2005) suggested that the high O_2 pressure caused the carry-over of excess oxygen from the

Jong Hyuk Hwang, Ph.D. Thesis

nitrification reactor to following denitrification reactor, resulting in the increase of nitrate concentration in the final effluent, however nitrate accumulation was not observed in this study. Effect of DO on hydrogenotrophic denitrification from consecutive operation was discussed in Chapter 7.3.

7.1.4 Conclusions

Total nitrogen removal in a two-step MBfR system, incorporating sequential nitrification and hydrogen-driven autotrophic denitrification was investigated, in order to achieve nitrogen removal by autotrophic bacteria alone. Consecutive operation of nitrification and autotrophic denitrification was operated for 230 days. Average specific nitrification rate of 1.87 g N/m^{2}d could be achieved and performance was very stable throughout the experimental periods. Nitrification performance from this study was comparable to previous studies, considering this work was conducted at slightly lower temperature than other studies. Performance of autotrophic denitrification was maintained stably throughout the experimental periods. Average specific denitrification rate was 1.77 g N/m^{2}d and maximum specific denitrification rate was 2.4 g N/m^{2}d. This study showed that, with an appropriate biofilm control, stable long-term operation of a fully autotrophic MBfR system for total nitrogen removal was possible without major membrane cleaning procedures.

Jong Hyuk Hwang, Ph.D. Thesis

7.2 Biofilm control

7.2.1 Introduction and objectives

One major concern when operating MBfR is the accumulation of excess (waste) biofilm (Syron and Casey, 2008). Excessive biofilm growth will not only cause non-uniform flow distribution and channelling, but also inhibit substrate or gas diffusion, eventually deteriorating the system performance (Semmens *et al.*, 2003). Some previous studies suffered from biofilm overgrowth, which prevented long-term steady operation. Excessive biofilm growth is typically found in heterotrophic organic removal applications where higher growth rates and higher yields lead to generation of larger mass of excess solids (Semmens *et al.*, 2003). To ensure stable operation, the detachment or sloughing of waste biomass needs to be balanced with accumulation of active biomass. Daily backwashing, which consists of compressed air scouring and the complete replacement of the bulk liquid to remove the detached heterotrophic biomass, was required to prevent channelling and remove excessive biomass in a MBfR, at a COD loading of 8.94 kg/m^{3}d (Pankhania *et al.*, 1994). The absence of backwashing for 6 days resulted in extensive biofilm growth and performance deterioration. High reactor recirculation with air scouring and bulk liquid replacement was beneficial, causing the increase of biofilm density and its adherence to the membrane surface (Pankhania *et al.*, 1999, Brindle *et al.*, 1999). In MBfR with loading of 5.1 kg COD/m^{3}d (Semmens *et al.*, 2003) which were lower than the 8.94 kg COD/m^{3}d reported by Pankhania *et al.* (1994), generous gas recycle (2 L/min) to mix

Jong Hyuk Hwang, Ph.D. Thesis

the reactor and control biofilm, was able to maintain performance for 3 months of operation due to lower biomass generation. Excessive biofilm growth was also found in MBfR for nitrogen removal without organic oxidation, mainly due to the overgrowth of heterotrophic denitrifiers, growing at the outer side of biofilm (Suzuki *et al.*, 2000).   No excessive biofilm growth was reported in the nitrification-only MBfR.

Hydrogen-based autotrophic MBfR are also vulnerable to biomass accumulation, especially due to the increase in the mineral component of biomass resulting from increased local pH generated by high denitrification activity (Lee and Rittmann, 2003; Celmer *et al.*, 2006; Padhye *et al.*, 2007).   The precipitated minerals inhibited hydrogen diffusion by covering portions of the membrane fiber (Chapter 6.1). Increased shear force including faster mixing and nitrogen sparging could reduce the biofilm thickness, thus ensuring higher biofilm performance (Celmer *et al.*, 2008a). Intermittent ultrasound treatment was also proven to be effective for biofilm control without damaging the membrane itself (Celmer *et al.*, 2008b).

Although autotrophic nitrification and autotrophic denitrification generate less biomass than heterotrophic denitrification, high removal rates and lack of biomass withdrawal mechanisms in an MBfR still cause the build-up of excessive biofilm. Therefore, the use of nitrogen sparging as a means of biofilm control of MBfR was evaluated for stable operation. This chapter will discuss the suitability of nitrogen sparging as the biofilm control strategy for stable operation and the detailed investigation of solids balance and biofilm thickness.

Jong Hyuk Hwang, Ph.D. Thesis

7.2.2 Materials and methods

This section was written using the data collected during 240 days of consecutive operation, which was shown in previous Chapter 7.1.

The denitrifying MBfR was sparged with nitrogen gas for 2 minutes out of every 100 min of operation at a 15 L/min flow-rate, starting at day 70 of operation. The purpose of sparging was to induce mild biofilm sloughing for biofilm thickness control. Nitrogen was applied through a coarse bubble diffuser to create shear force. Nitrogen sparging was specifically chosen (rather than air), because of possible adverse effect on denitrification due to oxygen in the air. Since sparging was done just for 2 minutes per every 2 hours, DO condition in denitrification was not changed by the nitrogen sparging. No biofilm control was conducted on the nitrification reactor, as no excessive biofilm growth was found during the experimental period.

This chapter aimed to achieve stable operation by removing the excessive biofilm build-up – defined as accumulation that begins to impact the performance negatively. A solids mass balance was established. Theoretical solids generation was calculated from the stoichiometries of each reaction below, and the solids balance was calculated based on these biomass yields.

- Autotrophic nitrification (Metcalf and Eddy, Equation (7-91), 2003)

$$NH_4^+ + 1.863O_2 + 0.098CO_2 \rightarrow 0.0196C_5H_7NO_2 + 0.98NO_3^- + 0.941 H_2O + 1.98H^+$$

- Autotrophic denitrification with hydrogen (WERF, 2003)

Jong Hyuk Hwang, Ph.D. Thesis

$$NO_3^- + H^+ + 2.86H_2 + 0.15CO_2 \rightarrow 0.0286C_5H_7NO_2 + 0.49N_2 + 3.14H_2O$$
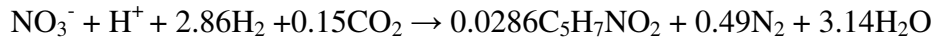
Theoretical yield of heterotrophic denitrification was estimated as follows.

- 0.40 g VSS yield/g COD consumed (Metcalf and Eddy, Table (8-18), 2003)

- 2.86 g COD consumed/ g NO_3-N (from stoichiometry of methanol)

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$$

$$5CH_3OH + 7.5O_2 \rightarrow 5CO_2 + 10H_2O$$
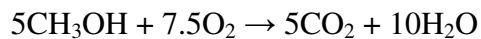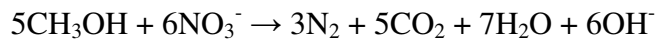
Theoretical biomass yields from above equations are 0.16 g VSS/g N for nitrification and 0.23 g VSS/g N for autotrophic denitrification. Rezania *et al.* (2006) reported similar true yield of 0.27 g VSS/g N in autotrophic denitrification. According to the stoichiometry of heterotrophic denitrification above, its yield can be estimated as 1.14 g VSS/g N, which is five times higher than that of autotrophic denitrification.

Biofilm thickness was measured to investigate whether nitrogen sparging can effectively prevent the overgrowth of biofilm. It was not measured before the nitrogen sparging was initiated as the biofilm was assumed to be still developing. After the deterioration of system performance was confirmed, the biofilm control and thickness measurements were started using the method described by Celmer *et al.* (2008). Biofilm thickness was measured by removing the membrane module with biofilm from the denitrifying MBfR and placing it in a vertical position for 20 minutes to allow excess water to drain. Then, the thickness was calculated based on the liquid volume displaced by the biomass and the membrane.

Jong Hyuk Hwang, Ph.D. Thesis

7.2.3 Results and discussions

1) Failed denitrification reactor case

Before showing the results regarding biofilm control, the case of the failed denitrification reactor due to improper biofilm control will be addressed. The denitrification reactor was operated between March 2008 and June 2008 as the second part of consecutive operation before the operation described in Chapter 7.1.

Figure 7.5 shows NOx-N concentration and SDNR from the failed denitrification reactor. Denitrification performance was stable for the first 35 days, and then started to deteriorate over time except some fluctuations around 60 days of operation. Average specific denitrification rate during 15 to 30 days of operation (when the performance was steady) was 1.42 g N/m^{2}d and maximum was 1.60 g N/m^{2}d.

Deterioration of denitrification reactor was caused by the excessive growth of biomass. Biomass generation, lack of proper biofilm control and additional biomass would accelerate performance deterioration.

Eventually, the membrane module started to float, possibly due to trapped nitrogen, which was generated from denitrification activity (inside of the membrane fibers) similarly to the rising sludge issues in conventional settling tanks. It could also have been caused by hydrogen gas, which could leak from the membrane fiber connections. Figure 7.6 shows that the bottom part of the membrane module was floated above the membrane support at the bottom of the reactor.

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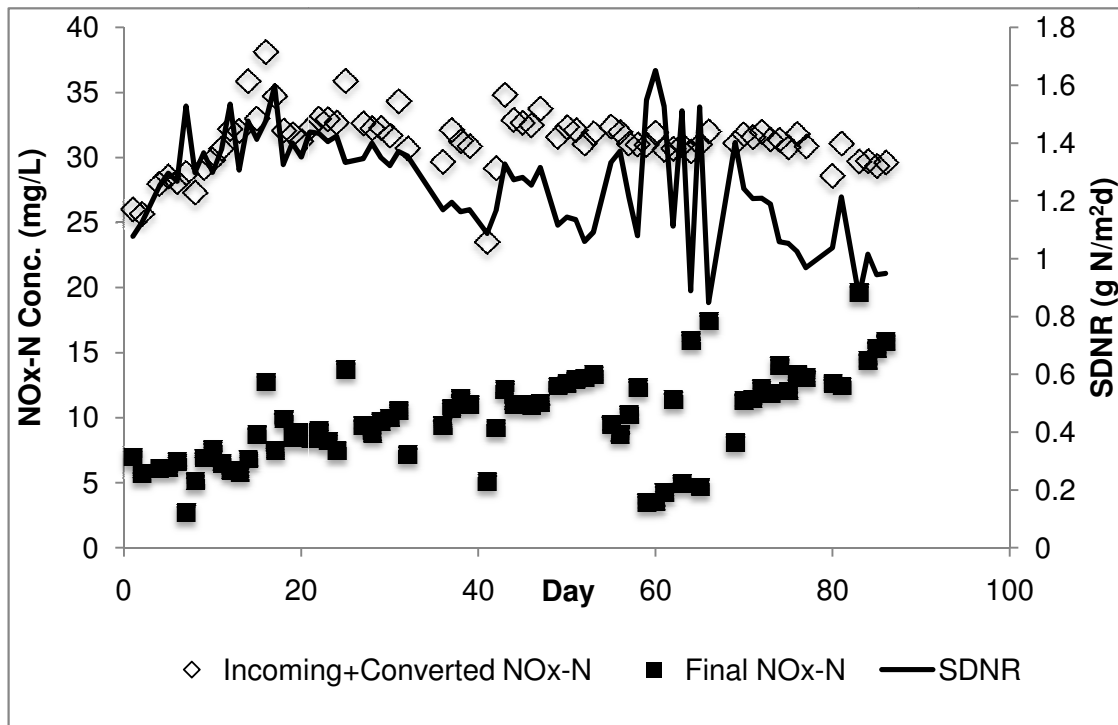
Figure 7.5 NOₓ-N concentration and SDNR from failed denitrification reactor

This floated membrane reactor had to be removed and replaced after 90 days of operation due to a visually detectable hydrogen leak, possibly caused by pressure build-up by the excessive biomass growth. Biomass from the failed membrane fibers was collected and the solids contents were measured. The amount of solids in the denitrification reactor at 90 days was measured as 4380 mg, which can be translated into 2740 mg/L, if it was suspended. Volatile portion of harvested solids was 81%, which indicates that mineral precipitation was not an issue in the denitrification reactor, receiving feed from the nitrification reactor.

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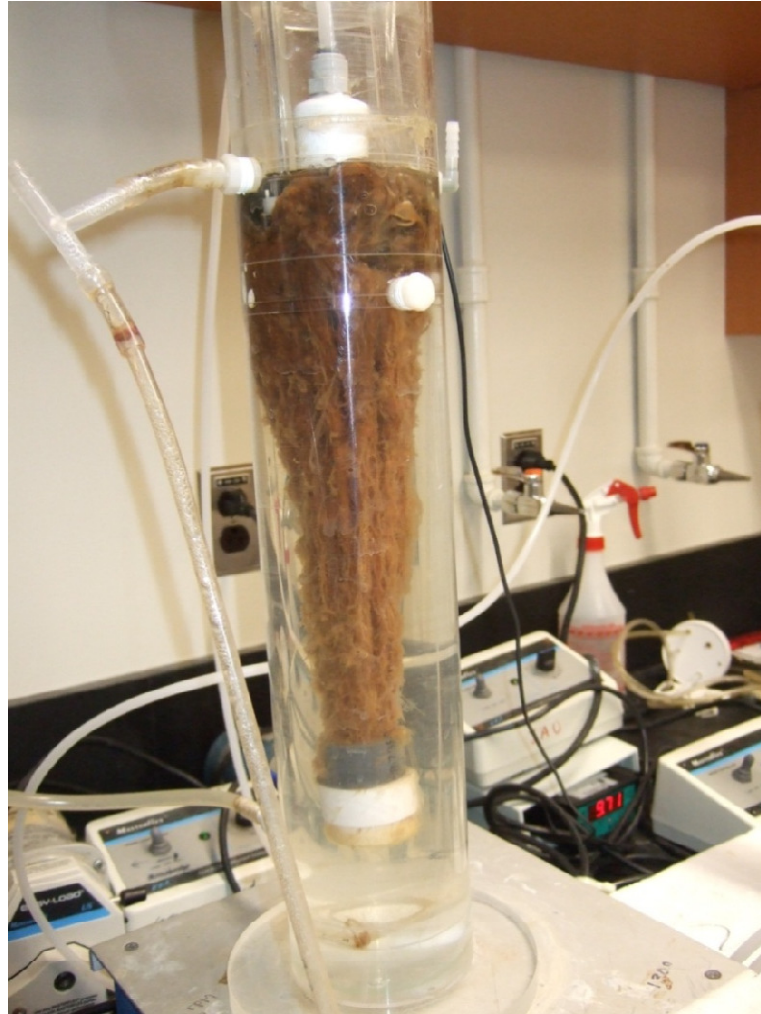
Figure 7.6 Picture of the denitrification reactor in a floated state

2) Performance of nitrification and denitrification reactor

Performance of consecutive operation during biofilm control was addressed in Chapter 7.1.

Nitrification rate reactor did not appear to be adversely affected by excessive biofilm growth. TSS concentration in the nitrification reactor effluent was measured only sporadically. In the five measurements done during the whole experiment, effluent solids were consistently lower than 5 mg TSS/L.

Jong Hyuk Hwang, Ph.D. Thesis

An average SNR of 1.87 g N/m^{2}d translates to 4.4 mg VSS/L of biomass generation according to the theoretical biomass yield and flowrate. The calculations and TSS measurements point to the phenomenon of the nitrifiers escaping the nitrification reactor and continuing to be active in the subsequent denitrification reactor. The presence of nitrifiers in the effluent from the nitrification reactor was confirmed by batch test – the results were shown in Chapter 7.1.

The denitrification reactor periodically suffered from excessive biofilm growth due to 30% higher yield (0.23 g VSS/g N) than nitrification (0.16 g VSS/g N), before the nitrogen sparging was initiated on Day 70. Additional nitrifier growth and perhaps even heterotrophic growth would further promote biofilm growth in the denitrification reactor.

No biofilm control was implemented until Day 70 of operation, when the SDNR stabilized at 1.5 g N/m^{2}d. Excessive biofilm growth was (visually) observed around Day 60. A sharp decline of SDNR on Day 66 led to the implementation of the biofilm control measures, which involved nitrogen gas sparging. SDNR increased to 1.92 g N/m^{2}d and average NO$_x$-N concentration in final effluent decreased from 7.4 mg/L to 3.7 mg/L following biofilm control. This suggested that the long-term stable operation of MBfR (exceeding 60 days in this study) would not be possible without proper biofilm control measures.

3) Biofilm control by N$_2$ gas sparging

Jong Hyuk Hwang, Ph.D. Thesis

Gas scouring was chosen as a biofilm control measure, as it was tested previously by other researchers (Pankhania *et al.*, 1994; Pankhania *et al.*, 1999; Brindle *et al.*, 1999; Semmens *et al.*, 2003). However, air sparging was excluded due to its possible adverse effect on hydrogenotrophic denitrification, so nitrogen gas was chosen due to its inertness. Nitrogen gas is generated in the process of denitrification and there may be an opportunity to re-use it in full scale (Rezania *et al.*, 2007).

Nitrogen sparging resulted in an improved denitrification performance. The average SDNR increased from 1.50 g N/m^{2}d to 1.92 g N/m^{2}d. Denitrification performance slightly declined around Day 150 due to local biofilm loss caused by local excessive shear force. The system did not experience any major performance deterioration (such as the one observed prior to nitrogen sparging) for over 6 months. Therefore, it was concluded that long-term stable operation could be achieved.

Since the synthetic influent used in this study did not contain any volatile solids, the solids in the final effluent originated from the detachment/washout from both reactors. Solids in the final effluent were measured from the effluent collected overnight. Figure 7.7 shows TSS and VSS concentration in the final effluent. Effluent TSS was mostly below 5 mg/L before nitrogen sparging, however it increased substantially with the shear force induced by nitrogen sparging.

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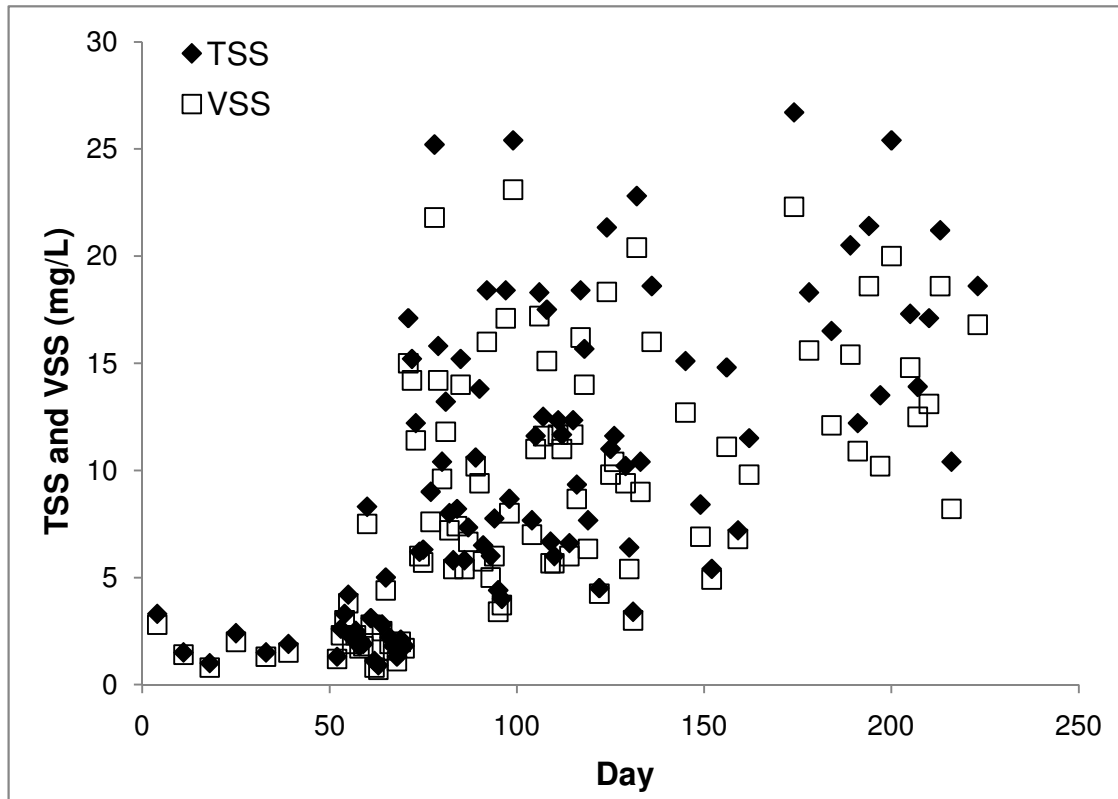
Figure 7.7 Total suspended solids (TSS) and volatile suspended solids (VSS) concentrations in the effluent from the denitrification reactor

Average TSS increased from 2.5 mg/L to 12.7 mg/L by nitrogen sparging. Average VSS/TSS ratio of 0.88 was higher than those from previous studies (Lee and Rittmann, 2003; Celmer *et al.*, 2007; Chapter 6.1 in this thesis) and indicated that the denitrification was not affected by inorganic precipitation. Effluent TSS concentration fluctuated from 2 mg/L to 26 mg/L depending on daily sparging events, which sometimes exerted only local shear force because of the location of the course-bubble diffuser.

4) Solids balance and biofilm thickness

Jong Hyuk Hwang, Ph.D. Thesis

In order to find out how much biomass generation would be expected inside the system, theoretical biomass generation was estimated – Figure 7.8, comprising of three biomass-generating reactions; 1) nitrification in the nitrification reactor, 2) denitrification in the denitrification reactor and 3) nitrification in the denitrification reactor. Theoretical biomass generation was calculated based on the biomass yield, as described in the Materials and Methods section of this chapter, the difference in related nitrogen concentrations, and daily flow-rates. Detailed equations are shown in Table 7.3.
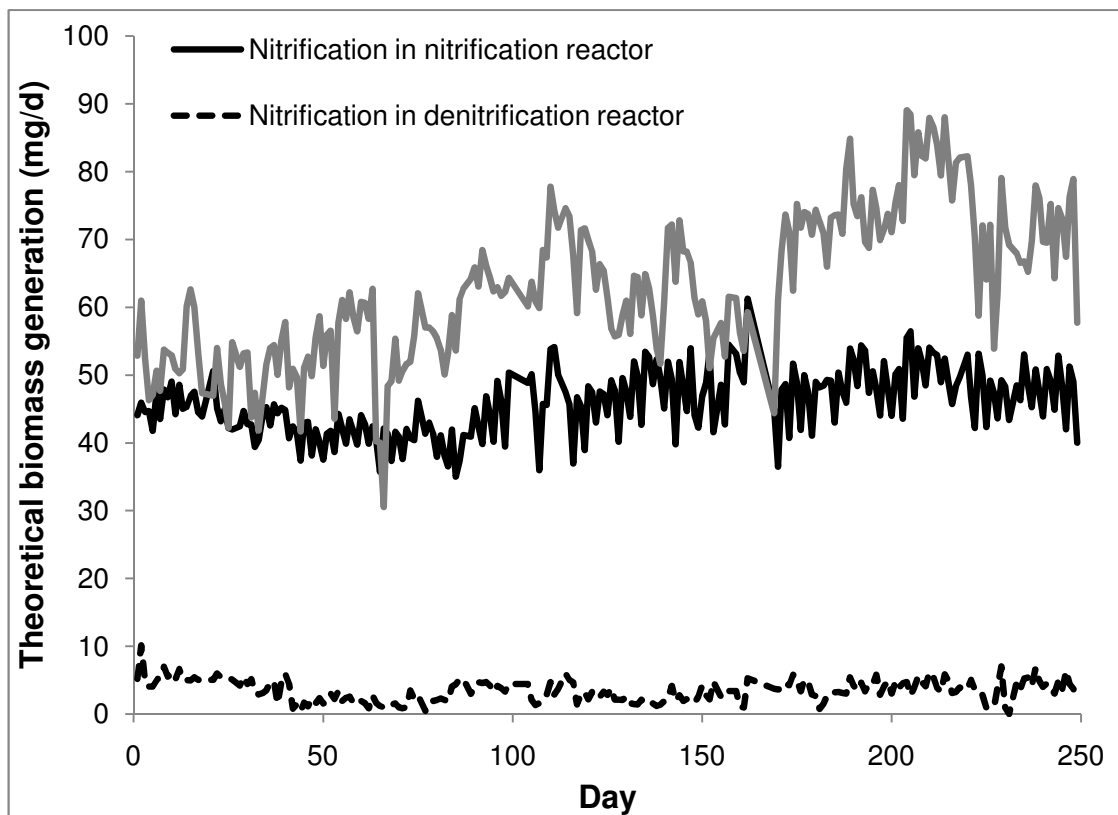
Figure 7.8 Theoretical biomass generation based on stoichiometry and nitrogen removal rate

Jong Hyuk Hwang, Ph.D. Thesis

Table 7.3 Equations for estimating biomass generation

Biomass-generating reaction	Equation
Nitrification in the nitrification reactor	$(NH_4\text{-}N_{influent} - NH_4\text{-}N_{nitrification\ effluent}) \times$ flowrate $\times$ 0.16
Denitrification in the denitrification reactor	$((NO_2\text{-}N_{nitrification\ effluent} + NO_3\text{-}N_{nitrification\ effluent} + (NH_4\text{-}N_{nitrification\ effluent} - NH_4\text{-}N_{final\ effluent})) - (NO_2\text{-}N_{final\ effluent} + NO_3\text{-}N_{final\ effluent)}) \times$ flowrate $\times$ 0.23
Nitrification in the denitrification reactor	$(NH_4\text{-}N_{nitrification\ effluent} - NH_4\text{-}N_{final\ effluent}) \times$ flowrate $\times$ 0.16

Figure 7.8 shows a similar trend to that of SNR in Figure 7.2 and SDNR in Figure 7.4. Although SNR was higher than or similar to SDNR, biomass generation by denitrification was higher than that by nitrification due to its higher yield, as shown from the stoichiometries. The fraction of biomass generated by nitrification in the denitrification reactor (dashed line) varied from 0.1 % to 6.4 % and accounted for an average of 3.0 % of total theoretical biomass generation.

Since no solids accumulation or solids breakthrough was observed in the nitrification reactor, it could be assumed that the nitrification reactor was balanced in terms of solids generation and discharge. This also indicated that the subsequent denitrification reactor would be more vulnerable to excessive solids accumulation. In summary, all the solids generation depicted in Figure 7.8 would be accumulated in the denitrification reactor. Therefore, biofilm thickness control would be essential in the denitrification reactor.

