Nutrient Removal from Slaughterhouse Wastewater and Oxygen-Limited Partial Nitrification Process in Sequencing Batch Reactors

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Dedicated to my parents and my wife Yangle Cao

Abstract

This project examined nutrient removal from slaughterhouse wastewater and partial nitrification under oxygen-limited conditions in sequencing batch reactors (SBRs).

Slaughterhouse wastewater contains high-strength organic mater and nutrients. Slaughterhouse wastewater, taken from a slaughtering and rendering wastewater treatment plant in Co. Mayo, was treated in 10-L laboratory-scale, intermittently aerated SBRs. The slaughterhouse wastewater contained 2,800–5,600 mg chemical oxygen demand (COD)/L, 220–400 mg total nitrogen (TN)/L and 26–40 mg total phosphorus (TP)/L. The aeration rate was found to be the most important process parameter and, at an aeration rate of 0.8 L air/min, the average COD, TN and TP removals were 96%, 96% and 99%, respectively. The addition of plastic biofilm carriers into the SBR did not significantly improve the nitrogen (N) removal efficiency. When the SBR was operated under low dissolved oxygen (DO) conditions with the maximum concentration of DO controlled at 1.0 mg/L, good effluent quality was obtained under both intermittent aeration and continuous aeration strategies, with TN removals of up to 94% and 91%, respectively. *In situ* measurements of oxygen utilization rate (OUR) can be used to control the aeration duration of SBRs.

In further experiments, partial nitrification was investigated in SBRs with synthetic wastewater simulating low COD/N-ratio wastewater. The synthetic wastewater contained readily biodegradable COD (rbCOD) of 100–300 mg/L and ammonium-N (NH_4^+-N) of 300 mg/L. Nitrous oxide (N_2O) emissions from this partial nitrification process were also evaluated. At 20 °C, a pH of 7.0–7.4 and a sludge retention time (SRT) of greater than 100 days, stable partial nitrification was achieved. A start-up period of 7 days was very important to encourage the growth of ammonium oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB). In the long-term partial nitrification period (180 days), over 90% of the influent NH_4^+-N was oxidized and the mass ratio of nitrite-N (NO_2^--N) to the total oxidized nitrogen (NO_2^--N + nitrate-N (NO_3^--N)) was over 90% in the effluent. Oxygen-limited conditions were suspected to be the main factor resulting in stable partial nitrification. Molecular analysis results show that AOB represented up to 10% of the total bacterial population in the sludge biomass, and the AOB/NOB ratio was generally over 100. With regard to N_2O emissions from the partial nitrification SBR, the heterotrophic denitrification pathway was considered to be the main contributor to elevated N_2O emissions. When the influent rbCOD was zero, N_2O emissions were equal to 1.7% of the influent nitrogen loading rate (NLR). N_2O emissions rose to 6% and 12% of the influent NLR, while rbCOD in the influent was 100 mg/L and 500 mg/L, respectively. Minimizing the influent rbCOD concentration, reducing the duration of the anoxic operation, and decreasing the free nitrous acid concentrations in the reactor could help to reduce the N_2O emissions from the oxygen-limited partial nitrification process.

Declaration

This dissertation is the result of my own work, except where explicit reference is made to the work of others, and has not been submitted for another qualification to this or any other university.

– Jianping Li

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Chapter 1

Introduction

1.1 Overview of the research background

Nitrogen (N) fixation by human activities converts around 120 million tonnes of nitrogen gas (N_2) from the atmosphere per year into reactive N, including ammonium-nitrogen (NH_4^+), organic nitrogen, oxidized nitrogen (mainly nitrite (NO_2^-), and nitrate (NO_3^-)) (Rockstrom et al., 2009). A significant part of the reactive N is lost or transported into water streams. An elevated N concentration in water bodies may cause serious environmental problems, such as eutrophication, depletion of dissolved oxygen (DO) in the receiving water body, taste and odour problems, and increased treatment costs for water supplies (Clabby et al., 2008). Therefore, N in wastewater should be removed to meet the discharge standards so as to avoid over-discharge of N into receiving water bodies.

High-strength wastewaters are generated in the agricultural, industrial and waste disposal industries. Generally, they are transported to local municipal wastewater treatment plants (WWTPs). This may cause overloading of the WWTPs and the final effluent may not meet discharge standards due to the overloading (Monaghan et al., 2009).

For on-site treatment of high-strength wastewater, the application of anaerobic digestion (AD) is a good option to remove high levels of organic matter at a low operational cost, and has the potential to produce biogas as a by-product. After removal of organic matter, the chemical oxygen demand (COD)/ N ratio of the wastewater becomes very low. This challenges conventional biological nitrogen removal (BNR) processes, which consist of successive nitrification and denitrification via NO_3^-, and is the most common approach applied in N removal in WWTPs. Conventional nitrification and denitrification has encountered difficulties in the treatment of low COD/N-ratio wastewaters to meet stringent discharge standards (Sheng-Peng Sun, 2010). For low COD/N-ratio, ammonium-rich wastewater, the aeration energy input for complete nitrification, the addition of external organic carbon (C) (e.g. methanol) for denitrification, and the recycling of wastewater from the nitrification section of a WWTP to the denitrification section (like in the A/O and A^2/O processes) leads to a significant increase in the operational cost of WWTPs. N removal from medium- to high-strength wastewaters has encouraged intensive research interest.

Wastewater generated in slaughterhouses and rendering plants contains high-strength organic matter and nutrients. Conventional activated sludge processes are used to treat slaughterhouse wastewater. Sequencing batch reactor (SBR) technology is among the best available technologies recommended by the European Commission. Typical COD, total nitrogen (TN) and total phosphorus (TP) removals from slaughterhouse wastewater achieved in SBRs are 95%, 60–80% and 40%, respectively (European Commission, 2005). To meet discharge standards, the operation strategy of a SBR should be optimized to improve the system performance and reduce the operational cost.

There is an urgent need for the slaughterhouse wastewater treatment sector to reduce the operational cost. By applying advanced technologies developed in recent years, current slaughterhouse wastewater treatment facilities may be upgraded to meet stricter emission standards at lower operational costs. Two options may be implemented: 1) upgrading of existing facilities and optimization of operational strategies; and 2) integration of an AD process to remove organic matter, with the production and recovery of biogas energy to offset the operational cost of WWTPs, and then application of efficient processes to remove N from the AD digestate, which has low COD/N ratios.

The newly developed anaerobic ammonium oxidation (anammox) process (Jetten et al., 1997) has the potential to significantly reduce the cost of N removal from low COD/N-ratio wastewater. In comparison with conventional biological nitrogen removal (BNR) systems, the anammox process can save up to 90% of the operational costs for N removal (Jetten et al., 2005).

Partial nitrification, whereby NH_4^+ is only oxidized to NO_2^-, but not NO_3^-, is the process used to supply NO_2^- as a substrate for the anammox process. However, to sustain long-term stable and efficient partial nitrification is challenging. The SHARON (Single reactor system for High activity Ammonium Removal Over Nitrite) process has been successfully developed and integrated with the anammox process to treat low COD/N-ratio wastewater in full-scale systems (van der Star et al., 2007). The relatively high temperature used (30–35 °C) is essential for the SHARON process to keep a faster growth rate of ammonium oxidizing bacteria (AOB) than nitrite oxidizing bacteria (NOB). Efficient operation of partial nitrification at a moderately low temperature (15–20 °C) is of great importance since most wastewaters have an ambient temperature in this range (Tchobanoglous et al., 2003). The integration of partial nitrification with the anammox process, operated at 15–20 °C, should be more practical.

This thesis investigates the development of technologies to efficiently remove N from slaughterhouse wastewater and the development of partial nitrification technologies for the treatment of low COD/N-ratio wastewater at a temperature of about 20 °C.

1.2 Objectives

The objectives of this study were to:

1) investigate nutrient removal from slaughterhouse wastewater using laboratory-scale SBRs.

2) optimize the operational conditions, including DO control and aeration strategy, so as to achieve good N and P removals, and to reduce the aeration energy input.

3) develop an oxygen-limited, partial nitrification process for low COD/N-ratio, ammonium-rich wastewater at moderately low temperatures; and to explore the mechanisms of partial nitrification.

1.3 Procedures

Raw slaughterhouse wastewater was treated in 10-L intermittently aerated SBRs (IA-SBR) operated at a constant aeration rate of 0.8 L air/min, with and without the addition of biofilm carriers. The effects of different aeration rates (0.2, 0.4, 0.8 and 1.2 L air/min) on the performance of the IA-SBRs were studied. DO control and aeration duration control were applied to a 10-L SBR under intermittent aeration (IA) and continuous aeration (CA) operation strategies. The efficiency of partial nitrification was investigated in a 2-L IA-SBR treating synthetic wastewater with COD/N ratios of 1:1 and 1:3. The emission of nitrous oxide (N_2O) from the partial nitrification reactor was examined by online measurement of dissolved N_2O in the liquid phase.

1.4 Thesis structure

This dissertation comprises 9 chapters:

Chapter 1 is the introduction. The main objectives and research procedures are presented.

Chapter 2 reviews previous research, practices and policies relating to the generation and treatment of slaughterhouse wastewater, biological nitrogen removal, and partial nitrification.

Chapters 3 to 6 details the research on slaughterhouse wastewater treatment in SBRs operated with an intermittent aeration pattern. Chapter 3 examines the performance of an IA-SBR operated at a fixed aeration rate. Chapter 4 examines the performance of an IA-SBBR operated at a fixed aeration rate. Chapter 5 examines the effects of the aeration rate on the performance of the IA-SBRs. Chapter 6 examines the effects of low DO and aeration duration control on the performance of a SBR operated with IA and CA strategies.

Chapter 7 presents the development of a partial nitrification process treating low COD/N-ratio, ammonium-rich wastewater at ambient temperature under oxygen-limited conditions.

Chapter 8 studies the N_2O emission from the oxygen-limited partial nitrification process.

Chapter 9 presents the main conclusions from this work and the recommendations for future studies.

Chapter 2

Literature Review

2.1 Overview

N removal from ammonium-rich wastewater (e.g. >100 mg N/L) is challenging. Slaughterhouse wastewater is a high-strength wastewater with both high COD and high N. Among a few available technologies, SBR technology is recommended as the best available technology for the removal of COD and N from slaughterhouse wastewater after preliminary and primary treatment (European Commission, 2005).

The high organic matter content of slaughterhouse wastewater makes it suitable for AD. In the AD process, the majority of the organic matter is removed and biogas is produced as a by-product. Anaerobically-treated slaughterhouse wastewater has a low COD/N ratio, which is a problem for conventional BNR processes. Low COD/N-ratio wastewater may be treated using a combination of partial nitrification and the anammox processes.

In this chapter, a literature review was carried out on BNR processes, SBR technology, slaughterhouse wastewater generation and treatment, and partial nitrification.

2.2 Meat industry and slaughterhouse wastewater

Meat is a valuable protein source for humans and meat consumption is one of the major indicators of the living quality of people. In 2007, per capita meat consumption was 64 kg in developed countries, but it was only 24 kg in developing countries (OCED-FAO, 2008).

During a 10-year period from 2007 to 2016, the increase for the per capita meat consumption in both developed countries and developing countries was 9% and 15%, respectively (OCED-FAO, 2008). In 2008, the carcass weight of livestock slaughtered for meat in Ireland was cattle, 537 ktonnes; pigs, 202 ktonnes; and sheep/lambs, 107 ktonnes (Central Statistical Office Ireland, 2009).

The slaughtering of livestock generates high-strength wastewater. Most of the wastewater generated in slaughterhouses is due to the cleaning process. Cleaning of the holding areas, slaughtering areas and carcasses accounts for 80% of the water used in slaughterhouses (European Commission, 2005). Depending on the management, the volume of wastewater generated in animal slaughtering varies: 1.6–9.0 m^3 of wastewater per tonne of cattle carcase, 1.6–8.3 m^3 of water per tonne of pig carcase, and 5.6–8.3 m^3 of wastewater per tonne of sheep carcase (European Commission, 2005).

Animal by-products are generated in slaughterhouses. A rendering plant is usually used to process the animal by-products. The European Union animal by-products regulation (1774/2002/EC;EC,2002) categorizes animal by-products into three categories: Categories 1, 2, and 3 (European Commission, 2002). Blood and other animal by products, belonging to Category 1 and Category 3, are sent to rendering plants. The cooking process of the rendering plant releases steam vapours. A condenser is used to strip ammonia (NH_3^+) from rendering vapours. The rendering vapours from the condenser represent the major pollution load in rendering wastewater which contains high concentrations of COD and NH_4^+-N and low concentrations of P (Johns, 1995). Rendering wastewater is usually treated together with the wastewater generated in the slaughtering process.

Depending on whether preliminary/primary treatment is carried out and its efficiency, the concentrations of contaminants in slaughterhouse wastewater are variable, with major pollutants such as suspended solids (SS), COD, TN, and TP concentrations ranging 250–5,000 mg/L, 1,000–20,000 mg/L, 150–10,000 mg/L and 22–217 mg/L, respectively (Fuchs et al., 2003; Cassidy and Belia, 2005; Pozo and Diez, 2005; Merzouki et al., 2005; Mittal, 2006).

2.3 Biological nitrogen removal

BNR is the most commonly applied approach to remove N from wastewater. Excluding the biomass assimilation, the conventional BNR process includes two steps: (1) aerobic autotrophic nitrification, and (2) anoxic heterotrophic denitrification. In the autotrophic nitrification process, NH_4^+ is oxidized to NO_2^- and then to NO_3^- with oxygen as the electron acceptor. In the denitrification process, the produced NO_3^- is reduced into inert N_2 gas with organic C as the electron donor. Anammox is a newly developed BNR process. The combination of partial nitrification and anammox is a new approach for ammonium-rich wastewater treatment. The major biochemical reactions associatied with BNR are now briefly described.

2.3.1 Biomass assimilation

The nitrogen content of cells is about 12.5% of the dry weight. $C_5H_7O_2N$ can be used as a stoichiometric formula to represent cell biomass (Tchobanoglous et al., 2003). The amount of N removed by microorganisms via biomass assimilation is controlled by the net growth of biomass. When treating municipal wastewater in a laboratory-scale bioreactor, the N removal via biomass assimilation in the activated sludge process is about 24% (Barker and Dold, 1995).

2.3.2 Nitrification

Autotrophic nitrification consists of two steps, each performed by individual group of autotrophic bacteria (Tchobanoglous et al., 2003).

The first step, called nitratation, is described using Eq. (2.1). Under aerobic conditions, NH_4^+ is oxidized to NO_2^- by autotrophic AOB, such as *Nitrosomonas.* NH_4^+ is the electron donor and oxygen (O_2) is the electron acceptor.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \tag{2.1}$$

The second step, called nitritation, is described using Eq. (2.2). With oxygen as the electron acceptor, NO_2^- is further oxidized into NO_3^- by autotrophic NOB, such as *Nitrobacter* and *Nitrospira.*

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \tag{2.2}$$

The overall nitrification process oxidizing NH_4^+ to NO_3^- is described in Eq. (2.3).

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \tag{2.3}$$

COD, DO, pH, and temperature are important parameters affecting the nitrification process. Generally, nitrification occurs after the removal of most of the readily biodegradable COD (rbCOD) in the wastewater. High-strength COD present in the reactor would cause the nitrifiers to be out-competed by fast-growing heterotrophic bacteria. A sufficient oxygen supply is required for complete nitrification. According to Eq. (2.3), stoichiometrically, 4.7 g of O_2 is needed to oxidize 1 g of NH_4^+-N into NO_3^--N. The operational

DO level in a conventional activated sludge process is suggested to be maintained at 2–3 mg/L during the nitrification process (Tchobanoglous et al., 2003). The optimal pH for nitrification is 7.5–8.0 (Tchobanoglous et al., 2003). When the operational temperature is lower than 10–12 °C, the nitrification rate may drop significantly to less than 50% of the maximum nitrification rate (Shammas, 1986).

2.3.3 Denitrification

In the denitrification process, heterotrophic microorganisms reduce NO_3^-/NO_2^- into N_2 in the absence of DO. For municipal wastewater treatment, about 4 g of biochemical oxygen demand (BOD) is required to reduce 1 g of NO_3^--N into N_2 (Tchobanoglous et al., 2003), and the organic C is mainly from the influent wastewater. If organic C from the influent is not sufficient, external C sources, such as methanol, should be supplemented to perform complete denitrification (Eq. (2.4)).

$$6CH_3OH + 6NO_3^- \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \tag{2.4}$$

The denitrification process leads to an increase in alkalinity. According to Eq. (2.4), the reduction of 1 equivalent of NO_3^--N produces 1 equivalent of alkalinity, meaning that the reduction of 1 g of NO_3^--N into N_2 can produce 3.57 g of alkalinity as $CaCO_3$.

