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TREATMENT OF PIG MANURE DIGESTATE LIQUID AND STUDY OF NITROUS OXIDE EMISSIONS FROM BIOREACTORS

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Acknowledgment

Abstract

Large amounts of pig manure containing high concentrations of nutrients are generated on pig farms. Anaerobic digestion is one of the sustainable technologies for pig manure management. However, most of the ammonium remains in the digestate liquid after anaerobic digestion of pig manure. This induces that the wastewater has a low ratio of readily biodegradable organic matter to nitrogen, which inhibits nitrogen removal when using conventional biological nitrogen removal processes and nitrous oxide (N_2O) emission. Thus, the overall aim of this PhD research was to find a novel and efficient method for further treatment of the pig manure digestate liquid.

During this research, a novel laboratory-scale intermittently aerated sequencing batch reactor (IASBR) system was constructed in the Environmental Engineering Laboratories at the National University of Ireland, Galway to treat pig manure digestate liquid for the purpose of organic matter removal, nitrogen removal and achievement of partial nitrification. In addition, the aerobic granular sludge sequencing batch reactors (SBRs), aerobic upflow biofilters, and chemical coagulation and struvite precipitation were also investigated for pig manure digestate liquid treatment. The specific objectives of this PhD research included: (1) to investigate pig manure digestate liquid treatment using IASBRs, aerobic granular sludge SBRs, aerobic upflow biofilters and chemical treatment, particularly for nitrogen removal; and (2) to study the characteristics of N_2O emissions from the IASBRs and aerobic granular sludge SBRs.

In the IASBRs, 75% - 90% of COD removal was achieved and the non-biodegradable COD in the effluent can be removed via chemical coagulation. The nitrogen removal efficiency and the nitrite accumulation efficiency were up to 76% and 80%, respectively. The intermittent aeration strategy, the aeration rate, readily biodegradable organic matter and denitrification were found to affect partial nitrification. In the aerobic granular sludge SBRs, the mean COD removal efficiencies were more than 99%. Under continuous aeration conditions, the high

nitrogen removal efficiency was 36% which confirmed that simultaneous nitrification and denitrification took place in aerobic granular sludge SBRs. In the upflow biofilters, the COD removal efficiency was up to 92%. The nitrogen removal efficiencies were 84% and 88% at two loading rates of 0.12 kg TN/ (m^3· d) and 0.07 kg TN/ (m^3· d), respectively. The attached biofilm had a more efficient nitrification and denitrification activity than that of suspended growth biomass in biofilters. Phosphorus removal efficiencies were 88%. In the chemical treatment study, more than 75% of COD and 85% of turbidity removals were achieved by coagulation when anion polyacrylamide and pH of 4.5 were adopted. In the struvite precipitation experiment, under optimal experimental conditions (pH= 10.5, and Mg^{2+}: NH_4^+-N: PO_4^{3-}-P= 1.3: 1: 1.3), more than 96% NH_4^+-N was removed from the separated pig manure digestate liquid.

The N_2O emission from the IASBRs and the aerobic granular sludge SBRs were investigated using N_2O microsensors. N_2O emissions were 12.0% of the influent nitrogen loading rate in IASBRs. The aeration period and non-aeration period contributed to 91.7% and 8.3% of N_2O emissions, respectively. The content of readily biodegradable organic matter in wastewater, dissolved oxygen, pH, nitrite concentrations and free nitrous acid affected the N_2O emissions in the IASBRs. As for the N_2O emission in aerobic granular sludge SBRs, the N_2O emission was 2.2% - 8.2% of the influent nitrogen loading rate, decreased with the increase in the COD: N ratio and the aeration rate.

The research results show that all the technologies examined were efficient in nutrient removal from the pig manure digestate liquid and can be used in practice. According to the experience obtained in this research, the best method for pig manure digestate liquid treatment is to combine the chemical treatment technology for nitrogen recovery and the biotechnologies.

Chapter One

Introduction

1.1 Background

In the European Union (EU), pig farming is a major agricultural enterprise and is carried out on large centralized pig farms (Molinuevo *et al.*, 2009). As a result, a large amount of pig manure containing high concentrations of nutrients and solids is produced annually. It is estimated that more than 3.2 million m^3 of pig manure is produced annually in Ireland (S.I. No.610, 2010). In Ireland, the European Communities (Good Agricultural Practice for the Protection of Waters) Regulations (S.I. No.610, 2010) have imposed immense restrictions on the direct use of pig manure on land.

Anaerobic digestion (AD) is one of the sustainable technologies for pig manure management. However, after AD treatment, most of the ammonium in pig manure still remains in the digestate liquid. In practice, the digestate containing high ammonium levels is used as fertilizer. However, this may not always be practical due to the restriction of soil conditions and land availability as required by the European Communities Regulations (S.I. No.610, 2010). In addition, inadequate digestate treatment results in a risk of breaches of the Urban Wastewater Treatment Directive (91/271/EEC; EEC, 1991) and the Water Framework Directive (2000/60/EC; EC, 2000) if the digestate is discharged into receiving water bodies. Thus, further treatment of the digestate, particularly nitrogen removal, is necessary before discharge into water bodies.

Biological nitrogen removal via partial nitrification is efficient for treating high ammonium wastewater due to the advantages as follow: (I) reduction of the carbon demand of denitrifying bacteria by 40% for denitrifying nitrite in comparison with nitrate; and (II) reduction of the aeration energy by 25% when nitrifying ammonium to nitrite rather than nitrate (Turk and Mavinic, 1986; Li, 2010). Meanwhile, t

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anaerobic ammonium oxidation (ANAMMOX) process can be used to treat nitrite accumulation if necessary.

The Department of Civil Engineering at National University of Ireland Galway (NUI Galway) have observed nitrogen removal via partial nitrification in intermittently aerated sequencing batch reactors (IASBRs) treating slaughterhouse wastewater (Li, 2010). Thus, in this PhD research, the IASBR technology will be used to examine the performance of IASBRs in organic matter and nitrogen removal, and achievement of partial nitrification when treating pig manure digestate liquid.

Biological nitrogen removal leads to nitrous oxide (N_2O) generation and emissions. Because N_2O is a potent greenhouse gas (GHG), it is important to study N_2O emissions and generation when treating pig manure digestate in bioreactors, which is a high ammonium wastewater.

In addition, it is necessary to evaluate pig manure digestate liquid treatment using other technologies, since it is difficult to treat pig manure digestate to reach the discharge standards with a single wastewater treatment technology. The valid technologies include aerobic granular sludge sequencing batch reactors, chemical treatment and aerobic upflow biofilters.

Up to date, there have been few successful applications of technologies into pig manure digestate liquid treatment, and no studies have been carried out on N_2O generation and emission during digestate treatment using biotechnologies. Therefore, this study will examine various technologies for pig manure digestate treatment, with the focus on the intermittently-aerated sequencing batch reactor technology, which was developed in the Environmental Engineering Laboratory, NUI Galway; provide operation experience and data for technology development; and investigate N_2O emissions from bioreactors.

1.2 Research aim and objectives

The overall aim of this PhD research project is to develop a methodology for pig

manure digestate management by biological or chemical technologies. The specific objectives of this study are:

1. To investigate the treatment of pig manure digestate liquid, to examine partial nitrification efficiency and operational strategy in IASBRs and to study nitrous oxide emissions from IASBRs.

2. To study treatment of pig manure digestate liquid using aerobic granular sludge sequencing batch reactors and nitrous oxide emission.

3. To study separated pig manure digestate liquid treatment using aerobic upflow biofilters.

4. To investigate separated pig manure digestate liquid treatment using chemical methods including coagulation and struvite precipitation.

1.3 Procedures

In order to address specific objectives of this research, the research procedures are given in brief below:

For examination of the IASBR technology, three 10 L laboratory-scale IASBRs were constructed in the Environmental Engineering Laboratories at NUI Galway. The IASBRs were operated as sequencing batch reactors with alternating aeration and non-aeration periods in the react phase. Raw pig manure digestate liquid was treated in the IASBRs at various organic loading conditions. The performance of the IASBRs in wastewater treatment was studied in detail by measuring the parameters in the influent and effluent and by conducting phase studies. Nitrous oxide emissions from the IASBRs were studied by measuring N_2O concentrations in the bulk fluid using N_2O sensors.

For examination of the aerobic granular sludge sequencing batch reactor technology, three 1.5 L aerobic granular sludge bioreactors were constructed and operated in School of Engineering, Beijing Normal University, China. The reactors trea

synthetic wastewater simulating a mixture of municipal wastewater and separated pig manure digestate liquid. The performance of the reactors in wastewater treatment, in terms of organic matter and nitrogen removal, and N_2O emissions were examined.

For examination of the aerobic upflow biofilter technology, six 2 L aerobic upflow biofilters were constructed and operated in the Environmental Engineering Laboratories at NUI Galway. The reactors treated the raw separated pig manure digestate liquid. The performance of the reactors in wastewater treatment was examined by measuring the parameters in the influent and effluent and by conducting phase studies.

For examination of chemical treatment, batch experiments of the separated pig manure digestate liquid were conducted. Chemicals were dosed into wastewater and the experiments were carried out under various testing conditions. The wastewater quality after coagulation and struvite precipitation was measured.

Parameters including chemical oxygen demand (COD), nitrogen (ammonium, nitrite, nitrate and total nitrogen), phosphorus (orthophosphate and total phosphorus) and solids contents were measured.
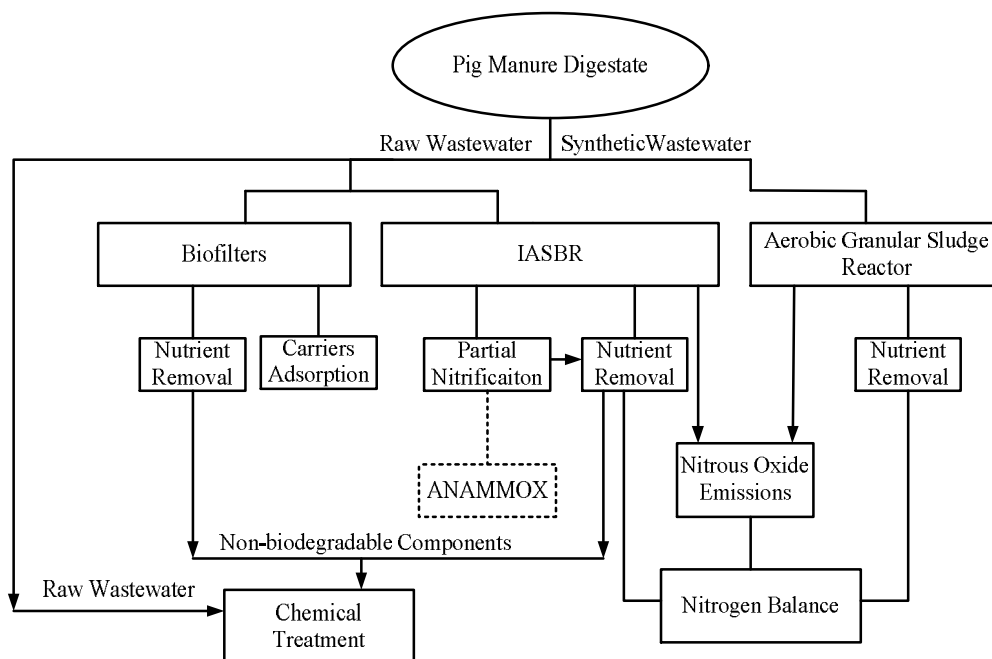
Figure 1.1 Research procedures and PhD thesis structure diagram (solid line shows the proposed research work)

The procedures of the research work and structure of the PhD thesis is given in Figure 1.1.

1.4 Structure of dissertation

The PhD thesis structure is given in brief below:

Chapter 2 presents a review of the literature concerning pig manure generation, characteristics of separated pig manure digestate liquid, biological nitrogen removal and partial nitrification, nitrous oxide emissions and chemical nitrogen treatment, and a review of the research work carried out on IASBR to-date. These areas are relevant to this PhD research.

In Chapter 3 and Chapter 4, a detailed description of the laboratory-scale IASBRs in treatment of separated digestate liquid of pig manure at various nitrogen loading rates, and mechanisms of partial nitrification are presented.

In Chapters 5, nitrous oxide generation and emission and nitrogen balance in the laboratory-scale IASBRs are detailed.

Chapter 6 details the treatment of a mixture of municipal wastewater and separated pig manure digestate liquid in aerobic granular sludge sequencing batch reactors and the associated nitrous oxide generation and emission.

Chapter 7 describes the treatment of separated pig manure digestate liquid in aerobic upflow biofilters.

In Chapter 8, the application of chemicals in the treatment of separated digestate liquid is discussed.

Finally, Chapter 9 gives the conclusions from this thesis with recommendations for further research.

Chapter Two

Literature Review

2.1 Introduction

After anaerobic digestion of pig manure (PM), high concentrations of nutrients still remain in the digestate. Without proper treatment, nitrogen and organic matter contained in the digestate can cause water pollution, odour problems and eutrophication. Thus, removal of organic matter, nutrients and solids from the digestate is necessary before it is discharged to water bodies, and therefore efficient treatment technologies are required.

This chapter briefly introduces the pig industry and the pig manure arising from it in Europe and Ireland along with the typical PM treatment technologies. Then, since this PhD research is focused on nitrogen removal from the separated pig manure digestate liquid, conventional nitrogen removal processes are reviewed in addition with the characteristics of partial nitrification, nitrous oxide emissions and anaerobic ammonium oxidisation. Finally, sequencing batch reactor (SBR) and intermittently aerated sequencing batch reactor (IASBR) technologies adopted for the treatment of separated pig manure digestate liquid are reviewed.

2.2 Pig manure

2.2.1 Pig industry

In most regions of the world, pig numbers are increasing as reported by the Food and Agriculture Organization (FAO). Table 2.1 shows the pig number in the period of 2005 - 2009 worldwide. Over 50% and 19% of the world's pig population is in Asia and Europe, respectively.

Table 2.1 Global population of pigs (unit: million heads) (FAOSTAT, 2011)

Regions	2005	2006	2007	2008	2009
World	907.8	926.6	919.6	936.4	941.8
Africa	25.0	25.6	26.4	26.7	27.4
Americas	151.8	154.6	156.2	159.0	160.1
Asia	534.6	547.4	534.0	554.4	561.2
Europe	190.8	193.5	197.4	191.0	187.7
Oceania	5.6	5.6	5.5	5.3	5.2

In the European Union (EU), pig farming is a major agricultural enterprise (Megens *et al*., 2008; Molinuevo *et al*., 2009). The pig industry is an important part of the Irish economy and is the third most important agricultural sector (Martin, 2007), and in 2011, the pig production sector contributed to 7.4% of the gross agricultural output. As a result, a large amount of pig manure containing high concentrations of nutrients and solids is generated. It is estimated using data of European Communities (Good Agricultural Practice for the Protection of Waters) Regulations (S. I. No. 610, 2010) that more than 3.2 million m^3 of pig manure is produced annually in Ireland.

2.2.2 Characteristics of pig manure

Table 2.2 Characteristics of pig manure (Moral *et al*., 2005; Masse *et al*., 2007; Martinez-Suller *et al*., 2008; Xie *et al*., 2011)

Parameters	Range
Total solids (TS)	0.78 - 9.95%
Total volatile solids (TVS)	0.30 - 8.16%
Total Kjeldahl nitrogen (TKN)	1220 - 6700 mg/L
Ammonium nitrogen (NH_4^+-N)	540 - 3880 mg/L
Total chemical oxygen demand (COD)	7110 - 174300 mg/L
Soluble COD	1110 - 74700 mg/L
Total phosphorus (TP)	350 - 2720 mg/L
pH	7.00 - 7.90

Pig manure contains high concentrations of organic matter and nitrogen (Table 2.2).

These components are potentially hazardous for the environment (Bonmati and Flotats, 2003; Holzel *et al*., 2010).

Discharging PM to the environment without proper treatment has led to water, soil and air pollution (Dambreville *et al*., 2006; Sleutel *et al*., 2006). Ammonium and solids in the PM causes acidification (Hobbs *et al*., 1999); nitrogen and phosphorus enrichment contributes to eutrophication in the water bodies (Lu *et al*., 2011; Song *et al*., 2011). Pig manure also contains pathogenic organisms that could contribute to microbial contamination of ground water (Moral *et al*., 2008; Carthy *et al*., 2011). Therefore, efficient management of PM has become imperative in Europe.

2.2.3 Technologies for pig manure treatment

Pig manure is traditionally spread on lands (Imbeah, 1998). Meade *et al.* (2011) show that PM can be successfully used in combination with inorganic fertilizers to improve nitrogen nutrition of winter wheat in spring as part of an integrated nutrient program. However, in Ireland, the European Communities (Good Agricultural Practice for the Protection of Waters) Regulations (S. I. No. 610, 2010) imposes restrictions on the use of PM on grass and cereal land. According to the legislation, the maximum amount that can be applied to land every year is equivalent to 170 kg organic N /ha. Many lands are no longer suitable for land spreading because organic nitrogen loading from grazing livestock is approaching or already at the 170 kg N /ha limit.

Recently, many technologies, such as anaerobic digestion, aerobic treatment, composting, flocculation, air stripping, etc., have been developed to treat PM (Kuai and Verstraete, 1998; Gilbert *et al*., 2008; Xie *et al*., 2011). Zhu (2007) reported composting, which stabilizes manure organic matter into humus-like product, can be used for PM treatment. Organic matter in terms of chemical oxygen demand (COD) can be decomposed when PM is composted with sawdust, shredded green waste and chopped straw (Nolan *et al*., 2011). Christensen *et al*. (2009) used flocculation to examine the physicochemical properties of PM and remove organic matter, and they achieved high flocculation efficiency by dosing with high molecular weight cationic polymers. Bonmati and Flotats (2003) removed ammonium from PM through stripping. In order to completely remove ammonium from fresh pig slurry, a hi

temperature of 80 °C and a high initial pH of 11.5 would be required. Suspended-growth activated sludge integrated with aerated biofilters was used by Gilbert et al. (2008) to treat PM, and efficient nitrate removal was achieved via denitrifying biomass. High-concentration pollutants in PM could limit the application of aerobic treatment systems due to high operational costs, so Dosta *et al*. (2008) added coagulants /flocculants in a sequencing batch reactor (SBR) for COD and nitrogen removal, and jar-tests showed that the optimal dosage of the coagulant $FeCl_3$ was 800 mg/L.

Currently, anaerobic digestion of pig manure is widely adopted in Europe for PM management (Bonmati *et al*., 2001; El-Mashad *et al*., 2004; Karim *et al*., 2005; Anceno *et al*., 2009). It is one of the sustainable technologies for PM management, through which methane gas is recovered with the COD removal and it is mitigation against GHG emissions. Anaerobic reactors can work at psychrophilic (5 °C - 20 °C), mesophilic (30 °C - 40 °C) and extreme thermophilic (55 °C - 82 °C) conditions (Nozhevnikova *et al*., 1999). Karakashev *et al*. (2008) adopted two-step anaerobic reactors to digest pig manure; 85% COD and 26.8% nitrogen were removed. The performance of anaerobic digestion is very dependent on the type and composition of the material to be digested. Panichnumsin *et al*. (2010) examined co-digestion of cassava pulp with PM and achieved a solids removal of 61%. Zarkadas and Pilidis (2011) adopted anaerobic co-digestion of table olive debittering and washing effluent, cattle manure and pig manure, and obtained a 50% increase in the specific methane yield and 80% total organic carbon removal. Murto *et al*. (2004) co-digested the potato processing waste with PM. Organic matter and volatile solids were removed but high amounts of ammonium were still left in the digestate. Deng *et al*. (2007) used an anaerobic sequencing batch reactor to digest PM and obtained stable operation, but almost no ammonium was removed.

Usually, pig manure digestate is land spread on farm for its fertilizer value. However, the legislation mentioned above may limit this practice. Furthermore, the COD: N ratio is very low in the pig manure digestate after AD (Murto *et al*., 2004; Karakashev *et al*., 2008; Xie *et al*., 2011). This limits nitrogen removal from the digestate using conventional biological wastewater treatment processes.

2.3 Nitrogen removal from wastewater

2.3.1 Nitrogen

Nitrogen in wastewater can be divided into five categories as shown in Eq. 2.1 (Henze *et al.*, 2000):

$$C_{TN} = S_{NOx} + S_{NH4} + S_{I,N} + X_{S,N} + X_{I,N} \qquad \text{(Eq. 2.1)}$$

where: C_{TN}, total nitrogen (TN); S_{NOx}, total oxidized nitrogen (TON), including nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N); S_{NH4}, ammonia nitrogen (NH_3-N) plus ammonium nitrogen (NH_4^+-N); $S_{I,N}$, dissolved inert organic nitrogen; $X_{S,N}$, suspended readily biodegradable organic nitrogen; and, $X_{I,N}$, suspended inert organic nitrogen.

In raw domestic/municipal wastewater, NH_4^+-N and organic N are the major parts of TN. NO_3^--N and NO_2^--N are usually not found in raw domestic/municipal wastewater or only found in low concentrations. Organic nitrogen is converted to NH_4^+-N in the early stage of wastewater treatment through ammonification and hydrolysis (Strock, 2008).

Conventional biological nitrogen removal (BNR) consists of two successive steps: nitrification and denitrification. The first step is nitrification, during which NH_4^+-N is oxidized to NO_2^--N and then NO_3^--N by autotrophic nitrifiers under aerobic conditions (Process 1 - 2 in Figure 2.1). The second step is denitrification, in which NO_2^--N and/or NO_3^--N are reduced to nitrogen gas (N_2) by heterotrophic denitrifiers under anoxic conditions (Process 3 - 6 in Figure 2.1), with organic carbon as the electron donor (Henze *et al.*, 1995; Obaja *et al.*, 2005).
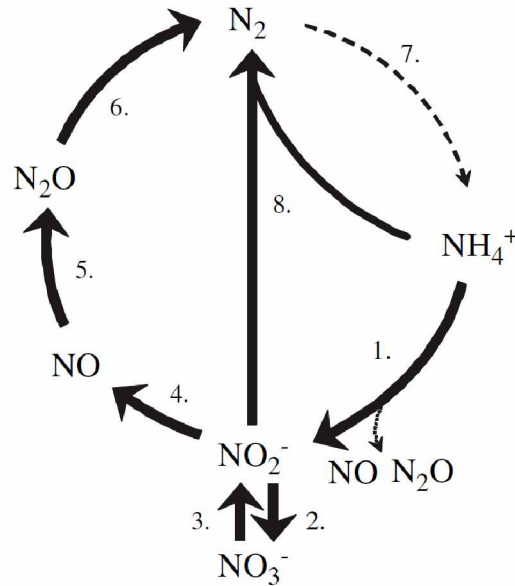
Figure 2.1 Biological nitrogen conversions (Kampschreur *et al.*, 2009): (1) aerobic ammonium oxidation; (2) aerobic nitrite oxidation; (3) nitrate reduction to nitrite; (4) nitrite reduction to nitric oxide; (5) nitric oxide reduction to nitrous oxide; (6) nitrous oxide reduction to dinitrogen gas; (7) nitrogen fixation; (8) ammonium oxidation with nitrite to nitrogen gas (ANAMMOX)

2.3.1.1 Nitrification

Nitrification is an important step in the nitrogen biogeochemical cycle and is conducted by two different autotrophic bacteria - ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) - with NO_3^- as the final product. The nitrifiers enriched in ammonium-rich liquids are *Nitrosomonas europaea* and *Nitrobacter*, and enriched in domestic wastewater with low ammonium concentrations are *Nitrosomonas oligotropha* and *Nitrospira* (Gujer, 2010). The first step of nitrification, namely nitritation/partial nitrification, is the oxidation of NH_4^+ to NO_2^- through hydroxylamine (NH_2OH) by AOB. O_2 is needed in this process as the electron accepter (Philips *et al.*, 2002). Individual steps involved in nitritation can be described as follows:

$$NH_4^+ + O_2 + H^+ + 2e^- \rightarrow NH_2OH + H_2O$$

$$NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$$

$$0.5O_2+2H^++2e^-\rightarrow H_2O$$

The overall nitritation reaction is:

$$NH_4^++1.5O_2\rightarrow NO_2^-+2H^++H_2O \hspace{4cm} \text{(Eq. 2.2)}$$
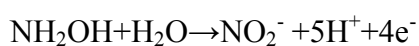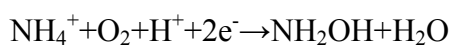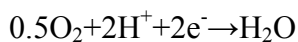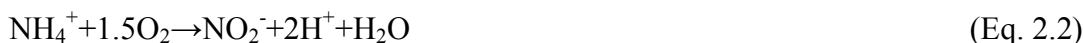
As Eq. 2.2 shows, two proton ions are released, so the nitritation process consumes alkalinity.

In the second step, NO_2^- is further oxidized to NO_3^- by NOB. Individual reaction steps involved in nitration can be described as follows:

$$NO_2^-+H_2O\rightarrow NO_3^-+2H^++2e^-$$

$$0.5O_2+2H^++2e^-\rightarrow H_2O$$

The overall nitration reaction is:

$$NO_2^-+0.5O_2\rightarrow NO_3^- \hspace{5cm} \text{(Eq. 2.3)}$$
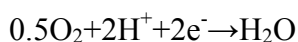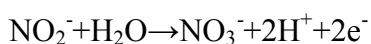
The overall reaction converting NH_4^+ to NO_3^- can be described in Eq. 2.4.

$$NH_4^++2O_2\rightarrow NO_3^-+2H^++H_2O \hspace{4cm} \text{(Eq. 2.4)}$$

In total, 350 kJ is generated with 1 mol NH_4^+ oxidation to NO_3^-. Sinha and Annachhatre (2007) reported that NH_3 is likely the real substrate for nitrification rather than NH_4^+.

2.3.1.2 Denitrification

With readily biodegradable carbon sources available, denitrification occurs through the activity of heterotrophic bacteria (*Proteus*, *Pseudomonas* or *Bacillus*) under anoxic conditions (Skiba, 2008) where carbon sources are the electron donor. The reaction pathway (Eq. 2.5) is described in Figure 2.1 (Tchobanoglous *et al*., 2003):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \hspace{4cm} \text{(Eq. 2.5)}$$

where: NO, nitric oxide; and, N_2O, nitrous oxide.

2.3.2 Partial nitrification

Usually, NO_2^- is undesirable in the water environment due to its toxicity to the permeability of cell membranes and inhibition of ATP synthesis (Almeida *et al*., 1995; Sijbesma *et al*., 1996). Recent studies have shown that biological nitrogen removal can be achieved via NO_2^-. This process has two advantages over conventional BNR via NO_3^-: (I) saving of 25% of the aeration energy, as NH_4^+ is only oxidized to NO_2^- in the nitrification process and (II) reduction of carbon demand of heterotrophs by 40% for denitrifying NO_2^--N in comparison with NO_3^--N (Sinha and Annachhatre, 2007).

Under normal nitrification conditions, the nitrite oxidation rate is coupled with the ammonia level, the accumulation of nitrite results from the inhibition of NOB growth kinetics and stable nitritation occurs by washing out of NOB from the reactor systems (Philips *et al*., 2002). Dissolved oxygen (DO), pH, and temperature are suggested to be important factors for the NOB activity and achieving partial nitrification (Blackburne *et al*., 2008a).

2.3.2.1 PH

Since partial nitrification always occurs in high ammonium wastewater, pH is one of the decisive parameters in inhibition of the NOB activity (Peng *et al*., 2008). Okabe *et al*. (2011) showed the important factors for successful partial nitrification were high ammonium loading rate and pH, giving high free ammonia (FA) or free nitrous acid (FNA) concentrations. FA and FNA can be calculated with Eq. 2.7 and Eq. 2.9, respectively (Philips *et al*., 2002):

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \tag{Eq. 2.6}$$

$$FA = \frac{[TAN]\ 10^{pH}}{K_a/K_w + 10^{pH}} \tag{Eq. 2.7}$$

$$H^+ + NO_2^- \leftrightarrow HNO_2 \tag{Eq. 2.8}$$

$$FNA = \frac{[NO_2\ N]\ 10^{pH}}{K_n + 10^{pH}} \tag{Eq. 2.9}$$

where: *TAN*, total ammonia nitrogen measured using the standard method, including NH_3-N and NH_4^+-N; K_a, the ionization constant for NH_4^+; K_w, the ionization constant for water; and, K_n, the ionization constant for NO_2^-.

FA and FNA can inhibit both AOB and NOB, but NOB react more sensitively to FA and FNA than AOB (Anthonisen *et al.*, 1976): when FA > 10 - 150 mg/L, both AOB and NOB are inhibited by FA; when 0.1 - 1.0 mg/L< FA < 10 - 150 mg/L, only NOB are inhibited; when FA < 0.1 - 1.0 mg/L and FNA < 0.2 - 2.8 mg/L, complete nitrification is possible; when FNA > 0.2 - 2.8 mg/L, NOB are inhibited by FNA. Because the concentrations of FA and FNA depend on pH (Eq. 2.7 and Eq. 2.9), FA is the main inhibitor of nitrification (Philips *et al.*, 2002) at high pH (>8), and FNA is the main inhibitor at low pH (<7.5). Successful partial nitrification has been found at high FA concentrations by Chen *et al.* (2010) and Zeng *et al.* (2011).

Ciudad *et al.* (2007) applied different pH values, in the range of 7.5 - 8.6, in nitrifying reactors and observed obvious partial nitrification (NO_2^--N was more than 80% of TON) for 249 days with the enrichment of AOB which was more than 95% of the total amount of nitrifiers. Wang and Yang (2004) applied pH values of 6.5, 7.5, 8.5, and 9.5 in membrane bioreactors and found that stable partial nitrification was obtained at pH of 7.5. Jenicek *et al.* (2004) reported that pH value and distribution was crucial in sequencing batch reactors treating wastewater with high NH_4^+ concentrations (above 1 g/L), and efficient partial nitrification was achieved with a minimal NO_3^- production. Sinha and Annachhatre (2007) concluded that a pH in the range of 7.5 - 8.5 is suitable for inhibition of NOB activity.

2.3.2.2 Dissolved oxygen

Eq. 2.2 and Eq. 2.3 show oxidation of NH_4^+ to NO_2^- and oxidation of NO_2^- to NO_3^- demand oxygen. Though the oxygen demand is 3.43 mg DO for oxidation of 1 mg NH_4^+-N to NO_2^--N and only 1.14 mg DO for oxidation of 1 mg NO_2^--N to NO_3^--N, the growth of AOB is more robust at low DO than NOB. Low DO levels prevent re-growth of NOB in the long-term operation but not AOB (Brockmann and Morgenroth, 2010) because AOB have a higher oxygen affinity than NOB. The oxygen half saturation constants of AOB and NOB according to the Monod kineti which represent the oxygen mass transfer resistances for enrichment of AOB a

NOB, are 0.033 ± 0.003 mg/L and 0.43 ± 0.08 mg/L, respectively (Blackburne et $al.$, 2008b).

This is confirmed by Garrido et $al.$ (1997) who found that NO_2^- build-up occurred when DO dropped and was not affected by microbial adaptation but associated with intrinsic characteristics of the microbial growth system. Chuang et $al.$ (2007) pointed out that partial nitrification was satisfactorily accomplished under oxygen limiting conditions at around 0.2 mg DO/L. Yang et $al.$ (2010) limited DO concentrations by controlling the air flow rate, and achieved a stable partial nitrification performance, which was more than 99.9%, during continuous operation for 80 days. Ruiz et $al.$ (2003) treated high ammonium synthetic wastewater and achieved a nitrite accumulation efficiency of more than 65% at a DO level around 0.7 mg/L. In conclusion, DO below 1 mg/L is good for partial nitrification, under which the AOB activity is 2.56 times faster than that of NOB (Hanaki et $al.$, 1990).

Partial nitrification can also be achieved if the aeration is controlled to terminate just before the complete oxidation of NH_4^+. Blackburne et $al.$ (2008b) obtained NO_2^- accumulation in a SBR using the aerobic duration control strategy, by which aeration was terminated when NH_4^+ oxidation was complete. This process was proved to be effective in achieving partial nitrification in steady-state.

2.3.2.3 Temperature

When temperature increases, the activity of NOB is more sensitive to the temperature change and the maximum specific growth rate of AOB rises faster than that of NOB (Philips et $al.$, 2002). Isaka et $al.$ (2008) observed that NOB in sludge was killed by heat shock, but AOB survived. No NOB was detected after a heat-shock treatment with a temperature higher than 60 °C.

Nitrite accumulation in wastewater treatment systems starts to occur at 20 °C, and becomes more efficient with increasing temperature from 20 °C to 30 °C (Kim et $al.$, 2008a). When the temperature is increased to 35 °C, immediate and long-term nitrite accumulation can occur even after reduction of the temperature to 30 °C (Bougard et $al.$, 2006). Wang et $al.$ (2011) suggest that water temperature should be kept at relatively high levels (at least 30 °C) in order to allow nitrification to occur. In the

SHARON (single reactor high activity ammonia removal over nitrite) process, the optimal biomass activity and partial nitrification efficiency occurred at temperatures ranging between 35 °C to 45 °C (Hulle *et al.*, 2007).

Besides DO, pH and temperature, the nutrient concentrations can also affect partial nitrification. Zafarzadeh *et al.* (2011) pointed out that nitrite accumulation took place at the DO concentration of 1 - 1.5 mg/L and the nitrite accumulation efficiency increased with decreasing COD: N ratio in the aerobic reactor at COD: N ratios lower than 6. Phillips *et al.* (2002) reported chlorate, cyanate, azide and hydrazine inhibited the oxidation of nitrite more than that of ammonium.

2.3.2.4 Kinetics of the partial nitrification process

The partial nitrification process can be evaluated using typical kinetics (Gujer *et al.*, 1999; Henze *et al.*, 2000). The nomenclature of kinetic parameters for nitrification is listed in Table 2.3.

Recently, a two-step nitrification model has been developed (Carrera *et al.*, 2004; Iacopozzi *et al.*, 2007) according to the Monod equation, and it divides the nitrification process into nitritation and nitration steps and analyzes the autotrophic biomass related to AOB and NOB, respectively (Eq. 2.10 and Eq. 2.11):

$$\mu_{AOB} = \mu_{AOB,MAX} \frac{S_{O_2}}{K_{O_2,AOB} + S_{O_2}} \frac{S_{NH_4}}{K_{NH_4,A} + S_{NH_4}} \frac{S_{ALK}}{K_{ALK,A} + S_{ALK}} \qquad \text{(Eq. 2.10)}$$

$$\mu_{NOB} = \mu_{NOB,MAX} \frac{S_{O_2}}{K_{O_2,NOB} + S_{O_2}} \frac{K_{NH_4,I}}{K_{NH_4,I} + S_{NH_4}} \frac{S_{NO_2}}{K_{NO_2,A} + S_{NO_2}} \qquad \text{(Eq. 2.11)}$$

For a completely mixed system (like the intermittent aerated sequencing batch reactor (IASBR) used in this study, which can be regarded as a completed mixed system during the react phase), when NH_4^+-N consumption for heterotrophic bacteria growth is ignored (Blackburne *et al.*, 2008a), the AOB and NOB biomass growth rate can be described by Eq. 2.12 and Eq. 2.13:

$$\frac{dX_{AOB}}{dt} = \mu_{AOB} X_{AOB} - b_{AOB} X_{AOB} - \frac{X_{AOB}}{} \qquad \text{(Eq. 2.1}$$

$$\frac{dX_{NOB}}{dt} = \mu_{NOB} X_{NOB} - b_{NOB} X_{NOB} - \frac{X_{NOB}}{\theta} \qquad \text{(Eq. 2.13)}$$

Table 2.3 Nomenclature of kinetic parameters for the nitrification process (Carrera *et al.*, 2004; Iacopozzi *et al.*, 2007; Blackburne *et al.*, 2008a)

Item	Nomenclature	Item	Nomenclature
X_{AOB}	Biomass concentration for AOB (g VSS/ L)	$\mu_{AOB, MAX}$	Maximum specific growth rate for AOB (/d)
X_{NOB}	Biomass concentration for NOB (g VSS/L)	$\mu_{NOB, MAX}$	Maximum specific growth rate for NOB (/d)
b_{AOB}	Decay rate of AOB (/d)	μ_{AOB}	Specific growth rate for AOB (/d)
b_{NOB}	Decay rate of NOB (/d)	μ_{NOB}	Specific growth rate for NOB (/d)
$K_{O2, AOB}$	Half saturation constant to DO for AOB (mg O_2/L)	S_{O2}	DO concentration (mg O_2/L)
$K_{O2, NOB}$	Half saturation constant to DO for NOB (mg O_2/L)	S_{NH4}	NH_4^+-N concentration (mg NH_4^+-N /L)
$K_{HN4, A}$	Half saturation constant to NH_4^+-N for autotrophs (mg NH_4^+-N /L)	S_{NO2}	NO_2^--N concentration (mg NO_2^--N /L)
$K_{NO2, A}$	Half saturation constant to NO_2^--N for autotrophs (mg NO_2^--N /L)	S_{ALK}	Alkalinity concentration (mg ALK/L)
$K_{NH4, I}$	Inhibition constant to NH_4^+-N for nitrite oxidation (mg NH_4^+-N /L)	$K_{ALK, A}$	Half saturation constant to alkalinity for autotrophs (mg ALK/L)
		θ	Sludge retention time (d)

2.3.3 Nitrous oxide generation and emission

Nitrous oxide (N_2O) is a GHG with a steady-state life-time of 114 years in the atmosphere, and its 100-years global warming potential is ~300 times higher than that of carbon dioxide (CO_2; IPCC, 2007).

Lo et $al.$ (2010) found in their research that N_2O was one of the main end products of nitrogen removal. N_2O emission from wastewater treatment facilities is mainly due to N_2O generation during heterotrophic denitrification and autotrophic nitrification processes. In the heterotrophic denitrification process, N_2O gas is an intermediate product as shown in Eq. 2.5 (Skiba, 2008). In the autotrophic nitrification process, some AOB form N_2O during NH_4^+ oxidation through: (1) chemical decomposition of the intermediate products, like NH_2OH or NO_2^- (Wrage et $al.$, 2001), or (2) reduction of NO_2^- by nitrite reductase (Kampschreur et $al.$, 2008b).

Generally, N_2O emissions from municipal wastewater treatment plants are estimated by IPCC (2006) to be ~0.5% of the total influent nitrogen loading rate (NLR). However, it can be emitted at a much higher amount, for example, ~1.7% of the NLR from nitrifying reactors (Kampschreur et $al.$, 2008a), 15% of the NLR in full-scale reactors, and 95% of the NLR in laboratory-scale bioreactors (Kampschreur et $al.$, 2009) were removed via N_2O.

Townsend-Small et $al.$ (2011) investigated wastewater treatment processes in urban southern California, and found N_2O generation was equal to 1.2% of total nitrogen removal. Foley et $al.$ (2010) reported N_2O generation ranged 0.006 - 0.253 kg N_2O-N/ kg N denitrified in the wastewater treatment plants. In long-term partial nitritation reactors, the emission of N_2O is 0.6 - 2.6% (average 1.9%) of the NLR (de Graaff et $al.$, 2010). Bhunia et $al.$ (2010) reviewed N_2O emissions in current main wastewater treatment facilities, the results of which are shown in Table 2.4.

According to a study conducted by Okamoto (2009) in Japan, the CO_2 equivalent of N_2O emissions in wastewater treatment plants was 7 million tonnes CO_2 equivalent in 2004 and was as high as 34% of the total GHG emissions from the wastewater treatment sector. In the same study, the CO_2 equivalent of CH_4 emissions was only 4%. Therefore, it is necessary to study N_2O emissions from wastewater treatment facilities so as to find an environmentally sound approach to mitigate against GHG emissions.

A variety of operational parameters affect N_2O emissions, such as DO, pH, COD: N ratio, etc (Zeng et $al.$, 2003; Tallec et $al.$, 2006; Kampschreur et $al.$, 2008a). N generation became significant at DO restriction conditions in the bioreactors (Park

al., 2000; Zhu and Chen, 2011; Rajagopal and Beline, 2011). The COD: N ratio is an important factor causing N_2O emissions in heterotrophic denitrification. Alinsafi *et al*. (2008) demonstrated that when the COD: N ratio decreased from 7 to 3, the N_2O emissions increased from 1.0% to 5.1% of the NLR. In a full-scale plant treating ammonium-rich wastewater with a COD: N ratio of 1.5, the dissolved N_2O concentration in the mixed liquor was as high as 6.27 mg N/L (Itokawa *et al*., 1996).

Table 2.4 N_2O emissions from wastewater treatment systems (Bhunia *et al*., 2010)

Description of wastewater treatment system	N_2O emissions as % of NLR
Biological nitrification process with synthetic wastewater	3.82 - 2.24
SBR with synthetic wastewater	2.5 - 4.62
SBR with domestic wastewater	2.45
Suspended growth process with real domestic wastewater	4.57
Biofilm process with domestic wastewater	3
Activated sludge process with urban wastewater	0.1 - 0.06
Nitrogen removal with swine wastewater	1.71 and 17.7
Draw and fill mode activated sludge process with swine wastewater	35
Aerobic treatment of swine slurry	30

2.3.4 Nitrogen removal by chemical methods

High concentrations of nitrogen and phosphorus contained in anaerobically digested swine wastewater can be removed by means of struvite crystallization (Song *et al*., 2011; Ye *et al*., 2011). Magnesium ammonium phosphate (MAP), named struvite, has a solubility is $10^{-13.24\pm0.5}$. It is a white crystalline substance (Figure 2.2) consisting of magnesium, ammonium and phosphorus in equal molar ratios ($MgNH_4PO_4 \cdot 6H_2O$). MAP precipitation is suggested as a promising physicochemical method for nitrogen recovery from wastewater (Roncal-Herrero and Oelkers, 2011).
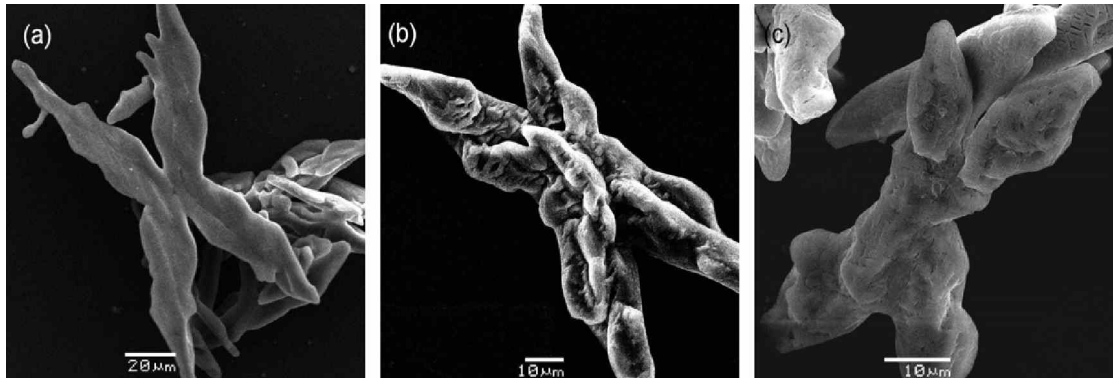
Figure 2.2 SEM photomicrographs of struvite powder (Roncal-Herrero and Oelkers, 2011)

Struvite forms according to the reaction shown as Eq. 2.14 (Doyle and Parsons, 2002):

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O \qquad (Eq.\ 2.14)$$
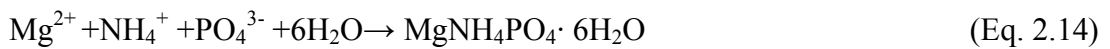
Struvite crystallization is efficient in recovering nitrogen from nitrogen-rich wastewater (Yetilmezsoy and Sapci-Zengin, 2009; Kabouris *et al.*, 2009). He *et al.* (2007) recovered more than 96% NH_4^+ when using the struvite precipitation process to treat landfill leachate. The pH value during the formation of MAP notably affects the MAP precipitation and nitrogen removal efficiency (Figure 2.3). The pH for the minimum struvite solubility is between 8.9 and 9.25 and is not affected by the Mg: P ratio (Nelson *et al.*, 2003). With an increase in pH, the struvite precipitation efficiency increases (Pastor *et al.*, 2008).
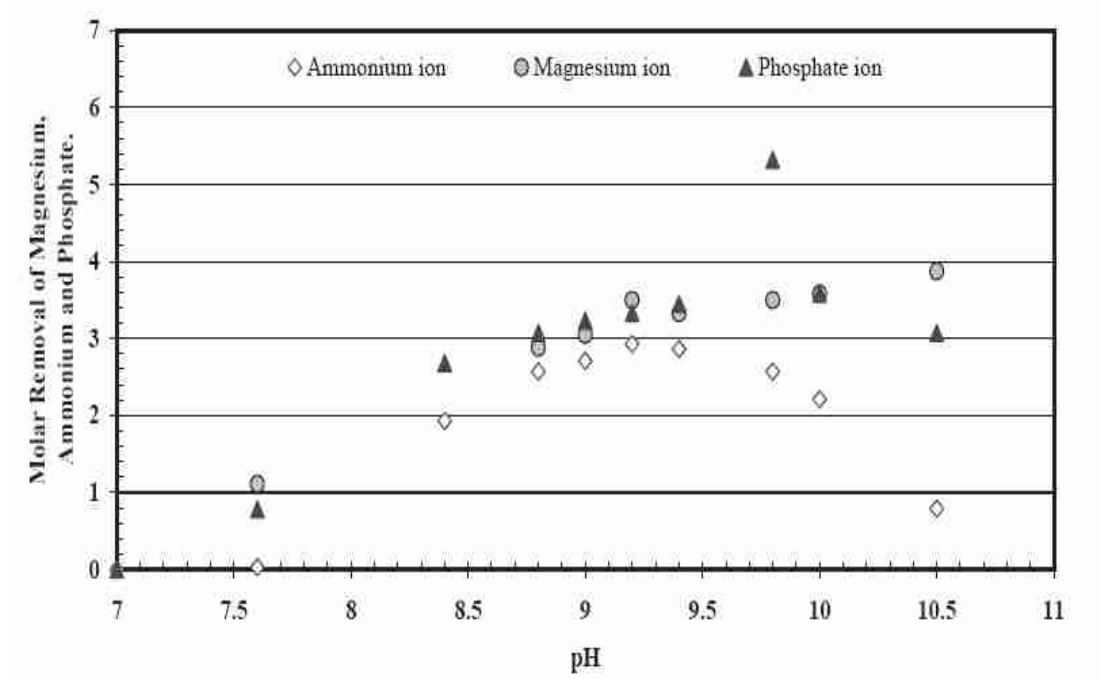
Figure 2.3 Effect of pH on soluble magnesium, ammonium and phosphate concentrations (Booker *et al*., 1999)

Yilmazel and Demirer (2011) treated anaerobically digested and solid-liquid separated manure via struvite precipitation, and found that the Mg: N: P molar ratio affected NH_4^+-N removal efficiencies. When the Mg: N: P molar ratio was 1: 1: 1 in the liquid phase, the average NH_4^+-N removal efficiency was 86.4%, compared with 97.4% at the Mg: N: P ratio of 1.5:1:1. Warmadewanthi and Liu (2009) also have found that efficiency of NH_4^+-N removal increased as the molar ratio of Mg to P increased.

The potential value of struvite is land application because it is a valuable slow release fertilizer (Carballa *et al*., 2009). Yetilmezsoy *et al*. (2009) selected grass *L. Perenne* as the model plant and assessed the effects of different doses of MAP precipitate on the grass growth. The best growth was observed at the MAP to seed ratio of 2. Plants receiving higher MAP doses did not demonstrate any inhibition on growth.

2.3.5 Anaerobic ammonium oxidation

It is well known that the oxidation of NH_4^+ can occur under aerobic or oxygen

limiting conditions. In theory, NH_4^+ can also react with NO_2^- or NO_3^- because the Gibbs free energy of these reactions is similar with that of oxidation of NH_4^+ by oxygen (Table 2.5; Jetten *et al.*, 1999). This means that it may be possible to oxidize NH_4^+ with NO_2^- or NO_3^- instead of oxygen.

Table 2.5 Gibbs free energy in the processes of NH_4^+ oxidation (Jetten *et al.*, 1999)

Reaction	Gibbs free energy
$3NO_3^-+5NH_4^+\rightarrow 4N_2+9H_2O+2H^+$	-297 kJ/ mol NH_4^+-N
$NO_2^-+NH_4^+\rightarrow N_2+2H_2O$	-358 kJ/ mol NH_4^+-N
$2O_2+NH_4^+\rightarrow NO_3^-+H_2O+2H^+$	-349 kJ/ mol NH_4^+-N
$6O_2+8NH_4^+\rightarrow 4N_2+12H_2O+8H^+$	-315 kJ/ mol NH_4^+-N

In the early 1990s, Mulder *et al.* (1995) first observed anaerobic NH_4^+ oxidation in a denitrification pilot plant, after noticing that NH_4^+ and NO_3^- simultaneously disappeared from the reactor effluent with a concomitant increase in N_2 gas production. This process was then named as anaerobic ammonium oxidation (ANAMMOX). Further studies have shown that NO_2^- rather than NO_3^- is the preferred electron acceptor in nature (van Niftrik *et al.*, 2004). Wang and Kang (2005) observed that after the exhaustion of NO_2^-, activated sludge performed the ANAMMOX process by using NO_3^- as the electron acceptor to oxidize NH_4^+.

The mechanisms for the ANAMMOX process are still unclear, but NH_2OH and hydrazine (N_2H_4) have been identified as important intermediates during NH_4^+ oxidation by NO_2^-. One of the possible metabolic pathways is that NH_4^+ is biologically oxidized with NH_2OH as the electron acceptor, which most likely comes from NO_2^-. This leads to a transient accumulation of N_2H_4, which is then converted to N_2 gas (Van de Graaf *et al.*, 1999; Jetten *et al.*, 1999; Schmidt *et al.*, 2002).

In the ANAMMOX process (Eq. 2.15), bacteria are enriched with bicarbonate as the only carbon source (Jetten *et al.*, 2010). Thus, without the need for organic matter, this technology has been developed to treat ammonium rich but low organic matter wastewater, such as landfill leachate, animal manure and the supernatant of digested sludge (Liang and Liu, 2008; Jin *et al.*, 2011).

$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow$

$0.066CH_2O_{0.5}N_{0.15} + 1.02N_2 + 0.26NO_3^- + 2.03H_2O$ (Eq. 2.15)
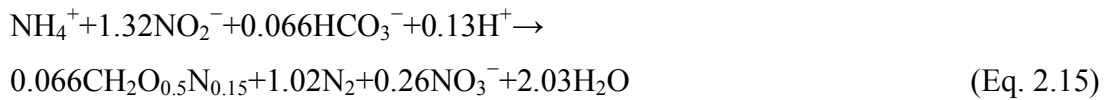
ANAMMOX bacteria are considered to be difficultly cultivated because of their low specific growth rate ($\mu_{max} = 0.065$ /d), which makes the start-up period much longer than other nitrogen removal technologies (Lopez *et al.*, 2008). ANAMMOX bacteria's activity is observed between pH = 6.5 - 9, with an optimum pH of 8 and an optimum temperature of 37 °C (Egli *et al.*, 2001). Upflow anaerobic sludge bed (UASB) reactors and SBR systems can be used for ANAMMOX cultivation (Strous *et al.*, 1998; Chamchoi *et al.*, 2008; Molinuevo *et al.*, 2009).

2.4 Reviews of the sequencing batch reactor technology

2.4.1 Sequencing batch reactor

A sequencing batch reactor (SBR) is efficient for biological nutrient removal from wastewaters. SBR is a time-based treatment technology compared with the conventional area-based activated sludge technologies (Liu and Liu, 2006) and is based on a cyclic sequence consisting of fill, react, settle, draw and idle phases (Figure 2.4) repeated over time (Magri *et al.*, 2009).
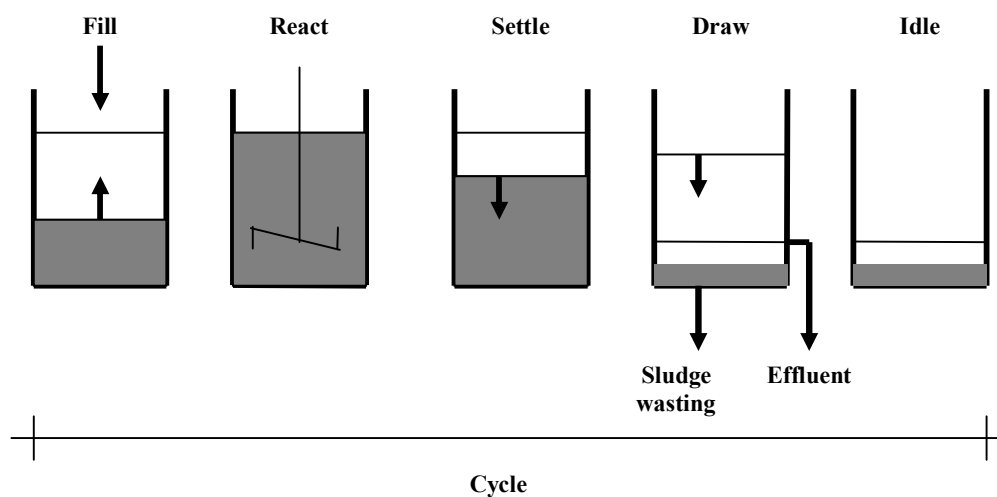
Figure 2.4 operation phases during one cycle of a typical SBR process (Wilderer *et al.*, 2000)

From the 1920s, SBRs have been used for municipal and industrial wastewater treatment worldwide. Various novel SBRs, such as anaerobic SBR, membrane SBR, moving bed SBR, two-phase SBR and intermittently aerated SBR have been developed in recent years (Mohan *et al*., 2007; Zhan *et al*., 2009; Fakhru'l-Razi *et al*., 2010; Tomei *et al*., 2010; Sreethawong *et al*., 2010; Lim *et al*., 2011) due to the advantages of this technology (Suresh *et al*., 2011) as follows:

(1) Simplicity and cost effectiveness;

(2) Combination of aerobic and anoxic phases in a single reactor;

(3) High flexibility in terms of sequence and cycle time;

(4) Near ideal quiescent settling conditions; and

(5) Resistant to fluctuating influent loading rates.

In a typical SBR, simultaneous carbon, nitrogen and phosphorus removals from the wastewater would be achieved (Fontenot *et al*., 2007; Roy *et al*., 2010). Nitrification and denitrification can occur in one SBR (Clippeleir *et al*., 2009), and the aerobic duration control strategy can be used for nitrogen removal from wastewater (Blackburne *et al*., 2008a). Tsuneda *et al*. (2006) observed that the SBR process had a much higher anoxic/aerobic phosphate uptake rate than the conventional A^2O (anaerobic/anoxic/aerobic) and AO (anaerobic/aerobic) processes.