Jong Hyuk Hwang, Ph.D. Thesis

It would be desirable to maintain a constant biofilm thickness when operating an MBfR that achieves an equilibrium between biomass accumulation and sloughing/endogenous decay. As TSS and VSS concentration in the final effluent increased by nitrogen sparging as shown in Figure 7.7, theoretical biomass generation in Figure 7.8 could be compared to measured VSS discharge in order to show that excessive solids accumulation can be controlled by nitrogen sparging and long-term stable operation can be achieved. Theoretical biomass generation in Figure 7.9 shows the sum of all the biomass generation, depicted individually in Figure 7.8. Discharged biomass in Figure 7.9 was calculated from daily VSS discharge and flowrate from Figure 7.7.
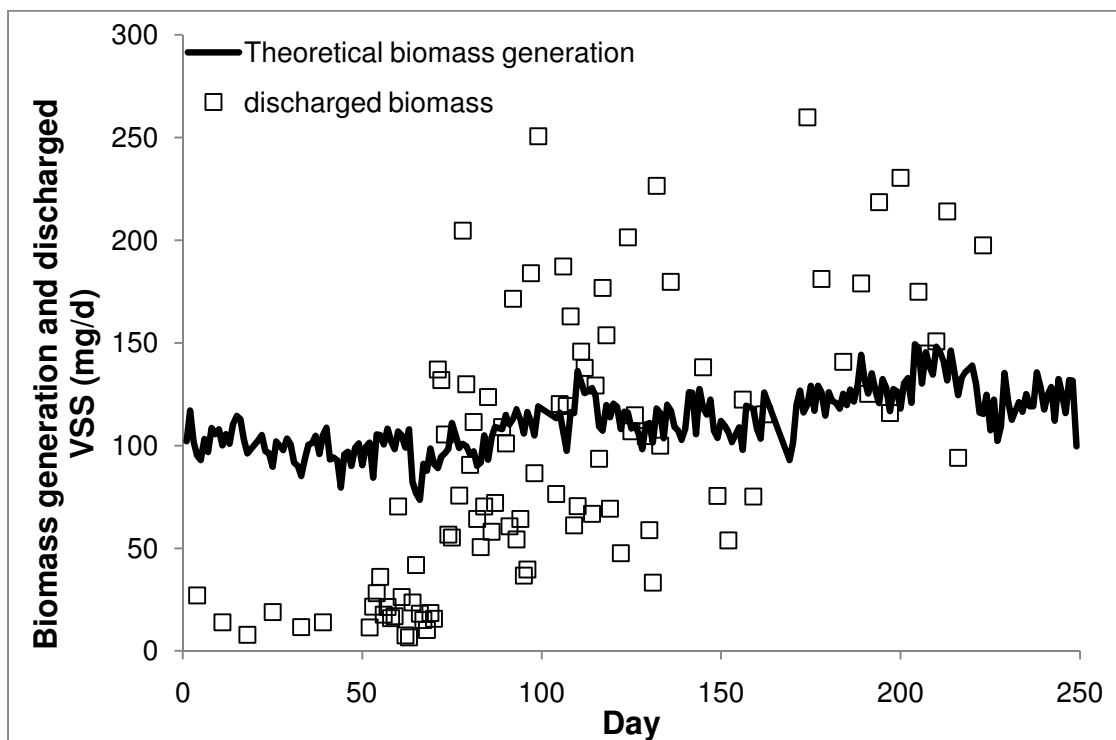
Figure 7.9 Theoretical biomass generation and discharged biomass from the denitrifying reactor (nitrogen sparging started on day 70)

Jong Hyuk Hwang, Ph.D. Thesis

Figure 7.9 shows the accumulation of biomass before nitrogen sparging as the amount of washed out biomass was far lower than the amount of generated biomass. After the initiation of periodic nitrogen sparging, the amount of discharged biomass often exceeded the theoretical biomass generation. It should also be noted that the theoretical solids generation in this study only includes the nitrification and denitrification reactions. Heterotrophic growth on the organic substrate generated by biomass decay was not accounted for in the calculation, although it is a very likely contribution to the total biomass growth. Coexistence of nitrifiers and heterotrophs has been found in autotrophic nitrifying suspended growth cultures (Rittmann *et al.*, 1994) and biofilms (Kindaichi *et al.,* 2004; Okabe *et al*., 2005; Lackner *et al*., 2008) maintained without an external organic carbon source. It was also reported that autotrophic nitrifying systems with long SRT, such as a biofilm systems, would have significant amount of heterotrophs (Nogueira *et al.*, 2005). Even though complete solids balance could not be explained in Figure 7.9, the discharged biomass amount was on a par with the generated biomass amount, in spite of fluctuations.

Average theoretical biomass generation of 112 mg VSS/d could be translated into 10.5 mg/L of effluent VSS concentration, resulting in 11.98 mg TSS/L based on VSS/TSS ratio of 0.88 from the data in Figure 7.7. Although high amount of biomass generation is naturally inevitable due to the high nitrogen removal rate, 12 mg/L of TSS in final effluent can be problematic, considering that this process was intended to achieve tertiary treatment. Therefore, measures for periodic biofilm thickness control

Jong Hyuk Hwang, Ph.D. Thesis

(similar to filter backwashing) are necessary to attain steady-state high-rate operation of MBfR. Solids analysis in this study was conducted from the samples containing the final effluent, accumulated over at least 12 hours, constituting a form of composite sampling. By visual observation, the largest solids discharge occurred within 10 minutes of nitrogen sparging. Therefore, other sparging schemes should also be explored, varying the sparging strength and interval in order to minimize and/or equalize the effluent solids discharge.

In order to show that excessive biofilm accumulation could be restrained by nitrogen sparging, the biofilm thickness was also monitored after the introduction of sparging. Biofilm thickness was not monitored before the introduction of nitrogen sparging as the biofilm was assumed to be developing. Figure 7.10 shows the biofilm thickness measured during the experimental period.

As seen in Figure 7.10, the biofilm thickness ranged from 220 µm to 300 µm with an average thickness of 270 µm throughout the experimental period. Continuous removal of accumulating biomass by nitrogen sparging was shown to maintain steady biofilm thickness, as well as the steady system performance over 190 days of experiment. However, more studies regarding various sparging rates and biofilm structure changes would be required to understand the full effect of biofilm control.

Excessive biofilm growth exerts several negative effects on MBfR performance. It decreases HRT, limits diffusion of substrate and electron donors through the biofilm, and results in an uncontrolled unloading of biomass from the biofilm.

Jong Hyuk Hwang, Ph.D. Thesis
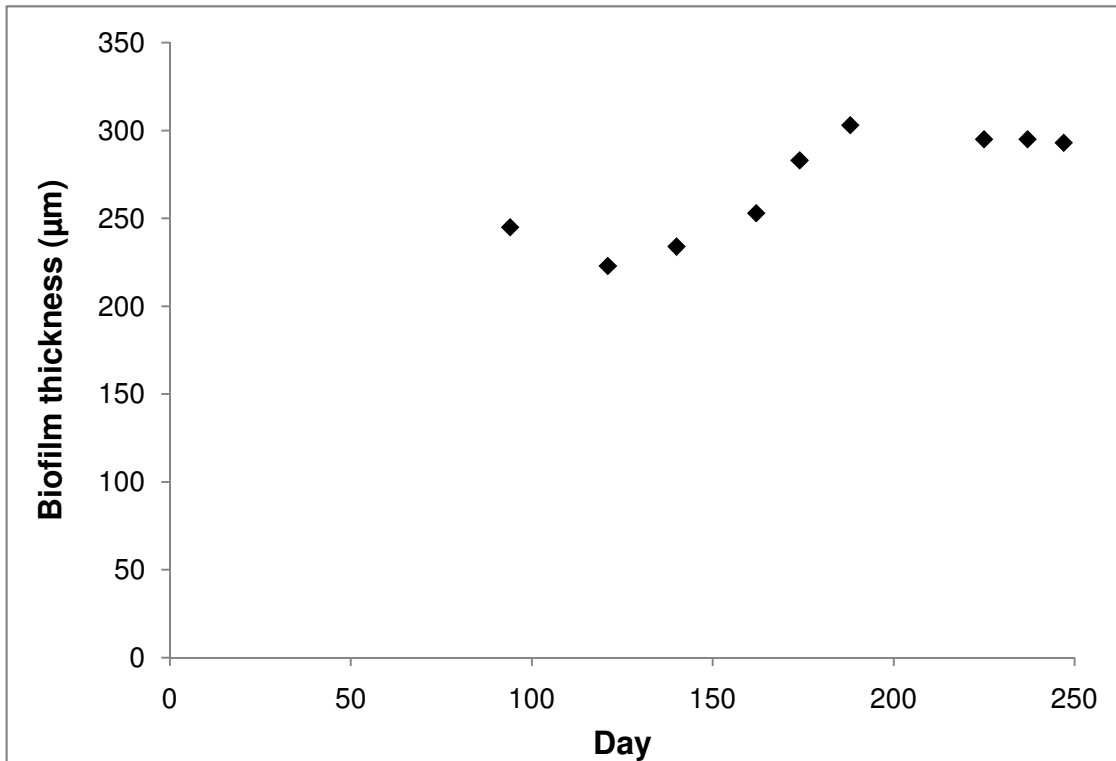
Figure 7.10 Biofilm thickness in the denitrification reactor after the initiation of periodic sparging

With a maximum 300 µm of biofilm thickness (occupying over 14% of denitrification reactor volume), the actual retention time of the influent would be less than the specified HRT of 3.62 hours, which was calculated from reactor volume and flowrate. With 400 µm of biofilm thickness experienced before Day 70 of operation, 23% of reactor volume would be occupied by the biofilm further reducing the effective HRT and leading to unexpected and uncontrolled biofilm unloading and thus unreliable and unsteady performance. Maintaining a thick biofilm with the intention of holding more biomass in the system would not improve the system performance due to diffusion limitations. For example, Nicolella *et al.* (2000) and Habiya *et al.*

Jong Hyuk Hwang, Ph.D. Thesis

(2004) reported that oxygen penetrated biofilms to depths of 150 μm and 300 μm, respectively, in their biofilm processes.

Optimum biofilm thickness would vary with substrate type, substrate concentration, gas type, gas pressure, fiber spacing and gas delivery mechanism. Although many studies have been conducted to find out optimum biofilm thickness in the fields of biological aerated filters (BAF) or moving bed biofilm reactors (MBBR), the differences in gas delivery mechanisms (co-diffusion vs. counter-diffusion) make the comparisons difficult with MBfR. Table 7.4 shows a significant diversity of biofilm thickness data from previous MBfR researches. The data refer to maximum biofilm thicknesses found suitable for operation in each study, with larger values causing deterioration of nitrogen removal rates.

Table 7.4 Comparison of various reported biofilm thickness in MBfR with this study

Reference	Biofilm Thickness (μm)	Length of Operation (d)	Process
Semmens *et al.* (2003)	600	190	COD removal & nitrification
Satoh *et al.* (2004)	2100	60	COD removal & nitrification
Sahu *et al.* (2007)	1000	120	Denitrification with H_2
Modin *et al.* (2008)	940	59	Denitrification with CH_4
Celmer *et al.* (2008)	500	>300	Denitrification with H_2
Downing and Nerenberg (2008)	125	270	COD removal & nitrification
This study	270	250	Denitrification with H_2

Jong Hyuk Hwang, Ph.D. Thesis

Although nitrogen removal could be improved in thick biofilms designed for COD and ammonia removal by developing heterotrophic denitrification in the anoxic part of the biofilm (Semmens *et al.*, 2003; Satoh *et al.*, 2004), system failure was unavoidable without proper biofilm control due to the increasing volume of biofilm (Semmens *et al.*, 2003). Downing and Nerenberg (2008) implemented nitrogen sparging specifically to limit oxygen transfer from the water surface to the bulk liquid in their reactor and reported the thinnest biofilm. Although the methods for biofilm thickness measurement varied from previous results shown in Table 7.4, the biofilm thickness of 270 µm found in this study falls into the lower range of the reported biofilm thicknesses. It has allowed continuous MBfR operation at a steady denitrification rate which was higher than the rates obtained during the periods with thicker biofilm.

7.2.4 Conclusions

Sequential nitrification and hydrogen-driven autotrophic denitrification was tested in a series of two MBfRs treating synthetic non-nitrified secondary wastewater, over a period of 250 days. Biofilm thickness control using nitrogen sparging in the denitrification reactor was initiated on Day 70, to ensure long-term stable operation.

The nitrification reactor had an average specific nitrification rate of 1.88 g N/m^{2}d and exhibited consistent performance over 250 days, without excessive biofilm growth. No biofilm control was found to be necessary.

Jong Hyuk Hwang, Ph.D. Thesis

The subsequent denitrification reactor suffered from excessive biofilm growth, resulting in the decline of the specific denitrification rate (SDNR). Excessive biofilm accumulation resulted from the higher biomass yield in denitrification and to a much lesser extent due to the carry-over of solids from the nitrifying reactor.

Biofilm control by nitrogen sparging, started on Day 70, resulted in an improved denitrification performance and ensured long-term stable operation. The average specific denitrification rate (SDNR) increased from 1.50 g N/m^{2}d to 1.92 g N/m^{2}d after sparging.

Biofilm thickness was maintained at an average of 270 µm, indicating biomass accumulation and nitrogen scouring was balanced and long-term (190 days) stable operation could be maintained.

Effluent solids concentration increased immediately after sparging. Average long-term effluent total suspended solids increased from 2.5 mg/L to 12.7 mg/L.

7.3 Effect of bulk dissolved oxygen in consecutive operation

7.3.1 Introduction and objectives

Heterotrophic denitrifying bacteria prefer oxygen as an electron acceptor over nitrate. It has been known that denitrification is inhibited in the presence of DO concentrations over 0.2 mg/L (Terai and Mori, 1975, Metcalf and Eddy, 2003). And it

Jong Hyuk Hwang, Ph.D. Thesis

also has been reported that the denitrification activity ceases with oxygen concentrations higher than 1.0 mg/L (Whitmyer *et al.*, 1991).

Even though DO might affect denitrification activity negatively, the mechanisms inhibiting denitrification differs between heterotrophic denitrification and autotrophic denitrification by hydrogen. In heterotrophic denitrification, the presence of DO would switch off the activity of heterotrophic denitrifiers biologically. The inhibition mechanism of presence of DO on autotrophic denitrification using hydrogen is still unclear. It is not even clear whether the presence of oxygen actually inhibits autotrophic denitrification.

There have been conflicting results regarding the effect of DO on the performance of hydrogen-driven denitrification as shown in Chapter 6.2.

Since the effect of DO on hydrogenotrophic denitrification was investigated in stand-alone denitrification reactor, the objective of this chapter is to investigate the effect of bulk DO, carried over from the nitrification reactor, on hydrogenotrophic denitrification performance during consecutive operation.

7.3.2 Materials and methods

The effect of DO was investigated in consecutive nitrification and denitrification MBfR for 85 days between June 2008 and September 2008. It was conducted simultaneously with the experiment, described in Chapter 7.1. Therefore, the experimental details were already described in Chapter 7.1.

Jong Hyuk Hwang, Ph.D. Thesis

Regarding DO control, the effluent from the nitrification reactor was diverted to a separate (middle) tank (0.3 L) before entering into denitrification reactor in order to reduce (or completely eliminate) residual DO coming from the nitrification reactor, during day 55 - 85 of operation. Nitrogen gas was introduced continuously through a diffuser in the middle tank. The system configuration is shown in Figure 7.11.

7.3.3 Results and discussion

Despite controlling the oxygen supply in the nitrification reactor to limit residual DO in the denitrification reactor (Brindle *et al.*, 1998), sufficient oxygen was supplied for effective nitrification (Chapter 5.4), also allowing the investigation of a DO effect on the denitrification reactor. Average DO from the effluent of the nitrification reactor was 15.8 mg O_2/L, which is higher than the oxygen saturation concentration (9.1 mg O_2/L at 20°C) due to the use of pure oxygen. The average DO inside the denitrification reactor was 1.0 mg O_2/L before DO control was started at day 55 of operation.

An oxygen balance suggests that most of DO difference was caused by the nitrification activity within the denitrification reactor. NH_4-N concentrations in the nitrification reactor effluent and final effluent were 4.3 mg NH_4-N/L and 0.9 mg NH_4-N/L, respectively. According to the stoichiometry of nitrification - Equation (7.3), 4.25 g of oxygen is required to oxidize 1 g of ammonia nitrogen. Therefore, 3.4 mg NH_4-N/L (difference between above two values) would require 14.5 mg O_2/L. The

Jong Hyuk Hwang, Ph.D. Thesis

existence of nitrifiers in denitrification reactor was also confirmed by batch tests (Chapter 7.1).

$$NH_4^+ + 1.863O_2 + 0.098CO_2 \rightarrow 0.0196C_5H_7NO_2 + 0.98NO_3^- + 0.941\ H_2O + 1.98H^+$$
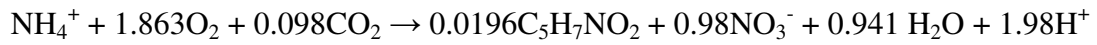
<div align="right">Equation (7.3)</div>
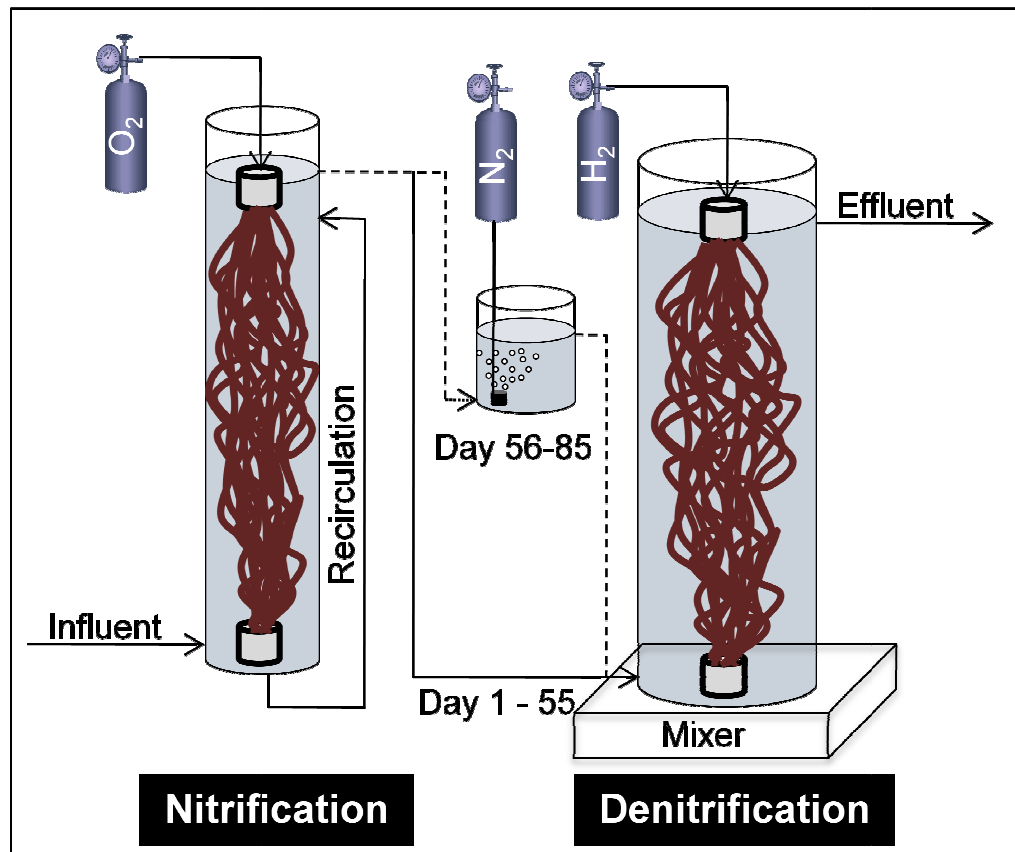
Figure 7.11 Reactor configurations

Although most of the DO was consumed by nitrifiers, the remaining (measured) 1 mg O_2/L (1.3 mg O_2/L from oxygen balance) may affect hydrogenotrophic

denitrification. Therefore, DO entering the denitrification reactor was artificially controlled to investigate this potential effect.

As explained in the materials and methods section, the effluent from the nitrification reactor was diverted to a middle tank (0.3 L) before entering the denitrification reactor in order to eliminate residual DO by nitrogen gas during day 55-85. Figure 7.12 shows the DO concentration entering the denitrification reactor as well as inside the denitrification reactor.
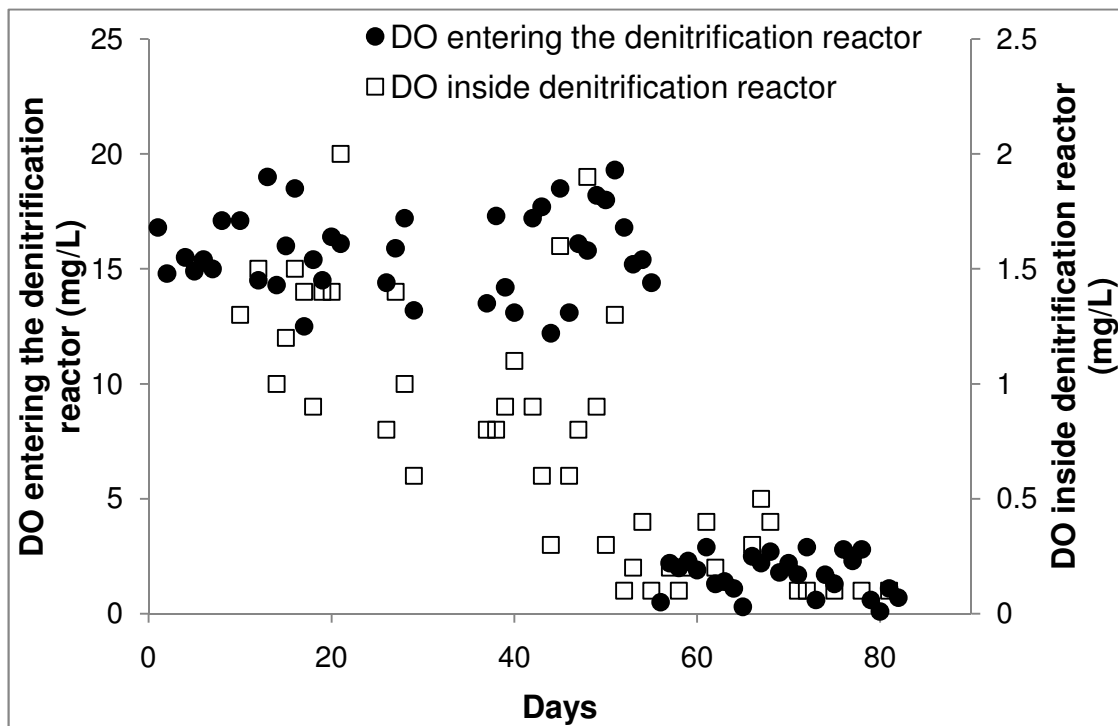
Figure 7.12 DO concentrations entering and inside the denitrification reactor

Jong Hyuk Hwang, Ph.D. Thesis

Average DO concentrations entering the denitrification reactor from day 1-55 and day 56-83 were 15.8 mg O_2/L and 1.7 mg O_2/L, respectively. Average DO concentrations within the denitrification reactor during the same operating periods were 1.0 mg O_2/L and 0.2 mg O_2/L, respectively. As shown in Figure 7.12, the DO in the influent and within the denitrification reactor, was substantially reduced by N_2 gas sparging, so the effect of oxygen on hydrogenotrophic denitrification could be investigated. Figure 7.13 shows the relationship between reactor DO and SDNR.
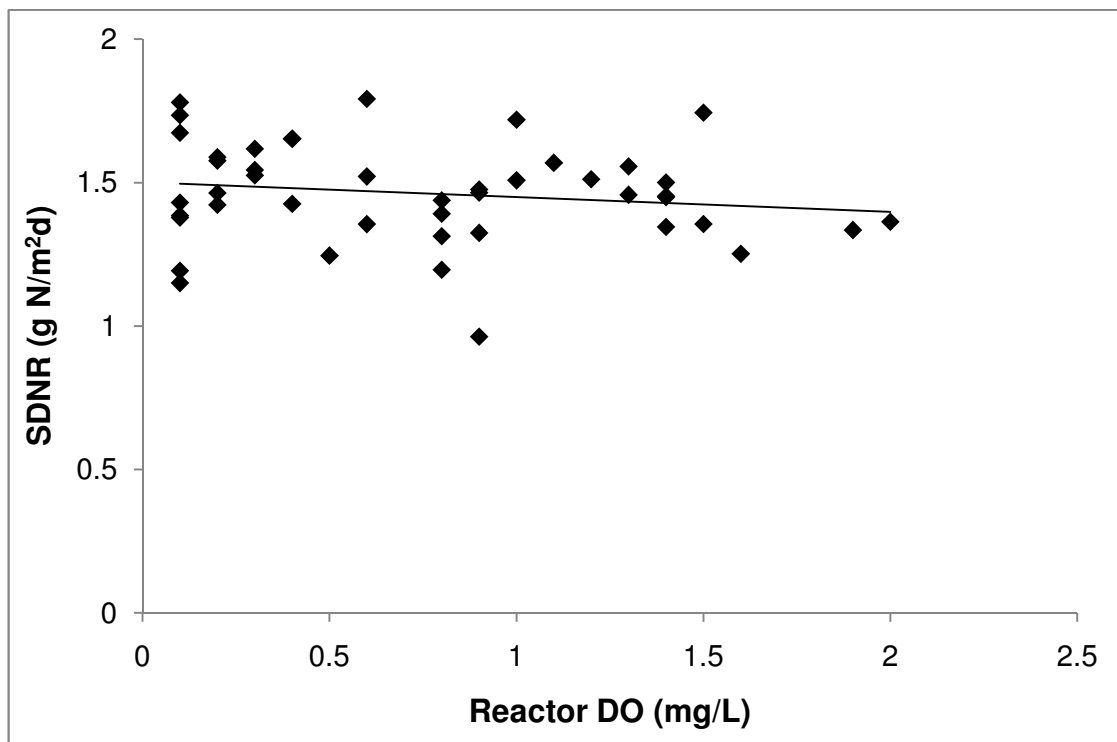
Figure 7.13 Relationship between measured reactor DO and SNDR

Although a slight downward trend of SDNR over increasing DO can be observed in Figure 7.13, the degree of DO effect was not as pronounced as observed in past studies

Jong Hyuk Hwang, Ph.D. Thesis

involving heterotrophic denitrification. Average SDNRs before and after DO control were 1.46 g N/m^{2}d and 1.47 g N/m^{2}d, respectively. Standard deviations were 0.19 and 0.14, respectively.

Based on the experimental data, it can be concluded that the effect of DO on hydrogenotrophic denitrification in consecutive operation is negligible.

As stated earlier, some previous studies (Kurt *et al*., 1987, Gross *et al.* 1986) suggested the possible adverse effect of DO on hydrogenotrophic denitrification. It was suggested that the presence of oxygen would inhibit the activity of hydrogenotrophic denitrification, based on the measurements of 5 % (Kurt *et al*., 1987) and up to 15% (Gross *et al.* 1986) more hydrogen consumption than the stoichiometric requirement (0.36 g H/g N) from Equation (7.4).

$$2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^-$$ (Equation 7.4)

These studies suggested that the reduction of oxygen with hydrogen, producing water - Equation 7.5, would demand more hydrogen supply than required for hydrogenotrophic denitrification.

$$2H_2 + O_2 \rightarrow 2H_2O$$ Equation (7.5)

However, there are two additional issues that need to be considered in such an analysis. Firstly, the stoichiometric equation (Equation (7.4)) used for theoretical

Jong Hyuk Hwang, Ph.D. Thesis

hydrogen consumption in these studies did not include cell growth, causing the underestimation of theoretical hydrogen requirements. A stoichiometric equation for hydrogenotrophic denitrification including cell growth (Equation (7.6), WERF, 2003) suggests 0.48 g H/g N of hydrogen is needed, which is 25% higher than the hydrogen requirement from Equation (7.4).

$$NO_3^- + H^+ + 2.86H_2 + 0.15CO_2 \rightarrow 0.0286C_5H_7NO_2 + 0.49N_2 + 3.14H_2O$$
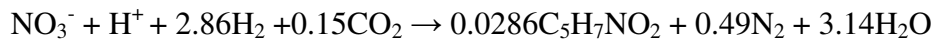
(Equation 7.6)

Secondly, although hydrogen-oxidizing bacteria can grow under micro-oxic conditions, the reaction shown in Equation (7.5) is highly exergonic and needs to be catalyzed by the specific enzyme, *hydrogenase* (Madigan *et al.*, 2009). Therefore, it is highly unlikely that the reaction shown in Equation (7.5) caused the over-requirement of hydrogen (as some studies claimed) without substantial increases in temperature or fire-related problem.

Ho *et al.* (2002) reported that there was an 8 % reduction in denitrification rate under saturated DO condition compared to 2 mg O_2, conditions, while nitrification was highly inhibited with the decrease of DO. The results, shown in Chapter 6.2 regarding the effect of DO from stand-alone operation, did not test denitrification under saturated DO conditions, primarily because achieving saturated DO conditions requires high intensity of air diffusion, which would cause serious scouring of the biofilm.