2.3.4 Anammox

In the anammox process, autotrophic anammox microorganisms take NH_4^+ and NO_2^- as main substrates to produce N_2 and a small amount of NO_3^- in the absence of dissolved oxygen (Eq. (2.5)) (Strous et al., 1998).

$$\begin{aligned} &NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \\ &\rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \end{aligned} \tag{2.5}$$

Partial nitrification is the process to supply NO_2^- for the anammox process. The SHARON process has been successfully used with the anammox process to treat the waste water at full-scale (van der Star et al., 2007). The application of partial nitrification (Eq. (2.1)) and the anammox process is a cost-effective way to treat ammonium-rich wastewater with low COD/N ratios (Jetten et al., 1997). Compared to the conventional BNR process, the anammox process can save up to 90% of the operational costs for N removal (Jetten et al., 2005).

2.4 Sequencing batch reactor (SBR)

A SBR is a fill-and-draw activated sludge system for wastewater treatment (US EPA, 1999). Mixed liquor remains in the reactor and all steps of the activated sludge process occur in a single reactor, thereby eliminating the need for a separate secondary clarifier. When using a SBR to treat municipal wastewater with a concentration SS of less than 400 mg/L, primary clarifiers are typically not required (US EPA, 1999). An equalization tank is needed when the effluent from the SBR needs to be further treated, for example, by means of filtration and disinfection.

The initial fill-and-draw batch system was first operated at full-scale between 1914 and 1920 (US EPA, 1999). With the development of advanced instruments and control technology, e.g., low cost automatic controllers, electrical valves and level sensors, research using the SBR technology commenced in the late 1950s. By the late 1970s, the SBR process was widely used worldwide, mainly for small-scale municipal or industrial wastewater treatment (Artan and Orhon, 2005).

The basic SBR configuration is a single tank with five phases operated in a sequential manner: fill, react, settle, draw and idle (Fig. 2.1).

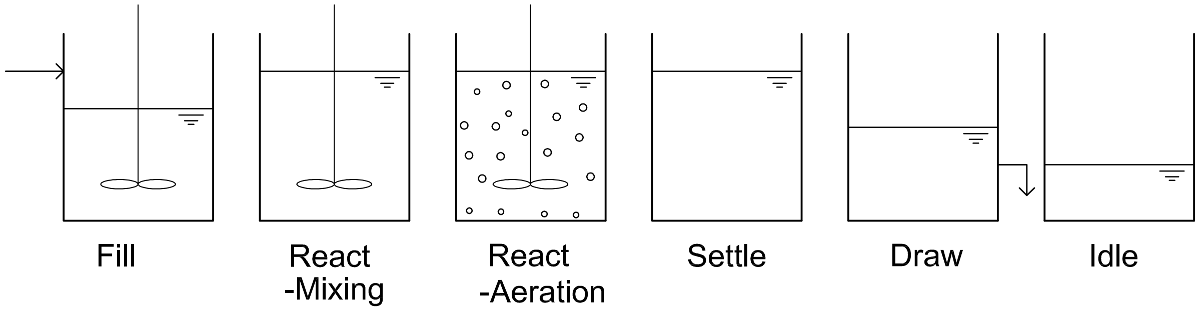


Figure 2.1: Typical sequence operation of a SBR

The phases are now described:

Fill: The influent wastewater is added into the reactor. The mode of static fill, mixed fill or aerated fill is applied, depending on the operational strategy. A static fill can create conditions where high COD concentrations relative to the sludge concentrations are present, which is favorable to better sludge settling, and can also create anaerobic conditions for P release. A mixed fill is favorable to denitrification under anoxic conditions and P release under anaerobic conditions if there is sufficient rbCOD in the influent. An aerated fill mode is used when the fill period is long, e.g, more than 20% of the cycle time. Aerated fill can reduce the aeration time required in the react step (US EPA, 1999).

React: The main biochemical reactions occur in the react phase. The react phase can shift between aeration periods and non-aerated mixing periods. The aeration creates aerobic conditions, and anoxic conditions are created in the non-aeration period with the mechanical stirrer, which creates uniform hydraulic conditions in the reactor. P uptake, COD removal, and nitrification can occur under aerobic conditions, while heterotrophic denitrification occurs under the anoxic mixing condition.

Settle: There is no mixing and flow change in the static settle phase. The biomass and supernatant are separated in the static settle phase.

Draw: The supernatant in the reactor is discharged as treated effluent in the draw phase. A floating decanter is usually used in a full-scale SBR to avoid decanting of the surface foam or scum. It also helps to avoid the disturbance on the sludge layer.

Idle: The idle phase is after the draw phase and before the fill phase of the next cycle. The reactor is static and no operation is conducted. The energy input is minimum. The duration of the idle phase is decided by the influent loading rate and the performance during the react phase. Sludge wasting can be operated during the idle period.

The COD and nutrient removal of a SBR is typically comparable to a conventional activated sludge process. When treating municipal wastewater, the SBR has a 5-day BOD (BOD_5) removal efficiency of 85–95%, with effluent TN $<$ 10 mg/L and TP $<$ 1–2 mg/L (US EPA, 1999).

Compared to the conventional activated sludge process, a small foot-print, low capital cost, and flexible operation to achieve a good nutrient removal performance are the main advantages of SBR technology. A higher requirement for control instruments and technologies, and for maintenance is the main disadvantages of SBR technology.

2.5 Slaughterhouse wastewater treatment

For small-scale slaughterhouses, the wastewater is normally transported to local municipal WWTPs, where it is treated. Due to economics of scale and hygienic regulations, the current trend is to operate large-scale slaughterhouses. For large-scale slaughterhouses, on-site biological wastewater treatment is recommended by the European Commission to remove organic pollutants and nutrients before the wastewater is discharged to surface water or local wastewater treatment plants (European Commission, 2005).

A typical on-site slaughterhouse wastewater treatment plant comprises preliminary treatment, primary treatment, secondary treatment and, in some instances, tertiary treatment.

2.5.1 Preliminary and primary treatment

For preliminary treatment and primary treatment, screening, settling and dissolved air floatation (DAF) are widely used for the removal of SS and fats, oils and greases (FOGs) from slaughterhouse wastewater (Johns, 1995). The solid particles in slaughterhouse wastewater include food residues, fat, bone, hair and meat lost during the slaughtering process.

Coarse screens can remove particles larger than 4 mm, and fine screens have an aperture size down to 0.24 mm (European Commission, 2005). Settling tanks remove FOGs and fine solid particles by gravity. A skimmer removes the floated FOGs and scum off the water surface, and a sludge scraper removes the settled solid particles from the bottom of the settling tank. The removed FOGs and solids can be processed in the rendering plants.

Large slaughterhouses and rendering plants use DAF to remove the FOGs and to reduce the organic loading rate to the subsequent secondary treatment process. Air is pressurized and dissolved into the wastewater. In the floatation tank, the dissolved air is released as fine bubbles. FOGs and solid particles are brought up to the water surface by the uprising air bubbles and are then scraped off. DAF can remove up to 50% of the BOD and 90% of SS from the preliminarily-treated slaughterhouse wastewater (European Commission, 2005).

Slaughterhouse wastewater, comprising wastewater generated in the slaughtering and rending processes, contains high-strength organic C, N and P. After preliminary and primary treatment, it contains SS of 400–2,000 mg/L, COD of 1,000–8,000 mg/L, TN of 150–400 mg/L, TP of 20–80 mg/L, and fats of 20–300 mg/L (Cassidy and Belia, 2005; Pozo and Diez, 2005; Merzouki et al., 2005; Mittal, 2006).

2.5.2 Secondary anaerobic treatment

Prior to aerobic treatment, anaerobic treatment can be applied to remove organic matter. Low-rate anaerobic lagoons are popular in the United States and Australia, while high-rate anaerobic reactors are popular in Europe and Asia (Mittal, 2006).

Low-rate anaerobic lagoons have a hydraulic retention time (HRT) of 5-10 days and the SS and BOD can be effectively removed in anaerobic lagoons (Mittal, 2006). They can handle a wide variety of wastewater and the FOGs normally do not cause operational problems. Covering the surface of anaerobic lagoons can help reduce odor emissions, reduce heat loss and collect biogas.

A high-rate up-flow anaerobic sludge blanket reactor (UASB) can retain high concentrations of anaerobic biomass in the reactor (Schmidt and Ahring, 1996). Under optimized operational conditions at a temperature of 30–35 °C, a high organic loading rate (OLR) of 3–10 kgCOD/(m^3.d) can be applied to UASB reactors treating slaughterhouse wastewater. The HRT of UASB reactors can be shortened to 10–20 hours and the average COD removal rates are 80–85% (Mittal, 2006).

2.5.3 Secondary aerobic treatment

Secondary aerobic treatment is essential to reduce pollutants in the slaughterhouse wastewater before discharge. The activated sludge process is a popular choice for the secondary treatment of slaughterhouse wastewater. The activated sludge process is capable of treatment of the primary effluent or anaerobic effluent. In the activated sludge process, organic matter (such as SS, COD, and fats) and NH_4^+-N are efficiently removed. In 1970s–1980s, the activated sludge process was used to treat slaughterhouse wastewater in the United States (Johns, 1995). An extended-aeration strategy was used in these activated sludge plants to achieve complete nitrification and to reduce sludge production (Johns, 1995).

The European Commission recommends that SBRs be amongst the best available techniques (BATs) for on-site slaughterhouse wastewater treatment, as SBRs are capable of removing organic C, nutrients and SS from wastewater, and have low capital and operational costs (European Commission, 2005). If the slaughterhouse wastewater has a low COD/N ratio, external C sources, such as fermented waste sludge (Ra et al., 2000), should be added to enhance denitrification. If simultaneous P and N removal is expected to be achieved in the reactor, P accumulating organisms (PAOs) will compete with denitrifiers for rbCOD. This competition between PAOs and denitrifiers will result in unstable biological P removal if the influent wastewater does not contain sufficient rbCOD (Filali-Meknassi et al., 2005b). Therefore, tertiary treatment, such as chemical precipitation, is required to guarantee a low concentration of P in the effluent. Typical COD, TN and TP removals from slaughterhouse wastewater achieved in SBRs are 95%, 60–80% and 40%, respectively (European Commission, 2005).

2.5.4 Current status of slaughterhouse wastewater treatment in Ireland

In 2008, there were 46 active licences for slaughterhouses processing > 50 tonnes of meat per day and 12 active licences for rendering plants in Ireland. There are more than 200 small slaughterhouses in Ireland, each of which processes less than 50 tonnes-a-day.

There are 27 on-site slaughterhouse WWTPs applying conventional activated sludge processes for C and N removal in Ireland. The treated wastewater is usually discharged into surface water bodies. Depending on the discharge volume of wastewater and the sensitivity of the receiving water bodies, the limit values for the discharge of treated slaughterhouse wastewater into rivers are: BOD_5, 10–40 mg/L; COD, 50–200 mg/L; SS, 10–60 mg/L; TN, 15–50 mg/L; TP, 0.5–2 mg/L; and fats, 10–15 mg/L (Irish EPA, 2009).

2.6 Partial nitrification

2.6.1 Overview of partial nitrification

In nitrification systems, only a small group of nitrifying species play dominant roles (Wittebolle et al., 2008). In both laboratory and full-scale WWTPs, the dominant AOB is *Nitrosomonas* and the major NOB are *Nitrobactor* and/or *Nitrospira* (Li et al., 2007; Dytczak et al., 2008). Operational temperature has a significant impact on the growth rate of AOB and NOB. NOB is more sensitive to environmental factors, such as pH, free ammonia (FA) and free nitrous acid (FNA), than AOB (Wiesmann et al., 2007).

The partial nitrification process biologically oxidizes NH_4^+-N into NO_2^--N, and avoids further oxidation to NO_3^--N. The production rate of NO_2^- by AOB is higher than the NO_2^- oxidation rate by NOB. Compared to conventional BNR via NO_3^-, the benefits of BNR via partial nitrification are: 1) savings of 25% of oxygen supply for NO_2^- oxidation; 2) savings of 40% of organic C supply for denitrification via NO_2^-; 3) a reduction of 55% of sludge production in denitrification; 4) less carbon dioxide (CO_2) emissions to the atmosphere in denitrification.

If the anammox process is combined with the partial nitrification process for N removal, the saving of oxygen supply for NH_4^+ oxidation is up to about 60% and no external C supply is needed for denitrification (Jetten et al., 1998). The combination of partial nitrification and the anammox process has been proved successful both in laboratory- and full-scale reactors.

Various wastewaters have been proved to be suitable for the partial nitrification process, especially when the wastewater contains high N but low COD concentrations, such as reject water (Meyer and Wilderer, 2004; Gali et al., 2007a), landfill leachate (Kim et al., 2006), anaerobic effluent of seafood processing wastewater (Mosquera-Corral et al., 2005; Lamsam et al., 2008), and supernatant from AD of municipal solid wastes (Mace et al., 2006). For wastewater with a high strength of COD and N, such as slaughterhouse wastewater, partial nitrification and denitrification via NO_2^- may be achieved without the addition of external C sources (Lemaire et al., 2008). For low-strength wastewater, such as municipal wastewater, partial nitrification and denitrification via NO_2^- has been studied in long-term operation (Peng et al., 2004; Blackburne et al., 2007b; Wu et al., 2007).

To sustain partial nitrification, it is necessary to achieve high AOB/NOB-ratio biomass. This means that a high AOB population is sustained in the reactor, and the population of NOB is at a low level due to the unfavoured growth conditions and washing-out of the reactor. Partial nitrification may be developed in three types of reactor systems (Wiesmann et al., 2007): 1) temperature-promoted sludge washing-out systems (like the SHARON process), 2) oxygen-limited, suspended-growth sludge systems, and 3) biofilm systems.

2.6.2 Microbiology of dominant AOB and NOB in nitrifying reactors

2.6.2.1 The dominant AOB: *Nitrosomonas*

Nitrosomonas is the dominant AOB in most laboratory-scale bio-reactors and full-scale WWTPs (Li et al., 2007; Dytczak et al., 2008). In some nitrifying systems, *Nitrosospira* (Schramm et al., 1998; Liang and Liu, 2007) and *Nitrosococcus* (Juretschko et al., 1998; Salem et al., 2004) are rarely observed to be the dominant AOB.

Nitrosomonas-AOB is most frequently detected in municipal WWTPs (Wagner and Loy, 2002). Recent studies show that the AOB only accounts for a very low fraction of the total bacteria population in the sludge, which is significantly lower than the common estimation of 5–8% (Dionisi et al., 2002b). By using molecular analysis (i.e. real-time polymerase chain reaction (PCR)) on samples from twelve WWTPs, Limpiyakorn et al. (2005) found that AOB only accounted for 0.01–2.8% of the total bacterial population. The AOB population size in sludge is mainly dependent on the sludge retention time (SRT), while DO concentrations primarily affect the NH_4^+ oxidizing activity per AOB cell. In a pilot-scale membrane bioreactor (MBR) treating reject water at nitrification rates of up to 2.5 kg N/(m^3.d) and a sludge concentration of 4–15 g SS/L, *Nitrosomonas* accounted for 5.4–13.4% of the total bacterial population (Liebig et al., 2001).

Nitrosomonas has been found to be the dominant AOB in many partial nitrification systems. For instance, *Nitrosomonas* was the dominant AOB and accounted for 50–70% of the total AOB population in high loading rate SHARON-type partial nitrification reactors (van Dongen et al., 2001; Egli et al., 2003; Mosquera-Corral et al., 2005). In suspended-growth partial nitrification reactors operated under low DO conditions (Wyffels et al., 2004; Sliekers et al., 2005) and in partial nitrification biofilm reactors (Han et al., 2003; Isaka et al., 2007b; Chuang et al., 2007b), *Nitrosomonas* was the dominant AOB. In a granular sludge partial nitrification SBR, *Nitrosomonas* was the dominant AOB in the sludge, and NOB was rarely detected in the granules (Kim and Seo, 2006).

2.6.2.2 The dominant NOB: *Nitrobacter* and *Nitrospira*

Nitrobacter and *Nitrospira* are the most detected NOB species in nitrifying bioreactors (Li et al., 2007; Dytczak et al., 2008). *Nitrobacter* has a high specific nitratation rate and its characteristics have been intensively studied with the enriched *Nitrobacter* cultures. *Nitrospira*-like NOB is unculturable and has a low specific nitrification rate. Spieck et al. (2006) enriched NOB-*Nitrospira* from activated sludge taken from a municipal WWTP and the NOB-*Nitrospira* population increased to approximately 86% of the total bacterial population.