2.4.2 Intermittently aerated sequencing batch reactor

2.4.2.1 Principles of the IASBR

Through the operation of SBR with intermittent aeration regimes (alternating aerobic and anoxic periods) in the react phase, NO_2^- and NO_3^- are produced by means of nitrification during the aeration periods, and are then reduced by means of denitrification in the subsequent non-aeration periods (Zhan *et al*., 2009). In addition, it is possible to achieve partial nitrification through intermittent aeration, and then enhance the reactor performance on nitrogen removal by means of denitrification via nitrite (Khanitchaidecha *et al*., 2010). AOB and NOB are all aerobic autotrop

bacteria and prefer constant aerobic conditions. Alternating aerobic and anoxic conditions leads to difference in the activity of AOB and NOB (Li *et al*., 2008a). After switching the anoxic period to the aerobic period, it takes NOB a longer time to recover activity than AOB due to difference in their growth and decay rates (Dytczak *et al*., 2008). Thus, frequent switching between the anoxic and aerobic periods can lead to nitrite accumulation (Yoo *et al*., 1999).). Li *et al*. (2011) analyzed AOB and NOB populations in IASBRs for partial nitrification when treating high ammonium wastewater. They found that under the intermittent aeration control conditions, a larger AOB population accumulated and was maintained in the IASBRs, while the NOB population was kept at a minimum level. The AOB population to the NOB population ratio was higher than 2000 folds in the stable operation periods (Figure 2.5).
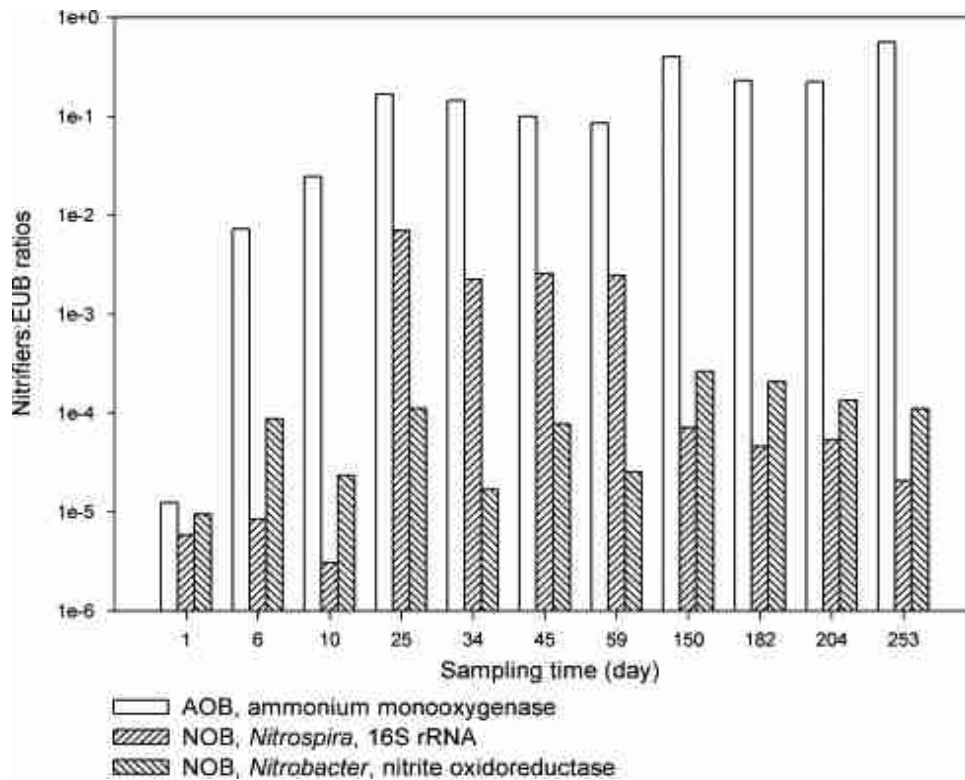
Figure 2.5 Population variations of AOB and NOB in the sludge in partial nitrification IASBR treating ammonium-rich wastewater (Li *et al*., 2011)

2.4.2.2 IASBR in practice

Nitrogen can be removed efficiently using IASBRs technology. Guo *et al*. (2008) compared a continuously-aerated reactor with an intermittently-aerated reactor in

treatment of synthetic wastewater. The efficiency of NH_4^+-N and TN removal was 80% and 70% in the continuously-aerated reactor, respectively; while, NH_4^+-N concentration was always under the detection limit and 86% of TN was removed in the intermittently-aerated reactor. Katsogiannis *et al*. (2003) studied nitrogen removal from a synthetic wastewater containing NH_4^+-N at 50 mg/L in an IASBR where aerobic and anoxic periods were maintained with a duration ratio of 1: 3 (20 min: 60 min). The efficiency of nitrogen removal via NO_2^- was 98.0 ±1.6%.

Efficient partial nitrification has been observed in IASBRs (Jiang *et al*., 2009; Cheng *et al*., 2001; Zeng *et al*., 2008; Magdalena *et al*., 2008). Using the IASBR technology to achieve partial nitrification has a major advantage as there is no need for the precise control of DO and temperature in the reactor (Ciudad *et al*., 2007; Pambrun *et al*., 2008). Zeng *et al*. (2008) adopted the real-time aerobic/anoxic duration control strategy to achieve nitrogen removal via NO_2^--N and the nitrite accumulation rate (NO_2^--N/ TON) was above 95%. Li *et al*. (2008b) used intermittent aeration with an air supply of 0.8 L/min at 50 minute/50 minute aeration and non-aeration intervals in the react phase (Figure 2.6), and the NO_2^--N accumulation rate during most of the operational cycle was over than 80%.
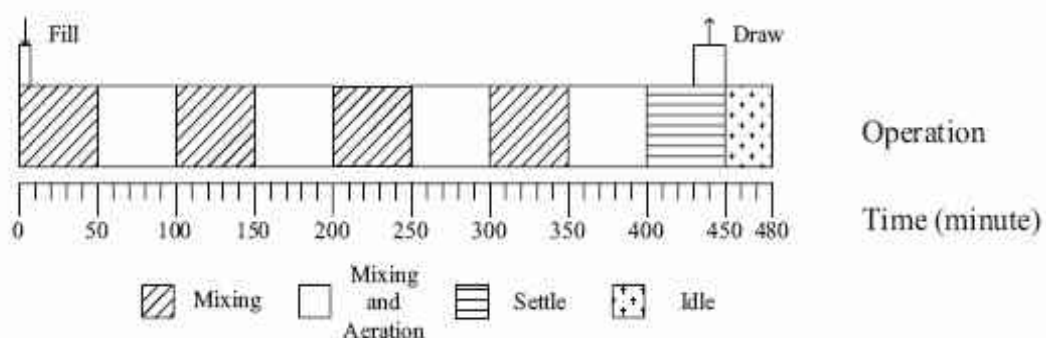
Figure 2.6 Complete operational cycle of the laboratory-scale SBR system (Li *et al*., 2008b)

However, it is reported that partial nitrification by this approach is sensitive to operational upsets, and long-term stable partial nitrification in IASBRs is likely unreliable when treating wastewater with fluctuating influent (Norton *et al*., 2009).

2.5 Summary

This chapter presents a brief review of the characteristics of pig manure, technologies for pig manure and its digestate treatment, issues specific to biological nitrogen removal, including nitrification, denitrification, partial nitrification, nitrous oxide emissions and chemical nitrogen removal. Sequencing batch reactor and intermittently aerated sequencing batch reactor technologies used in this research are also reviewed.

Chapter Three

Effects of Loading Rate on Nutrient Removal and Partial Nitrification in Intermittently Aerated Sequencing Batch Reactors Treating Pig Manure Digestate Liquid

3.1 Introduction

A novel wastewater treatment technology, intermittently-aerated sequencing batch reactors, was developed in Civil Engineering, NUI Galway. IASBRs are considered to be an efficient technology for partial nitrification in low COD: N ratio wastewater treatment.

The pig manure digestate liquid was treated under three loading rates in IASBRs. Over a study period of 257 days in two stages, the performance of IASBRs, in terms of COD and nitrogen removal and the partial nitrification efficiency, was examined. The effects of the operational parameters including DO, pH and the aeration rate on partial nitrification were investigated.

3.2 Materials and methods

3.2.1 Laboratory-scale intermittently-aerated sequencing batch reactors

Three identical laboratory-scale IASBRs (IASBR$_L$, IASBR$_M$ and IASBR$_H$ for different loading rates) were constructed in the laboratory (Figure 3.1). The cylindrical reactors were made from transparent Plexiglas, with an inner diameter of 194 mm and a height of 400 mm, and each had an effective volume of 10 litres. Each reactor was stirred with a rectangular mixing paddle (100 mm × 80 mm). Air was supplied using air pumps through air diffusers installed at the bottom of the reactor and the air flow rate was controlled by air flow meters. MasterFlex L/S peristal

pumps were used to feed the influent wastewater into the reactors and withdraw effluent. The operation of the IASBRs was controlled with programmable timers (Samson Electric Wire, Germany).

(a)
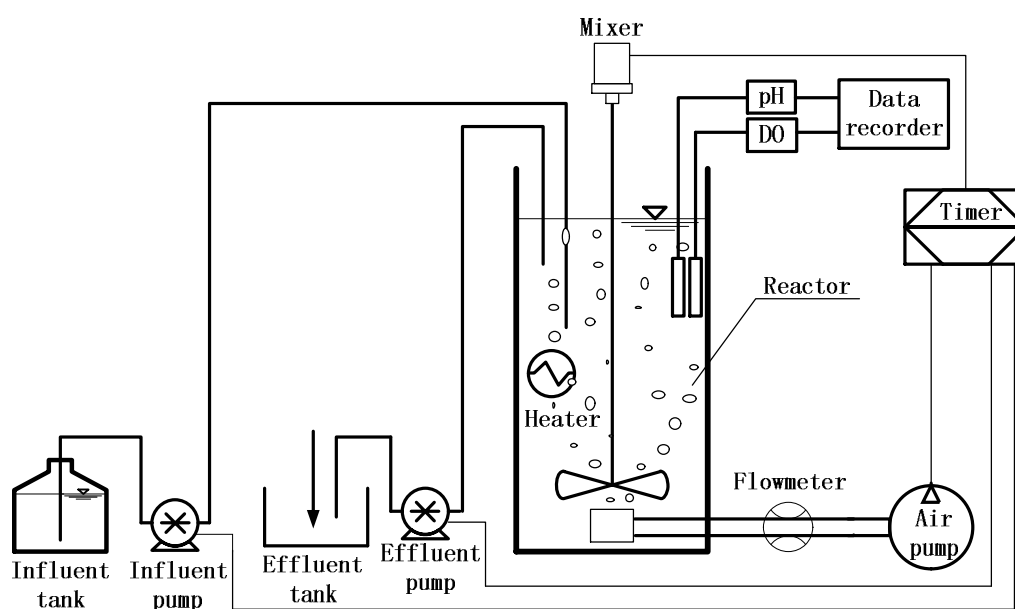
(b)

Figure 3.1 Photograph (a) and schematic diagram (b) of the IASBR systems

The IASBRs were seeded with activated sludge taken from a local municipal wastewater treatment plant (WWTP). Before seeding, the activated sludge was cultivated for 20 days in a constant aeration condition with synthetic wastewater containing 600 mg COD/L and 200 mg NH_4^+-N/L. The initial biomass concentrations in the IASBRs after seeding were 2.48 - 2.57 g suspended solids (SS)/L, and the volatile suspended solids (VSS) /SS ratio was 88%. During the entire operation period, the sludge volume index (SVI) was around 122 mL/g SS. In the steady state stage, 500 mL mixture liquor was discharged from the reactors at the end of the final aeration period in the react phase to maintain sludge retention time of 20 days.

3.2.2 Operation of the IASBR systems

The experiment consisted of two stages. In the first stage (Stage 1, Day 1 - Day 110), the duration of the operational cycle was 8 hours. In the second stage (Stage 2, Day 121 - Day 257), the three reactors were operated with a cycle of 12 hours.
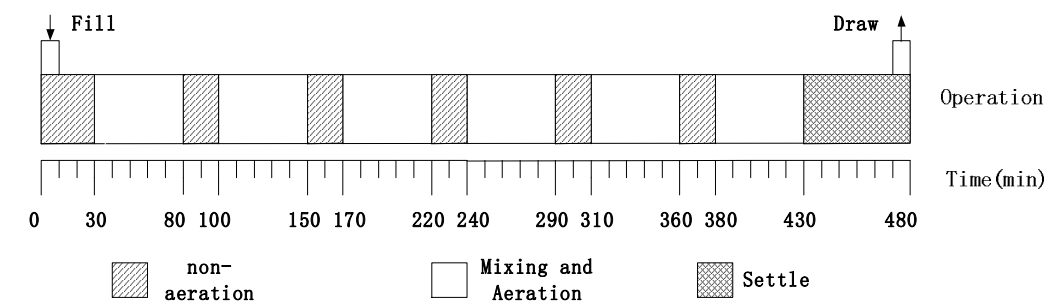
(a)
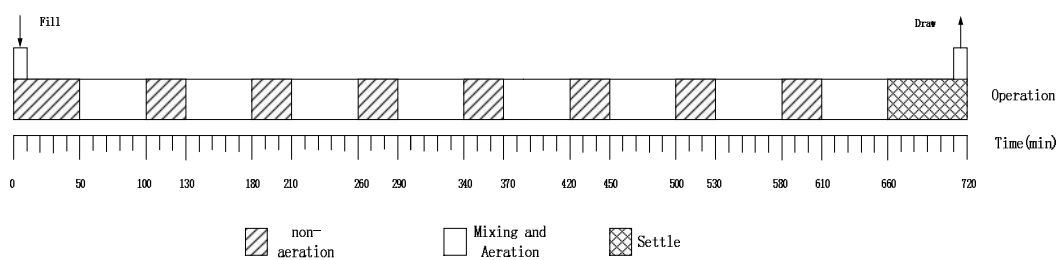
(b)

Figure 3.2 Complete operational cycle of the IASBR systems: (a) 8-hour cycle duration; (b) 12-hour cycle duration

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The operational phases in a cycle are shown in Figure 3.2. During the 10-minute fill phase, 0.5, 1.0 and 2 litres of wastewater were pumped into $IASBR_L$, $IASBR_M$ and $IASBR_H$, corresponding to the volume exchange ratios of 5%, 10% and 20%, respectively. During the react phase, the reactors were intermittently aerated with 6 successive 50-minute aeration/20-minute non-aeration periods for the 8 hour cycle duration or 8 successive 50-minute aeration/30-minute non-aeration periods for the 12 hour cycle duration. The aeration rate during the aeration period was 0.8 - 1.0 L air/min, which was based on previous research (Li *et al.*, 2008b). The mechanical stirrer worked continuously during the fill and react phases. The settle phase lasted for 40 - 50 minutes, and then, the effluent was withdrawn within 10 minutes.

The temperature in the three reactors was maintained at 31°C, because the temperature of the digestate withdrawn from the mesophilic anaerobic digester was 35°C and an experimental temperature of 31 °C was used to simulate practical wastewater treatment conditions. Moreover, this temperature would benefit partial nitrification (Kim and Kim, 2008).

3.2.3 Characteristics of the separated pig manure digestate liquid

The digestate used in this study was taken from a mesophilic anaerobic digester digesting pig manure in a pig farm in Co. Kerry, Ireland. After collection, it was stored in a refrigerator at 4 °C and was used within 2 months. The digestate taken from the digester contained 7, 500 - 14, 000 mg COD/L and 2, 000 - 3, 800 mg NH_4^+-N /L. The wastewater was diluted by 3 - 4 times before being fed into the IASBRs in order to investigate the effect of the loading rate on partial nitrification.

The characteristics of the wastewater treated in the IASBRs were: 2950±230 mg/L of COD; 790±182 mg/L of NH_4^+-N; 962±144 mg/L of total nitrogen (TN); 12.1 ±3.8 mg/L of orthophosphate (PO_4^{3-}-P); 1.25±0.18 g/L of suspended solids (SS); 1.17±0.10 g/L of VSS; and pH of 8.28±0.05. The 5-day biochemical oxygen demand (BOD_5) to COD ratio was only 0.24, indicating a low proportion of biodegradable organic matter in the wastewater. The wastewater had a COD to TN ratio of 2.9 and the loading rates in the three IASBRs were 0.44 kg COD/ ($m^3 \cdot$ d) and 0.14 kg TN/ ($m^3 \cdot$ d) in $IASBR_L$, 0.89 kg COD/ ($m^3 \cdot$ d) and 0.29 kg TN/ ($m^3 \cdot$ d) in $IASBR_M$, 1.77 kg COD/ ($m^3 \cdot$ d) and 0.58 kg TN/ ($m^3 \cdot$ d) in $IASBR_H$ in Stage 1; 0.30 kg COD/

$(m^3 \cdot d)$ and 0.10 kg TN/ $(m^3 \cdot d)$ in IASBR$_L$, 0.59 kg COD/ $(m^3 \cdot d)$ and 0.19 kg TN/ $(m^3 \cdot d)$ in IASBR$_M$, 1.18 kg COD/ $(m^3 \cdot d)$ and 0.38 kg TN/ $(m^3 \cdot d)$ in IASBR$_H$ in Stage 2.

3.2.4 Batch experiments

Batch experiments were designed to examine the effect of nitrification and denitrification on nitrite accumulation under different conditions. Activated sludge was taken from the three reactors at the end of the final aeration period. The biomass mixture was then washed twice with tap water and aerated to remove the remaining substrates. The washed activated sludge biomass mixture was added into 0.5 L beakers with an effective volume of 400 mL. NH$_4$Cl, NaNO$_2$ or KNO$_3$ solutions with designed concentrations of NH$_4^+$-N, NO$_2^-$-N or NO$_3^-$-N, were added into the beakers, respectively. The effect of the aeration rate and COD: N ratio on partial nitrification was studied. Air diffusers were fixed at the bottom of the beakers when aeration was needed. In anaerobic conditions for denitrification, the beakers were continuously stirred with magnetic stirrers, and anaerobic conditions were achieved by stripping DO out for 3 minutes using argon gas. After experiments commenced, liquid samples were taken from the batch reactors at intervals for the measurement of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N concentrations.

3.2.5 Water quality analysis

The methods used for water samples test are given in Appendix B.

3.2.6 Microbial structure analysis

The molecular analysis technique, polymerase chain reaction (PCR) - denaturing gradient gel electrophoresis (DGGE), was adopted to analyze the ecological structure of the sludge in IASBRs. 1-2 g activated sludge was taken from the reactors and was concentrated twice by centrifugation at 14,000 rpm for 2 minutes. Genomic DNA was extracted from the concentrated samples using commercial kits (MO-BIO, US). 1μL eukaryotic genes extracted were amplified using universal PCR primers (Bac341f: 5'-CCT ACG GGA GGC AGC AG-3')/ Bac534r: 5'-ATT ACC GCG GCT GCT GG-3') with 99 μL PCR reagent. The PCR mixture was heated to 94 °C fo

min, and reacted in 30 times' repeated cycle with 45 s denaturation at 94 °C, 45 s annealing at 56 °C and 120 s extension at 72 °C in each cycle. The final elongation step was 7 min at 72 °C, and then the PCR product was kept at 4 °C. After amplification, DGGE (DGGE-1001, C.B.S., US) was performed using gels containing 10% (wt/vol) acrylamide/bis-acrylamide solution with 70% - 40% denaturising grads. Gels were attained with SYBR-GREEN and visualized with UV transillumination. The major bands were excised, re-amplified and sequenced for identification of the microbial species. Sequences were compared with the Gene-bank database in order to indentify close relatives.

3.3. Results and discussion

3.3.1 Performance of IASBRs under the different loading rates

The three IASBRs were operated for 110 days (Days 1 - Day 110) in Stage 1 and 137 days (Days 121 - Day 257) in Stage 2 with the diluted digestate. The COD removal reached steady state after operation for 30 days but it took 60 - 80 days for the profiles of NH_4^+-N, NO_2^--N and NO_3^--N to reach pseudo-steady state. The performance of the three reactors is given in Figure 3.3.

In the three IASBRs, the total effluent COD concentrations were 650 - 750 mg/L. The effluent BOD_5 concentration was only 100 ± 10 mg/L. This shows that most of COD remaining in the effluent was not biologically degradable. After the effluent samples were centrifuged at 14,000 rpm, the soluble COD concentrations were about 500 - 600 mg/L and BOD_5 was lower than 20 mg/L. This means most of effluent BOD_5 was due to SS. It was calculated that about 120 - 140 mg/L COD had been removed during centrifugation with the simultaneous removal of 80 mg/L BOD_5, indicating that the effluent SS had a high BOD_5 to COD ratio.

In order to further remove COD from the effluent, a standard jar test was carried out to evaluate the possibility of coagulation in wastewater treatment (Gregor, *et al.*, 1997; Dosta *et al.*, 2008). 200 mg/L of $FeCl_3$ and 10 mg/L of polyacrylamide (PAM) was added into the jar after adjustment of the pH to 5.5. The effluent COD was

reduced to less than 30 mg/L and hardly any BOD_5 was detectable. It indicates the effluent from IASBRs can be further treated by means of coagulation to the COD levels acceptable for discharge.

Nitrogen removal and nitrite accumulation occurred immediately after the commencement of the experiments in the three IASBRs. The nitrite accumulation efficiency (η) is a term used to describe the performance of IASBRs in partial nitrification:

$$= \frac{S_{NO_2}}{S_{NO_2} + S_{NO_3}} \quad 100\%$$

(Eq. 3.1)

For the effluent of three IASBRs at the start-up periods, the NH_4^+-N concentration was high when the pig manure digestate was fed into the reactors. After 20 days, 25 days and 40 days, the effluent NH_4^+-N concentration was noticeably decreased in $IASBR_L$, $IASBR_M$ and $IASBR_H$, respectively.
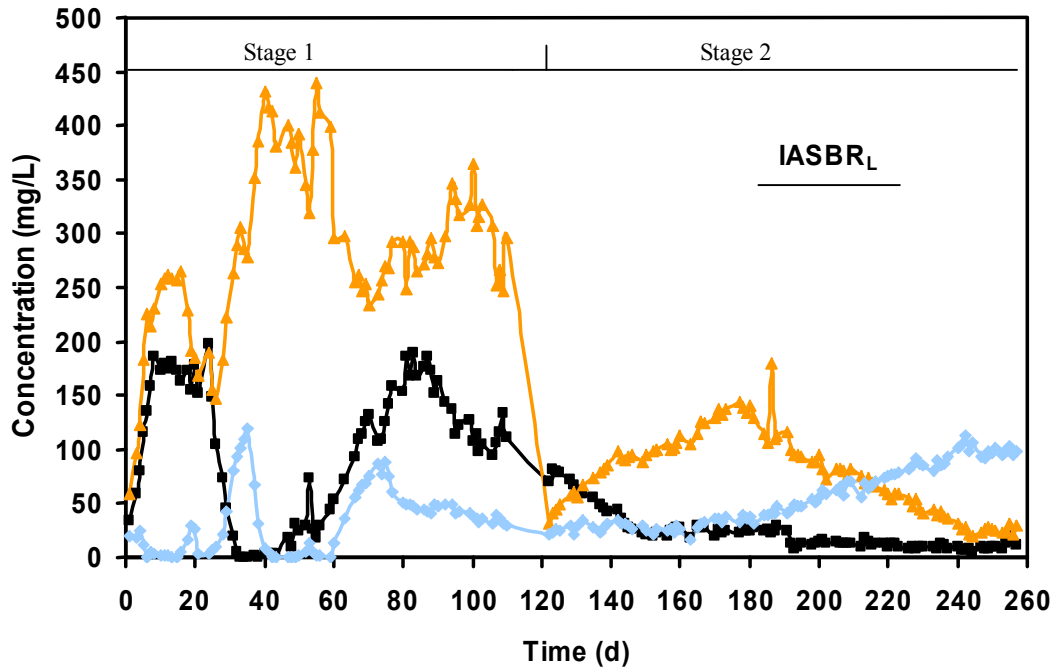
(a)
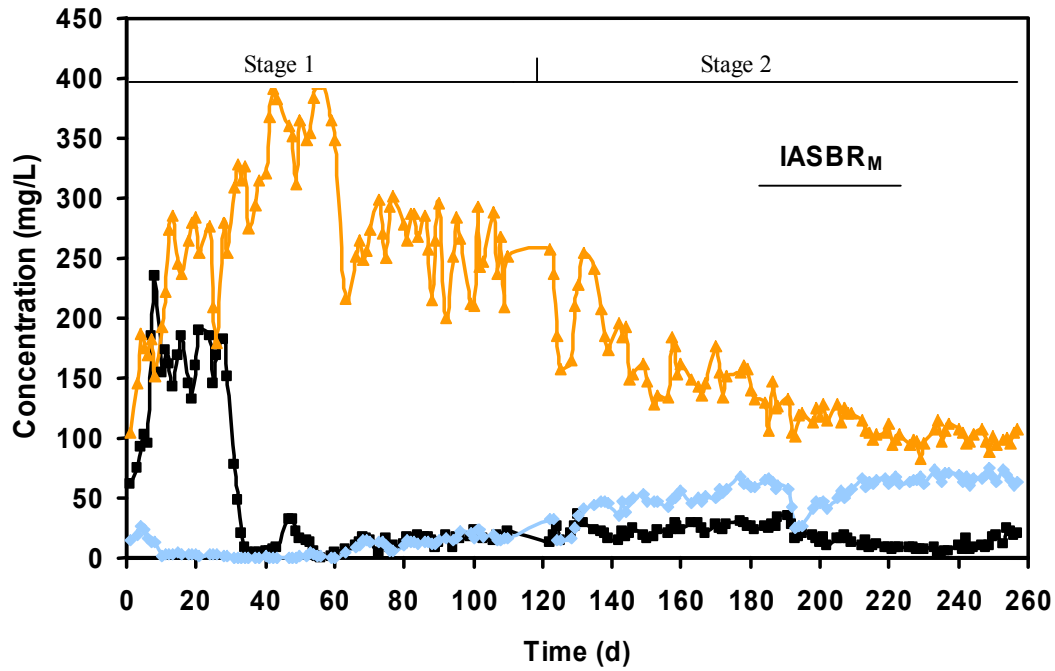
(b)
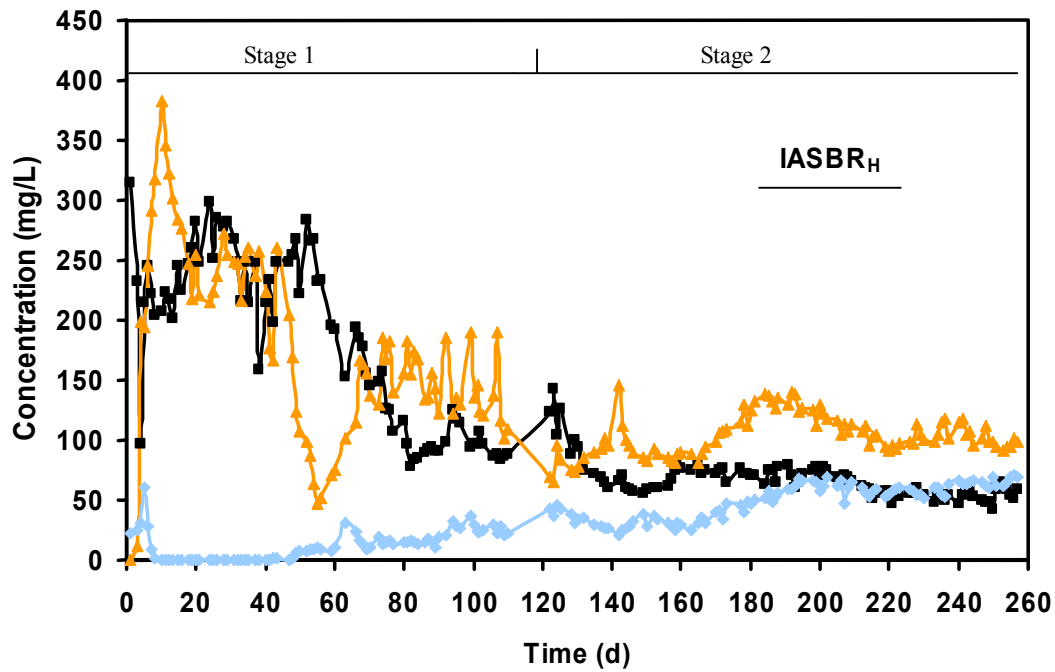
(c)

Figure 3.3 Effect of the loading rates on profiles of nitrogen in the effluent (■ ammonium; ▲ nitrite; ♦ nitrate)

In IASBR$_L$, 58.2 mg/L of NO$_2^-$-N appeared in the effluent as soon as the operation of reactor commenced and nitrite accumulation efficiency of 95% was achieved from Day 40 to Day 60. There was an evident increase in effluent nitrate in the periods of Day 32 - Day 38 due to a malfunction of the programmable timer which controlled aeration. Under the low loading rate, high DO concentrations of above 4.2 mg/L were maintained from the third aeration periods in the aerobic periods (Figure 3.4). Previous studies show that the nitration activity might rapidly increase and recover when the partial nitrification reactor is operated under high DO levels conditions (Isaka *et al.*, 2008). Therefore, the activity of NOB recovered and led to a slight increase in the effluent NO$_3^-$-N concentration from Day 60. In addition, after Day 60, pH dropped to 6.3, which greatly inhibited the activity of NOB and AOB. The inhibition of AOB, which oxidizes the NH$_4^+$-N to NO$_2^-$-N, also caused the effluent NH$_4^+$-N concentration to increase. This indicated that the operation strategy used in Stage 1 can not maintain long-term partial nitrification. When Stage 2 started, the pH in the reactor was adjusted to 7.6, and the activity of partial nitrification recovered immediately and the nitrite accumulation efficiency rose to 78%. However, after Day 180, effluent NO$_3^-$-N increased gradually. Due to the longer cycle duration, the loading rate applied in SBR$_L$ in Stage 2 was only 66% of that in Stage 1. In Stage 2, the pH value (7.05 - 7.15) did not drop to as low as in Stage 1, and high DO concentration phase in Stage2 was greater than that in Stage 1. Thus, suitable conditions were created for NOB activity to recover, and the effluent NO$_3^-$-N concentration was 98.5±6.4 mg/L at the end of Stage 2. The NO$_2^-$-N accumulation efficiency was 21%.

In IASBR$_M$, NO$_2^-$-N accumulated from the commencement of the experiment and NH$_4^+$-N dropped quickly from Day 25. The nitrite accumulation efficiency was steady at 91% - 97% until the end of Stage 1; the effluent NO$_2^-$-N concentrations were up to 249±43 mg/L and the NH$_4^+$-N and NO$_3$-N concentrations were 18.1±5.1 mg/L and 18.2±5.7 mg/L in the steady state, respectively. However, in Stage 2, the loading rate was reduced to 192.4 mg TN/ (L· d) from 288.6 mg TN/ (L· d), and DO levels in the last three aeration periods were over 2 mg/L, which encouraged the NOB recovery and the oxidation of NO$_2^-$-N to NO$_3^-$-N. Thus, it was observed that the effluent NO$_3^-$-N concentration rose in Stage 2. Finally, the NO$_2^-$-N accumulati efficiency was only 63%.

In IASBR$_H$, due to a high loading rate shock, NO$_2^-$-N accumulation occurred later than those in SBR$_L$ and SBR$_M$ during the initial start-up period and then increased rapidly. The effluent NO$_3^-$-N concentrations were low. From Day 50, NO$_3^-$-N slowly rose with the rise in biomass concentration from an initial concentration of 2.5 g/L to 5.6 g/L. After Day 80, NO$_2^-$-N and NO$_3^-$-N concentrations were maintained at 151±67 mg/L and 24.7±4.9 mg/L, respectively. The average NO$_2$-N accumulation efficiency was 84% and the remaining NH$_4^+$-N in the effluent was about 93 mg/L, with the TN removal efficiency of 72% in Stage 1. In Stage 2, the longer cycle duration benefited NOB growth, and NO$_3^-$-N increased in the effluent due to ammonium oxidation. The average NO$_2^-$-N accumulation efficiency was ~59%.

Table 3.1 performance of IASBRs in Stage 1 and Stage 2

		Stage 1	Stage 2
IASBR$_L$	Loading (mg TN/(L· d))	144.3	96.2
	TN removal efficiency (%)	52±6.1	84±1.7
	Effluent NH$_4^+$-N (mg/L)	112.8±11.4	10.1±3.4
	Nitrite accumulation efficiency (%)	88±7.5	21±2.5
IASBR$_M$	Loading (mg TN/(L· d))	288.6	192.4
	TN removal efficiency (%)	67±3.2	79±1.9
	Effluent NH$_4^+$-N (mg/L)	18.1±5.1	13.4±6.1
	Nitrite accumulation efficiency (%)	95±4.8	63±2.6
IASBR$_H$	Loading (mg TN/(L· d))	577.2	384.8
	TN removal efficiency (%)	72±3.5	75±1.1
	Effluent NH$_4^+$-N (mg/L)	92.8±7.4	53.8±5.5
	Nitrite accumulation efficiency (%)	84±5.5	59±1.3

In conclusion, all IASBRs achieved partial nitrification as soon as the reactor commenced. The highest nitrite accumulation appeared in IASBR$_M$ in both stages. The reason is that in the low loading rate reactor (IASBR$_L$), the high DO concentration improved the growth of NOB. In the high loading rate reactor (IASBR$_H$), high organic matter availability can inhibit partial nitrification (Zafarzadeh *et al*., 2011). It can be seen in Table 3.1 that, the nitrite accumulation was higher in Stage 1 than in Stage 2, whereas the total nitrogen removal efficiency

was higher in Stage 2 than in Stage 1. The longer cycle duration's (as a result a lower loading rate) allowed more time for nitrification and denitrification (Joo *et al.*, 2000).

3.3.2 Factors affecting partial nitrification

3.3.2.1 Dissolved oxygen and pH

DO and pH in the three IASBRs were measured in typical cycles (Figure 3.4). When COD and NH_4^+-N were oxidized, oxygen was consumed. Thus, DO concentrations did not increase unless a large amount of COD and NH_4^+-N had been removed. After several aeration periods, a significant increase in DO occurred in the three reactors. This corresponded to a significant reduction in biodegradable COD and NH_4^+-N in these periods. Consequently, denitrification was limited in the following non-aeration periods due to a lack of biodegradable COD in the three IASBRs.

Oxidation of NH_4^+-N led to decrease in pH; but denitrification in the non-aeration periods and air stripping of CO_2 in the aeration periods caused pH to rise. In Stage 1, the pH value continued to decrease before DO increased in the liquid phase. The low pH value of $IASBR_L$ and $IASBR_M$ indicates that alkalinity in the two reactors was not sufficient. In $IASBR_H$ where NH_4^+-N was not completely removed within the operational cycle and the applied loading rate was higher than in the other two reactors, alkalinity was enough to keep pH relatively steady.

In Stage 1, pH dropped to below 7 in $IASBR_L$ and $IASBR_M$. In $IASBR_L$, pH was lower than 6.4 after 4 hours in one cycle. Under low pH conditions (lower than 6.8), activities of nitrifiers are adversely affected (Kim and Kim, 2006). When pH is lower than 6.45 or higher than 8.95, complete inhibition of nitrification occurs (Ruiz *et al.*, 2003). Thus, at such a low pH level in $IASBR_L$, nitrification was inhibited with the effluent NH_4^+-N concentration of 110±24 mg NH_4^+-N /L in $IASBR_L$. As a consequence, TN removal was lower than in the other two reactors.

In Stage 2, in most of the cycle duration, the pH value in the IASBRs was higher than 6.9. Nitrification was not affected by pH. Thus, the effluent NH_4^+-N concentrations were lower than in Stage 1. However, the nitrite accumulation efficiency was lower due to higher DO concentrations.
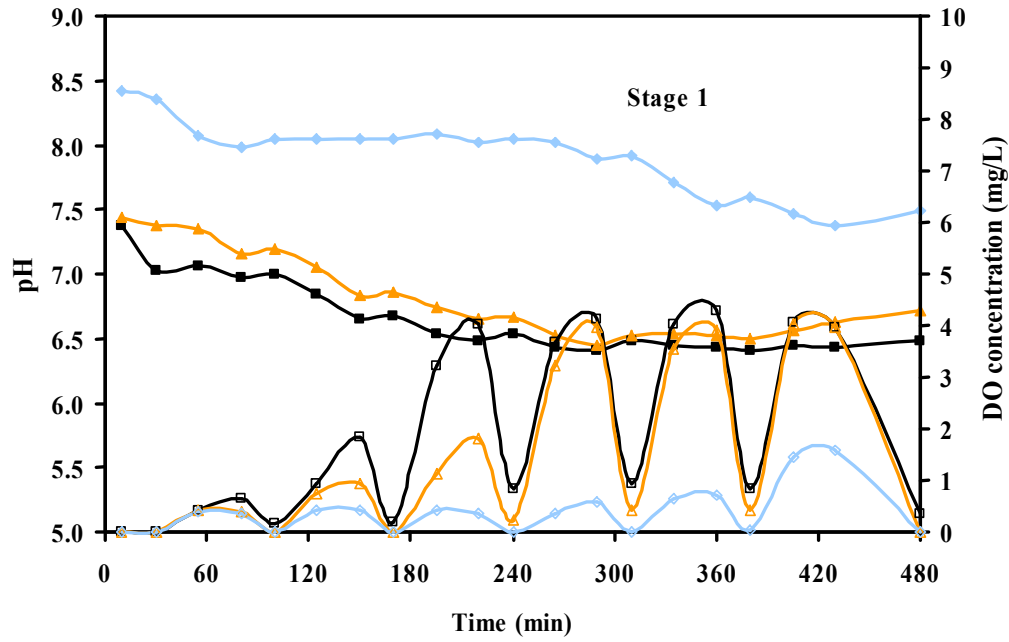
(a)
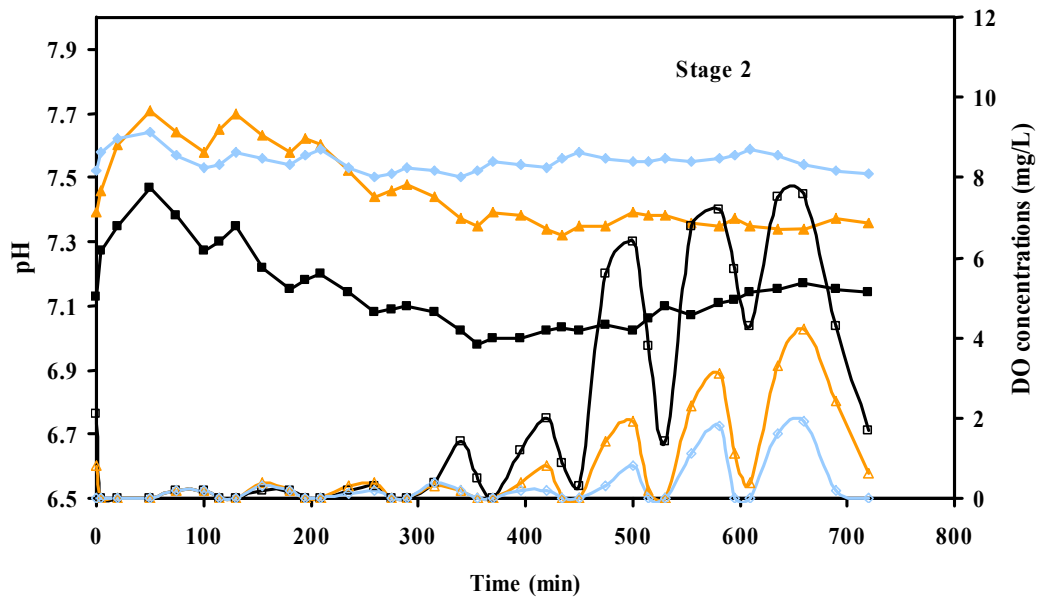
(b)

Figure 3.4 Profiles of pH and DO in typical cycles in the three IASBRs in Stage 1 and Stage 2 (■ pH in IASBR$_L$; ▲ pH in IASBR$_M$; ♦ pH in IASBR$_H$; □ DO in IASBR$_L$; Δ DO in IASBR$_M$; ◊ DO in IASBR$_H$)

3.2.2.2 Aeration rate

Batch experiments were carried out to study the influence of the aeration rate on nitrite accumulation. Two aeration rates were adopted (Figure 3.5): (I) 3 L air /min to supply sufficient oxygen and keep high DO levels; (II) 36 mL air /min (at this flow rate, the ratio of the air flow rate to the reactor effective volume was equal to that used in the three IASBRs). The initial NH_4^+-N concentration when the batch experiments commenced was 20 mg/L and pH was 7.4, so the effect of FA and FNA on partial nitrification can be ignored.

Under the high aeration rate, DO reached the saturation concentration within 2 minutes and NO_3^--N was generated after 10 minutes. The calculated specific ammonium oxidation rate (AOR) of biomass was 3.31 mg NH_4^+-N/ (g VSS· h) and the specific nitrite oxidation rate (NOR) was 1.87 mg NO_2^--N/ (g VSS· h). The ratio of AOR to NOR was 1.77: 1.
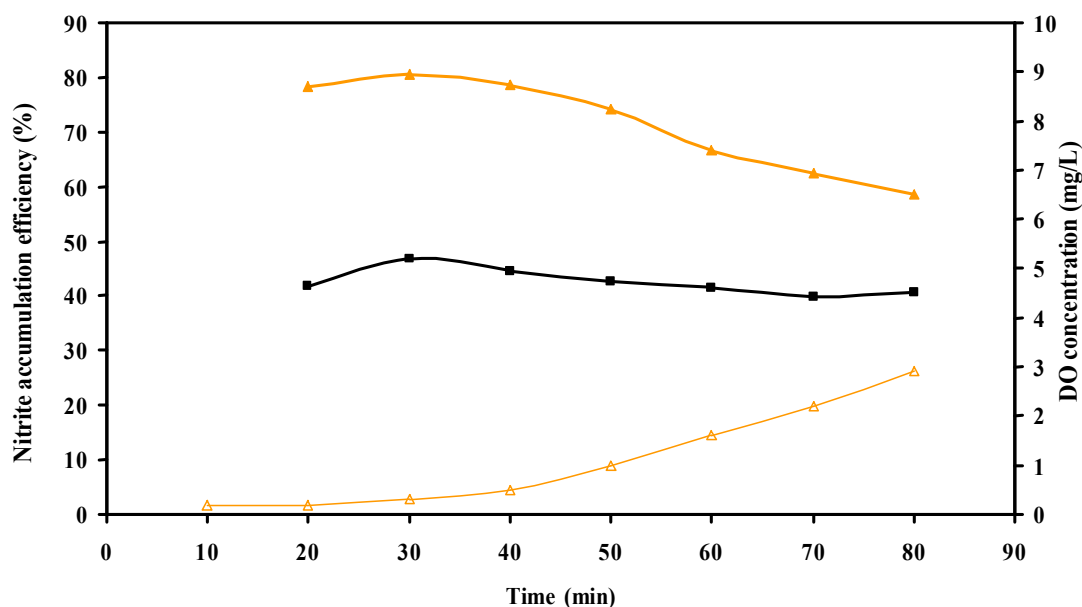
Figure 3.5 Effects of the aeration rate on partial nitrification (■: nitrite accumulation efficiency at the high aeration rate; ▲: nitrite accumulation efficiency at the low aeration rate; Δ: DO concentration at the low aeration rate)

Under the low aeration rate, DO was less than 0.3 mg/L in the first 30 minutes, and increased up to 1 mg/L after 1 hour. The low DO environment inhibits nitr

oxidation (Hanaki et $al.$, 1990). Under this aeration rate, AOR was 1.85 mg NH_4^+-N/ (g VSS· h) and NOR was 0.79 mg NO_2^--N / (g VSS· h), with AOR: NOR of 2.3: 1, thereby proving that the low aeration rate adopted in this study enhanced nitrite accumulation.

3.2.3.3 Denitrification

It was found that in some anoxic periods during the operation of IASBRs, the NO_2^--N concentrations were constant or increased, so nitrite production during denitrification might contribute to nitrite accumulation. Batch experiments were conducted at three different COD: N ratios - 2:1 (close to the COD: N ratio in the pig digestate); 5:1; and 10:1 (excessive carbon source for denitrification) with 40 mg/L NO_2^--N solution or 20 mg/L NO_2^--N and 20 mg/L NO_3^--N solution. The NO_2^--N changing rates are given in Table 3.2.

Table 3.2 Effects of denitrification on partial nitrification under different COD: N conditions (unit: mg N/ (g VSS· h))

COD: N	NO_2 added	NO_2+NO_3 added	
	NO_2^--N change	NO_2^--N change	NO_3^--N change
2: 1	-0.84	0.32	-1.33
5: 1	-2.36	-0.16	-2.19
10: 1	-2.96	-0.36	-2.56

It was found that when the COD: N ratio was 2: 1, NO_2^--N was produced during the denitrification of NO_2^--N + NO_3^--N. Some researches suggest that, NO_2^- is an intermediate of denitrification from NO_3^- to N_2, which proceeds in a pathway consisting of four reductive steps mediated by four individual enzymes (Eq. 2.5). However, not all denitrifiers can execute the entire pathway and generate all the necessary enzymes (Philip et $al.$, 2002). Thus nitrite accumulation can also be created in anoxic denitrification periods. Insufficient COD in the anoxic period can inhibit the further reduction of NO_2^--N. It can be concluded from Table 3.2 that the digestate with low COD: N ratios treated in IASBRs could lead to nitrite accumulation in the anoxic periods.

3.2.3. Microbial analysis of activated sludge in IASBRs

The DNA bands of the sludge samples taken from the IASBRs were obtained after the PCR-DGGE analysis (Fig. 3.6). After sequencing, the results are compared with the Gene-bank, and the detailed information is given in Table 3.3.
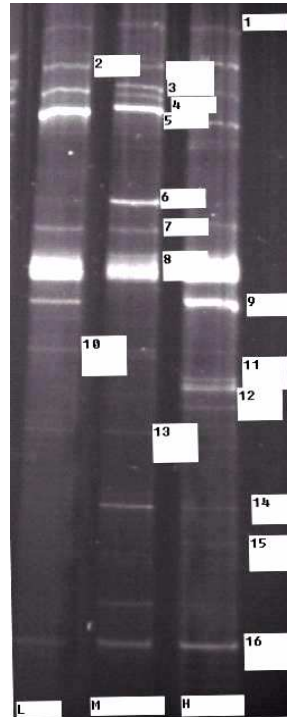
Figure 3.6 DGGE bands of the activated sludge samples taken from the three IASBRs

Band 8 (*Sphingobacteriales Bacteroidetes*) was dominant in the sludge samples, and is recognized to be capable of degrading a wide range of refractory pollutants (Martin *et al.*, 2011).

Band 2 was the key bacteria (*Nitrosomonas eutropha*) for nitrification in the three IASBRs and is recognized as one of the main AOB species. No evident NOB was detected in the three reactors. The reason may be that the concentration of NOB gene in the IASBRS was too low to be amplified by PCR with the protocol used in this study. Band 6 (*Comamonadaceae Beta-Proteobacteria*), Band 7 (*Comamonadaceae Beta-proteobacteria*), and Band 14 (*Rhodocyclaceae Betaproteobacteria*) were common denitrification bacteria in wastewater treatment processes (Harder a Probian, 1997).

Table 3.3 Clone library and affiliation of sequenced bands in IASBRs

Bands	Closest sequence	Gene Bank Access No	Phylogenetic group	Similarity
Band-1	Clone PIST-AGC02	AM982553	*Acholeplasma Palmae Acholeplasmatales*	445/464
Band-2	Nitrosomonas sp. 16S rRNA gene	AJ224410	*Nitrosomonas eutropha Beta-Proteobacteria*	441/474
Band-3	Clone PISD-AIC09	AM982601	*Rumen Bacterium Bacteroidetes*	457/464
Band-4	Clone PISD-AIC09	AM982601	*Rumen Bacterium Bacteroidetes*	467/474
Band-5	Clone: D05	AB241580	*Flavobacteriaceae Bacteroidetes*	462/469
Band-6	Bacterium rJ10 gene	AB021328	*Comamonadaceae Beta-Proteobacteria*	472/475
Band-7	Uncultured bacterium Clone MFC-GIST416	EU704625	*Comamonadaceae Beta-Proteobacteria*	437/486
Band-8	Uncultured bacterium Clone Dok36	FJ710755	*Sphingobacteriales Bacteroidetes*	474/480
Band-9	Uncultured Fluviicola sp. Clone PI6C	FJ439030	*Fluviicola Bacteroidetes*	369/383
Band-10	Uncultured Phenylobacterium sp. Clone AUVE-05D12	EF651222	*Phenylobacterium Alphaproteobacteria*	370/381
Band-11	Clone QEDN9DB09	CU926269	*Xanthomonas Gammaproteobacteria*	335/350
Band-13	Clone QEDN5CB12	CU925900	*Bacteroidetes*	253/313
Band-14	Uncultured bacterium Clone 3S1-21	GQ472379	*Rhodocyclaceae Betaproteobacteria*	375/431
Band-15	Clone EV818EB5CPSAJJ42	DQ337044	*Burkholderiales Betaproteobacteria*	402/430
Band-16	Uncultured Actinobacteria	CU927277	*Actinobacteria*	419/433

Band 1 (*Acholeplasma Palmae Acholeplasmatales*), Band 3 (*Rumen Bacterium Bacteroidetes*) and Band 4 (*Rumen Bacterium Bacteroidetes*) presented in these reactors are species of pig manure-indigenous bacteria (Castro *et al.*, 2005). Band 11 (*Xanthomonas Gammaproteo bacteria*), Band 13 (*Bacteroidetes*), Band 15 (*Burkholderiales Betaproteobacteria*) and Band 16 (*Actinobacteria*) are the important microorganisms involved in anaerobic digestion (Riviere *et al.*, 2009) and they were introduced into IASBRs from the digestate. Band 5 (*Flavobacteriaceae Bacteroidetes*) is one of the main microorganisms involved in the composting process (Watanabe *et al.*, 2007).

In $IASBR_M$ and $IASBR_H$, more species of microorganisms, especially anaerobes, existed than in $IASBR_L$. Based on the phase study data (Figure 3.4), the duration of low DO periods was longer when the loading rate increased. Thus, in the high loading rate reactors, the variety of anaerobic bacteria was more abundant.

3.4 Summary

Digestate after anaerobic digestion of pig manure was treated under three loading rates in IASBRs to achieve partial nitrification, carbonaceous oxidation and nitrogen removal.

The removal of COD was similar under the three loading rates. The COD removal efficiency was up to 75% and the remaining COD in the effluent can be further reduced to 30 mg/L by means of coagulation.

Partial nitrification was achieved in the three reactors with a nitrite accumulation efficiency of 89%, 93% and 84% in Stage 1; and 21%, 79% and 59% in Stage 2, respectively.

The batch experiments show that the pH, aeration and denitrification process can contribute to partial nitrification. *Nitrosomonas eutropha* was one of the main AOB species responsible for nitrification in the three IASBRs.

Chapter Four

Effects of Readily Biodegradable Organic Matter on Intermittently Aerated Sequencing Batch Reactors Treating Separated Pig Manure Digestate Liquid

4.1 Introduction

Two IASBRs as described in Chapter 3 (Figure 3.1) were used to treat the separated pig manure digestate liquid (IASBR-1) and synthetic wastewater (IASBR-2) in order to understand the influence of the low level of readily biodegradable organic matter on nitrogen removal and partial nitrification.

The difference of nitrogen removal in the two IASBRs was explained through the phase study and nitrification activity analysis. The mechanisms of partial nitrification under the intermittent aeration pattern were studied. Meanwhile, the change of the ecological structure in IASBRs during the 5-month operation was investigated by using the Fluorescence *in situ* hybridization technique.

4.2 Materials and methods

4.2.1 Intermittently aerated sequencing batch reactors

Two identical laboratory-scale IASBRs constructed in the Environmental Laboratory, NUI Galway, were used in this study as described in Chapter 3 (Figure 3.1). One reactor (IASBR-1) treated the separated digestate liquid and the other (IASBR-2) treated synthetic wastewater simulating the digestate. The temperature of the two IASBRs was controlled at 26±1 °C.
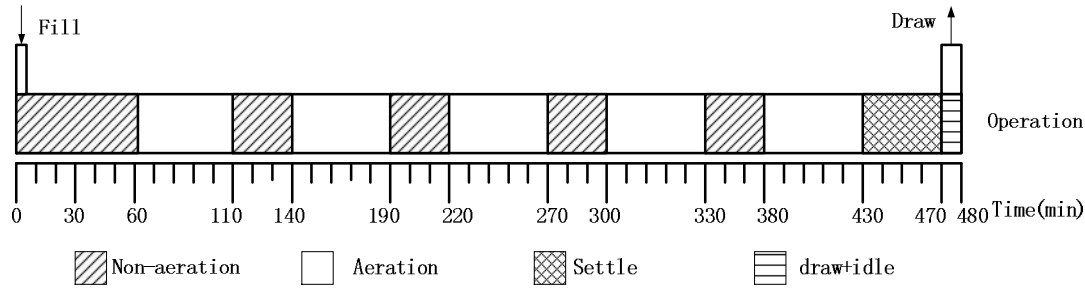
Figure 4.1 Complete operational cycle of the IASBR system

The IASBRs were operated in 8-hour cycles. The operational phases in a cycle are shown in Figure 4.1. 330 mL wastewater was filled each time, and after one hour non-aeration period in which the fill phase lasting 5 minutes was included, the reactors were intermittently aerated with 4 successive 50 minutes aeration/ 30 minutes non-aeration periods. Then, after the fifth 50 minutes aeration, the settle phase lasted 40 minutes. The effluent was withdrawn in the last 10 minutes. Every day, 660 mL of mixed liquor was discharged from each reactor immediately before the settle phase, to maintain a sludge retention time of 15 days.

4.2.2 Separated digestate liquid and synthetic wastewater

The separated digestate liquid was taken from a mesophilic pig manure digester on a pig farm in Co. Kerry, Ireland. A centrifuge, in addition with alum and polyacrylamide, was used to reduce solids from the digestate. The separated digestate liquid was delivered to the lab, and stored in a refrigerator at 4°C until use. The original wastewater was fed into IASBR-1 at the loading rates of 1.15 kg COD/ $(m^3 \cdot d)$ and 0.38 kg NH_4^+-N/ $(m^3 \cdot d)$. Table 4.1 lists the average water quality of the separated digestate liquid. The COD: N ratio was 2.9 and the NH_4^+-N concentration was up to 94.2% of TN. BOD_5 was only 25.1% of COD, indicating a low proportion of readily biodegradable organic matter in the separated digestate liquid.

To understand influence of the low level of readily biodegradable organic matter on nitrogen removal, organic matter removal and partial nitrification in the IASBR technology, synthetic wastewater which had similar COD, TN and NH_4^+-N levels as in the separated digestate liquid was treated in IASBR-2. The average volatile fatty acids (VFA) concentration in the separated digestate liquid was 2,330 mg/L. Thus,

the synthetic wastewater contained 2.8 g/L sodium acetate. The other components of the synthetic wastewater included 10 g/L glucose, 17.6 g/L $(NH_4)_2SO_4$, 1.2 g/L KH_2PO_4, 20 g/L $NaHCO_3$, 100 mg/L NaCl, 200 mg/l $MgCl_2$, 20 mg/L $FeCl_2$, 20 mg/L $MnSO_4$ and 2 g/L yeast. The synthetic wastewater had a much higher BOD_5 level than the separated digestate liquid.

Table 4.1 Characteristics of real and synthetic wastewater treated in IASBRs

	COD (mg/L)	BOD_5 (mg/L)	NH_4^+-N (mg/L)	TN (mg/L)	COD: N	BOD_5: N
Separated digestate liquid	11540±860	2900±200	3808±98	4041±59	2.9: 1	0.7: 1
Synthetic wastewater	12000±200	11500±100	3810±25	4015±90	3.0: 1	2.9: 1

The two IASBRs were seeded with activated sludge taken from a local municipal wastewater treatment plant and the cultivated partial nitrification sludge from Chapter 3 with the ratio of 1: 1. The initial biomass concentrations in the IASBRs after seeding were 2.4 g SS/L, and the volatile suspended solids (VSS)/SS ratio was 92%.

4.2.3 Water quality analysis

The methods used for water samples test are given in Appendix B.

4.2.4 Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization (FISH) is a cytogenetic technique used to detect and localize the presence or absence of specific DNA on chromosomes. In this study, the FISH technique was used to measure AOB and NOB in IASBRs. The probes are given in Table 4.2. The procedures used in this study are as follows (Wu *et al.*, 2009):

(1) Slides washing: new slides were washed for 30 s in 1% HCl + 70% ethanol solution. Then, slides were dipped into 0.1% gelatine + 0.01% CrK $(SO_4)_2$ solution for 30 s at 70 °C.

(2) Fixation: the sludge sample was centrifuged at 4000 rpm to remove wastewater and was placed into 2 - 3 volumes of 4% Paraformaldehyde (PFA) solution for 1 volume of the sample. The sample was fixed for 3 h and then the PFA was removed.