Jong Hyuk Hwang, Ph.D. Thesis

Cowman *et al.* (2005) reported that the high O_2 pressure for nitrification caused the carry-over of excess oxygen from the nitrification reactor to the subsequent denitrification reactor, resulting in the increase of nitrate concentration in the final effluent. Therefore, they suggested that proper O_2 control should be done to avoid inhibiting denitrification by high oxygen.

The present findings concurred with those of Schnobrich *et al.* (2007) who reported successful treatment of groundwater containing high DO in a pilot-scale study, and those of Sahu *et al.*(2007) who reported that the steady denitrification efficiency could be achieved, regardless of the DO in the influent, in a bench-scale MBfR.

7.3.4 Conclusions

The effect of oxygen on hydrogenotrophic denitrification in MBfR was investigated. The consecutive nitrification-denitrification reactor, representing the system treating non-nitrified secondary effluent from domestic wastewater treatment plants, was operated under various DO conditions.

Most of the residual oxygen from the nitrification reactor was consumed by nitrifiers in the denitrification reactor. Without any DO control, the remaining 1 mg O_2/L (during the first 55 days of experiment) did not result in lower denitrification performance than when the DO was controlled at 0.2 mg O_2/L. SDNR before and after DO control were 1.46 g N/m^{2}d and 1.47 g N/m^{2}d, respectively, suggesting the effect of DO on

Jong Hyuk Hwang, Ph.D. Thesis

hydrogenotrophic denitrification was negligible in the consecutive nitrification and denitrification system. Although this study observed the negligible effect of DO on hydrogenotrophic denitrification in MBfR configuration, it does not mean that the effect of DO on hydrogenotrophic denitrification would be negligible because most of denitrification activity in MBfR would happen at the surface of membrane fiber, where DO was not measured in this thesis. Therefore, the effect of DO should be confirmed in suspended biomass.

7.4 Effect of loading rate on nitrification and denitrification during consecutive operation

7.4.1 Introduction and objectives

Although the nitrification and denitrification performances shown in Table 7.1 and 7.2 were comparable or higher than those from previous similar studies, the reactor was not operated under substrate diffusion-limited condition and the maximum achievable removal rates have not been investigated using PMP membrane, which is newly developed and supposed to have high potential of gas delivery. Since all investigations regarding the effect of various operating parameters, such as DO (Chapter 7.3, Chapter 6.2, Chapter 5.4), temperature (Chapter 6.3), biofilm control (Chapter 7.2) and mineral

Jong Hyuk Hwang, Ph.D. Thesis

precipitation (Chapter 6.1) were completed, the investigation on maximum achievable nitrogen removal rate was initiated.

7.4.2 Materials and methods

The experimental details were as presented in Chapter 7.1, except the HRT. The loading rate was increased by increasing influent flowrate during consecutive operation. Effect of loading rate was investigated for 90 days from March 2009 to May 2009.

7.4.3 Results and discussions

Influent flowrate was raised from 11.6 L/d (HRT 1.03 hr) to 41.4 L/d (HRT 0.29 hr). Figure 7.14 shows the change of HRT during this test. Figure 7.15 shows the NH_4-N concentration in the influent, nitrification reactor effluent, final effluent and HRT over time.

The residual NH_4-N concentration from the nitrification reactor was low enough to be completely removed in following denitrification reactor at HRT of 1 hr. With the decrease of HRT, NH_4-N concentration from nitrification reactor started to increase over 10 mg/L at HRT of 0.3 hr. Even at very low HRT, some nitrification still occurred

Jong Hyuk Hwang, Ph.D. Thesis

in the denitrification reactor, indicating that residual oxygen was fed to the denitrification reactor and that the nitrification reactor was not under oxygen-limited.

Figure 7.16 shows NO_x-N concentration of the influent and effluent of the denitrification reactor, and HRT.
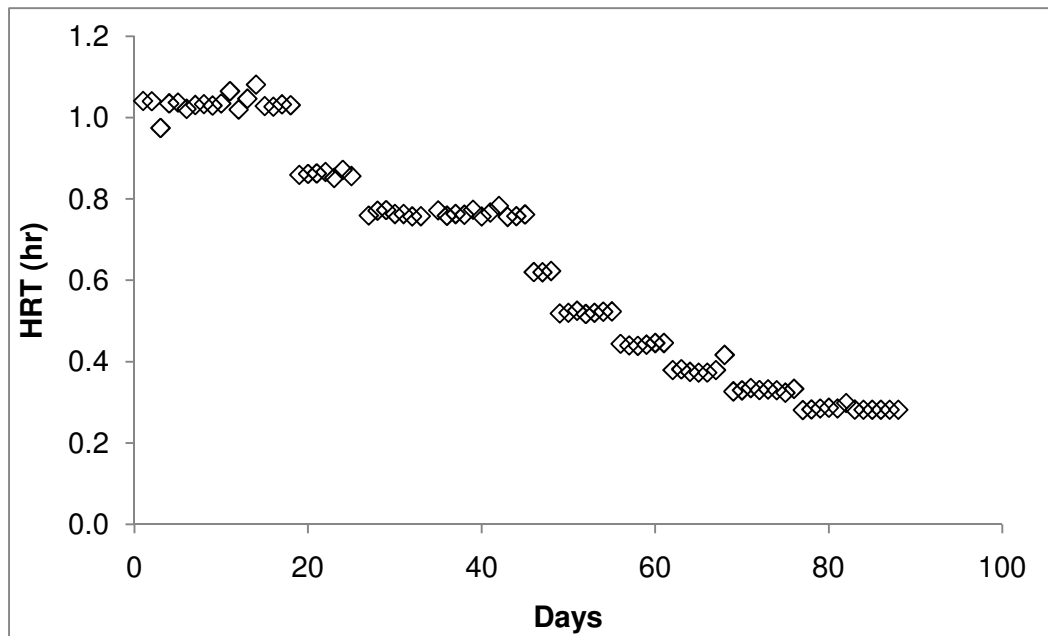
Figure 7.14 Change of HRT

Influent NO_x-N concentration decreased with increasing HRT as NH_4-N incoming from the nitrification reactor to the denitrification reactor decreased. However, the degree of incoming NO_x-N reduction was lower than the increase of NH_4-N from the nitrification reactor effluent as shown in Figure 7.15 indicating that nitrification took place consistently in the denitrification reactor regardless of HRT. Due to the decrease of incoming NO_x-N concentration to the denitrification reactor, the increase of loading rate to denitrification reactor would be less than anticipated by the HRT decrease.

Jong Hyuk Hwang, Ph.D. Thesis
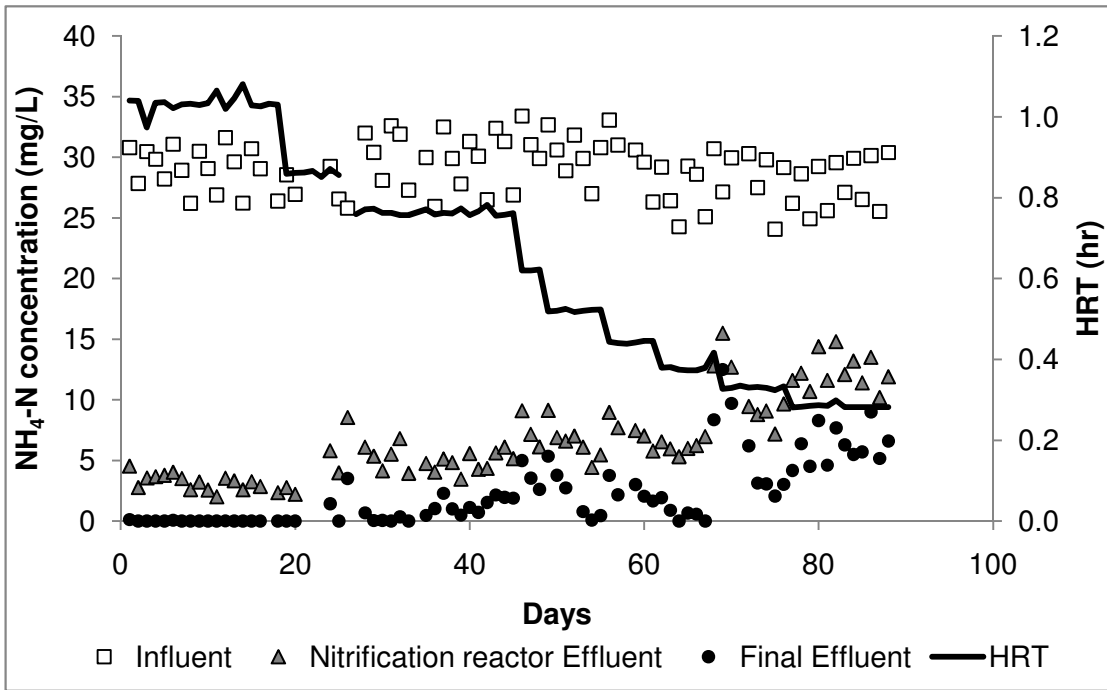
Figure 7.15 Nitrification reactor performance
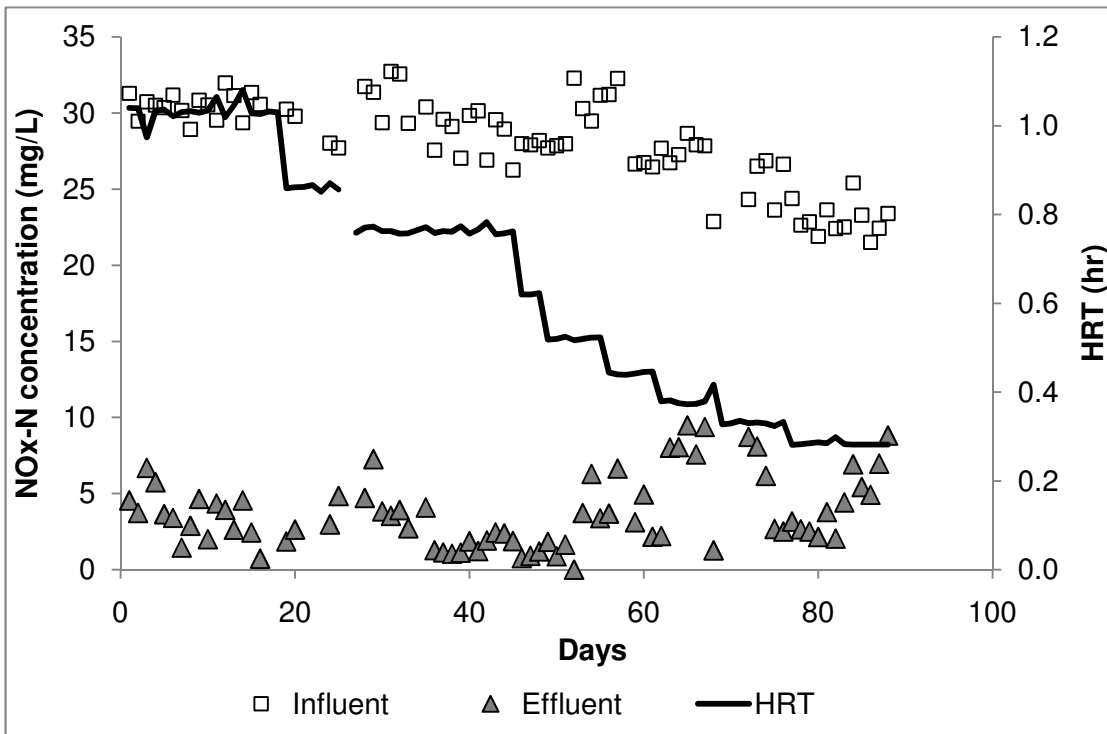
Figure 7.16 Denitrification reactor performance as affected by increasing load

Jong Hyuk Hwang, Ph.D. Thesis

Figure 7.17 shows the relationship between specific loading rate and both SNR and SDNR.
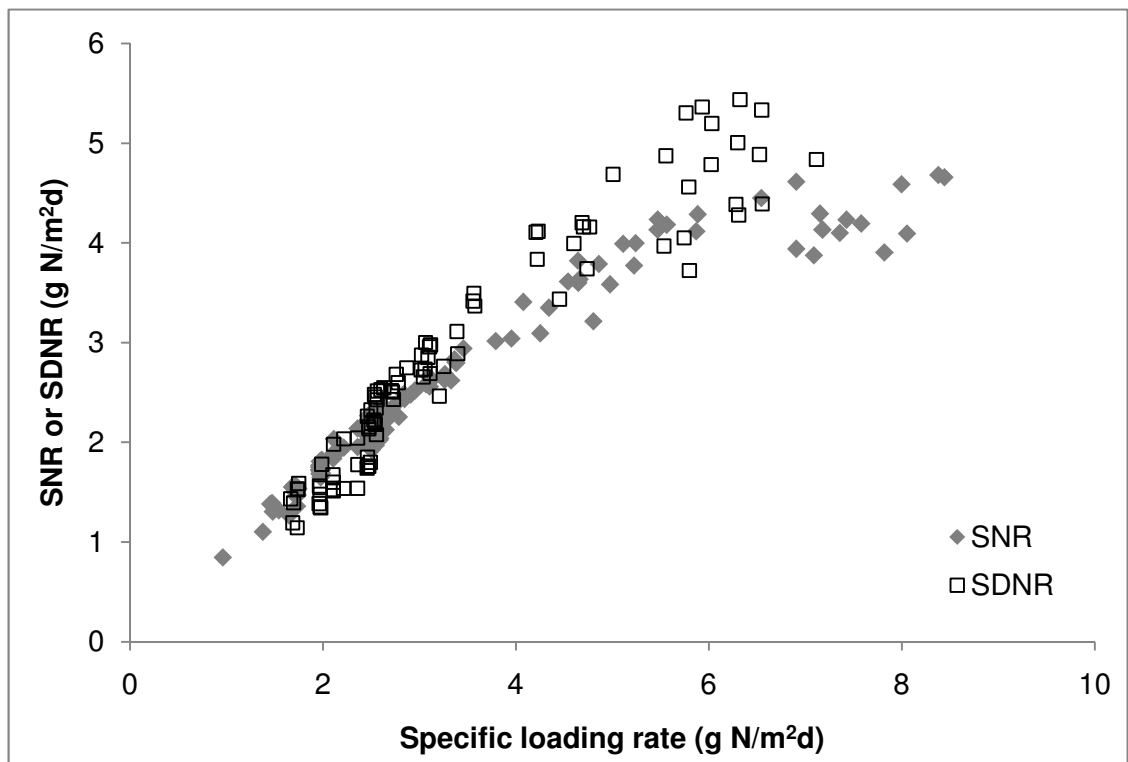
Figure 7.17 Relationship between specific loading rate and SNR, SDNR

Nitrification performance : The relationship between loading rate and SNR exhibits similar trend as in Figure 5.7. SNR increased linearly with specific loading rate, up to around 6 g N/m^{2}d, which was much higher than the 3.5 g N/m^{2}d shown in Figure 5.7. Difference of threshold from the two figures might originate from the difference of membrane used for the experiments. Substrate diffusion limitation seemed to occur at loads above 6 g NH$_4$-N/m^{2}d.

Jong Hyuk Hwang, Ph.D. Thesis

Denitrification performance : The relationship between loading rate and SDNR shows similar trend as SNR, however the substrate diffusion limitation did not seem to occur according to Figure 7.17. Specific loading rate was not as high as that from nitrification, as NH_4-N was not sufficiently nitrified at higher loading. At high loading rates, a more unsteady relationship between loading and SDNR was observed, possibly due to biofilm overgrowth. Apparently, the biofilm control using a steady shear force as described in previous chapter, was not enough to balance off the higher loading, which would induce high biomass generation.   If nitrification happened in the nitrification reactor, it is impossible that SDNR is higher than SNR, however SDNR was higher than SNR due to continuing nitrification in the denitrification reactor.

Although the substrate diffusion limitation did not occur in the denitrification reactor, the maximum achievable loading rate would be below 6 g NH_4-N/m^2d based on the nitrification reactor performance, as the denitrification is subject to the performance of the nitrification.

7.4.4 Conclusions

Effect of loading rate was investigated to find out the maximum achievable nitrogen removal rate in consecutive operation. Specific nitrification rate increased linearly with specific loading rate, up to around 6 g N/m^2d. Denitrification also showed similar trend as in nitrification, however, there was very unsteady relationship between loading and SDNR, due to biofilm overgrowth, in high loading rate. Maximum achievable loading

Jong Hyuk Hwang, Ph.D. Thesis

rate would be below 6 g NH_4-N/m^2d based on the nitrification reactor performance, as the denitrification is subject to nitrification.

Jong Hyuk Hwang, Ph.D. Thesis

8. ENGINEERING SIGNIFICANCE

8.1 Performance comparison with other tertiary treatment options

There are two ways to meet stringent effluent standards for municipalities, 1) reconstruct entire facilities or 2) retrofit or upgrade existing facilities. The topic of upgrading existing facilities has become increasingly important because many municipalities are considering the implementation of additional nutrient removal steps. Upgrading existing facilities rather than reconstructing whole plants has advantages of not incurring huge construction expense allows continuous delivery of service. Some add-on processes also can be done in smaller footprint.

To meet total nitrogen (TN) criteria near the limit of technology, fixed film post-denitrification processes are likely to be installed at a number of facilities. These processes include deep-bed denitrifying filters, fluidized bed reactors, submerged attached growth filters, and moving bed biofilm reactors (Bill *et al.*, 2008).

Since the technology studied in this research project was intended to be applied as a tertiary treatment for nitrogen removal from BOD-removal only facilities, this chapter will evaluate the available technologies and discuss the engineering significance of this study.

Trickling filters would be the conventional types for tertiary nitrification. Earlier experiments have shown that nitrification rates for trickling filters lie between 0.01

Jong Hyuk Hwang, Ph.D. Thesis

and 0.32 g N/m^{2}d for temperatures between 5 and 15 °C (Bovendeur and Klapwijk *et al.*, 1989). Experiments with municipal water report between 0.5 and 2 g N/m^{2}d (Parker *et al.*, 1997). Nitrification rate of 1.6 g N/m^{2}d was measured in an upstream moving bed bioreactor filled with Kaldnes rings, with temperatures varying from 8 to 16°C (Rusten *et al.*, 1995)

Using an integrated fixed-film activated sludge (IFAS) technology or a moving-bed biofilm reactor (MBBR) technology offers a small footprint and high performance.

According to a pilot-scale nitrification study for tertiary treatment with an MBBR, the linear relationship between loading rate and nitrification rate could be achieved up to the specific loading rate of 1.75 g N/m^{2}d (Kaldate *et al.*, 2008), which is lower than 4 g N/m^{2}d found from this study (Figure 7.17). 0.54 kg N/m^3-filter-bed·d (actual volumetric rate is expected to be lower when it is translated to kg N/m^{3}d)) was achieved from a laboratory scale MBBR reactor (Jeong *et al.*, 2006), as 1.22 kg N/m^{3}d (equivalent to 4 g N/m^{2}d) was achieved in this study.

Biological aerated filter (BAF) is more advantageous in terms of space saving over MBBR. Since more biomass could be packed in a BAF, it can have higher volumetric removal rates at similar specific nitrification rate with MBBR (Hansen *et al*., 2006).

The most common add-on option for tertiary treatment removing nitrate would be a denitrification filter. Denitrification filters require the smallest space requirement as an add-on process at existing facilities. The technology involves passing nitrified secondary effluent through a deep-bed filter that contains denitrifying organisms. Suspended solids can be removed through this process as it would act as a filter. The

Jong Hyuk Hwang, Ph.D. Thesis

process requires an external carbon source, typically methanol, for denitrification which results in a significant chemical cost. Despite some concerns associated with the required startup time and cold weather performance, methanol is usually the chemical of choice due to its relatively lower cost and lower yield (Regan *et al.*, 1998). However, adding methanol could cause toxicity in the final effluent due to the residual carbon or the build-up of by-products, requiring further treatment. Methanol also generates high amount of solids, which require backwashing of the filter and further wastage.

Denitrification rate of about 0.8 kg N/m^{3}d could be attained at temperatures of 12 - 15°C with such filters. The denitrification is reduced significantly after back-washing. Moreover, frequent back-washings (several times per day) lead to methanol breakthroughs, which is toxic, due to biofilm loss (Koch and Siegrist, 1997). The effluent TN concentration from a denitrification filter can be expected to be below 3 mg/L (USEPA, 2008).

MBBR has been implemented as well in a number of full-scale applications for secondary effluent polishing (Rusten *et al.*, 1997, Taljemark *et al.*, 2004, Odegaard, 2006). In Northern Europe, MBBR, which uses almost identical equipment to IFAS but has only an attached growth biomass, has proven to be a good nutrient removal choice for facilities with weak waste strength and low operating temperatures (Johnson *et al.*, 2007). Up to 1.4 g N/m^{2}d denitrification rate was attained in pilot scale application (Peric et al., 2008). Using ethanol (2.2 N/m^{2}d) or glycerol (1.9 N/m^{2}d) could boost the denitrification rate up to four times that of methanol (0.49 N/m^{2}d) in pilot-scale MBBR post-denitrification (Bill *et al.*, 2009).

Jong Hyuk Hwang, Ph.D. Thesis

According to Figure 7.17 from this thesis, the maximum achievable loading for nitrification and denitrification was 6 g N/m^2d, and the rates were over 4 g N/m^2d at that loading for both nitrification and denitrification. Volumetric loading rates could be translated to 1.22 kg N/m^3d for nitrification and 0.46 kg N/m^3d for denitrification. The difference of volumetric rates stems from the difference of reactor sizes used. In terms of specific or volumetric nitrification or denitrification rates, the performance from this thesis was comparable or higher than those from currently available tertiary options.

8.2 Cost comparison with existing technology of a denitrification filter

Since the performance of MBfR is similar or superior to other options, it is expected that MBfR can also be an economically feasible option. This chapter will compare the cost of installation, operation and maintenance of denitrifying H_2-driven MBfR and denitrification filter, the most common options for tertiary denitrification. Although the reactor construction cost, equipment prices (mixer, blower and piping), electricity and labour can be calculated by using equations for rough estimation, many assumptions needed to be made, resulting in some uncertainty of costs. Therefore, the cost comparison in this study will be conducted based on the reported cost estimation and case study cost data for denitrification filter by the USEPA (2008).   Theoretical installation (simulation) and case study cost data for denitrification filter were

Jong Hyuk Hwang, Ph.D. Thesis

compared with installation and operation of denitrifying H_2-driven MBfR for nitrate removal.

8.2.1 Simulation case

A model denitrification filter system serving the flowrate of 19,000 m^3/d (5 MGD) with a target NO_3-N effluent concentration of 3 mg/L was used for cost estimation.

Capital cost for theoretical installation case was simulated in the report, "Municipal nutrient removal technologies technical document – Volume 1" by USEPA (2008). Capital cost was estimated by CAPDETWorks software (Version 2.1, Hydromantis Corporation, 2008). This software uses a database to estimate the capital, labour, chemical and energy costs that would be incurred based on the input variables, such as process layout, wastewater characteristics and desired effluent quality. The CAPDET model provides planning-level costs, which estimates were within ± 20 % of the actual costs (Wright et al., 1988). The software includes engineering, site preparation, electrical and control installation and building costs as a capital cost. Land costs were not included.

O&M (operation and maintenance) costs in the simulation include operations labour, maintenance labour, maintenance materials, power consumption, chemical usage (3 g methanol / g N) and additional sludge generation.

Jong Hyuk Hwang, Ph.D. Thesis

Life-cycle costs were calculated for the CAPDETWorks results by first annualizing the capital cost for 20 years at 6 percent interest. The annualized capital cost was calculated according to Equation 5.1.

$$Annualized\_capital\_\cos t = Capital\_\cos t \times [i + \frac{i}{(1+i)^n - 1}] \quad \text{Equation (5.1)}$$

The annualized capital cost was then added to the annual O&M cost to obtain a total annual cost.

The cost obtained from the software, which was based on year 2000 dollar value, was then converted to year 2007 dollar value using the *Engineering News-Record* (ENR) Construction Cost Index, the Marshall & Swift Index, and the Pipe Index.

Capital cost, O&M cost and life-cycle cost from simulation was $4,750,000, $570,000/yr and $984,000/yr, respectively.

8.2.2 Case study cost data

Cost data from The Fiesta Village Advanced Wastewater Treatment Plant in Lee County, Florida, USA was used for cost comparison in this study. This plant consists of an extended aeration oxidation ditch process followed by denitrification filters with methanol feed. Nitrogen is being removed successfully down to 3 mg/L. Although it is permitted and designed for 19,000 m^3/d (5 MGD) capacity, it processed an average of 12,000 m^3/d (3.16 MGD) in 2006 (USEPA, 2008). Four denitrification filters (3 m

Jong Hyuk Hwang, Ph.D. Thesis

$\times$12 m or 10 ft $\times$ 40 ft) were installed at the hydraulic loading rate of 133 $m^3/m^2 \cdot d$ (2.2 gpm/ft^2). All the cost data were from USEPA report, "Municipal nutrient removal technologies reference document, Volume 2 – Appendices, USEPA, 2008".

Denitrification filter was installed at a cost of $930,000 in 1984, when the main upgrades of the plant for BNR occurred. Capital cost was converted to $1,780,000, 2007 dollar value based on the *Engineering News-Record* Capital Cost Index (ENR CCI).

O&M (operation and maintenance) in USEPA report costs include operations labour, maintenance labour, maintenance materials, the power consumption, methanol usage and disposal of additional sludge. Unfortunately, USEPA reported O&M costs associated with overall nitrogen removal, which included denitrification filter as well as nitrification that consumes lots of electricity. Therefore, $260,000/yr for O&M cost in the report cannot be used for direct comparison. However, the report states the costs associated with methanol usage and additional sludge disposal, which are the major concerns in this thesis.

As for other items, such as maintenance labour, maintenance materials and power consumption, assumptions which can not be fully verified without existing discrete data, are used to enable comparison with a model MBfR system.

Assumptions used for power consumption, maintenance labour and maintenance materials were based on the report, "Nutrient reduction technology cost estimations for point sources in the Chesapeake Bay Watershed", prepared by The Nutrient Reduction Technology Cost Task Force, A Stakehold Group of the Chesapeake Bay Program (2002).

Jong Hyuk Hwang, Ph.D. Thesis

Power consumption was estimated on the basis of pumping and other uses at $0.05/kWh. Labour was based on 5 hours/day for 19,000 m^3/d (5 MGD) plant. Labour was considered for a 5-day work and $30/hour to cover salary and fringe benefits. Maintenance costs were developed using 2 % of the capital costs.

Although O&M cost can be compared between denitrification filter and MBfR, the costs from the above option will not be changed. Therefore, only chemical usage and sludge production will be compared for O&M costs, assuming other costs will be same.

Out of $260,000 O&M cost actually spent for nitrogen removal in 2006, only $20,800 was used for methanol purchase and addition.

Methanol consumption and cost were 21,300 kg/yr (47,000 lb/yr) and $12,500 ($ 0.60/kg), respectively. 21,300 kg of methanol expected to generate 14.2 ton of solids/yr or 710 ton of sludge, based on the heterotrophic yield, 0.4 g VSS/g COD and 2% of solids concentration. Based on the operation data, 710 ton of sludge occupied 2.2% of total sludge generation from plant. At the plant's average sludge disposal charge of 4.9 cents/gallon, the total disposal cost of sludge generated from the denitrification filter was $8,300 per year.

$20,000 of chemical and sludge disposal cost seems too small compared to total O&M cost. The reasons were the followings. The first reason was the optimal use of the existing facilities. Some denitrification was achieved in the sludge blanket of the secondary clarifier. The second reason was the actual flow rate was much lower than the design flowrate. Average flow rate during the data collection was 12,000 m^3/d rather than 19,000 m^3/d. The third reason was the NO_3-N concentration incoming to

Jong Hyuk Hwang, Ph.D. Thesis

the denitrification filter was already lowered to 3 mg/L, which was further reduced to 1.45 mg/L through the denitrification filter.