Nitrobacter has been found to be the dominant NOB in most high-rate nitrification reactors. *Nitrobacter* out-competes *Nitrospira* at high substrate concentrations, such as high NH_4^+-N and DO conditions (Liang and Liu, 2007). *Nitrospira* accounted for 1–2% of the biomass in a bench-scale activated sludge reactor treating municipal wastewater (Dionisi et al., 2002b). In a nitrifying fluidized bed reactor loaded with inorganic nitrogenous wastewater, complete nitrification occurred, and the dominant NOB was *Nitrospira* (Schramm et al., 1998). In a pilot-scale MBR treating reject water at a nitrification rate of up to 2.5 kg N/(m^3.d), complete nitrification occurred, and NOB *Nitrospira* (17.8–14.5%) were the dominant NOB nitrifiers (Liebig et al., 2001). In some sustained partial nitrification systems operated under low DO conditions, a low level of NOB-*Nitrospira* can be detected using genetic methods, while NOB-*Nitrobacter* can not be detected (Han et al., 2003; Sliekers et al., 2003).

2.6.3 Kinetics of the dominant AOB and NOB in nitrifying reactors

When DO becomes limited, AOB is a better oxygen competitor than NOB, growth of NOB is limited, and NOB may out-compete in the system. The population dynamics are regulated by the main stoichiometric and kinetic parameters of the nitrifiers. The main stoichiometric and kinetic parameters for nitrifiers are yield rate (Y), maximum specific growth rate (μ_{max}), half saturation coefficient for substrate NH_4^+-N (Ks), half saturation coefficient for O_2 (Ko), and decay rate (b).

2.6.3.1 Yield rate (Y) and maximum specific growth rate (μ_{max})

The yield rate is an important stoichiometric coefficient for autotrophic nitrifiers. The NOB-*Nitrobacter* has a significantly lower yield rate than AOB (Table 2.1). Liebig et al. (2001) found that NO_3^--N was the dominant nitrification product in a pilot-scale nitrification reactor operated with a NLR of 0.18 g N/(L.d), 42% of total bacteria population was AOB, and NOB-*Nitrobacter* was less than 5%. The low population of *Nitrobacter* in the reactor was mainly due to the difference of yield rates.

The maximum specific growth rate, μ_{max}, of AOB is generally higher than that of NOB. The μ_{max} of AOB and NOB in a developing biofilm is slower than in suspended culture (Kindaichi et al., 2006).

Table 2.1: Key parameters of nitrifiers: *Nitrosomonas*, *Nitrobactor* and *Nitrospira*

Nitrifiers	OUR_{max} mg/(g VSS.h)	μ_{max} 1/h	Y 1/h
Nitrosomonas	943 [a]	0.043 [a]	0.12 [a]
		0.02–0.09 [a]	
		0.087 [c]	
		0.016 [d]	0.14 [d]
Nitrobactor	289±15 [b]		
		0.019 [d]	0.072 [d]
Nitrospira	32±2 [b]		0.15±0.04 [b]
		0.021 [c]	

[a] Vadivelu et al. (2006a), enriched suspended culture at 30 °C

[b] Blackburne et al. (2007b), enriched suspended culture at 30 °C

[c] Kindaichi et al. (2006), enriched suspended culture at 37 °C

[d] Blackburne et al. (2007a), values were adjusted to 20 °C

2.6.3.2 Ks

Under optimized conditions, both AOB and NOB have low Ks values for corresponding substrates (Wiesmann et al., 2007). For activated sludge in a membrane bioreactor and a conventional activated sludge WWTP, K_{NH_4} and K_{NO_2} were less than 0.2 mg N/L (Manser et al., 2005) in the nitrifying activated sludge processes. The Ks values tend to become lower when reactors are operated under low NLRs (Sliekers et al., 2005).

2.6.3.3 *Ko*

The *Ko* value of heteroptrphic biomass is considered to be lower than nitrifiers (Manser et al., 2005). Significant nitrification can only happen when organic C becomes limited for the growth of heterotrophs (Tchobanoglous et al., 2003). *Ko* values of AOB and NOB depend on the availability of the substrates (DO, NH_4^+ and NO_2^-) (Sliekers et al., 2005).

When there is limited NH_4^+-N supply and low DO, *Nitrospira* is the dominant NOB, and it has a higher O_2 affinity than AOB (Sliekers et al., 2005). The *Ko* of AOB-*Nitrosomonas* and NOB-*Nitrospira* was 0.20 mg O_2/L and 0.04 mg O_2/L, respectively.

Under sufficient NH_4^+-N but oxygen-limited conditions, AOB and NOB have to compete with each other for the limited oxygen available. Sliekers et al. (2005) found that under such oxygen-limited conditions, when the DO < 0.032 mg O_2/L, the *Ko* of AOB-*Nitrosomonas* was as low as 0.074 mg DO/L; and O_2/LNH_4^+ was mainly oxidized into NO_2^-, with the mass ratio of $NO_2^-/(NO_2^- + NO_3^-)$ up to 85% in the effluent.

When both DO and NH_4^+/NO_2^- are sufficient, NOB-*Nitrobacter* is usually the dominant NOB. AOB has higher oxygen affinity than NOB in activated sludge. Guisasola et al. (2005) found that the *Ko* of AOB and NOB for enriched nitrifying biomass were 0.74 mg O_2/L and 1.75 mg O_2/L, respectively, using the respirometric method. In municipal WWTPs, a *Ko* of AOB and NOB-*Nitrobacter* of 0.2 and 2.2 mg O_2/L, respectively, are used in the mathematical modeling of activated sludge processes (Matsumoto et al., 2007).

When DO is sufficient, for the enriched culture, NOB-*Nitrospira* and NOB-*Nitrobacter* have the same *Ko* of 0.54 mg/L (Blackburne et al., 2007b). The supply of sufficient DO is not a factor for differentiating NOB-*Nitrobacter* and NOB-*Nitrospira*.

2.6.3.4 Decay rate (*b*)

As shown in Table 2.2, the decay rate of pure culture of nitrifiers or laboratory-enriched nitrification sludge is significantly lower than in conventional activated sludge, especially under anoxic and anaerobic conditions. After 6 weeks of anoxic/anaerobic storage, the nitrification capability of activated sludge can be quickly recovered when a wastewater feed resumes (Yilmaz et al., 2007).

Table 2.2: Decay rates of AOB, NOB and heterotrophs

Conditions	AOB 1/d	NOB 1/d	Heterotrophs 1/d	References	Note
Aerobic	0.2	0.2		Salem et al. (2006)	Activated sludge
	0.02	0.08		Salem et al. (2006)	Enriched culture
	0.15	0.15	0.28	Manser et al. (2006)	Activated sludge
	0.044			Park and Noguera (2007)	Pure culture
		0.007		Vadivelu et al. (2006b)	Pure culture
Anoxic	0.1	0.1		Salem et al. (2006)	Activated sludge
	0.02	0.06		Salem et al. (2006)	Enriched culture
	0.015	<0.001	0.033	Manser et al. (2006)	Activated sludge
Anaerobic	0.06	0.06		Salem et al. (2006)	Activated sludge
	0.08	0.07		Salem et al. (2006)	Enriched culture

2.6.4 Temporary nitrite accumulation in activated sludge processes

In municipal WWTPs, the activity of AOB and NOB is balanced under practical operational conditions. DO is usually maintained at 2–3 mg/L to provide sufficient oxygen to AOB and NOB (Wiesmann et al., 2007). The NH_4^+-N in the influent wastewater is also generally less than 100 mg/L. Under this condition, the specific activity of NOB is higher than AOB (Hellinga et al., 1998). Therefore, NO_2^- is a limiting substrate and high levels of NO_2^- as an intermittent substrate is not present. Temporary NO_2^- accumulation is found when the balance is disturbed due to variation of the operation conditions, such as lower DO, or unexpectedly high NLR (Tchobanoglous et al., 2003).

2.6.5 Temperature promoted partial nitrification with biomass wash-out

2.6.5.1 Temperature effect on the growth rates of AOB and NOB

Temperature has a dramatic effect on the growth and activity of nitrifiers. At optimized conditions, with increased temperature in the range of 10–40 °C, the specific growth rates increase (Figure 2.2). When the temperature is over 25 °C, AOB grow significantly faster than the NOB-*Nitrobacter*.

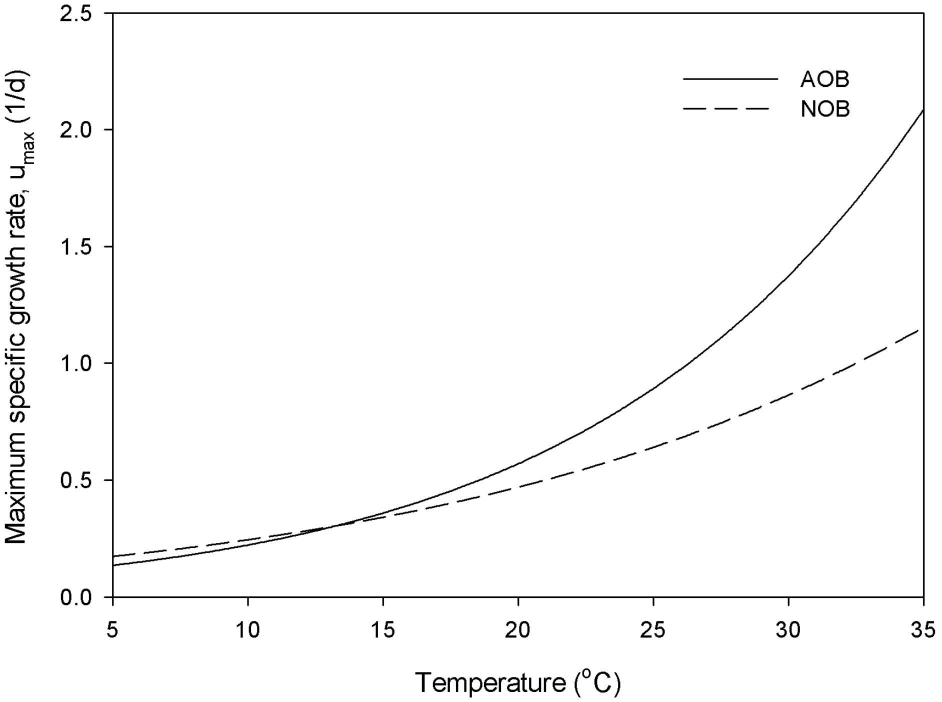


Figure 2.2: Maximum specific growth rates of AOB and NOB-*Nitrobacter* as a function of the temperature (Hunik et al., 1994)

2.6.5.2 SHARON process

The SHARON process was developed in the 1990s. It is a continuously stirred tank reactor (CSTR)-type process, where AOB grows faster than NOB with an unlimited substrate supply and optimized operational conditions at high temperatures of 30–40 °C, sufficient oxygen supply (DO >3 mg/L) and a pH of 7.0–7.5. By keeping the SRT equal to the HRT of 1.5–2 days in the reactor, the slower growing NOB is completely washed out of the reactor within a few days, but AOB still maintains a sufficient population size in the reactor (Egli et al., 2003). The MLSS in the CSTR-type SHARON process was reported to be 250–400 mg/L (Fux et al., 2002; Gali et al., 2007b). The selective growth of AOB and wash-out of NOB can ensure that NH_4^+ from the influent is only oxidized to NO_2^- (Hellinga et al., 1998).

The SHARON process shows very stable partial nitrification performance in treating high-strength wastewaters in the laboratory, such as reject water (Gali et al., 2007b), anaerobic effluent of fish-canning wastewater (Mosquera-Corral et al., 2005), and piggery waste (Hwang et al., 2005). Full-scale SHARON reactors have been installed to treat reject water from WWTPs (Hellinga et al., 1998; van Loosdrecht and Salem, 2006).

High temperature and high DO are essential for the SHARON process to promote the growth of AOB in the sludge. At temperatures of 19–23 °C and a DO of 0.4 mg/L, the SHARON process is very sensitive to process disruptions (Blackburne et al., 2008a).

Because the HRT should not be shorter than 1 day in the CSTR-type SHARON process, to sustain the growth of AOB in the reactor, the NH_4^+-N concentration in the influent should be higher than 500 mg/L (Mosquera-Corral et al., 2005).

Nitrification in the SHARON process is also influenced by competition between heterotrophic and autotrophic microorganisms. This competition depends on the COD/N ratio of wastewater (Carrera et al., 2004). Mosquera-Corral et al. (2005) found that when the COD/N ratio was higher than 0.3, the ammonium oxidization efficiency decreased to levels of around 10%

The SHARON and the anammox process have been shown to be very stable and efficient in N removal in the laboratory for a long period (van Dongen et al., 2001). The full-scale SHARON and the anammox process was started-up in 2006 (van der Star et al., 2007). In order to combine the SHARON process with the anammox process, the composition of the effluent from the SHARON reactor should have a NH_4^+-N/NO_2^--N ratio of 1 (Mosquera-Corral et al., 2005). This ratio is highly dependent on the pH, FA and HRT.

2.6.5.3 SBR-type partial nitrification process with short SRT

Because the HRT of the CSTR-type SHARON process should be higher than 1 day, the NLR of the CSTR-type SHARON process is limited compared to the high NLR of the anammox process. Using SBRs operated at a temperature of 30–35 °C, the HRT can be shortened to less than 1 day, and the SRT can also be increased up to 10 days to achieve partial nitrification at higher NLR (Pollice et al., 2002). In a SBR-type SHARON process treating reject water, with a temperature of 30 °C and a DO $>$ 3 mg/L, partial nitrification was sustained at an SRT of 5 days and a HRT of 0.35 days (Gali et al., 2007a). The mixed liquor volatile suspended solids (MLVSS) in the partial nitrification reactor can increase from 400 mg/L up to 1,200 mg/L, while the SBR was changed from 1 day to 4 days (Gali et al., 2007a). In an SBR treating landfill leachate at a NLR of 1.5 kg N/(m^3.d), a SRT of 3–7 days, a temperature of 36 °C, a pH of 6.8–7.1, and a DO of 2 mg/L, stable partial nitrification was achieved (Ganigue et al., 2007). With longer SRT, the SBR-type SHARON process is not as stable as the CSTR-type SHARON process, and once the partial nitrification performance is disrupted, it takes a long time to recover (Gali et al., 2007a).

2.6.6 Partial nitrification in oxygen-limited suspended-growth sludge reactors with a long SRT

2.6.6.1 Oxygen-limited conditions for partial nitrification

Sliekers et al. (2005) found that a reliable way to achieve sustained partial nitrification in suspended sludge reactors is to ensure that the oxygen supply is limited to oxidize all of the NH_4^+. When treating high NH_4^+ wastewater, the presence of NH_4^+ in the reactor as a substrate for AOB growth is generally sufficient. In a long SRT reactor, AOB in the sludge is kept at high concentrations. AOB is a better DO competitor and has a higher yield rate than NOB (Wiesmann, 1994). The oxygen flux into the reactor is not enough to oxidize most of the NH_4^+-N so oxygen supply becomes the limited substrate for the high population of AOB, and the DO in the bulk is relatively low. The activity of NOB-*Nitrobacter* is reduced at the low DO level (Hanaki et al., 1990). In oxygen-limited conditions, inhibition of NOB by FA or FNA, and minimization of NO_2^- concentrations in the reactor by denitrification or anammox, causing the substrate limitation for NOB, can sustain the partial nitrification process in the reactor (Blackburne et al., 2008a).

2.6.6.2 Oxygen-limited conditions together with inhibitions on NOB by FA and FNA

pH-related high FA and FNA concentrations can help to sustain partial nitrification (Pambrun et al., 2008). The pH itself can not be used to regulate the partial nitrification process. Complete nitrification only occurs in the pH range of 6.45–8.95 (Ruiz et al., 2003) and AOB are more sensitive to pH changes than NOB (Campos et al., 2007). NOB are more sensitive to FA and FNA than AOB under oxygen-limited conditions. Pambrun et al. (2008) found that in a SBR with influent NH_4^+-N of 1000 mg/L, SRT of 18 days, MLSS >4000 mg/L, pH of 8.25–8.5, temperature of 30 °C, complete NH_4^+ removal with OUR control, and stable partial nitrification can be achieved for 190 days. Gustavsson et al. (2008) found that low DO (DO $<$ 0.6 mg/L) and high FNA conditions sustained long-term stable partial nitrification in a SBR treating sludge liquor with a SRT of 29 days.