(3) Spot and dehydration: 20 - 30 µL fixed sludge sample was spotted onto a microscope slide at ambient temperature. Then the sample was dehydrated in an ethanol dilution (50% - 80% - 96%) by immersing for 3 min each.

(4) Hybridization: the hybridization buffer solution, probe and slide were preheated to 46 °C, and then, 8 µL hybridization buffer solution and 1 µL probe was added on each sample. A moisture chamber was prepared by soaking a piece of tissue paper with remaining hybridization buffer in a 50 mL centrifuge tube. Then, the slide was placed in the moisture chamber to incubate for at least 3 h.

(5) Washing: the washing buffer solution was preheated to 48 °C in a 50 mL centrifuge tube and the slide was transferred and immersed in the washing buffer solution at 48 °C for 20 min. Then, slide was rinsed with deionised water to remove the washing buffer solution.

(6) Evaluation: the slide was covered with a cover slip, and then, the sample was evaluated with epifluorescence or confocal laser microscopy.

Table 4.2 List of probes

Probe	Sequence	Specificity	Labelled
EUB338	GCTGCCTCCCGTAGGAGT	EUB bacteria	Cy5
NSO190	CGATCCCCTGCTTTTCTCC	AOB	Cy3
NIT3	CCTGTGCTCCATGCTCCG	NOB	Cy3

4.3 Results and discussion

4.3.1 Alkalinity adjustment and pH control

Nitrite accumulation appeared immediately in both IASBRs (Figure 4.2). However,

the effluent NH_4^+-N level rose and the nitrification efficiency worsened from Day 3.
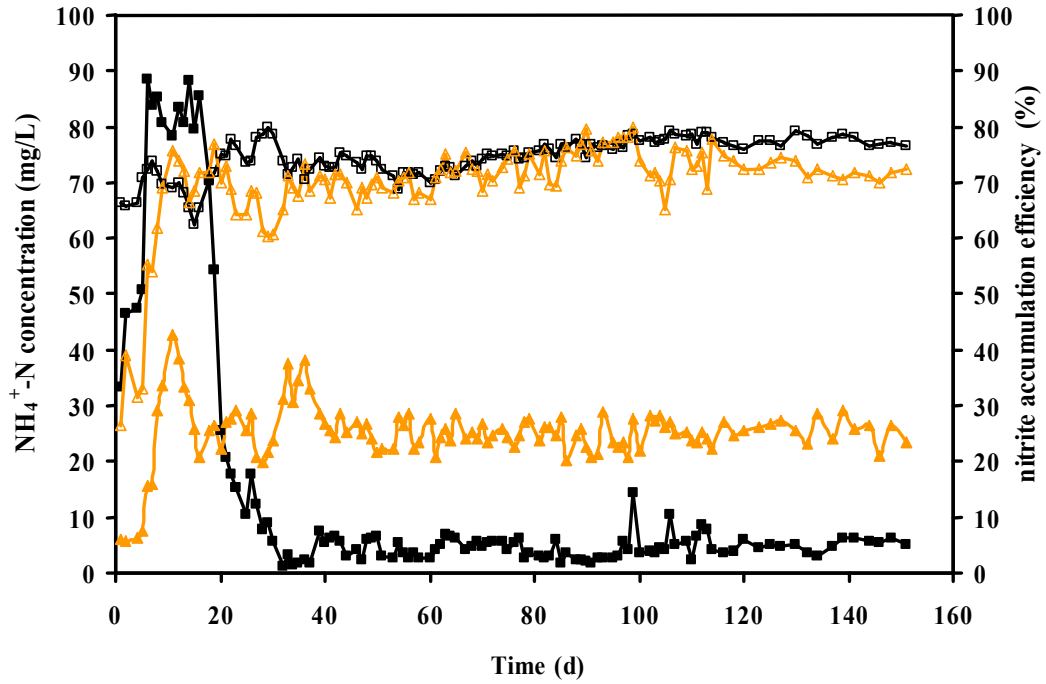
Figure 4.2 Performance of IASBRs in NH_4^+-N and partial nitrification (■: effluent NH_4^+-N in IASBR-1; ▲: effluent NH_4^+-N in IASBR-2; □: nitrite accumulation efficiency in IASBR-1; △: nitrite accumulation efficiency in IASBR-2)
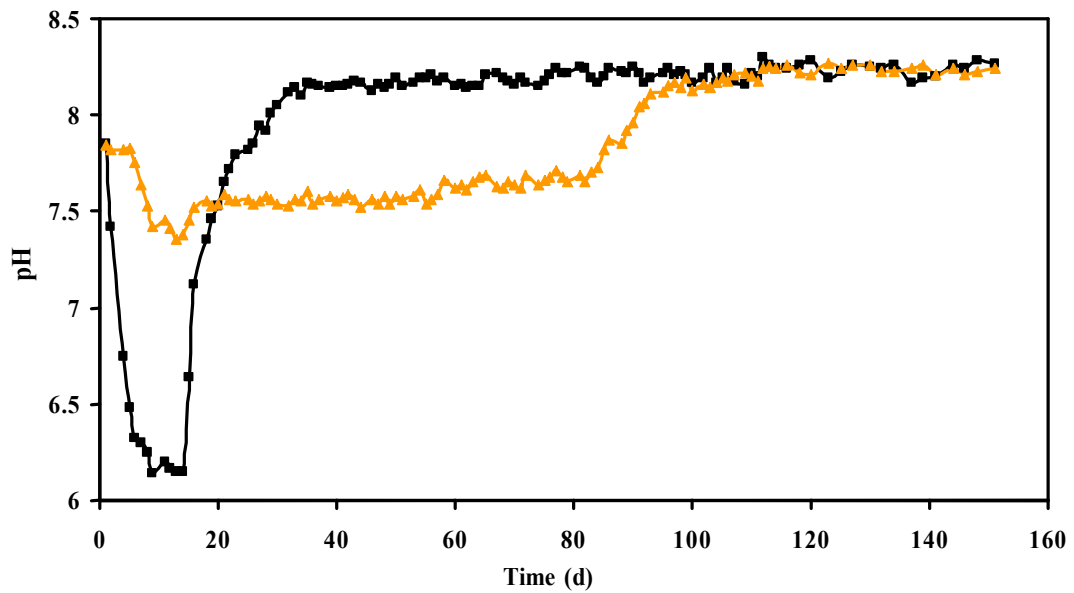
Figure 4.3 Profiles of pH in the IASBRs (■: IABSR-1; ▲: IABSR-2)

During this period, a serious pH decrease was observed in IASBR-1: the pH value was decreased from 7.9 to 6.2 in the first 10 days (Figure 4.3). That means the average alkalinity (21.6 g/L in the pig digestate, as $NaHCO_3$) was not sufficient to sustain stable pH because nitrification of high concentrations of NH_4^+-N consumed alkalinity (Henze *et al.*, 2002):

$$55NH_4^+ + 76O_2 \quad 109HCO_3 + \ C_5H_7NO_2 \quad 54NO_2 \quad 57H_2O \quad 104H_2CO_3 \quad \text{(Eq. 4.1)}$$

$$400NO_2 + NH_4 \quad 195O_2 + HCO_3^+ \quad 4H_2CO_3 + \ C_5H_7NO_2 \quad 400NO_3 + 3H_2O \ \text{(Eq. 4.2)}$$
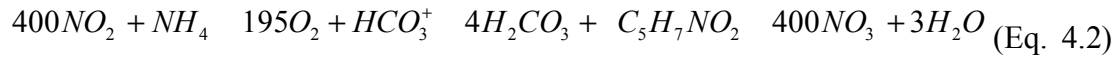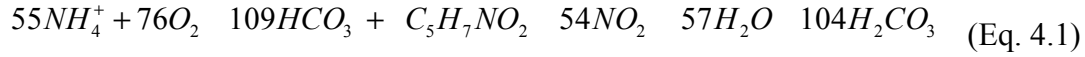
In the start-up period of IASBR-1, the total nitrogen removal efficiency was around 77% - 83%, and the addition of 4.1 - 5.3 g/L of alkalinity into the wastewater was required to sustain stable pH. An additional 7 g/L of alkalinity was added to the digestate from Day 13 in IASBR-1. The pH value decrease was immediately reversed and pH was increased to 8.0 on Day 32. After Day 32, the amount of alkalinity added was decreased from 7 g/L to 4.5 g/L. After pH adjustment, the NH_4^+-N oxidation rate increased, and the effluent NH_4^+-N concentration dropped to below 10 mg/L from Day 30.

In order to investigate and compare the effects of IASBR-2 with IASBR-1, 1.2 g/L alkalinity was added into IASBR-2 after Day 80 to achieve similar pH value with IASBR-1.

4.3.2 Overall performance of IASBRs in removals of organic matter and nitrogen

The two reactors were operated for 5 months. Figure 4.4 shows the performance of the two IASBRs in removing COD. The average COD removal efficiency in IASBR-1 was 89.8%, while it was over 99% in IASBR-2. Since the BOD_5:COD ratio was only 25.1% in the separated digestate liquid, the results clearly show that the IASBR technology was capable of removing slowly biodegradable organic matter under a long HRT, which was around 10 days. IASBR-1 effluent was added to flasks in addition with activated sludge taken from the reactor, and then air was continuously provided for 8 days; there was no evident decrease in COD, indicating that most of the remaining COD in IASBR-1 effluent was inert organic matter. The high COD

removal in IASBR-2 was understandable as the synthetic wastewater contained a much higher concentration of readily biodegradable organic matter compared with the separated digestate liquid.
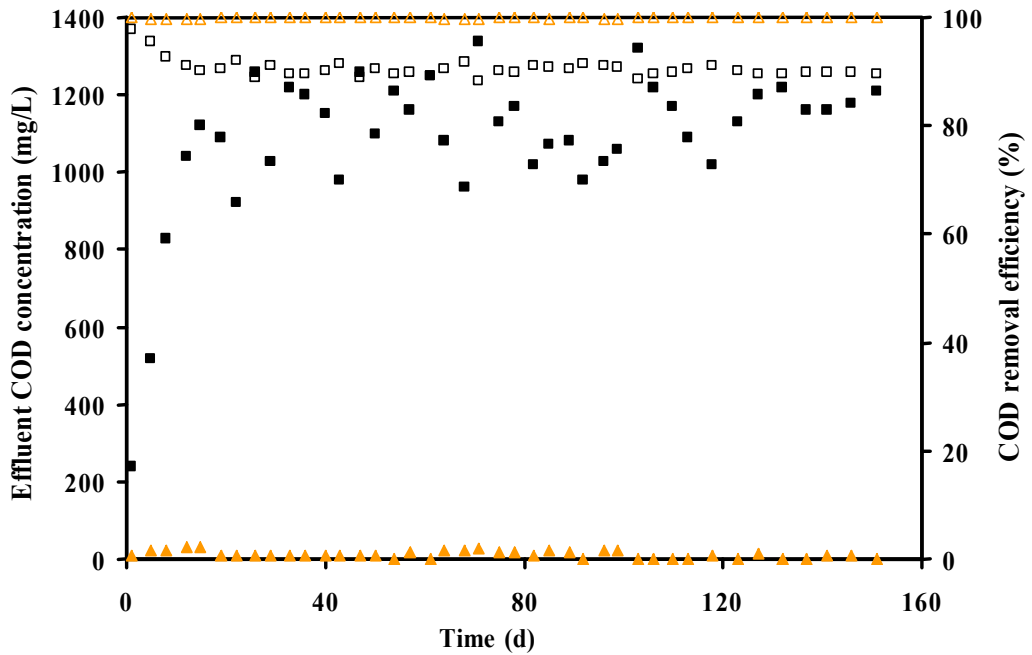
Figure 4.4 Performance of COD removal in IASBRs (■: effluent COD in IASBR-1; ▲: effluent COD in IASBR-2; □: COD removal efficiency in IASBR-1; Δ: COD removal efficiency in IASBR-2)

The effluent NH_4^+-N level in IASBR-2 was higher than in IASBR-1 (Figure 4.2), due to the facts as follow: (1) high levels of readily biodegradable organic matter existed in IASBR-2; and (2) there was a relatively lower DO in IASBR-2 operational cycle and DO was not above 1 mg/L until the end of the fifth aeration period (Figure 4.5). The activity of autotrophic bacteria in IASBR-2 was inhibited. The pH value in IASBR-2 was in the range of 7.6 - 7.7, which was very stable from the commencement of the experiment. The reasons were: (1) there was efficient denitrification in IASBR-2, with nitrogen removal of 97%, causing the recovery of alkalinity (Henze *et al.*, 2002):

$$C_{18}H_{19}O_9N + 14NO_3 \quad 14H \ + \ 7N_2 \quad 17CO_2 \quad HCO_3^+ \quad NH_4 + 14H_2O \qquad \text{(Eq. 4.2`}$$
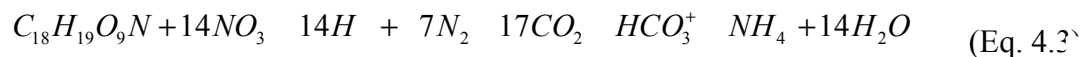
and (2) nitrification was not as efficient as in IASBR-1. After Day 80, an extra

g/L alkalinity was added to the synthetic wastewater. The pH value was increased to above 8, but there was no increase of the nitrification efficiency.
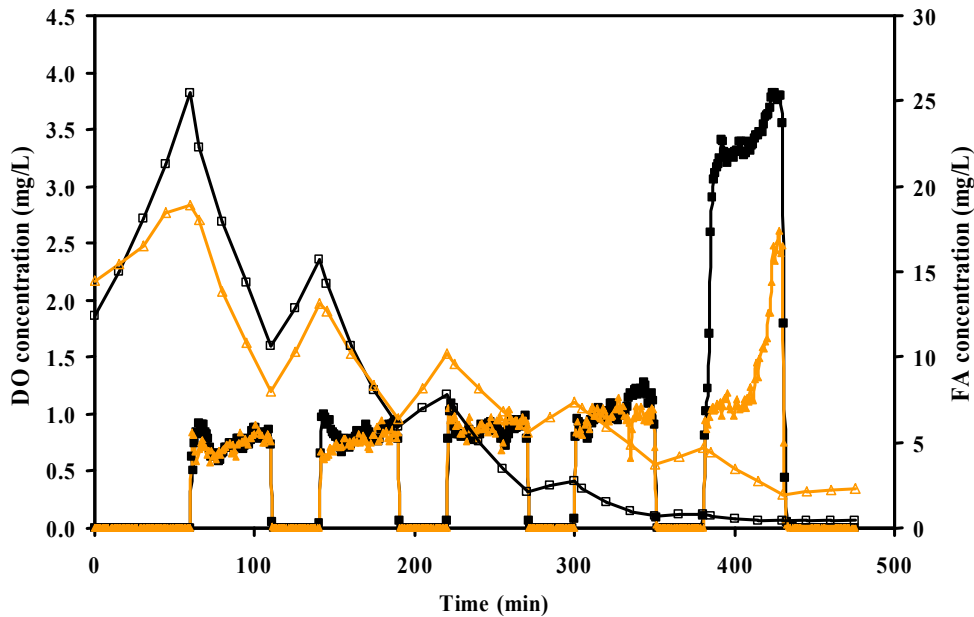
Figure 4.5 Profile of DO and FA in a typical cycle (■: DO in IASBR-1; ▲: DO in IASBR-2; □: FA in IASBR-1; ∆: FA in IASBR-2)

On average, 76.5 % of nitrogen removal was achieved in the stable period in IASBR-1. The nitrite accumulation efficiency was very stable, at 77 - 79%. There were several factors contributing to the efficient and stable partial nitrification. One was high pH in IASBR-1 and IASBR-2. The pH values in the two IASBRs ranged from 7.7 - 8.3. When pH is above 7.5, it may inhibit the growth and activity of nitrite oxidizing bacteria (NOB). Ciudad *et al*. (2007) reported that partial nitrification was achieved in the pH range of 7.5 - 8.6 and Sinha and Annachhatre (2007) have concluded that pH in the range of 7.5 - 8.5 is suitable for inhibition of NOB activity. The second factor was presence of FA in the two reactors. The FA level was from 0.5 to 27.5 mg/L in IASBR-1, and 2.3 to 18.9 mg/L in IASBR-2 (Figure 4.5). FA concentrations in the range of 0.1 - 150 mg/L can benefit partial nitrification (Philips *et al*., 2002).

In IASBR-2, the total nitrogen removal efficiency was 97%, higher than that in IASBR-1 due to more readily biodegradable organic matter in the synthetic wastewater. However, even in IASBR-2, the COD: N ratio was 3.0 and BOD_5: N

was 2.9, much lower than the optimal COD: N range, above 5 - 6, for the conventional BNR process (Henze, *et al.*, 2002). This shows that the IASBR technology is a promising technology for nitrogen removal from low COD: N ratio wastewaters. The nitrite accumulation efficiency was 71%, and it was lower relative to that in IASBR-1.

4.3.3 Lag time of NOB under the control of intermittent aeration

The intermittent aeration strategy applied in the SBR system could result in partial nitrification. Mota *et al.* (2005) observed that in intermittently aerated reactors, NO_2^--N accumulation occurred at aeration to non-aeration ratios of 4 hour: 1 hours. In order to confirm that the intermittent aeration strategy was the important factor for partial nitrification, batch experiments were carried out at two aeration strategies: 30 minutes non-aeration before aeration, and direct aeration. In batch experiments, activated sludge taken from the reactors (IASBRs) was washed with tap water and added into 0.5 litre beakers (effective volume of 400 mL), in addition with $NaNO_2$ solutions with the NO_2^--N concentration of 50 mg/L. Air was provided with air pumps through air diffusers located at the bottom of the beakers. For the first aeration strategy, there was no aeration (the mixed liquor was quiescent in the beakers) for 30 minutes before the aeration was turned on. For the second aeration strategy, the aeration commenced immediately when the experiment commenced. Water samples were taken from the beakers at intervals for the measurement of NO_2^--N and NO_3^--N concentrations.

Under the first aeration strategy, after commencement of aeration, the increase in nitrate was retarded for 15 - 18 minutes (the amount of NO_2^--N supply was ample) (Figure 4.6), indicating that NO_2^--N oxidation was not activated immediately after aeration started and the lag time was 15- 18 minutes. Tappe *et al.* (1999) attributed the lag time of NO_2^--N oxidation to the different mechanisms of AOB and NOB in maintenance energy demand and starvation recovery dynamics; AOB can be resuscitated more easily than NOB. The batch experiment result clearly shows that the intermittent aeration strategy can benefit partial nitrification.
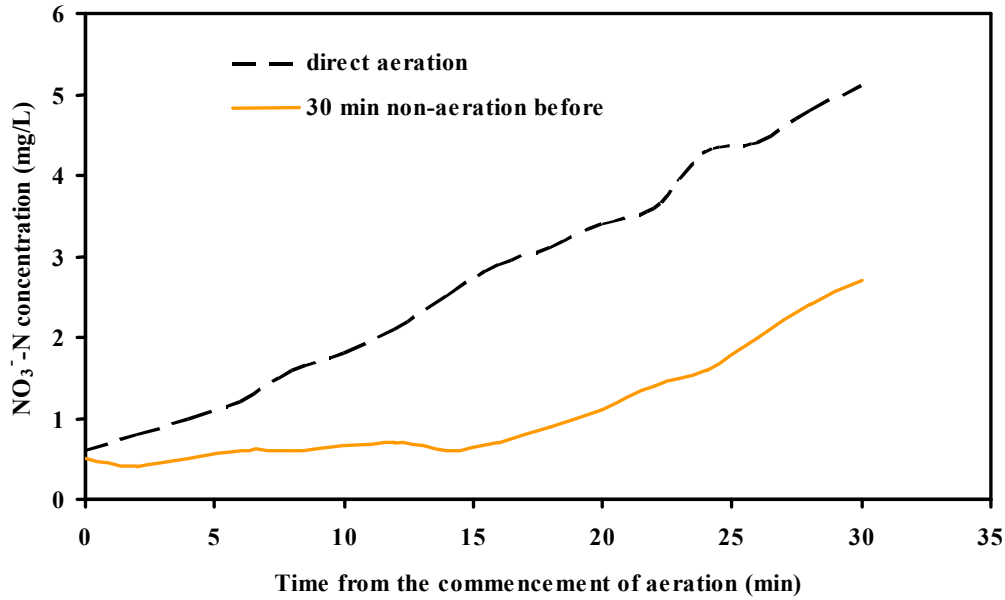
Figure 4.6 Effects of the aeration strategy on partial nitrification

4.3.4 Cycle performance and activity of nitrification

The cycle performance of the IASBR was investigated in a few operational cycles, and a typical cycle performance on Day 135 was described. In both IASBRs, COD reduction completed within five aeration periods (Figure 4.7). The difference was that COD degradation was accomplished in the last aerobic period in IASBR-2; while in IASBR-1, the COD reduction rate was very low in the third aeration period and the COD concentration did not change a lot in the remaining period of the cycle.

After 5-minutes fill, TON linearly decreased via denitrification (Figure 4.8). The denitrification rates were 29 mg N/ (L· h) and 36.9 mg N/ (L· h) in IASBR-1 and IASBR-2, respectively. It was higher in IASBR-2 due to a sufficient supply of readily biodegradable organic matter. The denitrification rates decreased gradually. In the third aeration period in IASBR-1 and the fifth aeration period in IASBR-2, when most biodegradable organic matter was consumed, while there was 25 mg NH_4^+-N /L remaining, the specific NH_4^+-N utilization rates were 13.4 NH_4^+-N/ (g VSS· h) and 8.5 NH_4^+-N/ (g VSS· h), respectively. It shows readily biodegradable organic matter in IASBR-2 could inhibit the activity of nitrification.
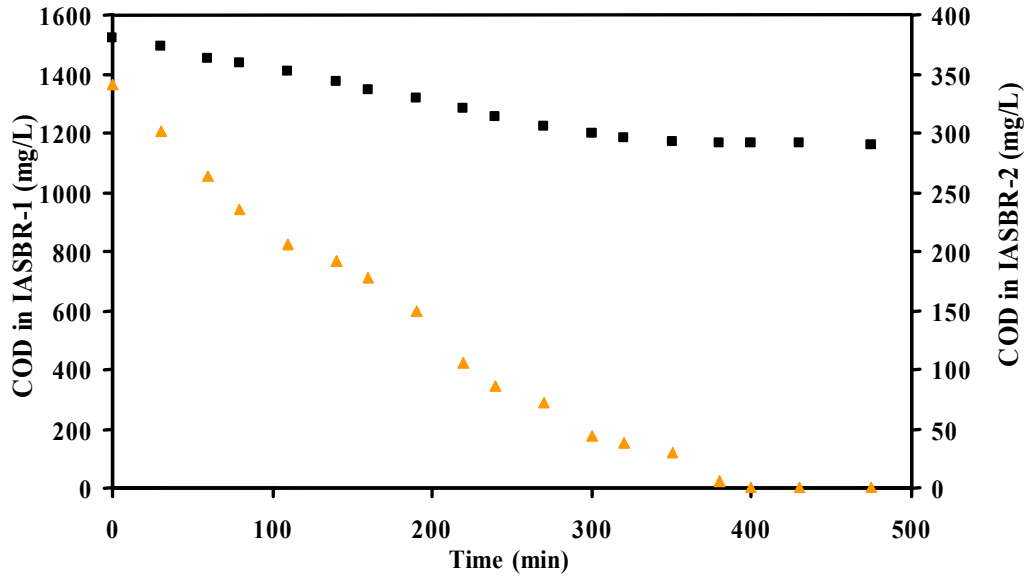
Figure 4.7 Profile of COD concentrations in a typical cycle (■: IASBR-1; ▲: IASBR-2)
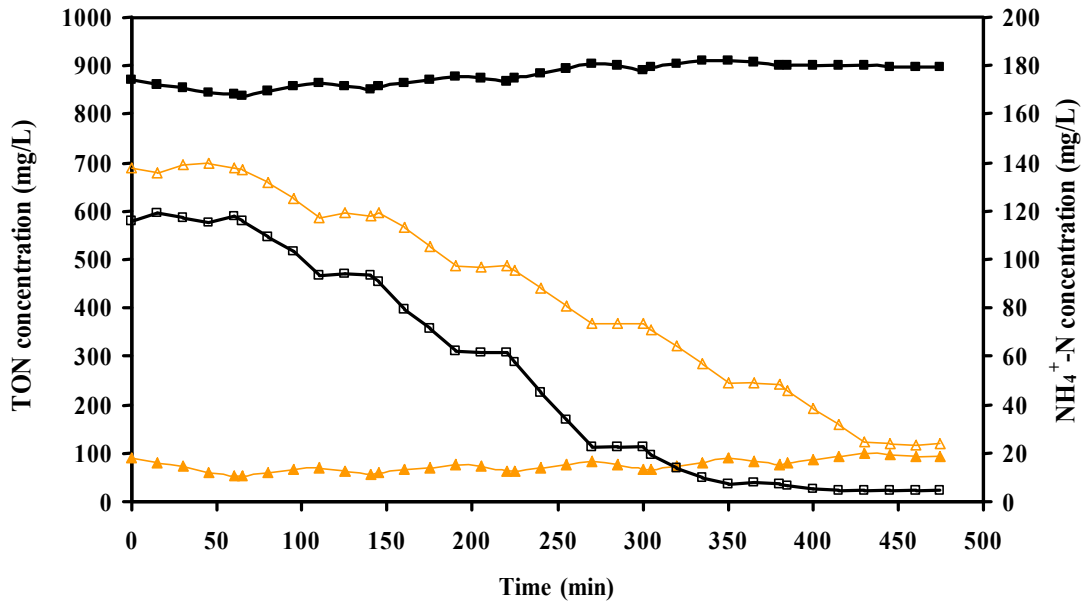
Figure 4.8 Profile of TON and NH_4^+-N concentrations in a typical cycle (■: TON in IASBR-1; ▲: TON in IASBR-2; □: NH_4^+-N in IASBR-1; Δ: NH_4^+-N in IASBR-2)

The pH and DO performance of the IASBRs were studied in a typical cycle. DO was one of the parameters to indicate organic matter oxidation and nitrification proce When COD, NH_4^+-N and NO_2^--N was oxidized, oxygen was consumed, so DO

not increase until a large amount of COD and NH_4^+-N had been removed. Significant increase in DO occurred in the fourth and fifth aerobic phase of these two reactors during intermittent aeration, respectively (Figure 4.5). This corresponded to significant reduction of biodegradable COD and NH_4^+-N in these periods. Marsili-Libelli (2006) proposed that DO data could be used to identify the end of the nitrification activity. The evident and fast increase of DO in our study can be used to identify that BOD_5 was exhausted and most of NH_4^+-N was oxidized. NO_2^--N oxidation was still going-on, but DO concentration increased rapidly and this was caused by low NOB activity. The ammonium oxidation rate (AOR) and nitrite oxidation rate (NOR) of biomass decreased under low DO condition, NO_2^--N oxidation decreased sharply. The ratios of the AOR to NOR were 4.27 and 2.18 when DO was 4 mg/L (in the last aeration period) in IASBR-1 and IASBR-2, respectively. When DO was less than 1.2 mg/L (in the first four aeration periods), the AOR: NOR ratios increases to 5.44 and 2.77 in IASBR-1 and IASBR-2, and the partial nitrification efficiency increased by 27.4% and 27.1%, respectively.

NH_4^+ and NO_2^- oxidation kinetics can be simulated using the Monod equation:

$$r = \frac{r_{max}\ S}{k_s + S}$$
(Eq. 4.4)

where: r is the specific uptake rate of the substrate, mg / (g VSS· h); r_{max} is the maximum specific uptake rate of the substrate, mg / (g VSS· h); K_S is the half-saturation coefficient for the substrate, mg/L; S is the concentration of the substrate (NH_4^+ or NO_2), mg/L.

With regard to the biomass in IASBR-1 and IASBR-2, K_S values for NH_4^+-N oxidation and NO_2^--N oxidation were calculated (Eq. 4.4) as 10 mg NH_4^+-N/L and 6.7 mg NH_4^+-N/L, 1.3 mg NO_2^--N /L and 2.7 mg NO_2^--N /L, respectively. r_{max} was calculated as 13.8 mg NH_4^+-N / (g VSS · h) and 3.0 mg NO_2^--N / (g VSS · h) in IASBR-1, and 9.3 mg NH_4^+-N / (g VSS· h) and 4.4 mg NO_2^--N / (g VSS · h) in IASBR-2, respectively. The ratios of the AOR to NOR were 4.27 and 2.18 when DO was 4 mg/L (in the last aeration period) in IASBR-1 and IASBR-2, respectively. When DO was less than 1.2 mg/L (in the first four aeration periods), the AOR: NOR ratios increases to 5.44 and 2.77 in IASBR-1 and IASBR-2, respectively.

4.3.5 Fluorescence *in situ* hybridization (FISH)

The development of AOB and NOB in IABSR-1 was studied using the FISH technique. The sludge was taken from the IASBR-1 reactor on Day 132. The contents of AOB and NOB in the sludge were compared with those in the seed sludge. The results are given as Figure 4.9:
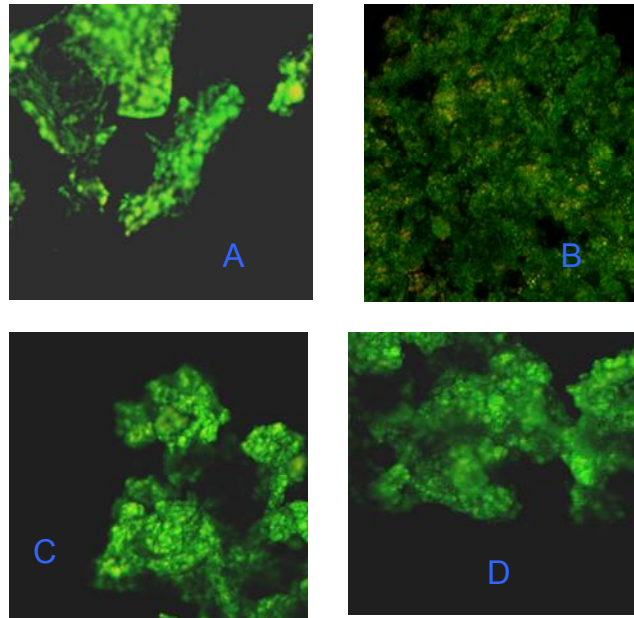
Figure 4.9 FISH results of AOB and NOB in the IABSR-1sludge and the seed sludge, ×100 (A: AOB in the seed sludge; B: AOB in IABSR-1 sludge; C: NOB in the seed sludge; and D: NOB in IABSR-1 sludge)

The yellow colour indicates that AOB and NOB existed in the sludge samples. According to the signal intensity, the percentages of AOB and NOB to EUB are calculated (Figure 4.10).

Because half of the seed sludge was taken from the IASBR described in Chapter 3, which had possessed efficient partial nitrification, there was a significant amount of nitrifiers in the seed sludge. The ratio of AOB: NOB in the seed sludge was 2.0. During the 132 days' operation, the ratio of AOB: NOB in IASBR-1 sludge rose to 4.5. The percentage of NOB in EUB did not change much, while, the AOB/EUB was nearly doubled. This means the intermittent aeration pattern and operational conditions used in this study can encourage the enrichment of AOB in the sludge.
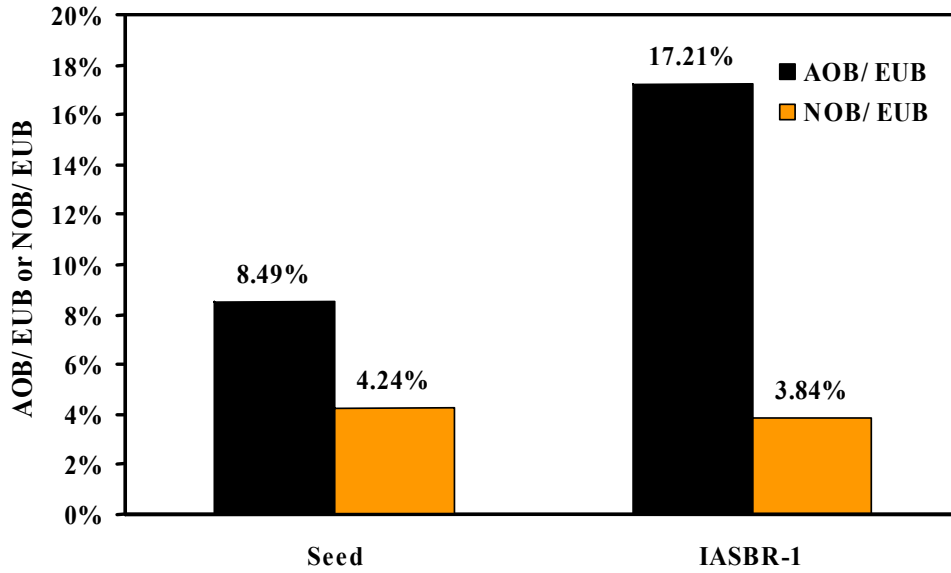
Figure 4.10 AOB/ EUB and NOB/ EUB percentages in the seed sludge and the IASBR-1 sludge

4.4 Summary

The effects of the low readily biodegradable organic matter on the performance of IASBR technology in achievement of partial nitrification, and organic matter and nitrogen removal from the separated pig manure digestate liquid were studied. Synthetic wastewater, simulating the separated digestate liquid with similar COD and nitrogen concentrations but BOD_5 of 11500 ± 100 mg/L, was also treated.

At the mean COD loading rate of 1.15 kg COD / $(m^3 \cdot d)$, the COD removal efficiency was 89.8% in IASBR-1 and 99% in IASBR-2. The effluent COD was inert organic matter and can be further reduced through coagulation.

The content of readily biodegradable organic matter in wastewater affected the IASBR performance in nitrogen removal and partial nitrification. At the mean nitrogen loading rate of 0.38 kg TN / $(m^3 \cdot d)$, nitrogen removal efficiencies were 76.5% and 97% in IASBR-1 and IASBR-2. The effluent NO_2^--N concentrations ranged 650 - 720 mg/L and 40 - 72 mg/L in IASBR-1 and IASBR-2 with nitrite

acclamation efficiencies of 77 - 79% and 71%, respectively.

The intermittent aeration strategy was proven to be one of the main factors causing partial nitrification. The FISH analysis results show that the ratio of AOB: NOB in the sludge of IASBR-1 was up to 4.5, which was much higher than 2.0 in the seed sludge.

Chapter Five

Characteristics of Nitrous Oxide Emissions from Intermittently Aerated Sequencing Batch Reactors Treating Separated Pig Manure Digestate Liquid

5.1 Introduction

High nitrite concentrations and partial nitrification could lead to high N_2O generation and emission in wastewater treatment systems (Foley *et al.*, 2010). In this study, the N_2O emission and generation from the IASBRs (two IASBRs described in Chapter 4) was investigated.

Dissolved N_2O concentrations in the bulk fluid of IASBRs were measured using a N_2O microsensor. The N_2O emission and generation rates were then calculated. The reasons for N_2O emission from the IASBRs were discussed. The nitrogen balance in an operational cycle was detailed, including nitrogen removal via N_2O emissions in the anaerobic and aerobic periods.

5.2 Materials and methods

5.2.1 N_2O Microsensor and N_2O measurement

Measurement of the N_2O emissions is widely conducted by means of off-gas detection, which is a direct measurement of N_2O emission in the gas phase. Recently, some researchers have carried out direct online measurement of dissolved N_2O in the liquid phase in bioreactors (Kampschreur *et al.*, 2008a) using a sensitive N_2O micro-sensor (Andersen *et al.*, 2001), which can measure the dissolved N_2O concentration in the liquid phase. Compared with the off-gas method, this method can not only quantify the amount of N_2O emissions, but also can quantify the generation of N_2O

A dissolved N$_2$O microsensor (Unisense, Denmark), which is the miniature Clark-type sensor constructed of glass (Figure 5.1) based on the diffusion of N$_2$O through a membrane in the sensor tip, was used for online N$_2$O measurement in the liquid phase of IASBRs.
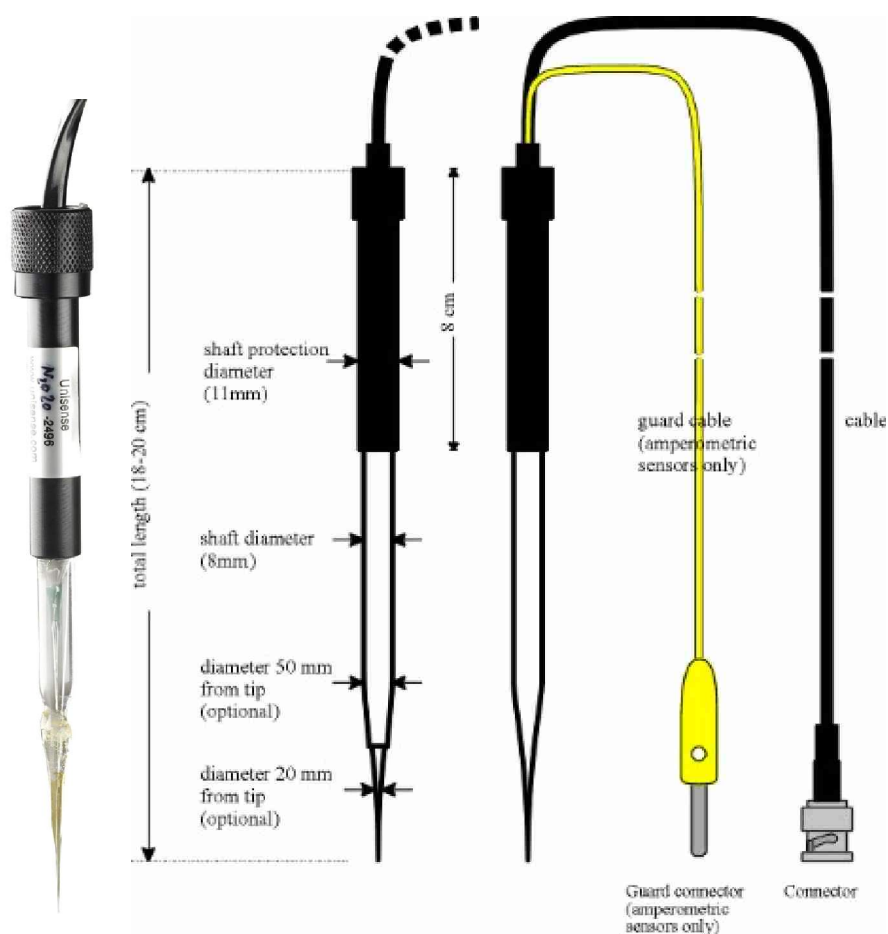
Figure 5.1 N$_2$O microsensor used in the N$_2$O study (Unisense, 2011)

In the sensor tip, the diffused N$_2$O is reduced at a cathode, generating an electric current which is converted to a signal. The signal is recordable using a Unisense picoammeter (PA2000, Unisense, Denmark) with an analogue/digital (A/D) converter which converts the signal to a digital format conveyed via a USB cable (Figure 5.2).

Figure 5.2 Picoammeter PA2000 and A/D converter

This N$_2$O microsensor is able to accurately measure N$_2$O concentrations ranging 0 - 30 mg/L with a detection limit of 0.04 mg/L with the software Profix V3.09 running on a personal computer (Figure 5.3).
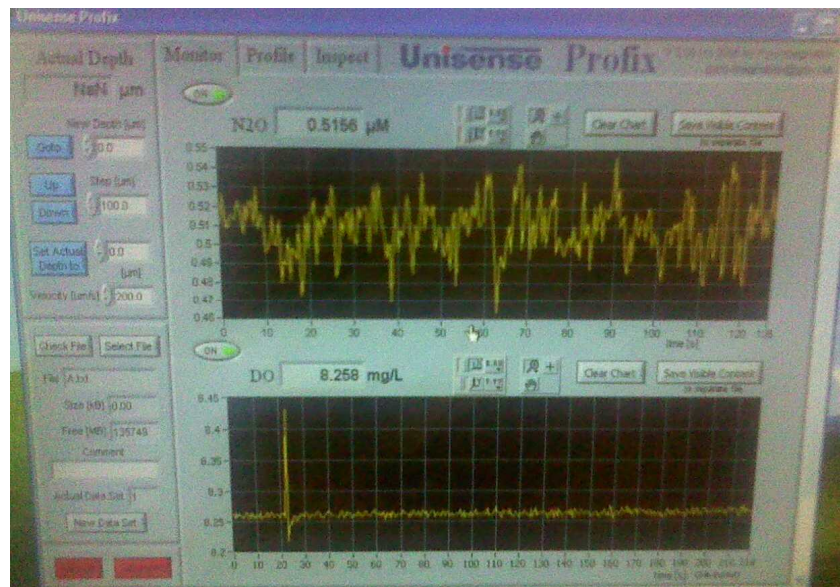
Figure 5.3 Profix V3.09 software used to calibrate and read signals from the N microsensor

A two point calibration was used to calibrate the microsensor. The zero reading was obtained by immersing the N$_2$O sensor in N$_2$O free water. The second point reading was obtained by allowing the N$_2$O sensor to take signal from a N$_2$O solution with a known N$_2$O concentration, which was diluted from the saturated N$_2$O solution.

5.2.2 Calculation of N$_2$O emission and generation

N$_2$O emission from the aqueous phase to the atmosphere is through diffusion and air stripping across the water-air interface. The clear water tests show that the profiles of the diffusion and air striping (including diffusion) rates of N$_2$O in tap water can be described using linear equations with respect to the dissolved N$_2$O concentration (Li, 2010).

$$r_e = \; KC_{N_2O} \tag{Eq. 5.1}$$

where, r_e is the N$_2$O emission rate, mg/ (L· min); C_{N_2O} is the soluble N$_2$O concentration, mg/L; and K is the mass transfer coefficient, /min. The negative sign means that N$_2$O is emitted from the liquid phase to the atmosphere.

Serial batch experiments were carried out to confirm this finding in the IASBRs using tap water. An IASBR was set up, which was identical to IASBR-1 and IASBR-2 with an effective volume of 10 litres. The reactor, without any biomass, was filled with a N$_2$O solution, made from tap water, with the initial N$_2$O concentration of 5.3 mg/L in the liquid phase. The reactor was aerated with an aeration rate of 0.9 L/min as used in IASBR-1 and IASBR-2. Under non-aeration (only stirring; to determine K value via diffusion) and aeration (with stirring; to determine K value via air stripping) conditions, the dissolved N$_2$O concentration was measured and recorded *in situ*, and the N$_2$O decrease rate, which was equal to the emission rate because no N$_2$O generation occurred, was calculated (Figure 5.4). The N$_2$O emission rates were linearly dependent on the N$_2$O concentration:

$$r_e = \; 0.0036C_{N_2O} \qquad (R^2 = 0.85, P<0.05) \quad \text{via diffusion} \tag{Eq. 5.2}$$

$$r_e = \; 0.0602C_{N_2O} \qquad (R^2 = 0.99, P<0.05) \quad \text{via air stripping} \tag{Eq. 5.3}$$
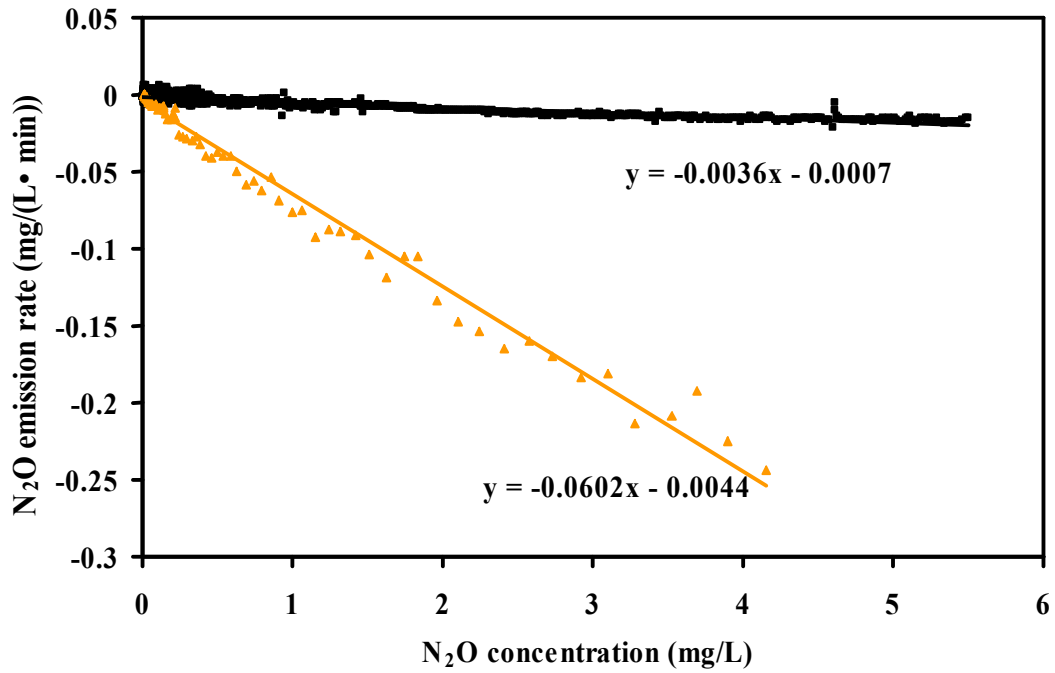
Figure 5.4 Relationship between the soluble N$_2$O concentration and the N$_2$O emission rate (■: N$_2$O emissions via diffusion; ▲: N$_2$O emissions via air stripping)

The calculated mass transfer coefficient K was 0.0036 /min for N$_2$O emissions via diffusion (in the non-aeration condition), and 0.0602 /min via air stripping (diffusion was included; in the aeration condition), respectively. The N$_2$O emissions from IASBR-1 and IASBR-2 in non-aeration periods were via N$_2$O diffusion and in aeration periods were via air stripping (diffusion was included).

The accumulation rate, r_c, of soluble N$_2$O in the reactors, at time t, can be calculated using the equation as follows:

$$r_c = \frac{dC_{N_2O}}{dt}$$

(Eq. 5.4)

where, r_c is the N$_2$O accumulation rate in the liquid phase, mg/ (L· min).

With regard to N$_2$O generation, emission and accumulation occurring in the reactors, a mass balance equation can be established:

$$r_g = r_c \quad r_e$$

(Eq. 5.5

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where, r_g is the N₂O generation rate, mg/ (L· min).

According to Eq. 5.5, the N₂O generation rate can be calculated. Then, the specific N₂O generation rate can be calculated as follows:

$$\mu_g = \frac{r_g}{MLVSS}$$ (Eq. 5.6)

where, μ_g is the specific N₂O generation rate, mg/ (g VSS· min); MLVSS is the mixed liquor volatile suspended solids concentration, g VSS/ L.

From the N₂O emission rate, the amount of N₂O emission during the period of t_1 - t_2 can be calculated:

$$Q = V \int_{t1}^{t2} r_e dt$$ (Eq. 5.7)

where, Q is the N₂O emission amount in the period of t_1 - t_2, mg; and V is the effective liquid volume, L.

From the N₂O generation rate, the amount of N₂O generation during the period of t_1 - t_2 can be calculated:

$$G = V \int_{t1}^{t2} r_g dt$$ (Eq. 5.8)

where, G is the N₂O generation amount in the period of t_1 - t_2, mg.

5.2.3 IASBRs operation

The operation of two reactors (IASBR-1 and IASBR-2) is described in Chapter 4.

5.2.4 Water quality analysis

The methods used for water samples test are given in Appendix B.

5.3 Results and discussion

5.3.1 Performance of N₂O in IASBRs

During the stable operation periods, N_2O concentrations were measured in a number of operational cycles. A typical cycle performance measured on Day 135 when both reactors were in pseudo-steady state is presented in Figure 5.5.

The soluble N_2O concentrations rose in the non-aeration periods, showing the accumulation of N_2O in the liquid phase. There was a significant decrease in N_2O concentrations in the aeration periods following commencement of the aeration. The N_2O concentrations were 2.8 mg/L and 4.0 mg/L in the effluent of IASBR-1 and IASBR-2, equal to 0.3% and 1.4% of TN contained in the effluent, respectively.
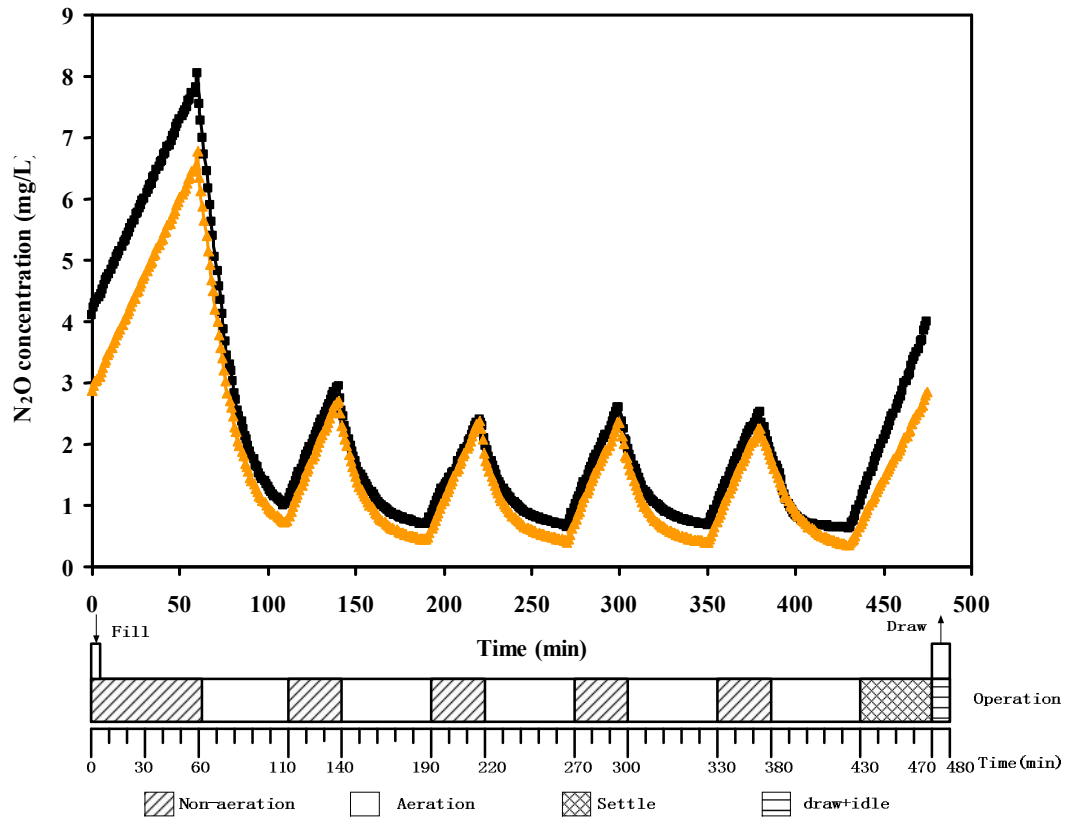
Figure 5.5 Typical cycle profile (Day 135) of soluble N_2O and COD concentrations (■: N_2O in IASBR-1; ▲: N_2O in IASBR-2)

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5.3.2 N₂O emissions and generation in IASBRs

It is hypothesized that the mass transfer coefficients, K, for N₂O emissions via diffusion and air stripping obtained in the clear water tests would be applied in the two reactors and K values would be identical: 0.0036 /min for the diffusion coefficient in non-aeration periods and 0.0602 /min for the air stripping coefficient (diffusion was included) in aeration periods. From this the N₂O emission rates in the two reactors, r_e, can be calculated according to Eq. 5.1, which are shown in Figure 5.6 (the negative sign means that N₂O was emitted from the liquid phase to the atmosphere). The emission rates in the non-aeration periods were much lower than those in the aeration periods, due to the air stripping effect. In the aeration periods, the N₂O emission rate increased immediately after aeration commenced.
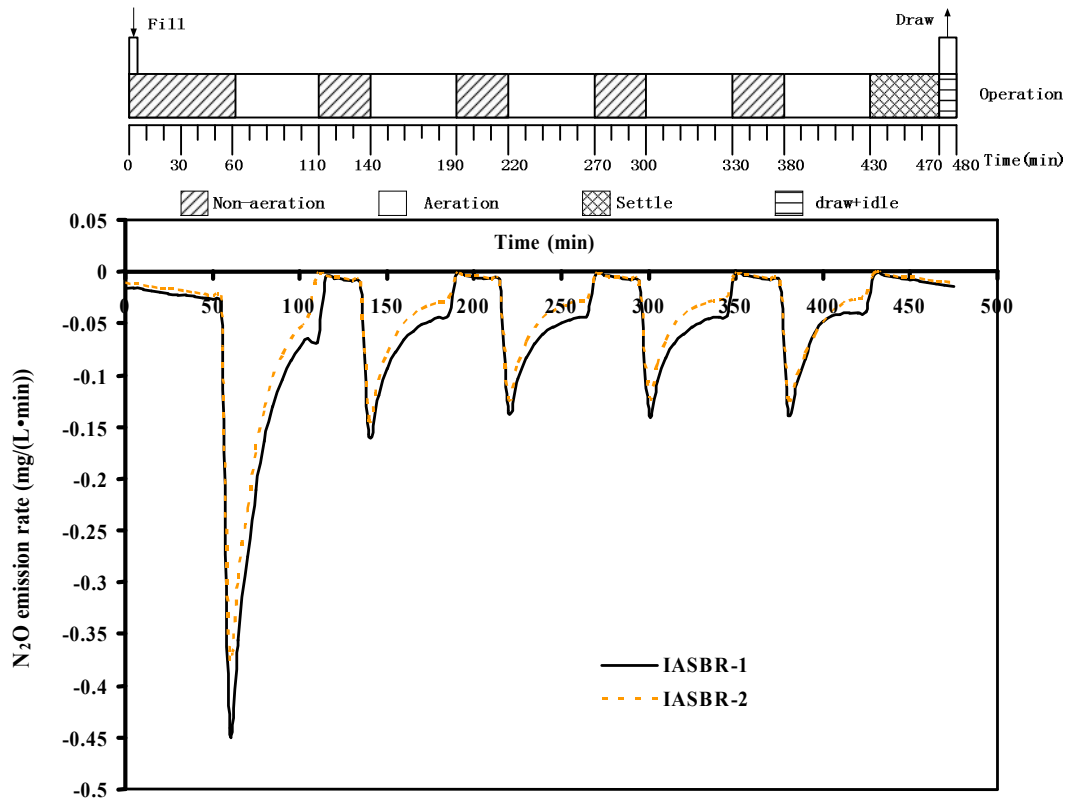
Figure 5.6 Profile of N₂O emission rates in the two intermittently aerated sequencing batch reactors (IASBRs) during a typical cycle (the negative sign means "emission")

From the N₂O emission rate (Figure 5.6), the amount of N₂O emissions can be calculated using Eq. 5.7. The N₂O emissions in this typical cycle were calculated as

253.6 mg and 205.3 mg in IASBR-1 and IASBR-2, respectively. The emission of N$_2$O in IASBR-1 was 23.5 % higher than that in IASBR-2. During the non-aeration periods, only 8.3% (21.0 mg) and 8.4% (17.2 mg) of total N$_2$O emissions occurred in IASBR-1 and IASBR-2, respectively, and the aeration periods contributed to 91.7% (232.6 mg) and 91.6% (188.1 mg) of total N$_2$O emissions in IASBR-1 and IASBR-2, respectively. More than 60% of total N$_2$O emissions took place in the first 20 minutes of the aeration periods. This indicates that air stripping was the principal mechanism for N$_2$O gas emission to the atmosphere in the IASBRs.

The results show that N$_2$O was generated both during aeration and non-aeration periods while the specific N$_2$O generation rates in the non-aeration periods were much higher than during the aeration periods. The average specific N$_2$O generation rates were 9.5 µg/ (g VSS· min) and 4.6 µg / (g VSS· min) in the aeration periods, 23.6 µg / (g VSS· min) and 20.6 µg / (g VSS· min) rates in the non-aeration periods in IASBR-1 and IASBR-2, respectively.