If the denitrification filter were to treat 19,000 m^3/d of secondary effluent (design flowrate) from 25 mg NO_3-N/L to 3 mg NO_3-N/L, the chemical cost and sludge disposal cost would be $290,000 and $193,000, respectively, based on the actual chemical cost and hauling cost, incurred during the plant operation and it will contribute more to O&M costs than before. Based on above estimation, total O&M cost would be $557,600/yr, which will be close to $570,000/yr from the simulation case, shown in Chapter 8.2.1.

8.2.3 H_2-driven MBfR for denitrification

For the estimation of capital cost for H_2-driven MBfR system, the reactor size had to be assumed since no pilot scale data was available from this thesis. Data from the thesis of a former student, Dr. Celmer were also referenced. The maximum achievable specific loading rate of 6 g N/m^2d and corresponding volumetric denitrification rate of 0.46 kg N/m^3d (from Figure 7.17) were used for the capital cost estimation.

As 6 g N/m^2d of loading rate was achieved in lab-scale, it was assumed that only 75% of 6 g N/m^2d (4.5 g N/m^2d) would be achievable in larger scale. The volumetric removal rate was unchanged because the value was found from a one-membrane module system. It was assumed that membrane modules can be more condensely spaced if multiple modules were used. Even though optimum spacing between

Jong Hyuk Hwang, Ph.D. Thesis

membrane modules, which should be close enough to save space but far enough to avoid biofilm clumping and inhibit the substrate diffusion, can be the topic of further research, 25% of volume saving by putting multiple membrane modules was assumed. Thus the assumption for a volumetric loading rate of 0.46 kg N/m^{3}d was not altered.

To treat 19,000 m^3/d of design flow rate containing 25 mg NO$_3$-N/L, 106,000 m^2 of membrane surface area and 1030 m^3 of reactor size (54 m^3/ML) were required. Although the membrane module used for this thesis has a low 0.152 m^2 of surface area, the membrane, which would be used for full-scale, will have higher surface area/membrane module. A 154 m^2 of surface area/membrane was assumed and 690 membrane modules were needed to satisfy the 106,000 m^2 of required surface area (36 modules/ML). Due to high diffusivity of GE's newly developed PMP membrane, the number of required membrane modules and reactor volume were highly reduced from that of Dr. Celmer's work (2009), who reported a minimum 52,500 membrane modules and 50,300 m^3 of reactor size from high ultrasound dosage case to treat 300,000 m^3 of wastewater (175 modules/ML and 167 m^3/ML).

Same capital cost, $1,780,000, as the case study shown in 5.2.2 was assumed, as the used volumetric denitrification rate, 0.46 kg N/m^{3}d, was similar to that of the denitrification filter (Polprasert and Pak, 1986). The membrane cost would contribute a major portion of the capital cost, as it was difficult to subscribe a cost as the membrane was not yet commercialized. An estimate of $1,780,000 ($94,000/ML) would be reasonable if it was compared with around $70,000,000 of capital cost, which was estimated for flowrate of a 300,000 m^3/d ($233,000/ML) by Celmer (2009), as only one third of membrane modules and reactor size was required in this thesis.

Jong Hyuk Hwang, Ph.D. Thesis

Regarding O&M, the expenses associated with labour, power consumption and maintenance of facilities were assumed to be those from Chapter 8.2.2. Only chemical (hydrogen) and additional sludge handling costs were compared. Cost, which would be required for biofilm control, was not included in this calculation.

Based on equation 1-7, in order to reduce 1 g of nitrate to nitrogen gas, 0.41 g of hydrogen gas would be required and 0.29 g of SS (0.23 g of VSS and VSS/SS ratio of 0.8) would be generated. If MBfR were to treat 19,000 m^3/d of secondary effluent from 25 mg NO_3-N/L to 3 mg NO_3-N/L, 63,000 kg of hydrogen gas/yr (41% of 153,000 kg of N/yr) would be required and 35,000 kg of dry solids/yr would be generated. Based on the actual data (2% solids content and 4.9 cent/gallon) from Chapter 5.2, hauling cost would be $22,700/yr. Cost of hydrogen gas varies from $1 to $20 per kilogram (MSNBC, http://www.msnbc.msn.com/id/4563676/, 2004). It may go up in the future due to the tight supply or go down due to the mass production, driven by hydrogen-related industry. Although Celmer used $1.37/kg of hydrogen gas, this thesis used $5/kg to estimate the cost conservatively. Based on $5/kg, the annual hydrogen cost would be $315,000. The sum of chemical and sludge disposal cost would be $337,700 for MBfR case, compared to $483,000 for the denitrification filter. Therefore, MBfR can be an economically feasible option for tertiary denitrification.

One thing to note in this calculation is that the economic feasibility of MBfR highly depends on the cost of hydrogen gas. If the cost of hydrogen exceeds $7.5/kg, MBfR would be more expensive to maintain, compared to denitrification filter.

Finally, Table 8.1 shows the comparison of capital costs, O&M costs and life-cycle cost for all three cases shown.

Jong Hyuk Hwang, Ph.D. Thesis

Table 8.1 Cost comparison table

	Simulated denitrification filter	Actual denitrification filter	MBfR
Capital cost ($)	4,750,000	1,780,000	1,780,000
O&M cost ($/yr)	570,000	557,600	412,300
Life-cycle cost ($/yr)	984,000	712,800	567,500

Table 8.1 shows that MBfR can be an economically viable option for tertiary treatment. However, it should be noted that the economic feasibility of MBfR is strongly correlated to the cost of hydrogen gas.

8.3 Engineering significances

Other than performance and cost perspectives found from this thesis, there are number of findings that would contribute to wide-scale application of MBfR in the future.

Long-term stable operation of sequential nitrification and denitrification was achieved in this study. As explained in Chapter 7.2, the accumulation of excess biofilm is the major concern while operating MBfR, except for nitrification. Some previous studies suffered from biofilm overgrowth, which prevented long-term steady operations. By employing proper biofilm control measures (N_2 gas sparging in this study), MBfR was operated over 400 days, combining continuous operations through

Jong Hyuk Hwang, Ph.D. Thesis

Chapter 7.4, without any major problems. Biofilm thickness was stably maintained over the operational days. System stability would be one of the crucial factors during pilot-or field scale reactor implementation. The long-term stable operation can be then achieved in pilot scale operation, although more studies regarding the degree of shear force such as sparging strength or duration should be conducted to ratify the feasibility of N_2 sparging in larger scale.

Effect of dissolved oxygen on hydrogenotrophic denitrification was investigated in this study and showed no impact on the denitrification performance. It is expected that this finding would make it possible to modify the system configuration further. For example, the nitrification biofilm and denitrification biofilm can be put in the same reactor, avoiding the flow channel or pumping, which would be required in sequential operation. Since the denitrification performance will not be affected by DO in MBfR, which might be leaked or left over from the nitrification reactor, a one-reactor configuration would be worthy of challenging in the future. Another system modification would be a hybrid biofilm process where the hydrogenotrophic bacteria grows on top of the membrane fiber with H_2 supply from the membrane fiber, and the nitrifiers grow in bulk liquid where the oxygen is supplied via blower for nitrification. Although the oxygen is supplied via blower in bulk solution, denitrification within the biofilm would not be significantly affected. It could be even possible to just put a H_2-driven membrane inside existing aeration tank for boosting denitrification. This setup would be similar to other hybrid biofilm systems, reported by Nerenberg (2008), who investigated the nitrification in MBfR and heterotrophic denitrification in bulk liquid, in one reactor. Biofilm control by air sparging rather than N_2 sparging could be

Jong Hyuk Hwang, Ph.D. Thesis

investigated as no significant effect by DO on hydrogenotrophic denitrification was observed.

pH control for denitrification was proven ineffective in this study, as it only increased the precipitation of minerals, which eventually inhibited the diffusion of gas and substrate. Denitrification performed well in pH over 8.5 without pH control. Nitrite accumulation was not observed.

In terms of denitrification performance with changing temperature, the room temperature of controlled lab condition showed the best denitrification performance, indicating that intentional control of temperature in order to improve the performance would be unnecessary. The denitrification would have to be tested at 5 – 20 °C temperature range, temperatures of conventional wastewater treatment in northern hemisphere.

Jong Hyuk Hwang, Ph.D. Thesis

9. FUTURE WORK

9.1 Pilot scale application with a robust membrane

To become a more viable option for tertiary treatment, MBfR needs to be tested in pilot scale. Since multiple membrane modules would be placed for operation, studies regarding optimum spacing between modules and details of biofilm control would be implemented.

As addressed in Chapter 8.3, a modified system configuration can be tested to save reactor volume or avoid pumping or piping by placing nitrification and denitrification membrane biofilm system in a single reactor. A hybrid tertiary system with bulk nitrification can also be tested. Inserting denitrification biofilm into an existing aeration tank would eliminate the needs of a separate reactor to achieve denitrification.

An increase in number of fibers per module from that of lab-scale modules should be tested to provide more surface area in a limited space. In terms of the membrane itself, more robust membranes need to be developed to sustain the conditions of pilot scale operation. One of the obstacles during lab-scale operation was the fragility of the membrane fibers. Once one of fiber was cut by accident, a gas leak from that point resulted in the replacement of the entire membrane module. Pilot-scale operation requires more robust membranes than the lab-scale environment. The membrane manufacturer is aware of this issue, which is expected to be resolved before commercialization.

Jong Hyuk Hwang, Ph.D. Thesis

9.2 Greenhouse gas emission

Heterotrophic denitrification which requires organic carbon inevitably generates carbon dioxide, which is one of the greenhouse gases (CO_2, N_2O and CH_4) arising from wastewater treatment. According to Equation 1-4, denitrification of 1 kg of NO_3-N would induce the emission of 2.62 kg of CO_2.

$$5CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \qquad \text{(Equation 1-4)}$$
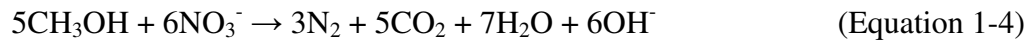
Meanwhile, hydrogenotrophic denitrification uses inorganic carbon as a carbon source for cell growth, therefore it does not generate carbon dioxide, according to Equation 1-7.

$$NO_3^- + H^+ + 2.86H_2 + 0.15CO_2 \rightarrow 0.0286C_5H_7NO_2 + 0.49N_2 + 3.14H_2O$$

$$\text{(Equation 1-7)}$$
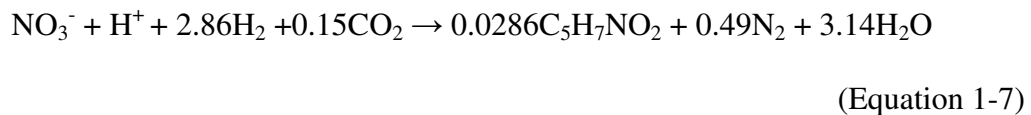
Avoiding the greenhouse gas (CO_2) generation can be considered as one additional advantage of autotrophic denitrification over heterotrophic denitrification nowadays, as it can contribute the reduction of greenhouse gas generation. If the reduction of greenhouse gas by hydrogenotrophic denitrification were estimated by monetary value, it can be estimated as $12,000/yr in the MBfR case from Chapter 8.2.3 using a $30/ton carbon credit (*http://en.wikipedia.org/wiki/Carbon_credit*).

Jong Hyuk Hwang, Ph.D. Thesis

N$_2$O is an important greenhouse gas, having a 300-fold stronger effect than CO$_2$ (IPCC, 2001). Although IPCC guidelines (2006) assume that direct N$_2$O emission from wastewater treatment plants (WWTPs) is minor compared to large N$_2$O emission from nitrification and denitrification in estuaries and rivers, the emission from WWTPs is expected to increase due to wider implementation of BNR systems in the coming years.

N$_2$O emission potential during hydrogenotrophic denitrification has not been explored so far, therefore it would be worthy of investigation. It is known that DO concentration is considered an important parameter controlling N$_2$O emission (Zhang *et al.*, 1994, Tallec *et al.*, 2006, Kampschreur *et al.*, 2008), with low DO concentrations leading to higher emissions.

Since hydrogenotrophic denitrification was successfully conducted in high DO condition, there is a chance of lower N$_2$O emission potential from hydrogenotrophic denitrification than from heterotrophic one. Nitrite, resulting from incomplete nitrification or denitrification, is also known to increase the N$_2$O emission (Schulthess *et al.*, 1995). Nitrite accumulation was not observed in this study.

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Jong Hyuk Hwang, Ph.D. Thesis

11. APPENDIX

Appendix 1. Data for Figure 5.2

	Day	HRT		NH$_4$-N			SNR (g N/m^{2}d)	
		N1	N2	Inf.	HRT 12hr	HRT 7hr	HRT 12hr	HRT 7hr
08/02/2007	1	12.4	6.9	8.7	7.9	7.7	0.020	0.047
09/02/2007	2	10.6	7.9	9.1	7.0	6.8	0.065	0.097
12/02/2007	5	10.1	7.7	8.5	6.3	6.4	0.069	0.089
13/02/2007	6	9.7	7.5	9.4	6.8	7.1	0.087	0.102
14/02/2007	7	9.6	7.1	8.6	7.4	7.7	0.039	0.041
15/02/2007	8	10.1	7.8	9.0	7.9	8.3	0.034	0.029
19/02/2007	12	10.0	7.9	10.6	6.8	8.3	0.124	0.093
20/02/2007	13	9.8	7.9	9.4	5.4	6.8	0.134	0.107
22/02/2007	15	8.6	7.2	9.0	5.4	5.3	0.137	0.165
23/02/2007	16	9.1	7.0	8.0	4.5	5.3	0.127	0.128
27/02/2007	20	9.8	7.4	8.6	4.1	3.1	0.150	0.244
28/02/2007	21	9.1	7.0	8.3	3.7	3.6	0.164	0.218
01/03/2007	22	9.2	6.9	8.3	2.6	3.0	0.202	0.250
05/03/2007	26	10.0	7.5	12.4	4.4	4.7	0.260	0.336
06/03/2007	27	9.3	7.2	11.9	3.9	4.6	0.283	0.333
07/03/2007	28	9.2	7.3	10.9	7.3	6.8	0.128	0.184
08/03/2007	29	9.2	7.2	18.5	11.0	10.7	0.268	0.355
09/03/2007	30	9.4	7.4	16.3	10.6	9.2	0.197	0.312
12/03/2007	33	8.8	7.0	16.0	8.7	10.1	0.269	0.287
13/03/2007	34	9.4	7.3	15.9	7.6	12.2	0.290	0.248
14/03/2007	35	12.3	7.3	16.1	5.9	11.8	0.272	0.254
20/03/2007	41	15.1	7.5	17.9	9.2	12.2	0.187	0.246
21/03/2007	42	16.3	7.9	20.7	16.6	18.1	0.082	0.107
22/03/2007	43	15.0	7.2	22.3	18.9	19.5	0.072	0.124
23/03/2007	44	14.0	7.1	21.7	19.0	20.0	0.063	0.078
26/03/2007	47	15.6	8.7	19.9	19.2	18.0	0.015	0.071
27/03/2007	48	15.3	7.2	20.1	14.6	18.3	0.117	0.081
28/03/2007	49	13.6	7.0	17.2	15.2	16.7	0.047	0.022
29/03/2007	50	15.5	6.4	21.6	16.9	19.8	0.099	0.093
30/03/2007	51	15.9	6.7	22.3	16.2	19.9	0.123	0.116
02/04/2007	54	13.0	7.0	23.4	17.1	18.4	0.158	0.231
03/04/2007	55	15.1	7.4	21.1	17.3	20.5	0.081	0.024
04/04/2007	56	16.8	7.6	20.0	16.3	17.3	0.071	0.113
05/04/2007	57	16.6	7.3	20.0	15.6	17.7	0.085	0.101
06/04/2007	58	16.3	7.0	20.2	15.3	17.8	0.098	0.112
08/04/2007	60	16.0	6.7	20.7	17.6	18.1	0.063	0.126
09/04/2007	61	15.6	6.9	20.7	18.2	18.4	0.052	0.109
10/04/2007	62	14.4	7.2	19.8	17.0	16.8	0.063	0.135
11/04/2007	63	14.9	7.1	19.7	17.9	18.0	0.039	0.078
12/04/2007	64	15.1	7.3	19.7	17.9	18.0	0.039	0.075
13/04/2007	65	14.8	7.1	20.6	15.0	18.1	0.122	0.113
14/04/2007	66	15.8	7.3	19.2	18.0	17.5	0.026	0.079
16/04/2007	68	15.6	6.9	21.3	21.0	18.6	0.007	0.129

Jong Hyuk Hwang, Ph.D. Thesis

17/04/2007	69	15.4	7.3	20.4	19.8	18.4	0.013	0.089
18/04/2007	70	16.1	7.4	19.9	18.8	17.5	0.021	0.103
22/04/2007	74	16.4	7.3	18.7	17.7	17.7	0.020	0.044
25/04/2007	77	7.4	7.3	18.8	14.9	17.1	0.172	0.076
26/04/2007	78	11.5	7.4	18.4	14.2	16.6	0.118	0.079
27/04/2007	79	12.3	7.5	18.8	14.6	16.7	0.111	0.091
28/04/2007	80	15.1	7.5	17.4	13.5	15.2	0.083	0.096
30/04/2007	82	15.2	7.4	17.8	14.4	15.8	0.073	0.089
01/05/2007	83	13.2	7.2	19.0	14.6	15.5	0.110	0.160
02/05/2007	84	13.3	6.9	18.5	14.0	16.3	0.110	0.105
03/05/2007	85	13.0	7.2	20.7	15.1	16.9	0.140	0.171
04/05/2007	86	12.8	7.3	19.7	15.3	18.2	0.112	0.067
05/05/2007	87	12.8	7.4	19.5	12.1	18.0	0.188	0.066
06/05/2007	88	12.7	7.4	19.9	11.6	17.3	0.211	0.113
07/05/2007	89	12.6	7.5	19.2	11.9	17.5	0.189	0.075
08/05/2007	90	12.6	7.5	19.3	11.9	17.4	0.191	0.087
09/05/2007	91	12.8	7.0	19.8	13.6	17.4	0.159	0.109
10/05/2007	92	12.8	6.9	19.8	15.5	17.6	0.110	0.104
11/05/2007	93	13.2	7.0	19.8	13.7	16.8	0.151	0.142
12/05/2007	94	12.8	7.0	19.8	12.6	17.1	0.185	0.130
14/05/2007	96	12.8	7.0	20.3	13.7	17.5	0.168	0.132
22/05/2007	104	12.7	7.2	19.4	16.5	17.1	0.073	0.103

Appendix 2. Data for Figure 4.2.3

Date	Day	HRT	Inf.	Eff.	SNR	Date	Day	HRT	Inf.	Eff.	SNR
24/09/2007	1	10.2	17.5	14.8	0.06	25/10/2007	32	5.6	41.2	23.8	0.76
25/09/2007	2	10.9	19.6	16.7	0.06	26/10/2007	33	5.5	38.4	29.6	0.39
26/09/2007	3	10.9	18.0	13.7	0.10	27/10/2007	34	5.6	27.2	18.0	0.40
27/09/2007	4	10.6	18.9	13.9	0.12	28/10/2007	35	5.7	25.6	15.8	0.42
28/09/2007	5	10.5	17.9	13.0	0.11	29/10/2007	36	5.6	25.3	14.0	0.49
30/09/2007	7	10.5	20.5	14.4	0.14	30/10/2007	37	5.5	30.0	16.8	0.58
01/10/2007	8	10.5	20.6	16.0	0.11	31/10/2007	38	5.5	26.3	9.8	0.72
02/10/2007	9	10.8	18.9	14.6	0.10	01/11/2007	39	5.5	34.2	16.2	0.80
03/10/2007	10	12.1	17.9	11.9	0.12	02/11/2007	40	5.7	33.8	18.2	0.67
04/10/2007	11	11.8	17.8	11.3	0.13	03/11/2007	41	5.5	33.5	15.3	0.80
10/10/2007	17	11.7	11.0	8.6	0.05	04/11/2007	42	5.5	34.4	17.9	0.73
11/10/2007	18	12.0	16.4	12.4	0.08	05/11/2007	43	5.5	33.8	16.3	0.77
12/10/2007	19	11.8	19.6	11.3	0.17	06/11/2007	44	5.7	24.0	6.4	0.76
13/10/2007	20	12.1	18.6	10.2	0.17	07/11/2007	45	5.6	22.2	4.1	0.79
14/10/2007	21	12.0	16.7	9.7	0.14	08/11/2007	46	5.9	23.8	6.7	0.71
15/10/2007	22	11.9	15.5	7.1	0.17	09/11/2007	47	5.6	23.9	8.8	0.66
19/10/2007	26	11.3	20.4	19.0	0.03	10/11/2007	48	5.5	17.1	6.6	0.47
20/10/2007	27	11.3	18.9	13.8	0.11	11/11/2007	49	5.5	18.6	4.2	0.64
21/10/2007	28	11.3	17.7	8.7	0.19	12/11/2007	50	5.4	22.6	4.5	0.81
22/10/2007	29	11.4	16.0	3.3	0.27	13/11/2007	51	5.5	23.3	4.4	0.83
23/10/2007	30	11.4	13.6	3.2	0.22	14/11/2007	52	4.9	22.1	5.9	0.81
24/10/2007	31	5.5	19.3	6.3	0.57	15/11/2007	53	5.1	22.9	1.4	1.02

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 3. Data for Figure 5.3

	Day	HRT	Inf.	Eff.	SNR		Day	HRT	Inf.	Eff.	SNR
30/11/2007	1	1.9	39.4	16.1	0.94	01/02/2008	64	1.4	30.2	3.6	1.53
01/12/2007	2	1.9	40.0	28.2	0.48	02/02/2008	65	1.4	30.9	3.2	1.61
02/12/2007	3	1.9	40.6	29.6	0.45	03/02/2008	66	1.4	31.0	3.0	1.60
03/12/2007	4	1.9	41.0	29.8	0.46	04/02/2008	67	1.4	30.2	2.9	1.59
04/12/2007	5	2.1	40.0	26.6	0.50	05/02/2008	68	1.5	31.3	2.8	1.51
05/12/2007	6	2.1	39.2	27.8	0.43	06/02/2008	69	1.4	32.2	3.1	1.63
06/12/2007	7	2.1	36.2	23.6	0.48	07/02/2008	70	1.4	30.9	4.5	1.50
07/12/2007	8	2.1	35.4	22.6	0.48	08/02/2008	71	1.4	33.4	4.6	1.65
08/12/2007	9	2.0	46.6	30.6	0.62	09/02/2008	72	1.4	31.9	3.4	1.63
09/12/2007	10	2.1	49.6	35.8	0.52	10/02/2008	73	1.4	32.3	3.6	1.64
10/12/2007	11	2.1	50.2	34.8	0.58	11/02/2008	74	1.4	31.1	3.1	1.61
11/12/2007	12	2.1	46.4	26.6	0.73	12/02/2008	75	1.4	32.5	2.8	1.70
13/12/2007	14	2.1	48.4	26.8	0.80	13/02/2008	76	1.4	29.6	2.6	1.54
14/12/2007	15	2.1	29.0	3.4	0.98	14/02/2008	77	1.4	33.1	3.4	1.72
15/12/2007	16	2.3	29.0	1.3	0.93	15/02/2008	78	1.4	32.1	3.3	1.64
16/12/2007	17	2.4	28.6	1.7	0.90	16/02/2008	79	1.4	31.4	3.2	1.62
17/12/2007	18	2.6	30.6	2.1	0.88	17/02/2008	80	1.4	31.0	3.4	1.55
18/12/2007	19	1.9	32.9	3.2	1.22	18/02/2008	81	1.4	31.0	2.8	1.61
19/12/2007	20	1.9	30.9	2.8	1.17	19/02/2008	82	1.4	30.9	2.4	1.62
20/12/2007	21	1.9	35.7	4.6	1.29	20/02/2008	83	1.4	30.7	2.9	1.61
26/12/2007	27	1.9	27.5	2.4	1.05	21/02/2008	84	1.4	29.0	2.4	1.53
27/12/2007	28	1.9	27.0	0.8	1.08	22/02/2008	85	1.4	30.1	2.8	1.56
28/12/2007	29	2.0	28.4	1.3	1.09	23/02/2008	86	1.4	32.8	1.3	1.78
29/12/2007	30	2.0	34.0	2.8	1.24	24/02/2008	87	1.4	32.4	3.6	1.66
30/12/2007	31	1.9	34.1	2.7	1.32	25/02/2008	88	1.4	32.3	3.6	1.64
31/12/2007	32	2.0	39.3	3.2	1.44	26/02/2008	89	1.4	31.8	3.4	1.65
01/01/2008	33	2.0	41.4	3.6	1.50	27/02/2008	90	1.4	32.3	5.0	1.53
02/01/2008	34	2.0	41.8	3.0	1.56	28/02/2008	91	1.4	32.9	3.7	1.65
03/01/2008	35	1.9	37.7	3.1	1.41	29/02/2008	92	1.4	29.8	2.4	1.54
04/01/2008	36	1.9	41.1	3.6	1.53	01/03/2008	93	1.4	31.4	5.3	1.47
05/01/2008	37	1.7	41.3	4.4	1.75	02/03/2008	94	1.4	30.0	4.4	1.45
07/01/2008	39	1.7	35.8	4.4	1.48	03/03/2008	95	1.4	32.8	5.1	1.57
08/01/2008	40	1.7	34.7	2.7	1.50	04/03/2008	96	1.4	32.6	3.1	1.67
09/01/2008	41	1.4	35.9	4.8	1.76	05/03/2008	97	1.6	31.7	4.3	1.33
10/01/2008	42	1.4	35.9	3.0	1.83	06/03/2008	98	1.4	31.2	3.3	1.61
11/01/2008	43	1.4	36.1	4.3	1.78	07/03/2008	99	1.4	33.1	3.4	1.69
12/01/2008	44	1.4	36.7	2.3	1.88	08/03/2008	100	1.4	30.0	3.3	1.52
13/01/2008	45	1.4	34.9	3.1	1.79	09/03/2008	101	1.4	33.3	2.8	1.74
14/01/2008	46	1.4	37.3	3.1	1.92	10/03/2008	102	1.4	31.8	2.5	1.67
15/01/2008	47	1.4	31.4	3.3	1.60	11/03/2008	103	1.4	31.3	3.8	1.59
16/01/2008	48	1.4	33.5	4.0	1.69	12/03/2008	104	1.4	28.4	2.9	1.44
17/01/2008	49	1.4	34.9	3.6	1.80	13/03/2008	105	1.4	34.3	3.7	1.77
18/01/2008	50	1.4	31.8	3.1	1.57	14/03/2008	106	1.4	33.2	3.2	1.72
19/01/2008	51	1.4	34.5	8.9	1.42	15/03/2008	107	1.4	31.1	3.0	1.61
20/01/2008	52	1.4	33.9	3.3	1.67	16/03/2008	108	1.4	31.3	2.7	1.65
21/01/2008	53	1.4	35.6	6.2	1.65	17/03/2008	109	1.4	32.8	2.5	1.75
22/01/2008	54	1.4	33.9	5.2	1.64	18/03/2008	110	1.3	32.3	2.7	1.79
23/01/2008	55	1.4	30.5	4.0	1.52	19/03/2008	111	1.3	31.7	5.8	1.56
24/01/2008	56	1.4	30.8	4.4	1.52	20/03/2008	112	1.4	31.8	9.4	1.29