The NOB-*Nitrobacter* can be acclimatized to high FA conditions, especially under high DO conditions. Villaverde et al. (2000) found that using FA inhibition as the only selection parameter, stable partial nitrification in the reactor can not be guaranteed. While still keeping high FA concentrations (7–54 mg NH_3-N/L), but with increased oxygen supply in the MBRs, NH_4^+-N from the influent was completely converted to NO_3^-

(Wyffels et al., 2003).

2.6.6.3 Oxygen-limited conditions together with limited NO_2^- by denitrification

Coupled with aeration duration control and denitrification in the SBRs, partial nitrification can be achieved in the treatment of high-strength and low-strength wastewater. In laboratory-scale and pilot-scale SBRs treating municipal wastewater, using a real-time aeration duration control, nitrification and denitrification via NO_2^- may be achieved for a long-run period, and the NO_2^- accumulation rate may be over 80% (Wu et al., 2007; Blackburne et al., 2008b). In a SBR treating supernatant from the anaerobic digestion of municipal solid wastes operated, at a temperature of 30 °C, a pH of between 7.4 and 8.6, a HRT of 3 days and a SRT of 12 days, with partial nitrification and denitrification, stable NO_2^- build-up was achieved (Mace et al., 2006). N removal via NO_2^- can be sustained in a step-feed, laboratory-scale SBR treating slaughterhouse wastewater, operated at a DO of 1.5–2.0 mg/L, a temperature of 20 °C, a SRT of 15 days, and a pH of 7.1–7.9, the highest NH_4^+-N concentration during a cycle was less than 20 mg/L (Lemaire et al., 2008). Lemaire et al. (2008) suggested that NOB are eliminated due to the gradual reduction of their energy source through the use of denitrification, rather than due to the inhibition of their growth kinetics. Any reduction of the NOB growth kinetics would help to speed-up the onset and establishment of the NO_2^- pathway (Lemaire et al., 2008).

2.6.6.4 Oxygen-limited conditions together with limited NO_2^- by anammox

In one-reactor autotrophic N removal systems, such as CANON (completely autotrophic removal of nitrogen over nitrite) or OLAND (oxygen-limited autotrophic nitrification-denitrification) reactors, NOB in the sludge is in competition for both substrates, O_2 and NO_2^-. Sliekers et al. (2003) found that in a gas-lift CANON reactor, partial nitrification and the anammox process occurred under low DO concentrations of 0.5 mg/L, NO_2^- in the reactor near was zero, and the activity of NOB was out-competed under stable operation. When NH_4^+ became the limited substrate, both NH_4^+ and NO_2^- concentrations were close to zero, and DO rose in the reactor. As a consequence, the anammox activity was inhibited by high DO, NOB developed in the reactor, and the N removal rate decreased to 57% (Sliekers et al., 2003). The changes caused by the NH_4^+ limitation were reversible. The NOB is out-competed when NLR is increased, and AOB and anammox bacteria become the stronger competitor for O_2 and NO_2^- consumption (Third et al., 2001).

2.6.7 Partial nitrification in biofilm nitrification systems

Maintaining low DO concentrations in the biofilm reactors can be a good choice to achieve partial nitrification (Bernet et al., 2001a). The major advantage of nitrifying biofilm reactors over suspended-growth reactors is to maintain high nitrifying biomass in the reactor so as to endure varying loading rates and environmental conditions, e.g., low temperatures of 5–10 °C (Hoilijoki et al., 2000).

In nitrifying biofilm reactors, most of the nitrifiers are immobilized in the biofilm, and nitrifiers in the suspended culture can be easily washed out due to a shorter SRT for the suspended culture than for the biofilms, and due to the slow growth of autotrophic nitrifying bacteria. The mass transfer is of great importance to the nitrifying biofilm process. Nitrification in biofilms is mass transfer-limited, rather than kinetically-limited (Rostron et al., 2001). A sharp DO gradient is expected in nitrifying biofilms due to oxygen diffusion and the simultaneous utilization of DO by nitrifiers (Bernet et al., 2005).

Kindaichi et al. (2006) found that in a sufficiently aerated biofilm reactor, when a high DO (i.e. >7 mg/L) was kept in the bulk liquid, O_2 penetrated into the biofilm up to a distance of 200 μm. Okabe et al. (1999) and Kim and Seo (2006) found that in complete nitrification biofilm reactors, under sufficient oxygen supply, AOB is present throughout the biofilm, and NOB is located in the inner part of the biofilm.

While treating ammonium-rich wastewater, under lower DO conditions, oxygen supply is the limiting factor for NOB in the inner layer of the biofilm and its growth rate is significantly inhibited (Kindaichi et al., 2006). Thus, NO_2^- accumulation can build up in the biofilm reactor, and AOB may out-compete and displace NOB in the biofilm surface due to its higher oxygen affinity and higher specific growth rate. A high FA and/or FNA concentration in the reactor may also suppress the growth of NOB in biofilms.

With a high NLR and oxygen limitation in combination with inhibition of FA and FNA, partial nitrification can be maintained in biofilm reactors treating high-strength N wastewater (Kim et al., 2003; Han et al., 2003; Kim and Seo, 2006; Liang and Liu, 2007). Five-month's continuous operation of a biofilm reactor showed that the NO_2^- accumulation ratio rose by decreasing DO concentrations (Chuang et al., 2007a). At a DO of 3.5 mg/L, a maximum NO_2^- accumulation ratio of 42% was obtained in an air-lift biofilm reactor at NLR of 3.3 kg N/(m^3.d) (Oyanedel-Craver et al., 2005).

Bernet et al. (2005) used the O_2/NH_4^+-N ratio as the control parameter, which ranged from 0.05–0.1, and at a DO in the reactor ranging 1–2 mg/L, observed that 60% of the influent NH_4^+-N was oxidized to NO_2^--N. While keeping FA of 3–4 mg N/L and DO of 1.0

mg/L, stable partial nitrification of 84–88% can be achieved for 270 days in a rotating disk biofilm SBR (Antileo et al., 2006). At a DO of 0.5 mg/L, stable partial nitrification was sustained in a completely-stirred biofilm reactor for more than 110 days with an NO_2^-/NO_3^- ratio of more than 90% (Bernet et al., 2001b).

Granular sludge is considered as a type of biofilm structure (Beun et al., 2002). By shortening the settling time in the SBR, AOB can be selectively enriched and granulated. Kim and Seo (2006) found that at a DO of 1.8–2.5 mg/L and a temperature of 25 °C, AOB-*Nitrosomonas* became the dominant nitrifying bacteria in granulated sludge, and NOB were gradually washed out of the reactor.

In stable biofilm systems, partial nitrification can occur under less strict operation conditions than in suspended-growth activated sludge systems. This is due to the thickness of biofilms and the restricted growth pattern of nitrifiers in the biofilm. At a NLR of 3.3 kg N/(m^3.d), a HRT of 4.3 hours, a temperature of 31 °C, and a pH of 7.8, Oyanedel-Craver et al. (2005) found that the maximum NO_2^- accumulation was achieved at a DO of 3.5 mg/L in a biofilm reactor.

Once stable NO_2^- accumulation is achieved, the partial nitrification process seems to be easily kept stable, unless there is a dramatic decrease of NLR or an increased oxygen supply (Bougard et al., 2006; Kim and Seo, 2006). Bernet et al. (2001b) show that once complete nitrification is established in the biofilm system, reducing the DO concentration in the reactor may help to recover partial nitrification.

2.7 Summary

This chapter reviewed the current status of BNR process, SBR technology, slaughterhouse wastewater generation and treatment, and partial nitrification. The microbiology of AOB and NOB, partial nitrification in three types of reactor systems (temperature promoted, oxygen-limited and biofilm) were also reviewed.

Chapter 3

Nitrogen Removal from Slaughterhouse Wastewater in an Intermittently Aerated Sequencing Batch Reactor (SBR)

3.1 Overview

The chapter describes slaughterhouse wastewater treatment in a SBR operated with an intermittent aeration strategy.

3.2 Introduction

Slaughterhouses produce high-strength wastewater (Johns, 1995). The discharge standards in Ireland for slaughterhouse wastewater are: COD $\geq$ 75% removal or 125–250 mg/L in the effluent; TN $\geq$ 80% removal or 15–40 mg/L in the effluent; and TP $\geq$ 80% removal or 2–5 mg/L in the effluent (Irish EPA, 2006).

The slaughterhouse and rendering plant at Ballyhaunis, County Mayo, is a typical large-scale operation in Ireland, slaughtering cattle and sheep. The typical flow rate of wastewater into the plant is 1000 m^3/d. After the preliminary and primary treatment by means of screening and DAF, a conventional activated sludge process (A/O) is applied to treat combined slaughtering and rendering wastewater with a conditioning tank and a series of 5 activated sludge aeration tanks. A total aeration tank capacity of 5,780 m^3 is provided in these five aeration tanks. Each aeration tank is equipped with a surface aerator. The return rate of the settled sludge liquid from the secondary clarifier to the aeration tanks to the influent is about $15:1$. The average HRT and SRT of the activated sludge process are 5–6 days and 20–30 days, respectively. A tertiary treatment—chemical precipitation—is applied to remove the remaining P from the secondary effluent. The final effluent meets the discharge limits and is discharged into a neighboring river. The discharge limit for the treated slaughterhouse wastewater from this plant is: SS, 25 mg/L; BOD, 20 mg/L; COD, 50 mg/L; NO_x^--N, 15 mg/L; NH_4^+-N, 10 mg/L; and TP, 3 mg/L.

SBRs are amongst the best available technologies recommended by the European Commission for slaughterhouse wastewater treatment (European Commission, 2005). A conventionally operated SBR can be changed to an intermittently aerated SBR (IA-SBR), where one complete operational cycle comprises four phases: fill, react (intermittently aerated), settle and draw. In the intermittently aerated react phase, the reactor is continuously stirred, and anoxic non-aeration (mixing) and aerobic aeration are alternatively applied. Intermittently aerated reactors have been used to remove N from domestic wastewater (Zhao et al., 1999) and swine wastewater (Mota et al., 2005b). During the aeration periods in an IA-SBR, DO is supplied for aerobic nitrification to oxidize NH_4^+-N to NO_x^--N. During the subsequent non-aeration period, the remaining DO is rapidly depleted to achieve anoxic conditions for heterotrophic denitrifiers to reduce NO_x^--N to N_2 gas. If the DO of the bulk liquid in the reactor is controlled by means of intermittent aeration, NH_4^+-N will be partially oxidized to NO_2^--N, and then in the non-aeration periods, NO_2^--N will be reduced to N_2 gas. This partial nitrification can reduce the operation cost by reducing oxygen supply and reducing the demands for rbCOD in the denitrification process (Khin and Annachhatre, 2004; Ahn, 2006; Paredes et al., 2007). In addition, in an IA-SBR, the organic C, stored by PAOs, can be used by denitrifiers for denitrification during subsequent anoxic periods (Akin and Ugurlu, 2005). This could result in improved denitrification efficiency without sacrificing biological P removal.

In this chapter, the performance of a laboratory-scale, intermittently aerated SBR for simultaneous N and P removal from slaughterhouse wastewater was investigated.

3.3 Materials and methods

3.3.1 Laboratory-scale SBR system

The laboratory-scale SBR system is illustrated in Fig. 3.1. The reactor tank was made from transparent Plexiglas and had a working volume of 10 L, with an inner diameter of 194 mm and a height of 400 mm. Two MasterFlex L/S (Cole-Parmer, US) peristaltic pumps were used. One filled the reactor with slaughterhouse wastewater from the influent tank during the fill phase and the other decanted the effluent from the reactor during the draw phase. A mechanical mixer with an 80 mm-deep by 100 mm-wide rectangular paddle was installed over the reactor. An air pump supplied air through a porous stone diffuser located at the bottom of the reactor. The air flow rate was regulated by an air flowmeter. The operational sequence of the SBR and the operation of all mechanical devices, including the peristaltic pumps, the mixer and the air pump was controlled by a programmable logic controller (PLC, S7-222, Siemens, Germany).

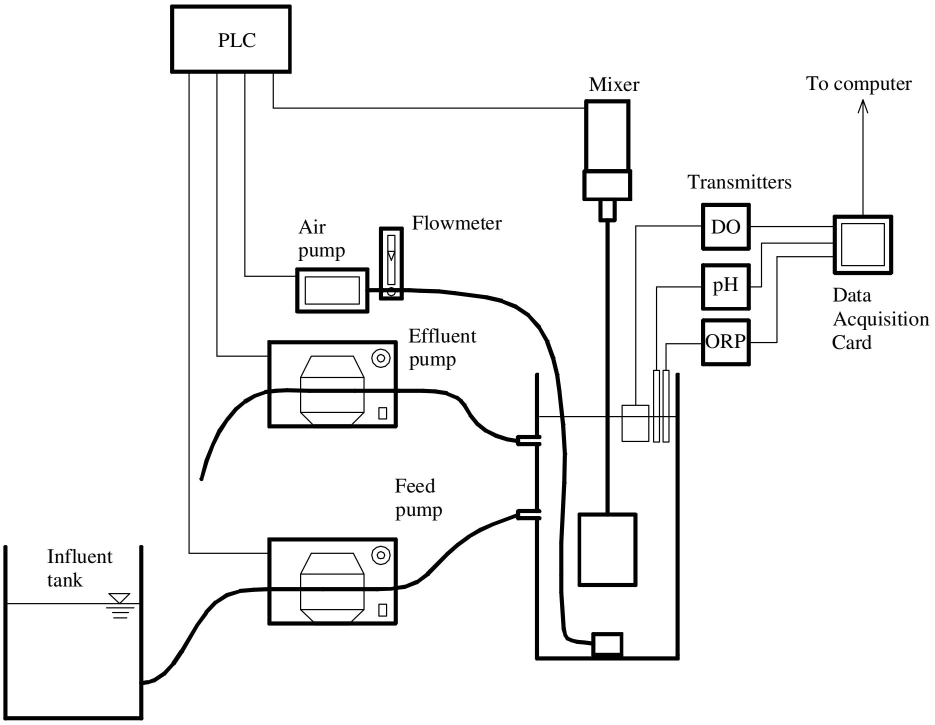


Figure 3.1: Schematic diagram of the laboratory-scale SBR system

DO, pH and oxidation-reduction potential (ORP) in the reactor were real-time monitored using electrodes. The DO electrode (EC-DOTPII-S, Eutech, Singapore), pH electrode (Sentix 20, WTW, Germany) and ORP electrode (Sentix ORP, WTW, Germany) were connected to corresponding transmitters (Eutech, Singapore) that transformed the signals from the three electrodes into 4–20 mA analog signals. A data acquisition card (USB-6009, National Instrument, USA) transformed analog signals into digital signals, which were processed by a LabVIEW computer programme (V7.1, National Instruments, USA).

The duration of a complete cycle was 480 minutes (6 hours), giving three cycles per day. The sequential operation of a complete cycle is shown in Fig. 3.2. During the fill phase (7 mins), 1.0 L of slaughterhouse wastewater was pumped from the influent tank into the reactor vessel. From Minute 50, the reactor was intermittently aerated with an air supply of 0.8 L air/min four times at 50-minutes intervals, 50 minutes each time. The mechanical mixer was operated continuously with a speed of 100 rpm from the beginning of the fill phase to the end of the react phase. During the draw phase, the supernatant wastewater was decanted until the liquid volume in the reactor decreased to 9.0 L. SRT was manually controlled by discharging a certain amount of mixed liquor from the reactor every day just before the settle phase commenced.

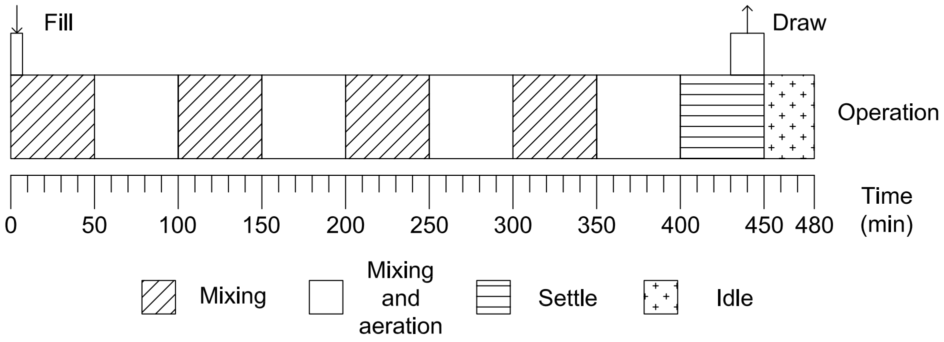


Figure 3.2: A complete operational cycle of the laboratory-scale SBR system

3.3.2 Slaughterhouse wastewater

The slaughterhouse wastewater was collected from the conditioning tank in the WWTP of the slaughterhouse and rendering plant at Ballyhaunis, Co. Mayo, Ireland. A conventional activated sludge process is applied in the plant. The aeration tanks of this plant are operated at an average OLR of 0.5 kg COD/(m^3.d). The SRT is 20–30 days and the concentration of MLSS is 5,000–6,000 mg/L.