5.3.3 Parameters affecting N$_2$O emissions

During the non-aeration periods, the low BOD$_5$: N ratio in the wastewater would cause N$_2$O generation because the organic matter source was one of the most important factors affecting heterotrophic denitrification. When organic matter is sufficient for heterotrophic denitrifiers, complete denitrification occurs, reducing oxidized nitrogen to N$_2$. When organic matter is insufficient in wastewater, incomplete heterotrophic denitrification occurs, leading to N$_2$O generation. The BOD$_5$: N ratio was 0.7:1 and 2.9:1 in the influent to IASBR-1 and IASBR-2, respectively. The separated digestate liquid of pig manure treated in IASBR-1 had a much lower BOD$_5$ concentration than the synthetic wastewater fed into IASBR-2 and this is one explanation for the higher specific N$_2$O generation rate in the anaerobic periods in IASBR-1 than in IASBR-2. This finding is confirmed by Itokawa et al. (2001) who found that high N$_2$O emission rates appeared under low organic matter conditions due to endogenous denitrification of NO$_2^-$-N in the anoxic phase.

During most of the aeration phase, DO concentrations in the reactors were less th 1.0 mg/L (Figure 5.7). The low DO concentrations would encourage N$_2$O generati

by nitrifiers through the denitrification pathway (Okayasu *et al.*, 1997). Hynes and Knowles (1984) observed that *Nitrosomonas europaea* produced N$_2$O under low DO concentrations. In the aeration periods, N$_2$O could also be formed through chemical decomposition of the intermediate products of ammonium oxidation, like NH$_2$OH. The activity of AOB in IASBR-1 was higher than in IASBR-2; the maximum specific NH$_4^+$-N oxidation rates (r_{max}) were 13.8 mg NH$_4^+$-N/ (g VSS· h) and 9.3 mg NH$_4^+$-N/ (g VSS· h) in IASBR-1 and IASBR-2, respectively (described as Chapter 4). It is reasonable to hypothesize that the generation rate of N$_2$O, as a by-product of the AOB ammonium oxidation process, would be higher in IASBR-1. The low DO concentrations would also encourage N$_2$O generation by heterotrophic denitrifiers through the heterotrophic denitrification pathway (Okayasu *et al.*, 1997). This is because the N$_2$O reductase is more sensitive to oxidative stress than other reductases and could be easily depressed by DO (Otte *et al.*, 1996). However, in the two IASBR reactors, DO concentrations during the non-aeration periods were as low as zero. Hence, the effect of DO on N$_2$O generations via heterotrophic denitrification in the non-aeration periods was negligible.
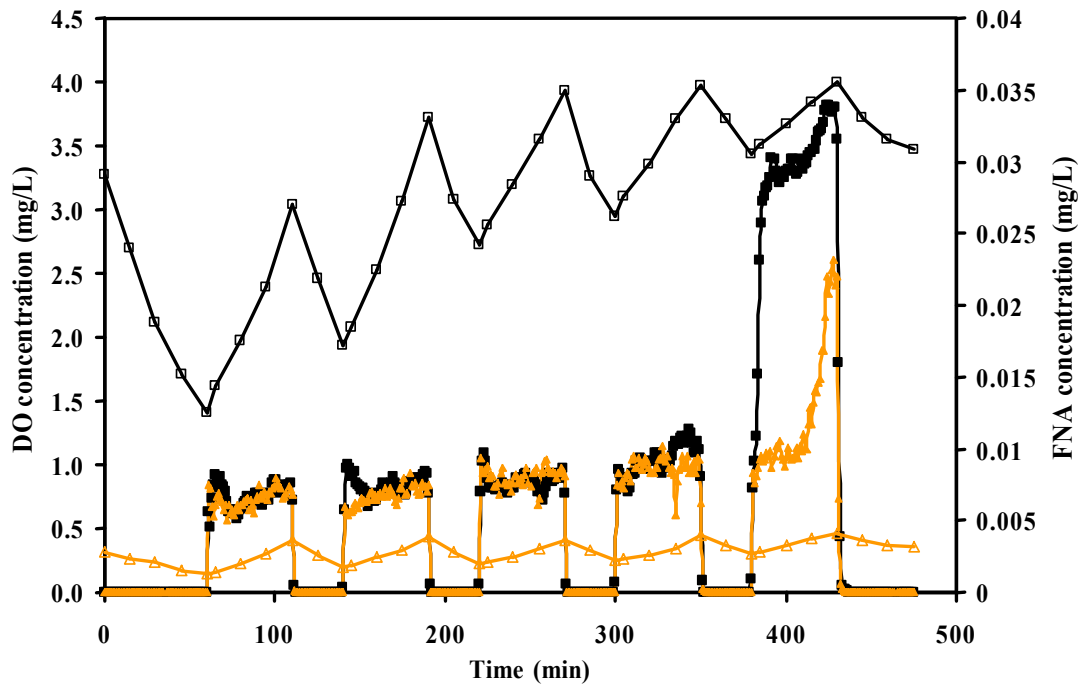

Figure 5.7 Profiles of DO and FNA concentrations in a typical cycle (■: DO in IASBR-1; ▲: DO in IASBR-2; □: FNA in IASBR-1; Δ: FNA in IASBR-2)

Another factor encouraging N_2O emissions in the two reactors might be the high NO_2^--N concentrations (Figure 5.8). High NO_2^- concentrations can induce N_2O generation in both conventional nitrification and denitrification processes. AOB can use NO_2^--N as the electron acceptor under low DO conditions, instead of oxygen, to oxide NH_4^+-N or NH_2OH, with a final product of N_2O (Kampschreur *et al.*, 2008b). As for heterotrophic denitrifiers, the nitrous oxide reductase is easily inhibited when the NO_2^--N level is above 2 mg/L (von Schulthess, 1996), leading to N_2O generation. In this study, in an operation cycle, NO_2^--N ranged 650 - 720 mg/L and 40 - 72 mg/L in IASBR-1 and IASBR-2, respectively (Figure 5.8), both being high enough to encourage N_2O emissions during the aeration and non-aeration periods.
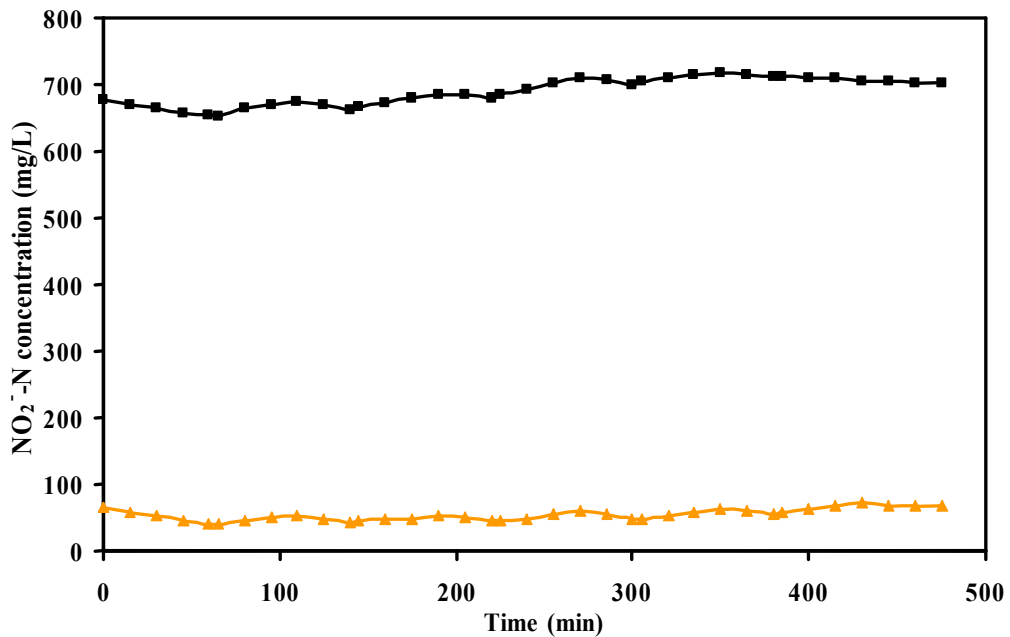
Figure 5.8 Profile of NO_2^--N concentrations in a typical cycle in the two reactors (■: IASBR-1; ▲: IASBR-2)

On the other hand, the facts that both NO_2^- and pH have been reported to have a significant impact on N_2O accumulation during denitrification suggests that free nitrous acid (FNA), rather than NO_2^-, might be the true factor responsible for N_2O generation. Zhou *et al.* (2008) have found when FNA concentrations were higher than 0.002 mg/L, N_2O reduction by heterotrophic denitrification was strongly depressed by FNA, inducing N_2O generation. In the present study, the FN concentrations were between 0.003 and 0.035 mg/L in the IASBRs (Figure 5.

which could result in a high specific N₂O generation rate, especially in IASBR-1. This could be another explanation for the higher average specific N₂O generation rate in IASBR-1.

5.3.4 Nitrogen balance

There were 253.6 mg and 205.3 mg N₂O emissions in the typical cycle, corresponding to 161.4 mg and 130.6 mg of N₂O-N in IASBR-1 and IASBR-2, respectively. Thus, the N₂O-N emissions were equal to 12.0% and 9.8% of the influent nitrogen loading rate (NLR) in IASBR-1 and IASBR-2, respectively. The total nitrogen (TN) removals were 76.9% and 96.8% in IASBR-1 and IASBR-2, respectively, so, 15.6% and 10.1% of N removals from IASBR-1 and IASBR-2, respectively, was via N₂O emissions. In order to investigate how nitrogen was removed or transformed, a nitrogen balance analysis using Eq. 5.9 and Eq. 5.10 was conducted:

$$TN_I = TN_E \quad TN_S + \quad TN_{D_i} + TN_N \quad TN_A \quad TN_O$$

(Eq. 5.9)

$$TD_{D_i} = \quad TD_{D_i}(N_2O) + \quad TD_{D_i}(N)$$

(Eq. 5.10)

where, TN_I: amount of nitrogen delivered into the reactor tank in the fill phase; TN_E: amount of nitrogen contained in the effluent withdrawn from the reactor tank in the draw phase; TN_S: amount of nitrogen used for biomass assimilation; ΣTN_{Di}: amount of nitrogen removed in non-aeration periods (i = 1, 2, 3, 4, 5), which included nitrogen removal through N₂O gas emissions (ΣTN_{Di} (N₂O)), and through N₂ and NO gases (ΣTN_{Di} (N)); TN_N: amount of nitrogen removed via N₂O emissions in aeration periods; TN_A: amount of nitrogen removed via air stripping of ammonia gas in aeration periods; and TN_O: nitrogen removal through other pathways, such as simultaneous conventional nitrification and denitrification in aeration periods.

In the typical cycle, 1347 mg and 1338 mg TN_I was fed into IASBR-1 and IASBR-2, respectively. After 8 hours treatment, TN_E was equal to 316 and 42 mg of N in IASBR-1 and IASBR-2, respectively. TN_S values due to sludge withdrawn calculated were 76 and 81 mg N in IASBR-1 and IASBR-2, respectively (Zhang *et al.*, 2011a).

Calculated from the reduction of the TON in the non-aeration periods, ΣTN_{Di} was 800 mg and 994 mg N in IASBR-1 and IASBR-2, respectively, equal to 59.4% and 74.3% of the total amount of influent nitrogen in the typical cycle. TON was reduced to NO, N$_2$O and N$_2$ gases, among which N$_2$O and N$_2$ were the main gaseous products (Philip et al., 2002). ΣTN_{Di}(N$_2$O) was calculated as 105 mg and 101 mg according to the N$_2$O generation rate in each non-aeration period, up to 7.8 % and 7.6 % of TN_I in IASBR-1 and IASBR-2, respectively.
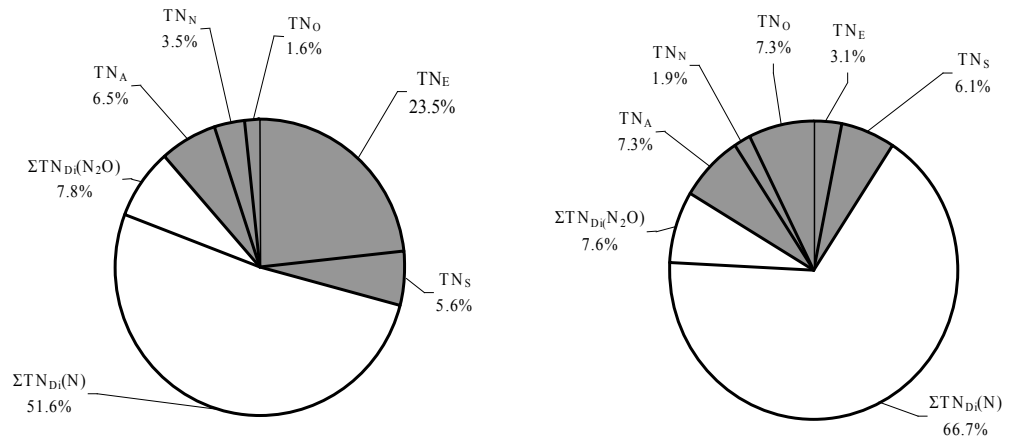
Figure 5.9 Nitrogen balance analysis in the intermittently-aerated sequencing batch reactors (left: IASBR-1; right: IASBR-2)

During the aeration periods, according to the N$_2$O generation rate in the aeration periods, TN_N was 47 mg and 25 mg of N$_2$O-N generation in IASBR-1 and IASBR-2, equal to 3.5% and 1.9% of TN_I, respectively. The nitrogen removal via air stripping of ammonia gas (TN_A) was up to 87 mg and 98 mg (Zhang et al., 2011a), so, TN_O were 21 mg and 98 mg nitrogen in IASBR-1 and IASBR-2, respectively, equating 1.6% and 7.3% of total nitrogen removed, respectively. The nitrogen balance analysis results, in consideration with N$_2$O emissions in the non-aeration periods and aeration periods, are summarized in Figure 5.9.

5.4 Summary

In this study, N$_2$O emission and generation from IASBRs treating the separated]

manure digestate liquid and the synthetic wastewater was investigated.

In steady state, N$_2$O was generated and accumulated in the non-aeration periods, with the highest N$_2$O concentrations measured at the end of the non-aeration periods. There was a significant reduction in N$_2$O concentrations during the aeration periods.

N$_2$O emissions were 12.0% and 9.8% of the total influent nitrogen loading rate, equal to 15.6% and 10.1% of the total amount of nitrogen removed from IASBR-1 and IASBR-2, respectively.

The mean specific N$_2$O generation rates were 9.5 µg / (g VSS· min) and 4.6 µg / (g VSS· min) in the aeration periods, 23.6 µg / (g VSS· min) and 20.6 µg / (g VSS· min) in the non-aeration periods for IASBR-1 and IABSR-2, respectively. The IASBR-1 with low influent BOD$_5$ concentrations induced more N$_2$O generation.

The mean N$_2$O emissions in an operational cycle were 253.6 mg and 205.3 mg for IASBR-1 and IASBR-2, respectively. During the non-aeration periods, only 8.3% and 8.4% of total N$_2$O emissions occurred in IASBR-1 and IASBR-2, respectively; while during the aeration periods, 91.7% and 91.6% of N$_2$O emissions took place in IASBR-1 and IASBR-2, respectively.

According to the nitrogen balance, 7.8 % and 7.6 % of total influent nitrogen was removed from IASBR-1 and IASBR-2 via N$_2$O emission in the non-aeration periods, respectively; 3.5% and 1.9% of total influent nitrogen was removed via N$_2$O emission in the aeration periods in IASBR-1 and IASBR-2, respectively.

Chapter Six

Nutrient Removal and Nitrous Oxide Emissions from Aerobic Granular Sludge Sequencing Batch Reactors Treating the Separated Pig Manure Digestate Liquid

6.1 Introduction

The application of the aerobic granular sludge process into wastewater treatment is promising due to the excellent settling ability of aerobic granules and the high microbial concentrations in the granules (Qin *et al*., 2004; Liu *et al*., 2005; Adav *et al*., 2007; Kim *et al*., 2008b). Aerobic bacteria are grown on the surface layer of the granules and nitrifiers are located at a depth of 70 µm to 100 µm from the granule surface, while anoxic denitrifiers have been detected at a depth of 800 µm - 900 µm from the granule surface (Tay *et al*., 2002; Adav *et al*., 2008; Gao *et al*., 2011). Therefore, this technology has a potential to achieve simultaneous nitrification and denitrification. However, this spatial structure may induce incomplete denitrification, which can lead to generation of N_2O.

In this chapter, synthetic wastewater simulating a mixture of separated digestate liquid and municipal wastewater was treated in aerobic granular sludge sequencing batch reactors. The performance of the reactors for nutrient removal was examined, and N_2O emissions were determined. The N_2O generation rate in the aerobic granular sludge SBRs was calculated and discussed.

6.2 Materials and methods

6.2.1 Sequencing batch reactor set-up

(a)
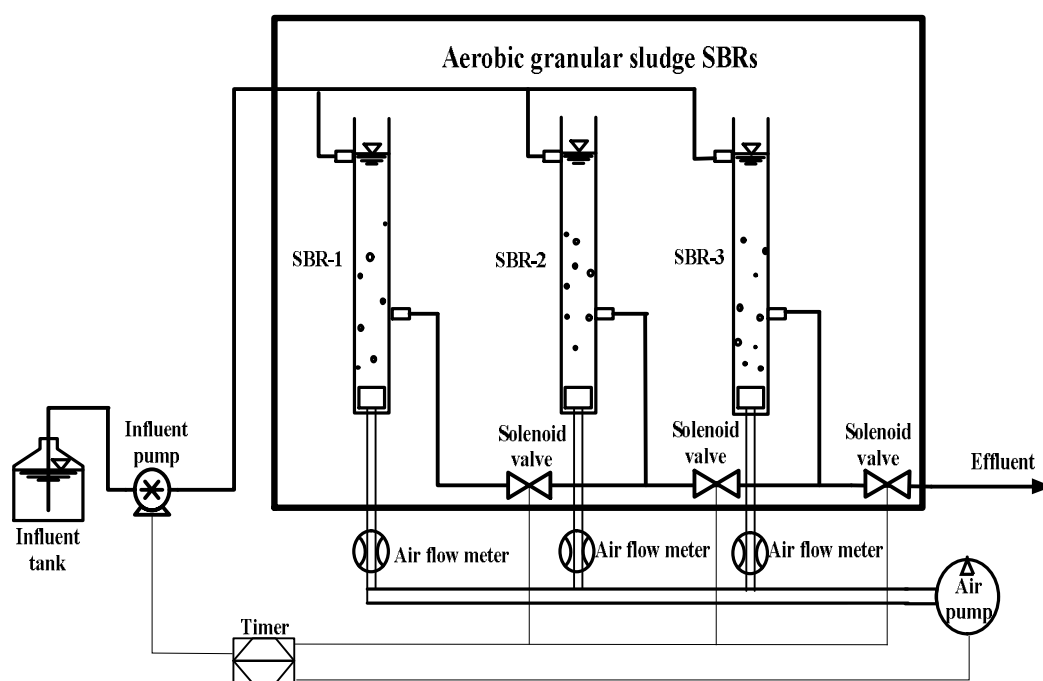
(b)

Figure 6.1 Photograph (a) and schematic diagram (b) of the aerobic granular sludge sequencing batch reactor systems

Three identical laboratory-scale SBRs were established (Figure 6.1). The cylindrical reactors were made from transparent Plexiglas, each having an effective volume of 1.5 litres with an inner diameter of 5 cm and a depth of 100 cm. Air was supplied using aquarium air pumps through air diffusers installed at the bottom of the reactors, and the air flow rates to the three identical SBR reactors, SBR1, SBR2 and SBR3, were controlled at 0.2 L/min, 0.6 L/min and 1.0 L/min by air flow meters, respectively. The ambient temperature was 14±4 °C. A peristaltic pump (Longer, China) was used to feed the same amount of influent wastewater into the reactors, and the treated wastewater was withdrawn through solenoid valves.

The SBRs were operated in 4-hour cycles with a hydraulic retention time (HRT) of 8 hours, ensuring a 50% volume exchange ratio each time, controlled by programmable timers (Samson Electric Wire, Germany). In a cycle, after a 5-minutes fill phase, the reactors were aerated continuously for 220 minutes, followed by 5 minutes settlement. The effluent was withdrawn during the last 10 minutes of the cycle. In every operational cycle, half of the reactor working volume was exchanged with influent wastewater.

6.2.2 Synthetic wastewater

Synthetic wastewater simulating a mixture of the separated pig manure digestate liquid and municipal wastewater which was characterized with low COD: N ratios (particularly low BOD: N ratios) was treated in the three reactors. In order to determine the effects of COD: N ratios on N$_2$O emissions in the aerobic granular sludge SBR reactors, N$_2$O measurement consisted of three operational periods (10 days for each period) successively, with three NH$_4^+$-N concentrations of 148 mg/L, 106 mg/L and 74 mg/L in each period, each corresponding to COD: N ratios of 1: 0.22, 1: 0.15 and 1: 0.11, respectively. The carbon source in the synthetic wastewater was glucose (COD was 700 mg/L). The nitrogen source was (NH$_4$)$_2$SO$_4$ (for NH$_4^+$-N concentrations of 148 mg/L, 106 mg/L and 74 mg/L, the synthetic wastewater contained 700 mg/L, 500 mg/L and 350 mg/L of (NH$_4$)$_2$SO$_4$, respectively). Other components of the synthetic wastewater were 100 mg/L KH$_2$PO$_4$, 500 mg/L K$_2$HPO$_4$, 100 mg/L NaCl, 200 mg/l MgCl$_2$, 20 mg/L FeCl$_2$ and 20 mg/L CaCl$_2$. The synthetic wastewater was fed to the SBRs at a mean loading rate of 2.1 kg COD/ (m^3· d).

6.2.3 Aerobic granular sludge

The reactors were seeded with aerobic granular sludge taken from a SBR which had been operated stably for more than half a year treating synthetic high strength domestic wastewater. The initial suspended solids (SS) concentration in the three SBRs after seeding was 20 g/L, and the three reactors were fed with the synthetic wastewater with a COD: N ratio of 1: 0.22 for two weeks before the measurement of N$_2$O commenced. Then, the wastewater was switched to the other two synthetic wastewaters with COD: N ratios of 1:0.15 and 1:0.11 successively. The biomass concentrations in the SBRs during this experimental period were 18.9± 0.8 g SS/L, and the main particle diameter was 300 - 2000 µm (Figure 6.2), with a mean diameter of 710±85 µm. In the steady state, no sludge was withdrawn from the reactors, except the suspended solids exiting the reactors with the effluent in the draw phase. This led to a sludge age of more than 45 days.
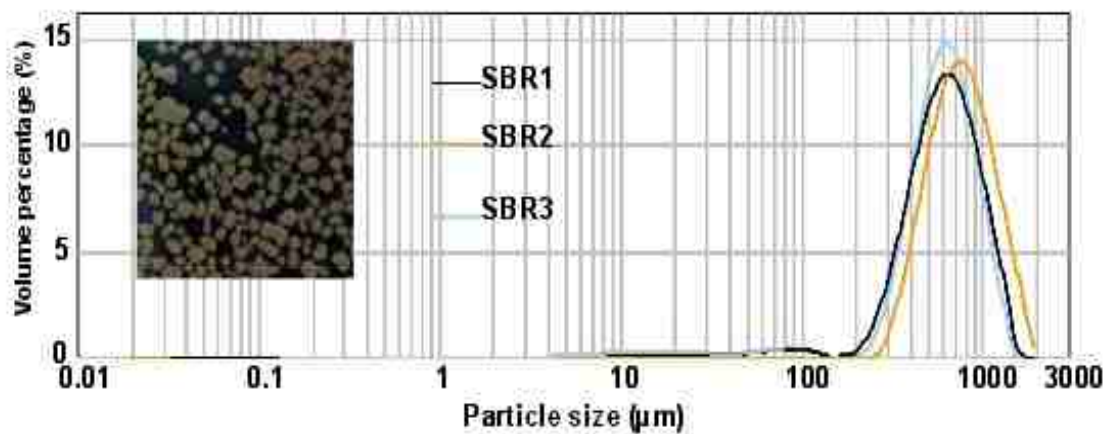
Figure 6.2 Aerobic granular sludge and particle diameter

6.2.4 Water quality analysis

The methods used for water samples test are given in Appendix B.

6.2.5 Poly-β-hydroxybutyrate measurement

The poly-β-hydroxybutyrate (PHB) content in granular sludge biomass was measured using the spectrophotometric method (Karr *et al*., 1983; Panda *et al*., 2008) with the modified procedure as follows: (1) 25 ml of mixed liquor taken from the

reactors was ground and centrifuged at 4,000 rpm; (2) the centrifuged biomass was washed with 50%, 80% and 99.5% ethanol successively (each for 3 minutes); (3) the biomass was washed with 0.5 ml concentrated sulphuric acid twice and transferred to screw topped glass tubes; (4) 1 ml 98.5% concentrated sulphuric acid was added to each tube and heated at 105 °C for 30 minutes; (5) The concentration of PHB was determined at the wavelength of 235 nm using a UV-VIS (DR2800, Hach, USA). Sodium 3-hydroxybutyrate was used as a standard PHB and was digested using the procedure mentioned above for calibration.

6.2.6 N$_2$O concentration measurement

The dissolved N$_2$O micro-sensor, capable of measuring N$_2$O concentrations accurately within a range of 0 - 30 mg/L, was adopted for online N$_2$O measurement. The detail of N$_2$O micro-sensor is described in Chapter 5.

6.2.7 N$_2$O emission coefficient

According to Eq. 5.1 and Eq. 5.4, N$_2$O emission coefficients (K) must be determined before the calculation of N$_2$O emissions from the aerobic granular sludge SBRs. Clear water test experiments mimicking the operation of the aerobic granular sludge SBR reactors were designed to find K values (Figure 6.3). A N$_2$O solution made of distilled water with a N$_2$O concentration of 6.2 mg/L in the liquid phase was added into the three reactors where biomass had been completely removed. The reactors were aerated at three aeration rates of 0.2 L/min, 0.6 L/min and 1.0 L/min, respectively. The N$_2$O concentrations were recorded using the N$_2$O sensor. The N$_2$O emission rate, which was equal to r_c due to no N$_2$O generation in the clear water test, was calculated. The N$_2$O emission rates were linearly dependent on the N$_2$O concentration:

$$r_e = 0.106C_{N_2O} \qquad (R^2 = 0.99) \qquad \text{at the aeration rate of 0.2 L/min} \qquad \text{(Eq. 6.1)}$$

$$r_e = 0.131C_{N_2O} \qquad (R^2 = 0.98) \qquad \text{at the aeration rate of 0.6 L/min} \qquad \text{(Eq. 6.2)}$$

$$r_e = 0.150C_{N_2O} \qquad (R^2 = 0.99) \qquad \text{at the aeration rate of 1.0 L/min} \qquad \text{(Eq. 6.3}$$

Thus, the measured coefficient, *K*, was 0.106 /min, 0.131 /min and 0.150 /min at the three aeration rates, receptively. It is hypothesized that the *K* value, obtained through the clear water experiments could be applied to aerobic granular sludge SBRs treating the synthetic wastewater (Zhang et al., 2011).
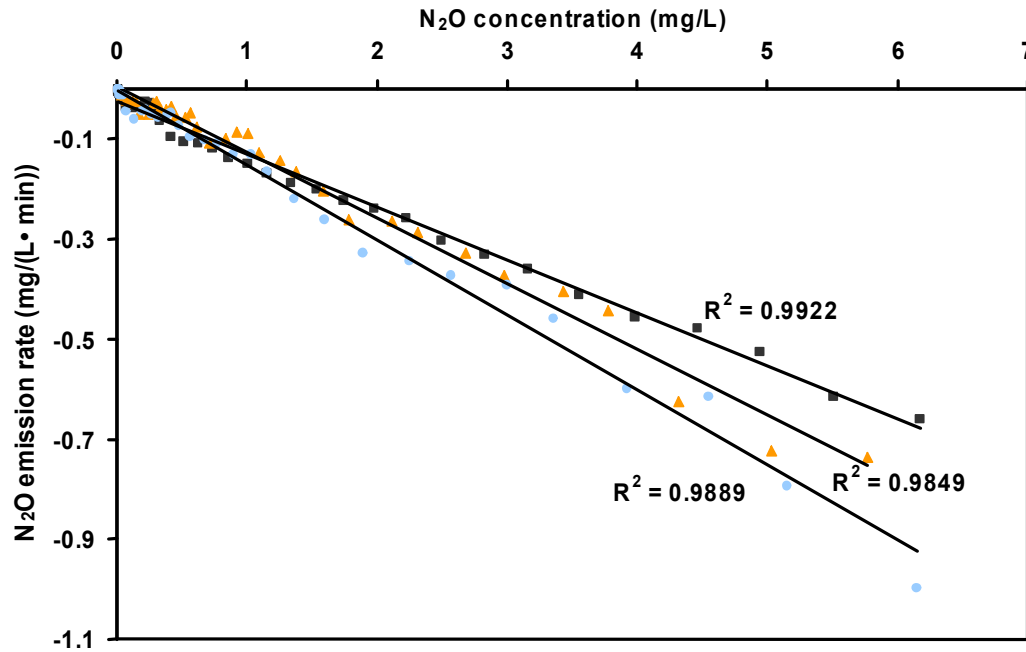
Figure 6.3 Linear dependence of N₂O emission rates on the dissolved N₂O concentration in the clear water test (■: 0.2 L/min, ▲: 0.6 L/min, ♦: 1.0 L/min)

6.3 Results and discussion

6.3.1 Performance of aerobic granular sludge SBRs

The performance of SBRs operated under the three different COD: N ratios (or different NLR) are shown in Table 4.1 (COD: N ratios were 1: 0.22, 1: 0.15 and 1: 0.11). The mean COD removal efficiencies were more than 99% in the three SBRs at the three aeration rates and three COD: N ratios.

No more than 40% TN removal was obtained under the continuous aeration operation. On average, the highest nitrogen removal efficiency was 36% in SBR1 and SBR2 at the COD: N ratio of 1: 0.11. The results confirm that simultaneous

nitrification and denitrification took place in aerobic granular sludge SBRs even though continuous aeration was provided. The nitrogen removal efficiency increased when the ratio of COD: N decreased. It decreased when the air flow rate increased.

Table 1 Effluent of aerobic granular sludge sequencing batch reactors (unit: mg/L)

	COD: N = 1: 0.11			COD: N = 1: 0.15			COD: N = 1: 0.22		
	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3
NH_4^+-N	0.4± 0.2	0.4± 0.1	0.5± 0.1	2.5± 0.1	1.3± 0.7	2.1± 0.5	83.5± 3.0	64.4± 1.5	43.8± 0.7
NO_2^--N	0.1± 0.1	0.1± 0.1	0.1± 0.1	0.5± 0.1	0.2± 0.1	0.7± 0.6	4.1± 0.2	1.3± 0.2	0.3± 0.1
NO_3^--N	50.8± 2.8	50.9± 1.7	64.5± 0.2	72.1± 1.3	78.2± 0.1	86.7± 1.8	47.2± 1.0	72.1± 1.3	96.0± 0.1

When the COD: N ratio was 1: 0.22 with the highest influent nitrogen concentration, there was 53%, 42% and 28% of influent NH_4^+-N remaining in the effluent, showing that the duration of aeration was not sufficiently long to complete NH_4^+-N oxidation under the high NLRs.

6.3.2 N$_2$O emission and generation in the aerobic granular sludge SBRs

During the stable operation periods, real-time N$_2$O concentrations were measured. The typical cycle performance at different COD: N ratios when SBRs were in pseudo-steady state are presented in Figure 6.4.

The dissolved N$_2$O concentration increased sharply immediately after wastewater was delivered into the reactors. In this period, the wastewater had enough organic matter (COD) for heterotrophic denitrification inside the granules with the DO concentrations in the bulk fluid lower than 1.5 mg/L. However, the low DO concentrations in the bulk fluid would lead to heterotrophic denitrification inside the aerobic granules, thereby encouraging N$_2$O generation via the heterotrophic denitrification pathway (Okayasu et al., 1997). This is because the N$_2$O reductase is more sensitive to oxidative stress than other reductases and could be easily inhibit by DO (Otte et al., 1996). Therefore, N$_2$O was obviously generated in the fill phase

(a)

(b)

(c)
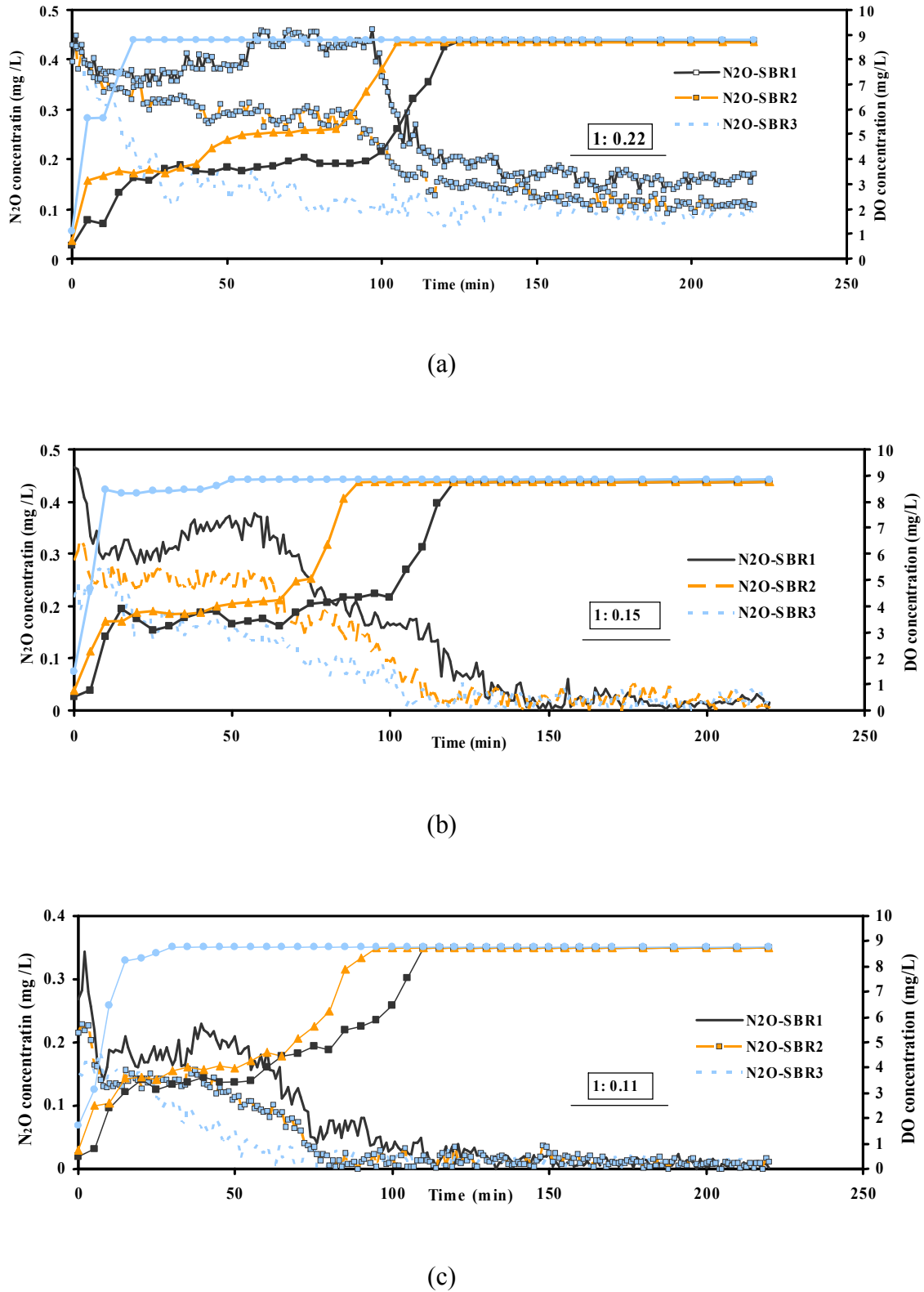
Figure 6.4 Profiles of N₂O and DO in a typical cycle at different COD: N ratios (■:
DO in SBR1; ▲: DO in SBR2; ♦: DO in SBR3)
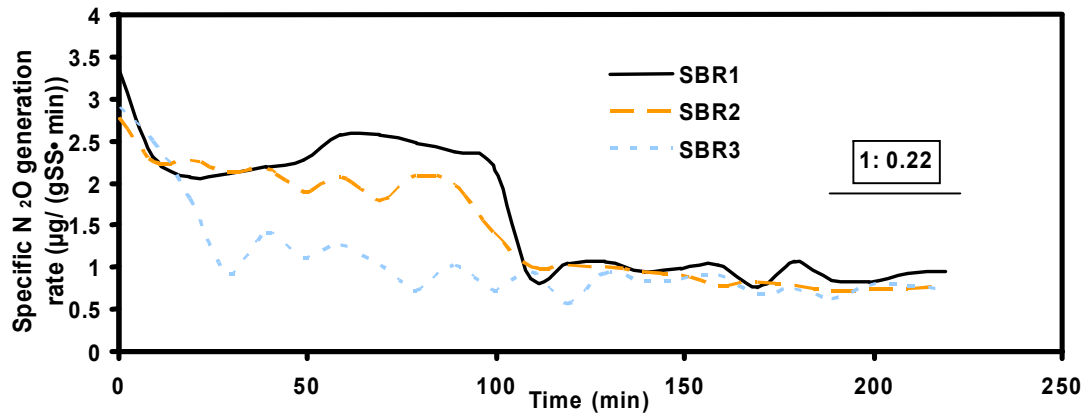
(a)
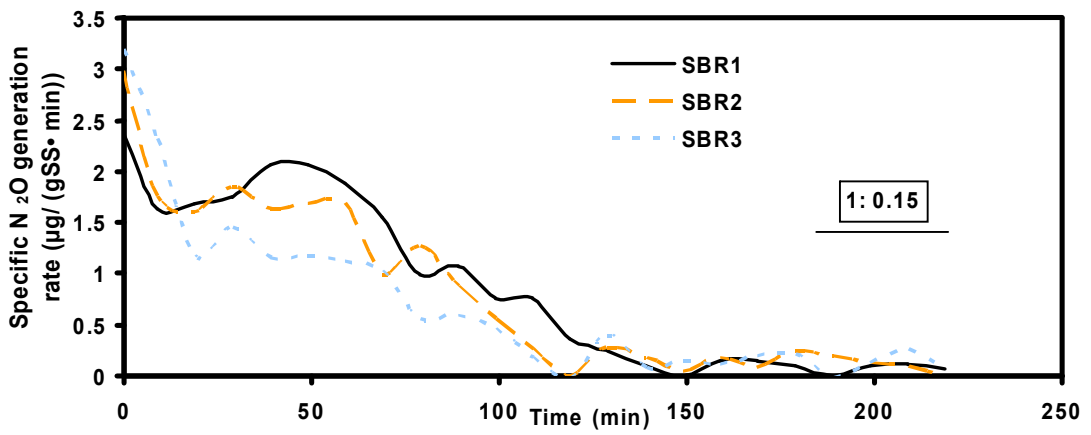
(b)
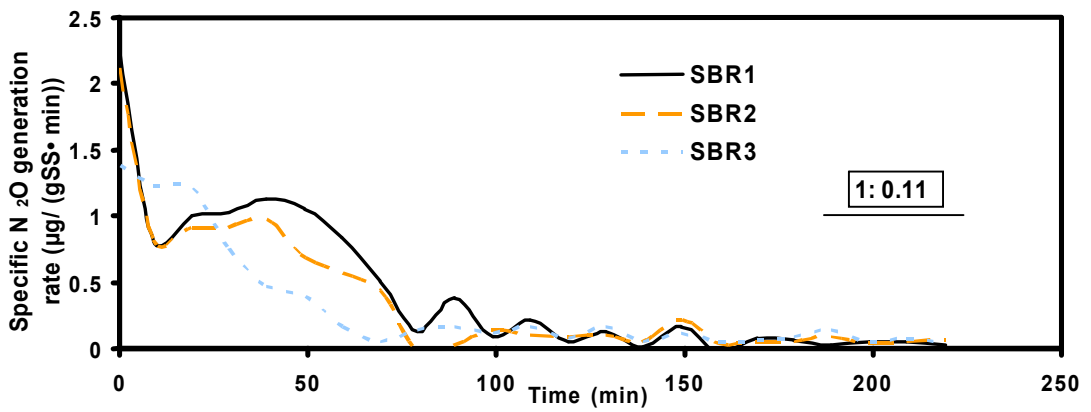
(c)

Figure 6.5 Specific N$_2$O generation rate in a typical cycle at different COD: N ratio

At 2 - 5 minutes after the aeration commenced, the N$_2$O concentration decreased and levelled off for 30 - 50 min, indicating the N$_2$O generation was in a balance with the N$_2$O emission, before it dropped in SBR1 and SBR2 at the three COD: N ratios. As shown in Figure 6.5, in the aeration stage, in SBR1 and SBR2, the average specific N$_2$O generation rate calculated using Eq. 5.6 was 2.1 μg N$_2$O / (g SS· min) and 2.2 μg N$_2$O / (g SS· min) at COD: N ratio of 1: 0.22, 1.8 μg N$_2$O / (g SS· min) and 1.7 μg N$_2$O / (g SS· min) at COD: N ratio of 1: 0.15, 1.1 μg N$_2$O / (g SS· min) and 0.9 μg N$_2$O / (g SS· min) at COD: N ratio of 1: 0.11. This shows that even though DO concentrations were as high as the saturation concentrations, due to the dense structure of aerobic granules, low DO was possible to be maintained inside the granules, leading to N$_2$O generation via heterotrophic denitrification.

However, in SBR3, DO concentrations increased sharply after the aeration commenced and were saturated in the liquid phase after 10 - 20 minutes' aeration at the three COD: N ratios. The N$_2$O concentration dropped after the aeration commenced. The average specific N$_2$O generation rate decreased to 1.1 μg N$_2$O / (g SS· min) from 2.9 μg N$_2$O / (g SS· min) at the beginning of the fill phase at the COD: N ratio of 1: 0.22, and 1.1 μg N$_2$O / (g SS· min) from 3.2 μg N$_2$O / (g SS· min) at the COD: N ratio of 1: 0.15. When the COD: N ratio was 1: 0.11, because of the low influent nitrogen concentration, the average specific N$_2$O generation rate decreased from 1.3 μg N$_2$O / (g SS· min) to 0.2 μg N$_2$O / (g SS· min) continuously (Figure 6.5). This indicates that the high aeration rate in SBR3 led to high DO levels. Consequently, N$_2$O generation via heterotrophic denitrification was inhibited.

In SBR1, SBR2 and SBR3, when treating wastewater with the COD: N ratio of 1: 0.22, the N$_2$O concentration was 0.17 mg/L, 0.11 mg/L and 0.09 mg/L, equal to 0.13%, 0.08% and 0.06% of the total nitrogen in effluent, respectively. When treating synthetic wastewater with the other two COD: N ratios, the effluent N$_2$O concentrations were all lower than 0.01 mg/L.

According to Eq. 5.7, the N$_2$O emission amounts in a typical cycle when treating synthetic wastewater with different COD: N ratios at the three aeration rates were calculated (Figure 6.6). The proportion of N$_2$O emissions to the NLR in SBR1, SRB2 and SBR3 was 8.2%, 6.1% and 3.8% at the COD: N ratio of 1: 0.22; 7.0%, 5.1% and 3.5% at the COD: N ratio of 1: 0.15; and 4.4%, 2.9% and 2.2% at the COD:

N ratio of 1: 0.11, respectively. N$_2$O emissions from municipal wastewater treatment plants are estimated to be ~0.5% of the NLR (IPCC, 2006) and ~1.7% of the NLR in nitrifying reactors (Kampschreur *et al.*, 2008a). Thus, the aerobic granular sludge process used for nitrogen removal from wastewater could induce significantly higher N$_2$O emissions. N$_2$O emissions can be minimised by increasing the ratio of COD: N and the aeration rate.
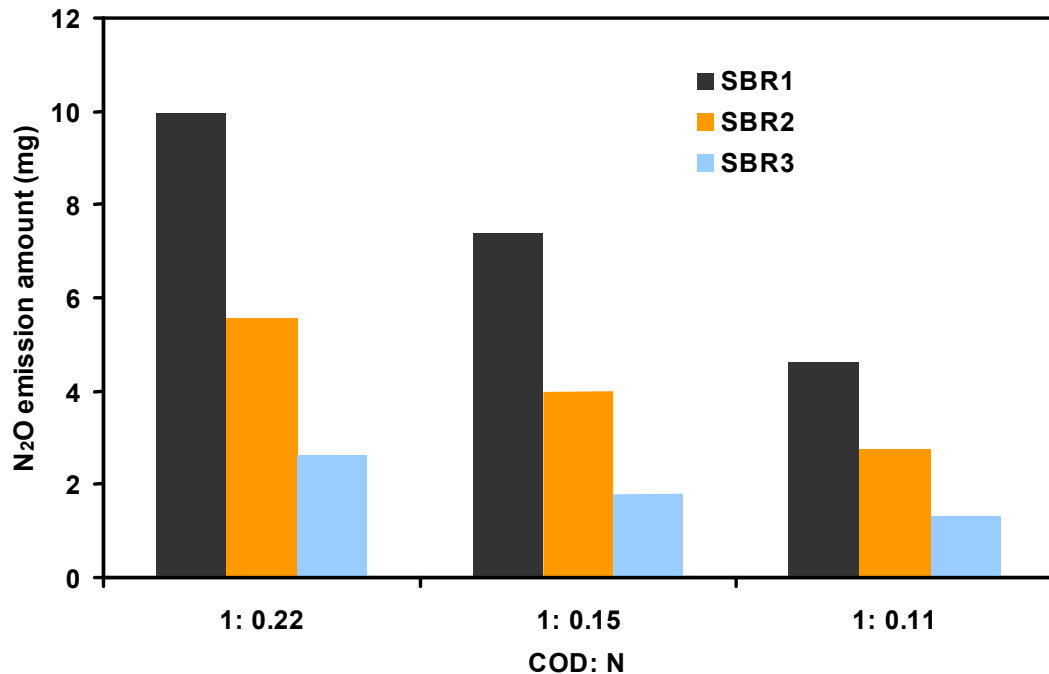
Figure 6.6 N$_2$O emission amount in a typical cycle

6.3.3 Relationship between N$_2$O emissions and nutrient removals

DO was consumed for COD and NH$_4^+$-N oxidation (namely carbonaceous oxidation and nitrification, respectively) in the three SBRs. Figure 6.7 shows the profiles of COD and NH$_4^+$-N in a typical cycle. It shows that when COD was completely consumed, DO concentration began to increase until saturation in SBR1 and SBR2. When DO increased and COD was exhausted, it can be seen from Figure 6.4 and Figure 6.5 that soluble N$_2$O concentrations decreased suddenly in SBR1 and SBR2. The heterotrophic denitrification process generating N$_2$O and demanding organic carbon sources (COD) under low DO concentrations were inhibited in SBR1 a SBR2 during this period.
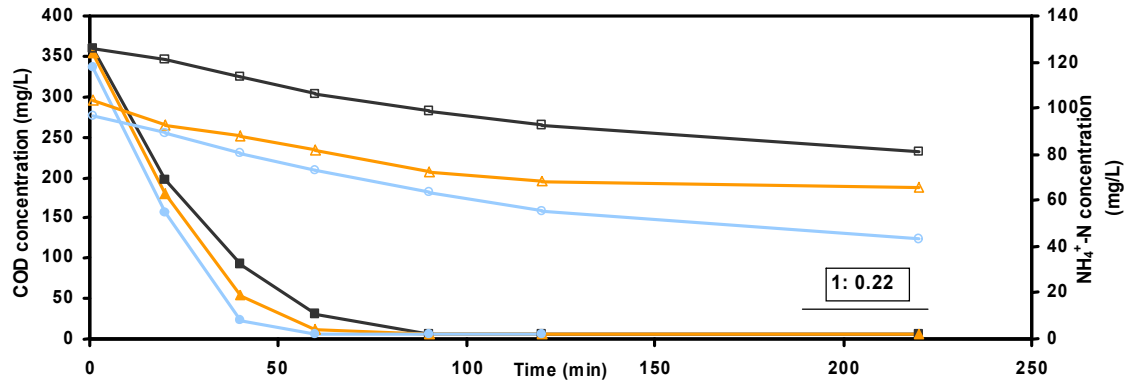
(a)
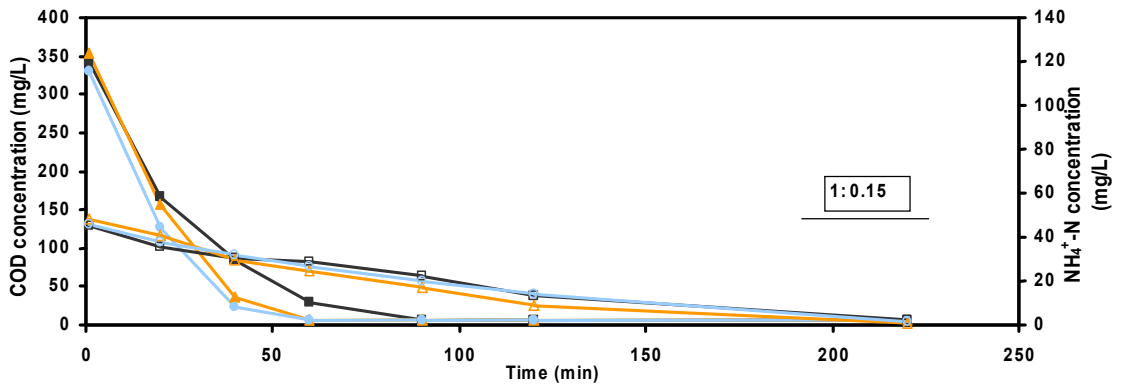
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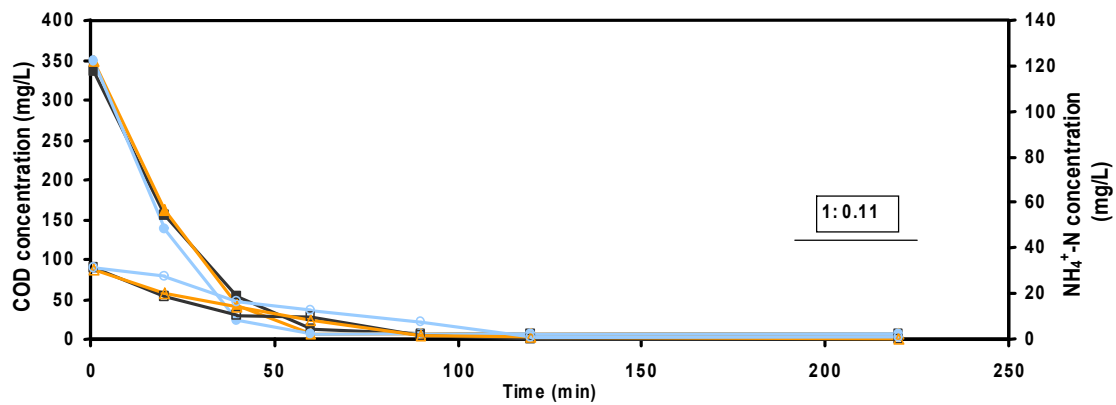
(c)

Figure 6.7 Profiles of COD and ammonium in a typical cycle (■: COD in SBR1; ▲: COD in SBR2; ♦: COD in SBR3; □: NH$_4^+$-N in SBR1; Δ: NH$_4^+$-N in SBR2; ◊: NH$_4^+$-N in SBR3)

However, the N$_2$O concentration did not decrease to zero after DO increased (the same phenomenon also appeared in SBR3 where DO rose quickly as soon as aeration commenced). This indicates that another pathway of N$_2$O generation under aerobic conditions, namely autotrophic nitrification during NH$_4^+$-N oxidation, existed in the aerobic granular sludge SBRs. When treating the synthetic wastewater with the COD: N ratio of 1: 0.22, the NH$_4^+$-N concentration was higher than 40 mg/L at the end of the operation cycle (Figure 6.7). Unlike what happened while treating the synthetic wastewater with the COD: N ratios of 1:0.15 and 1:0.11, the N$_2$O concentration and generation rate was not decreased to zero at the end of aeration period. When treating the synthetic wastewater with the COD: N ratios of 1:0.15 and 1:0.11, after NH$_4^+$-N was lower than 10 mg/L, the soluble N$_2$O concentration gradually fell to zero.

6.3.4 Factors affecting N$_2$O generation in the aerobic granular sludge SBRs

The factors affecting N$_2$O aerobic generation in the aerobic granular sludge were analyzed using batch experiments. Aerobic granular sludge in the three SBRs was aerated for 30 minutes, and then the liquid in the SBRs was replaced with tap water, so there was no nutrient in the liquid phase. NaNO$_2$ or KNO$_3$ was added to the SBRs separately to achieve an initial concentration of 50 mg/L NO$_2^-$-N or 50 mg/L NO$_3^-$-N, respectively, so as to measure N$_2$O emissions in the reactors with NO$_2^-$ or NO$_3^-$ as the only nitrogen component in the wastewater. After the aeration commenced, dissolved N$_2$O concentration was recoded. No N$_2$O accumulation occurred with NO$_2^-$-N and NO$_3^-$-N being the only N component in the wastewater. However, when glucose (equal to 350 mg/L COD) was added in the reactors, the soluble N$_2$O concentration increased immediately and was stable after 5 minutes. When the N$_2$O concentration was stable, the average specific N$_2$O generation rates were calculated using Eq. 5.6 and the results were presented in Figure 6.8.

In the heterotrophic denitrification pathway, with NO$_2^-$-N as the nitrogen component, the specific N$_2$O generation rates were 1.73 µg N$_2$O / (g SS· min), 1.55 µg N$_2$O / (g SS· min) and 1.26 µg N$_2$O / (g SS· min), which were 40.9%, 44.8%, 39.9% higher than those with NO$_3^-$-N as the only nitrogen component, respectively. As the aeration rate increased, N$_2$O generation rates declined, because high aeration rate can inhi the oxidized-nitrogen denitrification process. This also indicates that N$_2$O generati

was more sensitive to NO$_2^-$-N concentrations in the aerobic granular sludge. And this phenomenon was related to the oxidized nitrogen denitrification process. Satoh *et al.* (2003) reported that nitrous oxide reductase which can catalyze N$_2$O denitrification, could be inhibited by high concentrations of NO$_2^-$-N.
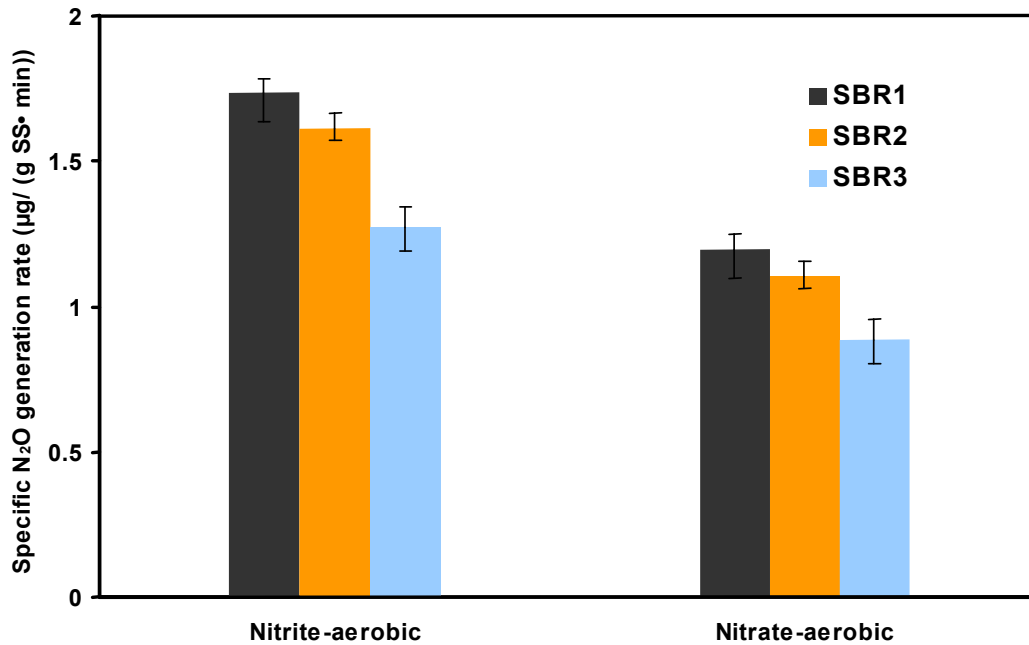
Figure 6.8 Specific N$_2$O generation rate with nitrite or nitrate being the only nitrogen component in aerobic conditions

The denitrification activity of the granular sludge biomass reducing NO$_2^-$ or NO$_3^-$ was measured using batch experiments. Washed aerobic granular sludge taken from aerobic granular sludge SBRs was added into 500 mL effective volume beakers. Synthetic wastewater made from tap water containing 210 mg/L COD (from glucose) and 30 mg/L NO$_2^-$-N or 30 mg/L NO$_3^-$-N was added in the beakers where anoxic conditions were maintained. Magnetic stirrers were used to keep the mixed liquor suspension. Liquid samples were taken at intervals to measure the change of NO$_2^-$-N or NO$_3^-$-N concentrations. The results show that the specific denitrification rate of the aerobic granular sludge with NO$_2^-$ as the only nitrogen component was 0.073 mg NO$_2^-$-N / (g SS· min), and was more than 50% faster than that measured with NO$_3^-$ as the only nitrogen component, 0.048 mg NO$_3^-$-N / (g SS· min). A faster NO$_2^-$-N reduction rate and the inhibition of the N$_2$O denitrification by NO$_2^-$-N resulted in

higher N_2O generation rates.