Jong Hyuk Hwang, Ph.D. Thesis

25/01/2008	57	1.4	30.2	3.8	1.53		21/03/2008	113	1.4	34.1	7.4	1.52
26/01/2008	58	1.4	29.1	3.1	1.47		22/03/2008	114	1.4	33.6	5.0	1.66
27/01/2008	59	1.4	32.1	3.1	1.65		23/03/2008	115	1.4	34.9	5.2	1.66
28/01/2008	60	1.4	32.0	2.4	1.68		24/03/2008	116	1.4	34.0	3.6	1.69
29/01/2008	61	1.4	31.1	2.7	1.56		25/03/2008	117	1.4	31.2	4.8	1.50
30/01/2008	62	1.4	30.6	3.0	1.51		26/03/2008	118	1.4	29.3	4.0	1.43
31/01/2008	63	1.4	34.5	2.5	1.80							

Appendix 4. Data for Figure 5.9

Date	Day	HRT	DO	SNR		Day	HRT	DO	SNR
15/02/2008	1	2.9	0.9	1.42	12/05/2008	88	3.9		1.50
16/02/2008	2	2.9		1.13	13/05/2008	89	3.9		1.51
17/02/2008	3	2.9		0.92	14/05/2008	90	3.9		1.57
18/02/2008	4	2.9	0.3	1.14	15/05/2008	91	3.9		1.60
19/02/2008	5	2.9	0.5	1.24	16/05/2008	92	3.9		1.57
20/02/2008	6	2.9	1.5	1.22	17/05/2008	93	3.9		1.56
21/02/2008	7	2.9	0.3	0.92	18/05/2008	94	3.9		1.55
22/02/2008	8	2.9		1.08	19/05/2008	95	3.9		1.55
23/02/2008	9	2.8	0.1	1.16	20/05/2008	96	3.9		1.42
24/02/2008	10	2.9		1.09	21/05/2008	97	3.9		1.39
26/02/2008	12	2.9	0.0	1.22	22/05/2008	98	3.9		1.37
27/02/2008	13	2.9	0.1	1.08	23/05/2008	99	3.9		1.45
28/02/2008	14	2.9		1.10	24/05/2008	100	3.9		1.60
29/02/2008	15	2.9	0.2	1.06	25/05/2008	101	3.9		1.54
01/03/2008	16	2.9	0.3	0.97	26/05/2008	102	3.9		1.59
02/03/2008	17	2.9	0.2	1.05	27/05/2008	103	3.9		1.60
03/03/2008	18	2.9		1.05	28/05/2008	104	3.9		1.60
05/03/2008	20	2.8	0.0	0.90	29/05/2008	105	3.9		1.61
06/03/2008	21	2.9		1.00	30/05/2008	106	2.3	8.4	1.88
07/03/2008	22	2.9		1.19	31/05/2008	107	2.3	7.9	2.14
08/03/2008	23	2.9	0.1	1.11	01/06/2008	108	2.3	6.4	2.49
09/03/2008	24	2.8	0.2	1.22	02/06/2008	109	2.3	12.7	2.30
10/03/2008	25	2.9		1.13	03/06/2008	110	2.3		2.41
11/03/2008	26	2.8	0.4	1.16	04/06/2008	111	2.3		2.31
12/03/2008	27	2.9	0.2	1.36	05/06/2008	112	2.3		2.28
13/03/2008	28	2.9		1.00	06/06/2008	113	2.3		2.37
14/03/2008	29	2.7	0.0	1.17	07/06/2008	114	2.3		2.47
18/03/2008	33	2.9	0.0	1.16	08/06/2008	115	2.3		2.43
19/03/2008	34	2.9	0.0	0.94	09/06/2008	116	2.3		2.45
20/03/2008	35	2.9		0.86	10/06/2008	117	2.3		2.35
21/03/2008	36	2.9		1.14	11/06/2008	118	2.3		2.37
22/03/2008	37	2.8	0.1	0.83	12/06/2008	119	2.3		2.28
23/03/2008	38	3.0	0.3	0.91	13/06/2008	120	2.3		2.32
24/03/2008	39	2.9	0.0	1.34	14/06/2008	121	2.3		2.36
25/03/2008	40	2.9		0.90	15/06/2008	122	2.3		2.44
26/03/2008	41	2.8		0.94	16/06/2008	123	2.3		2.42
27/03/2008	42	3.0	0.0	0.96	17/06/2008	124	2.3		2.38

Jong Hyuk Hwang, Ph.D. Thesis

28/03/2008	43	2.8		1.84	18/06/2008	125	2.3		2.41
29/03/2008	44	2.9	5.8	1.95	19/06/2008	126	2.3		2.41
30/03/2008	45	2.9	8.0	2.15	20/06/2008	127	1.7		2.67
31/03/2008	46	3.0	0.6	2.12	22/06/2008	129	1.7	6.3	2.90
01/04/2008	47	2.9	0.8	1.89	23/06/2008	130	1.7	15.6	2.43
02/04/2008	48	2.8	0.4	1.99	24/06/2008	131	1.7	14.4	2.36
03/04/2008	49	2.8		1.96	25/06/2008	132	1.7	13.4	2.39
04/04/2008	50	2.9		1.95	26/06/2008	133	1.7		2.80
05/04/2008	51	2.8	0.0	2.01	27/06/2008	134	1.7		2.59
06/04/2008	52	2.9	0.7	1.93	28/06/2008	135	1.7		2.82
07/04/2008	53	2.9	0.3	1.93	29/06/2008	136	1.7	4.2	2.77
08/04/2008	54	2.9	0.2	1.86	01/07/2008	138	1.7	10.8	2.88
09/04/2008	55	2.9	0.3	2.11	02/07/2008	139	1.7	11.4	2.69
10/04/2008	56	2.9	0.8	2.07	03/07/2008	140	1.8		2.32
11/04/2008	57	2.9	0.5	2.04	04/07/2008	141	1.7		2.46
12/04/2008	58	2.9	0.7	1.98	05/07/2008	142	1.8		2.50
13/04/2008	59	2.9		1.95	06/07/2008	143	1.8		2.51
14/04/2008	60	2.9	0.6	2.05	07/07/2008	144	1.7		2.42
15/04/2008	61	2.9	0.6	2.04	08/07/2008	145	1.8		2.54
16/04/2008	62	2.9	0.6	2.00	09/07/2008	146	1.8		2.52
17/04/2008	63	2.9	2.6	1.96	10/07/2008	147	1.8		2.47
18/04/2008	64	2.9	0.5	1.98	11/07/2008	148	1.4	6.4	2.50
19/04/2008	65	2.9	0.4	2.03	12/07/2008	149	1.4	6.2	2.58
21/04/2008	67	2.9	0.4	2.03	13/07/2008	150	1.4	7.4	2.64
22/04/2008	68	2.9	0.5	2.02	14/07/2008	151	1.4	8.5	2.62
23/04/2008	69	2.9	0.7	2.06	15/07/2008	152	1.4		2.48
24/04/2008	70	2.9	4.8	2.14	16/07/2008	153	1.4	9.3	2.61
25/04/2008	71	2.9	7.9	2.20	17/07/2008	154	1.5	12.3	2.39
26/04/2008	72	2.9	5.1	2.01	18/07/2008	155	1.5		2.73
30/04/2008	76	2.9		1.96	19/07/2008	156	1.4		2.69
01/05/2008	77	2.9	1.7	1.88	20/07/2008	157	1.4		2.47
02/05/2008	78	2.9	2.1	1.93	21/07/2008	158	1.4		2.73
03/05/2008	79	2.9	4.3	2.06	22/07/2008	159	1.4		2.47
05/05/2008	81	2.9		1.95	23/07/2008	160	1.4		2.62
06/05/2008	82	2.9	3.7	1.95	24/07/2008	161	1.4		2.46
07/05/2008	83	2.9	3.1	2.07	25/07/2008	162	1.4		2.69
08/05/2008	84	2.9	3.5	2.01	26/07/2008	163	1.5		2.76
09/05/2008	85	3.9	14.0	1.56	27/07/2008	164	1.4		2.66
10/05/2008	86	3.9		1.75	28/07/2008	165	1.4		2.69
11/05/2008	87	3.9		1.47	01/08/2008	169	1.4		2.67

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 5. Data for Figure 5.10(a)

Time (hr)	Time (d)	pH	NH_4-N	NO_2-N	NO_3-N	NOx-N	TIN	Estimated NOx-N
0.00	0.00	7.30	17.70	0.29	13.50	13.79	31.49	13.80
3.00	0.13	7.71	17.00	0.38	13.43	13.81	30.81	13.89
6.13	0.26	7.90	17.60	0.41	13.55	13.96	31.56	14.00
24.10	1.00	8.24	16.90	1.45	13.51	14.96	31.86	14.95
25.60	1.07	8.25	16.80	1.05	13.55	14.60	31.40	15.06
28.50	1.19	8.25	15.50	1.98	13.55	15.53	31.03	15.30
47.00	1.96	8.15	13.60	4.02	14.10	18.12	31.72	17.64
50.70	2.11	8.08	12.90	4.20	14.12	18.32	31.22	18.34
54.70	2.28	7.98	12.50	4.45	14.09	18.54	31.04	19.22
71.30	2.97	7.54	6.60	10.87	14.80	25.67	32.27	24.74
77.00	3.21	7.45	3.20	13.55	15.20	28.75	31.95	27.61

Appendix 6. Data for Figure 5.10(b)

Time (h)	Time (d)	pH	NH_4-N	NO_2-N	NO_3-N	NOx-N	TIN	Estimated NOx-N
0.00	0.00	8.13	36.50	3.80	22.60	26.40	62.90	26.40
1.83	0.08	8.13	35.95	3.80	22.60	26.40	62.35	26.52
6.17	0.26	8.17	33.94	5.96	22.62	28.58	62.51	26.84
22.50	0.94	8.20	31.36	7.54	22.84	30.38	61.74	28.74
25.83	1.08	8.19	30.69	7.85	22.23	30.08	60.76	29.31
29.67	1.24	8.20	30.19	8.61	22.47	31.08	61.27	30.07
46.50	1.94	8.05	26.10	13.90	22.28	36.18	62.27	35.32
50.50	2.10	7.92	24.64	15.70	21.87	37.57	62.21	37.21
51.92	2.16	7.88	23.98	16.60	21.67	38.27	62.25	37.96
53.33	2.22	7.88	22.62	17.50	21.57	39.07	61.69	38.75
60.25	2.51	7.70	17.70	22.80	20.76	43.56	61.26	43.37
71.33	2.97	7.37	4.18	34.30	20.85	55.15	59.33	54.18
73.67	3.07	7.38	1.06	35.70	20.65	56.35	57.41	57.17
77.02	3.21	7.84	0.11	35.90	21.76	57.66	57.77	61.99

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 7. Data for Figure 6.2

Date	Day	HRT (D1)	HRT (D2)	TIN Inf.	DN-1	DN-2	NO3-N Inf.	DN-1	DN-2	NO2-N Inf.	DN-1	DN-2	SDNR (D1)	SDNR (D2)
20/03/2007	1	8.5	17.4	19.4	0.0	0.9	19.4	0.0	0.0	0.0	0.0	0.0	0.76	0.36
21/03/2007	2	9.8	17.8	18.5	0.0	0.0	18.5	0.0	0.0	0.0	0.0	0.0	0.63	0.35
22/03/2007	3	9.9	18.1	29.0	8.1	0.0	29.0	8.1	0.0	0.0			0.71	0.54
23/03/2007	4	9.4	16.1	30.6	8.9	1.5	30.6	8.9	1.5	0.0			0.78	0.61
26/03/2007	7	9.5	18.3	27.1	14.1	5.7	27.1	14.1	5.7	0.0			0.46	0.39
27/03/2007	8	9.1	17.9	29.7	12.8	5.7	29.7	8.7	5.4	0.0	4.1	0.3	0.62	0.45
28/03/2007	9	9.8	16.1	26.3	10.6	7.4	26.3	7.0	6.9	0.0	3.6	0.5	0.54	0.40
29/03/2007	10	12.7	16.9	31.4	14.8	3.9	31.4	9.3	3.7	0.0	5.5	0.2	0.44	0.55
30/03/2007	11	8.1	17.6	30.0	20.0	6.1	30.0	14.4	5.8	0.0	5.6	0.3	0.42	0.46
02/04/2007	14	8.1	17.1	30.6	25.0	8.4	30.6	17.8	7.2	0.0	7.2	1.2	0.23	0.44
03/04/2007	15	7.9	18.4	28.6	20.5	11.4	28.6	14.2	11.4	0.0	6.3	0.0	0.35	0.32
04/04/2007	16	8.2	18.2	31.5	20.5	15.1	31.5	15.3	15.1	0.0	5.2	0.0	0.45	0.30
05/04/2007	17	7.8	18.3	35.1	20.9	19.7	35.1	11.7	19.7	0.0	9.2	0.0	0.61	0.28
06/04/2007	18	7.6	18.1	32.2	19.0	16.1	32.2	12.9	16.1	0.0	6.0	0.0	0.59	0.30
08/04/2007	20	7.3	17.9	31.6	19.0	15.6	31.6	12.9	15.6	0.0	6.0	0.0	0.58	0.30
09/04/2007	21	6.9	17.8	32.0	19.7	16.1	32.0	13.4	16.1	0.0	6.3	0.0	0.60	0.30
10/04/2007	22	6.7	17.4	32.3	20.7	16.8	32.3	14.0	16.8	0.0	6.7	0.0	0.59	0.30
11/04/2007	23	6.6	17.8	26.0	18.2	15.2	26.0	12.1	15.2	0.0	6.1	0.0	0.40	0.21
12/04/2007	24	6.7	18.0	25.4	18.1	13.0	25.4	15.1	11.1	0.0	3.0	1.9	0.37	0.23
13/04/2007	25	6.6	18.0	26.1	20.3	15.7	26.1	18.3	12.7	0.0	1.9	3.0	0.30	0.19
14/04/2007	26	6.6	17.9	24.9	20.0	15.4	24.9	18.1	12.5	0.0	2.0	2.9	0.25	0.18
16/04/2007	28	6.9	17.1	26.5	20.4	17.4	26.5	18.9	14.3	0.0	1.5	3.1	0.30	0.18
17/04/2007	29	7.2	18.0	27.5	20.6	17.8	27.5	17.5	13.0	0.0	3.2	4.8	0.32	0.18
18/04/2007	30	7.7	17.9	28.6	22.4	19.4	28.6	18.5	14.9	0.0	4.0	4.5	0.27	0.17
19/04/2007	31	7.8	17.9	26.3	19.4	20.0	26.3	14.9	14.8	0.0	4.5	5.1	0.30	0.12
22/04/2007	34	7.7	17.9	26.3	19.4	16.1	26.3	14.9	10.8	0.0	4.5	5.3	0.30	0.19
25/04/2007	37	8.5	18.0	26.4	18.4	16.2	29.3	16.6	14.0	0.0	3.8	4.0	0.32	0.19
01/05/2007	43	7.5	17.7	27.0	16.1	12.9	26.0	16.1	6.2	0.0	0.0	6.7	0.49	0.27
02/05/2007	44	8.1	16.8	26.7	16.2	12.8	25.5	16.2	6.2	0.0	0.0	6.5	0.44	0.28
03/05/2007	45	8.4	17.9	31.1	18.4	14.1	31.1	18.4	7.6	0.0	0.0	6.6	0.51	0.32
04/05/2007	46	8.3	17.2	30.6	18.3	14.3	30.6	18.3	7.8	0.0	0.0	6.5	0.50	0.32
05/05/2007	47	7.5	17.7	30.5	19.4	14.1	30.5	19.4	8.1	0.0	0.0	6.0	0.50	0.31
06/05/2007	48	7.4	17.6	29.8	19.3	13.3	29.8	17.8	9.4	0.0	1.5	3.9	0.48	0.32
07/05/2007	49	6.9	17.4	28.6	20.0	16.3	28.6	19.0	15.1	0.0	1.0	1.3	0.42	0.24
08/05/2007	50	8.1	18.0	29.2	20.8	17.3	29.2	20.8	17.3	0.0	0.0	0.0	0.35	0.22
09/05/2007	51	10.2	18.5	27.3	18.5	15.6	27.3	18.5	15.6	0.0	0.0	0.0	0.29	0.21
10/05/2007	52	10.3	18.5	26.7	11.4	14.9	26.7	8.9	14.9	0.0	2.6	0.0	0.50	0.22
11/05/2007	53	6.0	18.4	26.5	19.5	14.8	26.5	18.7	9.8	0.0	0.9	5.0	0.39	0.21
12/05/2007	54	6.0	18.9	27.3	21.7	16.0	27.3	21.2	13.7	0.0	0.5	2.3	0.32	0.20
14/05/2007	56	5.9	18.5	28.1	22.5	17.8	28.1	22.5	15.2	0.0	0.0	2.6	0.32	0.19
15/05/2007	57	5.7	18.6	29.8	24.3	21.0	29.8	24.3	13.2	0.0	0.0	7.8	0.33	0.16
16/05/2007	58	5.4	18.9	29.7	24.7	21.1	29.7	24.7	13.0	0.0	0.0	8.1	0.31	0.15
17/05/2007	59	5.2	18.0	29.5	24.7	20.9	29.5	24.7	12.7	0.0	0.0	8.2	0.31	0.16
18/05/2007	60	5.1	17.9	27.9	24.1	20.0	27.9	24.1	8.8	0.0	0.0	11.2	0.25	0.15
19/05/2007	61	6.0	18.1	29.5	24.0	21.0	29.5	24.0	16.2	0.0	0.0	4.8	0.31	0.16
20/05/2007	62	6.7	18.0	29.5	22.3	19.7	29.5	22.3	19.3	0.0	0.0	0.4	0.36	0.18
22/05/2007	64	6.6	17.7	30.8	22.4	19.4	30.8	22.0	18.8	0.0	0.4	0.5	0.43	0.22
23/05/2007	65	5.9	17.3	26.5	19.1	15.8	26.5	19.1	15.8	0.0	0.0	0.0	0.42	0.21

Jong Hyuk Hwang, Ph.D. Thesis

24/05/2007	66	6.1	17.3	28.9	20.3	17.3	28.9	20.3	17.3	0.0	0.0	0.0	0.47	0.23
25/05/2007	67	7.0	17.9	31.9	20.3	18.2	31.9	20.3	18.2	0.0	0.0	0.0	0.56	0.26
26/05/2007	68	7.0	17.8	32.0	22.7	19.2	32.0	22.7	19.2	0.0	0.0	0.0	0.45	0.24
28/05/2007	70	7.1	17.7	29.1	20.6	17.4	29.1	20.6	17.4	0.0	0.0	0.0	0.40	0.22
29/05/2007	71	7.1	17.4	28.4	19.2	13.3	28.4	19.2	13.3	0.0	0.0	0.0	0.44	0.29
30/05/2007	72	6.8	17.7	28.3	20.2	15.1	28.3	20.2	15.1	0.0	0.0	0.0	0.40	0.25
31/05/2007	73	6.7	17.4	27.8	14.1	12.9	27.8	10.3	4.8	0.0	3.9	8.1	0.68	0.29
01/06/2007	74	6.7	17.4	27.3	14.5	14.1	27.3	9.3	8.0	0.0	5.1	6.2	0.64	0.25
03/06/2007	76	6.7	17.3	27.6	16.8	7.8	27.6	9.4	0.2	0.0	7.3	7.7	0.54	0.39
04/06/2007	77	6.9	17.2	26.2	14.0	6.6	26.2	11.1	0.2	0.0	2.9	6.4	0.60	0.38
05/06/2007	78	7.6	18.1	27.9	13.6	7.7	27.9	8.6	0.0	0.0	5.0	7.7	0.64	0.38
06/06/2007	79	7.5	17.8	30.9	13.9	5.7	30.9	13.9	0.0	0.0	0.0	5.7	0.77	0.48
07/06/2007	80	8.3	18.1	29.6	14.8	5.5	29.6	14.1	0.2	0.0	0.7	5.2	0.60	0.45
08/06/2007	81	6.8	17.8	30.3	17.6	6.2	30.3	17.6	0.0	0.0	0.0	6.2	0.63	0.46
09/06/2007	82	8.3	18.0	32.3	16.5	5.5	32.3	16.5	0.6	0.0	0.0	4.9	0.64	0.50
12/06/2007	85	5.9	17.2	35.1	22.1	6.9	35.1	21.2	5.9	0.0	0.9	1.0	0.75	0.55
13/06/2007	86	6.5	17.5	32.4	18.8	3.4	32.4	18.8	3.4	0.0	0.0	0.0	0.71	0.56
14/06/2007	87	6.6	17.5	28.9	18.4	4.3	28.9	18.4	0.5	0.0	0.0	3.8	0.54	0.47
15/06/2007	88	6.6	17.4	26.3	16.8	5.2	26.3	16.0	0.0	0.0	0.8	5.2	0.49	0.41
17/06/2007	90	5.9	17.5	28.8	20.5	4.1	28.8	20.5	0.0	0.0	0.0	4.1	0.47	0.48
18/06/2007	91	5.8	17.4	27.6	19.9	2.8	27.6	19.9	0.0	0.0	0.0	2.8	0.45	0.48
19/06/2007	92	5.9	17.8	28.2	19.2	2.7	28.2	19.2	0.1	0.0	0.0	2.6	0.51	0.48
20/06/2007	93	6.3	18.1	28.2	17.9	1.1	28.0	17.6	0.3	0.2	0.3	0.8	0.55	0.51
21/06/2007	94	6.8	18.4	30.2	16.0	4.1	30.2	15.8	0.2	0.0	0.2	3.9	0.71	0.48
22/06/2007	95	6.6	18.2	29.6	18.7	5.2	29.6	18.6	3.5	0.0	0.1	1.7	0.55	0.45
23/06/2007	96	6.2	18.1	27.8	20.7	1.5	27.8	20.7	0.0	0.0	0.0	1.5	0.38	0.49
25/06/2007	98	6.3	18.0	28.2	17.9	1.6	28.2	17.9	0.0	0.0	0.0	1.6	0.55	0.50
26/06/2007	99	7.3	18.1	27.6	16.7	2.1	27.6	16.7	1.5	0.0	0.0	0.6	0.50	0.47
27/06/2007	100	7.7	15.1	28.4	16.7	3.9	28.4	16.7	3.0	0.0	0.0	0.9	0.51	0.54
28/06/2007	101	8.1	15.3	28.4	15.8	4.6	28.4	15.8	4.0	0.0	0.0	0.6	0.52	0.52
29/06/2007	102	7.8	15.4	28.2	15.6	8.7	28.0	15.4	8.2	0.0	0.1	0.4	0.54	0.43
30/06/2007	103	7.3	15.1	27.4	15.6	11.0	27.3	15.6	10.9	0.0	0.0	0.1	0.55	0.37
01/07/2007	104	7.3	15.2	26.0	17.8	8.7	25.9	17.8	8.7	0.0	0.0	0.1	0.38	0.38
04/07/2007	107	7.0	15.0	26.2	16.4	0.1	26.2	16.4	0.0	0.0	0.0	0.1	0.47	0.59
05/07/2007	108	6.6	15.1	29.2	17.4	0.2	29.2	17.3	0.1	0.0	0.1	0.1	0.60	0.65
06/07/2007	109	6.9	13.4	28.0	16.2	1.3	27.8	16.1	0.4	0.0	0.1	0.8	0.57	0.67
07/07/2007	110	6.5	13.4	31.0	18.2	8.3	31.0	17.9	1.6	0.0	0.3	6.6	0.67	0.57
09/07/2007	112	7.4	12.7	31.9	14.2	5.7	31.8	13.4	1.5	0.0	0.8	4.2	0.81	0.70
10/07/2007	113	7.3	13.2	33.6	19.8	12.0	33.4	19.3	6.2	0.0	0.3	5.6	0.64	0.55
11/07/2007	114	7.7	13.3	30.6	16.8	6.6	30.6	16.0	2.4	0.0	0.7	4.1	0.61	0.61
12/07/2007	115	8.5	13.2	29.3	13.5	9.2	29.2	12.3	7.3	0.0	1.1	1.9	0.63	0.51
13/07/2007	116	8.2	13.4	29.1	11.9	9.8	29.1	10.6	9.8	0.1	1.3	0.1	0.71	0.48
15/07/2007	118	5.2	13.0	31.3	18.4	4.0	31.2	18.2	2.6	0.0	0.1	1.3	0.84	0.71
16/07/2007	119	5.6	13.1	31.7	17.9	4.2	31.6	17.7	3.5	0.0	0.1	0.6	0.83	0.71
17/07/2007	120	7.3	13.2	29.3	13.5	7.3	29.1	13.4	7.1	0.0	0.1	0.2	0.73	0.56
18/07/2007	121	7.6	13.3	30.0	12.5	13.5	29.7	12.1	13.2	0.0	0.3	0.1	0.77	0.42
19/07/2007	122	7.5	13.4	30.4	14.1	17.2	30.1	13.6	16.8	0.0	0.3	0.1	0.73	0.33
20/07/2007	123	8.2	13.4	30.8	12.8	17.8	30.8	12.6	17.7	0.0	0.2	0.1	0.74	0.33
21/07/2007	124	5.9	13.6	29.3	17.9	17.8	29.3	17.8	17.6	0.1	0.1	0.1	0.66	0.29
23/07/2007	126	5.8	13.4	30.6	18.4	16.8	30.5	18.3	16.7	0.1	0.1	0.1	0.71	0.35
24/07/2007	127	5.0	13.1	28.4	19.1	9.0	28.3	17.5	8.0	0.0	1.6	0.9	0.62	0.50
25/07/2007	128	4.8	13.1	28.9	18.9	9.7	28.6	18.4	1.5	0.1	0.4	8.3	0.70	0.49
26/07/2007	129	4.8	12.9	28.9	19.4	8.9	28.7	19.3	1.8	0.0	0.1	7.2	0.67	0.52