The raw wastewater was collected in 10-L plastic containers and stored in a refrigerator at approximately 4 °C for 10–20 days before use. The wastewater in the influent tank was prepared daily by filtering the raw wastewater through a 0.6 mm mesh screen and a submerged aquarium pump was placed in the influent tank to stir the wastewater. The average concentrations of SS, COD, BOD_5, TN, TP and NH_4^+-N in the influent wastewater over the study period were 1,403 ± 596 mg/L, 4,672 ± 952 mg/L, 2,895 ± 585 mg/L, 256 ± 46 mg/L, 29 ± 10mg/L and 342 ± 87 mg/L, respectively.

3.3.3 Operation of the IA-SBR

The reactor was continuously operated for 132 days. The slaughterhouse wastewater feeded into the reactor was 3.0 L/d, so the HRT of the reactor was 3.3 days. pH values in the reactor ranged from 7.0–8.0. During Days 88–102 and Days 116–132, influent COD concentrations were as high as 6,620 and 5,710 mg/L, respectively. Excluding these two periods, the average influent COD and SS concentrations were 3,931 and 983 mg/L, respectively, giving an average OLR of 1.2 g COD/(L.d). TN was relatively stable during the study period with an average value of 356 mg/L. During Days 94–109, the aeration rate was only 0.1–0.3 L air/min because of blockage of the air diffuser. This was replaced on Day 110.

Figure 3.3 shows the variation of the sludge concentration in terms of MLSS, MLVSS, and sludge volume index (SVI) during the study period. The average VSS/SS ratio was 0.92. SVI ranged from 87–107 mL/g, indicating that the sludge had a good settlement capability. As a SRT of 20–30 days was used in the aeration tanks of the local slaughterhouse WWTP, the initial SRT of the SBR was set at 25 days. It was observed that MLSS in the reactor rapidly rose to over 6,500 mg/L. Therefore, from Day 17, the SRT was decreased to 20 days, but a rapid increase in MLSS was also measured. Consequently, from Day 40, the SRT was kept at 14.5 days, so that MLSS concentrations in the reactor were almost stable at 6,000 mg/L. During Days 88–102 and Days 116–132, due to high influent SS and COD concentrations, MLSS concentrations in the reactor tank were over 7,000 mg/L. This SRT of 14.5 days was shorter than the optimum SRT of 20–25 days obtained by Tremblay et al. (1999) treating slaughterhouse wastewater in a conventional SBR.

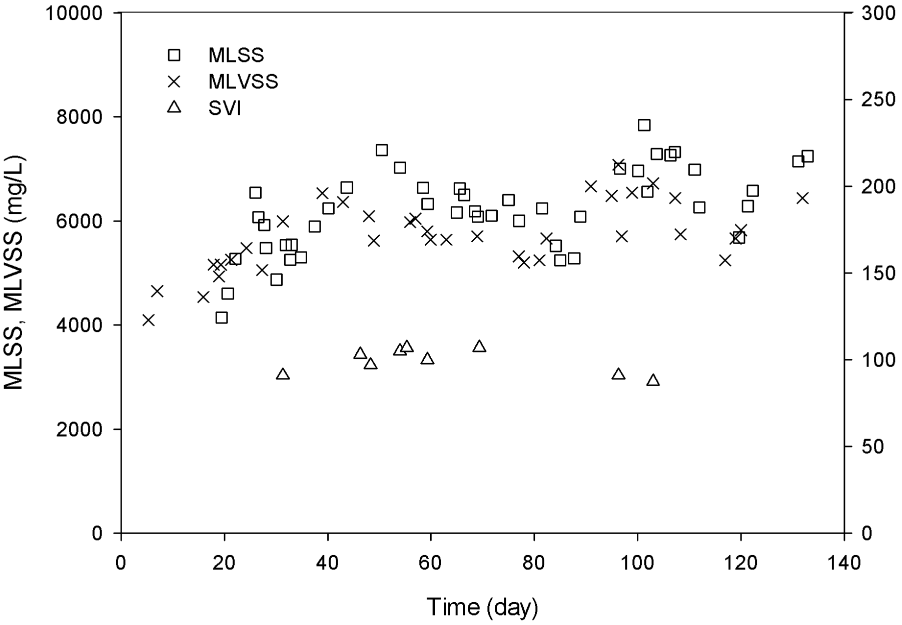


Figure 3.3: Profiles of MLSS, MLVSS and SVI in the reactor

3.3.4 Analytical methods

COD, BOD_5, MLSS and MLVSS were measured in accordance with the standard APHA methods (Eaton et al., 1995). TN and NO_3^--N were measured using Hach TN test-kits and nitrate test-kits (Hach, Germany). After filtration with 0.8 μm filter papers, PO_4^{3-}-P, NH_4^+-N, NO_2^--N and total oxidized nitrogen (TON) in the water samples were measured with a nutrient analyzer (Konelab 20, Thermo, USA).

The N balance in a cycle can be analyzed as:

$$TN_{in} = TN_{out} + TN_M + \sum_i (TN_D)_i \tag{3.1}$$

where TN_{in} is the amount of N (mg) contained in the influent wastewater fed into the reactor during the fill phase; TN_{out} is the amount of N (mg) contained in the effluent wastewater leaving the reactor during the draw phase; TN_M is the amount of N (mg) utilized by microorganisms for biomass synthesis; $\sum_i (TN_D)_i$ is the amount of N (mg) removed from the reactor by means of denitrification in the i^{th} non-aeration periods ($i =$ 1, 2, 3, 4).

3.4 Results and discussion

3.4.1 Overall performance of the IA-SBR

The performance of the IA-SBR in slaughterhouse wastewater treatment is illustrated in Fig. 3.4–Fig. 3.6. Excluding Days 88–102 and Days 116–132, when influent SS was over 1,500 mg/L, the effluent SS concentrations were below 60 mg/L with an average concentration of 33 mg/L (Fig. 3.4). Before Day 87, the effluent contained an average of 120 mg/L total COD (CODt) and 62 mg/L BOD_5, representing an organic matter removal of 97%. Due to the increase of influent COD and SS during Days 88–132, effluent COD concentrations during this period were high. The highest effluent CODt was 320 mg/L, representing a CODt removal of 96%; however, effluent BOD_5 remained at approximately 60 mg/L. On average, the effluent soluble COD (CODs) was less than CODt by 25 mg/L.

Excluding four periods (Days 19–25, Days 43–45, Days 63–68 and Days 94–109), the soluble TN (TNs) in the effluent was below 20 mg/L, giving a TN removal of 96% and an effluent NH_4^+-N of less than 1.0 mg/L, indicating complete ammonium oxidation (Fig.

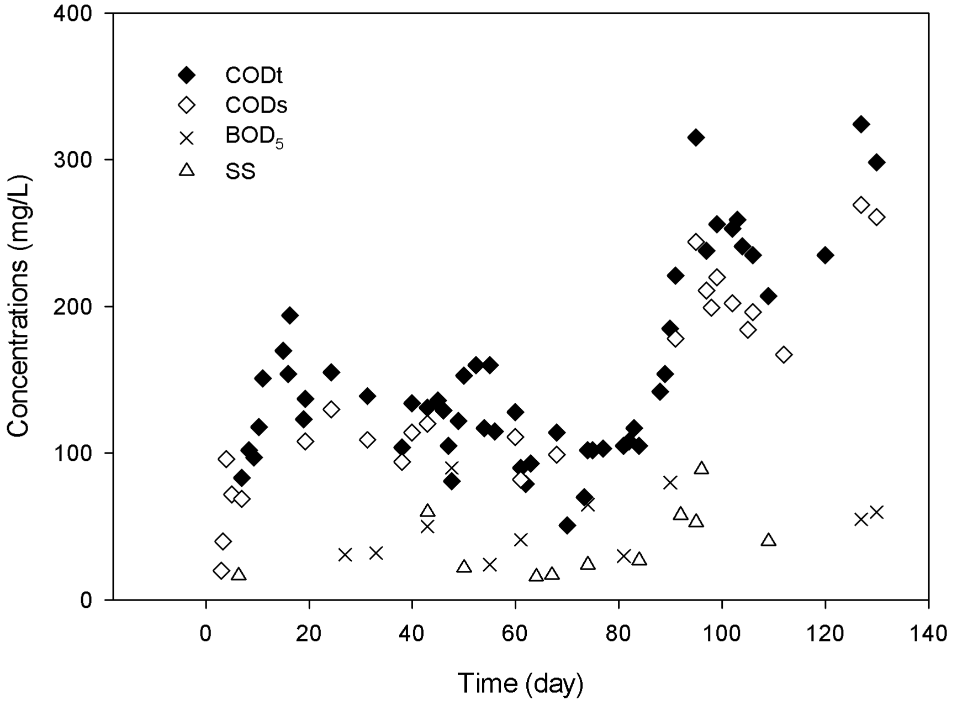


Figure 3.4: Variations of effluent COD, BOD_5 and SS

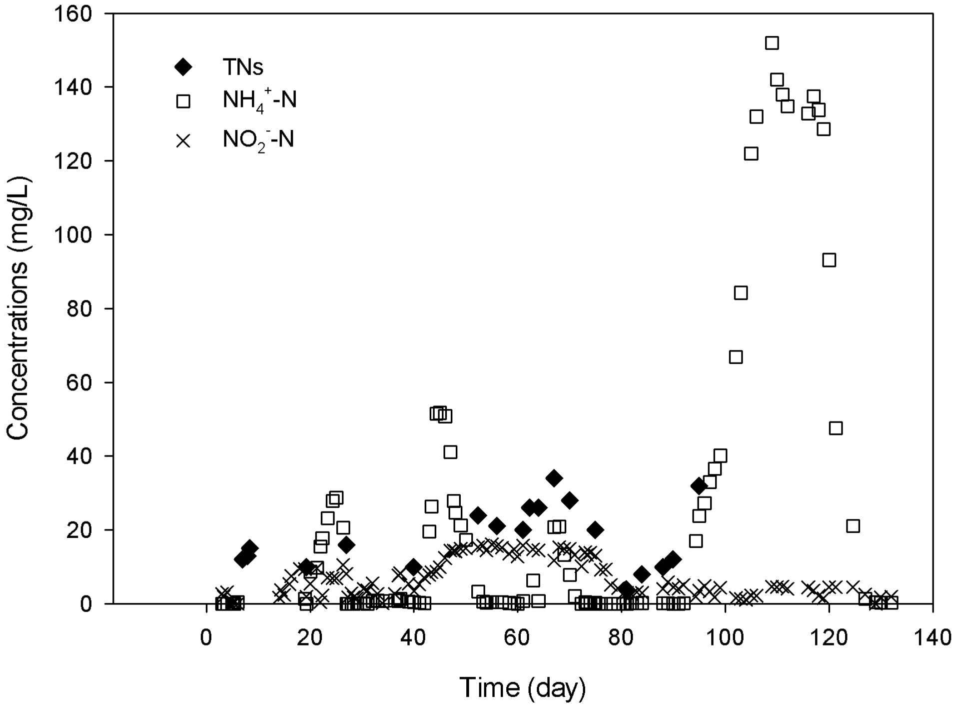


Figure 3.5: Variations of effluent N matter

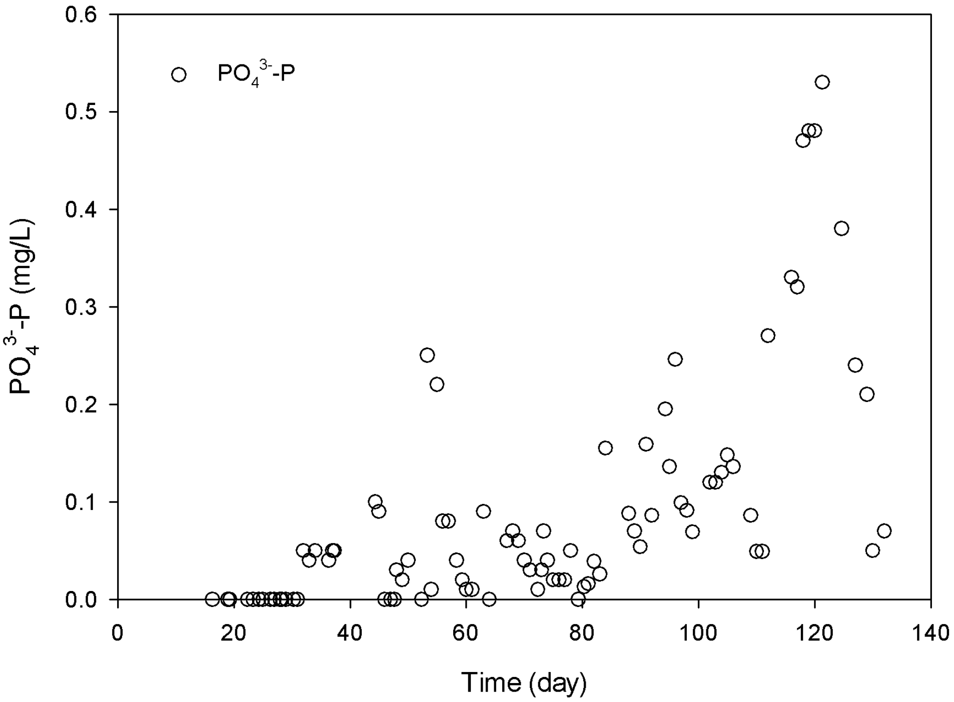


Figure 3.6: Varitations of effluent PO_4^{3-}-P

3.5). Generally, DO concentrations during the fourth aeration period were 3.5–5.5 mg/L. During Days 94–109, due to blockage of the air diffuser, the aeration rate decreased to 0.1–0.3 L air/min and the effluent NH_4^+-N rose to 152 mg/L. After replacement of the diffuser, NH_4^+-N quickly decreased to less than 1.0 mg/L. The rises in the effluent NH_4^+-N that occurred on three other occasions (Days 19–25, Days 43–45 and Days 63–68) were caused by a temporary blockage of the air diffuser, resulting in low DO concentrations during the aeration periods in the react phase (data are not shown).

The SBR had satisfactory biological P removal (Fig. 3.6). Effluent PO_4^{3-}-P concentrations were less than 0.6 mg/L and effluent TP ranged from 0.4 to 1.25 mg/L. The low effluent P concentrations showed that tertiary treatment was not necessary. When the SRT was 14.5 days, the P content of the sludge was 15–20 mg TP/g VSS, which was comparable to the typical P content of biomass in conventional activated sludge systems (Tchobanoglous et al., 2003).

The efficient removals of COD, TN and TP (96%, 96% and 99%, respectively) by the IA-SBR indicate that an intermittent aeration strategy is advantageous over conventionally operated SBRs, in which 95–97% TN removals from slaughterhouse wastewater were achieved only with the addition of sodium acetate as an external C source for complete denitrification (Filali-Meknassi et al., 2004, 2005a).

3.4.2 Cycle performance

The cycle performance of the IA-SBR was investigated in several operational cycles. A typical cycle performance, which took place on Day 77, is now discussed. MLVSS in the reactor was 5,320 mg/L. In the react phase, the average DO concentrations during the four aeration periods were 0.7, 1.3, 2.6 and 6.0 mg/L, respectively. During non-aeration periods, DO rapidly decreased to less than 0.1 mg/L.

P release occurred during the first non-aeration period and a maximum PO_4^{3-}-P concentration of 11.8 mg/L was reached at Minute 50 (Fig. 3.7). Considering that the influent PO_4^{3-}-P concentration was 27 mg/L, it was estimated that 91 mg PO_4^{3-}-P was released from the sludge. P uptake commenced at the start of the first aeration period and was complete by 20 minutes. Throughout the cycle, the average P uptake rate was 0.54 mg P/(L.min). CODs decreased during the fill phase and the first non-aeration period, and it leveled to around 100 mg/L until the end of the cycle (Fig. 3.7).