In order to study N_2O generation via NH_4^+-N oxidation in aerobic autotrophic nitrification, NH_4Cl was added to pervious batch test systems (measuring N_2O generation from NO_2^- or NO_3^-) to measure the total N_2O generation rate through NH_4^+-N plus NO_2^--N or NH_4^+-N plus NO_3^--N. Compared with the specific N_2O generation rates shown in Figure 6.8, the increased specific N_2O generation rates obtained in this batch experiment were due to NH_4^+-N oxidation. The average increased specific N_2O generation rates were similar, regardless of the different oxidized nitrogen substrates (NO_2^--N or NO_3^--N) or different aeration rates, and the value was 0.82 ± 0.02 μg N_2O / (g SS· min). The results show that N_2O generation via NH_4^+-N oxidation in aerobic granular sludge reactors was not sensitive to the aeration rate applied in the study. The obtained specific NH_4^+-N oxidation rate was 0.022 mg NH_4^+-N / (g SS· min), so, ~3.7% reduced NH_4^+-N was transferred to N_2O gas.

Under different COD: N ratios (1: 0.22, 1: 0.15 and 1: 0.11) and in the anoxic condition, the N_2O generation rate was also investigated using batch experiments. Washed aerobic granular sludge was added into 500 mL effective volume beakers. 350 mg/L COD with 77 mg/L, 52 mg/L and 38 mg/L NO_2^--N or NO_3^--N was added in the beakers when anoxic conditions were achieved, respectively. Magnetic stirrers were used to keep the mixed liquor suspension and the soluble N_2O concentration was recorded. Under the anaerobic conditions, the specific N_2O generation rate was 41 μg N_2O / (g SS· min), 38 μg N_2O / (g SS· min) and 10 μg N_2O / (g SS· min) with NO_2^--N as the nitrogen substrate, and 12 μg N_2O / (g SS· min), 8 μg N_2O / (g SS· min) and 5 μg N_2O / (g SS· min) with NO_3^--N as the substrate by the aerobic granular sludge at the COD: N ratio of 1: 0.22, 1: 0.15 and 1: 0.11, respectively. The results explain why the N_2O concentration increased suddenly at the start of one SBR cycle where the DO concentration was very low.

During a typical cycle, the specific N_2O generation rate increased in SBR1, and there was no obvious decrease in SBR2 (Figure 6.5) at the time between when COD was used-up and DO started to increase. Schalk-Otte *et al*. (2000) observed that as soon as carbon compounds were exhausted, the culture entered the starvation phase and PHB stored in the biomass turned into the growth substrate. Thus, in a typical cy

at COD: N ratio of 1: 0.15, the PHB content in the aerobic granular sludge was analyzed (Figure 6.9).
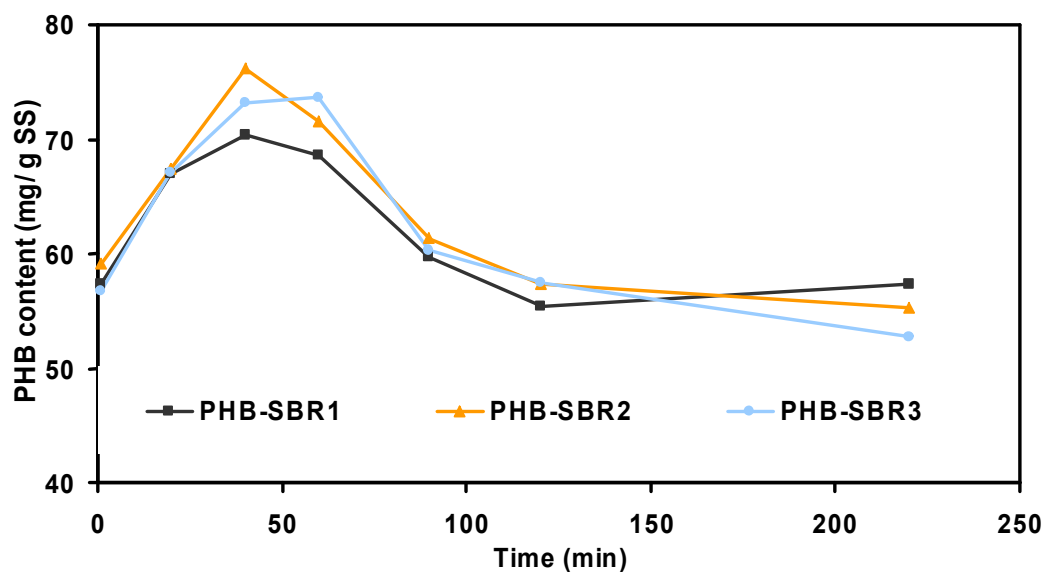
Figure 6.9 Profiles of poly-β-hydroxybutyrate (PHB) content in granular sludge in a typical cycle (■: PHB in SBR1; ▲: PHB in SBR2; ♦: PHB in SBR3)

From Figure 6.9, PHB accumulation occurred as soon as the commencement of aeration. When most of COD was utilized, the PHB content in the granular sludge rose to 70.4 mg/g SS, 76.2 mg/g SS and 73.2 mg/g SS in SBR1, SBR2 and SBR3, respectively; and it gradually decreased after 40 minutes. This indicates that PHB was utilized. Inadequate organic matter could lead to incomplete heterotrophic denitrification, and N$_2$O could generate continuously via denitrification (Itokawa *et al*. 2001). Before the PHB content decreased to the lowest level, the specific N$_2$O generation rate (Figure 6.5) was kept at ~2.0, 1.7 and 1.2 μg N$_2$O / (g SS• min) in SBR1, SBR2 and SBR3, respectively. This means that PHB was used for heterotrophic denitrification after most of the carbon source was used-up.

6.4 Summary

At a temperature of 14±4 °C, three identical laboratory-scale aerobic granular sludge SBRs were established to treat synthetic wastewater simulating a mixture of

separated pig manure digestate liquid and municipal wastewater at three different aeration rates (0.2 L/min, 0.6 L/min and 1.0 L/min corresponding to SBR1, SBR2 and SBR3, respectively) and three COD: N ratios (1: 0.22, 1: 0.15 and 1: 0.11).

The mean COD removals were more than 99% in three SBR under all conditions. Almost all of NH_4^+-N was removed under the COD: N ratio of 1: 0.15 and 1: 0.11. When the COD: N ratio was 1: 0.22, there was 53%, 42% and 28% of influent NH_4^+-N remaining in SBR1, SBR2 and SBR3, respectively. Under continuous aeration conditions, the highest total nitrogen removal efficiency was 36% at a COD: N rate of 1: 0.11 which confirmed that simultaneous nitrification and denitrification took place in aerobic granular sludge SBRs.

The measurement shows the proportions of N₂O emissions to the influent nitrogen loading rate in SBR1, SBR2 and SBR3 were 8.2%, 6.1% and 3.8% at the COD: N ratio of 1: 0.22; 7.0%, 5.1% and 3.5% at the COD: N ratio of 1: 0.15; and 4.4%, 2.9% and 2.2% at the COD: N ratio of 1: 0.11, respectively.

With NO_2^--N being the only nitrogen component in the liquid phase, specific N₂O generation rates were 1.73 µg N₂O / (g SS• min), 1.55 µg N₂O / (g SS• min) and 1.26 µg N₂O / (g SS• min) in three SBRs, which were 40.9%, 44.8% and 39.9% higher than those with NO_3^--N being the only nitrogen component, respectively. With NH_4^+-N being the nitrogen component, the average specific N₂O generation rate was 0.82±0.02 µg N₂O / (g SS• min).

After most of the carbon source was used-up, PHB can be used as the carbon source for heterotrophic denitrification, causing N₂O emissions under the aerobic conditions in the aerobic granular sludge SBRs.

Chapter Seven

Nutrient Removals in Upflow Biofilters Treating Separated Pig Manure Digestate Liquid

7.1 Introduction

Biofiltration is considered a valid technology for wastewater treatment due to its capability in maintaining a high biomass concentration in the reactors (Gebara, 1999). In this study, separated pig manure digestate liquid was treated with upflow biofilters.

Six laboratory-scale upflow biofilters were constructed in the Environmental Engineering Laboratories at NUI Galway to treat the separated digestate liquid at two loading rates. The biofilters were operated for 136 days, during which the removal efficiency of nutrients was studied. When biofiltration experiments finished, reactors were decommissioned in order to investigate the capacity of the suspended-growth activated sludge and biofilm biomass in nitrification and denitrification. The mechanisms of phosphorus removal in the biofilters were also researched.

7.2 Materials and methods

7.2.1 Characteristics of the separated pig manure digestate liquid

In this study, the separated pig manure digestate liquid was taken from a mesophilic anaerobic digester at a pig farm in Co. Kerry, Ireland in September, 2010. After collection, it was stored in a refrigerator at 4°C and was used within 5 months. The separated digestate liquid contained 7670 ± 370 mg COD/L, 720 ± 82 mg NH_4^+-N /L, 814 ± 52 mg TN/L, 213 ± 13 mg TP/L and 1.1 ± 0.3 g SS /L, with pH of 8.05 ± 0.10. The mean BOD_5 concentration was 2550 ± 50 mg/L so the BOD_5 to COD ratio was 0.?? The wastewater had a COD to TN ratio of 9.4, which was more suitable for nitrog

removal compared with 2.9 in the previous separated pig manure digestate liquid treated in Chapter 3 and Chapter 4. This shows that the fluctuating nature of the pig manure digestate due to the variation of the characteristics of feedstock digested in the anaerobic digester.

7.2.2 Laboratory-scale biofilters
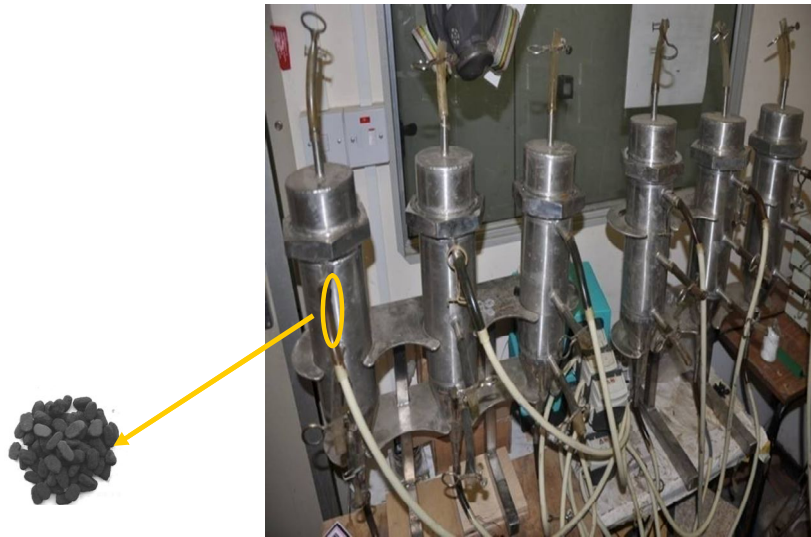
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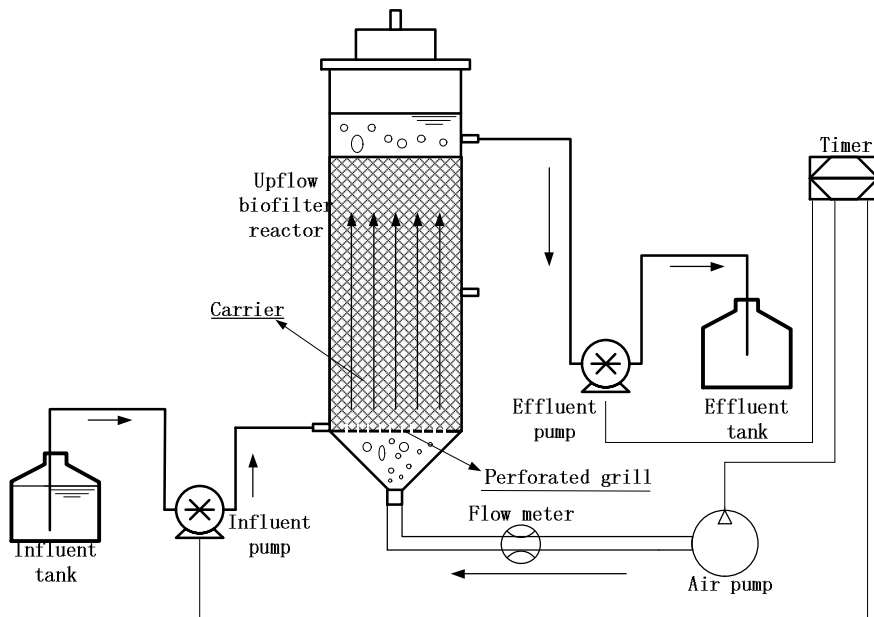
(b)

Figure 7.1 Photograph (a) and schematic diagram (b) of the upflow biofilter systems

Six identical laboratory-scale column-type stainless steel reactors were constructed to form hybrid (co-existence of attached-growth biofilm and suspended-growth activated sludge) biofilters (Figure 7.1). The reactors consisted of a lid, a main reactor zone and a funnel bottom made from stainless steel, and had an effective volume of 2 litres with an inner diameter of 100 mm and a height of 300 mm. 1.2 litres activated sludge taken from IASBRs as described in Chapter 4 was seeded into biofilters with an initial biomass of 4.8 g and VSS/ SS ratio of 91%. Then clay carriers were added into each reactor until the liquid top surface reached the 2 litres level in the biofilters, so the volume of carriers was equal to 40% of the effective biofilter volume. The amount of clay carriers added in each biofilter was 2200 g. The air was supplied using air pumps through stainless steel mesh installed at the bottom of the reactors, which was used to maintain the media particles in the reactors, and the air flow rate was controlled by air flow meters. Peristaltic pumps (MasterFlex L/S) were used to feed the wastewater into the reactors and withdraw effluent. The influent was fed from the bottom of the reactors (Figure 7.1). The operation of the biofilters was controlled with programmable timers (Samson Electric Wire, Germany). No sludge was intended to be discharged from the biofilters during the whole operation period.

7.2.3 Operation of the upflow biofilters

The six laboratory-scale biofilter reactors were operated at ambient temperature. One reactor malfunctioned after 5 days of commissioning the experiment, so only five biofilter reactors were continuously operated (R1, R2, R3, R4 and R5).

During the 2-minute fill phase, 100 ml and 60 mL of wastewater was pumped into R1, R2 and R3 (high loading rate biofilters) and R4 and R5 (low loading rate biofilters), respectively. The calculated loading rates were 1.15 kg COD/ ($m^3 \cdot$ d) and 0.12 kg TN/ ($m^3 \cdot$ d) in high loading rate biofilters; 0.69 kg COD/ ($m^3 \cdot$ d) and 0.07 kg TN/ ($m^3 \cdot$ d) in low loading rate biofilters based on the 2 litre effective biofilter capacity. Biofilters were operated as sequencing batch reactors with a cycle duration of 8 hours. In a cycle, after 120 minutes non-aeration, the reactors were aerated for 350 minutes with an aeration rate of 0.4 L air/min. The settle phase lasted for ² minutes, followed by the effluent withdrawal in last 5 minutes of the cycle.

7.2.4 Batch experiments

Batch experiments were carried out to examine the nitrification rate, the denitrification rate, and adsorption of phosphorus by biomass in the biofilters. After 136 days' operation, suspended-growth sludge and biofilm carriers were taken out of the reactors. According to the location inside the biofilters, the biofilm carriers were grouped into three layers: top layer, middle layer and bottom layer. Suspended-growth sludge and three layers of carriers were then washed twice with tap water.

In the nitrification rate and denitrification rate experiment, washed activated sludge and carriers were added into 0.5 L beakers with an effective volume of 400 mL separately. For the NH_4^+-N and NO_2^--N nitrification activity experiments, NH_4Cl or $NaNO_2$ used to make solutions with NH_4^+-N or NO_2^--N concentrations of 40mg/L, respectively. Air diffusers were fixed at the bottom of the beakers with an aeration rate of 0.4 L air/min. After the experiment commenced, liquid samples were taken from the batch reactors at intervals for the measurement of NH_4^+-N and NO_2^--N concentrations to determine the NH_4^+-N and NO_2^--N nitrification activity, receptively. For the NO_3^--N denitrification activity experiment, glucose and KNO_3 was added into beakers to achieve 375 mg/L COD and 40 mg NO_3^--N /L. Anaerobic conditions in the batch experiment were achieved by stripping DO out of the beakers for 3 minutes using N_2. The beakers were then placed on a shaker (SSL2, Stuart, UK) with a rotation speed of 120 rpm. After the experiment commenced, liquid samples were taken at intervals for measurement of NO_3^--N concentrations. When the nitrification and denitrification batch experiment finished, the amount of the biomass in the beakers was measured.

Phosphorus adsorption by the clay carriers was also evaluated. 30 g, 50 g, 70 g, 90 g and 110 g of fresh carriers were added into the 250 mL beakers to study the carriers' adsorption capacity. 100 mL K_2HPO_4 solution was added into each beaker to achieve an initial PO_4^{3-}-P concentration of 120 P mg/ L, and then pH was adjusted to be close to that in the biofilters (pH= 8.1). The beakers were placed on the shaker with a rotation speed of 180 rpm. The samples were taken from the beakers after 1 day to measure the final PO_4^{3-}-P concentration in the liquid phase. 110 g carriers with biofilm growth taken from biofilters were also investigated using the above procedure.

7.2.5 Water quality analysis

The methods used for water samples test are given in Appendix B.

7.2.6 Extracellular polymeric substance extraction

Extracellular polymeric substance (EPS) in the suspended sludge was extracted using the following procedure (Zhang *et al.*, 1999). After the biofilters were decommissioned, suspended growth sludge was taken out and centrifuged at 4000 rpm for 15 min. The solids obtained were then mixed with 1×PBS buffer solution. The EPS was then extracted from the solids by placing the mixture in an incubator (HIM20, Boekel, England) at 80 °C for 1 hour. As a blank, distilled water without sludge was extracted using the procedure mentioned above. After cooling to ambient temperature, the mixed liquor was centrifuged at 14,000 rpm for 20 min and filtered with 0.45 μm pore size GC/F filter papers (Whatman, UK). The filtrate contained EPS extracted from the sludge samples. The total phosphorus level in the filtrate was measured. After subtracting the blank value, the phosphorus content in the extracted EPS was obtained.

7.3 Results and discussion

7.3.1 Overall performance of upflow biofilters in removal of organic matter and nitrogen

The five upflow biofilters were operated successfully for 136 days. Because the seed sludge was taken from IASBRs which had been run for more than 5 months to treat pig digestate liquid, the COD removal reached steady state as soon as operation of the biofilters commenced. The profiles of effluent NH_4^+-N, NO_2^--N and NO_3^--N took 15 - 20 days to become stable.

Statistics analysis using a T-test for two groups (Johnson and Bhattacharyya, 2009)-$t_{OBS,}$ was calculated to compare parameters analyzed for two individual reactors. As for TN, the results were $t_{OBS, R1-R2}= 2.54$, $t_{OBS, R1-R3}= 2.43$, and $t_{OBS, R2-R3}= 2.52$, $t_{C R4-R5}=0.67$; As for TP, the results were $t_{OBS, R1-R2}= 0.01$, $t_{OBS, R1-R3}=0.37$, $t_{OBS, R2-F}$

0.26, and $t_{OBS, R4-R5}= 1.66$. All of the t_{OBS} values were less than $t_{\infty, 0.01}= 2.58$ at the level of significance of 0.01. This means that there is no statistical evidence to conclude that there were differences among R1, R2 and R3 which operated at the high loading rate and between R4 to R5 which operated at the low loading rate. Thus, the mean profiles calculated for R1-R3 and R4-R5 can describe the performance of the biofilters operating at the two loading rates, respectively and the results are given in Table 7.1. It can be seen that effluent COD and TN concentrations were influenced by the loading rates.

Table 7.1 Average performance of the upflow biofilters

Nutrients	High loading rate	Low loading rate	t_{OBS}
Effluent COD (mg/L)	614±28.8	563±18.6	5.01
COD removal rate	92.0%	92.7%	
Effluent TN (mg/L)	129±17.9	97.7±5.1	15.16
TN removal rate	84.2%	88.0%	
Effluent TP (mg/L)	25.5±1.3	25.3±2.5	1.26
TP removal rate	88.0%	88.1%	

The profiles of NH_4^+-N, NO_2^--N, and NO_3^--N in effluent at the two loading rates are shown in Figure 7.2. The data from Day 71 to Day 74 was not obtained because the reactors were moved to a new environmental engineering laboratory in the New Engineering Building at NUI Galway. After the experiment recommenced, no evident upsets were observed in any of the upflow biofilters. This shows that the biofilters recovered quickly after non-operation for a short period.

Because the seed sludge was taken from IASBRs, the ability of partial nitrification was maintained after it was seeded to upflow biofilters and the NO_2^--N concentration in the effluent sharply increased. The ratio of the aeration rate (0.4 L/ min) to the volume (2 L) of upflow biofilters was 0.2 on average and it was much higher than the ratio of 0.08 in the IASBRs (Chapter 4). Therefore, as Figure 7.3 shows, the DO concentration in the bulk fluid in biofilters increased quickly to 3 - 4 mg/L after the aeration commenced. According to Figure 4.5, the highest DO concentration in IASBRs was only 4.0, but the DO level reached the saturation level after 3 hours aeration in the biofilters. Such a high DO concentration did not inhibit the growth of

NOB (Brockmann and Morgenroth, 2010). After 15 - 25 days' operation, the effluent NO_2^--N concentration was decreased to lower than 5 mg/L.
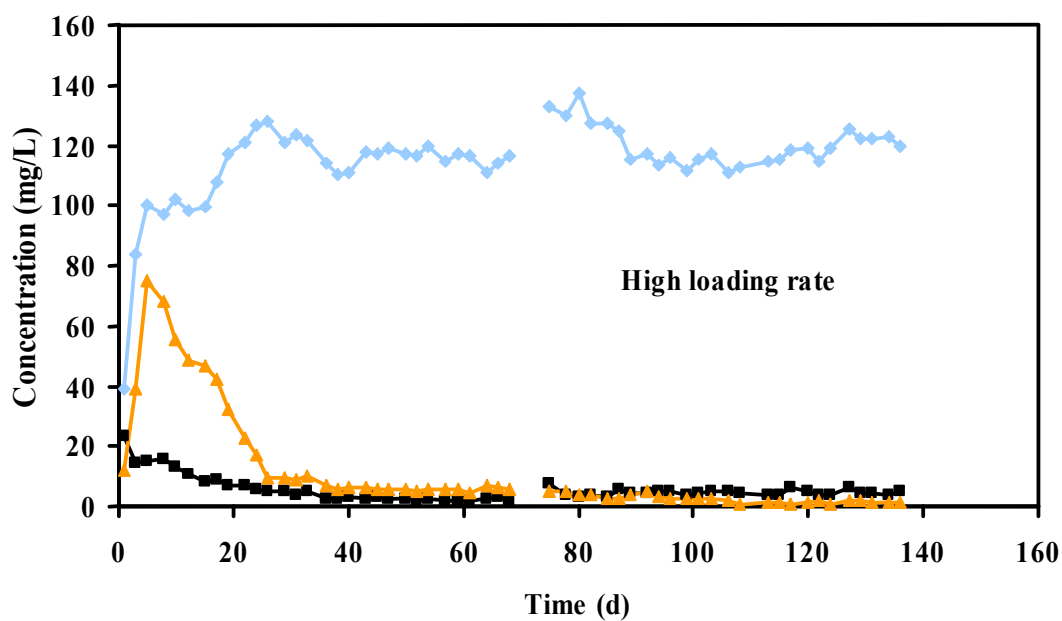
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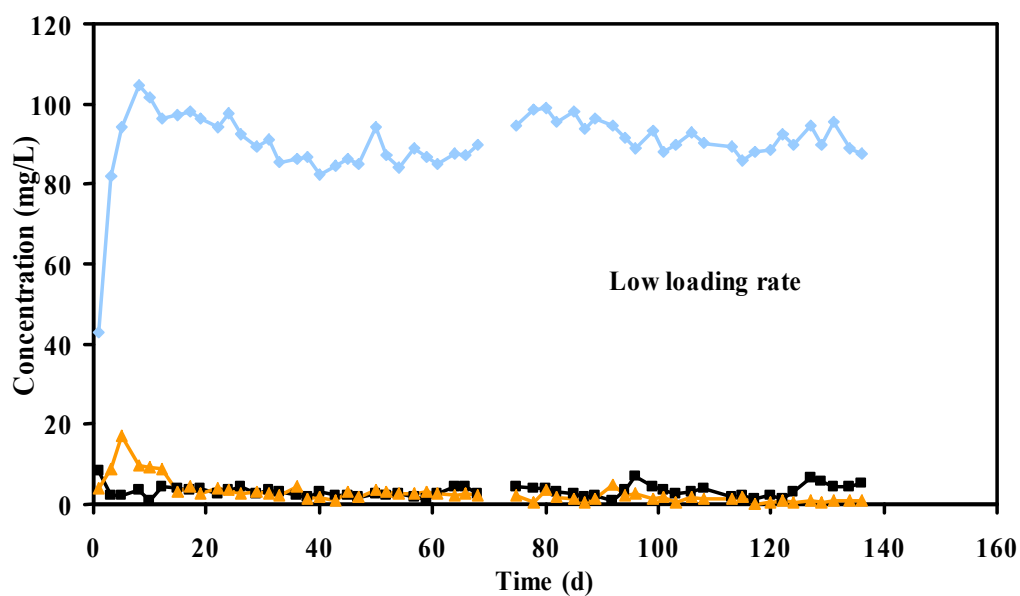
(b)

Figure 7.2 Mean nitrogen performance of the upflow biofilters (■:NH_4^+-N; ▲: NO N; ♦: NO_3^--N)

Meanwhile, it was found that in the high loading rate biofilters (R1, R2 and R3) during the start-up period, the average effluent NO_2^--N concentrations were up to 68 mg/L and was much higher than those in the low loading rate reactors (R4 and R5). The reason for this was that after wastewater was fed to the reactors, there was 4.74 mg free ammonia (FA) /L in high loading rate reactors, whereas it was only 3.02 mg FA/L in low loading rate reactors as calculated using the Eq. 2.7. The higher FA concentration in R1, R2 and R3 inhibited the NOB activity more strongly than in R4 and R5. Effluent NO_2^--N dropped fast in the low loading rate biofilters. With the recovery of the NOB activity, the NO_3^--N concentration increased quickly and levelled off. On average in the steady state, there were 119.1 ± 14.5 mg NO_3^--N /L and 91.7 ± 5.2 mg NO_3^--N /L in the effluent of high and low loading biofilters, respectively. Almost all NH_4^+-N was removed in upflow biofilters.
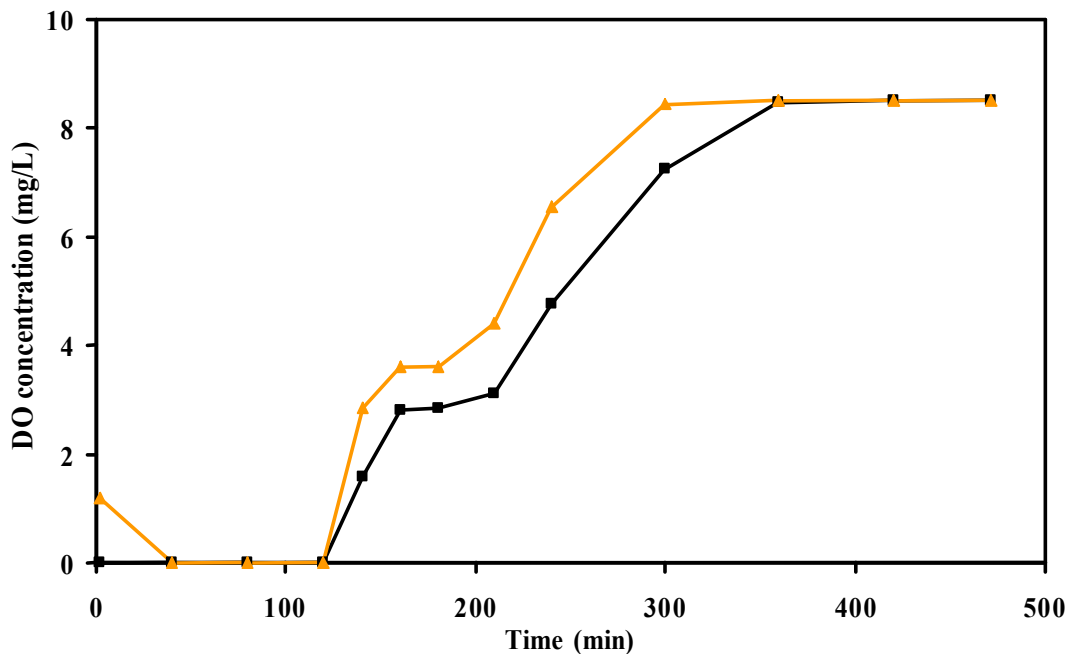
Figure 7.3 Profile of mean DO concentration in a typical cycle (■: DO in high loading rate biofilters; ▲: DO in low loading rate biofilters)

7.3.2 Phase study and nitrogen profiles of upflow biofilters

Phase studies were carried out in upflow biofilters on Day 136. During these studies, liquid samples were taken from each biofilter at intervals during a whole operation cycle, centrifuged and analysed for measurement of NH_4^+-N, NO_2^--N, NO_3^--N

(Figure 7.4) and COD concentrations. It was found from phase studies that all the biodegradable COD was removed within the first 2 hour of aeration. And from Figure 7.4, the nitrogen removal can be divided into three phases: anoxic phase, rapid aerobic NH_4^+-N oxidation phase, and extended aeration phase.
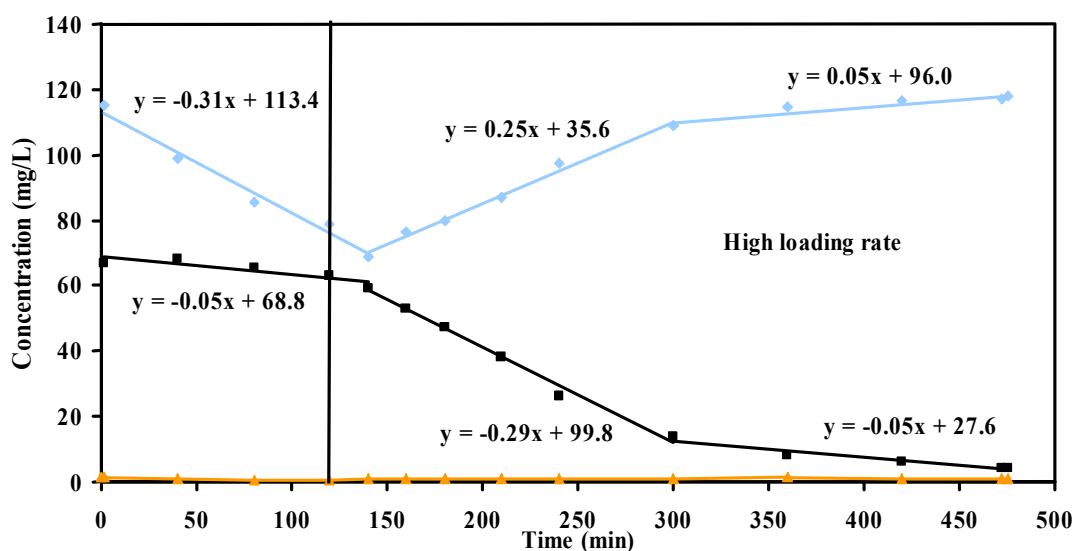
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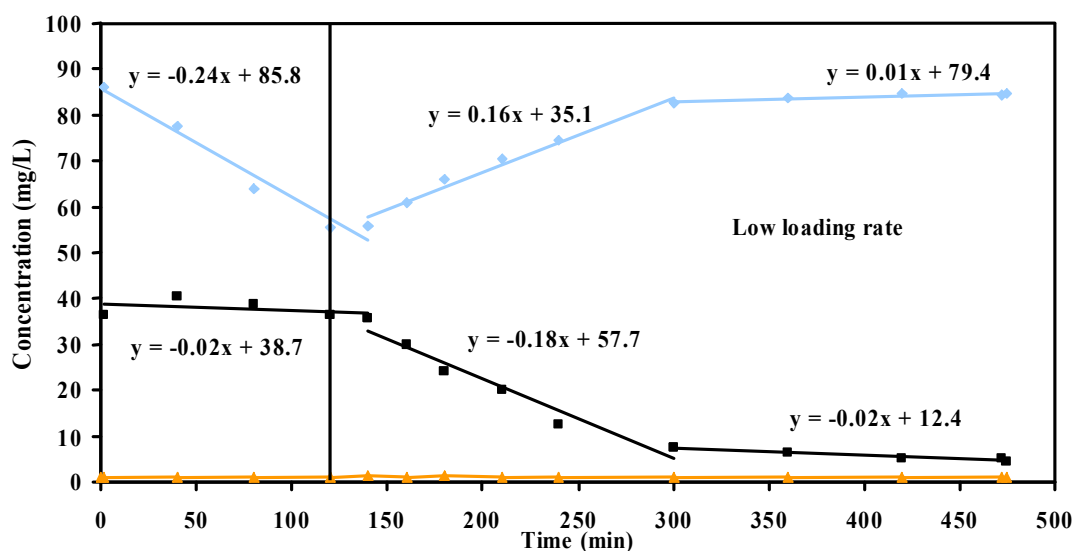
(b)

Figure 7.4 Profiles of NH_4^+-N, NO_2^--N and NO_3^--N concentrations in a typical cy (■:NH_4^+-N; ▲: NO_2^--N; ♦: NO_3^--N)

During the anoxic phase, the NH_4^+-N concentration decreased slightly. The kinetics of NH_4^+-N reduction in this phase can be described as follows, respectively:

S_{NH4}= -0.05t + 68.8   (R^2= 0.79)   (High loading rate)   (Eq. 7.1)

S_{NH4}= -0.02t + 38.7   (R^2= 0.18)   (Low loading rate)   (Eq. 7.2)

where, S_{NH4} is the NH_4^+-N concentration in the bulk liquid phase, mg NH_4^+-N /L; t, time, min.

Normally, some of the organic nitrogen contained in the influent wastewater can be converted into NH_4^+-N through ammonification, causing the NH_4^+-N concentration to increase. In the biofilters, the adsorption of NH_4^+ onto the carriers was a possible reason for a reduction in NH_4^+-N. In addition, the anabolism of heterotrophic micro-organisms consumed NH_4^+-N in the wastewater for cell synthesis.

Within the first 2 hours anoxic phase, NO_3^--N was reduced in the reactors, indicating denitrification occurred. The reduction kinetics of NO_3^--N via denitrification in this period can be described as follows:

S_{NO3}= -0.31t + 113.4   (R^2= 0.98)   (High loading rate)   (Eq. 7.3)

S_{NO3}= -0.24t + 85.8   (R^2= 0.97)   (Low loading rate)   (Eq. 7.4)

where, S_{NO3}, the NO_3^--N concentration in the bulk fluid in the anaerobic phase, mg NO_3^--N /L.

From Eq. 7.3 and Eq. 7.4, the NO_3^--N changing rate in high loading rate reactors was 0.31 mg/ (L· min) and 32% higher than that in R4 - R5, 0.24 mg/ (L· min). The reasons could be: (1) the higher NO_3^--N concentrations in high loading rate reactors than low loading rate reactors improved denitrification process; and (2) higher biomass concentrations (the total biomass was 12.2 g in high loading rate reactors, compared with 10.1 g in low loading rate reactors). Mean NO_3^--N concentrations were 74.5 mg/L and 55.5 mg/L at the end of the anaerobic phase in the higher and low loading rate reactors, respectively. After the aeration phase started, the NO_3^--N concentration continued to decrease slightly in the low bulk fluid DO concentration

period. The DO concentration in biofilm was much lower than the bulk fluid, where denitrification can occur.

Within 3 hours after the aeration commenced, NH_4^+-N was nitrified to NO_3^--N in the rapid aerobic NH_4^+-N oxidation phase. The nitrification kinetics of NH_4^+-N can be described as follows:

$$S_{NH_4} = -0.29t + 99.8 \quad (R^2 = 0.99) \qquad \text{(High loading rate)} \qquad \text{(Eq. 7.5)}$$

$$S_{NH_4} = -0.18t + 57.7 \quad (R^2 = 0.95) \qquad \text{(Low loading rate)} \qquad \text{(Eq. 7.6)}$$

Eq. 7.5 and Eq. 7.6 show that in the rapid aerobic NH_4^+-N oxidation phase, NH_4^+-N nitrification rates were 0.29 mg NH_4^+-N /(L· min) and 0.18 mg NH_4^+-N /(L· min) in the two loading rate biofilters, respectively. Eq. 7.7 and Eq. 7.8 describes the increase of NO_3^--N in the aerobic NH_4^+-N oxidation phase. It was found that, the NO_3^--N increase rate (0.25 mg NO_3^--N / (L· min) and 0.16 mg NO_3^--N / (L· min)) were less than corresponding NH_4^+-N reduction rates, respectively. The possible reason was that simultaneous nitrification and denitrification occurred in the biofilters (Puznava *et al.*, 2001).

$$S_{NO_3} = -0.25t + 35.6 \quad (R^2 = 0.99) \qquad \text{(High loading rate)} \qquad \text{(Eq. 7.7)}$$

$$S_{NO_3} = -0.16t + 35.1 \quad (R^2 = 0.98) \qquad \text{(Low loading rate)} \qquad \text{(Eq. 7.8)}$$

When the NH_4^+-N concentration in the reactors was lower than 10 - 15 mg/L, the upflow biofilters entered an extended aeration phase when the NH_4^+-N oxidation occurred slowly. The nitrification kinetics of NH_4^+-N in this phase can be described as follows:

$$S_{NH_4} = -0.05t + 27.6 \quad (R^2 = 0.93) \qquad \text{(High loading rate)} \qquad \text{(Eq. 7.9)}$$

$$S_{NH_4} = -0.02t + 12.4 \quad (R^2 = 0.95) \qquad \text{(Low loading rate)} \qquad \text{(Eq. 7.10)}$$

In the extended aeration phase, the NH_4^+-N oxidation rates were only 0.05 mg NH_4^+-N /(L· min) and 0.02 mg NH_4^+-N /(L· min) in the high and low loading rate biofilters, respectively. The increased rates of NO_3^--N were very close to the NH_4^+-N oxidati rates (Eq. 7.11 and Eq. 7.12). In all biofilters, NH_4^+-N concentrations at the end

the aerobic phase were less than 5 mg/L.

$$S_{NO_3} = -0.05t + 90.6 \quad (R^2 = 0.90) \qquad \text{(High loading rate)} \qquad \text{(Eq. 7.11)}$$

$$S_{NO_3} = -0.01t + 79.5 \quad (R^2 = 0.83) \qquad \text{(Low loading rate)} \qquad \text{(Eq. 7.12)}$$

In the upflow biofilters, two types of biomass were contained. One was suspended growth sludge trapped between carrier particles in the reactors, and the other was biofilm attached on the carriers. After Day 136, two group reactors were decommissioned to measure the two types of biomass in the reactors. The mean total suspended growth sludge was weighed 7.3 g and 5.6 g, and the mass of biofilm on the carriers was 4.8 g and 4.5 g in the high and low loading rate biofilters, respectively. The weight of biofilm biomass was similar at the two loading rates.

In order to evaluate the capacity of nitrification and denitrification of the two kinds of biomass, batch experiments were carried out. The specific NH_4^+-N nitrification rates of the suspended-growth sludge and of the three layers of biofilm (top layer, middle layer and bottom layer) were calculated as 0.025 mg NH_4^+-N /(g VSS· min), 0.049 mg NH_4^+-N /(g VSS· min), 0.059 mg NH_4^+-N /(g VSS· min) and 0.059 mg NH_4^+-N /(g VSS· min) in high loading rate reactors, and 0.027 mg NH_4^+-N /(g VSS· min), 0.050 mg NH_4^+-N /(g VSS· min), 0.052 mg NH_4^+-N /(g VSS· min) and 0.058 mg NH_4^+-N /(g VSS· min) in low loading rate reactors. The specific nitrification rate tended to be higher at the bottom layer than the top layer and middle layer. The reason for this was that wastewater was fed to the biofilters from the bottom and thus the bottom layer received the higher nitrogen loading rates.

The NO_3^--N denitrification rates of the suspended growth sludge and three layer biofilm were 0.040 mg NO_3^--N /(g VSS· min), 0.066 mg NO_3^--N /(g VSS· min), 0.067 mg NO_3^--N /(g VSS· min) and 0.070 mg NO_3^--N /(g VSS· min) in high loading rate reactors and 0.039 mg NO_3^--N /(g VSS· min), 0.062 mg NO_3^--N /(g VSS· min), 0.065 mg NO_3^--N /(g VSS· min) and 0.064 mg NO_3^--N /(g VSS· min) in low loading rate reactors. Similar to the NH_4^+-N nitrification activity (120% and 190% more than suspended sludge), the attached biofilm had a much higher specific NO_3^--N denitrification activity (63% and 69% more than that of suspended growth biomass), respectively. The reason for this might be that the suspend-growth biomass consisted

of the low activity microorganism which was sloughed from the attached biofilm.

7.3.3 Phosphorus removal

The performance of the laboratory-scale upflow biofilters for phosphorus removal from the separated pig manure digestate liquid was monitored. The average TP removals in the steady state were up to 88.0% in high loading rate reactors and 88.1% in the low loading rate reactors (Table 7.1). Phosphorus removal mechanisms included: biological phosphorus removal and chemical phosphorus removal.

As for biological phosphorus removal, C: P ratios of 90 - 150: 1 is considered as the optimum microorganism growth nutrient condition (Gray, 2004). And enhanced biological phosphorus removal (EBPR) is main pathway to remove phosphorus (Henze *et al.*, 2002). In the present study, there was no sludge discharged during the whole operational period, but, on average 0.3 g/L - 0.5 g/L sludge remained in the effluent, contributing to 47 mg/L - 81 mg/L phosphorus in the effluent each time.

As for the chemical phosphorus removal, the metal ions existing in the wastewater could react with soluble PO_4^{3-} to form precipitates (Parson and Berry, 2004). In this study, the sludge constituent in the biofilters was digested and TP concentration was measured. The phosphorus contents in biomass were up to 15.7% and 16.3% on average in the high and low loading reactors, respectively. The phosphorus content in biomass is found to be 2% - 8% in EBPR sludge (Panswad *et al.*, 2007; Zhou *et al.*, 2009; Zhang and Huang, 2011). The phosphorus content in EPS of biomass was accounted for 4.6% and 4.3% of the TP content in the biomass taken from the high and low loading reactors, respectively. This indicates that the remaining 3% - 9% phosphorus content in biomass would be due to phosphorus precipitates or phosphate adsorbed onto the clay carriers during the entire operational periods.

The adsorption capacity of the carriers can not be ignored. In order to investigate the adsorption of phosphorus of fresh carrier particles, batch experiments were conducted. The adsorption of phosphorus onto the adsorbent can be calculated in accordance with the equation:

$$q_e = \frac{V(C_0 - C_e)}{m}$$

(Eq. 7.1

where, q_e is adsorption amount of PO_4^{3-}-P, mg P/g adsorbent; V is the bulk liquid volume in the beaker, L; C_0 is the initial PO_4^{3-}-P concentration, mg/L; C_e is the equilibrium concentration of PO_4^{3-}-P, mg/L.

The relationship between q_e and C_e is given in Figure 7.5. The adsorption isotherm can be expressed with a Langmuir Equation as follows (Febrianto $et\ al.$, 2009):

$$q_e = q_{max} \frac{C_e}{K + C_e}$$

(Eq. 7.14)

where, q_{max} is the maximal adsorption amount of phosphorus onto the adsorbent, mg P/g adsorbent; K is the Langmuir adsorption constant, mg/L.
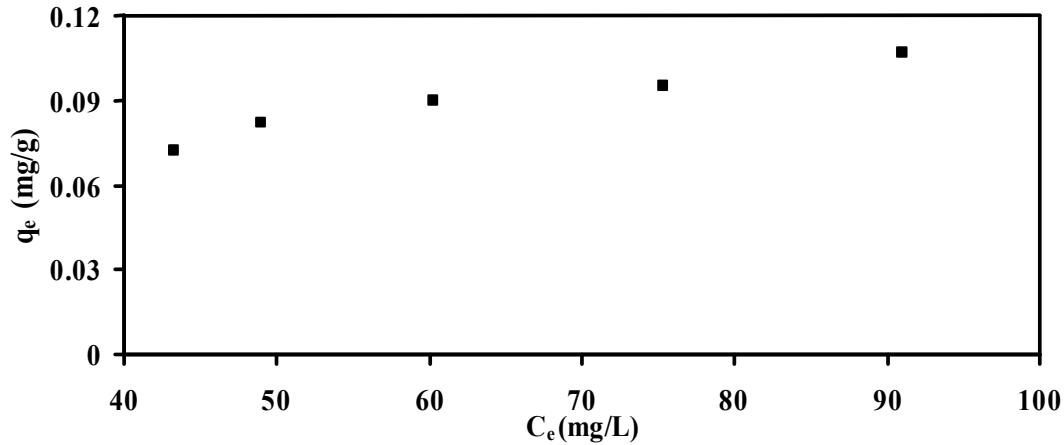
Figure 7.5 Adsorption isotherm of PO_4^{3-}-P (C_0=123 mg PO_4^{3-}-P /L)

Eq. 7.14 can be converted to:

$$\frac{1}{q_e} = \frac{K}{q_{max}} C_e \quad \frac{1}{q_{max}}$$

(Eq. 7.14)

Through the intercept of Eq. 7.14, q_{max} of the carriers was calculated as 0.173 mg P/g carrier (R^2= 0.97, P<0.05). It proved that in the upflow biofilters, the carriers were able to adsorb phosphorus and to remove it from wastewater.

After 136 days' operation, the carriers were taken from high and low loading rate reactors and the capacity for phosphorus removal was also investigated. At the initial

PO_4^{3-}-P concentration of 124 mg/L, after 1 day's adsorption, 43.5±0.3% of TP was removed, with a removal capacity of 0.095 mg P /g carrier onto both of two loading rate carriers. The results indicated that the carriers with biofilm grown were still available for phosphorus adsorption. However, it is hard to differentiate phosphorus adsorption, chemical precipitation and EBPR from the hybrid biofilter systems.

7.4 Summary

Separated pig manure digestate liquid with a COD to TN ratio of 9.4 was treated over 136 day in upflow biofilters at two loading rates of 0.12 kg TN/ (m^3· d) and 0.07 kg TN/ (m^3·d).

The total nitrogen removal efficiency was 84.2% and 88.0%, respectively, and NH_4^+-N and biodegradable COD was completely removed at the two loading rates in upflow biofilters.

The phase studies show that in the anoxic phase, the NO_3^--N reduction rates were 0.304 mg NO_3^--N / (L· min) and 0.237 mg NO_3^--N / (L· min). In rapid aerobic NH_4^+-N oxidation phase, NH_4^+-N nitrification rates were 0.293 mg NH_4^+-N / (L· min) and 0.176 mg NH_4^+-N / (L· min) at the high loading rate and low loading rate biofilters, respectively. Attached biofilm had a much higher nitrification and denitrification activity than that of suspended growth biomass in biofilters.

Phosphorus removal rates were up to 88% in the biofilters, respectively. Phosphorus removal might be due to biological P removal, chemical precipitation and adsorption.

Chapter Eight

Chemical Treatment of Separated Pig Manure Digestate Liquid

8.1 Introduction

Chemical method is considered as an effective treatment for high nutrient wastewater. In this chapter, two chemical technologies, coagulation and struvite precipitation, were applied to treat separated pig manure digestate liquid.

The separated digestate liquid was treated in a series of coagulation experiments. The factors, including coagulant dosage, polyacrylamide type and dosage, and pH value, were examined with an orthogonal experimental design.

In the struvite precipitation experiment, two factors, which influenced nutrient removal, pH and Mg: NH_4^+-N: PO_4^{3-}-P ratio, were investigated.

8.2 Pig manure digestate treatment using coagulation

8.2.1 Coagulation experimental design

The separated digestate liquid contained a large amount of non-settable solids. This would affect the performance of the IASBR reactors and other bioreactors. Therefore, it was proposed to use coagulants to remove solids. COD and nutrients would also be reduced.

Coagulation is an effective method to remove suspended solids from wastewater by adding coagulants (such as metallic ion and cationic PAM) into the wastewater in addition with proper stirring and settlement conditions. In order to examine the efficiency of coagulation on the separated digestate liquid treatment, Al_2 $(SO_4)_3$ a

PAM were adopted as coagulant. An orthogonal array experiment L_{18} (2^1*3^7) was designed (1 factor - 2 levels with 7 factors - 3 levels in 18 array experiments) as Table 8.1 (John, 1998). Among the orthogonal array experiments, 4 factors were regarded as error bar; other 4 factors were investigated: two kinds of PAM (anion LT25 PAM and cation FLO410 PAM), three pH values, three $Al_2(SO_4)_3$ dosages and three PAM dosages.

Table 8.1 Orthogonal array experimental design of the coagulation experiment (e1-e4: errors control)

Experiment No.	PAM type	$Al_2(SO_4)_3$ dosage	pH	PAM dosage	e1	e2	e3	e4
1	LT25	0.5 g/L	6.5	5 mg/L	1	1	1	1
2	LT25	0.5 g/L	5.5	10 mg/L	2	2	2	2
3	LT25	0.5 g/L	4.5	15mg/L	3	3	3	3
4	LT25	1 g/L	6.5	5 mg/L	2	2	3	3
5	LT25	1 g/L	5.5	10 mg/L	3	3	1	1
6	LT25	1 g/L	4.5	15mg/L	1	1	2	2
7	LT25	1.5 g/L	6.5	10 mg/L	1	3	2	3
8	LT25	1.5 g/L	5.5	15mg/L	2	1	3	1
9	LT25	1.5 g/L	4.5	5 mg/L	3	2	1	2
10	FLO410	0.5 g/L	6.5	15mg/L	3	2	2	1
11	FLO410	0.5 g/L	5.5	5 mg/L	1	3	3	2
12	FLO410	0.5 g/L	4.5	10 mg/L	2	1	1	3
13	FLO410	1 g/L	6.5	10 mg/L	3	1	3	2
14	FLO410	1 g/L	5.5	15mg/L	1	2	1	3
15	FLO410	1 g/L	4.5	5 mg/L	2	3	2	1
16	FLO410	1.5 g/L	6.5	15mg/L	2	3	1	2
17	FLO410	1.5 g/L	5.5	5 mg/L	3	1	2	3
18	FLO410	1.5 g/L	4.5	10 mg/L	1	2	3	1

The coagulation experiments were conducted using the standard jar-test. The separated pig manure digestate liquid used in this study had the same characteristics as the wastewater used in Chapter 4 (Table 4.1). The separated digestate liquid was added into beakers with an effective volume of 1 L. The digestate was kept in

suspension with calculated dosages of $Al_2(SO_4)_3$ and PAM as designed in Table 8.1 at a stirring speed of 120 rpm for 5 minutes. When mixed completely, the pH value in the bulk fluid was adjusted to the desired value. After 5 minutes of pH adjustment, the speed decreased to 30 rpm for another 25 minutes' mixing to promote the formation of flocs. Finally, after the mixing stopped, the bulk fluid in the beakers was settled for 20 min, and supernatant samples were taken from the beakers for the measurement of COD, turbidity and TN concentrations. All procedures were conducted at room temperature.

8.2.2 Factors influencing the coagulation efficiency

Table 8.2 Results of the coagulation experiment

Experiment No.	COD removal efficiency (%)	Turbidity removal efficiency (%)	TN removal efficiency (%)
1	16.7	29.0	2.0
2	70.4	57.8	25.9
3	76.8	89.8	31.5
4	23.7	42.5	4.0
5	83.7	93.7	36.2
6	77.5	92.0	31.1
7	62.9	81.3	26.5
8	86.0	88.2	39.2
9	76.8	90.3	36.2
10	7.1	13.6	1.4
11	23.4	44.9	20.4
12	39.7	73.6	24.4
13	33.2	63.0	24.4
14	55.6	78.9	22.6
15	25.8	52.4	22.8
16	26.1	46.1	6.3
17	21.4	37.7	3.4
18	51.3	82.1	26.9

The results of the orthogonal array experiments are shown in Table 8.2. More th

75% of COD and 85% of turbidity removals were achieved in No. 3, No. 5, No. 6, No. 8 and No. 9 experiments. The TN removal efficiency did not exceed 39.2% in any experiments. Therefore, most of the nitrogen which was soluble NH_4^+-N in this study was not removed efficiently via coagulation.