Jong Hyuk Hwang, Ph.D. Thesis

27/07/2007	130	6.7	13.1	28.4	14.1	9.6	28.3	14.0	3.3	0.0	0.1	6.4	0.72	0.48
29/07/2007	132	6.4	13.1	27.8	18.1	11.4	27.7	18.0	8.7	0.1	0.1	2.7	0.51	0.42
30/07/2007	133	6.7	13.1	25.5	17.2	10.3	25.4	17.1	5.8	0.0	0.1	4.5	0.42	0.39
31/07/2007	134	6.0	13.2	31.3	21.0	12.3	31.0	20.8	12.2	0.0	0.0	0.0	0.57	0.48
01/08/2007	135	6.3	13.3	29.1	19.1	11.3	29.1	19.0	5.9	0.0	0.1	5.4	0.54	0.45
02/08/2007	136	6.5	13.4	29.9	18.3	11.5	29.9	18.2	6.7	0.0	0.1	4.8	0.60	0.46
03/08/2007	137	7.0	13.0	29.4	16.4	9.3	29.4	16.4	5.5	0.0	0.0	3.7	0.62	0.52
04/08/2007	138	6.7	13.3	28.5	17.4	6.9	28.4	17.4	6.2	0.0	0.0	0.6	0.56	0.55
06/08/2007	140	6.4	13.2	27.0	18.6	8.1	26.9	18.6	7.6	0.0	0.0	0.5	0.44	0.48
07/08/2007	141	6.6	13.2	28.4	17.1	10.9	28.3	17.1	10.4	0.0	0.0	0.5	0.58	0.45
08/08/2007	142	6.5	13.5	29.3	17.2	9.4	29.3	17.2	8.3	0.0	0.0	1.1	0.63	0.50
09/08/2007	143	6.1	13.0	29.1	14.0	11.7	28.8	13.8	11.1	0.0	0.2	0.6	0.83	0.45
13/08/2007	147	7.2	13.1	27.9	12.8	8.3	27.8	12.7	7.9	0.0	0.1	0.4	0.71	0.51
14/08/2007	148	7.3	13.7	28.6	14.0	12.1	28.3	13.7	11.0	0.0	0.2	1.0	0.67	0.41
15/08/2007	149	7.2	12.7	27.8	13.6	9.2	27.6	13.5	9.0	0.0	0.1	0.1	0.66	0.49
16/08/2007	150	6.9	12.2	28.4	12.1	9.6	28.3	12.0	9.4	0.0	0.1	0.1	0.79	0.52
17/08/2007	151	8.0	13.7	28.9	13.9	12.5	28.8	13.8	12.4	0.1	0.1	0.1	0.63	0.40
19/08/2007	153	7.2	13.4	29.0	21.2	11.3	28.7	21.1	3.7	0.0	0.1	7.6	0.36	0.44
20/08/2007	154	7.4	13.7	29.4	19.9	12.2	29.3	19.5	3.7	0.0	0.3	8.5	0.44	0.43
21/08/2007	155	7.0	13.7	30.6	26.4	18.2	30.2	26.1	8.9	0.0	0.0	8.9	0.20	0.31
22/08/2007	156	6.3	13.7	30.8	18.7	22.6	30.4	16.0	22.3	0.1	2.6	0.2	0.65	0.20
23/08/2007	157	7.3	13.6	29.8	17.0	20.7	29.7	10.0	19.9	0.0	7.0	0.9	0.59	0.23
24/08/2007	158	7.6	13.6	26.9	12.5	15.3	26.9	8.6	6.4	0.0	3.9	8.9	0.64	0.29
25/08/2007	159	7.5	13.6	27.8	17.3	14.6	27.6	10.6	0.2	0.1	6.7	14.3	0.47	0.33
27/08/2007	161	6.3	13.3	28.8	20.3	12.6	28.7	18.0	0.3	0.1	2.3	12.3	0.45	0.41
28/08/2007	162	5.8	13.6	28.4	21.0	12.1	28.2	16.7	0.2	0.2	4.2	11.9	0.43	0.40
29/08/2007	163	8.1	13.6	28.3	19.7	12.3	28.2	11.4	0.2	0.1	8.3	12.1	0.36	0.40
30/08/2007	164	8.1	13.5	29.2	19.8	13.0	28.9	9.8	0.5	0.2	10.0	12.4	0.39	0.41
31/08/2007	165	8.5	13.5	29.2	20.7	13.0	29.1	11.2	0.3	0.0	9.5	12.7	0.34	0.40
02/09/2007	167	7.4	13.3	29.6	22.0	12.4	29.6	18.3	0.7	0.0	3.7	11.7	0.35	0.44
03/09/2007	168	7.9	13.1	29.2	21.9	13.8	29.2	15.1	1.2	0.0	6.8	12.6	0.31	0.40
04/09/2007	169	7.8	13.2	28.9	21.1	12.0	28.9	14.9	0.8	0.0	6.2	11.2	0.34	0.43
05/09/2007	170	7.8	13.1	29.4	21.9	10.9	29.3	19.2	0.0	0.0	2.7	10.9	0.33	0.48

Appendix 8. Data for Figure 6.6

		TSS generation (mg)			VSS generation (mg)			ISS generation (mg)		
	Day	HRT 7	HRT 13.5	HRT 18	HRT 7	HRT 13.5	HRT 18	HRT 7	HRT 13.5	HRT 18
	0	0.0		0.0	0.0		0.0	0.0		0.0
30/03/2007	11	N.A			N.A			N.A		
25/04/2007	37	1384.8		1391.1	249.3		278.2	1135.5		1112.9
09/05/2007	51	645.7		1419.6	297.0		333.6	348.7		1086.0
29/05/2007	71	712.6		1049.2	407.2		312.7	305.4		736.5
14/06/2007	87	528.6		967.6	389.0		388.0	139.6		579.6
03/07/2007	106	617.0	824.5		393.0	387.5		224.0	437.0	
23/07/2007	126	892.9	631.4		523.2	353.6		369.7	277.8	
08/08/2007	142	670.9	661.6		387.2	345.2		283.7	316.3	
21/08/2007	155	574.0	686.1		342.1	330.7		231.9	355.4	

Jong Hyuk Hwang, Ph.D. Thesis

06/09/2007	170	871.2	725.2		407.7	254.5		463.5	470.7	

Appendix 9. Data for Figure 6.11

Date	Day	Feed	Reactor	SDNR	Date	Day	Feed	Reactor	SDNR
11/11/2008	1				15/01/2009	66	6.5	6.0	0.85
12/11/2008	2				16/01/2009	67		6.3	0.84
13/11/2008	3	5.8	0.0		17/01/2009	68			0.88
14/11/2008	4	6.6	0.1		18/01/2009	69		6.2	0.86
15/11/2008	5	5.6	0.1	0.73	19/01/2009	70	7.1	5.4	0.81
16/11/2008	6	4.9	0.3	0.62	20/01/2009	71		5.5	1.06
17/11/2008	7	6.5	0.5	0.57	21/01/2009	72	6.0	3.7	0.95
18/11/2008	8	6.3	0.2	0.55	22/01/2009	73	6.7	4.8	0.99
19/11/2008	9	5.6	0.1	0.53	23/01/2009	74			0.82
20/11/2008	10	6.6	0.2	0.63	24/01/2009	75			0.87
21/11/2008	11	6.9	0.2	0.70	25/01/2009	76			0.96
22/11/2008	12	6.5	0.2	0.76	26/01/2009	77			0.90
23/11/2008	13	6.2	0.5	0.66	27/01/2009	78		5.4	0.89
24/11/2008	14			0.65	28/01/2009	79	1.4	0.0	
25/11/2008	15	7.4		0.58		80	0.7	0.0	0.63
26/11/2008	16	5.6	0.4	0.63	30/01/2009	81	0.1	0.0	0.70
27/11/2008	17	6.9	0.4	0.57	31/01/2009	82	0.7	0.0	0.78
28/11/2008	18	4.8	0.6	0.69	01/02/2009	83			1.02
29/11/2008	19	5.0	0.9	0.70	02/02/2009	84	0.0	0.0	0.75
30/11/2008	20			0.69	04/02/2009	86			1.02
01/12/2008	21			0.68	05/02/2009	87			0.80
02/12/2008	22			0.62	07/02/2009	89			0.84
03/12/2008	23			0.62	08/02/2009	90	0.3	0.0	0.78
04/12/2008	24			0.64	09/02/2009	91			0.82
05/12/2008	25			0.73	10/02/2009	92			0.74
06/12/2008	26			0.75	11/02/2009	93	0.4	0.0	0.79
07/12/2008	27			0.64	12/02/2009	94			0.88
08/12/2008	28			0.85	13/02/2009	95			0.87
09/12/2008	29			0.90	15/02/2009	97			0.88
10/12/2008	30			0.70	16/02/2009	98			0.87
11/12/2008	31			0.81	17/02/2009	99			0.86
12/12/2008	32			0.82	18/02/2009	100			0.92
13/12/2008	33			0.89	19/02/2009	101			0.94
14/12/2008	34	8.6	0.4	0.81	20/02/2009	102			0.94
15/12/2008	35			0.77	21/02/2009	103	1.6	0.0	0.92
17/12/2008	37			0.88	22/02/2009	104	1.1	0.3	0.86
18/12/2008	38			0.90	23/02/2009	105			0.85
23/12/2008	43	5.6	1.0	0.79	24/02/2009	106	5.3	0.6	0.79
25/12/2008	45	8.4	0.9	0.80	25/02/2009	107	6.4	1.2	0.88
27/12/2008	47	6.0	4.4	0.76	26/02/2009	108			0.87
28/12/2008	48	5.2	5.7	0.73	27/02/2009	109			0.90
29/12/2008	49	5.6	5.2	0.65	01/03/2009	111			0.83
30/12/2008	50	6.5	5.6	0.62	02/03/2009	112			0.80
31/12/2008	51	5.6	6.0	0.52	03/03/2009	113			0.95
01/01/2009	52	6.5	4.9	0.74	04/03/2009	114	7.8	0.8	0.92
02/01/2009	53			0.82	05/03/2009	115			0.90

Jong Hyuk Hwang, Ph.D. Thesis

Date	Day			
03/01/2009	54			0.85
04/01/2009	55			0.88
05/01/2009	56			0.98
06/01/2009	57			0.90
07/01/2009	58			0.88
08/01/2009	59			0.94
09/01/2009	60			0.96
10/01/2009	61	5.8	5.4	0.98
11/01/2009	62			0.87
12/01/2009	63			0.90
13/01/2009	64	5.3	5.2	0.94
14/01/2009	65		5.9	0.94

Date	Day			
06/03/2009	116			0.88
07/03/2009	117		0.6	0.87
08/03/2009	118			0.87
09/03/2009	119		0.8	0.81
10/03/2009	120	4.6	1.4	0.89
11/03/2009	121			0.84
12/03/2009	122			0.92
13/03/2009	123		2.2	0.81
14/03/2009	124		1.3	0.87
15/03/2009	125		3.1	0.76
16/03/2009	126			0.65

Appendix 10. Data for Figure 6.12

Date	Day	HRT	Temp.	TIN (mg/L) INF	EFF	SDNR (g N/m2d)
03/04/2009	1	3.6		31.2	17.0	1.00
04/04/2009	2	3.6	23.0	31.2	16.4	1.03
05/04/2009	3	3.9	25.0	30.3	11.9	1.18
06/04/2009	4	3.7	21.5	30.7	15.0	1.06
07/04/2009	5	3.7	22.5	30.9	17.1	0.94
08/04/2009	6	3.7	22.5	31.6	17.3	0.98
09/04/2009	7	3.8	13.0	32.0	20.6	0.77
10/04/2009	8	3.8	14.0	32.1	20.8	0.75
11/04/2009	9	3.8	13.0	31.9	20.5	0.76
12/04/2009	10	3.9	12.0	30.9	20.1	0.71
13/04/2009	11	3.8	13.0	30.7	20.0	0.71
14/04/2009	12	3.8	13.0	31.0	20.2	0.73
15/04/2009	13	3.8	13.0	30.4	19.5	0.73
16/04/2009	14	3.9	8.0	31.2	25.2	0.39
17/04/2009	15	3.6	9.0	31.4	26.4	0.35
18/04/2009	16	3.6	8.0	32.1	27.2	0.35
19/04/2009	17	3.6	8.5	30.4	26.2	0.30
20/04/2009	18	3.6	8.0	30.4	26.7	0.26
21/04/2009	19	3.6	8.0	31.6	27.5	0.29
22/04/2009	20	3.6	8.0	31.6	27.8	0.27
23/04/2009	21	3.4	13.0	30.4	20.2	0.74
24/04/2009	22	3.5	13.5	30.7	20.5	0.74
25/04/2009	23	3.5	13.0	31.7	21.2	0.77
26/04/2009	24	3.5	13.0	30.4	20.6	0.71
27/04/2009	25	3.4	13.0	29.1	19.8	0.68
28/04/2009	26	3.6	13.0	31.6	21.0	0.76
29/04/2009	27	3.4	12.5	31.2	20.7	0.77
30/04/2009	28	3.4	21.0	31.7	17.1	1.10
01/05/2009	29	3.3	21.0	32.8	19.0	1.04
02/05/2009	30	3.2	22.0	32.1	18.0	1.10
03/05/2009	31	3.4	22.0	33.4	19.3	1.05

Date	Day	HRT	Temp.	TIN (mg/L) INF	EFF	SDNR (g N/m2d)
04/05/2009	32	3.5	22.0	33.3	18.5	1.07
05/05/2009	33	3.6	22.0	30.2	16.8	0.95
06/05/2009	34	3.9	22.0	31.6	16.3	0.99
07/05/2009	35	3.8	27.0	31.0	14.7	1.09
08/05/2009	36	3.8	28.5	31.3	14.0	1.14
09/05/2009	37	3.8	28.5	30.9	14.0	1.12
10/05/2009	38	3.8	28.0	30.8	14.2	1.10
11/05/2009	39	3.9	28.0	31.6	15.6	1.05
12/05/2009	40	3.9	28.5	32.4	15.6	1.09
13/05/2009	41	3.9	28.5	31.8	15.3	1.07
14/05/2009	42	3.8	28.0	32.7	17.1	1.05
15/05/2009	43	3.7	34.5	31.7	15.8	1.09
16/05/2009	44	3.7	34.0	32.3	16.1	1.10
17/05/2009	45	3.7	34.0	32.5	16.1	1.11
18/05/2009	46	3.8	33.5	32.9	17.3	1.05
19/05/2009	47	3.8	35.0	33.0	15.8	1.14
20/05/2009	48	3.7	34.5	32.5	16.4	1.09
21/05/2009	49	3.7	34.0	32.0	15.5	1.11
22/05/2009	50	3.9	29.0	33.1	15.3	1.16
23/05/2009	51	3.8	28.0	32.8	16.3	1.10
24/05/2009	52	3.8	28.5	32.6	16.6	1.06
25/05/2009	53	3.9	28.0	32.7	16.0	1.09
26/05/2009	54	3.8	28.5	32.3	15.3	1.12
27/05/2009	55	3.9	28.0	31.8	15.5	1.07
28/05/2009	56	3.9	28.5	32.1	15.3	1.10
29/05/2009	57	3.8	23.0	32.7	16.4	1.08
30/05/2009	58	3.9	23.0	34.7	17.9	1.08
31/05/2009	59	3.9	23.0	32.5	16.3	1.06
01/06/2009	60	3.9	23.0	32.4	16.8	1.02
02/06/2009	61	3.9	23.0	31.8	16.3	1.02
03/06/2009	62	3.9	23.0	31.8	15.8	1.05

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 11. Data for Figure 7.2

	Day	Inf	Nitrification reactor effluent	Final effluent	SNR		Day	Inf	Nitrification reactor effluent	Final effluent	SNR
09/07/2008	1	32.1	3.7	0.3	1.81	08/11/2008	122		3.6	0.9	1.77
10/07/2008	2		6.9	0.5	1.89	09/11/2008	123	32.8	5.8	1.4	1.96
11/07/2008	3	31.6	3.3	0.2	1.83	10/11/2008	124	31.5	4.6	1.3	1.94
12/07/2008	4	31.6	2.9	0.3	1.84	11/11/2008	125	29.0	3.8	0.4	1.81
13/07/2008	5	29.7	2.8	0.2	1.72	12/11/2008	126	33.3	5.4	1.1	2.02
14/07/2008	6	33.6	3.3	0.2	1.96	13/11/2008	127	30.8	4.2	1.5	1.97
15/07/2008	7	31.4	3.7	0.1	1.79	14/11/2008	128		2.8	0.6	1.65
16/07/2008	8	34.9	4.7	0.3	1.97	15/11/2008	129		7.6	0.9	2.04
17/07/2008	9	33.3	3.6	0.1	1.92	16/11/2008	130	30.6	3.4	0.5	1.88
18/07/2008	10		4.0	0.2	2.02	17/11/2008	131		3.1	0.3	1.74
19/07/2008	11	31.5	3.5	0.2	1.82	18/11/2008	132	33.2	3.9	0.3	2.06
20/07/2008	12		4.6	0.4	2.00	20/11/2008	133	31.0	2.9	0.2	1.98
21/07/2008	13	31.9	3.5	0.3	1.85	21/11/2008	134	28.1	3.7	1.2	1.69
22/07/2008	14	32.4	3.9	0.7	1.86	22/11/2008	135	33.0	2.8	0.4	2.12
23/07/2008	15	33.4	3.4	0.2	1.93	23/11/2008	136	32.6	3.3	0.0	2.09
24/07/2008	16	33.9	3.9	0.4	1.95	24/11/2008	137	30.4	3.1	0.6	1.93
25/07/2008	17	31.8	3.3	0.0	1.83	25/11/2008	138	31.9	3.1	1.1	2.08
26/07/2008	18	32.9	3.9	0.7	1.81	26/11/2008	139	31.4	3.1	0.9	1.97
27/07/2008	21		3.8	0.6	2.08	27/11/2008	140	28.1	2.7	0.8	1.79
28/07/2008	22	35.0	5.2	1.2	1.86	28/11/2008	141	32.1	3.0	0.9	2.06
29/07/2008	23	33.2	4.5	0.8	1.78	29/11/2008	142	31.4	3.2	0.6	1.96
01/08/2008	24	34.0	4.3	0.8	1.85	30/11/2008	143		2.5	0.7	1.58
02/08/2008	25	32.2	4.0	0.5	1.73	01/12/2008	144	32.8	3.5	1.4	2.06
03/08/2008	26	31.9	3.9	0.4	1.72	02/12/2008	145	30.6	2.9	1.0	1.91
04/08/2008	28	30.9	3.3	0.6	1.74	03/12/2008	146	28.4	2.8	0.4	1.77
05/08/2008	29	34.3	4.4	0.7	1.84	04/12/2008	147	33.0	3.2	1.1	2.14
06/08/2008	30	32.7	4.0	1.0	1.85	05/12/2008	148	28.8	3.4	1.3	1.75
08/08/2008	31	32.8	4.3	0.9	1.85	06/12/2008	149		2.5	0.6	1.68
09/08/2008	32	28.5	2.7	0.5	1.70	07/12/2008	150	29.3	3.4	0.2	1.86
10/08/2008	33	31.5	3.1	1.0	1.75	08/12/2008	151	30.4	3.3	0.4	1.94
11/08/2008	34	30.5	2.7	0.8	1.90	09/12/2008	152	33.7	3.4	0.1	2.12
12/08/2008	35	32.9	2.9	0.6	1.96	10/12/2008	153		2.8	0.5	1.65
13/08/2008	36	31.8	3.4	0.3	1.84	11/12/2008	154	32.9	3.3	0.0	2.07
14/08/2008	37	33.6	3.7	0.6	1.97	12/12/2008	155	30.0	2.5	0.2	1.93
15/08/2008	38	31.9	2.6	1.6	1.92	13/12/2008	156		2.7	0.3	1.70
16/08/2008	39	34.1	3.8	0.6	1.95	14/12/2008	157		4.6	0.8	2.16
17/08/2008	40	33.9	4.7	0.8	1.94	15/12/2008	159	34.6	4.6	0.8	2.11
18/08/2008	41	30.7	3.0	0.0	1.76	16/12/2008	160	32.0	3.4	0.0	2.00
19/08/2008	42	32.5	4.0	2.1	1.83	17/12/2008	161	29.5	2.1	0.0	1.94
20/08/2008	43	31.8	3.7	1.4	1.80	18/12/2008	162		5.6	1.2	1.83
21/08/2008	44	28.6	3.6	2.2	1.61	19/12/2008	169	32.1	6.0	1.0	1.74
22/08/2008	45	32.9	4.4	0.7	1.86	20/12/2008	170	28.6	5.0	0.5	1.83
23/08/2008	46	32.6	4.0	0.4	1.86	27/12/2008	171	33.1	5.5	0.7	1.90
24/08/2008	47	29.3	3.7	0.2	1.65	28/12/2008	172	31.1	4.9	0.8	1.93
25/08/2008	48	32.6	4.8	1.2	1.81	29/12/2008	173		4.3	0.4	1.62
26/08/2008	49	31.1	4.5	0.7	1.73	30/12/2008	174	32.9	5.1	0.6	2.05

Jong Hyuk Hwang, Ph.D. Thesis

Date	#					Date	#				
27/08/2008	50	29.2	3.7	0.6	1.62	31/12/2008	175	30.6	4.5	0.2	1.94
28/08/2008	51	32.3	4.4	1.7	1.79	01/01/2009	176		4.2	0.4	1.66
29/08/2008	52	31.1	3.6	0.3	1.80	02/01/2009	177	31.1	4.5	0.3	1.98
30/08/2008	53	29.3	3.6	0.2	1.67	03/01/2009	178	29.5	4.0	0.1	1.88
31/08/2008	54	33.9	4.4	0.0	1.91	04/01/2009	179		3.6	0.2	1.63
01/09/2008	55	33.3	5.6	1.1	1.82	05/01/2009	180	29.2	2.7	0.0	1.94
02/09/2008	56	31.4	4.5	0.3	1.72	06/01/2009	181	28.1	2.3	0.9	1.91
03/09/2008	57	33.1	4.0	1.0	1.88	07/01/2009	182		1.3	0.0	1.92
04/09/2008	58	32.2	4.3	0.1	1.81	08/01/2009	183	30.8	4.1	0.1	1.96
05/09/2008	59	29.5	3.0	0.1	1.72	09/01/2009	184	30.2	3.8	0.6	1.95
06/09/2008	60	33.9	4.5	0.4	1.90	10/01/2009	185		2.7		1.71
07/09/2008	61	32.5	3.9	0.0	1.85	11/01/2009	186	30.2	2.9		2.00
08/09/2008	62	30.4	3.7	0.2	1.72	12/01/2009	187	28.1	2.7		1.88
09/09/2008	63	33.2	4.8	0.3	1.83	13/01/2009	188	28.4	3.5	0.3	1.82
10/09/2008	64	31.4	4.6	0.8	1.75	14/01/2009	189	34.0	5.1	0.7	2.14
11/09/2008	65		4.3	1.7	1.54	15/01/2009	190	32.2	3.9	0.4	2.07
12/09/2008	66	32.8	5.2	2.1	1.82	16/01/2009	191	29.8	3.5	0.4	1.92
13/09/2008	67	31.6	4.1	0.3	1.79	17/01/2009	192	34.1	4.6	0.4	2.16
14/09/2008	68	28.6	3.5	0.2	1.61	18/01/2009	193	33.4	4.3	0.4	2.13
15/09/2008	69	32.8	4.7	0.4	1.80	19/01/2009	194	28.9	3.7	0.0	1.88
16/09/2008	70	31.8	4.1	0.3	1.77	20/01/2009	195	32.2	4.9	0.3	2.01
17/09/2008	71	30.2	4.4	0.4	1.63	21/01/2009	196	32.5	6.7	0.2	1.90
18/09/2008	72	32.5	4.3	0.3	1.82	22/01/2009	197	28.4	4.2	0.7	1.75
19/09/2008	73	31.6	4.1	0.5	1.76	23/01/2009	198	32.5	4.5	0.4	2.07
20/09/2008	74	30.1	3.3	0.3	1.75	24/01/2009	199	29.9	4.1	0.1	1.90
21/09/2008	75	33.6	3.7	0.3	2.00	25/01/2009	200		3.4	0.2	1.75
22/09/2008	77		1.9	0.1	1.79	26/01/2009	201	31.0	3.7		1.99
23/09/2008	78	31.8	3.2	0.3	1.86	27/01/2009	202	32.2	5.1	0.5	2.02
26/09/2008	79	30.8	2.5	0.3	1.79	28/01/2009	203		3.6	0.2	1.73
27/09/2008	80		2.6	0.2	1.64	29/01/2009	204	34.5	4.4	0.4	2.20
28/09/2008	81	32.0	4.8	1.3	1.78	30/01/2009	205	32.9	3.1	0.3	2.24
29/09/2008	82	30.5	3.8	1.0	1.64	31/01/2009	206	29.9	3.6	0.2	1.86
30/09/2008	83		3.3	0.6	1.58	01/02/2009	207	33.4	4.3	0.2	2.14
01/10/2008	84	32.0	4.4	0.4	1.81	02/02/2009	208	32.7	4.4	0.2	2.06
02/10/2008	85	30.5	5.7	1.2	1.51	03/02/2009	209	30.2	4.0	0.3	1.92
03/10/2008	86		5.4	1.5	1.61	04/02/2009	210	33.9	4.6	0.3	2.14
04/10/2008	87	30.6	6.8	2.8	1.78	05/02/2009	211	33.8	5.1	0.1	2.12
05/10/2008	89		3.4	0.9	1.77	06/02/2009	212	32.5	3.8	0.2	2.10
06/10/2008	90	31.8	5.6	0.9	1.95	07/02/2009	213	29.8	3.2	0.1	1.94
08/10/2008	91	29.8	4.7	1.0	1.83	08/02/2009	214	32.5	4.5	0.0	2.08
09/10/2008	92		3.7	0.1	1.72	09/02/2009	215	30.4	4.0	0.0	1.95
10/10/2008	93	32.0	5.0	0.6	2.02	10/02/2009	216	28.3	3.4	0.0	1.82
11/10/2008	94	30.3	4.6	0.4	1.90	11/02/2009	217	29.7	3.9	0.0	1.92
12/10/2008	95		3.9	0.5	1.74	12/02/2009	218	30.7	3.8	0.0	1.97
13/10/2008	96	34.0	5.2	0.6	2.12	13/02/2009	220	31.6	3.0	0.3	2.10
14/10/2008	97	31.1	4.3	0.0	2.00	14/02/2009	222		3.5	0.0	1.73
15/10/2008	98		3.7	0.5	1.70	16/02/2009	223	33.0	4.7	1.1	2.19
16/10/2008	99	33.2	4.2	0.4	2.07	17/02/2009	224	30.7	3.7	0.9	2.05
17/10/2008	104	33.1	5.1	1.6	2.01	18/02/2009	225	29.2	5.2	4.5	1.90
18/10/2008	105	33.1	4.4	2.1	2.06	19/02/2009	226	31.5	4.6	3.5	2.01
23/10/2008	106	29.8	4.2	1.9	1.83	20/02/2009	227	30.2	4.5	3.5	1.94
24/10/2008	107		2.5	0.7	1.48	21/02/2009	228	28.6	4.9	0.7	1.79
25/10/2008	108	29.8	3.3	0.5	1.88	22/02/2009	229	32.3	5.8	0.4	2.02

Jong Hyuk Hwang, Ph.D. Thesis

26/10/2008	109	30.3	4.0	1.0	1.88	23/02/2009	230	30.7	4.8	3.8	1.99
27/10/2008	110	32.0	5.0	1.5	2.21	24/02/2009	231	28.1	4.5	3.8	1.78
28/10/2008	111	31.8	4.7	2.7	2.23	25/02/2009	233	30.8	4.5	0.1	1.99
29/10/2008	112	30.0	5.0	3.9	2.06	26/02/2009	234		2.8	0.0	2.03
30/10/2008	114	32.2	5.6	0.8	1.95	27/02/2009	235	30.5	3.6	0.0	2.05
31/10/2008	115	30.9	5.1	0.6	1.88	01/03/2009	236	29.8	3.7	0.0	1.99
02/11/2008	116		4.7	3.1	1.52	02/03/2009	237	28.2	3.8	0.0	1.89
03/11/2008	117	34.9	8.1	5.6	1.92	03/03/2009	238	31.1	4.0	0.1	2.07
04/11/2008	118	31.5	5.7	2.3	1.87	04/03/2009	239	28.9	3.5	0.0	1.95
05/11/2008	119		3.7	0.7	1.60	05/03/2009	240		2.6	0.0	1.81
06/11/2008	120	31.7	4.3	1.0	1.99	06/03/2009	241	30.5	3.2	0.0	2.09
07/11/2008	121	31.2	4.6	1.5	1.95						

Appendix 12. Data for Figure 7.3

Time (d)	NH_4-N	NOx-N	Estimated NOx-N
0.0	21.8	11.6	10.9
0.1	21.2	11.9	11.2
0.3	20.9	12.1	11.7
1.0	19.4	14.4	14.4
1.0	18.9	14.5	14.8
1.1	18.5	15.1	15.3
1.2	18.0	15.6	15.8
1.3	18.0	15.8	16.2
1.3	17.3	16.3	16.5
1.5	15.9	17.8	17.9
2.0	10.1	23.4	23.1
2.0	9.2	24.3	23.6
2.1	7.9	25.4	25.3
2.2	6.2	26.9	27.0
2.3	5.2	27.9	28.1