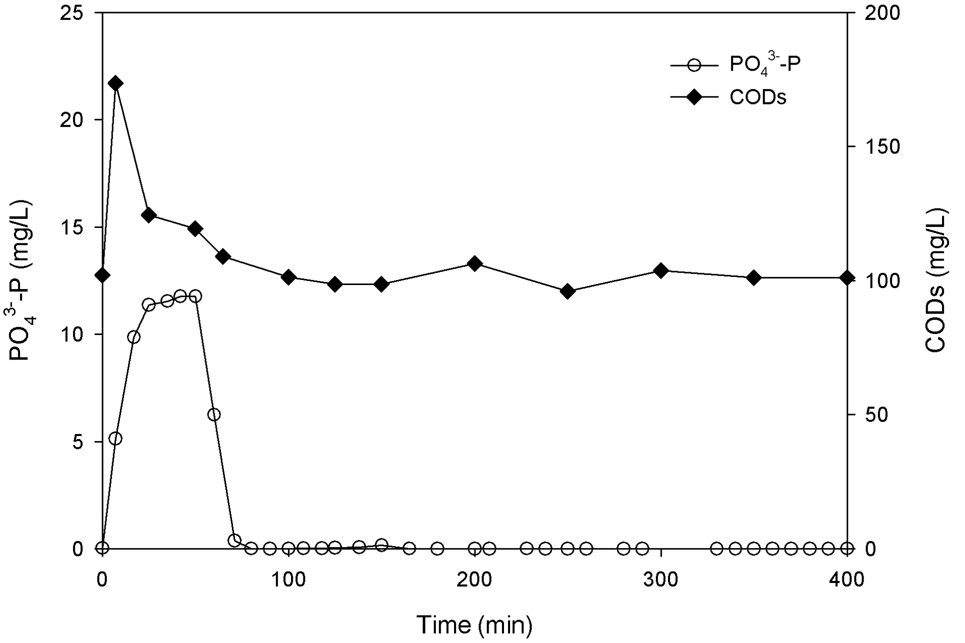


Figure 3.7: Profiles of COD and PO_4^{3-}-P in a typical react phase on Day 77

During the 7-min fill time, the remaining NO_x^--N (NO_2^--N and NO_3^--N) in the IA-SBR from the preceding cycle was rapidly removed by denitrification, with a denitrification rate of over 1.25 mg N/(L.min) (Fig. 3.8). After the fill phase, a soluble TN (TNs) concentration of 35 mg/L was measured in the bulk mixed liquor phase. NH_4^+-N decreased during the aeration periods, resulting from nitrification and biomass synthesis. A release of around 7 mg NH_4^+-N/L was observed during the second anoxic period (100–150 min),

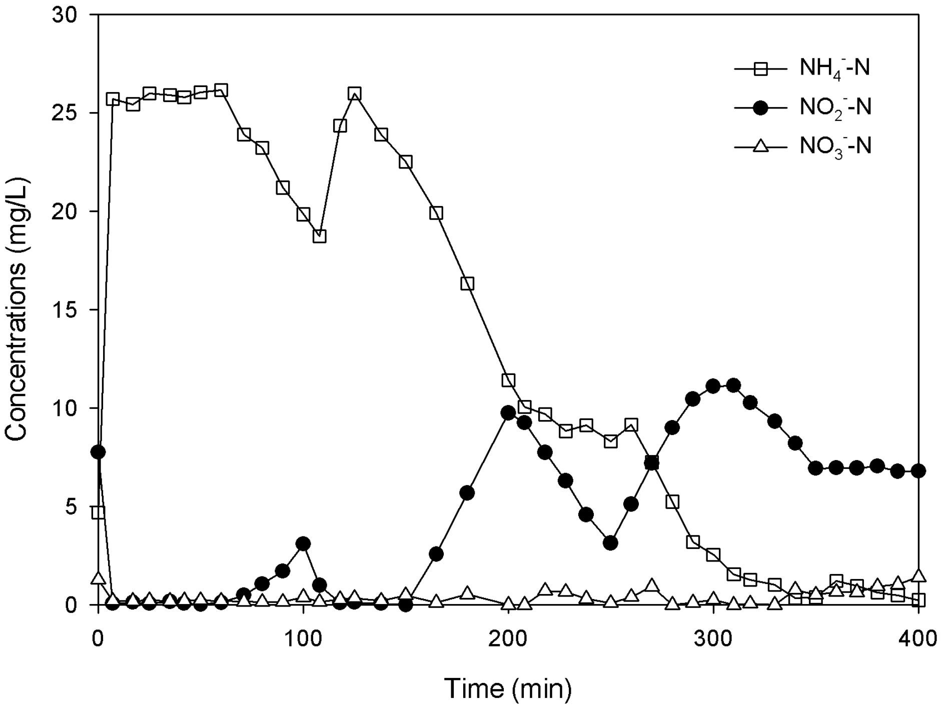


Figure 3.8: Profiles of N components in a typical react phase on Day 77

because of ammonification of organic N. After P uptake was complete, NO_2^--N was measured in the reactor from Minute 71. NO_2^--N increased linearly in the first three aeration periods (50–100 min, 150–200 min and 250–300 min, respectively) and NO_2^--N production rates were 0.065, 0.207 and 0.178 mg/(L.min). During the last aeration period (350–400 min), the nitrification rate was close to zero due to the depletion of NH_4^+-N.

If a pseudo-steady state is assumed, then

$TN_{in} = 340$ mg/L × 1 L = 340 mg N

$TN_{out} = 9$ mg/L × 1 L = 9 mg N

Using NO_x^--N concentrations at the start and end of the non-aeration periods, N removals through denitrification in the first, second, third and fourth non-aerated periods were 81 mg, 31 mg, 66 mg and 42 mg, respectively. Hence, $\sum_i (TN_D)_i = 220$ mg N.

Substituting TN_{in}, TN_{out} and $\sum_i (TN_D)_i$ into Eq. (3.1), TN_M was calculated to be 111 mg N. Therefore, 66% of TN removed from the wastewater was due to denitrification and 34% was utilized for biomass synthesis. To verify that 111 mg N was used for biomass synthesis, the TN content in the sludge biomass was measured and it was 94 mg N/g VSS. Since SRT was 14.5 days and the volume of the mixed liquor desludged was 0.69

L/d, the amount of TN removed from the reactor by means of desludging was calculated as 345 mg N/day. Therefore, in every operational cycle, the amount of TN removed from the reactor by means of desludging was 115 mg N. This was very close to 111 mg N, calculated from Eq. (3.1).

In this complete cycle, pre-denitrification during the fill phase accounted for 37% of the amount of N removed by denitrification (81 mg/220 mg), and post-denitrification during the non-aeration periods accounted for 63% (139 mg/220 mg). This shows that an intermittent aeration strategy employed in the SBR can enhance N removal.

In the aeration periods of the react phase, NO_2^--N comprised 64–96% of the TON, indicating that partial nitrification occurred in the IA-SBR. Many researchers have found that the major selective pressures for partial nitrification are DO and NH_4^+-N concentrations in the bulk liquid phase. Low DO concentrations, ranging 0.2–0.7 mg/L, favour partial nitrification (Ruiz et al., 2003; Chuang et al., 2007a). It also have been found that when DO concentrations were in the medium range (i.e. 1.4 mg/L), NO_2^--N accumulation can still take place (Ciudad et al., 2005). In biofilm reactors, NO_2^--N accumulation occurs at DO concentrations of up to 3.5 mg/L (Oyanedel-Craver et al., 2005). When the mass ratio of bulk DO to FA is lower than 10, NO_2^--N is the dominant form of NO_x^--N (Cecen, 1996). However, in the IA-SBR under examination, the average DO concentrations during the four aeration periods were 0.7, 1.3, 2.6, and 6 mg/L, and the FA concentration was less than 0.45 mg N/L. Both DO and the DO/FA ratio were not consistent with the findings of the other researchers mentioned above. The NO_2^--N/NO_x^--N ratios were 96% and 89% in the third and fourth aeration periods, respectively. These were higher than the ratios in the first and second aeration periods—64% and 72%, respectively.

It is possible that the aeration pattern applied in the SBR caused partial nitrification. Mota et al. (2005a) found that in intermittently aerated reactors, NO_2^--N accumulation occurred at an aeration-to-non-aeration ratio (ANA) of 1 h/4 h. This ANA ratio was different to this study (50 min/50 min). In order to examine the effect of the aeration pattern on NO_2^--N accumulation, a batch experiment was carried out. A certain amount of mixed liquor was taken from the reactor just before the settle phase commenced. The mixed liquor was added at a concentration of 1,481 mg/L into a 2-L jar, along with effluent from the SBR and 50 mg N/L ammonium chloride (NH_4Cl), and aeration and mixing commenced immediately. During the experiment, pH was in the range of 7.5–8.0, DO was more than 5 mg/L and temperature was controlled at 20 °C. Water samples were taken from the jar at regular intervals and the concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were measured. The production of NO_2^--N was 3.63 mg N/(g VSS.L); the production of NO_3^--N was not detectable in the first hour, but it increased to 0.98 mg N/ (g VSS.L) later. This may indicate that autotrophic NOB existed in the SBR at a lower population size. It took more than 1 hour for NOB to recover the activity after the operational condition of the changed from the anaerobic or anoxic to aerobic conditions. As in this study the aeration duration of 50 minutes was shorter than the lag time of NOB (1 hour), the activity of NOB was inhibited, which caused the accumulation of NO_2^--N. However, further research should be carried out to study the mechanisms triggering NO_2^--N accumulation in the SBR.

3.5 Summary

A laboratory study on slaughterhouse wastewater treatment using an intermittently aerated SBR system resulted in the following conclusions:

1. At an influent OLR of 1.2 g COD/(L.d), average effluent concentrations of COD, TN and TP were 150 mg/L, 15 mg/L and 0.8 mg/L, respectively. This represented COD, TN and TP removals of 96%, 96% and 99%, respectively.

2. A nitrogen balance was carried out and showed that 66% of N removed was due to denitrification and 34% was used for biomass synthesis.

3. Partial nitrification and denitrification occurred in the system, possibly due to the intermittent aeration pattern employed. 95% of N removal by means of denitrification was via NO_2^--N.

Chapter 4

Performance of an Intermittently Aerated Sequencing Batch Biofilm Reactor (SBBR) Treating Slaughterhouse Wastewater

4.1 Overview

This chapter describes slaughterhouse wastewater treatment in a laboratory-scale sequencing batch biofilm reactor (SBBR) operated with an intermittent aeration strategy.

4.2 Introduction

As described in Chapter 3, a laboratory-scale intermittently aerated SBR was used to successfully remove COD and nutrients from slaughterhouse wastewater. The addition of small, floating biofilm carriers into a SBR, forming a SBBR, may enhance its nitrogen removal capacity. The synthetic biofilm carriers may improve volumetric nitrification rates, and denitrification may be accomplished under aerobic conditions because anoxic zones can be formed within the biofilm depth. In this study, the performance of an intermittently-aerated SBBR (IA-SBBR) operated with a constant aeration rate for slaughterhouse wastewater treatment was investigated.

4.3 Materials and methods

4.3.1 Laboratory-scale IA-SBBR system

The schematic diagram of the IA-SBBR is the same as Fig. 3.1 and the setting up of the IA-SBBR was similar to the IA-SBR as described in Section 3.3.1. The IA-SBBR had a working volume of 10 L. A bulk volume of 4.0 L of Kaldnes K1 plastic biofilm carriers (Anoxkaldnes AS, Norway) was added into the reactor. The biofilm carrier elements have a nominal diameter and length of 9.1 mm and 7.2 mm, respectively, and a specific weight of approximately 0.95 kg/L. The effective specific surface area of the biofilm carriers, available for biofilm growth, is 500 m^2/m^3.

4.3.2 Slaughterhouse wastewater

As described in Section 3.3.2, the slaughterhouse wastewater used to feed the IA-SBBR was collected from the conditioning tank of a slaughterhouse wastewater treatment plant. The raw wastewater was collected with 10-L plastic containers and stored at 4 °C for up to 10–20 days before use. The average SS, COD, TN and TP concentrations of the influent used in this study were 1010 ± 150, 3930 ± 148, 379 ± 44 and 23 ± 4 mg/L, respectively.

4.3.3 Operation of the IA-SBBR

The reactor was operated at an ambient temperature of 18–24 °C. As shown in Fig. 3.2, during the 7-min fill phase, 1.0 L of slaughterhouse wastewater was pumped into the reactor. From Minute 50, the reactor was intermittently aerated with an air flowrate of 0.8 L/min. The mechanical mixer was operated continuously with a speed of 100 rpm from the beginning of the fill phase to the end of the react phase. During the draw phase, the supernatant was decanted until the liquid volume in the reactor decreased to 9.0 L. The SRT was manually controlled by daily discharging a certain amount of mixed liquor from the reactor at the end of the react phase. Since 3.0 L of wastewater was treated every day, the HRT was 3.3 days. The MLSS concentration in the reactor was kept at approximately 5000 mg/L by retaining the SRT at 11–12 days. The average OLR was 1.18 ± 0.04 kg COD/(m^3.d) and the NLR was 0.11 ± 0.01 kg N/(m^3.d). The pH in the reactor ranged from 7.0 to 8.0, so external alkalinity addition was not necessary.

4.3.4 Fluorescence *in situ* hybridization (FISH) analysis

Fluorescence *in situ* hybridization (FISH) was performed to detect AOBs, PAOs and total general bacteria using Amx368, PAO651, and EUB338 probes, respectively (Daims et al., 1999; Crocetti et al., 2000; Schmid et al., 2005).

4.4 Results and discussion

4.4.1 Overall performance of the IA-SBBR

The study lasted for 120 days. Because the carrier elements floated during the settling phase, a final clarifier should have been used to settle sloughed sludge. In this study, a final clarifier was not used, so the effluent quality was tested after filtration through 0.8 μm pore-diameter filter paper. The settleability of the suspended sludge in the IA-SBBR was more poor than that in the control SBR as described in Chapter 3. The suspended sludge in the IA-SBBR had a SVI ranging from 200–225 mL/g SS, while in the control SBR, the SVI ranged from 87–107 mL/g SS. The average floc size of the suspended sludge in the IA-SBBR and the IA-SBR (examined in Chapter 3) was 13.8 and 23.7 μm, respectively.

During the 4-month study period, the IA-SBBR had good performance in organic matter and nutrient removals. COD, TN and TP removals from the slaughterhouse wastewater were over 94%, 92% and 95%, respectively (Table 4.1). From Table 4.1, the effluent from the IA-SBBR can satisfy the Irish national emission standard (Irish EPA, 2006). The IA-SBBR had good P removal. The effluent PO_4^{3-}-P concentration was generally less than 0.4 mg/L (Fig. 4.1). TN ranged from 5 to 22 mg N/L. NO_2^--N, at 0.1–15 mg/L, was the main form of oxidized N in the effluent. Generally, the effluent NO_3^--N concentration was less than 1.0 mg/L, and the effluent NH_4^+-N concentration was always less than 1.0 mg/L (Fig. 4.2).

After 1 month, the color of the biofilm carrier elements changed to light brown and the thickness of the biofilm was less than 10 μm. After 2 months, the thickness of the biofilm on the carrier elements was around 10–15 μm. At the end of 4 months, the thickness of biofilm attached on the inside surface of the elements, which was protected from water flushing, increased to 100–500 μm. On the outside surface, the biofilm thickness was less than 50 μm.

4.4.2 Cycle performance of the IA-SBBR

The performance of the IA-SBBR was examined in several operational cycles. A typical cycle performance study, carried out on Day 109, is now detailed. The cycle variation in the DO concentration is shown in Fig. 4.3. During the intermittently aerated process, the DO dropped to less than 0.1 mg/L during the non-aerated mixing periods. The maximum DO concentration in each aeration period was 2.3, 3.4, 3.7 and 6.3 mg/L, respectively.