All of the four factors (PAM type, pH value, $Al_2(SO_4)_3$ dosage and PAM dosage) influenced the efficiency of coagulation. F-analysis which states statistical significance of each factor was calculated according to orthogonal experiment results using the following equations (John, 1998):

$$S_{i,j,p} = \sum_{k=1}^{k_j} X_{i,k,p} \tag{Eq. 8.1}$$

$$M_p = \frac{1}{i_j \, k_j} \sum_{i=1}^{i_j} S_{i,j,p} \tag{Eq. 8.2}$$

$$\sigma_{j,p} = k_j \sum_{i=1}^{i_j} (\frac{S_{i,j,p}}{k_j} - M_p)^2 \tag{Eq. 8.3}$$

$$V_{j,p} = \frac{\sigma_{j,p}}{i_j - 1} \tag{Eq. 8.4}$$

$$V_{e,p} = \sum_{n=1}^{n_e} \frac{\sigma_{e,p,n}}{i_j - 1} \tag{Eq. 8.5}$$

$$F_{j,p} = \frac{V_{j,p}}{V_{e,p}} \tag{Eq. 8.6}$$

where, i is level of orthogonal experiment; j is factor of orthogonal experiment; k is code of result in a level; p is wastewater quality in terms of COD, turbidity and TN; k_j is number of k in a level of Factor j; i_j is number of i in Factor j; e is error of the orthogonal experiment; n_e is number of e; X is result of the orthogonal experiment; S is sum; M is mean of value; σ is sum of squares of deviations; V is variation.
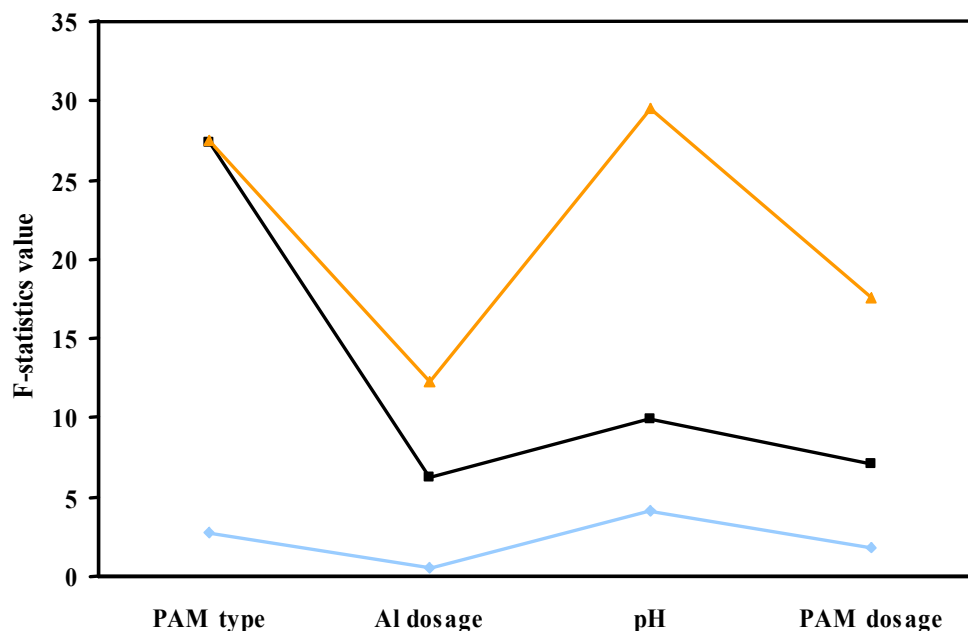
Figure 8.1 F-statistics for significance of tested factors (■: COD removal; ▲: turbidity removal; and ♦: total nitrogen removal)

F-statistics analysis results in Figure 8.1 show that the type of PAM used affected COD and turbidity removals significantly. Choosing a suitable PAM (anion LT25 PAM in this study) was the most important course before adopting the coagulation treatments to the separated digestate liquid. The pH value influenced turbidity more significantly, because the formation of flocs can be affected by pH adjustment (Gergor *et al.*, 1997). However, in practice, such low pH values would limit the successive secondary biological treatment by inhibiting the activity of bacteria. Al_2 $(SO_4)_3$ and PAM dosage also affected the coagulation efficiency, but, they had lower significance than the other two factors. None of the factors significantly affected total nitrogen removal, and this can help explain why the TN removal efficiency was lower than that of COD and turbidity.

8.3 Nitrogen removal from separated pig manure liquid digestate using struvite precipitation

8.3.1 Materials and methods

In the struvite precipitation test, the effects of different pH values on the formation of struvite precipitate in the separated pig manure digestate liquid were examined. The separated digestate liquid was added into 125 mL effective volume glass flasks. Then, $MgCl_2 \cdot 6H_2O$ and KH_2PO_4 were added to the flaks to achieve the desired stoichiometric ratio (Mg^{2+}: NH_4^+-N: PO_4^{3-}-P = 1: 1: 1). The mixture was continuously stirred using a magnetic stirrer at a speed of 120 rpm. After 15 minutes, 5 mol/L or 1 mol/L NaOH was added into the flasks to adjust the pH values of the liquid phase to designated values (8.5, 9.0, 9.5, 10.0, 10.5 and 11.0), and then the mixture was continuously stirred at a speed of 40 rpm for 10 min. After 20 min settling, struvite precipitate was collected and the NH_4^+-N, PO_4^{3-}-P and COD concentration in the supernatant phase was analysed. All procedures were conducted at room temperature ~18 °C.

After obtaining the optimal pH for struvite precipitation, nine different chemical dosing treatments were carried out under this optimal pH value. As well as the stoichiometric ratio (Mg^{2+}: NH_4^+-N: PO_4^{3-}-P = 1: 1: 1), other eight ratios (Mg^{2+}: NH_4^+-N: PO_4^{3-}-P= 1.3: 1: 1.3, 0.7: 1: 0.7, 1.3: 1: 1, 1.3: 1: 0.7, 1: 1: 0.7, 1: 1: 1.3, 0.7: 1: 1.3 and 0.7: 1: 1) were also investigated.

8.3.2 Optimal pH for struvite precipitation

The NH_4^+-N recovery through struvite precipitation was described as Eq. 2.15. In the initial experimental conditions without pH adjustment, almost no struvite precipitation was observed. Struvite precipitation was observed after pH adjustment. The NH_4^+-N concentration was recorded and its removal efficiency (Figure 8.2) was calculated at each of the following pH (8.5, 9.0, 9.5, 10.0, 10.5 and 11.0).

The NH_4^+-N removal efficiency increased with increasing pH. At pH 8.5, 85.1% of NH_4^+-N was recovered via struvite precipitation. When pH was above 10, the NH_4^+-N removal efficiency gradually levelled off. When pH was 10.5, the NH_4^+-N removal efficiency was up to 91.6%, and after pH increased to 11.0, the NH_4^+-N removal efficiency only rose by 0.3%.

The amount of NaOH required for each pH adjustment increased with pH increase (Figure 8.2). 4 g/L NaOH was needed to obtain a pH value of 10.5. From an

economic perspective, an additional 7.2% NaOH (260 mg/L NaOH) consumed for only 0.3% increase of the NH_4^+-N removal efficiency under the pH of 11.0 was not considered justifiable. Thus, 10.5 was considered the optimal pH for struvite precipitation treatment of the separated pig manure digestate liquid.
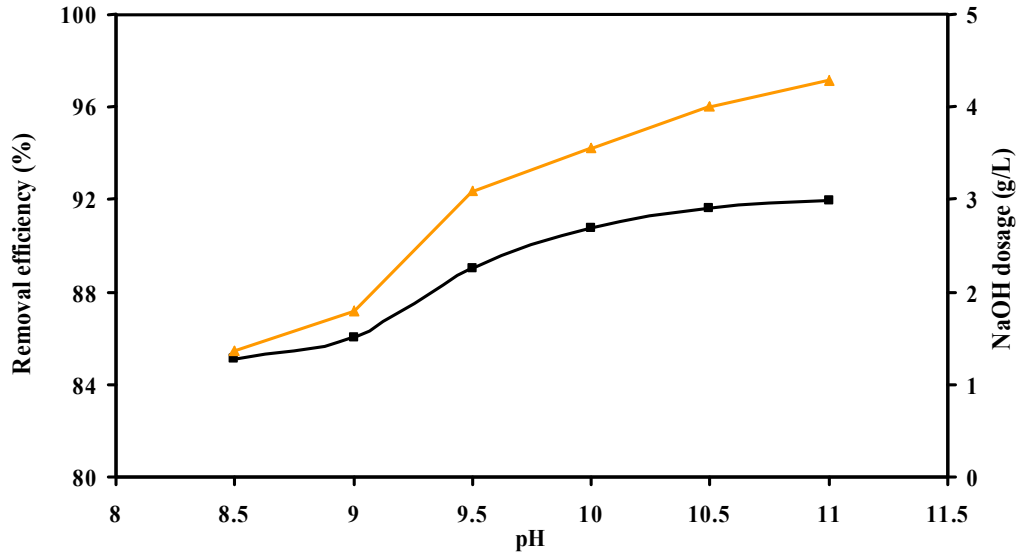
Figure 8.2 NH_4^+-N removal efficiency and the amounts of NaOH consumed under different pH (■: NH_4^+-N removal efficiency; ▲: NaOH dosage based on the wastewater volume)

8.3.3 Effects of the mole ratio of Mg^{2+}: NH_4^+-N: PO_4^{3-}-P on struvite precipitation

In order to study the effects of different Mg^{2+}: NH_4^+-N: PO_4^{3-}-P ratios on the struvite precipitation, a series of batch experiments were conducted at an optimal pH of 10.5 obtained in the previous section. Nine different ratios (Mg^{2+}: NH_4^+-N: PO_4^{3-}-P= 1: 1: 1, 1.3: 1: 1.3, 0.7: 1: 0.7, 1.3: 1: 1, 1.3: 1: 0.7, 1: 1: 0.7, 1: 1: 1.3, 0.7: 1: 1.3 and 0.7: 1: 1) were investigated (Figure 8.3). The ratio of "0.7" and "1.3" were considered as under-dose and over-dose of Mg^{2+} or PO_4^{3-}-P concentration relative to the NH_4^+-N concentration, respectively.

According to Figure 8.3, when at least one of Mg^{2+} and PO_4^{3-}-P was over-dosed (Mg^{2+}: NH_4^+-N: PO_4^{3-}-P = 1.3: 1: 1.3, 1.3: 1: 1, and 1: 1: 1.3), efficient NH_4^+-N removal (more than 90%) via struvite precipitation was achieved. The reason v that a portion of Mg^{2+} and PO_4^{3-}-P can form precipitates with ions in the wastewa

or between themselves at the high pH, so the over-dose of Mg^{2+} and PO_4^{3-}-P can enhance struvite precipitation and NH_4^+-N recovery. T-statistics was conducted to evaluate the effects of the over-dose of Mg^{2+} or overdose of PO_4^{3-}-P (Table 8.3).
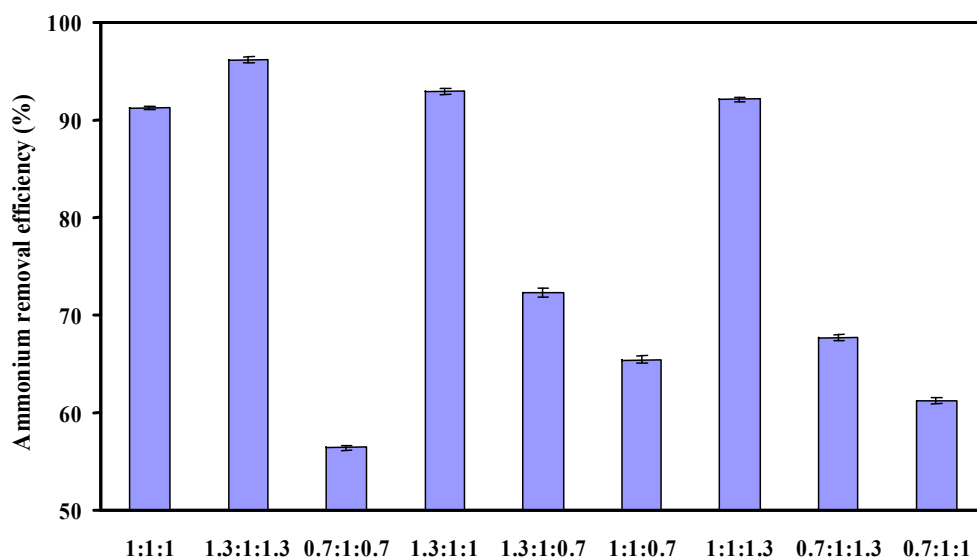
Figure 8.3 Effects of the Mg^{2+}: NH_4^+-N: PO_4^{3-}-P molar ratio on struvite precipitation treatments at pH of 10.5

Table 8.3 T-statistics for the effects of over-dose of Mg^{2+} and overdose of PO_4^{3-}-P

Groups	t_{OBS}
Mg^{2+}: NH_4^+-N: PO_4^{3-}-P= 1.3: 1: 1 to 1: 1: 1.3	3.87
Mg^{2+}: NH_4^+-N: PO_4^{3-}-P= 1.3: 1: 0.7 to 0.7: 1: 1.3	13.6
Mg^{2+}: NH_4^+-N: PO_4^{3-}-P= 1: 1: 0.7 to 0.7: 1: 1	15.4

All of the t_{OBS} values were larger than $t_{6, 0.01}$= 3.71 at the level of significance of 0.01 and that means, it can be concluded that there was statistical difference between over-dose of Mg^{2+} and the over-dose of PO_4^{3-}-P. Figure 8.3 shows that over-doses of Mg^{2+} was more efficient for the NH_4^+-N removal than the over-dose of PO_4^{3-}-P. Meanwhile, under-dose of both the two chemicals for struvite precipitation treatments adversely affected NH_4^+-N removal (less than 75%). On the basis of the most efficient experimental conditions (pH= 10.5, Mg^{2+}: NH_4^+-N: PO_4^{3-}-P= 1.3: 1: 1.3; Figure 8.3), the NH_4^+-N concentration can be reduced to 27.7 mg/L.

However, in these struvite precipitation experiments, the highest COD removal efficiency obtained was only 8.8%, indicating struvite precipitation had almost no effects on the organic matter removal, and additional treatment of separated pig manure digestate liquid after struvite precipitation would be necessary. In this case, a suitable technology would be to use secondary biological wastewater treatment, such as the SBR technology.

8.4 Summary

Coagulation and struvite precipitation methods were investigated for treatment of the pig manure digestate liquid.

More than 75% of COD and 85% of turbidity was removed through chemical coagulation with $Al_2(SO_4)_3$ as the coagulant. The highest nitrogen removal achieved was 39.2%. The results of F-statistics analysis show that use of the anion coagulant (LT25 PAM) and low pH (4.5 or 5.5) were the two most important factors affecting COD and turbidity removal.

In the struvite precipitation experiment, the NH_4^+-N removal efficiency increased with the increase in pH. The optimal pH was considered to be 10.5. Increasing Mg^{2+} and PO_4^{3-}-P dosage could enhance the NH_4^+-N recovery efficiency. On the basis of the optimal experimental conditions (pH= 10.5, and Mg^{2+}: NH_4^+-N: PO_4^{3-}-P = 1.3: 1: 1.3), more than 96% NH_4^+-N was removed from the separated pig manure digestate liquid.

Chapter Nine

Conclusions and Recommendations

9.1 Overview

During this study, pig manure digestate liquid was treated using various technologies: (1) intermittently aerated sequencing batch reactors (IASBRs); (2) aerobic granular sludge sequencing batch reactors; (3) aerobic upflow biofilters; and (4) chemical coagulation and struvite precipitation. Partial nitrification using IASBRs was studied. Nitrous oxide emission and generation was also examined in the IASBRs and the aerobic granular sludge sequencing batch reactors. The primary objectives of this research were to provide best available technology and operation for pig manure digestate treatment. Conclusions obtained from all laboratory-scale studies with recommendations made are presented in the following sections.

9.2 Conclusions

9.2.1 Laboratory-scale IASBRs study

This research studied the performance of the IASBR technology regarding organic matter and nitrogen removals from the pig manure digestate liquid and achievement of partial nitrification. It was found that the IASBR technology is promising for nitrogen removal from pig manure digestate liquid with low COD: N ratios. The results obtained are as follows:

1. In the IASBRs, biodegradable COD in the influent was efficiently removed and the effluent COD remaining in IASBRs was considered as inert organic matter which could be further reduced through coagulation.

 2. Stable and long-term partial nitrification was achieved in IASBRs. Adjustment

alkalinity and pH can improve nitrogen removal and partial nitrification efficiency. The nitrite accumulation efficiency was in the range of 20% - 80% appeared in IASBRs.

3. The content of readily biodegradable organic matter in wastewater affected the IASBR performance regarding nitrogen removal and partial nitrification. Nitrogen removal efficiencies were 76.5% and 97%, and partial nitrification efficiencies were 77 - 79% and 71%, in two IASBRs treating raw separated digestate liquid and synthetic wastewater, respectively.

4. NOB activity can be inhibited by the intermittent aeration strategy. Aeration rate and denitrification also led to partial nitrification.

5. The ecological analysis results show that the ratio of AOB: NOB in the sludge of IASBR was up to 4.5, which was much higher than that found in the seed sludge (ratio of AOB: NOB was 2.0). And *Nitrosomonas eutropha* was one of the main AOB species for nitrification in the IASBRs.

9.2.2 N_2O emissions from IASBRs study

In this study, the N_2O emission in the IASBRs treating the separated pig manure digestate liquid (IASBR-1) and the synthetic wastewater (IASBR-2) was investigated. The results include:

1. N_2O emissions were 12.0% and 9.8% of the total influent nitrogen loading rate, and equal to 15.6% and 10.1% of total removed nitrogen from IASBR-1 and IASBR-2, respectively.

2. The mean specific N_2O generation rates were 9.5 μg/ (g VSS· min) and 4.6 μg/ (g VSS· min) in the aeration periods, 23.6 μg/ (g VSS· min) and 20.6 μg/ (g VSS· min) in the non-aeration periods for IASBR-1 and IABSR-2, respectively. The IASBR-1 with low influent BOD_5 concentrations induced more N_2O generation.

3. The mean N_2O emissions in an operational cycle were 253.6 mg and 205.3 mg for IASBR-1 and IASBR-2, respectively. During the non-aeration periods, only 8.3% and 8.4% of total N_2O emissions occurred in IASBR-1 and IASBR-2, respectively;

while during the aeration periods, 91.7% and 91.6% of N_2O emissions took place in IASBR-1 and IASBR-2, respectively.

4. According to the nitrogen balance, 7.8 % and 7.6 % of total influent nitrogen in the non-aeration periods and 3.5% and 1.9% of total influent nitrogen in the aeration periods was removed via N_2O emission, respectively.

9.2.3 Nutrient removal and N_2O emission in aerobic granular sludge study

In this study, laboratory-scale aerobic granular SBRs were established to treat synthetic wastewater simulating a mixture of separated pig manure digestate liquid and municipal wastewater at three different aeration rates (0.2 L/min, 0.6 L/min and 1.0 L/min, corresponding to SBR1, SBR2 and SBR3, respectively) and three COD: N ratios (1:0.22, 1:0.15 and 1:0.11). The results include:

1. The mean COD removal efficiencies were more than 99% in three SBR under all aeration rates and COD: N ratio conditions. Almost all NH_4^+-N was removed under the COD: N ratio of 0.15: 1 and 0.11: 1. When the COD: N ratio was 1: 0.22, the NH_4^+-N removal efficiency was only 47%, 58% and 72% in SBR1, SBR2 and SBR3, respectively.

2. Under the continuous aeration conditions, the highest nitrogen removal efficiency was 36% at a COD: N rate of 1: 0.11 which confirmed that simultaneous nitrification and denitrification took place in aerobic granular sludge SBRs.

3. The proportions of N_2O emission relative to the influent nitrogen loading rate in SBR1, SBR2 and SBR3 were 8.2%, 6.1% and 3.8% at a COD: N ratio of 1: 0.22; 7.0%, 5.1% and 3.5% at a COD: N ratio of 1: 0.15; and 4.4%, 2.9% and 2.2% at a COD: N ratio of 1: 0.11, respectively.

4. With NO_2^--N being the only nitrogen component in the liquid phase, the N_2O generation rates were 1.73, 1.55 and 1.26 µg N_2O / (g SS• min) in SBR1, SBR2, and SBR3, respectively, which were much higher than those with NO_3^--N being the only nitrogen component. With NH_4^+-N being the nitrogen component, the average N_2O generation rate was 0.82±0.02 µg N_2O / (g SS• min) via nitrification.

5. After the carbon source in the wastewater was used-up, PHB can be used as the carbon source for heterotrophic denitrification, causing N_2O emissions under the aerobic conditions in the aerobic granular sludge SBRs.

9.2.4 Nutrient removals in upflow biofilter study

In this study, separated pig manure digestate liquid with a COD to TN ratio of 9.4 was treated in upflow biofilters at two loading rates (1.15 kg COD/ ($m^3 \cdot$ d) and 0.69 kg COD/ ($m^3 \cdot$ d)) for 136 days. The results include:

1. The nitrogen removal efficiency was 84.2% and 88.0%, respectively, and NH_4^+-N and biodegradable COD was completely removed at the two loading rates.

2. The phase studies show that in the anaerobic phase, the NO_3^--N reduction rates were 0.304 mg NO_3^--N / (L$\cdot$ min) and 0.237 mg NO_3^--N / (L$\cdot$ min). In the NH_4^+-N oxidation phase, NH_4^+-N nitrification rates were 0.293 mg NH_4^+-N / (L$\cdot$ min) and 0.176 mg NH_4^+-N / (L$\cdot$ min) for the high loading rate and low loading rate biofilters, respectively.

3. Attached biofilm had a much higher nitrification and denitrification activity than that of suspended growth biomass in biofilters.

4. Phosphorus removal rates were up to 88% in the biofilters. Phosphorus removal included biological phosphorus removal, chemical precipitation and adsorption.

9.2.5 Chemical treatments study

In this study, coagulation and struvite precipitation methods were investigated for treatment of the pig manure digestate liquid. The results include:

1. More than 75% of COD and 85% of turbidity was removed through chemical coagulation with $Al_2 (SO_4)_3$. The highest nitrogen removal achieved was 39.2%. The results of F-statistics analysis show that the use of the anion coagulant (LT25 PAM) and low pH (4.5 or 5.5) were the two most important factors influencing COD and turbidity removal.

2. In the struvite precipitation experiment, the NH_4^+-N removal efficiency increased

with the increase in pH. The optimal pH was determined to be 10.5. Increasing Mg^{2+} and PO_4^{3-}-P dosage enhanced the NH_4^+-N recovery efficiency. Under optimal experimental conditions (pH= 10.5, and Mg^{2+}: NH_4^+-N: PO_4^{3-}-P = 1.3: 1: 1.3), more than 96% of NH_4^+-N was removed from the separated pig manure digestate liquid.

9.2.6 Summary

The results show that all the technologies studied were efficient in treatment of pig manure digestate liquid, in terms of COD and nitrogen removals, and are applicable in practice. As for the IASBR technology, it is considered as an efficient nitrogen removal technology by achieving nitrogen removal through nitrite when treating low COD: N pig manure digestate liquid; however, the effluent would contain undesirable NO_2^--N, which needs to be further reduced using the ANAMMOX process. The IASBR is also a complicated system which needs precise control. As for the aerobic granular sludge SBR, nitrogen removal can be achieved under the aerobic condition by means of simultaneous nitrification and denitrification due to the spatial structure of aerobic granules; however, the high liquid exchange volume required in operation hinders its application into raw pig manure digestate liquid treatment unless it is diluted with municipal wastewater. As for the upflow biofilter technology, compared with conventional biological technologies, it is more effective in high nitrogen wastewater treatment, but is not for low COD: N wastewater treatment, and is only suitable for pig manure digestate liquid with an adequate COD: N ratio. The results obtained in this research show that it is difficult to meet the wastewater discharge standards with a single wastewater treatment technology. If biotechnologies are used, N_2O emission will also be a concern due to the high nitrogen concentration contained in the pig manure digestate. The aeration strategy and COD: N ratio are two important factors affecting N_2O emission. The COD: N ratio can be adjusted via chemical treatment including coagulation and struvite precipitation. Thus, the best option for pig manure digestate liquid treatment is to use chemical treatment technologies for nitrogen recovery, followed by biological technologies.

9.3 Recommendations for future research

1. The ANAMMOX process was proposed to treat the IASBRs effluent containi

high NO_2^--N. Ten reactors have been built-up to cultivate ANAMMOX bacteria. However, due to the long enrichment period necessary (ANAMMOX bacteria have a low specific growth rate) and the relocation of the laboratory to the New Engineering Building, the ANAMMOX bacteria have not been successfully cultivated up to now. This suggests that cultivation of ANAMMOX bacteria should continue. In future research, the operational conditions of IASBRs should be adjusted to achieve compatible effluent for the ANAMMOX reactors. The effects of the non-biodegradable organic matter contained in pig manure digestate liquid on the ANAMMOX bacteria should be examined.

2. Nitrous oxide analysis in the air phase has not been conducted in this research. It is recommended to use gas chromatography to measure the nitrous oxide gas content in the gas phase *in situ*, which could be combined with the dissolved N_2O data measured with micro-sensors in the liquid phase to detail the mechanism of N_2O generation and emission in biological reactors.

3. Mathematical modelling to simulate the process of nutrient removal and partial nitrification in IASBRs needs to be developed to predict the effects of operational conditions on the performance of the bioreactors systems.

4. Further research on phosphorus removal mechanisms in upflow biofilters should be carried out, particularly on the differentiation of chemical precipitation and adsorption onto the carriers.

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Appendices

Appendix A: Publications and Presentation during PhD Research

1. **Zhang, M.**, Lawlor, P. G., Wu, G., and Lynch, B., 2011. Partial nitrification and nutrient removal in intermittently aerated sequencing batch reactors treating separated digestate liquid after anaerobic digestion of pig manure. Bioprocess and Biosystem Engineering, 34, 1049 - 1056. (Chapter 4)

2. **Zhang, M.**, Lawlor, P. G., Li, J. and Zhan, X., 2011. Characteristics of nitrous oxide (N_2O) emissions from intermittently-aerated sequencing batch reactors treating the separated liquid fraction of anaerobically digested pig manure. Water, Air and Soil Pollution, DOI: 10.1007/s11270-011-0998-z. (Chapter 5)

3. **Zhang, M.**, Lawlor, P. G., Wu, G., Lynch, B., Shen, Z., and Zhan, X., 2010. Partial nitrification in intermittently-aerated sequencing batch reactors (IASBRs) treating separated liquid after anaerobic digestion of pig Manure. 3rd World Congress of Industrial Biotechnology, China. (Chapter 3)

4. **Zhang, M.**, Quan, X., Shen, Z., Lawlor, P. G. and Zhan, X., 2011. Characteristics of nitrous oxide emissions from aerobic granular sludge-sequencing batch reactors (SBRs) treating synthetic wastewater. The 21st Irish Environmental Researchers' Colloquium, Ireland. (Chapter 6)

5. **Zhang, M.**, Lawlor, P. G., Li, J. and Zhan, X., 2011. Nitrous oxide emission in the intermittently-aerated sequencing batch reactors (IASBRs) treating separated digestate liquid after anaerobic digestion of pig manure. Agricultural Research Forum, Ireland. (Chapter 5)

6. **Zhang, M.**, Quan, X. Lawlor, P. G., Yang, Z. and Zhan, X., 2011. Characteristics of nitrous oxide emissions from aerobic granular sludge sequencing batch reactors International Conference on Environment Simulation and Pollution Control, China. (Chapter 6)

Appendix B: Analytical Methods

The analytical methods and procedures used for examination of contaminants during the course of the study are presented below:

B.1 Chemical oxygen demand (COD)

COD were tested using the Closed Reflux Titrimetric Method in accordance with the standard APHA methods (APHA, 1998). Results are given in units of mg COD /L.

B.2 5-day biochemical oxygen demand (BOD$_5$)

BOD$_5$ was measured in accordance with the standard APHA methods (APHA, 1998) using Wissenschaftlich Technische Werkstatten (WTW) OxiTop manometric respirometers. The results are given in units of mg BOD$_5$ /L.

B.3 Ammonium-nitrogen (NH$_4^+$-N)

NH$_4^+$-N was measured using a Thermo Clinical Labsystems - Konelab 20 Nutrient Analyser (Thermo, USA) according to the APHA standard methods (APHA, 1998). The results are given in units of mg NH$_4^+$-N /L.

B.4 Nitrite-nitrogen (NO$_2^-$-N)

NO$_2^-$-N was measured using a Thermo Clinical Labsystems - Konelab 20 Nutrient Analyser (Thermo, USA) according to the APHA standard methods (APHA, 1998). The results are given in units of mg NO$_2^-$-N /L.

B.5 Nitrate-nitrogen (NO$_3^-$-N)

NO$_3^-$-N was measured using a Thermo Clinical Labsystems - Konelab 20 Nutrient Analyser (Thermo, USA) according to the APHA standard methods (APHA, 1998). The results are given in units of mg NO$_3^-$-N /L.

B.6 Total nitrogen (TN)

TN was digested in accordance with the standard APHA methods (APHA, 1998).

Digested nitrogen was measured using a Thermo Clinical Labsystems - Konelab 20 Nutrient Analyser (Thermo, USA) according to the APHA standard methods (APHA, 1998). The results are given in units of mg TN /L.

B.7 Orthophosphate (PO$_4^{3-}$-P)

PO$_4^{3-}$-P was measured using a Thermo Clinical Labsystems - Konelab 20 Nutrient Analyser (Thermo, USA) according to the APHA standard methods (APHA, 1998). The results are given in units of mg PO$_4^{3-}$-P /L.

B.8 Total phosphorus (TP)

TP was measured using the HACH PHOSPHORUS, TOTAL, HR method (Method 10127). The results are given in units of mg TP /L.

B.9 Suspended sludge (SS)

SS was measured in accordance with the standard APHA methods (APHA, 1998) using 1.2 µm pore size GC/F filter papers (Whatman, UK). The results are given in units of g SS /L.

B.10 Volatile suspended solid (VSS)

VSS was measured in accordance with the standard APHA methods (APHA, 1998). The results are given in units of g VSS /L.

B.11 Dissolved oxygen (DO)

DO was measured using a WTW - CellOx 325 electrode which was connected to a WTW 330 meter according to the manufacturer's protocol. The results are given in units of mg DO /L.

B.12 pH

pH was measured using a WTW - pH 320 electrode which was connected to a WTW 3210 meter according to the manufacturer's protocol.

Appendix C: Effects of Loading Rate on Nutrient Removal and Partial Nitrification Efficiency of IASBR Study Data

Associated with Chapter 3

C.1 Effect of the loading rates on profiles of nitrogen in the effluent

Time (d)	IABSR$_L$ NH$_4^+$-N (mg/L)	NO$_2^-$-N (mg/L)	NO$_2^-$-N (mg/L)	IASBR$_M$ NH$_4^+$-N (mg/L)	NO$_2^-$-N (mg/L)	NO$_2^-$-N (mg/L)	IASBR$_H$ NH$_4^+$-N (mg/L)	NO$_2^-$-N (mg/L)	NO$_2^-$-N (mg/L)
1	34.3	58.2	19.2	61.4	105	14.3	315	0.15	22.6
3	58.6	97.0	18.5	75.4	146	18.6	232	12.5	24.5
4	80.1	123	23.9	92.5	187	26.9	97.3	199	30.7
5	116	183	11.7	102.6	175	23.4	215	194	60.7
6	136	226	0.52	95.6	169	16.2	246	245	28.3
7	159	214	4.52	185	182	12.8	222	291	9.54
8	187	230	2.67	235	151	12.6	204	318	1.19
10	173	254	1.12	154	193	2.01	207	382	0.05
11	179	258	1.07	173	222	2.97	224	346	0.01
12	175	262	1.05	162	274	2.59	217	322	0.03
13	182	258	0.09	143	286	3.01	202	302	0.01
15	173	256	0.01	169	245	5.03	245	284	0.05
16	164	264	4.52	185	237	2.73	225	277	0.01
18	173	228	15.7	145	264	2.49	247	247	0.01
19	156	191	30.2	133	279	2.38	260	218	0.01
20	178	184	25.8	161	284	2.41	283	254	0.45
21	152	169	2.53	189	254	2.15	248	221	0.06
24	198	189	2.56	186	277	2.21	299	215	0.19
25	149	155	5.98	145	209	2.36	251	223	0.24
26	105	147	10.4	169	180	3.05	286	237	0.01
28	73.6	183	20.5	183	280	2.03	278	272	0.01
29	45.9	222	41.7	152	255	2.05	283	254	0.01
31	19.7	263	79.3	78.6	309	0.01	267	248	0.01
32	5.32	289	92.4	49.2	328	0.01	248	247	0.01
33	0.01	305	102	20.8	315	0.35	216	216	0.01
34	0.13	286	109	9.04	327	0.01	249	253	0.01
35	0.01	278	119	4.19	275	0.01	214	261	0.01
37	1.05	352	67.2	4.95	294	0.29	248	237	0.01
38	0.13	386	31.6	4.65	315	0.01	159	257	0.01
40	3.42	431	8.16	5.34	321	0.83	215	223	0.01
41	2.89	416	5.67	6.51	367	0.29	234	176	0.54
42	3.03	413	0.25	7.84	391	0.01	199	166	1.35
43	3.16	381	0.16	8.49	382	0.01	249	260	1.06
47	18.2	401	0.01	31.8	361	0.01	248	204	0.19
48	10.5	384	0.01	31.9	352	0.23	254	169	1.01
49	31.6	361	0.01	22.5	312	0.56	268	123	5.23
50	25.2	392	1.22	15.8	364	0.74	222	108	7.76
52	29.4	344	4.34	14.6	349	1.59	284	98.4	7.22
53	72.9	319	12.6	13.2	354	4.95	266	86.1	8.15
54	26.9	377	5.09	6.43	384	2.62	268	62.9	8.67

55	17.9	439	0.94	0.85	395	1.59	232	47.1	9.89
56	28.6	412	2.06	0.32	405	2.09	234	50.9	8.62
59	43.4	398	0.59	1.46	364	0.01	196	71.2	7.26
60	53.8	296	13.1	4.23	348	0.34	192	74.6	10.7
63	72.7	297	35.6	6.62	216	4.12	153	102	30.6
66	92.6	255	56.3	10.9	251	8.56	194	115	23.5
67	109	261	62.3	12.9	264	10.9	186	166	15.6
68	115	246	66.9	17.1	249	14.7	178	163	13.8
69	126	253	68.4	16.3	256	14.1	156	156	9.21
70	133	234	75.2	15.2	274	13.9	145	137	10.6
73	108	244	86.8	4.52	298	13.3	148	130	19.5
74	109	256	76.8	10.7	271	12.9	158	186	13.6
75	126	269	88.3	15.6	250	10.7	126	168	15.3
76	142	268	75.2	9.27	292	6.29	125	183	15.6
77	159	292	60.3	9.96	302	5.22	107	139	13.2
80	154	293	50.2	12.5	278	10.8	116	156	14.8
81	187	249	48.4	17.4	265	14.3	97.6	183	14.5
82	169	293	49.1	14.4	287	13.7	77.7	155	15.9
83	189	287	47.8	16.4	287	13.5	83.5	173	14.1
84	168	264	43.8	18.7	268	12.4	85.9	168	13.7
86	176	272	43.5	17.5	285	13.7	89.5	134	14.7
87	186	281	42.9	17.3	257	12.9	92.3	135	17.3
88	174	295	40.8	15.3	214	12.5	93.4	156	14.5
89	152	277	48.3	9.38	265	11.6	90.5	142	10.8
90	164	273	47.6	12.6	296	15.1	91.5	122	18.5
92	143	298	49.2	19.6	200	16.6	98.5	185	20.5
94	138	346	48.6	9.22	252	15.4	125	122	32.6
95	115	331	41.5	18.2	284	16.8	117	135	28.1
96	123	317	42.5	16.3	266	22.5	114	129	26.7
99	127	327	40.8	18.1	212	20.4	94.8	190	36.8
100	108	364	38.0	23.2	210	16.4	97.4	135	29.7
101	115	308	35.7	17.5	292	22.8	97.5	146	23.9
102	98.6	316	33.4	17.4	242	23.8	108	124	22.8
103	105	326	36.2	16.7	247	18.3	96.4	120	24.3
106	95.1	307	30.4	14.0	288	18.9	86.5	137	29.4
107	106	252	39.5	19.3	237	15.3	88.4	189	21.6
108	116	267	35.4	15.6	267	15.1	83.9	116	27.7
109	134	247	30.8	17.8	209	16.1	86.6	102	20.6
110	111	295	29.4	21.5	252	16.8	88.1	109	22.3
122	70.5	31.2	21.1	13.2	257	32.5	123	69.5	42.0
123	82.3	40.2	22.4	19.3	237	31.9	142	65.2	36.7
124	80.6	43.5	25.8	26.3	186	15.4	104	95.8	46.1
125	78.4	49.1	24.5	14.9	157	14.9	127	83.5	42.1
128	72.5	57.2	29.8	21.2	164	15.6	88.0	75.1	38.6
129	66.4	60.4	20.9	25.9	211	25.3	99.4	73.5	31.1
130	62.7	55.9	28.7	37.1	228	35.6	94.5	78.4	34.2
132	58.6	66.3	33.6	29.7	255	41.3	75.6	85.3	35.7
135	55.9	73.4	27.5	23.6	241	44.2	72.3	90.4	29.7
137	48.6	78.6	23.6	22.7	208	47.1	68.4	92.3	28.0
138	46	82.4	27.8	20.0	186	46.9	65.2	101	27.1
139	42.7	85.0	31.0	16.5	173	45.5	60.4	95.8	26.3
142	43.5	98.7	34.2	14.3	195	35.8	66.2	145	
143	36.5	91.2	30.8	22.6	184	47.3	71.2	112	
144	31.7	90.3	30.4	18.2	192	38.7	60.7	99.4	

145	28.4	93.5	29.3	20.6	149	46.8	58.6	95.3	28.5
146	26.6	94.8	26.8	24.6	153	49.4	57.3	89.4	32.2
149	22.5	87.5	28.6	19.6	162	53.2	56.3	84.6	38.3
150	21.4	95.4	25.2	16.7	147	46.4	58.6	82.1	27.5
152	19.6	98.0	21.0	17.3	128	47.5	59.7	92.7	36.3
153	22.3	98.9	26.7	23.5	136	47.7	60.4	88.4	33.5
156	18.9	105	24.6	20.8	134	42.4	62.3	85.5	31.2
157	24.2	99.6	25.9	23.4	184	47.9	67.5	81.8	27.6
158	25.6	101	22.4	28.7	176	50.5	74.7	81.4	24.8
159	28.5	107	29.7	26.9	153	52.4	80.8	87.9	30.4
160	27.3	112	25.9	23.2	162	55.7	76.4	89.0	29.3
163	18.5	104	16.4	29.6	148	45.3	75.6	88.2	25.6
165	28.6	115	27.6	28.7	142	49.8	79.6	81.1	30.7
166	27.4	126	30.8	24.6	135	47.6	72.7	88.1	35.4
167	27.1	124	32.3	20.7	146	51.7	75.0	93.8	31.6
170	19.3	129	35.3	28.6	177	50.4	71.6	98.7	40.3
171	25.5	137	36.7	25.3	154	57.0	75.4	106	41.6
172	22.9	132	32.9	23.6	134	54.6	76.3	108	34.5
173	24.6	138	37.5	27.5	152	56.7	64.1	109	47.2
177	23.2	143	33.2	30.5	154	67.4	77.2	114	46.3
178	27.8	139	39.7	29.4	160	62.3	72.6	129	39.6
179	25.1	134	35.4	26.2	157	61.9	71.5	112	48.8
180	24.7	141	36.8	27.1	140	58.7	70.4	125	46.9
181	28.3	129	30.6	23.9	133	59.3	70.6	132	50.3
184	24.2	115	38.4	26.8	129	64.5	63.0	138	51.9
185	25.6	106	39.6	24.8	106	66.8	74.6	137	57.4
186	22.4	180	41.3	27.5	147	63.4	75.2	133	48.7
187	23.7	109	40.3	26.7	125	57.3	64.9	127	53.6
188	29.4	112	47.5	33.7	126	60.3	77.8	135	56.4
191	24.2	116	40.3	35.4	132	56.9	79.5	129	59.4
192	12.5	100	44.1	27.5	105	42.3	70.3	139	58.7
193	7.50	94.6	48.7	15.6	102	25.7	60.4	138	62.0
194	10.4	95.5	47.6	17.4	119	25.8	65.3	123	67.5
195	13.6	92.6	48.2	20.6	120	27.1	71.4	127	65.5
198	10.9	88.6	53.8	18.9	113	40.6	75.5	125	66.0
199	12.6	87.5	50.3	16.1	125	46.8	77.3	112	64.7
200	15.2	95.1	55.7	12.6	117	44.9	70.9	129	56.9
201	15.6	81.5	61.9	17.4	128	46.7	78.6	122	62.1
202	13.5	72.4	58.6	10.1	114	41.4	75.4	117	68.7
205	14.5	81.0	62.7	16.9	128	50.5	68.9	116	62.4
206	13.3	81.5	64.7	15.6	113	55.9	66.4	105	65.9
207	12.9	79.3	56.8	18.7	125	50.6	71.3	113	46.8
208	12.4	75.6	69.7	16.9	121	53.8	68.9	108	60.7
209	12.7	81.5	70.3	13.0	120	57.2	66.9	113	64.3
212	10.5	75.2	55.5	10.7	115	66.7	62.4	107	60.4
213	17.4	68.4	68.2	15.2	106	58.2	60.7	112	55.7
214	14.6	73.5	63.9	9.30	105	63.4	58.4	95.8	52.5
215	11.5	68.4	66.8	10.6	98.7	61.4	51.6	103	56.8
216	13.4	65.3	70.2	11.3	103	65.2	55.7	105	58.2
219	12.6	62.4	73.5	9.50	105	66.7	57.0	93.8	52.9
220	10.3	58.7	75.4	8.40	112	62.1	57.1	91.4	54.8
221	13.7	54.6	77.3	7.70	94.6	64.8	47.7	95.2	57.9
222	12.6	59.2	76.9	8.20	98.7	67.9	56.5	92.3	58.7
223	10.4	58.1	78.4	8.60	103	61.6	52.6	96.5	59.6

226	8.60	54.6	81.4	8.90	94.5	63.6	56.2	98.2	58.5
227	9.30	47.5	84.6	8.50	98.7	64.5	56.0	97.5	57.5
228	7.50	54.2	90.9	7.80	96.6	64.9	59.6	113	54.2
229	10.5	43.9	86.4	7.50	82.6	67.7	54.2	104	54.5
230	9.60	41.6	85.5	7.60	96.2	62.4	54.8	99.3	51.5
233	9.50	44.2	79.4	8.10	108	73.1	49.0	99.2	59.8
234	7.40	38.5	89.5	2.90	115	69.2	52.3	104	60.6
235	13.0	42.6	84.6	4.90	97.5	62.7	54.3	115	54.4
236	11.2	37.4	85.8	5.20	106	70.2	49.6	117	52.7
237	8.50	35.2	93.5	6.40	112	71.3	53.9	102	63.1
240	9.50	31.7	102	10.2	107	68.5	47.5	114	65.9
241	6.80	25.9	105	7.90	104	63.5	54.3	117	63.7
242	7.30	27.1	112	16.7	95.7	67.9	58.2	104	63.8
243	8.20	21.7	98.6	7.40	97.4	60.7	57.0	107	65.0
244	5.70	19.6	107	9.50	103	66.5	52.6	95.5	66.2
247	10.3	22.4	93.0	9.60	108	66.8	50.3	102	64.3
248	8.40	25.9	91.3	9.30	97.5	69.3	48.6	112	62.0
249	8.60	27.4	97.7	9.80	87.7	74.6	48.0	98.1	60.8
250	9.70	26.1	96.5	17.3	101	67.6	43.3	98.6	69.5
251	9.20	24.3	101	18.6	93.4	63.3	58.6	93.4	61.5
253	8.30	22.5	94.2	11.9	99.1	73.1	64.6	91.6	62.4
254	13.8	23.8	99.7	25.4	99.8	68.5	59.2	94.6	65.1
255	19.1	31.7	103	22.3	95.2	65.4	54.4	93.8	68.7
256	15.2	20.6	95.3	19.7	104	60.7	50.9	101	70.2
257	10.9	29.4	98.6	20.6	108	62.9	58.2	99.2	68.7

C.2 Profiles of pH and DO in typical cycles in the three IASBRs in Stage 1 and Stage 2

Time (min)	Stage 1					
	pH in IASBR$_L$	DO in IASBR$_L$ (mg/L)	pH in IASBR$_M$	DO in IASBR$_M$ (mg/L)	pH in IASBR$_H$	DO in IASBR$_H$ (mg/L)
10	7.38	0.00	7.44	0.00	8.42	0.00
30	7.03	0.00	7.38	0.00	8.36	0.00
55	7.07	0.42	7.35	0.42	8.07	0.38
80	6.98	0.66	7.15	0.38	7.98	0.35
100	7.00	0.15	7.20	0.00	8.04	0.00
125	6.85	0.95	7.05	0.73	8.05	0.42
150	6.65	1.84	6.83	0.94	8.04	0.41
170	6.68	0.18	6.86	0.00	8.05	0.00
195	6.53	3.22	6.74	1.14	8.09	0.41
220	6.49	4.02	6.65	1.82	8.02	0.35
240	6.53	0.84	6.66	0.24	8.05	0.00
265	6.43	3.69	6.52	3.24	8.02	0.35
290	6.41	4.12	6.45	3.96	7.89	0.59
310	6.48	0.92	6.52	0.42	7.92	0.00
335	6.44	4.02	6.54	3.56	7.71	0.63
360	6.43	4.28	6.52	3.89	7.53	0.72
380	6.41	0.84	6.50	0.43	7.59	0.04

405	6.45	4.07	6.56	4.02	7.47	1.46
430	6.43	3.96	6.62	3.97	7.38	1.58
480	6.49	0.34	6.72	0.00	7.49	0.00

	Stage 2					
Time (min)	pH in IASBR$_L$	DO in IASBR$_L$ (mg/L)	pH in IASBR$_M$	DO in IASBR$_M$ (mg/L)	pH in IASBR$_H$	DO in IASBR$_H$ (mg/L)
0	7.13	2.1	7.39	0.8	7.52	0.0
5	7.27	0.0	7.46	0.0	7.58	0.0
20	7.35	0.0	7.6	0.0	7.62	0.0
50	7.47	0.0	7.71	0.0	7.64	0.0
75	7.38	0.2	7.64	0.2	7.57	0.2
100	7.27	0.2	7.58	0.2	7.53	0.2
115	7.30	0.0	7.65	0.0.	7.54	0.0
130	7.35	0.0	7.7	0.0	7.58	0.0
155	7.22	0.2	7.63	0.4	7.56	0.3
180	7.15	0.2	7.58	0.2	7.54	0.2
195	7.18	0.0	7.62	0.0	7.57	0.0
210	7.20	0.0	7.6	0.0	7.59	0.0
235	7.14	0.2	7.52	0.3	7.53	0.1
260	7.08	0.3	7.44	0.4	7.5	0.2
275	7.09	0.0	7.46	0.0	7.51	0.0
290	7.10	0.0	7.48	0.0	7.53	0.0
315	7.08	0.4	7.44	0.3	7.52	0.4
340	7.02	1.4	7.37	0.2	7.5	0.2
355	6.98	0.5	7.35	0.0	7.52	0.0
370	7.00	0.0	7.39	0.0	7.55	0.0
395	7.00	1.2	7.38	0.4	7.54	0.2
420	7.02	2.0	7.34	0.8	7.53	0.2
435	7.03	0.9	7.32	0.0	7.56	0.0
450	7.02	0.3	7.35	0.0	7.58	0.0
475	7.04	5.6	7.35	1.4	7.56	0.3
500	7.02	6.4	7.39	1.9	7.55	0.8
515	7.06	3.8	7.38	0.2	7.55	0.0
530	7.10	1.4	7.38	0.0	7.56	0.0
555	7.07	6.8	7.36	2.3	7.55	1.1
580	7.11	7.2	7.35	3.1	7.56	1.8
595	7.12	5.7	7.37	1.1	7.57	0.0
610	7.14	4.3	7.35	0.4	7.59	0.0.
635	7.15	7.5	7.34	3.3	7.57	1.6
660	7.17	7.6	7.34	4.2	7.54	1.9
690	7.15	4.3	7.37	2.4	7.52	0.2
720	7.14	1.7	7.36	0.6	7.51	0.0

8

C.3 Effects of the aeration rate on partial nitrification

Time (min)	Nitrite accumulation efficiency under the sufficient oxygen condition (%)	Nitrite accumulation efficiency under the IASBR aeration condition (%)	DO concentration under the IASBR aeration condition (mg/L)
10			0.2
20	41.8	78.3	0.2
30	46.9	80.6	0.3
40	44.4	78.8	0.5
50	42.6	74.0	1.0
60	41.4	66.7	1.6
70	39.8	62.3	2.2
80	40.6	58.4	2.9

Appendix D: Effects of Readily Biodegradable Organic Matter on IASBRs Study Data

Associated with Chapter 4

D.1 Performance of IASBRs in nitrogen removal and partial nitrification

Time (d)	IASBR-1		IASBR-2	
	NH_4^+-N (mg/L)	Nitrite accumulation efficiency (%)	NH_4^+-N (mg/L)	Nitrite accumulation efficiency (%)
1	33.3	66.29	5.9	26.37
2	46.6	65.89	5.8	38.89
4	47.4	66.37	6.3	31.54
5	50.9	70.78	7.4	33.11
6	88.7	72.49	15.7	55.17
7	83.7	73.81	15.9	54.14
8	85.2	71.92	29.2	62.01
9	80.7	69.73	33.6	68.98
11	78.5	69.09	42.5	75.58
12	83.4	69.91	38.5	73.87
13	80.7	68.21	33.4	72.00
14	88.2	65.57	30.9	66.33
15	79.6	62.48	25.7	68.61
16	85.5	65.51	20.7	71.76
18	70.6	70.16	25.4	71.70
19	54.3	71.77	26.4	76.77
20	25.5	75.00	22.3	69.85
21	20.8	74.82	26.9	73.04
22	17.7	77.65	27.5	68.73
23	15.3	76.51	29	64.33
25	10.5	73.55	25.5	64.38
26	17.7	73.86	28.4	68.59
27	12.3	78.07	20.6	68.21
28	7.8	78.78	19.7	61.24
29	9.1	79.87	21.6	60.29
30	5.8	78.61	23.8	60.52
32	1.2	73.88	31.2	65.12
33	3.4	71.47	37.4	71.18
34	1.6	72.68	30.5	68.95
35	1.9	74.10	34.6	67.54
36	2.4	70.48	38.2	73.35
37	1.7	72.49	33	68.53
39	7.6	74.50	28.5	71.14
40	5.4	73.11	26.7	70.49
41	6.2	72.67	25.6	67.19
42	6.5	72.77	24.4	71.60
43	5.8	75.52	28.4	71.54
44	3.1	74.83	25.3	70.01
46	4.3	73.53	27.1	65.26
47	2.5	72.32	25	69.03

48	5.9	74.78	26.6	67.37
49	6.4	74.89	23.9	69.61
50	6.5	73.97	21.6	70.73
51	3.1	72.50	22.3	68.93
53	2.8	71.17	22.1	68.03
54	5.5	68.84	27.9	70.41
55	3.5	71.67	26.4	70.90
56	2.6	71.92	28.5	71.72
57	3.6	71.40	22.3	66.82
58	2.7	71.73	23.5	68.26
60	2.7	70.05	27.5	66.98
61	4.1	70.70	20.6	71.02
62	5.2	72.08	24.3	72.45
63	6.8	72.98	25.7	75.00
64	6.7	72.65	23.8	71.89
65	6.4	71.03	28.6	72.14
67	4.1	73.03	24.1	75.50
68	4.7	73.94	25.3	72.32
69	5.6	72.85	24	72.19
70	4.8	74.12	26.7	68.54
71	5.4	75.20	23.3	71.55
72	5.8	74.80	24.7	70.32
74	5.6	75.06	25.7	72.60
75	4.1	74.70	24.3	74.19
76	5.3	75.77	22.5	75.62
77	6.4	74.26	24.6	68.98
78	2.7	74.44	27.1	71.19
79	3.5	75.27	27.5	75.00
81	2.9	75.60	23.6	71.59
82	2.7	76.89	26.1	75.74
83	2.9	75.83	26.2	69.80
84	5.9	74.46	24.5	69.51
85	1.8	76.94	27.8	73.75
86	3.6	76.30	20.1	76.31
88	2.4	77.75	24.6	74.68
89	2.5	77.02	25.8	76.97
90	2.2	74.53	22.5	79.47
91	1.9	76.88	20.7	75.65
92	2.7	76.32	21.4	73.83
93	2.6	76.50	28.7	77.04
95	2.8	75.91	23.3	77.03
96	2.9	77.02	22.5	78.05
97	5.8	76.39	23.5	78.19
98	4.2	78.49	20.6	77.98
99	14.5	78.58	27.6	79.76
100	3.7	77.43	22	73.82
102	3.9	78.19	28.3	71.12
103	3.6	77.04	27.3	71.73
104	4.5	77.51	28.1	70.29
105	4.3	77.73	26	65.18
106	10.5	79.16	26.9	70.68
107	5.1	78.80	24.8	76.20
109	5.8	78.28	25.1	75.64
110	2.4	78.72	23.6	72.27

111	6.7	76.97	23.5	72.98
112	8.6	78.90	25.3	75.39
113	7.8	79.03	24.1	68.83
114	4.1	78.12	22.2	77.79
116	3.5	77.28	27.1	74.81
118	3.8	76.68	24.6	73.76
120	6.1	76.04	25.4	72.48
123	4.5	77.43	26.2	72.35
125	5.2	77.62	26.8	73.53
127	4.7	76.60	27.2	74.40
130	5	79.16	25.6	73.89
132	3.5	78.31	23.1	70.79
134	2.9	76.93	28.6	72.31
137	4.8	78.10	24.1	71.03
139	6.4	78.75	29	70.42
141	6.2	78.07	25.8	71.91
144	5.8	76.64	26.3	71.23
146	5.3	76.87	21.1	70.12
148	6.2	77.17	26.4	71.66
151	5.1	76.59	23.5	72.30

D.2 Profiles of pH in the IASBRs

Time (d)	pH in IABSR-1	pH in IABSR-2	Time (d)	pH in IABSR-1	pH in IABSR-1
1	7.85	7.84	68	8.19	7.62
2	7.42	7.82	69	8.18	7.65
4	6.75	7.82	70	8.16	7.64
5	6.48	7.83	71	8.19	7.62
6	6.32	7.75	72	8.17	7.69
7	6.3	7.64	74	8.15	7.64
8	6.25	7.53	75	8.18	7.66
9	6.14	7.42	76	8.21	7.68
11	6.2	7.45	77	8.24	7.71
12	6.17	7.41	78	8.22	7.68
13	6.15	7.35	79	8.22	7.65
14	6.15	7.38	81	8.25	7.69
15	6.64	7.45	82	8.24	7.65
16	7.12	7.52	83	8.19	7.7
18	7.35	7.55	84	8.17	7.73
19	7.46	7.53	85	8.2	7.82
20	7.53	7.54	86	8.24	7.87
21	7.65	7.59	88	8.23	7.85
22	7.72	7.56	89	8.22	7.92
23	7.79	7.55	90	8.25	7.96
25	7.82	7.56	91	8.22	8.04
26	7.85	7.54	92	8.17	8.06
27	7.94	7.55	93	8.19	8.11
28	7.92	7.58	95	8.22	8.12
29	8.01	7.56	96	8.24	8.15
30	8.05	7.54	97	8.21	8.17
32	8.12	7.53	98	8.23	8.14

33	8.14	7.56	99	8.21	8.19
34	8.1	7.55	100	8.17	8.13
35	8.17	7.6	102	8.19	8.16
36	8.16	7.54	103	8.24	8.14
37	8.15	7.56	104	8.17	8.17
39	8.14	7.58	105	8.19	8.19
40	8.15	7.55	106	8.24	8.18
41	8.15	7.57	107	8.18	8.21
42	8.16	7.59	109	8.16	8.22
43	8.18	7.56	110	8.22	8.2
44	8.17	7.52	111	8.2	8.18
46	8.13	7.56	112	8.3	8.24
47	8.16	7.54	113	8.26	8.25
48	8.14	7.58	114	8.24	8.24
49	8.16	7.54	116	8.24	8.26
50	8.19	7.58	118	8.26	8.22
51	8.15	7.56	120	8.28	8.21
53	8.17	7.58	123	8.19	8.27
54	8.19	7.61	125	8.23	8.24
55	8.19	7.54	127	8.26	8.26
56	8.21	7.56	130	8.25	8.26
57	8.18	7.59	132	8.24	8.23
58	8.19	7.66	134	8.26	8.23
60	8.15	7.62	137	8.17	8.24
61	8.16	7.64	139	8.19	8.26
62	8.14	7.61	141	8.2	8.21
63	8.15	7.65	144	8.26	8.24
64	8.15	7.68	146	8.24	8.21
65	8.21	7.69	148	8.28	8.23
67	8.22	7.63	151	8.27	8.24