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 13. Data for Figure 7.4

	Day	Influent	Final effluent	SDNR		Day	Influent	Final effluent	SDNR
09/07/2008	1	33.1	9.4	1.51	08/11/2008	122	28.4	3.5	1.84
10/07/2008	2	35.8	8.9	1.74	09/11/2008	123	31.6	3.7	1.96
11/07/2008	3	31.6	8.5	1.50	10/11/2008	124	29.9	3.2	1.93
12/07/2008	4	29.8	9.1	1.32	11/11/2008	125	29.6	4.1	1.81
13/07/2008	5	29.1	8.0	1.35	12/11/2008	126	31.2	8.2	1.67
14/07/2008	6	31.3	8.8	1.45	13/11/2008	127	30.6	7.7	1.64
15/07/2008	7	28.7	7.6	1.36	14/11/2008	128	28.7	6.6	1.65
16/07/2008	8	34.0	10.4	1.54	15/11/2008	129	35.7	7.8	1.73
17/07/2008	9	32.6	9.0	1.52	16/11/2008	130	31.6	6.3	1.80
18/07/2008	10	33.2	9.9	1.51	17/11/2008	131	29.9	7.2	1.65
19/07/2008	11	31.6	9.2	1.45	18/11/2008	132	33.7	7.0	1.91
20/07/2008	12	34.3	12.3	1.44	20/11/2008	133	32.5	7.0	1.90
21/07/2008	13	32.7	10.4	1.45	21/11/2008	134	29.7	8.0	1.85
22/07/2008	14	32.7	6.3	1.72	22/11/2008	135	32.9	7.5	1.75
23/07/2008	15	33.7	5.9	1.79	23/11/2008	136	32.9	8.0	1.75
24/07/2008	16	33.7	7.4	1.72	24/11/2008	137	31.6	7.4	1.68
25/07/2008	17	32.2	8.4	1.52	25/11/2008	138	31.6	10.7	1.54
26/07/2008	18	31.6	9.9	1.35	26/11/2008	139	31.9	10.5	1.48
27/07/2008	21	30.8	9.9	1.34	27/11/2008	140	30.6	6.1	1.74
28/07/2008	22	34.1	9.2	1.54	28/11/2008	141	32.0	3.9	2.05
29/07/2008	23	32.9	10.4	1.39	29/11/2008	142	32.3	3.8	2.07
01/08/2008	24	33.5	12.4	1.31	30/11/2008	143	30.2	1.3	1.82
02/08/2008	25	32.7	17.0	0.96	01/12/2008	144	32.3	3.2	2.08
03/08/2008	26	34.6	9.2	1.57	02/12/2008	145	31.5	3.6	1.95
04/08/2008	28	34.0	10.8	1.47	03/12/2008	146	30.9	2.9	1.95
05/08/2008	29	33.2	8.5	1.52	04/12/2008	147	31.5	5.6	1.90
06/08/2008	30	31.3	6.4	1.53	05/12/2008	148	30.8	5.3	1.75
08/08/2008	31	30.3	10.0	1.25	06/12/2008	149	29.7	7.3	1.69
09/08/2008	32	28.1	6.5	1.36	07/12/2008	150	33.1	10.2	1.74
10/08/2008	33	30.1	9.6	1.20	08/12/2008	151	31.9	9.7	1.66
11/08/2008	34	29.7	9.1	1.34	09/12/2008	152	31.3	10.5	1.46
12/08/2008	35	32.2	8.4	1.48	10/12/2008	153	29.6	8.2	1.58
13/08/2008	36	31.9	6.9	1.54	11/12/2008	154	31.1		1.70
14/08/2008	37	33.3	8.4	1.56	12/12/2008	155	30.0	6.3	1.65
15/08/2008	38	30.6	7.7	1.43	13/12/2008	156	28.9	7.4	1.51
16/08/2008	39	33.4	7.7	1.58	14/12/2008	157	33.9	8.8	1.76
17/08/2008	40	33.4	7.1	1.65	15/12/2008	159	33.9	8.8	1.76
18/08/2008	41	31.2	8.4	1.38	16/12/2008	160	32.0	7.3	1.61
19/08/2008	42	31.4	6.2	1.46	17/12/2008	161	29.9	7.7	1.53
20/08/2008	43	30.4	5.8	1.42	18/12/2008	162	35.2	12.0	1.70
21/08/2008	44	27.4	6.9	1.19	19/12/2008	169	30.0	2.7	1.47
22/08/2008	45	33.5	7.3	1.46	20/12/2008	170	27.1	2.3	1.75
23/08/2008	46	33.4	6.2	1.51	27/12/2008	171	27.8	1.9	1.88
24/08/2008	47	31.0	5.6	1.43	28/12/2008	172	29.7	1.6	2.02
25/08/2008	48	32.6	4.4	1.59	29/12/2008	173	29.4	1.5	1.95
26/08/2008	49	32.4	3.1	1.68	30/12/2008	174	30.8	6.8	1.70
27/08/2008	50	29.5	3.2	1.47	31/12/2008	175	30.3	1.8	2.06
28/08/2008	51	29.8	2.2	1.60	01/01/2009	176	29.5	1.4	1.97
29/08/2008	52	30.4	3.2	1.62	02/01/2009	177	30.6	2.0	2.03

Jong Hyuk Hwang, Ph.D. Thesis

30/08/2008	53	29.2	7.1	1.25	03/01/2009	178	29.7	0.9	2.02
31/08/2008	54	35.2	6.3	1.65	04/01/2009	179	29.0	0.9	1.94
01/09/2008	55	34.0	2.8	1.75	05/01/2009	180	29.2	0.9	2.04
02/09/2008	56	32.7	2.8	1.67	06/01/2009	181	27.9	0.5	1.99
03/09/2008	57	32.4	2.2	1.78	07/01/2009	182	27.2	0.5	1.95
04/09/2008	58	34.1	3.9	1.67	08/01/2009	183	31.3	4.9	1.81
05/09/2008	59	30.6	2.9	1.62	09/01/2009	184	29.8	0.6	2.01
06/09/2008	60	35.3	4.4	1.74	10/01/2009	185	28.6	0.4	2.02
07/09/2008	61	35.0	4.0	1.73	11/01/2009	186	29.2	1.1	2.02
08/09/2008	62	33.1	3.1	1.68	12/01/2009	187	28.5	1.9	1.94
09/09/2008	63	34.6	2.8	1.79	13/01/2009	188	31.2	0.4	2.21
10/09/2008	64	30.2	9.1	1.15	14/01/2009	189	33.7	1.8	2.33
11/09/2008	65	27.7	7.5	1.14	15/01/2009	190	32.8	4.2	2.07
12/09/2008	66	29.7	13.2	0.87	16/01/2009	191	31.9	3.9	2.02
13/09/2008	67	31.8	6.6	1.39	17/01/2009	192	34.5	4.8	2.09
14/09/2008	68	30.3	4.9	1.40	18/01/2009	193	34.0	6.0	1.91
15/09/2008	69	33.0	3.7	1.59	19/01/2009	194	32.1	5.9	1.89
16/09/2008	70	32.2	5.9	1.41	20/01/2009	195	32.4	1.7	2.12
17/09/2008	71	31.2	3.6	1.45	21/01/2009	196	33.5	3.0	1.92
18/09/2008	72	32.2	4.8	1.48	22/01/2009	197	30.6	2.3	1.92
19/09/2008	73	30.2	4.6	1.49	23/01/2009	198	32.5	4.1	1.96
20/09/2008	74	31.3	4.1	1.59	24/01/2009	199	31.1	2.8	2.02
21/09/2008	75	33.7	4.2	1.88	25/01/2009	200	30.0	1.9	1.95
22/09/2008	77	29.7	3.3	1.71	26/01/2009	201	31.7	2.4	2.07
23/09/2008	78	30.9	2.2	1.71	27/01/2009	202	33.0	1.8	2.14
26/09/2008	79	29.2	1.5	1.70	28/01/2009	203	30.0	2.1	2.00
27/09/2008	80	28.2	1.6	1.67	29/01/2009	204	34.6	1.1	2.44
28/09/2008	81	29.5	2.7	1.61	30/01/2009	205	34.0	0.9	2.43
29/09/2008	82	28.4	2.6	1.50	31/01/2009	206	33.0	1.3	2.18
30/09/2008	83	28.3	2.0	1.60	01/02/2009	207	33.7	1.1	2.36
01/10/2008	84	31.0	2.8	1.77	02/02/2009	208	33.6	1.1	2.26
02/10/2008	85	29.9	2.1	1.61	03/02/2009	209	32.7	1.0	2.24
03/10/2008	86	28.3	2.7	1.84	04/02/2009	210	34.1	0.6	2.41
04/10/2008	87	28.4	2.1	1.88	05/02/2009	211	34.0	0.7	2.38
05/10/2008	89	29.2	2.3	1.93	06/02/2009	212	32.2	0.4	2.31
06/10/2008	90	31.4	2.2	1.98	07/02/2009	213	31.8	1.1	2.18
08/10/2008	91	29.2	2.2	1.89	08/02/2009	214	34.3	2.5	2.42
09/10/2008	92	30.4	1.7	2.04	09/02/2009	215	34.2	2.7	2.25
10/10/2008	93	32.3	4.1	1.99	10/02/2009	216	30.3	0.4	2.08
11/10/2008	94	31.4	3.6	1.93	11/02/2009	217	32.0	2.2	2.23
12/10/2008	95	29.8	3.2	1.87	12/02/2009	218	32.2	0.1	2.25
13/10/2008	96	33.2	5.4	1.89	13/02/2009	220	31.8	0.4	2.26
14/10/2008	97	33.3	6.3	1.85	14/02/2009	222	30.3	2.1	2.01
15/10/2008	98	31.3	7.0	1.89	16/02/2009	223	31.0	8.1	1.68
16/10/2008	99	32.1	5.1	1.93	17/02/2009	224	30.3	2.6	2.06
17/10/2008	104	28.9	4.0	1.72	18/02/2009	225	27.5	4.6	1.99
18/10/2008	105	30.5	4.0	1.82	19/02/2009	226	28.7	1.7	2.29
23/10/2008	106	29.3	3.4	1.74	20/02/2009	227	28.2	8.1	1.77
24/10/2008	107	28.5	2.4	1.71	21/02/2009	228	30.8	5.8	1.77
25/10/2008	108	31.0	1.7	1.96	22/02/2009	229	32.3	1.6	2.26
26/10/2008	109	30.8	2.6	1.93	23/02/2009	230	28.2	1.6	2.05
27/10/2008	110	30.9	2.3	2.22	24/02/2009	231	27.8	1.3	1.98
28/10/2008	111	29.2	2.9	2.12	25/02/2009	233	31.3	4.6	1.95

Jong Hyuk Hwang, Ph.D. Thesis

29/10/2008	112	27.3	1.8	2.05	26/02/2009	234	29.5	3.7	2.03
30/10/2008	114	31.6	1.9	2.13	27/02/2009	235	30.7	6.7	1.80
31/10/2008	115	30.6	1.0	2.10	01/03/2009	236	30.5	5.7	1.86
02/11/2008	116	27.3	0.4	1.93	02/03/2009	237	30.4	3.6	2.02
03/11/2008	117	29.5	3.1	1.69	03/03/2009	238	31.2	3.4	2.21
04/11/2008	118	30.6	0.9	2.04	04/03/2009	239	30.2	1.4	2.17
05/11/2008	119	28.8	0.3	2.05	05/03/2009	240	28.9	2.9	1.99
06/11/2008	120	30.9	2.1	2.00	06/03/2009	241	30.9	4.6	1.99
07/11/2008	121	29.4	2.0	1.95					

Appendix 14. Data for Figure 7.7

Date	Day	TSS	VSS	Date	Day	TSS	VSS
15/07/2008	4	3.3	2.8	25/11/2008	137		
22/07/2008	11	1.5	1.4	26/11/2008	138		
29/07/2008	18	1.0	0.8	27/11/2008	139		
05/08/2008	25	2.4	2.0	28/11/2008	140		
13/08/2008	33	1.5	1.3	29/11/2008	141		
19/08/2008	39	1.9	1.5	30/11/2008	142		
01/09/2008	52	1.3	1.2	01/12/2008	143		
02/09/2008	53	2.6	2.3	02/12/2008	144		
03/09/2008	54	3.3	3.0	03/12/2008	145	15.1	12.7
04/09/2008	55	4.2	3.8	04/12/2008	146		
05/09/2008	56	2.2	1.9	05/12/2008	147		
06/09/2008	57	2.5	2.3	06/12/2008	148		
07/09/2008	58	1.8	1.7	07/12/2008	149	8.4	6.9
08/09/2008	59	1.9	1.8	08/12/2008	150		
09/09/2008	60	8.3	7.5	09/12/2008	151		
10/09/2008	61	3.1	2.8	10/12/2008	152	5.4	4.9
11/09/2008	62	1.1	0.8	11/12/2008	153		
12/09/2008	63	0.9	0.7	12/12/2008	154		
13/09/2008	64	2.8	2.5	13/12/2008	155		
14/09/2008	65	5.0	4.4	14/12/2008	156	14.8	11.1
15/09/2008	66	2.2	1.9	15/12/2008	157		
16/09/2008	67	1.8	1.6	16/12/2008	158		
17/09/2008	68	1.3	1.1	17/12/2008	159	7.2	6.8
18/09/2008	69	2.1	2.0	18/12/2008	160		
19/09/2008	70	1.8	1.7	19/12/2008	161		
20/09/2008	71	17.1	15.0	20/12/2008	162	11.5	9.8
21/09/2008	72	15.2	14.2	27/12/2008	169		
22/09/2008	73	12.2	11.4	28/12/2008	170		
23/09/2008	74	6.2	6.0	29/12/2008	171		
24/09/2008	75	6.3	5.7	30/12/2008	172		
26/09/2008	77	9.0	7.6	31/12/2008	173		
27/09/2008	78	25.2	21.8	01/01/2009	174	26.7	22.3
28/09/2008	79	15.8	14.2	02/01/2009	175		
29/09/2008	80	10.4	9.6	03/01/2009	176		
30/09/2008	81	13.2	11.8	04/01/2009	177		
01/10/2008	82	8.0	7.2	05/01/2009	178	18.3	15.6
02/10/2008	83	5.8	5.4	06/01/2009	179		
03/10/2008	84	8.2	7.4	07/01/2009	180		

Jong Hyuk Hwang, Ph.D. Thesis

04/10/2008	85	15.2	14.0	08/01/2009	181		
05/10/2008	86	5.8	5.4	09/01/2009	182		
06/10/2008	87	7.3	6.7	10/01/2009	183		
08/10/2008	89	10.6	10.2	11/01/2009	184	16.5	12.1
09/10/2008	90	13.8	9.4	12/01/2009	185		
10/10/2008	91	6.5	5.7	13/01/2009	186		
11/10/2008	92	18.4	16.0	14/01/2009	187		
12/10/2008	93	6.0	5.0	15/01/2009	188		
13/10/2008	94	7.7	6.0	16/01/2009	189	20.5	15.4
14/10/2008	95	4.4	3.4	17/01/2009	190		
15/10/2008	96	4.0	3.7	18/01/2009	191	12.2	10.9
16/10/2008	97	18.4	17.1	19/01/2009	192		
17/10/2008	98	8.7	8.0	20/01/2009	193		
18/10/2008	99	25.4	23.1	21/01/2009	194	21.4	18.6
23/10/2008	104	7.7	7.0	22/01/2009	195		
24/10/2008	105	11.6	11.0	23/01/2009	196		
25/10/2008	106	18.3	17.2	24/01/2009	197	13.5	10.2
26/10/2008	107	12.5	11.6	25/01/2009	198		
27/10/2008	108	17.5	15.1	26/01/2009	199		
28/10/2008	109	6.7	5.7	27/01/2009	200	25.4	20.0
29/10/2008	110	6.0	5.7	28/01/2009	201		
30/10/2008	111	12.3	11.7	29/01/2009	202		
31/10/2008	112	11.7	11.0	30/01/2009	203		
02/11/2008	114	6.6	6.0	31/01/2009	204		
03/11/2008	115	12.3	11.7	01/02/2009	205	17.3	14.8
04/11/2008	116	9.3	8.7	02/02/2009	206		
05/11/2008	117	18.4	16.2	03/02/2009	207	13.9	12.5
06/11/2008	118	15.7	14.0	04/02/2009	208		
07/11/2008	119	7.7	6.3	05/02/2009	209		
08/11/2008	120			06/02/2009	210	17.1	13.1
09/11/2008	121			07/02/2009	211		
10/11/2008	122	4.5	4.2	08/02/2009	212		
11/11/2008	123			09/02/2009	213	21.2	18.6
12/11/2008	124	21.3	18.3	10/02/2009	214		
13/11/2008	125	11.0	9.8	11/02/2009	215		
14/11/2008	126	11.6	10.4	12/02/2009	216	10.4	8.2
15/11/2008	127			13/02/2009	217		
16/11/2008	128			14/02/2009	218		
17/11/2008	129	10.2	9.4	16/02/2009	220		
18/11/2008	130	6.4	5.4	18/02/2009	222		
19/11/2008	131	3.4	3.0	19/02/2009	223	18.6	16.8
20/11/2008	132	22.8	20.4	20/02/2009	224		
21/11/2008	133	10.4	9.0	21/02/2009	225		
22/11/2008	134			22/02/2009	226		
23/11/2008	135			23/02/2009	227		
24/11/2008	136	18.6	16.0				

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 15. Data for Figure 7.8

Date	Day	Nitrification in nitrification reactor	Nitrification in denitrification reactor	Denitrification in denitrification reactor	Date	Day	Nitrification in nitrification reactor	Nitrification in denitrification reactor	Denitrification in denitrification reactor
12/07/2008	1	44.1	5.2	52.9	14/11/2008	126	49.2	3.8	56.8
13/07/2008	2	45.9	10.1	61.0	15/11/2008	127	47.8	2.1	55.7
14/07/2008	3	44.5	4.9	52.5	16/11/2008	128	40.2	2.1	55.9
15/07/2008	4	44.7	4.0	46.3	17/11/2008	129	49.6	2.1	58.8
16/07/2008	5	41.8	4.0	47.1	18/11/2008	130	47.5	2.6	61.0
17/07/2008	6	47.6	4.9	50.7	19/11/2008	131	43.9	1.6	56.1
18/07/2008	7	43.5	5.6	47.7	20/11/2008	132	52.0	1.4	64.7
19/07/2008	8	47.8	7.0	53.8	21/11/2008	133	49.9	1.4	64.5
20/07/2008	9	46.7	5.5	53.3	22/11/2008	134	42.7	2.1	58.8
21/07/2008	10	49.0	6.0	52.9	23/11/2008	135	53.4	1.6	64.9
22/07/2008	11	44.2	5.1	50.9	24/11/2008	136	52.7	1.9	62.7
23/07/2008	12	48.6	6.7	50.3	25/11/2008	137	48.6	1.6	58.8
24/07/2008	13	45.0	5.1	50.8	26/11/2008	138	52.3	1.2	53.8
25/07/2008	14	45.3	5.0	60.1	27/11/2008	139	49.6	1.4	51.7
26/07/2008	15	46.9	5.0	62.7	28/11/2008	140	45.1	1.9	60.7
27/07/2008	16	47.5	5.4	60.0	29/11/2008	141	52.0	2.3	71.7
28/07/2008	17	44.5	5.1	53.3	30/11/2008	142	49.4	4.2	72.2
29/07/2008	18	43.9	5.0	47.3	01/12/2008	143	39.8	2.0	63.8
01/08/2008	21	50.6	5.0	47.0	02/12/2008	144	51.9	2.8	72.8
02/08/2008	22	45.1	6.0	54.0	03/12/2008	145	48.2	1.9	68.2
03/08/2008	23	43.2	5.5	48.6	04/12/2008	146	44.7	2.2	68.2
04/08/2008	24	45.0	5.3	45.9	05/12/2008	147	54.0	1.9	66.6
05/08/2008	25	42.2	5.3	42.3	06/12/2008	148	44.2	1.7	61.3
06/08/2008	26	41.9	5.1	54.9	07/12/2008	149	42.2	2.6	59.0
08/08/2008	28	42.4	4.2	51.2	08/12/2008	150	46.8	4.1	60.9
09/08/2008	29	44.7	5.5	53.2	09/12/2008	151	48.8	2.8	58.0
10/08/2008	30	42.8	4.5	53.3	10/12/2008	152	53.4	2.2	51.0
11/08/2008	31	42.7	5.1	43.8	11/12/2008	153	41.6	4.7	55.4
12/08/2008	32	39.4	3.4	47.4	13/12/2008	155	48.6	2.6	57.7
13/08/2008	33	40.5	2.9	41.8	14/12/2008	156	42.7	2.5	52.7
14/08/2008	34	44.0	3.1	46.7	15/12/2008	157	54.5	3.4	61.6
15/08/2008	35	45.3	3.5	51.6	17/12/2008	159	53.1	3.4	61.4
16/08/2008	36	42.6	4.7	54.0	18/12/2008	160	50.4	1.1	56.2
17/08/2008	37	45.7	4.7	54.4	19/12/2008	161	48.9	0.9	53.6
18/08/2008	38	44.4	1.6	50.0	20/12/2008	162	61.3	5.3	59.3
19/08/2008	39	45.2	4.7	55.1	27/12/2008	169	44.9	3.7	44.4
20/08/2008	40	44.9	6.0	57.8	28/12/2008	170	36.5	3.6	61.1
21/08/2008	41	40.7	4.4	48.2	29/12/2008	171	47.9	3.7	68.4
22/08/2008	42	42.4	0.8	50.9	30/12/2008	172	48.7	4.5	73.7
23/08/2008	43	41.6	1.4	49.7	31/12/2008	173	40.8	4.2	71.1
24/08/2008	44	37.4	0.5	41.7	01/01/2009	174	51.7	5.8	62.4
25/08/2008	45	43.0	1.7	51.2	02/01/2009	175	48.8	5.0	75.2
26/08/2008	46	43.1	1.1	52.7	03/01/2009	176	41.9	3.3	71.7
27/08/2008	47	38.1	2.2	49.9	04/01/2009	177	50.0	5.0	74.0
28/08/2008	48	42.0	1.5	55.6	05/01/2009	178	47.4	4.8	73.7
29/08/2008	49	40.1	2.3	58.6	06/01/2009	179	41.0	2.9	70.7
30/08/2008	50	37.5	1.6	51.4	07/01/2009	180	49.0	2.6	74.4

Jong Hyuk Hwang, Ph.D. Thesis

31/08/2008	51	41.3	2.0	55.9	08/01/2009	181	48.2	0.8	72.7
01/09/2008	52	41.8	3.0	56.6	09/01/2009	182	48.4	1.5	71.0
02/09/2008	53	38.6	2.2	43.5	10/01/2009	183	49.3	2.7	66.0
03/09/2008	54	44.3	3.4	57.8	11/01/2009	184	49.2	2.8	73.2
04/09/2008	55	42.1	1.9	61.1	12/01/2009	185	43.0	3.2	73.6
05/09/2008	56	39.9	2.4	58.3	13/01/2009	186	50.3	3.3	73.7
06/09/2008	57	43.5	2.6	62.2	14/01/2009	187	47.5	3.1	70.8
07/09/2008	58	41.8	1.6	58.5	15/01/2009	188	45.9	3.0	80.4
08/09/2008	59	39.7	2.1	56.5	16/01/2009	189	53.9	5.4	84.9
09/09/2008	60	44.1	1.9	60.8	17/01/2009	190	52.3	4.2	75.4
10/09/2008	61	42.8	1.6	60.7	18/01/2009	191	48.4	3.5	73.4
11/09/2008	62	39.9	0.9	58.3	19/01/2009	192	54.4	4.6	76.2
12/09/2008	63	42.4	2.7	62.7	20/01/2009	193	53.6	3.4	69.6
13/09/2008	64	40.5	1.6	40.2	21/01/2009	194	47.4	4.5	68.8
14/09/2008	65	35.7	1.2	40.0	22/01/2009	195	50.5	4.4	77.3
15/09/2008	66	42.1	1.0	30.6	23/01/2009	196	48.0	5.8	74.6
16/09/2008	67	41.4	1.4	48.4	24/01/2009	197	44.1	2.8	69.9
17/09/2008	68	37.3	1.4	49.1	25/01/2009	198	52.1	4.0	71.4
18/09/2008	69	41.6	1.5	55.4	26/01/2009	199	47.9	4.8	73.8
19/09/2008	70	40.9	1.0	49.2	27/01/2009	200	44.0	3.0	71.1
20/09/2008	71	37.6	0.8	50.6	28/01/2009	201	50.1	4.8	75.5
21/09/2008	72	42.0	1.0	51.7	29/01/2009	202	50.9	3.9	78.0
22/09/2008	73	40.8	3.5	51.9	30/01/2009	203	43.6	4.5	72.7
23/09/2008	74	40.4	2.3	55.7	31/01/2009	204	55.5	4.7	89.0
24/09/2008	75	46.2	2.8	62.1	01/02/2009	205	56.4	2.8	88.4
26/09/2008	77	41.4	0.5	57.0	02/02/2009	206	46.8	3.9	79.5
27/09/2008	78	43.0	0.9	57.0	03/02/2009	207	54.0	5.6	85.8
28/09/2008	79	41.4	2.0	56.5	04/02/2009	208	51.8	4.7	82.3
29/09/2008	80	38.0	2.1	55.5	05/02/2009	209	48.5	4.1	81.9
30/09/2008	81	41.1	2.3	53.8	06/02/2009	210	54.0	6.2	87.9
01/10/2008	82	38.1	2.1	50.1	07/02/2009	211	53.3	5.5	86.7
02/10/2008	83	36.5	1.9	53.3	08/02/2009	212	53.0	3.9	84.0
03/10/2008	84	42.0	4.1	58.9	09/02/2009	213	48.9	3.3	79.4
04/10/2008	85	35.0	4.4	53.6	10/02/2009	214	52.4	5.8	88.0
05/10/2008	86	37.4	5.1	61.2	11/02/2009	215	49.1	5.0	82.0
06/10/2008	87	41.2	5.2	62.8	12/02/2009	216	45.8	3.1	75.8
08/10/2008	89	40.9	3.0	64.2	13/02/2009	217	48.4	3.3	81.3
09/10/2008	90	45.1	3.8	65.9	14/02/2009	218	49.7	3.9	82.1
10/10/2008	91	42.4	4.7	63.1	16/02/2009	220	53.0	3.6	82.2
11/10/2008	92	39.8	4.6	68.4	17/02/2009	221	46.8	5.1	78.1
12/10/2008	93	46.8	4.7	66.1	18/02/2009	222	42.2	3.6	70.3
13/10/2008	94	44.1	4.2	64.3	19/02/2009	223	53.2	3.6	58.7
14/10/2008	95	40.2	3.3	62.4	20/02/2009	224	49.9	2.8	72.1
15/10/2008	96	49.1	4.2	63.0	21/02/2009	225	42.3	1.0	64.1
16/10/2008	97	46.2	3.8	61.7	22/02/2009	226	49.2	1.1	72.2
17/10/2008	98	39.4	3.3	62.3	23/02/2009	227	47.3	1.1	53.9
18/10/2008	99	50.4	4.4	64.3	24/02/2009	228	43.6	3.9	62.0
23/10/2008	104	48.9	4.5	60.1	25/02/2009	229	49.2	7.0	79.0
24/10/2008	105	50.1	2.1	63.8	26/02/2009	230	48.3	0.9	71.8
25/10/2008	106	44.6	1.3	60.9	27/02/2009	231	43.4	0.0	69.2
26/10/2008	107	36.0	1.6	59.9	01/03/2009	233	48.5	4.7	68.0
27/10/2008	108	45.7	2.1	68.4	02/03/2009	234	46.3	3.7	66.5
28/10/2008	109	45.6	2.9	67.4	03/03/2009	235	53.0	5.2	66.8