Table 4.1: Effluent quality of the IA-SBBR treating slaughterhouse wastewater

Items	Effluent quality
COD	100–250 mg/L (94% removal)
TN	5–22 mg N/L (92% removal)
TP	0.5–1.5 mg P/L (95% removal)
NH_4^+-N	< 1.0 mg N/L

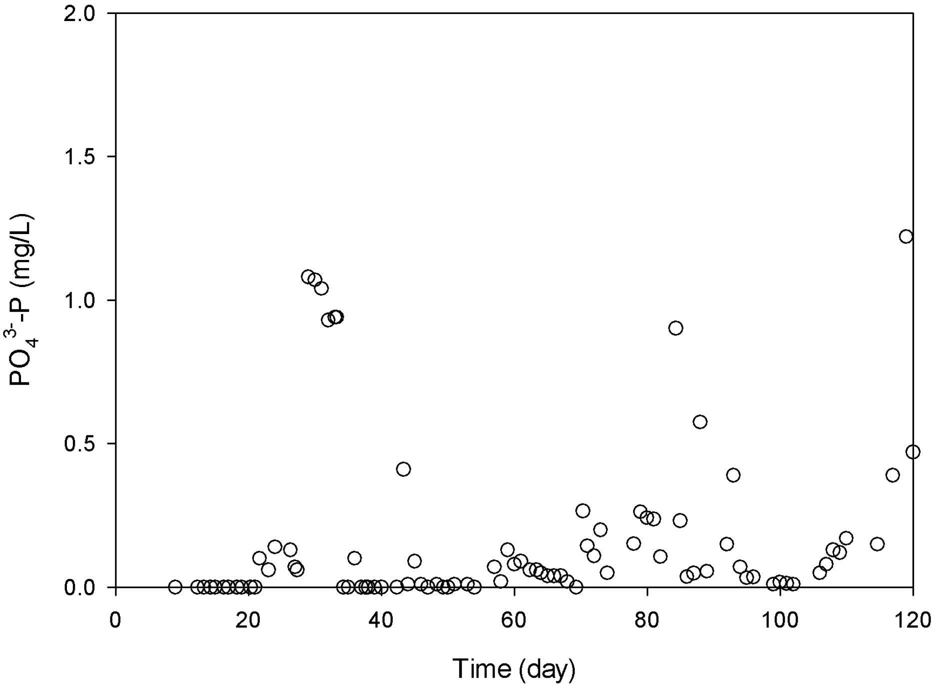


Figure 4.1: Variations of effluent PO_4^{3-}-P concentration throughout the study period

The CODs ranged from 185 to 145 mg/L in this cycle. At the end of the 1st and 2nd mixing periods, the CODs concentration was 173 and 185 mg/L, respectively.

P release occurred in the first mixing period and the highest PO_4^{3-}-P concentration, 16.8 mg/L, was achieved at Minute 50 (Fig. 4.4). The mass of PO_4^{3-}-P released from sludge was almost 5 times the mass of PO_4^{3-}-P in the influent. P uptake occurred during the first two aeration periods. During the rest of the react phase, the PO_4^{3-}-P concentration in the reactor was less than 0.2 mg/L. The estimated P uptake rate of 13.5 mg P/(L.h) was much lower than that measured in the control reactor −32.4 mg/(L.h). This may be due to the lower MLSS in the SBBR compared to the control SBR.

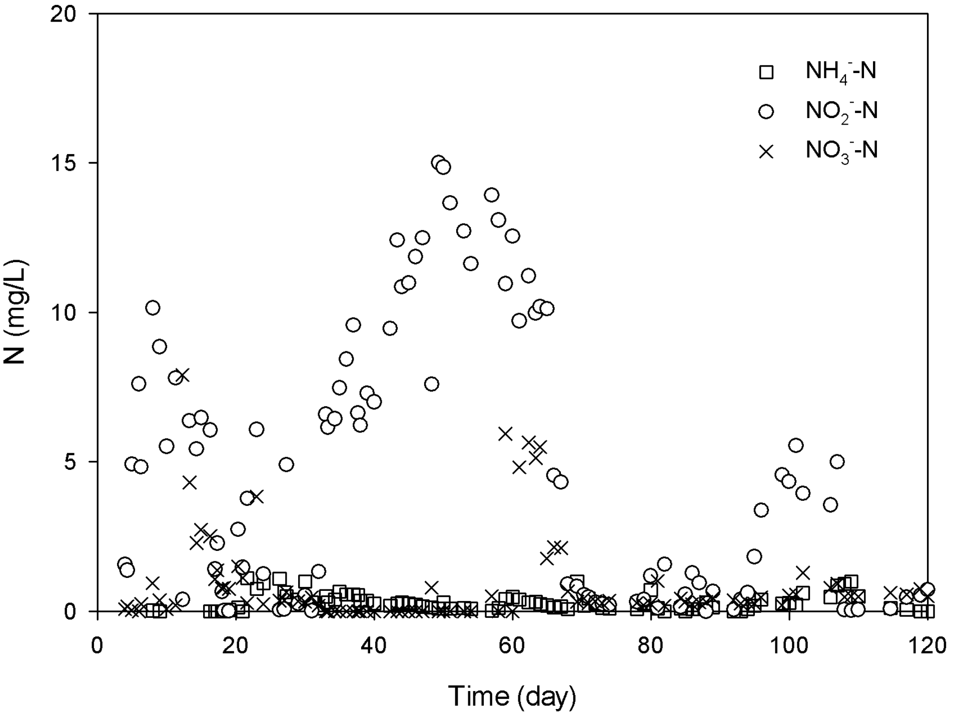


Figure 4.2: Variations of effluent N concentration throughout the study period

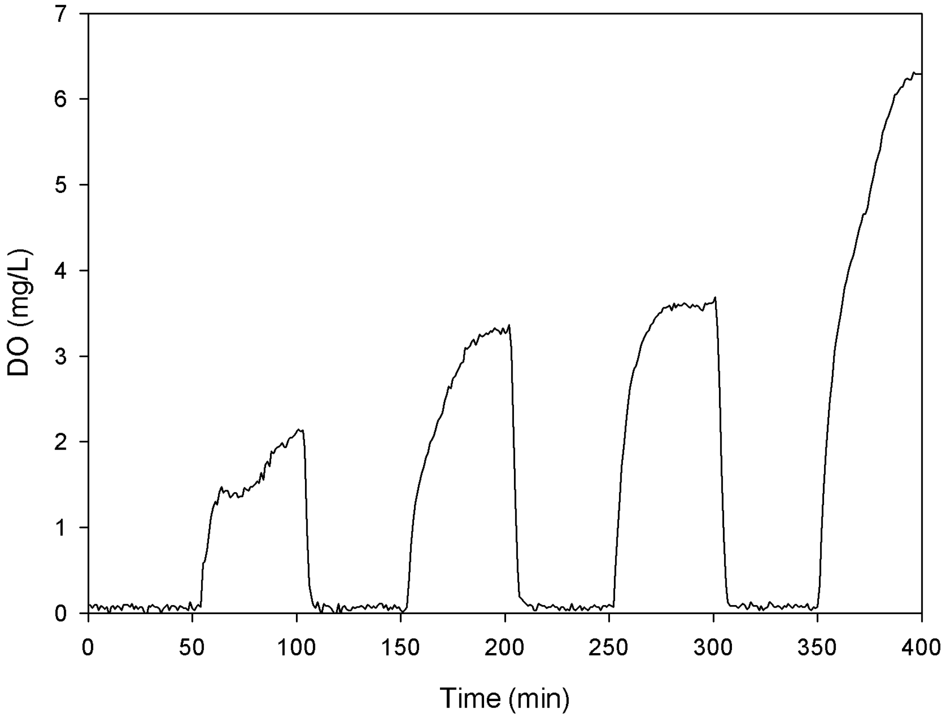


Figure 4.3: Profile of DO in a typical react phase

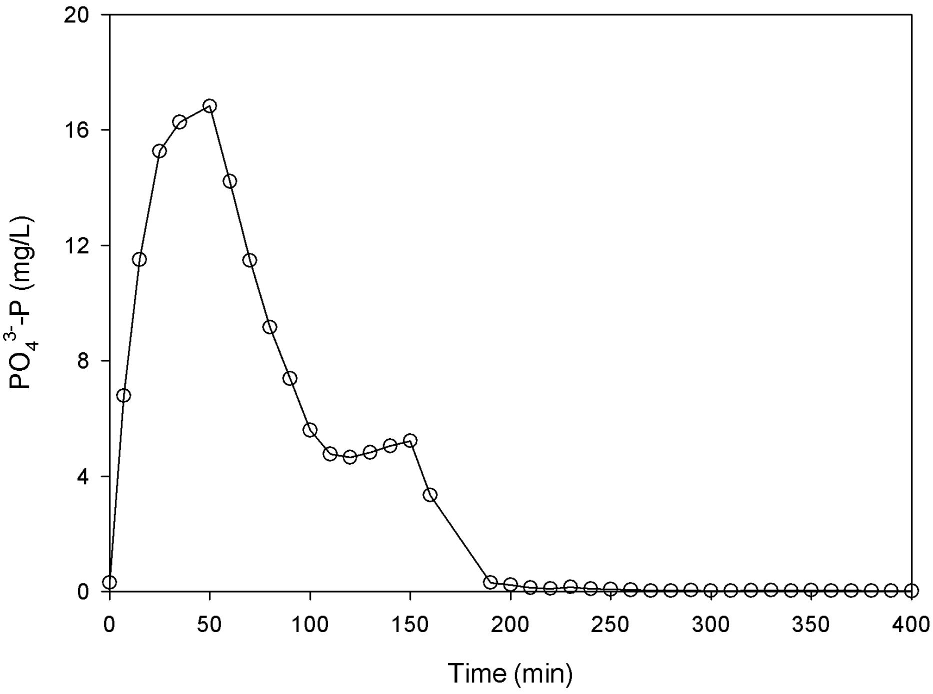


Figure 4.4: Profile of PO_4^{3-}-P in a typical react phase

Partial nitification/denitrification occurred in the IA-SBBR. NO_2^--N was the main NO_x^--N produced in the nitrification process (Fig. 4.5). The NO_3^--N concentration was less than 1.0 mg/L and its production in the reactor was negligible. The NO_2^--N production began from Minute 70. The NO_2^--N production rates in the four aeration periods were 0.079, 0.153, 0.187 and 0.048 mg N/(L.min), respectively. The difference in the four nitrification rates was due to DO concentration variations and substrate limitation. From the cycle study results, the highest nitrification rate occurred when DO $>$ 2.0 mg/L and NH_4^+-N $>$ 5 mg/L.

In the 2nd, 3rd and 4th mixing periods, post-denitrification occurred at rates of 0.288, 0.182 and 0.156 mg N/(L.min), respectively. In total, 237 mg N was removed in the three non-aerated periods by means of post-denitrification.

In this cycle, influent and effluent TN was 360 mg N/L and 6 mg N/L, respectively. Hence, 354 mg TN was removed from the wastewater. Therefore, 66% (237 mg/360 mg) of the influent TN was removed by partial nitrification/denitrification. The other 32% (117 mg/360 mg) was synthesized into biomass.

FISH results indicate that the suspended sludge in the IA-SBBR contained more PAOs (15.6% vs. 5.5%), but less aerobic AOBs (1.2% vs. 3.0%), compared with the sludge in the IA-SBR studied in Chapter 3. The P uptake rate in the IA-SBBR was lower than

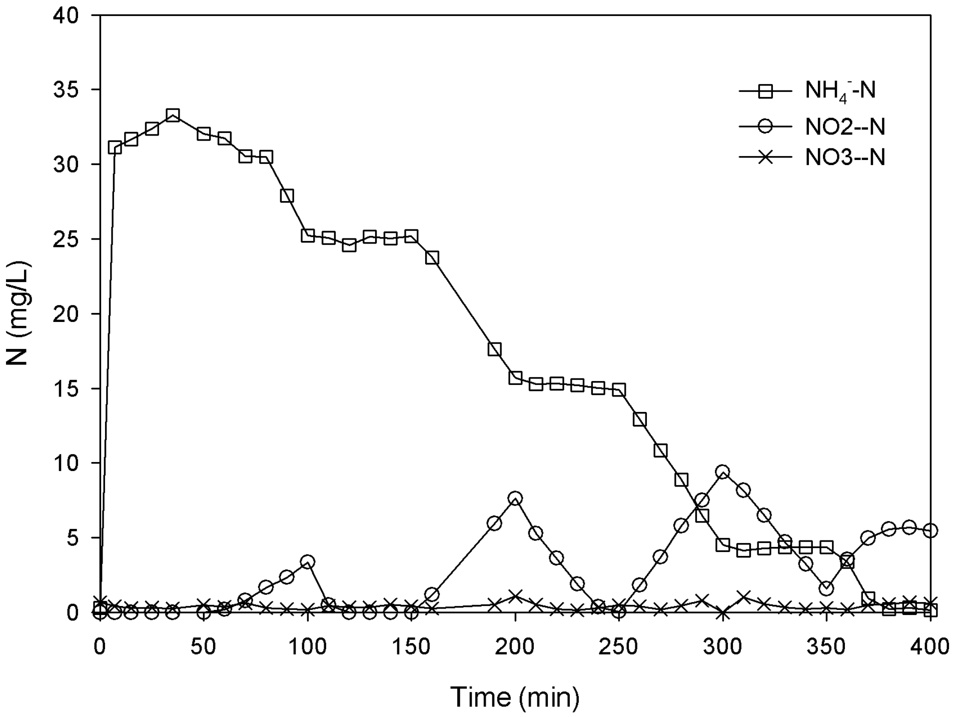


Figure 4.5: Profiles of N matter in a typical react phase

that in the control reactor. Biological P release was completed in the first mixing period, but 2–3 aeration periods were needed for complete P uptake.

4.4.3 Comparison of the IA-SBR and IA-SBBR treating slaughterhouse wastewater

The IA-SBBR can have a stable N removal performance due to the forming of nitrifying biofilm on the surface of the suspended carriers. Under the same aeration rate, due to lower MLSS in the IA-SBBR, the DO in the bulk liquid of IA-SBBR was higher than in the IA-SBR. In the IA-SBBR, partial nitrification/denitrification could occur under significantly higher DO levels in the bulk liquid. The mass transfer is of great importance to the nitrification activity in the biofilm. The nitrification in biofilm reactor is mass transfer limited, rather than kinetically limited (Rostron et al., 2001). A sharp DO gradient was expected in the nitrifying biofilm due to oxygen diffusion and simultaneous utilization of DO by nitrifiers. AOB may out-compete and displace NOB in the surface of the biofilm due to higher oxygen affinity and higher specific growth rate. With a limited oxygen supply, the growth rate of NOB in the inner layer of the biofilm may be significantly inhibited (Kindaichi et al., 2006).

P removal was not improved by adding the suspended plastic biofilm carriers. Although the MLSS concentrations in both reactors were about 5,000 mg/L, and the suspended sludge in the IA-SBBR had a higher population ratio of PAOs than in the SBR, the overall P uptake rate during the initial aeration period in the IA-SBBR was lower than in the IA-SBR.

The floatation of the biofilm carriers during the settle and draw phases caused high SS concentrations in the effluent. Therefore, an additional clarifier will be needed to separate the suspended activated sludge flocs from the IA-SBBR effluent. The results of this study also show that the suspended sludge in the IA-SBBR had a smaller floc size and poor settlement property, with SVI values over 200 mL/g SS.

Considering the cost of the plastic carriers and the lack of significant improvements in the effluent water quality in the IA-SBBR, it is not recommended to add plastic biofilm carriers into an IA-SBR system treating slaughterhouse wastewater to improve its performance.

4.5 Summary

Over a 120-day study, an IA-SBBR efficiently removed organic carbonaceous pollutants and nutrients from slaughterhouse wastewater. At an OLR of 1.25 kg COD/(m^3.d), the removals of COD, TN and TP were over 94%, 92% and 95%, respectively. Stable partial nitrification/denitrification occurred in the reactor and removed approximately 66% of the influent TN. The overall performance of the IA-SBBR was comparable to the IA-SBR, as presented in Chapter 3. It is not recommended to added plastic biofilm carriers into the reactor to improve the performance of the IA-SBR treating slaughterhouse wastewater.

Chapter 5

Effect of Aeration Rate on Nutrient Removal from Slaughterhouse Wastewater in Intermittently Aerated SBRs

5.1 Overview

The chapter describes the effects of the aeration rate on nutrient removal from slaughterhouse wastewater in IA-SBRs.

5.2 Introduction

As found in Chapter 3, in a 10-L laboratory-scale IA-SBR treating slaughterhouse wastewater at an influent OLR of 1.2 g COD/(L.d), with an aeration rate of 0.8 L air/min, the effluent can meet the discharge standards required by Irish EPA (Irish EPA, 2006). The aeration rate was found to have a significant effect on the effluent quality: with a decreased aeration rate due to blockage of the air diffuser, effluent COD and NH_4^+-N increased significantly.

In this chapter, the performances of two IA-SBRs were examined at four aeration rates — 0.2, 0.4, 0.8 and 1.2 L air/min. The nutrient removal efficiencies at the four aeration rates were then compared.

5.3 Materials and methods

5.3.1 Laboratory-scale IA-SBRs

Two identical laboratory-scale SBRs were used in this study. The setting up of the two SBRs is described in Section 3.3.1.

5.3.2 Slaughterhouse wastewater

The wastewater was collected from the same place as described in Section 3.3.2.

The average wastewater quality in the influent tank over the study period is given in Table 5.1.