D.3 Performance of COD removal in IASBRs

	IASBR-1		IASBR-2	
		COD removal efficiency		COD removal efficiency
Time (d)	COD(mg/L)	(%)	COD(mg/L)	(%)
1	240	97.92	10.5	99.91
5	520	95.49	21.0	99.83
8	830	92.81	21.0	99.83
12	1040	90.99	31.5	99.74
15	1120	90.29	31.5	99.74
19	1090	90.55	10.5	99.91
22	920	92.03	10.4	99.91
26	1260	89.08	10.4	99.91
29	1030	91.07	10.4	99.91
33	1220	89.43	10.4	99.91
36	1200	89.60	10.4	99.91
40	1150	90.03	10.4	99.91
43	980	91.51	10.4	99.91
47	1260	89.08	10.6	99.91
50	1100	90.47	10.4	99.91

54	1210	89.51	0.0	100.00
57	1160	89.95	15.6	99.87
61	1250	89.17	0.0	100.00
64	1080	90.64	21.0	99.83
68	960	91.68	20.8	99.83
71	1340	88.39	26.0	99.78
75	1130	90.21	15.8	99.87
78	1170	89.86	15.8	99.87
82	1020	91.16	10.4	99.91
85	1070	90.73	20.8	99.83
89	1080	90.64	15.6	99.87
92	980	91.51	0.0	100.00
96	1030	91.07	20.8	99.83
99	1060	90.81	20.8	99.83
103	1320	88.56	0.0	100.00
106	1220	89.43	0.0	100.00
110	1170	89.86	0.0	100.00
113	1090	90.55	0.0	100.00
118	1020	91.16	10.8	99.91
123	1130	90.21	0.0	100.00
127	1200	89.60	14.4	99.88
132	1220	89.43	0.0	100.0
137	1160	89.95	0.0	100.00
141	1160	89.95	9.6	99.92
146	1180	89.77	9.6	99.92
151	1210	89.51	0	100.00

D.4 Profile of DO and FA in a typical cycle

Time (min)	FA (mg/L) in IASBR-1	FA (mg/L) in IASBR-2	Time (min)	DO (mg/L) in IASBR-1	DO (mg/L) in IASBR-2
0	12.40	14.44	0	0.00	0.00
15	15.02	15.48	1	0.00	0.00
30	18.11	16.49	2	0.00	0.00
45	21.27	18.42	3	0.00	0.00
60	25.46	18.91	4	0.00	0.00
65	22.31	18.02	5	0.00	0.00
80	17.91	13.81	6	0.00	0.00
95	14.41	10.81	7	0.00	0.00
110	10.64	7.98	8	0.00	0.00
125	12.90	10.29	9	0.00	0.00
140	15.71	13.15	10	0.00	0.00
145	14.32	12.72	11	0.00	0.00
160	10.66	10.19	12	0.00	0.00
175	8.11	8.33	13	0.00	0.00
190	5.97	6.36	14	0.00	0.00
205	7.03	8.16	15	0.00	0.00
220	7.77	10.16	16	0.00	0.00
225	6.97	9.57	17	0.00	0.00
240	5.02	8.14	18	0.00	0.00
255	3.47	6.82	19	0.00	0.00

Time (min)					
270	2.10	5.58	20	0.00	0.00
285	2.51	6.49	21	0.00	0.00
300	2.71	7.36	22	0.00	0.00
305	2.26	6.97	23	0.00	0.00
320	1.50	5.91	24	0.00	0.00
335	0.97	4.92	25	0.00	0.00
350	0.70	3.74	26	0.00	0.00
365	0.79	4.21	27	0.00	0.00
380	0.76	4.66	28	0.00	0.00
385	0.72	4.39	29	0.00	0.00
400	0.56	3.41	30	0.00	0.00
415	0.46	2.71	31	0.00	0.00
430	0.40	1.97	32	0.00	0.00
445	0.44	2.09	33	0.00	0.00
460	0.47	2.24	34	0.00	0.00
475	0.48	2.31	35	0.00	0.00
Time (min)	DO (mg/L) in IASBR-1	DO (mg/L) in IASBR-2	36	0.00	0.00
256	0.79	0.93	37	0.00	0.00
257	0.72	0.95	38	0.00	0.00
258	0.78	1.02	39	0.00	0.00
259	0.83	1.04	40	0.00	0.00
260	0.89	0.97	41	0.00	0.00
261	0.94	0.95	42	0.00	0.00
262	0.92	0.93	43	0.00	0.00
263	0.87	0.95	44	0.00	0.00
264	0.88	0.94	45	0.00	0.00
265	0.94	0.89	46	0.00	0.00
266	0.91	0.93	47	0.00	0.00
267	0.89	0.93	48	0.00	0.00
268	0.94	0.97	49	0.00	0.00
269	0.98	0.95	50	0.00	0.00
270	0.78	0.91	51	0.00	0.00
271	0.06	0.00	52	0.00	0.00
272	0.00	0.00	53	0.00	0.00
273	0.00	0.00	54	0.00	0.00
274	0.00	0.00	55	0.00	0.00
275	0.00	0.00	56	0.00	0.00
276	0.00	0.00	57	0.00	0.00
277	0.00	0.00	58	0.00	0.00
278	0.00	0.00	59	0.00	0.00
279	0.00	0.00	60	0.00	0.00
280	0.00	0.00	61	0.63	0.84
281	0.00	0.00	62	0.51	0.81
282	0.00	0.00	63	0.74	0.67
283	0.00	0.00	64	0.84	0.59
284	0.00	0.00	65	0.92	0.69
285	0.00	0.00	66	0.82	0.75
286	0.00	0.00	67	0.90	0.77
287	0.00	0.00	68	0.91	0.72
288	0.00	0.00	69	0.84	0.71
289	0.00	0.00	70	0.83	0.64
290	0.00	0.00	71	0.79	0.68
291	0.00	0.00	72	0.71	0.57

292	0.00	0.00	73	0.63	0.68
293	0.00	0.00	74	0.65	0.62
294	0.00	0.00	75	0.65	0.64
295	0.00	0.00	76	0.62	0.60
296	0.00	0.00	77	0.62	0.65
297	0.00	0.00	78	0.58	0.68
298	0.00	0.00	79	0.60	0.64
299	0.00	0.00	80	0.65	0.68
300	0.08	0.00	81	0.66	0.69
301	0.80	0.84	82	0.70	0.71
302	0.96	0.93	83	0.71	0.73
303	0.93	0.83	84	0.75	0.74
304	0.90	0.87	85	0.74	0.65
305	0.88	0.81	86	0.71	0.73
306	0.90	0.90	87	0.74	0.68
307	0.82	0.94	88	0.72	0.62
308	0.79	0.92	89	0.78	0.73
309	0.82	0.87	90	0.78	0.75
310	0.94	1.04	91	0.74	0.84
311	0.92	1.06	92	0.71	0.76
312	0.94	0.98	93	0.69	0.74
313	1.02	0.98	94	0.72	0.79
314	1.04	1.05	95	0.78	0.76
315	1.05	1.01	96	0.74	0.73
316	1.00	0.96	97	0.73	0.79
317	0.96	1.00	98	0.77	0.78
318	0.97	1.05	99	0.85	0.81
319	1.01	1.03	100	0.88	0.83
320	1.03	0.98	101	0.88	0.90
321	1.03	0.94	102	0.83	0.85
322	1.03	0.93	103	0.78	0.83
323	1.09	0.97	104	0.80	0.81
324	1.09	0.98	105	0.84	0.74
325	0.99	1.04	106	0.83	0.75
326	0.96	1.05	107	0.83	0.77
327	0.94	1.15	108	0.84	0.83
328	0.98	1.06	109	0.86	0.80
329	1.00	1.03	110	0.73	0.76
330	1.02	1.07	111	0.05	0.01
331	1.04	0.98	112	0.00	0.00
332	1.03	0.94	113	0.00	0.00
333	1.07	0.97	114	0.00	0.00
334	1.15	0.92	115	0.00	0.00
335	1.18	0.61	116	0.00	0.00
336	1.17	0.87	117	0.00	0.00
337	1.16	0.96	118	0.00	0.00
338	1.21	0.98	119	0.00	0.00
339	1.22	1.06	120	0.00	0.00
340	1.17	0.97	121	0.00	0.00
341	1.18	0.98	122	0.00	0.00
342	1.23	1.06	123	0.00	0.00
343	1.28	0.94	124	0.00	0.00
344	1.25	0.98	125	0.00	0.00
345	1.17	0.93	126	0.00	0.00

346	1.09	1.02	127	0.00	0.00
347	1.09	0.94	128	0.00	0.00
348	1.18	1.05	129	0.00	0.00
349	1.12	1.04	130	0.00	0.00
350	0.91	0.70	131	0.00	0.00
351	0.09	0.02	132	0.00	0.00
352	0.00	0.01	133	0.00	0.00
353	0.00	0.00	134	0.00	0.00
354	0.00	0.00	135	0.00	0.00
355	0.00	0.00	136	0.00	0.00
356	0.00	0.00	137	0.00	0.00
357	0.00	0.00	138	0.00	0.00
358	0.00	0.00	139	0.00	0.00
359	0.00	0.00	140	0.04	0.00
360	0.00	0.00	141	0.65	0.67
361	0.00	0.00	142	0.97	0.63
362	0.00	0.00	143	1.00	0.60
363	0.00	0.00	144	0.97	0.63
364	0.00	0.00	145	0.91	0.63
365	0.00	0.00	146	0.94	0.64
366	0.00	0.00	147	0.95	0.70
367	0.00	0.00	148	0.86	0.69
368	0.00	0.00	149	0.84	0.66
369	0.00	0.00	150	0.83	0.70
370	0.00	0.00	151	0.80	0.74
371	0.00	0.00	152	0.81	0.72
372	0.00	0.00	153	0.79	0.71
373	0.00	0.00	154	0.71	0.71
374	0.00	0.00	155	0.67	0.72
375	0.00	0.00	156	0.72	0.78
376	0.00	0.00	157	0.78	0.79
377	0.00	0.00	158	0.73	0.73
378	0.00	0.00	159	0.71	0.76
379	0.00	0.00	160	0.73	0.72
380	0.10	0.00	161	0.76	0.76
381	0.81	0.94	162	0.78	0.77
382	1.02	0.86	163	0.80	0.75
383	1.23	0.91	164	0.85	0.76
384	1.71	0.97	165	0.83	0.82
385	2.60	1.04	166	0.82	0.75
386	2.90	1.07	167	0.83	0.77
387	3.06	1.03	168	0.81	0.81
388	3.11	1.04	169	0.85	0.77
389	3.17	1.09	170	0.91	0.76
390	3.19	1.06	171	0.86	0.71
391	3.25	1.11	172	0.84	0.69
392	3.41	0.98	173	0.80	0.74
393	3.40	1.07	174	0.77	0.81
394	3.30	1.15	175	0.78	0.81
395	3.26	1.13	176	0.77	0.89
396	3.21	1.19	177	0.76	0.76
397	3.27	1.07	178	0.79	0.78
398	3.29	1.04	179	0.87	0.94
399	3.25	0.99	180	0.87	0.84

400	3.30	1.06	181	0.86	0.83
401	3.31	1.13	182	0.88	0.85
402	3.32	1.06	183	0.89	0.79
403	3.39	1.04	184	0.90	0.76
404	3.40	1.05	185	0.87	0.78
405	3.30	1.07	186	0.90	0.86
406	3.28	1.13	187	0.92	0.83
407	3.29	1.05	188	0.95	0.83
408	3.40	1.14	189	0.93	0.79
409	3.38	1.11	190	0.78	0.84
410	3.31	1.24	191	0.06	0.00
411	3.36	1.13	192	0.00	0.00
412	3.37	1.12	193	0.00	0.00
413	3.42	1.31	194	0.00	0.00
414	3.45	1.45	195	0.00	0.00
415	3.45	1.32	196	0.00	0.00
416	3.48	1.49	197	0.00	0.00
417	3.47	1.58	198	0.00	0.00
418	3.54	1.57	199	0.00	0.00
419	3.61	1.64	200	0.00	0.00
420	3.62	1.67	201	0.00	0.00
421	3.63	1.89	202	0.00	0.00
422	3.69	1.89	203	0.00	0.00
423	3.78	2.16	204	0.00	0.00
424	3.82	2.48	205	0.00	0.00
425	3.82	2.34	206	0.00	0.00
426	3.78	2.44	207	0.00	0.00
427	3.76	2.52	208	0.00	0.00
428	3.80	2.61	209	0.00	0.00
429	3.80	2.41	210	0.00	0.00
430	3.55	2.47	211	0.00	0.00
431	1.80	0.74	212	0.00	0.00
432	0.44	0.06	213	0.00	0.00
433	0.05	0.02	214	0.00	0.00
434	0.02	0.01	215	0.00	0.00
435	0.02	0.00	216	0.00	0.00
436	0.01	0.00	217	0.00	0.00
437	0.00	0.00	218	0.00	0.00
438	0.00	0.00	219	0.00	0.00
439	0.00	0.00	220	0.07	0.00
440	0.00	0.00	221	0.79	1.05
441	0.00	0.00	222	1.03	0.94
442	0.00	0.00	223	1.09	0.94
443	0.00	0.00	224	1.03	0.96
444	0.00	0.00	225	0.89	0.99
445	0.00	0.00	226	0.84	0.89
446	0.00	0.00	227	0.82	0.92
447	0.00	0.00	228	0.82	0.91
448	0.00	0.00	229	0.83	0.83
449	0.00	0.00	230	0.80	0.83
450	0.00	0.00	231	0.81	0.85
451	0.00	0.00	232	0.81	0.76
452	0.00	0.00	233	0.84	0.93
453	0.00	0.00	234	0.86	0.97

454	0.00	0.00	235	0.83	0.86
455	0.00	0.00	236	0.82	0.86
456	0.00	0.00	237	0.77	0.84
457	0.00	0.00	238	0.81	0.85
458	0.00	0.00	239	0.84	0.79
459	0.00	0.00	240	0.82	0.86
460	0.00	0.00	241	0.82	0.91
461	0.00	0.00	242	0.90	0.92
462	0.00	0.00	243	0.88	0.86
463	0.00	0.00	244	0.86	0.88
464	0.00	0.00	245	0.86	0.89
465	0.00	0.00	246	0.89	0.84
466	0.00	0.00	247	0.92	0.92
467	0.00	0.00	248	0.93	0.97
468	0.00	0.00	249	0.87	0.86
469	0.00	0.00	250	0.85	0.83
470	0.00	0.00	251	0.88	0.94
471	0.00	0.00	252	0.88	0.95
472	0.00	0.00	253	0.84	0.97
473	0.00	0.00	254	0.79	0.68
474	0.00	0.00	255	0.80	0.74

D.5 Effects of the aeration strategy on partial nitrification

Time (min)	NO_3^--N for direct aeration (mg/L)	NO_3^--N for 30 min non-aeration before (mg/L)
0	0.6	0.5
2	0.8	0.4
6	1.2	0.6
8	1.6	0.6
12	2.1	0.7
14	2.5	0.6
16	2.9	0.7
18	3.1	0.9
20	3.4	1.1
22	3.6	1.4
24	4.3	1.6
26	4.4	2
28	4.8	2.4
30	5.1	2.7

D.6 Profile of COD concentrations in a typical cycle

Time (min)	COD(mg/L) in IASBR-1	COD(mg/L) in IASBR-1
0	1522	341
30	1493	302
60	1454	264
80	1435	235
110	1406	206
140	1373	192
160	1349	178
190	1320	149
220	1286	106
240	1258	86
270	1224	72
300	1200	43
320	1181	38
350	1171	29
380	1166	5
400	1166	0
430	1166	0
475	1162	0

D.7 Profile of TON and NH_4^+-N concentrations in a typical cycle

Time (min)	NH_4^+-N in IASBR-1 (mg/L)	TON in IASBR-1 (mg/L)	NH_4^+-N in IASBR-2 (mg/L)	TON in IASBR-2 (mg/L)
0	116	870	138	89.7
15	119	861	136	79.2
30	117	854	139	71.8
45	115	845	140	60.1
60	118	841	138	52.8
65	116	839	137	54.1
80	109	849	132	58.8
95	103	858	125	65.5
110	93.5	864	117	71.1
125	94.1	859	119	63.3
140	93.7	850	118	56.9
145	90.7	856	119	58.9
160	79.4	863	113	64.8
175	71.3	872	105	69.4
190	62.1	879	97.4	76.6
205	61.8	875	96.5	71.9
220	61.6	868	97.1	62.9
225	57.6	874	95.4	63.3
240	45.1	884	88.3	68.4
255	33.9	893	80.6	76.2
270	22.3	904	73.5	82.4
285	22.6	900	73.5	76.1
300	22.4	891	73.4	65.7
305	19.5	897	71.0	67.2

320	13.8	904	64.1	73.0
335	9.7	911	56.9	79.2
350	7.5	909	49.2	88.2
365	7.9	907	48.7	84.1
380	7.2	900	48.5	77.2
385	6.9	901	45.7	78.2
400	5.6	900	38.7	85.9
415	4.8	902	32.1	92.4
430	4.4	901	24.4	98.9
445	4.5	898	23.7	94.7
460	4.6	897	23.3	93.1
475	4.6	897	23.6	92.0

D.8 AOB/ EUB and NOB/ EUB percentages in the seed sludge and the IASBR-1 sludge

	Seed	IASBR-1
AOB to EUB	8.49%	17.21%
NOB to EUB	4.24%	3.84%

Appendix E: Characteristics of Nitrous oxide Emissions from IASBRs Study Data

Associated with Chapter 5

E.1 Relationship between the soluble N₂O concentration and the N₂O emission rate

				Diffusion			
N₂O (mg/L)	N₂O emission (mg/(L· min))	N₂O (mg/L)	N₂O emission (mg/(L· min))	N₂O (mg/L)	N₂O emission (mg/(L· min))	N₂O (mg/L)	N₂O emission (mg/(L· min))
5.496	-0.01496	1.556	-0.00713	0.248	-0.0059	0.040	-0.00078
5.482	-0.01474	1.548	-0.0075	0.240	-0.00189	0.046	0.001223
5.467	-0.01606	1.541	-0.00768	0.244	-0.00011	0.043	-0.00089
5.449	-0.01694	1.533	-0.00794	0.240	-0.00378	0.044	0.001558
5.433	-0.01716	1.525	-0.00719	0.237	-0.00156	0.046	0.003784
5.415	-0.0176	1.519	-0.00638	0.236	-0.00156	0.051	-0.00267
5.398	-0.0176	1.512	-0.00726	0.234	0.002446	0.040	-0.00134
5.380	-0.01694	1.504	-0.00752	0.241	0.002781	0.049	0.002666
5.364	-0.0176	1.497	-0.00638	0.239	-0.00211	0.046	0.001223
5.345	-0.01826	1.491	-0.00673	0.237	-0.00256	0.051	-0.00178
5.328	-0.01738	1.484	-0.00702	0.234	-0.00122	0.042	-0.00378
5.310	-0.01694	1.477	-0.01067	0.235	-0.00089	0.044	-0.00189
5.294	-0.01562	1.462	-0.00733	0.232	-0.00301	0.038	0.00044
5.279	-0.01672	1.463	-0.00299	0.229	0.001558	0.044	0.002332
5.260	-0.01628	1.456	-0.00695	0.236	0.001672	0.043	-0.00223
5.246	-0.0154	1.449	-0.00656	0.232	-0.00411	0.040	-0.00189
5.229	-0.01584	1.443	-0.00565	0.227	-0.00334	0.039	-0.00233
5.214	-0.01562	1.437	-0.00669	0.225	0.00044	0.035	-0.00122
5.198	-0.01562	1.430	-0.00673	0.228	0.001558	0.037	0.004224
5.183	-0.01584	1.424	-0.00598	0.228	-0.003	0.044	0.002341
5.166	-0.01782	1.418	-0.00554	0.222	-0.00356	0.042	-0.00256
5.148	-0.01914	1.413	-0.0057	0.221	-0.00312	0.039	-0.003
5.128	-0.01584	1.406	-0.00645	0.216	-0.00189	0.036	0.001558
5.116	-0.01386	1.400	-0.00673	0.218	0.001223	0.042	0.002552
5.100	-0.0154	1.393	-0.00649	0.218	-0.00044	0.041	0.001338
5.085	-0.01672	1.387	-0.00601	0.217	0.001778	0.044	0.000669
5.067	-0.01782	1.381	-0.00627	0.222	0.00022	0.042	-0.00211
5.049	-0.01782	1.374	-0.00618	0.217	-0.002	0.040	-0.00312
5.031	-0.01672	1.368	-0.00607	0.218	-0.00267	0.036	-0.00278
5.016	-0.01496	1.362	-0.00642	0.212	-0.0029	0.035	-0.00334
5.001	-0.0165	1.356	-0.00678	0.212	-0.00345	0.029	-0.00144
4.983	-0.01628	1.349	-0.00671	0.205	-0.00011	0.032	0.00022
4.969	-0.01518	1.342	-0.00594	0.212	-0.00077	0.030	0.000774
4.953	-0.01628	1.337	-0.00614	0.203	-0.00267	0.033	0.00367
4.936	-0.01474	1.330	-0.00627	0.207	0.001109	0.037	-0.00089
4.923	-0.01584	1.324	-0.00572	0.206	-0.00044	0.032	-0.00089
4.905	-0.01584	1.319	-0.00559	0.206	-0.00067	0.035	0.001558
4.891	-0.0165	1.313	-0.00554	0.204	-0.00134	0.035	-0.00178
4.872	-0.01738	1.307	-0.0053	0.203	-0.00245	0.032	0.00022

4.857	-0.01474	1.303	-0.00618	0.199	-0.00356	0.035	0.001338
4.842	-0.01452	1.295	-0.01129	0.196	-0.00055	0.034	0.001118
4.828	-0.01584	1.280	-0.0112	0.198	0.001223	0.037	-0.00089
4.811	-0.0176	1.273	-0.0064	0.198	-0.00022	0.032	0.002781
4.792	-0.01628	1.267	-0.0055	0.198	-0.00356	0.043	0.000554
4.778	-0.01584	1.262	-0.00458	0.191	-0.00434	0.034	-0.00268
4.761	-0.01672	1.258	-0.00521	0.189	0.000774	0.038	0.001558
4.745	-0.01672	1.251	-0.00691	0.193	-0.00078	0.037	0.001778
4.727	-0.01584	1.244	-0.0051	0.188	-0.00211	0.041	0.000774
4.713	-0.01672	1.241	-0.0059	0.189	-0.00077	0.038	-0.006
4.694	-0.01672	1.232	-0.00717	0.186	-0.00011	0.029	-0.00467
4.679	-0.01584	1.227	-0.00704	0.188	-0.00033	0.029	0.002561
4.662	-0.01628	1.218	-0.00601	0.185	-0.00457	0.034	0.000334
4.647	-0.01518	1.215	-0.0068	0.179	0.001223	0.030	0.001223
4.632	-0.01408	1.205	-0.00849	0.188	-0.00111	0.037	0.002112
4.619	-0.00506	1.198	-0.00796	0.177	-0.00634	0.034	-0.00278
4.622	-0.01056	1.189	-0.01008	0.175	-0.00033	0.031	-0.00233
4.598	-0.02068	1.178	-0.00799	0.176	-0.00144	0.029	-0.00033
4.580	-0.01584	1.173	-0.00627	0.172	0.000554	0.030	0.001672
4.566	-0.01606	1.165	-0.00796	0.177	0.002006	0.032	-0.00145
4.548	-0.01738	1.157	-0.00966	0.176	-0.00255	0.028	-0.00145
4.531	-0.01562	1.146	-0.00717	0.172	-0.00378	0.030	0.002341
4.517	-0.01452	1.143	-0.00444	0.169	-0.00122	0.032	0.000449
4.502	-0.01562	1.137	-0.0051	0.170	0.004444	0.030	-0.00067
4.486	-0.0154	1.132	-0.0077	0.177	0.001558	0.031	-0.00189
4.471	-0.01518	1.121	-0.00667	0.173	-0.00444	0.027	-0.00044
4.455	-0.01628	1.119	-0.00627	0.169	-0.00412	0.030	-0.00167
4.439	-0.0154	1.109	-0.00627	0.165	0.001223	0.023	0.000889
4.425	-0.01562	1.106	-0.0059	0.171	0.00367	0.032	0.004004
4.407	-0.01542	1.097	-0.00757	0.172	-0.00033	0.031	-0.00145
4.394	-0.01553	1.091	-0.00482	0.170	-0.004	0.029	-0.00222
4.376	-0.01672	1.087	-0.00563	0.164	-0.00201	0.027	0.002561
4.360	-0.01467	1.080	-0.00823	0.166	0.000889	0.034	0.003441
4.347	-0.01472	1.071	-0.00587	0.166	-0.00289	0.034	-0.00445
4.331	-0.01641	1.068	-0.00693	0.161	-0.00223	0.025	-0.00511
4.314	-0.0172	1.057	-0.00693	0.161	-0.00112	0.024	-0.00011
4.297	-0.01624	1.054	-0.00563	0.158	-0.00133	0.025	0.004224
4.282	-0.01571	1.046	-0.00678	0.159	0.000783	0.032	0.004224
4.265	-0.01621	1.041	-0.00574	0.160	0.003555	0.033	-0.00367
4.249	-0.01659	1.034	-0.00772	0.166	-0.00144	0.025	-0.00089
4.232	-0.01718	1.025	-0.00796	0.157	-0.00156	0.032	0.001223
4.215	-0.01672	1.018	-0.00667	0.163	0.001443	0.027	-0.003
4.198	-0.01496	1.012	-0.00587	0.160	-0.00456	0.026	0.001892
4.185	-0.01428	1.007	-0.00548	0.154	-0.00134	0.031	0.001778
4.170	-0.01483	1.001	-0.0068	0.157	0.000449	0.029	-0.00178
4.155	-0.01434	0.993	-0.00823	0.155	-0.00278	0.027	-0.00167
4.141	-0.01428	0.985	-0.00653	0.152	-0.0009	0.026	0.00345
4.127	-0.01448	0.980	-0.00431	0.153	0	0.034	0.000669
4.112	-0.01626	0.976	-0.00706	0.152	-0.00422	0.027	-0.00378
4.094	-0.01628	0.966	-0.00796	0.144	-0.00289	0.027	0.00022
4.080	-0.015	0.960	-0.00548	0.146	0.002561	0.028	0.000334
4.064	-0.01465	0.955	-0.00576	0.149	0.002332	0.027	0.0007
4.050	-0.01421	0.949	0.001166	0.151	0.000106	0.029	-0.000
4.036	-0.0163	0.957	-0.0064	0.150	-0.00156	0.027	-0.001

4.018	-0.01531	0.936	-0.01437	0.147	-0.00545	0.026	0.003221
4.005	-0.01549	0.929	-0.0064	0.139	-0.00223	0.033	-0.00066
3.987	-0.01725	0.923	-0.00561	0.143	-0.00122	0.024	-0.004
3.971	-0.01472	0.917	-0.00442	0.136	0.000669	0.025	-0.001
3.957	-0.01483	0.914	-0.00484	0.144	0.002226	0.022	0.001223
3.941	-0.01551	0.908	-0.00576	0.141	-0.00011	0.028	0.002446
3.926	-0.01487	0.903	-0.00653	0.144	0.001109	0.027	-0.00089
3.911	-0.01575	0.895	-0.00249	0.143	-0.00233	0.026	-0.003
3.895	-0.01637	0.898	-0.00273	0.139	0.001118	0.021	-0.00156
3.879	-0.01538	0.889	-0.00535	0.145	0.000554	0.023	0.001558
3.864	-0.01516	0.887	-0.00524	0.141	-0.00189	0.024	0.001443
3.848	-0.0158	0.879	-0.00757	0.141	-0.002	0.026	0.00044
3.833	-0.01582	0.872	-0.00733	0.137	-0.00323	0.025	-0.00089
3.817	-0.01531	0.864	-0.00471	0.135	0.000334	0.024	0.00022
3.802	-0.01518	0.862	-0.00286	0.137	-0.00255	0.026	0.002781
3.786	-0.01621	0.858	-0.00653	0.130	-0.00245	0.030	0.002675
3.769	-0.01536	0.849	-0.00693	0.132	0.005447	0.031	-0.00289
3.756	-0.01351	0.844	-0.00275	0.141	0	0.024	-0.00323
3.742	-0.01456	0.844	-0.00326	0.132	-0.00323	0.025	-0.00033
3.726	-0.01492	0.838	-0.00548	0.134	0	0.023	0.002561
3.713	-0.01573	0.833	-0.00444	0.132	-0.00099	0.030	0.000889
3.695	-0.01538	0.829	-0.00458	0.132	0.000774	0.025	-0.00167
3.682	-0.01324	0.824	-0.00497	0.134	-0.00179	0.026	-0.00189
3.669	-0.01397	0.819	-0.00535	0.129	-0.00422	0.021	-0.00111
3.654	-0.01564	0.813	-0.00484	0.125	-0.00033	0.024	0.003001
3.637	-0.01465	0.809	-0.00484	0.128	0.001329	0.027	0.00044
3.625	-0.01399	0.803	-0.00299	0.128	0.000669	0.025	-0.00223
3.609	-0.01476	0.803	-0.00627	0.129	-0.00022	0.023	-0.00111
3.595	-0.01349	0.791	-0.00706	0.128	-0.00255	0.023	-0.00099
3.582	-0.01329	0.789	-0.00405	0.124	-0.00055	0.021	0.002666
3.569	-0.01404	0.783	-0.00535	0.127	-0.0009	0.028	0.002112
3.554	-0.01397	0.779	-0.00392	0.123	0.001338	0.025	-0.00344
3.541	-0.01474	0.775	-0.00431	0.129	0.001003	0.021	0.002886
3.525	-0.01536	0.770	-0.00407	0.125	-0.0029	0.031	0.00367
3.510	-0.01428	0.767	-0.00418	0.123	-0.00267	0.029	-0.00311
3.496	-0.01445	0.762	-0.00508	0.119	-0.00223	0.024	-0.00234
3.481	-0.015	0.757	-0.00471	0.119	0.004673	0.024	0.001223
3.466	-0.01384	0.752	-0.00431	0.129	0.002895	0.027	-0.00144
3.453	-0.01074	0.748	-0.00352	0.125	-0.00323	0.021	-0.00122
3.445	-0.01366	0.745	-0.00431	0.122	-0.00055	0.024	0.000449
3.426	-0.01705	0.739	-0.0055	0.124	-0.00166	0.022	0.003775
3.411	-0.0141	0.734	-0.00601	0.119	-0.00512	0.032	0.002112
3.398	-0.01371	0.727	-0.00548	0.113	-0.00546	0.026	-0.00267
3.383	-0.01472	0.723	-0.00497	0.108	0.001778	0.027	0.001329
3.368	-0.01511	0.717	-0.0064	0.117	0.00601	0.029	-0.00356
3.353	-0.01434	0.710	-0.00365	0.120	0.001003	0.020	-0.00255
3.340	-0.0132	0.710	-0.00444	0.119	-0.00033	0.024	0.00623
3.327	-0.01366	0.701	-0.00587	0.119	0.000334	0.032	-0.00089
3.312	-0.01311	0.698	-0.00444	0.120	-0.007	0.022	-0.00545
3.300	-0.01252	0.693	-0.0068	0.105	-0.00312	0.021	0.001443
3.287	-0.01316	0.685	-0.00653	0.113	0.00367	0.025	0.001109
3.274	-0.01287	0.680	-0.00315	0.113	-0.004	0.023	-0.00045
3.262	-0.01243	0.678	-0.00352	0.105	-0.00067	0.024	-0.00055
3.249	-0.01258	0.672	-0.00667	0.111	0.001118	0.022	-0.00099

3.236	-0.01379	0.665	-0.00587	0.108	0.00022	0.022	0.002895
3.222	-0.01393	0.661	-0.00508	0.112	0.00022	0.028	0.001883
3.209	-0.01351	0.655	-0.00407	0.108	-0.00289	0.026	-0.00278
3.195	-0.01263	0.653	-0.00235	0.106	-0.00078	0.022	-0.00255
3.183	-0.01272	0.650	-0.00548	0.107	-0.00033	0.021	0.002781
3.169	-0.01417	0.642	-0.0081	0.105	-0.00055	0.028	-0.00078
3.155	-0.01362	0.634	-0.00301	0.105	-0.00056	0.019	-0.00489
3.142	-0.01375	0.636	-0.00222	0.104	-0.00077	0.018	0.001118
3.127	-0.01443	0.630	-0.00548	0.104	0.002895	0.021	0.002446
3.113	-0.0132	0.625	-0.00405	0.110	-0.00134	0.023	0.004338
3.101	-0.01192	0.622	-0.00288	0.101	-0.0029	0.030	0.002218
3.089	-0.01287	0.619	-0.00288	0.104	0.002666	0.028	-0.00268
3.075	-0.01366	0.616	-0.00521	0.107	-0.00022	0.025	-0.00222
3.062	-0.0123	0.608	-0.00667	0.104	-0.00133	0.023	-0.00278
3.051	-0.01175	0.602	-0.00405	0.104	0.000669	0.019	0.000669
3.038	-0.0132	0.600	-0.00326	0.105	-0.00367	0.024	0.002226
3.024	-0.01269	0.596	-0.00693	0.097	-0.00067	0.024	-0.00011
3.013	-0.01243	0.587	-0.00497	0.104	0.00367	0.024	0.001443
2.999	-0.01338	0.586	-0.00352	0.104	-0.00245	0.026	-0.003
2.986	-0.01307	0.579	-0.00561	0.099	-0.00489	0.018	-0.00267
2.973	-0.01333	0.575	-0.00405	0.094	0.002226	0.021	0.002886
2.960	-0.01294	0.571	-0.00341	0.103	0.003555	0.024	0.001558
2.947	-0.01311	0.568	-0.00367	0.101	-0.00467	0.024	-0.002
2.933	-0.01346	0.564	-0.00405	0.094	-0.00089	0.020	-0.00211
2.921	-0.01223	0.560	-0.00561	0.099	0.001778	0.020	0.00044
2.909	-0.0117	0.553	-0.00482	0.097	-0.00412	0.021	0.000774
2.897	-0.01256	0.550	-0.00431	0.091	-0.00022	0.022	-0.00022
2.884	-0.01243	0.544	-0.00576	0.097	0.002675	0.020	0.000229
2.872	-0.01236	0.539	-0.00275	0.097	-0.001	0.022	-0.00011
2.859	-0.01274	0.539	-0.00209	0.095	0.001892	0.020	0.001778
2.847	-0.01197	0.535	-0.00469	0.100	-0.00156	0.026	0.003898
2.835	-0.01228	0.529	-0.00574	0.092	-0.004	0.028	0.000334
2.822	-0.01296	0.523	-0.00471	0.092	0.003335	0.026	-0.00423
2.809	-0.01324	0.520	-0.00405	0.099	0.00066	0.020	-0.00045
2.796	-0.01263	0.515	-0.00196	0.094	-0.00456	0.025	0.002781
2.784	-0.01285	0.516	-0.00301	0.089	-0.00289	0.025	-0.0029
2.770	-0.01322	0.509	-0.0055	0.088	0.001118	0.020	-0.00011
2.757	-0.01239	0.505	-0.00273	0.092	0.004338	0.025	-0.00033
2.745	-0.0128	0.503	-0.00326	0.097	-0.00056	0.019	-0.001
2.732	-0.01225	0.498	-0.00418	0.091	-0.00211	0.023	0.00066
2.721	-0.01287	0.495	-0.00548	0.092	0.001786	0.020	-0.00245
2.706	-0.01344	0.488	-0.00458	0.094	-0.00078	0.018	-0.00011
2.694	-0.01221	0.486	-0.00211	0.091	-0.00301	0.020	0.002895
2.682	-0.01252	0.483	-0.00378	0.088	-0.00089	0.024	-0.00122
2.669	-0.01338	0.478	-0.00352	0.089	-0.00178	0.018	-0.00011
2.655	-0.0123	0.476	-0.00262	0.085	-0.00055	0.024	0.002112
2.644	-0.01129	0.473	-0.00392	0.088	0	0.022	-0.00178
2.632	-0.01186	0.468	-0.00458	0.085	0.000669	0.020	-0.00189
2.621	-0.01265	0.464	-0.00312	0.089	0.002781	0.018	-0.00145
2.607	-0.01234	0.462	-0.00301	0.090	0.002666	0.017	0.002561
2.596	-0.01188	0.458	-0.00341	0.095	-0.00055	0.023	0.002455
2.583	-0.01283	0.455	-0.00183	0.089	-0.00189	0.022	-0.003
2.570	-0.0114	0.454	-0.00222	0.091	-0.00367	0.016	-0.00
2.561	-0.01146	0.451	-0.00431	0.082	-0.00322	0.020	0.0041

2.547	-0.01256	0.446	-0.00405	0.084	-0.00089	0.024	0.000554
2.535	-0.01175	0.443	-0.00037	0.080	-0.00156	0.021	-0.0039
2.524	-0.01168	0.445	-0.00261	0.081	0.000554	0.017	0.001892
2.512	-0.01258	0.438	-0.0068	0.081	0.000106	0.025	0.000449
2.499	-0.0128	0.431	-0.00431	0.081	0.002666	0.018	-0.00268
2.486	-0.01239	0.429	-0.00274	0.086	0.00345	0.020	0.002552
2.474	-0.01276	0.426	-0.00196	0.088	-0.00111	0.023	0.000783
2.461	-0.01195	0.425	-0.00065	0.084	-0.00456	0.021	-0.00011
2.450	-0.01115	0.425	-0.00314	0.079	-0.00189	0.022	0.003001
2.439	-0.01102	0.419	-0.00353	0.080	-0.00055	0.027	0.000106
2.428	-0.01142	0.417	-0.00261	0.078	-0.00323	0.023	-0.00312
2.416	-0.01199	0.414	-0.00222	0.074	0.000334	0.021	-0.00222
2.404	-0.01186	0.413	-0.00679	0.079	-0.00055	0.018	0.002341
2.392	-0.01181	0.400	-0.00575	0.073	0.001109	0.026	-0.00145
2.380	-0.01131	0.402	0.000653	0.081	0.002561	0.015	-0.00367
2.369	-0.01107	0.401	0.000132	0.078	-0.00234	0.018	-0.00267
2.358	-0.01113	0.402	-0.00209	0.076	0.000449	0.010	-0.00201
2.347	-0.01082	0.397	-0.00183	0.079	0.001232	0.014	0.002332
2.336	-0.01019	0.398	-0.0017	0.079	-0.00123	0.015	-0.00022
2.327	-0.01122	0.394	-0.00314	0.076	0.001329	0.014	0.001452
2.314	-0.01206	0.392	-0.00248	0.081	0.000669	0.018	-0.00245
2.303	-0.0108	0.389	-0.00287	0.078	-0.00312	0.009	-0.00189
2.292	-0.01016	0.386	-0.00379	0.075	-0.002	0.014	0.006116
2.282	-0.01067	0.381	-0.00431	0.074	-0.00011	0.021	0.00367
2.271	-0.00988	0.377	-0.00183	0.075	0.001223	0.021	-0.00345
2.263	-0.01005	0.377	-0.00131	0.076	0.002552	0.014	0.000554
2.251	-0.0106	0.375	-0.00263	0.080	-0.00512	0.022	0.002446
2.241	-0.00959	0.372	-0.00215	0.066	-0.004	0.019	-0.00233
2.232	-0.00988	0.371	-0.00116	0.072	0.003564	0.018	-0.00211
2.222	-0.01032	0.370	-0.00089	0.073	-0.00222	0.015	-0.00045
2.211	-0.01001	0.369	0.003898	0.068	-0.00256	0.017	-0.00356
2.202	-0.00972	0.378	0.001338	0.068	0.002218	0.008	0.000554
2.192	-0.01005	0.371	-0.00434	0.072	0.002341	0.018	0.002781
2.182	-0.00992	0.369	-0.00578	0.073	-0.00144	0.013	-0.00011
2.172	-0.00939	0.360	-0.00278	0.069	-0.00089	0.018	0.000554
2.163	-0.01005	0.363	-0.00056	0.071	0.001003	0.014	-0.00023
2.152	-0.01038	0.359	-0.00078	0.071	-0.00278	0.017	0.002226
2.142	-0.00997	0.362	0.002121	0.065	0.001892	0.019	-0.00089
2.132	-0.01032	0.363	-0.00089	0.075	-0.00123	0.015	0
2.121	-0.00966	0.360	-0.00112	0.063	-0.00489	0.019	-0.00166
2.113	-0.00959	0.361	-0.00367	0.065	0.002561	0.012	-0.00122
2.102	-0.01038	0.353	-0.00534	0.068	0.000326	0.016	0.003555
2.092	-0.0106	0.350	-0.00434	0.066	-0.00166	0.019	-0.00278
2.081	-0.01014	0.344	-0.00178	0.065	0.000114	0.011	-0.00189
2.071	-0.00997	0.347	0.000783	0.066	-0.00011	0.015	0.000889
2.061	-0.00992	0.346	-0.00356	0.064	-0.00055	0.013	0.000229
2.052	-0.0097	0.339	-0.00278	0.065	-0.00267	0.016	0.000889
2.042	-0.0101	0.340	-0.00134	0.059	0.002226	0.014	-0.00011
2.031	-0.01005	0.337	-0.00033	0.069	0.001558	0.016	-0.00189
2.021	-0.01012	0.339	-0.00233	0.062	-0.00434	0.011	0.003555
2.011	-0.00964	0.332	-0.00734	0.061	0.003555	0.023	0.004118
2.002	-0.00981	0.325	-0.001	0.069	0.000106	0.019	-0.00489
1.992	-0.01056	0.330	0.003441	0.061	-0.00434	0.013	-0.00134
1.981	-0.00968	0.332	-0.00167	0.060	0.000563	0.016	-0.00022

1.972	-0.00911	0.327	-0.00811	0.062	-0.00044	0.012	-0.00134
1.963	-0.00946	0.315	-0.00289	0.060	-0.00112	0.014	-0.00033
1.953	-0.00981	0.321	0.003221	0.060	0.001549	0.012	0.002226
1.943	-0.01014	0.322	0.000889	0.063	0.000334	0.018	0.001778
1.933	-0.0099	0.323	-0.00077	0.060	-0.00044	0.015	-0.00089
1.923	-0.00959	0.320	-0.00467	0.062	-0.00278	0.016	0.001223
1.914	-0.00939	0.313	-0.00512	0.055	0.001672	0.018	-0.00033
1.905	-0.0097	0.310	-0.00033	0.065	0.002446	0.016	0.000783
1.894	-0.00955	0.313	-0.00099	0.060	-0.0049	0.019	0.000774
1.886	-0.00953	0.308	-0.00077	0.055	-0.00111	0.017	-0.0029
1.875	-0.00944	0.311	-0.00256	0.058	-0.00111	0.014	-0.00022
1.867	-0.00895	0.303	-0.00011	0.053	0.001778	0.017	0.00323
1.857	-0.01027	0.311	-0.00234	0.061	0.003335	0.020	-0.00189
1.846	-0.01016	0.298	-0.00501	0.060	-0.00356	0.013	-0.00089
1.837	-0.00924	0.301	0.000229	0.054	-0.00223	0.018	0.000449
1.828	-0.00986	0.299	-0.00312	0.055	0.00022	0.014	-0.00201
1.817	-0.0092	0.295	-0.00145	0.054	0.000669	0.014	0.001338
1.809	-0.00783	0.296	0.002112	0.057	0	0.016	0.00022
1.802	-0.00878	0.299	-0.00222	0.054	-0.00278	0.015	-0.00223
1.792	-0.00887	0.291	-0.00422	0.051	-0.00211	0.012	-0.00022
1.784	-0.00785	0.291	-0.00078	0.050	0.001672	0.014	0.001663
1.776	-0.00867	0.290	0.00022	0.054	0.002218	0.015	-0.004
1.767	-0.00906	0.291	-0.00256	0.055	-0.00256	0.006	-0.00099
1.758	-0.0086	0.285	-0.00578	0.049	0.000669	0.013	0.005896
1.749	-0.00794	0.279	-0.00277	0.056	-0.00077	0.018	0
1.742	-0.00845	0.279	0.002446	0.048	-0.00312	0.013	-0.00022
1.733	-0.00893	0.284	-0.001	0.050	0.002886	0.018	0.001109
1.724	-0.00862	0.277	-0.00178	0.054	0.002446	0.016	-0.00078
1.715	-0.00847	0.281	-0.00201	0.055	0.001786	0.016	-0.00167
1.707	-0.00823	0.273	0.000889	0.057	0.000898	0.012	0.001672
1.699	-0.00792	0.282	0.001892	0.056	-0.00356	0.019	0.001452
1.691	-0.00739	0.277	-0.00634	0.050	-0.00156	0.015	-0.00278
1.684	-0.00713	0.270	-0.00233	0.053	0.003564	0.014	-0.00189
1.677	-0.00726	0.272	-0.00345	0.057	-0.00223	0.011	0.001109
1.670	-0.00744	0.263	-0.0039	0.049	-0.00479	0.016	0.001223
1.662	-0.00719	0.264	0.001663	0.048	-0.00178	0.014	-0.00067
1.655	-0.00719	0.266	-0.00089	0.045	0.000889	0.015	-0.00055
1.648	-0.00715	0.263	0.001672	0.049	0.003775	0.013	0.000229
1.641	-0.0073	0.270	-0.00089	0.053	-0.00044	0.015	0.002218
1.633	-0.00722	0.261	-0.0029	0.048	-0.005	0.017	-0.00122
1.626	-0.00675	0.264	-0.00067	0.043	-0.00023	0.013	-0.00166
1.620	-0.00763	0.260	-0.00334	0.048	0.002666	0.014	0.004338
1.611	-0.00733	0.257	0.000334	0.048	0.000783	0.021	0.000669
1.605	-0.00678	0.260	0.001778	0.050	-0.00089	0.015	-0.00479
1.598	-0.00673	0.261	-0.00111	0.046	-0.0039	0.012	0.001443
1.592	-0.00695	0.258	-0.00311	0.042	0.000554	0.018	-0.00077
1.584	-0.00704	0.254	-0.001	0.048	0.002226	0.010	-0.00156
1.578	-0.00675	0.256	-0.00033	0.046	-0.002	0.015	0.001663
1.570	-0.00748	0.254	-0.00223	0.044	0.000449	0.014	-0.00125
1.563	-0.00728	0.252	-0.00278	0.047	-0.00167	0.012	-0.00116

			Air stripping				
N₂O (mg/L)	N₂O emission (mg/(L· min))	N₂O (mg/L)	N₂O emission (mg/(L· min))	N₂O (mg/L)	N₂O emission (mg/(L· min))	N₂O (mg/L)	N₂O emission (mg/(L· min))
4.148	-0.24376	1.001	-0.07656	0.170	-0.01628	0.026	-0.00198
3.902	-0.22484	0.906	-0.06886	0.155	-0.01232	0.023	-0.00242
3.699	-0.19228	0.864	-0.05346	0.145	-0.00924	0.021	-0.00176
3.527	-0.20878	0.799	-0.06226	0.136	-0.0077	0.020	-0.00154
3.281	-0.2134	0.739	-0.05676	0.130	-0.0088	0.018	-0.00176
3.101	-0.18128	0.686	-0.0583	0.119	-0.00858	0.016	-0.0011
2.918	-0.1837	0.623	-0.0506	0.113	-0.00858	0.016	-0.00044
2.733	-0.16962	0.585	-0.04004	0.102	-0.00946	0.015	-0.00044
2.579	-0.15994	0.543	-0.04004	0.094	-0.00572	0.015	-0.00044
2.413	-0.16478	0.504	-0.03762	0.090	-0.00594	0.015	-0.00044
2.250	-0.15356	0.467	-0.0407	0.082	-0.00792	0.014	-0.00066
2.106	-0.14718	0.423	-0.04048	0.074	-0.00726	0.013	-0.00044
1.955	-0.13332	0.386	-0.03256	0.068	-0.00704	0.013	-0.00022
1.840	-0.10538	0.358	-0.02772	0.060	-0.00704	0.013	-0.00022
1.745	-0.1056	0.331	-0.0297	0.054	-0.00572	0.013	0
1.628	-0.11814	0.299	-0.02904	0.049	-0.00396	0.013	-0.00022
1.508	-0.10318	0.273	-0.02794	0.046	-0.0033	0.012	-0.00022
1.422	-0.09086	0.243	-0.0264	0.042	-0.00374	0.012	0
1.327	-0.08866	0.220	-0.01518	0.038	-0.00462	0.012	0
1.245	-0.08756	0.212	-0.00902	0.033	-0.00418		
1.152	-0.09262	0.202	-0.01254	0.030	-0.00286		
1.059	-0.07502	0.187	-0.01584	0.027	-0.00198		

E.2 Typical cycle profile (Day 135) of soluble N₂O and COD concentrations

Time (min)	COD in IASBR-1 (mg/L)	COD in IASBR-2 (mg/L)	Time (min)	N₂O in IASBR-1 (mg/L)	N₂O in IASBR-2 (mg/L)
0	1522	341	0	4.09112	2.86528
30	1493	302	1	4.21916	2.93788
60	1454	264	2	4.29968	3.0184
80	1435	235	3	4.34808	3.0624
110	1406	206	4	4.38812	3.10728
140	1373	192	5	4.4506	3.1702
160	1349	178	6	4.53112	3.25072
190	1320	149	7	4.5936	3.3132
220	1286	106	8	4.66444	3.38448
240	1258	86	9	4.71856	3.4386
270	1224	72	10	4.7718	3.49228
300	1200	43	11	4.84308	3.56356
320	1181	38	12	4.9236	3.64452
350	1171	29	13	4.96804	3.68896
380	1166	5	14	5.05736	3.77872
400	1166	0	15	5.10224	3.8236
430	1166	0	16	5.15548	3.87684
475	1162	0	17	5.21796	3.93976
Time (min)	N₂O (mg/L) in IASBR-1	N₂O (mg/L) in IASBR-2	18	5.30728	4.02952

247	0.8866	0.63668	19	5.33368	4.05592
248	0.87208	0.62172	20	5.40452	4.1272
249	0.8536	0.60324	21	5.45776	4.18044
250	0.83952	0.58916	22	5.52024	4.24336
251	0.8228	0.57244	23	5.59196	4.31508
252	0.81488	0.56408	24	5.65444	4.37844
253	0.80608	0.55528	25	5.7442	4.46776
254	0.7964	0.54604	26	5.77896	4.50252
255	0.78452	0.53372	27	5.85992	4.58392
256	0.77836	0.52756	28	5.90392	4.62792
257	0.76604	0.51524	29	5.9664	4.69084
258	0.75592	0.50512	30	6.0104	4.74364
259	0.74932	0.49852	31	6.09136	4.81624
260	0.7392	0.4884	32	6.1622	4.88752
261	0.72864	0.4774	33	6.21632	4.94164
262	0.72292	0.47212	34	6.26032	4.98564
263	0.715	0.4642	35	6.34084	5.06616
264	0.71148	0.46068	36	6.37604	5.1018
265	0.70356	0.45232	37	6.4658	5.19156
266	0.6974	0.4466	38	6.51904	5.24524
267	0.6886	0.4378	39	6.6	5.3306
268	0.67584	0.4246	40	6.62552	5.36096
269	0.66528	0.41404	41	6.71572	5.44236
270	0.649	0.39776	42	6.74124	5.46832
271	0.71148	0.46068	43	6.8398	5.56732
272	0.78496	0.53416	44	6.88468	5.6122
273	0.8734	0.62304	45	6.95552	5.68348
274	0.9438	0.69388	46	7.02724	5.7552
275	1.01816	0.76824	47	7.08048	5.80888
276	1.089	0.83952	48	7.17948	5.90788
277	1.16072	0.91168	49	7.21468	5.94352
278	1.23156	0.98296	50	7.2952	6.02404
279	1.30108	1.05248	51	7.3392	6.06804
280	1.36972	1.12156	52	7.40168	6.13096
281	1.43616	1.18844	53	7.45492	6.1842
282	1.50436	1.25664	54	7.50816	6.23788
283	1.57168	1.3244	55	7.58912	6.31884
284	1.63636	1.38952	56	7.62388	6.35404
285	1.69884	1.45244	57	7.71408	6.44424
286	1.76836	1.52196	58	7.722	6.45216
287	1.83304	1.58708	59	7.82056	6.55116
288	1.8942	1.64868	60	8.06168	6.77732
289	1.95976	1.71424	61	7.546	6.34876
290	2.02444	1.77892	62	7.27232	6.11292
291	2.08956	1.84448	63	6.99776	5.8762
292	2.15292	1.90828	64	6.72408	5.64036
293	2.13884	1.8942	65	6.44996	5.40408
294	2.2198	1.97516	66	6.17628	5.15944
295	2.29196	2.04776	67	5.90172	4.93196
296	2.36808	2.12388	68	5.61572	4.68512
297	2.44816	2.2044	69	5.3878	4.50208
298	2.5168	2.27348	70	5.05032	4.19804
299	2.59204	2.34916	71	4.81448	3.9952
300	2.59292	2.34916	72	4.56852	3.78312

301	2.38436	2.13576	73	4.34016	3.586
302	2.2418	1.98924	74	4.11356	3.39064
303	2.06448	1.80752	75	3.88124	3.19088
304	2.04468	1.78728	76	3.68148	3.0184
305	1.92984	1.66936	77	3.44476	2.81468
306	1.8326	1.56992	78	3.30792	2.69676
307	1.75208	1.48764	79	3.19792	2.60216
308	1.661	1.39436	80	3.01752	2.4464
309	1.59368	1.32528	81	2.8248	2.28052
310	1.51932	1.24872	82	2.73636	2.20396
311	1.45332	1.18096	83	2.56212	2.05392
312	1.38732	1.1132	84	2.45828	1.9646
313	1.32968	1.05468	85	2.36588	1.88496
314	1.25972	0.98296	86	2.255	1.78948
315	1.22804	0.9504	87	2.11904	1.672
316	1.1858	0.90728	88	2.06316	1.62404
317	1.14708	0.86724	89	1.97428	1.54748
318	1.1088	0.82808	90	1.87088	1.45816
319	1.07448	0.79288	91	1.80224	1.3992
320	1.0428	0.76032	92	1.74812	1.35256
321	1.01816	0.73524	93	1.65792	1.27512
322	0.99132	0.70752	94	1.61348	1.2364
323	0.96272	0.67848	95	1.51844	1.15456
324	0.9438	0.65868	96	1.48544	1.1264
325	0.92444	0.63932	97	1.43352	1.08152
326	0.90464	0.61908	98	1.38952	1.04368
327	0.8888	0.6028	99	1.35168	1.01112
328	0.87296	0.58652	100	1.29316	0.96052
329	0.85976	0.57288	101	1.26104	0.9328
330	0.84612	0.5588	102	1.22584	0.90244
331	0.83028	0.54252	103	1.17612	0.87296
332	0.81972	0.53152	104	1.15236	0.83908
333	0.80608	0.51832	105	1.1242	0.81488
334	0.79552	0.50732	106	1.09956	0.79376
335	0.78364	0.495	107	1.06524	0.76428
336	0.7744	0.48576	108	1.03796	0.74052
337	0.76516	0.47608	109	1.0098	0.71632
338	0.7568	0.46728	110	1.02564	0.72468
339	0.74888	0.45936	111	1.05688	0.75724
340	0.74228	0.45232	112	1.12288	0.825
341	0.7348	0.44528	113	1.19592	0.90068
342	0.72512	0.43472	114	1.26588	0.97328
343	0.72116	0.43076	115	1.33452	1.04412
344	0.71632	0.42592	116	1.4036	1.1154
345	0.71236	0.42196	117	1.47048	1.18448
346	0.70928	0.41888	118	1.54044	1.25708
347	0.70532	0.41448	119	1.60864	1.32748
348	0.6974	0.40656	120	1.67816	1.3992
349	0.69784	0.407	121	1.74724	1.47092
350	0.682	0.39116	122	1.81324	1.53912
351	0.73216	0.4422	123	1.88012	1.6082
352	0.7876	0.4994	124	1.94568	1.67596
353	0.85976	0.57288	125	2.01124	1.74416
354	0.93104	0.64592	126	2.07592	1.8106