Jong Hyuk Hwang, Ph.D. Thesis

29/10/2008	110	53.8	4.7	77.8	04/03/2009	236	48.6	5.4	65.2
30/10/2008	111	54.1	2.9	74.2	05/03/2009	237	45.3	4.2	69.7
31/10/2008	112	50.0	3.7	71.8	06/03/2009	238	50.8	6.8	78.0
02/11/2008	114	47.5	6.0	74.6	07/03/2009	239	47.3	4.9	76.0
03/11/2008	115	45.8	5.1	73.4	08/03/2009	240	43.9	4.0	69.7
04/11/2008	116	37.0	4.7	67.6	09/03/2009	241	50.9	4.5	69.5
05/11/2008	117	46.7	1.4	59.1	10/03/2009	242	49.2	4.1	75.2
06/11/2008	118	45.4	3.0	71.4	11/03/2009	243	44.9	3.0	64.3
07/11/2008	119	39.0	3.2	71.7	12/03/2009	244	52.9	4.9	74.6
08/11/2008	120	48.3	2.2	69.9	13/03/2009	245	48.3	3.6	73.1
09/11/2008	121	47.5	3.2	68.2	14/03/2009	246	42.0	6.4	67.4
10/11/2008	122	43.0	2.4	62.6	15/03/2009	247	51.3	4.4	76.3
11/11/2008	123	47.6	2.5	66.4	16/03/2009	248	49.0	3.7	78.9
12/11/2008	124	47.2	3.3	65.5	17/03/2009	249	40.0	2.0	57.7
13/11/2008	125	44.1	2.8	61.4					

Appendix 16. Data for Figure 7.9

Date	Day	Theoretical biomass generation	discharged biomass	Date	Day	Theoretical biomass generation	discharged biomass
12/07/2008	1	102.1		14/11/2008	126	109.9	114.7
13/07/2008	2	117.1		15/11/2008	127	105.6	
14/07/2008	3	101.9		16/11/2008	128	98.2	
15/07/2008	4	95.1	27.1	17/11/2008	129	110.5	104.6
16/07/2008	5	92.9		18/11/2008	130	111.0	58.8
17/07/2008	6	103.2		19/11/2008	131	101.5	33.2
18/07/2008	7	96.8		20/11/2008	132	118.1	226.5
19/07/2008	8	108.6		21/11/2008	133	115.8	99.9
20/07/2008	9	105.6		22/11/2008	134	103.6	
21/07/2008	10	108.0		23/11/2008	135	119.9	
22/07/2008	11	100.2	13.9	24/11/2008	136	117.3	179.7
23/07/2008	12	105.5		25/11/2008	137	109.0	
24/07/2008	13	101.0		26/11/2008	138	107.4	
25/07/2008	14	110.4		27/11/2008	139	102.7	
26/07/2008	15	114.5		28/11/2008	140	107.7	
27/07/2008	16	113.0		29/11/2008	141	126.0	
28/07/2008	17	102.9		30/11/2008	142	125.7	
29/07/2008	18	96.2	7.8	01/12/2008	143	105.5	
01/08/2008	21	102.6		02/12/2008	144	127.5	
02/08/2008	22	105.1		03/12/2008	145	118.4	138.1
03/08/2008	23	97.3		04/12/2008	146	115.1	
04/08/2008	24	96.2		05/12/2008	147	122.4	
05/08/2008	25	89.7	18.9	06/12/2008	148	107.2	
06/08/2008	26	101.9		07/12/2008	149	103.8	75.4
08/08/2008	28	97.8		08/12/2008	150	111.9	
09/08/2008	29	103.4		09/12/2008	151	109.6	
10/08/2008	30	100.5		10/12/2008	152	106.5	53.8
11/08/2008	31	91.6		11/12/2008	153	101.7	

Jong Hyuk Hwang, Ph.D. Thesis

12/08/2008	32	90.2		13/12/2008	155	108.8	
13/08/2008	33	85.2	11.6	14/12/2008	156	98.0	122.4
14/08/2008	34	93.7		15/12/2008	157	119.4	
15/08/2008	35	100.4		17/12/2008	159	117.9	75.2
16/08/2008	36	101.3		18/12/2008	160	107.7	
17/08/2008	37	104.8		19/12/2008	161	103.5	
18/08/2008	38	95.9		20/12/2008	162	125.8	115.2
19/08/2008	39	105.0	14.0	27/12/2008	169	93.0	
20/08/2008	40	108.6		28/12/2008	170	101.2	
21/08/2008	41	93.3		29/12/2008	171	119.9	
22/08/2008	42	94.2		30/12/2008	172	126.8	
23/08/2008	43	92.7		31/12/2008	173	116.1	
24/08/2008	44	79.5		01/01/2009	174	119.9	259.8
25/08/2008	45	95.8		02/01/2009	175	129.1	
26/08/2008	46	97.0		03/01/2009	176	117.0	
27/08/2008	47	90.2		04/01/2009	177	129.0	
28/08/2008	48	99.1		05/01/2009	178	126.0	181.2
29/08/2008	49	101.0		06/01/2009	179	114.6	
30/08/2008	50	90.5		07/01/2009	180	126.0	
31/08/2008	51	99.2		08/01/2009	181	121.6	
01/09/2008	52	101.4	11.4	09/01/2009	182	120.9	
02/09/2008	53	84.4	21.6	10/01/2009	183	118.0	
03/09/2008	54	105.4	28.2	11/01/2009	184	125.2	140.8
04/09/2008	55	105.1	36.0	12/01/2009	185	119.8	
05/09/2008	56	100.6	17.6	13/01/2009	186	127.3	
06/09/2008	57	108.3	21.5	14/01/2009	187	121.4	
07/09/2008	58	101.9	15.9	15/01/2009	188	129.3	
08/09/2008	59	98.3	16.9	16/01/2009	189	144.2	179.0
09/09/2008	60	106.8	70.3	17/01/2009	190	131.8	
10/09/2008	61	105.0	26.2	18/01/2009	191	125.3	125.1
11/09/2008	62	99.0	7.5	19/01/2009	192	135.2	
12/09/2008	63	107.9	6.5	20/01/2009	193	126.6	
13/09/2008	64	82.3	23.6	21/01/2009	194	120.6	218.6
14/09/2008	65	77.0	41.9	22/01/2009	195	132.3	
15/09/2008	66	73.7	18.1	23/01/2009	196	128.4	
16/09/2008	67	91.1	15.0	24/01/2009	197	116.8	115.9
17/09/2008	68	87.8	10.2	25/01/2009	198	127.5	
18/09/2008	69	98.6	18.5	26/01/2009	199	126.5	
19/09/2008	70	91.1	15.7	27/01/2009	200	118.1	230.4
20/09/2008	71	89.1	137.1	28/01/2009	201	130.4	
21/09/2008	72	94.7	131.9	29/01/2009	202	132.9	
22/09/2008	73	96.2	105.4	30/01/2009	203	120.8	
23/09/2008	74	98.4	56.6	31/01/2009	204	149.3	
24/09/2008	75	111.0	55.2	01/02/2009	205	147.6	174.9
26/09/2008	77	98.8	75.7	02/02/2009	206	130.2	
27/09/2008	78	101.0	204.6	03/02/2009	207	145.4	144.8
28/09/2008	79	99.8	130.0	04/02/2009	208	138.8	
29/09/2008	80	95.5	90.7	05/02/2009	209	134.5	
30/09/2008	81	97.1	111.5	06/02/2009	210	148.1	150.8
01/10/2008	82	90.2	64.2	07/02/2009	211	145.5	
02/10/2008	83	91.7	50.6	08/02/2009	212	140.9	
03/10/2008	84	104.9	70.3	09/02/2009	213	131.7	214.1
04/10/2008	85	93.1	123.7	10/02/2009	214	146.3	

Jong Hyuk Hwang, Ph.D. Thesis

05/10/2008	86	103.8	58.0	11/02/2009	215	136.1	
06/10/2008	87	109.1	72.1	12/02/2009	216	124.7	94.1
08/10/2008	89	108.1	108.9	13/02/2009	217	133.0	
09/10/2008	90	114.9	101.0	14/02/2009	218	135.7	
10/10/2008	91	110.2	60.7	16/02/2009	220	138.9	
11/10/2008	92	112.8	171.4	17/02/2009	221	130.0	
12/10/2008	93	117.7	54.3	18/02/2009	222	116.1	
13/10/2008	94	112.6	64.3	19/02/2009	223	115.5	197.5
14/10/2008	95	105.8	36.7	20/02/2009	224	124.7	
15/10/2008	96	116.2	39.6	21/02/2009	225	107.4	
16/10/2008	97	111.7	183.9	22/02/2009	226	122.4	
17/10/2008	98	105.0	86.6	23/02/2009	227	102.3	
18/10/2008	99	119.1	250.7	24/02/2009	228	109.6	
23/10/2008	104	113.4	76.4	25/02/2009	229	135.3	
24/10/2008	105	116.0	120.2	26/02/2009	230	121.0	
25/10/2008	106	106.8	187.2	27/02/2009	231	112.6	
26/10/2008	107	97.5	119.2	01/03/2009	233	121.1	
27/10/2008	108	116.3	162.8	02/03/2009	234	116.5	
28/10/2008	109	115.8	61.2	03/03/2009	235	125.1	
29/10/2008	110	136.3	70.4	04/03/2009	236	119.2	
30/10/2008	111	131.2	145.8	05/03/2009	237	119.1	
31/10/2008	112	125.6	137.9	06/03/2009	238	135.6	
02/11/2008	114	128.0	66.8	07/03/2009	239	128.2	
03/11/2008	115	124.2	129.2	08/03/2009	240	117.6	
04/11/2008	116	109.3	93.5	09/03/2009	241	124.9	
05/11/2008	117	107.3	176.7	10/03/2009	242	128.5	
06/11/2008	118	119.8	153.7	11/03/2009	243	112.2	
07/11/2008	119	113.8	69.3	12/03/2009	244	132.4	
08/11/2008	120	120.4		13/03/2009	245	125.0	
09/11/2008	121	119.0		14/03/2009	246	115.8	
10/11/2008	122	108.0	47.6	15/03/2009	247	132.0	
11/11/2008	123	116.5		16/03/2009	248	131.6	
12/11/2008	124	115.9	201.4	17/03/2009	249	99.7	
13/11/2008	125	108.3	107.0				

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 17. Data for Figure 7.12

Date	Day	DO entering the denitrification reactor	DO inside denitrification reactor	SDNR	Date	Day	DO entering the denitrification reactor	DO inside denitrification reactor	SDNR
28/06/2008	1	16.8		1.46	11/08/2008	45	18.5	1.6	1.25
29/06/2008	2	14.8		1.51	12/08/2008	46	13.1	0.6	1.36
01/07/2008	4	15.5		1.29	13/08/2008	47	16.1	0.8	1.20
02/07/2008	5	14.9		0.86	14/08/2008	48	15.8	1.9	1.34
03/07/2008	6	15.4		1.72	15/08/2008	49	18.2	0.9	1.48
04/07/2008	7	15		1.57	16/08/2008	50	18	0.3	1.54
05/07/2008	8	17.1		1.42	17/08/2008	51	19.3	1.3	1.56
06/07/2008	9			1.39	18/08/2008	52	16.8	0.1	1.43
07/07/2008	10	17.1	1.3	1.46	19/08/2008	53	15.2	0.2	1.58
08/07/2008	11			1.56	20/08/2008	54	15.4	0.4	1.65
09/07/2008	12	14.5	1.5	1.36	21/08/2008	55	14.4	0.1	1.38
10/07/2008	13	19		0.95	22/08/2008	56	0.5		1.46
11/07/2008	14	14.3	1	1.51	23/08/2008	57	2.2	0.2	1.42
12/07/2008	15	16	1.2	1.51	24/08/2008	58	2	0.1	1.19
13/07/2008	16	18.5	1.5	1.74	25/08/2008	59	2.3	0.2	1.46
14/07/2008	17	12.5	1.4	1.50	26/08/2008	60	1.9		1.51
15/07/2008	18	15.4	0.9	1.32	27/08/2008	61	2.9	0.4	1.43
16/07/2008	19	14.5	1.4	1.35	28/08/2008	62	1.3	0.2	1.59
17/07/2008	20	16.4	1.4	1.45	29/08/2008	63	1.4		1.68
18/07/2008	21	16.1	2	1.36	30/08/2008	64	1.1		1.47
19/07/2008	22			1.54	31/08/2008	65	0.3		1.60
20/07/2008	23			1.52	01/09/2008	66	2.5	0.3	1.62
21/07/2008	24			1.51	02/09/2008	67	2.2	0.5	1.25
22/07/2008	25			1.45	03/09/2008	68	2.7	0.4	1.65
23/07/2008	26	14.4	0.8	1.44	04/09/2008	69	1.8		1.75
24/07/2008	27	15.9	1.4	1.45	05/09/2008	70	2.2		1.67
25/07/2008	28	17.2	1	1.72	06/09/2008	71	1.7	0.1	1.78
26/07/2008	29	13.2	0.6	1.79	07/09/2008	72	2.9	0.1	1.67
27/07/2008	30			1.72	08/09/2008	73	0.6		1.62
28/07/2008	31			1.52	09/09/2008	74	1.7		1.74
29/07/2008	32			1.35	10/09/2008	75	1.3	0.1	1.73
01/08/2008	35			1.34	11/09/2008	76	2.8		1.68
02/08/2008	36			1.54	12/09/2008	77	2.3		1.79
03/08/2008	37	13.5	0.8	1.39	13/09/2008	78	2.8	0.1	1.15
04/08/2008	38	17.3	0.8	1.31	14/09/2008	79	0.6		1.14
05/08/2008	39	14.2	0.9	0.96	15/09/2008	80	0.1		0.87
06/08/2008	40	13.1	1.1	1.57	16/09/2008	81	1.1	0.1	1.39
08/08/2008	42	17.2	0.9	1.47	17/09/2008	82	0.7		1.40
09/08/2008	43	17.7	0.6	1.52	18/09/2008	83			1.59
10/08/2008	44	12.2	0.3	1.53					

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 18. Data for Figure 7.15

Date	Day	HRT	Influent	Nitrification reactor Effluent	Final Effluent	Date	Day	HRT	Influent	Nitrification reactor Effluent	Final Effluent
01/03/2009	1	1.0	30.8	4.5	0.1	15/04/2009	46	0.6	33.4	9.1	5.0
02/03/2009	2	1.0	27.8	2.8	0.0	16/04/2009	47	0.6	31.0	7.2	3.6
03/03/2009	3	1.0	30.5	3.6	0.0	17/04/2009	48	0.6	29.9	6.1	2.6
04/03/2009	4	1.0	29.8	3.7	0.0	18/04/2009	49	0.5	32.7	9.1	5.4
05/03/2009	5	1.0	28.2	3.8	0.0	19/04/2009	50	0.5	30.6	6.9	3.8
06/03/2009	6	1.0	31.1	4.0	0.1	20/04/2009	51	0.5	28.9	6.6	2.7
07/03/2009	7	1.0	28.9	3.5	0.0	21/04/2009	52	0.5	31.8	7.0	
08/03/2009	8	1.0	26.2	2.6	0.0	22/04/2009	53	0.5	29.9	6.1	0.8
09/03/2009	9	1.0	30.5	3.2	0.0	23/04/2009	54	0.5	27.0	4.4	0.1
10/03/2009	10	1.0	29.1	2.6	0.0	24/04/2009	55	0.5	30.8	5.5	0.5
11/03/2009	11	1.1	26.9	2.0	0.0	25/04/2009	56	0.4	33.1	9.0	3.8
12/03/2009	12	1.0	31.6	3.5	0.0	26/04/2009	57	0.4	31.0	7.7	2.2
13/03/2009	13	1.0	29.6	3.3	0.0	27/04/2009	58	0.4			
14/03/2009	14	1.1	26.2	2.6	0.0	28/04/2009	59	0.4	30.6	7.5	3.0
15/03/2009	15	1.0	30.7	3.3	0.0	29/04/2009	60	0.4	29.6	7.0	2.1
16/03/2009	16	1.0	29.1	2.9	0.0	30/04/2009	61	0.4	26.3	5.8	1.7
17/03/2009	17	1.0				01/05/2009	62	0.4	29.2	6.5	1.9
18/03/2009	18	1.0	26.4	2.3	0.0	02/05/2009	63	0.4	26.4	6.0	0.9
19/03/2009	19	0.9	28.6	2.8	0.0	03/05/2009	64	0.4	24.3	5.3	0.0
20/03/2009	20	0.9	27.0	2.2	0.0	04/05/2009	65	0.4	29.3	6.0	0.7
21/03/2009	21	0.9				05/05/2009	66	0.4	28.6	6.2	0.6
22/03/2009	22	0.9				06/05/2009	67	0.4	25.1	7.0	0.0
23/03/2009	23	0.9				07/05/2009	68	0.4	30.7	12.8	8.4
24/03/2009	24	0.9	29.3	5.8	1.4	08/05/2009	69	0.3	27.1	15.5	12.5
25/03/2009	25	0.9	26.6	4.0	0.0	09/05/2009	70	0.3	30.0	12.7	9.7
26/03/2009	26		25.8	8.5	3.5	10/05/2009	71	0.3			
27/03/2009	27	0.8				11/05/2009	72	0.3	30.3	9.5	6.2
28/03/2009	28	0.8	32.0	6.1	0.7	12/05/2009	73	0.3	27.5	8.8	3.1
29/03/2009	29	0.8	30.4	5.4	0.0	13/05/2009	74	0.3	29.8	9.1	3.1
30/03/2009	30	0.8	28.1	4.2	0.1	14/05/2009	75	0.3	24.1	7.2	2.1
31/03/2009	31	0.8	32.6	5.5	0.0	15/05/2009	76	0.3	29.1	9.7	3.0
01/04/2009	32	0.8	31.9	6.8	0.4	16/05/2009	77	0.3	26.2	11.6	4.2
02/04/2009	33	0.8	27.3	3.9	0.0	17/05/2009	78	0.3	28.6	12.2	6.4
04/04/2009	35	0.8	30.0	4.8	0.5	18/05/2009	79	0.3	24.9	10.7	4.5
05/04/2009	36	0.8	26.0	4.1	1.0	19/05/2009	80	0.3	29.3	14.4	8.3
06/04/2009	37	0.8	32.5	5.1	2.3	20/05/2009	81	0.3	25.6	11.6	4.6
07/04/2009	38	0.8	29.9	4.8	1.0	21/05/2009	82	0.3	29.6	14.8	7.7
08/04/2009	39	0.8	27.8	3.5	0.5	22/05/2009	83	0.3	27.1	12.1	6.3
09/04/2009	40	0.8	31.3	5.6	1.1	23/05/2009	84	0.3	29.9	13.2	5.5
10/04/2009	41	0.8	30.1	4.3	0.7	24/05/2009	85	0.3	26.5	11.4	5.7
11/04/2009	42	0.8	26.5	4.4	1.5	25/05/2009	86	0.3	30.1	13.5	9.0
12/04/2009	43	0.8	32.4	5.6	2.2	26/05/2009	87	0.3	25.5	10.2	5.2
13/04/2009	44	0.8	31.3	6.1	2.0	27/05/2009	88	0.3	30.4	11.9	6.6
14/04/2009	45	0.8	26.9	5.2	1.9						

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 19. Data for Figure 7.16

Date	Day	HRT	Influent	Effluent	Date	Day	HRT	Influent	Effluent
01/03/2009	1	1.0	31.3	4.6	15/04/2009	46	0.6	28.0	0.8
02/03/2009	2	1.0	29.5	3.7	16/04/2009	47	0.6	27.9	0.9
03/03/2009	3	1.0	30.7	6.7	17/04/2009	48	0.6	28.2	1.2
04/03/2009	4	1.0	30.5	5.7	18/04/2009	49	0.5	27.7	1.8
05/03/2009	5	1.0	30.4	3.6	19/04/2009	50	0.5	27.9	0.9
06/03/2009	6	1.0	31.2	3.4	20/04/2009	51	0.5	28.0	1.6
07/03/2009	7	1.0	30.2	1.4	21/04/2009	52	0.5	32.3	0.0
08/03/2009	8	1.0	28.9	2.9	22/04/2009	53	0.5	30.3	3.7
09/03/2009	9	1.0	30.9	4.6	23/04/2009	54	0.5	29.5	6.3
10/03/2009	10	1.0	30.5	2.0	24/04/2009	55	0.5	31.2	3.4
11/03/2009	11	1.1	29.5	4.3	25/04/2009	56	0.4	31.2	3.7
12/03/2009	12	1.0	32.0	3.9	26/04/2009	57	0.4	32.3	6.6
13/03/2009	13	1.0	31.2	2.6	27/04/2009	58	0.4		
14/03/2009	14	1.1	29.4	4.5	28/04/2009	59	0.4	26.7	3.1
15/03/2009	15	1.0	31.3	2.4	29/04/2009	60	0.4	26.8	4.9
16/03/2009	16	1.0	30.6	0.7	30/04/2009	61	0.4	26.5	2.2
17/03/2009	17	1.0			01/05/2009	62	0.4	27.7	2.2
18/03/2009	18	1.0			02/05/2009	63	0.4	26.7	8.0
19/03/2009	19	0.9	30.3	1.9	03/05/2009	64	0.4	27.3	8.1
20/03/2009	20	0.9	29.8	2.6	04/05/2009	65	0.4	28.7	9.5
21/03/2009	21	0.9			05/05/2009	66	0.4	27.9	7.6
22/03/2009	22	0.9			06/05/2009	67	0.4	27.8	9.4
23/03/2009	23	0.9			07/05/2009	68	0.4	22.9	1.3
24/03/2009	24	0.9	28.0	3.0	08/05/2009	69	0.3		
25/03/2009	25	0.9	27.7	4.8	09/05/2009	70	0.3		
26/03/2009	26				10/05/2009	71	0.3		
27/03/2009	27	0.8			11/05/2009	72	0.3	24.3	8.7
28/03/2009	28	0.8	31.8	4.7	12/05/2009	73	0.3	26.5	8.1
29/03/2009	29	0.8	31.4	7.3	13/05/2009	74	0.3	26.9	6.2
30/03/2009	30	0.8	29.4	3.8	14/05/2009	75	0.3	23.6	2.7
31/03/2009	31	0.8	32.7	3.5	15/05/2009	76	0.3	26.6	2.5
01/04/2009	32	0.8	32.6	3.9	16/05/2009	77	0.3	24.4	3.2
02/04/2009	33	0.8	29.3	2.7	17/05/2009	78	0.3	22.6	2.7
04/04/2009	35	0.8	30.4	4.1	18/05/2009	79	0.3	22.9	2.5
05/04/2009	36	0.8	27.6	1.3	19/05/2009	80	0.3	21.9	2.1
06/04/2009	37	0.8	29.6	1.1	20/05/2009	81	0.3	23.6	3.8
07/04/2009	38	0.8	29.1	1.0	21/05/2009	82	0.3	22.4	2.0
08/04/2009	39	0.8	27.0	1.1	22/05/2009	83	0.3	22.5	4.4
09/04/2009	40	0.8	29.9	1.9	23/05/2009	84	0.3	25.4	6.9
10/04/2009	41	0.8	30.1	1.2	24/05/2009	85	0.3	23.3	5.4
11/04/2009	42	0.8	26.9	1.9	25/05/2009	86	0.3	21.5	4.9
12/04/2009	43	0.8	29.6	2.5	26/05/2009	87	0.3	22.4	7.0
13/04/2009	44	0.8	29.0	2.4	27/05/2009	88	0.3	23.4	8.8
14/04/2009	45	0.8	26.3	1.9					

Jong Hyuk Hwang, Ph.D. Thesis

Appendix 20. Data for Figure 7.17

Loading	SNR	SDNR	Loading	SNR	SDNR	Loading	SNR	SDNR	Loading	SNR	SDNR
0.96	0.85		2.11	2.03		2.60	2.22		4.25	3.09	
1.38	1.10		2.21		1.54	2.61	2.23		4.34	3.35	
1.46	1.38		2.22	1.95		2.62	2.28		4.45		3.44
1.47	1.39		2.22		2.03	2.62	2.21		4.54	3.61	
1.48	1.30		2.36		1.54	2.62	2.37		4.60		3.99
1.54	1.32		2.36		2.04	2.63		2.55	4.64	3.82	
1.65	1.26		2.36		1.78	2.65	2.13		4.65	3.60	
1.67		1.43	2.36	2.14		2.67	2.23		4.66	3.64	
1.68	1.55		2.36	1.95		2.70	2.28		4.68		4.20
1.69		1.19	2.45	2.08		2.71		2.52	4.70		4.16
1.70		1.39	2.46	2.22		2.71		2.51	4.74		3.74
1.70	1.57		2.46		1.74	2.72		2.50	4.76		4.16
1.71	1.50		2.46		2.26	2.73		2.43	4.80	3.21	
1.71	1.51		2.46		1.85	2.74	2.37		4.86	3.79	
1.72	1.46		2.46	2.13		2.76		2.68	4.97	3.58	
1.72	1.56		2.46	2.02		2.78		2.60	5.01		4.69
1.73	1.36		2.47	2.18		2.79	2.25		5.11	3.99	
1.73		1.14	2.47		1.76	2.84	2.49		5.22	3.77	
1.74		1.53	2.47	2.05		2.85	2.43		5.24	4.00	
1.74	1.47		2.47	2.27		2.87		2.75	5.47	4.13	
1.74		1.53	2.47	2.06		2.91	2.48		5.47	4.24	
1.75	1.54		2.47		1.76	2.95	2.52		5.53		3.97
1.75		1.59	2.47		2.16	3.02		2.72	5.55		4.87
1.96	1.72		2.48		2.13	3.02		2.88	5.56	4.18	
1.96	1.75		2.48	1.97		3.04		2.66	5.74		4.05
1.96	1.72		2.48	2.09		3.06		2.74	5.76		5.30
1.96		1.39	2.49	2.14		3.06		3.00	5.79		4.56
1.96		1.56	2.49		1.80	3.07	2.58		5.80		3.72
1.96	1.68		2.50		2.33	3.09		2.85	5.87	4.12	
1.97	1.77		2.50	2.16		3.10	2.66		5.88	4.29	
1.97	1.81		2.50		2.19	3.10	2.60		5.93		5.36
1.97	1.77		2.50	1.92		3.11		2.96	6.02		4.79
1.97	1.70		2.51	1.94		3.11	2.56		6.03		5.20
1.97		1.54	2.52	2.13		3.11		2.69	6.28		4.38
1.97		1.48	2.52	2.18		3.11		2.98	6.30		5.01
1.97		1.35	2.53		2.22	3.21		2.46	6.31		4.28
1.98	1.65		2.53	2.21		3.25		2.76	6.32		5.44
1.98		1.34	2.53		2.48	3.26	2.63		6.52		4.89
1.98	1.74		2.54		2.20	3.27	2.68		6.54	4.45	
1.98	1.73		2.54		2.45	3.28	2.65		6.55		5.33
1.98	1.81		2.54		2.18	3.33	2.62		6.55		4.39
1.99	1.72		2.55	2.22		3.36	2.83		6.90	4.61	
1.99	1.73		2.55	1.97		3.37	2.80		6.90	3.94	
1.99		1.78	2.55	2.19		3.38	2.80		7.09	3.88	
1.99	1.82		2.55		2.35	3.39		3.11	7.11		4.84
1.99	1.69		2.56		2.08	3.40		2.89	7.15	4.29	
1.99	1.73		2.56	2.32		3.46	2.94		7.18	4.13	
2.10		1.67	2.56		2.43	3.56		3.42	7.36	4.10	
2.11		1.60	2.56		2.51	3.56		3.49	7.42	4.23	

Jong Hyuk Hwang, Ph.D. Thesis

2.11	1.88			2.57	2.22			3.57		3.37
2.11		1.51		2.58	2.24			3.79	3.01	
2.11	1.84			2.58	2.19			3.95	3.04	
2.11		1.52		2.59	2.24			4.08	3.41	
2.11		1.98		2.59	2.02			4.21		4.11
2.11	1.89			2.59	2.04			4.22		3.84
2.11	1.86			2.60		2.53		4.23		4.12

7.58	4.19	
7.82	3.90	
8.00	4.59	
8.05	4.09	
8.38	4.68	
8.44	4.66	

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