355	1.00188	0.71852	127	2.14016	1.87748
356	1.07316	0.79156	128	2.20836	1.94788
357	1.1396	0.85976	129	2.27348	2.0152
358	1.2056	0.92752	130	2.34476	2.08868
359	1.27512	0.99836	131	2.40812	2.15424
360	1.34244	1.06744	132	2.46752	2.2154
361	1.4102	1.13696	133	2.5344	2.28492
362	1.47488	1.2034	134	2.59996	2.35268
363	1.5422	1.27248	135	2.662	2.41692
364	1.60512	1.33672	136	2.73152	2.48864
365	1.66848	1.4014	137	2.794	2.55332
366	1.72832	1.463	138	2.85164	2.61272
367	1.7952	1.53164	139	2.9216	2.68532
368	1.85724	1.595	140	2.94228	2.70116
369	1.92192	1.66144	141	2.7478	2.5058
370	1.97912	1.7204	142	2.55244	2.30956
371	2.04116	1.78376	143	2.45256	2.20924
372	2.10144	1.84536	144	2.31132	2.06712
373	2.1626	1.90828	145	2.20748	1.96284
374	2.222	1.969	146	2.07504	1.82996
375	2.28272	2.03148	147	1.96592	1.7204
376	2.29328	2.04204	148	1.881	1.63504
377	2.35752	2.10804	149	1.80356	1.56156
378	2.43012	2.1824	150	1.71864	1.4718
379	2.50448	2.25852	151	1.63372	1.38688
380	2.52428	2.24972	152	1.53868	1.2914
381	2.35796	2.11596	153	1.50832	1.26104
382	2.27436	2.04248	154	1.44584	1.19812
383	2.16216	1.96812	155	1.39788	1.15016
384	2.0526	1.89464	156	1.34684	1.09868
385	1.95756	1.82072	157	1.29228	1.04368
386	1.88012	1.74724	158	1.24828	0.99968
387	1.79696	1.67332	159	1.21484	0.9658
388	1.72304	1.59632	160	1.17304	0.924
389	1.64912	1.53472	161	1.13256	0.88352
390	1.59368	1.44408	162	1.10176	0.85228
391	1.53428	1.38072	163	1.06128	0.8118
392	1.41636	1.31428	164	1.0296	0.78012
393	1.28084	1.25268	165	0.99572	0.74624
394	1.18008	1.14752	166	0.96888	0.71896
395	1.09692	1.12904	167	0.95524	0.70532
396	1.03092	1.07536	168	0.9284	0.67848
397	0.97064	1.01156	169	0.90376	0.6534
398	0.92576	0.9746	170	0.88616	0.6358
399	0.8844	0.94512	171	0.87516	0.6248
400	0.84876	0.89672	172	0.85316	0.6028
401	0.81444	0.8448	173	0.83908	0.58828
402	0.78804	0.8206	174	0.81884	0.56804
403	0.76692	0.77396	175	0.81136	0.56056
404	0.748	0.7458	176	0.80388	0.55352
405	0.73436	0.72116	177	0.79464	0.54384
406	0.7216	0.69124	178	0.7788	0.52844
407	0.71192	0.65472	179	0.7722	0.5214
408	0.70092	0.63932	180	0.76296	0.51216

409	0.69564	0.61556	181	0.7458	0.495
410	0.68948	0.58784	182	0.74536	0.49456
411	0.68288	0.56936	183	0.72512	0.47432
412	0.6798	0.55484	184	0.71984	0.4686
413	0.67584	0.53064	185	0.71764	0.46684
414	0.67188	0.51832	186	0.71104	0.4598
415	0.6688	0.4928	187	0.704	0.4532
416	0.66616	0.484	188	0.69608	0.44528
417	0.66308	0.46992	189	0.69476	0.44352
418	0.66264	0.45804	190	0.69828	0.44704
419	0.66088	0.44792	191	0.73128	0.48004
420	0.65912	0.43208	192	0.79156	0.5412
421	0.6578	0.42372	193	0.86328	0.61292
422	0.65516	0.41404	194	0.93192	0.682
423	0.65472	0.40084	195	1.00012	0.7502
424	0.65296	0.39424	196	1.06656	0.81708
425	0.6512	0.38676	197	1.13256	0.88352
426	0.64988	0.38016	198	1.2012	0.9526
427	0.64812	0.37092	199	1.26588	1.01728
428	0.6468	0.36344	200	1.28788	1.08372
429	0.64548	0.35596	201	1.35388	1.15016
430	0.627	0.3586	202	1.3728	1.21264
431	0.64768	0.40656	203	1.44012	1.2804
432	0.71324	0.42548	204	1.46124	1.34596
433	0.7502	0.45232	205	1.52416	1.40888
434	0.82456	0.50732	206	1.54308	1.47268
435	0.91916	0.57684	207	1.60996	1.53956
436	1.00232	0.63844	208	1.7138	1.59984
437	1.08504	0.6996	209	1.73316	1.6632
438	1.16644	0.75944	210	1.79432	1.7248
439	1.24652	0.8184	211	1.85856	1.78904
440	1.3266	0.87736	212	1.92324	1.85416
441	1.4058	0.93544	213	1.93996	1.91488
442	1.48368	0.99308	214	2.04644	1.9778
443	1.55892	1.04852	215	2.06888	2.04512
444	1.63592	1.10528	216	2.156	2.13224
445	1.71336	1.16204	217	2.23476	2.21144
446	1.7886	1.21748	218	2.30736	2.28448
447	1.86208	1.2716	219	2.35048	2.3276
448	1.9316	1.32308	220	2.38788	2.365
449	2.0042	1.37676	221	2.35048	2.23916
450	2.08032	1.43264	222	2.31044	2.06668
451	2.156	1.48808	223	2.10232	1.85768
452	2.22904	1.5422	224	1.96372	1.7182
453	2.30076	1.595	225	1.8942	1.64868
454	2.37336	1.64868	226	1.80664	1.56068
455	2.44508	1.70148	227	1.71468	1.46784
456	2.51108	1.74988	228	1.64384	1.397
457	2.58104	1.80136	229	1.56508	1.3178
458	2.64968	1.85196	230	1.49908	1.25136
459	2.71832	1.903	231	1.43616	1.18844
460	2.77244	1.9426	232	1.3838	1.13564
461	2.88024	2.0218	233	1.32396	1.07536
462	2.99772	2.10848	234	1.27688	1.02828

463	3.0206	2.1252	235	1.23156	0.98296
464	3.12004	2.19868	236	1.18976	0.94072
465	3.14556	2.2176	237	1.15368	0.9042
466	3.2758	2.31352	238	1.11496	0.86592
467	3.38492	2.39404	239	1.08548	0.836
468	3.43112	2.42792	240	1.05512	0.80564
469	3.50944	2.48512	241	1.02476	0.77528
470	3.55608	2.51988	242	0.99308	0.7436
471	3.6652	2.6004	243	0.9702	0.72028
472	3.71228	2.63472	244	0.94468	0.69476
473	3.84208	2.73064	245	0.92972	0.6798
474	3.93052	2.79576	246	0.9064	0.65604
475	3.98772	2.83756			

E.3 Profile of N_2O emission rates in the two intermittently aerated sequencing batch reactors (IASBRs) during a typical cycle

Time (min)	N_2O emission (mg/(L·min)) in IASBR-1	N_2O emission (mg/(L·min)) in IASBR-2	Time (min)	N_2O emission (mg/(L·min)) in IASBR-1	N_2O emission (mg/(L·min)) in IASBR-2	Time (min)	N_2O emission (mg/(L·min)) in IASBR-1	N_2O emission (mg/(L·min)) in IASBR-2
0	-0.01537	-0.01079	160	-0.0662	-0.05119	320	-0.0597	-0.04262
5	-0.01653	-0.01192	165	-0.05721	-0.04217	325	-0.05359	-0.03637
10	-0.01769	-0.01308	170	-0.05144	-0.03636	330	-0.04934	-0.03201
15	-0.0188	-0.0142	175	-0.04769	-0.0326	335	-0.0461	-0.0287
20	-0.01989	-0.0153	180	-0.04454	-0.02943	340	-0.04382	-0.02636
25	-0.02106	-0.01647	185	-0.04242	-0.02731	345	-0.04241	-0.02491
30	-0.02213	-0.01755	190	-0.00289	-0.00199	350	-0.00287	-0.00184
35	-0.02326	-0.01867	195	-0.00408	-0.00318	355	-0.0041	-0.00309
40	-0.02434	-0.01976	200	-0.00498	-0.00437	360	-0.00531	-0.00433
45	-0.02553	-0.02095	205	-0.00585	-0.00553	365	-0.00646	-0.00551
50	-0.02664	-0.02206	210	-0.00689	-0.00667	370	-0.00756	-0.00664
55	-0.0277	-0.02313	215	-0.008	-0.00792	375	-0.00855	-0.00765
60	-0.44069	-0.3703	220	-0.13382	-0.12337	380	-0.13691	-0.12366
65	-0.35556	-0.29718	225	-0.10384	-0.089	385	-0.10844	-0.1008
70	-0.27556	-0.22819	230	-0.08332	-0.06838	390	-0.08434	-0.07873
75	-0.21086	-0.17245	235	-0.06954	-0.05454	395	-0.0591	-0.06183
80	-0.16373	-0.13183	240	-0.06005	-0.04502	400	-0.04775	-0.04915
85	-0.12976	-0.10256	245	-0.05356	-0.0385	405	-0.04292	-0.04
90	-0.10466	-0.08093	250	-0.04912	-0.03404	410	-0.04093	-0.03324
95	-0.08643	-0.06522	255	-0.04616	-0.03107	415	-0.03999	-0.02833
100	-0.07355	-0.05427	260	-0.04355	-0.02845	420	-0.03949	-0.02486
105	-0.06425	-0.04611	265	-0.04131	-0.02619	425	-0.03903	-0.02236
110	-0.06823	-0.00301	270	-0.00285	-0.00195	430	-0.00257	-0.00155
115	-0.0053	-0.00427	275	-0.00418	-0.00328	435	-0.0039	-0.00251
120	-0.00653	-0.00554	280	-0.00541	-0.00452	440	-0.00534	-0.00357
125	-0.00771	-0.00676	285	-0.00659	-0.00571	445	-0.0067	-0.00457
130	-0.0089	-0.00799	290	-0.00765	-0.00677	450	-0.00802	-0.00555
135	-0.01005	-0.00918	295	-0.0088	-0.00792	455	-0.00929	-0.00649

140	-0.1566	-0.14199	300	-0.13639	-0.12123	460	-0.01065	-0.00749
145	-0.11959	-0.10487	305	-0.10558	-0.08966	465	-0.01206	-0.00852
150	-0.09446	-0.07958	310	-0.08367	-0.06719	470	-0.01347	-0.00956
155	-0.07826	-0.06331	315	-0.06916	-0.05232	475	-0.01436	-0.01022

E.4 Profiles of DO and FNA concentrations in a typical cycle

Time (min)	FNA in IASBR-1 (mg/L)	FNA in IASBR-2 (mg/L)
0	0.0291	0.0029
15	0.0240	0.0024
30	0.0189	0.0021
45	0.0151	0.0015
60	0.0125	0.0013
65	0.0144	0.0014
80	0.0176	0.0020
95	0.0213	0.0027
110	0.0270	0.0037
125	0.0219	0.0026
140	0.0172	0.0018
145	0.0185	0.0019
160	0.0224	0.0024
175	0.0272	0.0029
190	0.0330	0.0038
205	0.0274	0.0028
220	0.0242	0.0020
225	0.0256	0.0021
240	0.0284	0.0024
255	0.0316	0.0030
270	0.0349	0.0037
285	0.0290	0.0029
300	0.0262	0.0022
305	0.0276	0.0023
320	0.0298	0.0026
335	0.0329	0.0030
350	0.0353	0.0039
365	0.0329	0.0033
380	0.0306	0.0027
385	0.0313	0.0028
400	0.0326	0.0033
415	0.0342	0.0037
430	0.0356	0.0041
445	0.0331	0.0036
460	0.0316	0.0032
475	0.0309	0.0031

E.5 Profile of NO$_2^-$-N concentrations in a typical cycle in the two reactors

Time (min)	NO$_2^-$-N (mg/L)	NO$_2^-$-N (mg/L)
0	678	65.0
15	671	58.7
30	665	53.6
45	657	45.2
60	654	39.7
65	652	40.6
80	664	44.8
95	670	49.1
110	675	52.5
125	671	47.7
140	663	43.1
145	668	44.4
160	673	46.6
175	680	48.4
190	686	52.2
205	684	50.4
220	679	45.1
225	685	44.8
240	693	48.3
255	702	54.7
270	709	59.0
285	707	55.4
300	701	47.2
305	705	48.5
320	711	51.7
335	716	56.4
350	717	63.2
365	716	60.4
380	712	55.7
385	712	57.2
400	709	62.7
415	709	66.8
430	706	71.6
445	704	68.5
460	703	67.3
475	703	66.3

Appendix F: Nutrient Removal and Nitrous Oxide Emissions from Aerobic Granular Sludge SBRs Study Data

Associated with Chapter 6

F.1 Linear dependence of N_2O emission rates on the dissolved N_2O concentration in the clear water test

N_2O in (mg/L)			N_2O emission rates (mg/(L• min))		
SBR1	SBR2	SBR3	SBR1	SBR2	SBR3
6.173948	5.767696	6.146668	-0.65912	-0.73621	-0.99678
5.514828	5.031488	5.149892	-0.61389	-0.72274	-0.79482
4.946172	4.322252	4.557036	-0.52633	-0.62511	-0.61552
4.462216	3.781316	3.91886	-0.47863	-0.44431	-0.59906
3.988908	3.433672	3.358916	-0.45509	-0.40436	-0.46068
3.552032	2.97264	2.997544	-0.41285	-0.37404	-0.39147
3.163204	2.685584	2.576024	-0.35917	-0.32824	-0.37237
2.833732	2.316204	2.252844	-0.33167	-0.28622	-0.34346
2.499904	2.113144	1.88914	-0.30272	-0.264	-0.32899
2.228292	1.788248	1.594868	-0.25859	-0.26083	-0.26237
1.982772	1.59148	1.36444	-0.23949	-0.2042	-0.21908
1.749352	1.37984	1.15676	-0.22238	-0.16733	-0.16667
1.53802	1.25686	1.03114	-0.20161	-0.14322	-0.13059
1.346136	1.0934	0.895576	-0.18872	-0.12668	-0.13042
1.160588	1.003508	0.770308	-0.16918	-0.08813	-0.10899
1.00782	0.91718	0.6776	-0.14885	-0.08562	-0.10111
0.862884	0.832304	0.568128	-0.13644	-0.10001	-0.09715
0.734932	0.7172	0.483296	-0.11946	-0.10745	-0.07225
0.624008	0.617408	0.423676	-0.10881	-0.0752	-0.04778
0.517308	0.566808	0.387728	-0.10512	-0.04712	-0.05034
0.41382	0.523204	0.323048	-0.09486	-0.05724	-0.05636
0.327624	0.452364	0.275	-0.06371	-0.05408	-0.05003
0.28644	0.415052	0.222992	-0.03762	-0.0363	-0.04017
0.252384	0.379808	0.1947	-0.02974	-0.04096	-0.04338
0.226996	0.333168	0.136224	-0.02627	-0.0374	-0.06208
0.199892	0.305052	0.070532	-0.03709	-0.02583	-0.04374
0.152812	0.281556	0.048752	-0.03652	-0.02939	-0.0136
0.126852	0.246268	0.043384	-0.037	-0.05192	-0.01016
0.078848	0.17776	0.028424	-0.03516	-0.04959	-0.0114
0.056584	0.147136	0.020636	-0.02253	-0.02583	-0.00396
0.033792	0.126104	0.020548	-0.01562	-0.02394	-0.00198
0.025388	0.099264	0.016676	-0.0092	-0.02869	-0.0033
0.015444	0.068728	0.013948	-0.00682	-0.02218	-0.0022
0.011792	0.054912	0.01232	-0.00383	-0.0128	-0.00132
0.007788	0.043164	0.011308	-0.0026	-0.01188	-0.00123
0.006644	0.031196	0.0099	-0.00114	-0.01197	-0.00141

F.2 Profiles of N₂O and DO in a typical cycle at different COD: N ratios

Time (min)	N₂O (mg/L)								
	COD: N= 1: 0.22			COD: N= 1: 0.15			COD: N= 1: 0.11		
	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3
0	0.3964	0.4307	0.4296	0.4663	0.2856	0.2161	0.2687	0.2159	0.1511
1	0.4483	0.4272	0.4184	0.4619	0.3063	0.2438	0.2829	0.2301	0.1462
2	0.4221	0.3825	0.4346	0.4417	0.3284	0.1886	0.3441	0.2200	0.1678
3	0.4305	0.4148	0.4149	0.4098	0.3123	0.2261	0.2805	0.2277	0.1690
4	0.3982	0.3928	0.3697	0.3958	0.2799	0.2394	0.2568	0.2040	0.1695
5	0.3899	0.3912	0.3373	0.3333	0.2528	0.2110	0.2174	0.1646	0.1514
6	0.3752	0.3896	0.3504	0.3197	0.2457	0.2652	0.1709	0.1621	0.1684
7	0.4026	0.3636	0.3685	0.3434	0.2438	0.2752	0.1529	0.1408	0.1857
8	0.3574	0.3626	0.3211	0.3018	0.2554	0.2657	0.1421	0.1320	0.1414
9	0.3813	0.3615	0.3200	0.2917	0.2656	0.2756	0.1605	0.1302	0.1405
10	0.3769	0.3357	0.3402	0.2994	0.2365	0.2771	0.1884	0.1356	0.1541
11	0.3722	0.3604	0.3062	0.2992	0.2368	0.2662	0.1848	0.1320	0.1580
12	0.3763	0.3433	0.3069	0.3091	0.2740	0.2437	0.1878	0.1350	0.1522
13	0.3763	0.3487	0.3333	0.3140	0.2541	0.2161	0.1860	0.1332	0.1599
14	0.3739	0.3406	0.3006	0.2886	0.2708	0.2481	0.1840	0.1312	0.1640
15	0.3724	0.3429	0.2882	0.3111	0.2341	0.2158	0.2098	0.1570	0.1517
16	0.3780	0.3429	0.2487	0.3266	0.2621	0.1780	0.1912	0.1384	0.1385
17	0.3709	0.3362	0.2403	0.3277	0.2414	0.2231	0.1766	0.1502	0.1455
18	0.3702	0.3341	0.2119	0.3096	0.2493	0.2080	0.1631	0.1434	0.1343
19	0.3787	0.3219	0.2493	0.2965	0.2354	0.1405	0.1612	0.1408	0.1536
20	0.3584	0.3454	0.2338	0.2824	0.2368	0.1908	0.1796	0.1268	0.1434
21	0.3619	0.3472	0.1871	0.3073	0.2369	0.1584	0.1684	0.1434	0.1408
22	0.3580	0.3477	0.1824	0.2830	0.2663	0.1701	0.1683	0.1375	0.1340
23	0.3630	0.3126	0.1623	0.3048	0.2441	0.1599	0.1811	0.1503	0.1216
24	0.3761	0.3146	0.1830	0.2888	0.2720	0.1752	0.1948	0.1386	0.1338
25	0.3512	0.3006	0.1966	0.3029	0.2554	0.1349	0.1733	0.1425	0.1122
26	0.3759	0.3206	0.2121	0.3093	0.2697	0.1434	0.1891	0.1434	0.1095
27	0.3633	0.3144	0.2141	0.2855	0.2712	0.1595	0.1719	0.1411	0.0955
28	0.3536	0.3212	0.1604	0.3212	0.2617	0.1978	0.1918	0.1408	0.1069
29	0.3795	0.3150	0.1404	0.3010	0.2698	0.1805	0.1808	0.1355	0.1122
30	0.3710	0.3192	0.1216	0.3112	0.2455	0.1940	0.1644	0.1336	0.1056
31	0.3754	0.3146	0.1321	0.3090	0.2673	0.1865	0.1723	0.1415	0.0945
32	0.3568	0.3231	0.1092	0.3150	0.2617	0.1786	0.2034	0.1408	0.1019
33	0.3737	0.3216	0.1170	0.3080	0.2379	0.1462	0.1620	0.1312	0.0748
34	0.3584	0.3204	0.1140	0.3447	0.2404	0.1821	0.1649	0.1341	0.0692
35	0.3710	0.3285	0.1477	0.3390	0.2353	0.1426	0.1894	0.1586	0.0982
36	0.3849	0.3261	0.1530	0.3269	0.2542	0.1506	0.1817	0.1509	0.0924
37	0.4134	0.3158	0.1539	0.3546	0.2389	0.1534	0.2241	0.1571	0.0822
38	0.3946	0.3135	0.1624	0.3661	0.2321	0.1599	0.2056	0.1496	0.0706
39	0.3835	0.3141	0.1693	0.3384	0.2690	0.1610	0.2296	0.1487	0.0648
40	0.3813	0.3073	0.1943	0.3568	0.2299	0.1556	0.2188	0.1355	0.07
41	0.4101	0.2868	0.1386	0.3432	0.2313	0.1619	0.2163	0.1338	0.05
42	0.3810	0.3038	0.1489	0.3595	0.2618	0.1617	0.2091	0.1285	0.05

43	0.4077	0.2766	0.1659	0.3432	0.2401	0.1716	0.2186	0.1452	0.0714
44	0.3989	0.2728	0.1540	0.3475	0.2679	0.1645	0.2037	0.1206	0.0814
45	0.4060	0.2780	0.1752	0.3630	0.2504	0.1758	0.1895	0.1285	0.0662
46	0.3809	0.2857	0.1424	0.3520	0.2387	0.1615	0.2034	0.1219	0.0455
47	0.3847	0.2881	0.1761	0.3603	0.2534	0.1513	0.2001	0.1219	0.0380
48	0.3821	0.3120	0.1723	0.3688	0.2512	0.1606	0.1929	0.1232	0.0495
49	0.3921	0.2911	0.1455	0.3725	0.2621	0.1322	0.2010	0.1104	0.0502
50	0.3893	0.2936	0.1448	0.3513	0.2378	0.1449	0.2097	0.1144	0.0626
51	0.3792	0.2891	0.1170	0.3629	0.2377	0.1568	0.1825	0.1153	0.0392
52	0.3891	0.2895	0.1422	0.3531	0.2637	0.1496	0.1833	0.0990	0.0154
53	0.3882	0.2970	0.1371	0.3270	0.2674	0.1527	0.1911	0.1021	0.0423
54	0.3831	0.2959	0.1481	0.3668	0.2361	0.1387	0.1943	0.1087	0.0472
55	0.4048	0.3106	0.1452	0.3566	0.2683	0.1522	0.1569	0.1069	0.0395
56	0.3913	0.2919	0.1489	0.3546	0.2495	0.1337	0.1704	0.0968	0.0312
57	0.4307	0.3140	0.1399	0.3780	0.2614	0.1337	0.1600	0.1003	0.0381
58	0.4272	0.2928	0.1801	0.3729	0.2474	0.1287	0.1813	0.0937	0.0367
59	0.4485	0.2997	0.1602	0.3696	0.2441	0.1240	0.1616	0.0906	0.0235
60	0.4368	0.2973	0.1591	0.3131	0.2544	0.1535	0.1864	0.0920	0.0367
61	0.4588	0.3062	0.1334	0.3711	0.2299	0.1627	0.1573	0.0823	0.0238
62	0.4572	0.2651	0.1449	0.3266	0.2547	0.1265	0.1548	0.1020	0.0089
63	0.4556	0.2963	0.1113	0.3333	0.2392	0.1382	0.1425	0.0897	0.0084
64	0.4296	0.2807	0.1385	0.3301	0.1852	0.1265	0.1615	0.0854	0.0376
65	0.4286	0.2778	0.1577	0.3000	0.2056	0.1442	0.1115	0.0906	0.0169
66	0.4275	0.2708	0.1158	0.3359	0.2185	0.1348	0.1199	0.0671	0.0211
67	0.4325	0.2778	0.1245	0.3003	0.1744	0.1156	0.1304	0.0776	0.0340
68	0.4264	0.2826	0.1295	0.3060	0.1701	0.1216	0.1013	0.0805	0.0353
69	0.4533	0.2641	0.1447	0.2963	0.1690	0.1263	0.1176	0.0648	0.0073
70	0.4587	0.2772	0.1450	0.2959	0.1662	0.1299	0.1265	0.0737	0.0024
71	0.4506	0.2843	0.1603	0.2984	0.1878	0.0991	0.1144	0.0616	0.0052
72	0.4529	0.2977	0.1437	0.2688	0.1639	0.0926	0.0946	0.0418	0.0288
73	0.4529	0.2967	0.1393	0.2615	0.1461	0.0909	0.0905	0.0377	0.0253
74	0.4462	0.2692	0.1105	0.2543	0.1837	0.0760	0.0513	0.0323	0.0312
75	0.4441	0.3031	0.1108	0.2390	0.1618	0.0959	0.0493	0.0343	0.0383
76	0.4319	0.3072	0.0960	0.2177	0.1800	0.1038	0.0419	0.0241	0.0024
77	0.4554	0.2949	0.0898	0.2314	0.1747	0.1051	0.0670	0.0142	0.0178
78	0.4572	0.2864	0.1035	0.2363	0.1741	0.0851	0.0473	0.0231	0.0017
79	0.4577	0.3014	0.0912	0.2262	0.1931	0.0926	0.0563	0.0211	0.0006
80	0.4226	0.2735	0.1060	0.2043	0.1847	0.0836	0.0769	0.0057	0.0164
81	0.4246	0.2705	0.0958	0.2114	0.1734	0.0640	0.0765	0.0128	0.0040
82	0.4106	0.2886	0.0867	0.2042	0.1608	0.0922	0.0574	0.0046	0.0297
83	0.4306	0.2691	0.1099	0.2011	0.1842	0.1008	0.0704	0.0141	0.0174
84	0.4244	0.2979	0.1188	0.2138	0.1547	0.1098	0.0494	0.0034	0.0129
85	0.4312	0.2655	0.1182	0.2152	0.1441	0.0808	0.0763	0.0264	0.0018
86	0.4250	0.2992	0.1095	0.1932	0.1340	0.0664	0.0600	0.0072	0.0297
87	0.4292	0.2904	0.1082	0.1804	0.1716	0.0863	0.0757	0.0075	0.0176
88	0.4246	0.2740	0.1076	0.1984	0.1694	0.1087	0.0747	0.0110	0.0271
89	0.4331	0.2929	0.1295	0.2089	0.1332	0.0674	0.0534	0.0006	0.0082
90	0.4316	0.2948	0.1209	0.1976	0.1649	0.0750	0.0796	0.0127	0.0210
91	0.4304	0.2913	0.1152	0.1772	0.1431	0.0757	0.0805	0.0150	0.0077
92	0.4385	0.2757	0.1055	0.1633	0.1403	0.0481	0.0595	0.0221	0.0106
93	0.4361	0.2431	0.0928	0.1638	0.1475	0.0795	0.0378	0.0092	0.0083

94	0.4258	0.2611	0.0957	0.1663	0.1107	0.0660	0.0389	0.0231	0.0023
95	0.4367	0.2500	0.1056	0.1866	0.1185	0.0811	0.0493	0.0047	0.0152
96	0.4373	0.2586	0.1043	0.1811	0.1279	0.0709	0.0501	0.0298	0.0262
97	0.4613	0.2339	0.1039	0.1585	0.1008	0.0937	0.0304	0.0205	0.0073
98	0.3968	0.2394	0.1012	0.1591	0.0972	0.0798	0.0412	0.0086	0.0268
99	0.4138	0.2237	0.0906	0.1694	0.0960	0.0527	0.0282	0.0094	0.0107
100	0.3663	0.2277	0.1187	0.1636	0.0906	0.0458	0.0330	0.0172	0.0200
101	0.3561	0.2240	0.0849	0.1654	0.1003	0.0832	0.0435	0.0059	0.0156
102	0.3351	0.2041	0.0935	0.1628	0.0824	0.0455	0.0339	0.0068	0.0262
103	0.3087	0.1923	0.0829	0.1656	0.0805	0.0287	0.0304	0.0218	0.0188
104	0.2962	0.1867	0.1530	0.1613	0.0534	0.0361	0.0339	0.0326	0.0170
105	0.2888	0.1841	0.0963	0.1565	0.0645	0.0075	0.0264	0.0039	0.0121
106	0.2452	0.1721	0.0859	0.1745	0.0482	0.0233	0.0224	0.0038	0.0253
107	0.2277	0.1693	0.1174	0.1734	0.0574	0.0109	0.0411	0.0036	0.0029
108	0.2941	0.1592	0.0796	0.1750	0.0782	0.0157	0.0411	0.0130	0.0221
109	0.2310	0.1691	0.1360	0.1377	0.0710	0.0302	0.0483	0.0142	0.0159
110	0.2482	0.1682	0.1102	0.1369	0.0250	0.0400	0.0208	0.0223	0.0227
111	0.2707	0.1631	0.1124	0.1481	0.0529	0.0277	0.0251	0.0135	0.0243
112	0.2181	0.1848	0.1333	0.1664	0.0168	0.0328	0.0106	0.0115	0.0093
113	0.2241	0.1713	0.1317	0.1320	0.0336	0.0079	0.0130	0.0060	0.0143
114	0.2133	0.1683	0.1247	0.1325	0.0315	0.0070	0.0004	0.0015	0.0080
115	0.2185	0.1361	0.0927	0.1342	0.0191	0.0086	0.0145	0.0210	0.0096
116	0.2062	0.1371	0.0858	0.0659	0.0035	0.0205	0.0191	0.0250	0.0078
117	0.1940	0.1287	0.0785	0.1181	0.0195	0.0143	0.0356	0.0061	0.0072
118	0.1975	0.1534	0.0947	0.1081	0.0175	0.0125	0.0188	0.0219	0.0323
119	0.2151	0.1606	0.0774	0.0948	0.0158	0.0007	0.0362	0.0045	0.0020
120	0.1872	0.1573	0.0621	0.0572	0.0206	0.0040	0.0088	0.0352	0.0230
121	0.1975	0.1504	0.0792	0.0737	0.0071	0.0371	0.0393	0.0118	0.0240
122	0.2016	0.1552	0.0838	0.0686	0.0351	0.0249	0.0176	0.0099	0.0001
123	0.2050	0.1459	0.1082	0.0661	0.0096	0.0530	0.0307	0.0220	0.0225
124	0.2048	0.1517	0.0855	0.0705	0.0101	0.0085	0.0298	0.0140	0.0242
125	0.2064	0.1423	0.0634	0.0606	0.0364	0.0015	0.0255	0.0198	0.0060
126	0.1985	0.1433	0.1163	0.0647	0.0427	0.0176	0.0330	0.0065	0.0069
127	0.1940	0.1452	0.0886	0.0909	0.0091	0.0459	0.0215	0.0048	0.0084
128	0.1835	0.1534	0.0981	0.0646	0.0215	0.0279	0.0215	0.0264	0.0160
129	0.1965	0.1525	0.1086	0.0739	0.0394	0.0325	0.0210	0.0224	0.0289
130	0.1855	0.1572	0.1013	0.0266	0.0428	0.0428	0.0191	0.0132	0.0122
131	0.2008	0.1486	0.0879	0.0208	0.0297	0.0092	0.0125	0.0120	0.0125
132	0.1987	0.1510	0.0874	0.0528	0.0301	0.0385	0.0140	0.0179	0.0073
133	0.1981	0.1470	0.0763	0.0395	0.0415	0.0066	0.0008	0.0150	0.0214
134	0.2037	0.1473	0.0784	0.0490	0.0424	0.0095	0.0105	0.0196	0.0209
135	0.2080	0.1414	0.0977	0.0376	0.0103	0.0353	0.0103	0.0216	0.0046
136	0.1925	0.1417	0.1330	0.0338	0.0369	0.0054	0.0216	0.0181	0.0207
137	0.2020	0.1503	0.1348	0.0340	0.0080	0.0057	0.0202	0.0083	0.0193
138	0.1775	0.1430	0.0873	0.0204	0.0329	0.0217	0.0127	0.0064	0.0020
139	0.1580	0.1418	0.1131	0.0200	0.0384	0.0325	0.0088	0.0085	0.0179
140	0.1687	0.1397	0.1103	0.0478	0.0300	0.0097	0.0078	0.0125	0.0012
141	0.1674	0.1335	0.1191	0.0518	0.0058	0.0340	0.0159	0.0069	0.0017
142	0.1677	0.1371	0.1203	0.0308	0.0012	0.0122	0.0194	0.0075	0.01
143	0.1704	0.1400	0.1146	0.0082	0.0244	0.0073	0.0097	0.0217	0.00
144	0.1721	0.1568	0.1158	0.0156	0.0036	0.0321	0.0070	0.0067	0.02

145	0.1734	0.1490	0.1010	0.0230	0.0248	0.0018	0.0224	0.0036	0.0231
146	0.1560	0.1349	0.0999	0.0113	0.0114	0.0269	0.0120	0.0139	0.0029
147	0.1601	0.1452	0.1158	0.0018	0.0143	0.0282	0.0132	0.0188	0.0052
148	0.1713	0.1232	0.1057	0.0108	0.0292	0.0127	0.0197	0.0372	0.0040
149	0.1744	0.1364	0.1080	0.0059	0.0267	0.0249	0.0202	0.0359	0.0172
150	0.1630	0.1236	0.0983	0.0191	0.0191	0.0173	0.0262	0.0264	0.0173
151	0.1680	0.1164	0.0938	0.0033	0.0174	0.0114	0.0035	0.0108	0.0213
152	0.1679	0.1215	0.1261	0.0093	0.0117	0.0290	0.0119	0.0249	0.0141
153	0.1781	0.1289	0.1180	0.0076	0.0219	0.0283	0.0043	0.0180	0.0100
154	0.1836	0.1243	0.1088	0.0052	0.0406	0.0261	0.0128	0.0075	0.0090
155	0.1796	0.1223	0.1085	0.0111	0.0277	0.0429	0.0052	0.0209	0.0010
156	0.1762	0.1323	0.1187	0.0604	0.0327	0.0159	0.0097	0.0194	0.0168
157	0.1892	0.1155	0.0764	0.0061	0.0128	0.0301	0.0166	0.0092	0.0029
158	0.1847	0.1263	0.1071	0.0136	0.0095	0.0282	0.0047	0.0168	0.0099
159	0.1688	0.1120	0.1078	0.0224	0.0307	0.0073	0.0053	0.0226	0.0141
160	0.1748	0.1270	0.1119	0.0253	0.0303	0.0052	0.0119	0.0107	0.0064
161	0.1821	0.1216	0.0879	0.0245	0.0081	0.0184	0.0058	0.0150	0.0146
162	0.1797	0.1190	0.1010	0.0128	0.0271	0.0346	0.0120	0.0175	0.0088
163	0.1466	0.1255	0.0962	0.0120	0.0011	0.0031	0.0062	0.0220	0.0013
164	0.1577	0.1194	0.1044	0.0154	0.0293	0.0368	0.0051	0.0036	0.0203
165	0.1591	0.1120	0.1043	0.0432	0.0303	0.0334	0.0152	0.0134	0.0126
166	0.1698	0.1118	0.1028	0.0266	0.0243	0.0068	0.0051	0.0144	0.0005
167	0.1496	0.0988	0.0931	0.0304	0.0106	0.0231	0.0147	0.0188	0.0099
168	0.1578	0.1162	0.0941	0.0411	0.0211	0.0225	0.0174	0.0033	0.0203
169	0.1451	0.1367	0.0884	0.0285	0.0302	0.0008	0.0097	0.0084	0.0090
170	0.1409	0.1076	0.0909	0.0191	0.0092	0.0288	0.0129	0.0125	0.0028
171	0.1433	0.1265	0.0880	0.0299	0.0243	0.0331	0.0118	0.0105	0.0200
172	0.1423	0.0982	0.0995	0.0183	0.0277	0.0184	0.0030	0.0090	0.0089
173	0.1538	0.1364	0.0994	0.0071	0.0009	0.0406	0.0094	0.0109	0.0008
174	0.1722	0.1168	0.0843	0.0229	0.0242	0.0088	0.0090	0.0094	0.0113
175	0.1469	0.1293	0.0850	0.0329	0.0143	0.0192	0.0147	0.0128	0.0074
176	0.1782	0.1166	0.0823	0.0271	0.0432	0.0016	0.0110	0.0126	0.0216
177	0.1683	0.0978	0.0731	0.0262	0.0513	0.0228	0.0019	0.0147	0.0117
178	0.1729	0.1153	0.0907	0.0101	0.0388	0.0060	0.0167	0.0117	0.0141
179	0.1778	0.1267	0.0967	0.0235	0.0251	0.0379	0.0110	0.0119	0.0001
180	0.1694	0.1074	0.0873	0.0168	0.0425	0.0070	0.0148	0.0011	0.0164
181	0.1628	0.1174	0.0951	0.0175	0.0169	0.0097	0.0128	0.0055	0.0249
182	0.1675	0.1031	0.0713	0.0099	0.0271	0.0211	0.0027	0.0043	0.0123
183	0.1360	0.1181	0.0996	0.0103	0.0444	0.0070	0.0057	0.0154	0.0149
184	0.1563	0.1156	0.1008	0.0089	0.0302	0.0213	0.0143	0.0060	0.0010
185	0.1648	0.1025	0.0871	0.0086	0.0394	0.0026	0.0081	0.0197	0.0196
186	0.1624	0.1066	0.0948	0.0102	0.0253	0.0155	0.0059	0.0050	0.0125
187	0.1488	0.1257	0.0763	0.0017	0.0233	0.0004	0.0056	0.0112	0.0091
188	0.1625	0.1311	0.0967	0.0105	0.0449	0.0398	0.0045	0.0004	0.0167
189	0.1657	0.1012	0.0737	0.0064	0.0318	0.0018	0.0061	0.0117	0.0191
190	0.1605	0.1111	0.0744	0.0097	0.0263	0.0018	0.0121	0.0174	0.0037
191	0.1372	0.1212	0.0675	0.0048	0.0046	0.0048	0.0226	0.0066	0.0014
192	0.1508	0.0929	0.0903	0.0094	0.0155	0.0232	0.0127	0.0099	0.0029
193	0.1507	0.1014	0.0929	0.0178	0.0260	0.0026	0.0098	0.0073	0.0073
194	0.1393	0.0986	0.0970	0.0136	0.0112	0.0158	0.0137	0.0068	0.0071
195	0.1459	0.1162	0.0928	0.0114	0.0198	0.0020	0.0128	0.0151	0.0145

196	0.1513	0.1073	0.0945	0.0074	0.0216	0.0041	0.0106	0.0173	0.0071
197	0.1558	0.1130	0.0887	0.0124	0.0228	0.0109	0.0005	0.0082	0.0072
198	0.1700	0.1103	0.0756	0.0167	0.0338	0.0139	0.0157	0.0050	0.0055
199	0.1440	0.1123	0.0902	0.0152	0.0293	0.0162	0.0077	0.0079	0.0103
200	0.1672	0.1004	0.0854	0.0118	0.0223	0.0100	0.0022	0.0098	0.0108
201	0.1599	0.0951	0.0816	0.0030	0.0101	0.0042	0.0042	0.0120	0.0160
202	0.1545	0.1078	0.0864	0.0125	0.0117	0.0143	0.0154	0.0070	0.0089
203	0.1688	0.1223	0.0826	0.0139	0.0011	0.0261	0.0110	0.0090	0.0016
204	0.1610	0.1168	0.0995	0.0170	0.0270	0.0388	0.0004	0.0002	0.0162
205	0.1643	0.1051	0.0869	0.0138	0.0216	0.0202	0.0083	0.0007	0.0102
206	0.1608	0.1156	0.0837	0.0167	0.0197	0.0261	0.0058	0.0119	0.0121
207	0.1682	0.1003	0.0920	0.0149	0.0205	0.0051	0.0045	0.0107	0.0157
208	0.1507	0.1070	0.1046	0.0130	0.0349	0.0385	0.0099	0.0058	0.0035
209	0.1569	0.1059	0.0959	0.0199	0.0191	0.0381	0.0072	0.0103	0.0080
210	0.1545	0.1077	0.1016	0.0197	0.0213	0.0191	0.0079	0.0095	0.0100
211	0.1542	0.1069	0.0923	0.0293	0.0211	0.0170	0.0004	0.0021	0.0150
212	0.1506	0.1113	0.0832	0.0242	0.0042	0.0181	0.0090	0.0054	0.0062
213	0.1601	0.1069	0.0923	0.0212	0.0127	0.0023	0.0087	0.0046	0.0095
214	0.1639	0.1129	0.0862	0.0158	0.0112	0.0403	0.0094	0.0094	0.0116
215	0.1654	0.1123	0.0905	0.0249	0.0122	0.0269	0.0055	0.0027	0.0055
216	0.1709	0.1057	0.0920	0.0304	0.0046	0.0275	0.0125	0.0090	0.0073
217	0.1691	0.1153	0.0818	0.0238	0.0091	0.0331	0.0037	0.0107	0.0085
218	0.1697	0.1048	0.0960	0.0223	0.0106	0.0086	0.0086	0.0006	0.0009
219	0.1541	0.1112	0.0969	0.0022	0.0070	0.0167	0.0081	0.0168	0.0029
220	0.1715	0.1091	0.0901	0.0143	0.0054	0.0031	0.0018	0.0111	0.0061

DO (mg/L)								
Time (min)	COD: N= 1: 0.22			COD: N= 1: 0.15			COD: N= 1: 0.11		
	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3
0	0.51	0.73	1.13	0.55	0.76	1.48	0.5	0.73	1.7
5	1.55	3.13	5.66	0.78	2.24	4.67	0.77	2.51	3.15
10	1.42	3.33	5.65	2.83	3.4	8.46	2.39	2.61	6.47
15	2.66	3.51	7.45	3.9	3.42	8.32	3.02	3.56	8.25
20	3.24	3.41	8.78	3.53	3.76	8.34	3.52	3.61	8.34
25	3.15	3.56	8.78	3.07	3.79	8.39	3.13	3.55	8.55
30	3.61	3.44	8.78	3.2	3.71	8.43	3.35	3.87	8.79
35	3.76	3.66	8.78	3.57	3.72	8.44	3.45	4.01	8.79
40	3.54	3.83	8.78	3.74	3.74	8.48	3.65	3.94	8.79
45	3.47	4.46	8.78	3.78	3.98	8.61	3.41	4.05	8.79
50	3.67	4.78	8.78	3.32	4.07	8.84	3.45	3.99	8.79
55	3.55	4.97	8.78	3.43	4.14	8.84	3.47	4.28	8.79
60	3.68	5.02	8.78	3.53	4.17	8.84	4.01	4.59	8.79
65	3.74	5.07	8.78	3.22	4.24	8.84	4.44	4.48	8.79
70	3.92	5.08	8.78	3.76	4.94	8.84	4.57	5.16	8.79
75	4.06	5.17	8.78	4.08	5.03	8.84	4.86	5.65	8.79
80	3.83	5.16	8.78	4.14	6.36	8.84	4.69	6.24	ˆ˜
85	3.8	5.2	8.78	4.35	8.11	8.84	5.5	7.9	
90	3.83	5.74	8.78	4.33	8.76	8.84	5.63	8.35	

95	3.92	6.73	8.78	4.46	8.76	8.84	5.86	8.74	8.79
100	4.3	7.65	8.78	4.32	8.76	8.84	6.45	8.74	8.79
105	5.21	8.71	8.78	5.38	8.76	8.84	7.56	8.74	8.79
110	6.44	8.71	8.78	6.26	8.76	8.84	8.73	8.74	8.79
115	7.11	8.71	8.78	7.95	8.76	8.84	8.73	8.74	8.79
120	8.51	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
125	8.72	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
130	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
135	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
140	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
145	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
150	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
155	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
160	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
165	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
170	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
180	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
190	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
200	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
210	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79
220	8.73	8.71	8.78	8.75	8.76	8.84	8.73	8.74	8.79

F.3 Specific N$_2$O generation rate in a typical cycle at different COD: N ratios

	N$_2$O generation rate (μg N$_2$O / (g SS$\bullet$ min))								
Time (min)	COD: N= 1: 0.22			COD: N= 1: 0.15			COD: N= 1: 0.11		
	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3
0	3.3500	2.7809	2.8852	2.3601	2.9836	3.1931	2.2595	2.1153	1.3800
9	2.3193	2.2538	2.4917	1.6400	1.7447	2.3102	0.8180	0.8161	1.2342
19	2.0706	2.2636	1.8174	1.6813	1.6030	1.1763	1.0084	0.9169	1.2100
29	2.1038	2.1402	0.9227	1.7474	1.8502	1.4451	1.0228	0.9184	0.7528
39	2.1863	2.1640	1.3988	2.0595	1.6295	1.1635	1.1330	0.9822	0.4704
49	2.2735	1.8992	1.1133	2.0580	1.6770	1.1666	1.0539	0.6815	0.3970
59	2.5674	2.0766	1.2696	1.8901	1.6886	1.1279	0.8407	0.5663	0.1592
69	2.5736	1.7918	1.0253	1.5495	0.9961	1.0182	0.5023	0.4338	0.0487
79	2.4752	2.0940	0.7171	0.9830	1.2751	0.5604	0.1219	-0.0400	0.1419
89	2.3755	1.9729	1.0313	1.0662	0.8797	0.5949	0.3765	0.0273	0.1637
99	2.2204	1.4114	0.7311	0.7660	0.5538	0.4583	0.0883	0.1344	0.1123
109	0.8704	0.9996	0.9527	0.7565	0.2568	0.1850	0.2197	0.1047	0.1612
119	1.0468	1.0297	0.5575	0.3667	0.0055	-0.0372	0.0454	0.0951	0.0736
129	1.0674	1.0077	0.9186	0.2492	0.2734	0.3892	0.1268	0.1026	0.1700
139	0.9451	0.9361	0.8479	0.1005	0.1752	0.0770	0.0112	0.0558	0.0522
149	0.9837	0.9031	0.8569	0.0029	0.0575	0.1515	0.1616	0.2102	0.1177
159	1.0157	0.7862	0.9019	0.1473	0.1897	0.1092	-0.0211	0.0432	0.0556
169	0.7673	0.8303	0.6907	0.1444	0.0880	0.1926	0.0723	0.0523	0.0594
179	1.0701	0.7786	0.7367	0.1017	0.2467	0.2137	0.0657	0.0559	0.0911
189	0.8504	0.7186	0.6207	-0.0076	0.1948	-0.0091	0.0250	0.0926	0.1336
199	0.8152	0.7466	0.7776	0.0943	0.1256	0.1360	0.0445	0.0377	0.0485

| 209 | 0.9159 | 0.7423 | 0.7906 | 0.1090 | 0.0978 | 0.2587 | 0.0523 | 0.0557 | 0.0740 |
| 219 | 0.9537 | 0.7702 | 0.7143 | 0.0583 | 0.0050 | 0.0676 | 0.0278 | 0.0666 | 0.0378 |

F.4 N₂O emission amount in a typical cycle

	N_2O emission amount (mg/L)		
	COD: N= 1: 0.22	COD: N= 1: 0.15	COD: N= 1: 0.11
SBR1	9.961643	5.557118	2.63268
SBR2	7.39063	3.998086	1.783091
SBR3	4.621484	2.739556	1.300221

F.5 Profiles of COD and ammonium in a typical cycle

	COD (mg/L)								
Time (min)	COD: N= 1: 0.22			COD: N= 1: 0.15			COD: N= 1: 0.11		
	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3
1	360	354	336	342	354	330	336	348	348
20	198	180	156	168	156	126	156	162	138
40	92	54	24	84	36	24	54	42	24
60	30	12	6	30	6	6	12	6	6
90	6	6	6	6	6	6	6	6	6
120	6	6	6	6	6	6	6	6	6
220	6	6	6	6	6	6	6	6	6

	NH_4^+-N (mg/L)								
Time (min)	COD: N= 1: 0.22			COD: N= 1: 0.15			COD: N= 1: 0.11		
	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3	SBR1	SBR2	SBR3
1	125.47	103.5	96.463	45.326	47.941	45.671	31.54	30.201	31.14
20	121.182	92.547	89.364	35.322	41.016	37.908	18.528	19.944	27.186
40	113.544	88.23	80.526	30.6	29.466	31.734	10.716	14.37	16.596
60	106.02	82.122	72.852	29.088	24.45	26.934	10.034	8.532	12.528
90	98.406	72.33	63.732	22.368	16.716	19.752	1.77	1.656	7.182
120	92.586	68.59	55.38	13.134	8.658	14.328	0.318	0.504	1.086
220	81.388	65.44	43.322	2.376	0.762	1.758	0.3	0.366	0.432

F.6 Specific N₂O generation rate with nitrite or nitrate being the only nitrogen component in aerobic conditions

	Special N_2O generation rate (µg/ (g SS· min))	
	Nitrite	nitrate
SBR1	1.733860193	1.198272956
SBR2	1.614337528	1.103421855
SBR3	1.271274609	0.88501184

F.7 Profiles of poly-β-hydroxybutyrate (PHB) content in granular sludge in a typical cycle

Time (min)	PHB (mg/g SS)		
	SBR1	SBR2	SBR2
1	57.4	59.1	56.8
20	66.9	67.4	67.1
40	70.4	76.2	73.2
60	68.6	71.6	73.7
90	59.7	61.4	60.3
120	55.4	57.3	57.5
220	57.3	55.2	52.7

Appendix G: Nutrient Removals in Upflow Biofilters Study Data

Associated with Chapter 8

G.1 Mean nitrogen performance of the upflow biofilters

Time (d)	High loading rate (mg/L)			Low loading rate (mg/L)		
	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N
1	23.4	12.3	39.0	8.5	3.8	43.1
3	14.6	39.4	83.5	2.2	8.7	82.0
5	15.3	74.8	100.2	2.4	17.1	94.0
8	15.6	68.0	96.7	3.3	9.5	104.6
10	13.1	55.7	101.8	0.8	9.1	101.4
12	10.6	48.6	98.2	4.4	8.7	96.5
15	8.5	46.5	99.7	3.8	3.3	97.4
17	8.7	42.1	107.8	3.7	4.4	98.0
19	6.8	32.0	117.0	4.1	2.6	96.2
22	7.1	22.4	120.8	2.7	3.8	94.4
24	5.6	17.3	126.8	3.3	3.4	97.5
26	4.7	9.2	127.7	4.3	2.7	92.6
29	4.9	9.7	121.2	2.7	3.3	89.6
31	3.9	8.8	123.7	3.3	2.7	90.9
33	4.9	9.8	121.3	3.1	2.0	85.3
36	2.4	6.6	114.0	2.1	4.6	86.5
38	2.5	5.8	110.2	1.8	1.3	86.9
40	3.4	6.2	111.2	2.9	1.7	82.4
43	2.5	6.2	117.7	2.4	1.0	84.6
45	3.1	5.5	116.9	2.4	3.1	86.2
47	2.4	5.8	118.8	1.9	1.6	85.0
50	2.2	5.5	117.0	2.7	3.5	94.4
52	2.1	5.1	116.5	2.2	3.0	87.1
54	2.4	5.8	119.6	2.7	2.6	84.0
57	2.0	6.0	114.6	1.7	2.6	89.1
59	1.8	5.7	117.0	1.5	3.2	86.9
61	2.0	4.7	116.7	2.5	2.5	84.8
64	2.6	7.2	110.7	4.5	2.3	87.6
66	3.2	6.3	113.9	4.3	2.7	87.3
68	1.6	5.6	116.3	2.6	2.4	89.9
75	7.3	4.9	132.8	4.3	2.1	94.7
78	3.9	5.2	129.6	4.0	0.4	98.6
80	3.0	3.5	137.1	4.1	3.4	99.1
82	3.9	3.7	127.4	3.0	1.7	95.4
85	3.2	2.5	127.2	2.8	1.4	98.1
87	5.6	2.4	124.8	1.6	0.4	93.6
89	4.4	4.0	115.5	2.3	1.4	96.3
92	4.4	5.2	116.9	0.7	4.9	94.4
94	5.1	3.4	113.6	3.6	2.3	91.4
96	4.7	2.4	116.0	6.9	2.7	88.7
99	3.6	2.6	111.6	4.6	1.2	93.2

101	4.3	2.7	115.6	3.5	1.8	88.1
103	4.8	2.5	117.0	2.5	0.3	89.7
106	4.9	1.9	110.7	2.9	1.6	92.8
108	4.7	0.8	112.6	3.9	1.4	90.1
113	3.9	1.3	114.4	2.0	1.5	89.6
115	3.7	1.2	115.4	2.3	1.8	86.0
117	6.4	0.7	118.7	1.3	0.2	88.2
120	5.1	1.2	119.3	2.3	0.3	88.6
122	3.6	1.9	114.5	1.4	0.7	92.3
124	3.7	0.8	119.3	3.0	0.6	89.7
127	6.1	1.9	125.3	6.6	1.1	94.6
129	4.1	1.7	122.4	5.6	0.6	89.8
131	4.3	1.1	122.2	4.4	0.7	95.7
134	3.5	1.5	122.6	4.4	0.9	88.9
136	5.3	1.4	119.6	5.5	1.1	87.7

G.2 Profile of mean DO concentration in a typical cycle

Time (min)	DO in high loading rate (mg/L)	DO in low loading rate (mg/L)
0	3.53	6.05
2	0.00	1.20
40	0.00	0.00
80	0.00	0.00
120	0.00	0.00
140	1.60	2.85
160	2.80	3.60
180	2.83	3.60
210	3.10	4.40
240	4.77	6.55
300	7.27	8.45
360	8.47	8.50
420	8.50	8.50
472	8.50	8.50
475	4.63	5.55

G.3 Profiles of NH_4^+-N, NO_2^--N and NO_3^--N concentrations in a typical cycle

	High loading rate (mg/L)			Low loading rate (mg/L)		
Time (d)	NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N
0	5.27	1.40	119.60	3.95	1.05	87.70
2	67.03	1.20	115.13	36.50	1.05	86.15
40	68.20	1.00	98.70	40.35	1.05	77.40
80	65.27	0.63	85.63	38.75	0.90	63.85
120	63.27	0.53	79.07	36.50	0.90	55.45
140	59.43	0.77	69.03	35.65	1.30	55.75
160	53.17	0.83	76.43	30.05	1.00	60.95
180	47.47	0.90	79.97	24.15	1.25	66.10

210	38.33	1.03	86.93	20.10	1.10	70.35
240	26.27	1.10	97.70	12.55	0.95	74.35
300	13.77	1.17	108.73	7.55	0.90	82.60
360	8.03	1.20	114.47	6.60	0.95	83.65
420	6.00	1.17	116.37	5.10	0.95	84.80
472	4.50	1.03	117.30	5.00	0.90	84.40
475	4.20	0.97	117.90	4.50	0.95	84.80

G.4 Adsorption isotherm of PO_4^{3-}-P

Equilibrium concentration of phosphorus (mg/L)	Adsorption ratio of phosphorus (mg/g)
91.00	0.106667
75.33	0.095333
60.33	0.089524
49.00	0.082222
43.33	0.072424

Appendix H: Chemical Treatments Study Data

Associated with Chapter 7

H.1 F-statistics for significance of tested factors

Parameters	PAM type	Al dosage	pH	PAM dosage
COD	27.43	6.25	9.89	7.05
Turbidity	27.5	12.3	29.5	17.6
TN	2.74	0.5	4.13	1.84

H.2 NH_4^+-N removal efficiency and the amounts of NaOH consumed under different pH

pH	NH_4^+-N removal rate (%)	NaOH dosage (g/L)
8.5	85.12	1.366
9	86.06	1.802
9.5	89.00	3.094
10	90.80	3.552
10.5	91.61	4.000
11	91.93	4.288

H.3 Effects of the Mg^{2+}: NH_4^+-N: PO_4^{3-}-P molar ratio on struvite precipitation treatments at pH of 10.5

Mg^{2+}: NH_4^+-N: PO_4^{3-}-P	NH_4^+-N removal efficiency (%)
1:1:1	91.24
1.3:1:1.3	96.17
0.7:1:0.7	56.44
1.3:1:1	92.97
1.3:1:0.7	72.31
1:1:0.7	65.44
1:1:1.3	92.12
0.7:1:1.3	67.68
0.7:1:1	61.25