Table 5.1: Characteristics of the slaughterhouse wastewater filled in the reactors

Parameter	Unit	Days 1–120 (Runs 1, 2, 3)	Days 121–230 (Run 4)
pH	–	7.0–8.0	7.0–8.0
SS	mg/L	1,400 ± 600	720 ± 160
COD	mg/L	4,700 ± 950	2,850 ± 780
BOD_5	mg/L	2,900 ± 600	1,000 ± 150
TN	mg N/L	350 ± 45	250 ± 45
TP	mg P/L	28 ± 5	20 ± 5

5.3.3 Operation of the reactors

Two SBRs (SBR1 and SBR2) were seeded with the return sludge taken from the secondary clarifier of the local slaughterhouse WWTP. After seeding, the two SBRs had an initial MLSS of about 3,500 mg/L.

SBR1 was operated at 0.2 L air/min during Days 1–70, 0.4 L air/min during Days 71–120 and 1.2 L air/min during Days 121–230. SBR2 was operated at 0.8 L air/min during Days 1–120. During Days 1–120, COD and SS in the influent wastewater fluctuated greatly and had average influent COD and SS concentrations of 4,700 and 1,400 mg/L, respectively, giving an average OLR of 1.4 g COD/(L.d). TN was relatively stable during this period and had an average value of 350 mg TN/L. During Days 121–230, raw slaughterhouse wastewater contained lower COD, SS and TN. Therefore, when SBR1 was operated at an aeration rate of 1.2 L air/min, the average OLR was 0.86 g COD/(L.d) and the average NLR was 0.075 g N/(L.d).

At aeration rates of 0.2 and 0.4 L air/min, relatively high SRTs of 30 and 20 days were used in order to maintain MLSS in the reactors in the range of 4,000–6,000 mg/L (5,100 ± 400 mg/L at an aeration rate of 0.2 L air/min and 5,700 ± 100 mg/L at an aeration rate of 0.4 L air/min). During the periods when 0.8 and 1.2 L air/min aeration rates were applied, both the reactors had a SRT of 14.5 days. The average MLSS during the 0.8 L air/min aeration rate was 4,400 ± 550 mg/L. Due to the low influent SS and COD concentrations over the period when the 1.2 L air/min aeration rate was applied, MLSS was around 3,900 ± 850 mg/L.

The average SVI of activated sludge in this study ranged from 90 to 140 mg/L, indicating that the sludge had a good settling property.

5.3.4 Analytical methods

The analytical methods are described in Section 3.3.4.

5.4 Results and discussion

5.4.1 Effects of the aeration rate on the overall performance of the IA-SBRs

The performance of the IA-SBRs at the four aeration rates is given in Table 5.2. At aeration rates of 0.2 and 0.4 L/min, the effluent contained high concentrations of COD and nutrients, and the effluent quality did not reach the emission standards required by the Irish EPA (Table 5.2). The effluent quality at the 0.4 L air/min aeration rate was much better than that at the 0.2 L air/min aeration rate, and produced lower effluent COD, TN and TP concentrations. Enhanced biological phosphorus removal (EBPR) did not occur at the 0.2 L air/min aeration rate but took place when the aeration rate was increased to 0.4 L air/min. This was because no P release occurred during the non-aeration periods at the aeration rate of 0.2 L air/min. At 0.4 L air/min, effluent PO_4^{3-}-P was less than 0.6 mg/L and effluent TP was around 1.8 mg P/L.

At high aeration rates of 0.8 and 1.2 L air/min, the effluent quality met the discharge standards (Table 5.2). COD removals were up to 97% and effluent SS concentrations were less than 60 mg/L. Biological nitrification and denitrification reduced TN in the effluent to 19 mg/L, representing TN removals of up to 95% and 92%, respectively. Effluent PO_4^{3-}-P and TP was less than 0.2 mg P/L and 1.0 mg P/L, respectively. TP

Table 5.2: Performance of SBR1 and SBR2 at the four aeration rates

Parameter	Unit	discharge standard*	0.2 L air/min	0.4 L air/min	0.8 L air/min	1.2 L air/min
SS	mg/L	60	220±73	100±42	33±18	24±11
COD	mg/L	125–250	1,500±316	330±165	115±13	96±27
COD removal rate		>75%	68%	93%	97%	97%
TN	mg N/L	15–40	343±52	232±22	19±8	19±7
TN removal rate		>80%	<5%	34%	95%	92%
TP	mg P/L	2–5	16±2.5	1.8±0.2	0.7±0.3	0.6±0.2
TP removal rate		>80%	43%	94%	97%	97%

* Discharge standard given by Irish EPA (2006)

removals were up to 97%. Filali-Meknassi et al. (2005b) applied a step-feed SBR to treat slaughterhouse wastewater and the average PO_4^{3-}-P concentration in the effluent was 10 mg/L. Consequently, ferric chloride was required to further reduce effluent PO_4^{3-}-P. The present study illustrates the possible advantages of IA-SBRs over step-feed SBRs in enhanced biological P removal.

The effects of the aeration rate on the performance of the IA-SBRs were due to different DO concentrations during the aerobic periods in the reactors at the four aeration rates (Fig. 5.1). Since the cyclic analysis associated with individual aeration rates was carried out at different influent wastewater conditions, the absolute values of DO at the four aeration rates were not comparable. At the 0.2 L air/min aeration rate, DO was negligible during the react phase (0–400 min). Because of the almost completely anaerobic conditions in the bulk water phase in the reactor, processes that required oxygen, including carbonaceous oxidation, nitrification and enhanced biological P uptake, were unable to occur. The constant ORP value of −12 mV during the whole react period (Fig. 5.2) revealed that the bulk wastewater in the reactor remained anaerobic.

When the 0.4 L air/min aeration rate was applied, during the 1st, 2nd and 3rd aeration periods, DO concentrations were relatively stable at 1.76 mg/L. In the last aeration period (350–400 min), DO gradually rose from 1.76 mg/L to 3.52 mg/L. Although the DO concentrations were supposed to be good for nitrification, high concentrations of organic matter in the water phase — 330 mg COD/L at the end of the react phase (at Minute 400) — inhibited nitrification. A stable pH in the reactor (between 7.7 and 7.8) indicates that no significant nitrification occurred during the react phase (Fig. 5.3). The low DO concentration during aeration periods resulted in PO_4^{3-}-P uptake and anaerobic conditions during the non-aeration periods contributed to P removal. This can explain the difference in P removal during the operation at 0.2 and 0.4 L air/min aeration rates.

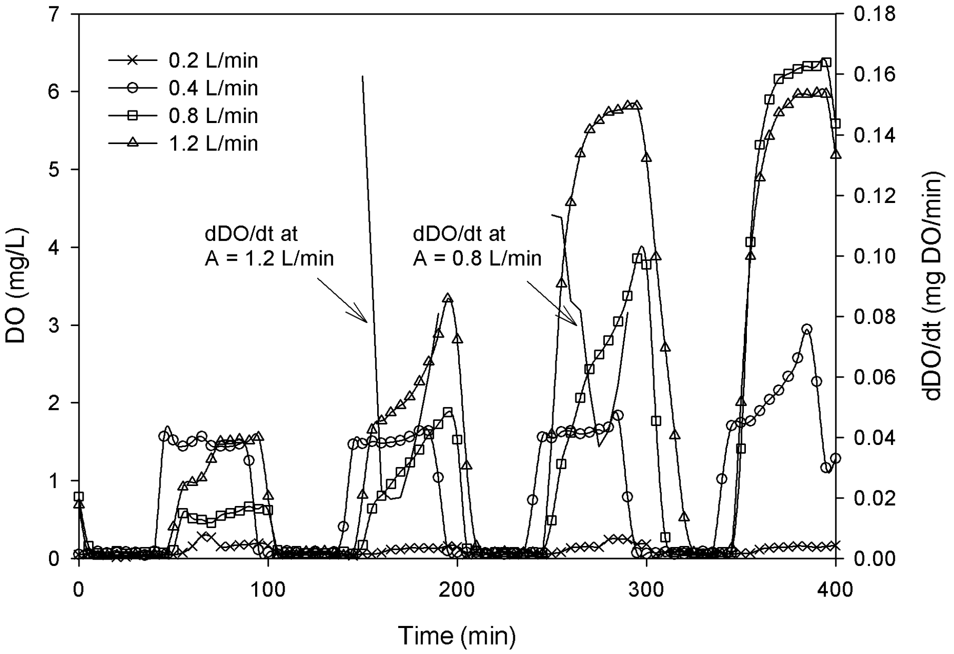


Figure 5.1: Typical cycle profiles of DO at different aeration rates

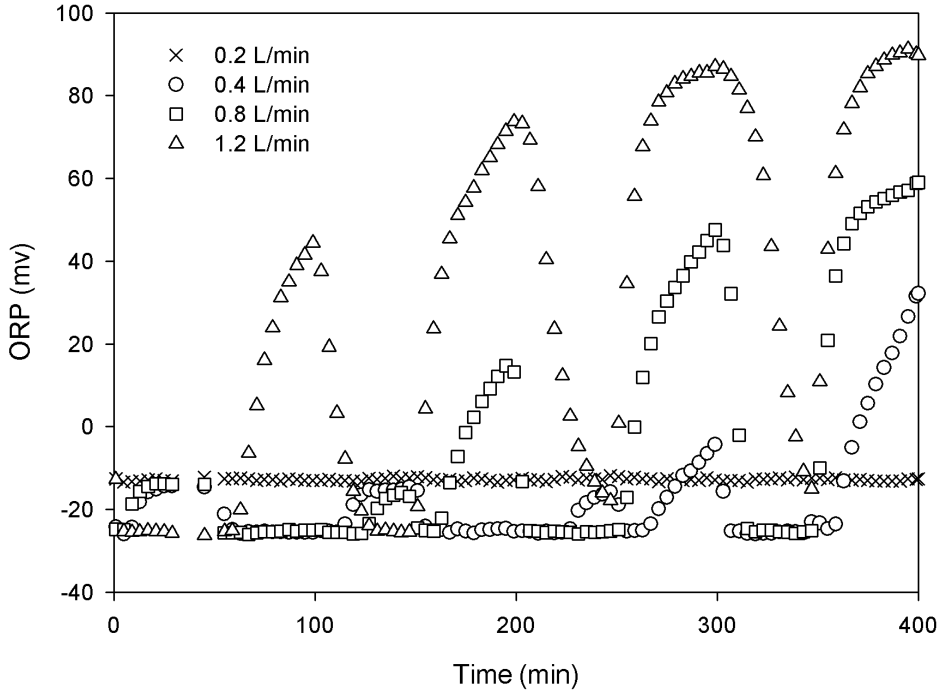


Figure 5.2: Typical cycle profiles of ORP at different aeration rates

At the 0.8 L/min aeration rate, the average DO concentrations during the four aeration periods were 0.7, 1.3, 2.6 and 6.0 mg/L (Fig. 5.1). During the non-aeration mixing

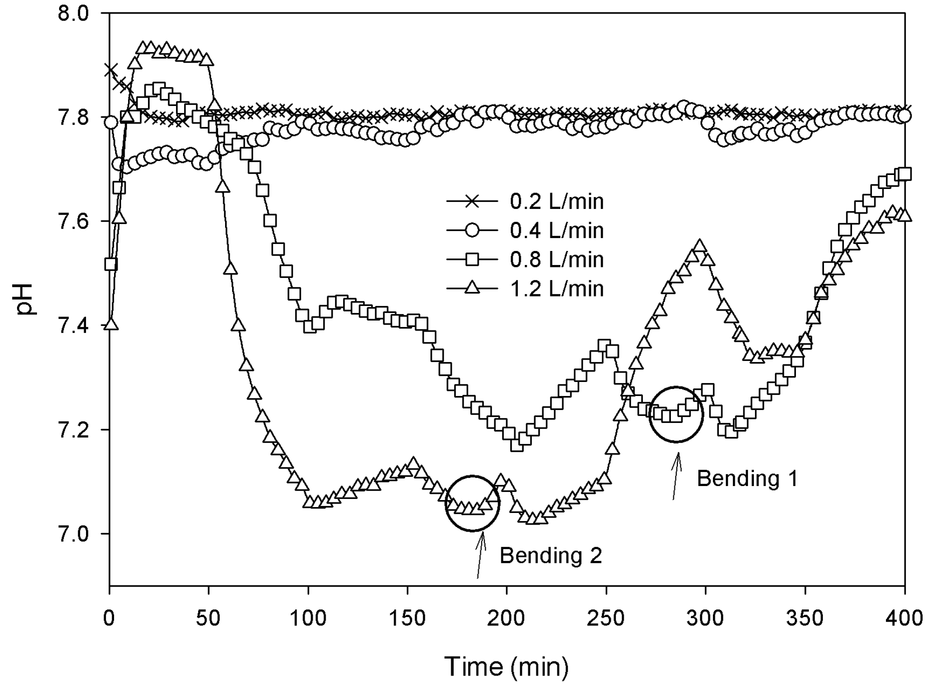


Figure 5.3: Typical cycle profiles of pH at different aeration rates

periods, DO was rapidly decreased to nearly zero. The high DO fostered nitrification and the NH_4^+-N concentration in the 3rd aeration period was less than 5 mg/L.

During the 7-min fill phase, the remaining NO_2^--N and NO_3^--N from the preceding cycle were rapidly removed by means of denitrification, with a denitrification rate over 1.25 mg N/(L.min) (Fig. 5.4a). After the fill phase, a soluble TN (TNs) of 35 mg N/L was measured in the bulk mixed liquor phase. NH_4^+-N decreased during the aeration periods, resulting from nitrification and biomass synthesis. A release of around 7 mg NH_4^+-N/L was observed during the second anoxic period (100–150 min), probably because of ammonification of organic N. After P uptake was complete, NO_2^--N appeared in the reactor from the 71st min. NO_2^--N increased linearly in the first three aeration periods (50–100 min, 150–200 min and 250–300 min, respectively) and NO_2^--N production rates were 0.065, 0.207 and 0.178 mg/(L.min). During the last aeration period (350–400 min), the nitrification rate was close to zero due to the depletion of NH_4^+-N. NO_2^--N did not decrease during the last aeration period.

P release occurred during the first non-aeration period, and a maximum PO_4^{3-}-P concentration of 11.8 mg/L was reached at Minute 50. Given an influent PO_4^{3-}-P concentration of 27 mg/L, it is calculated that 91 mg PO_4^{3-}-P was released from the sludge.

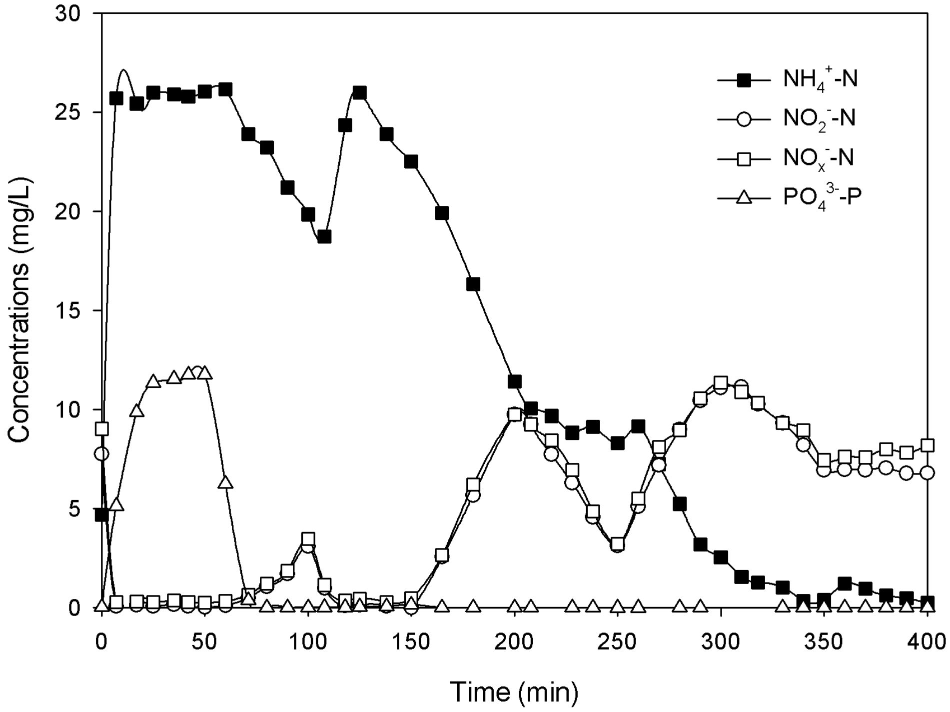
P uptake commenced at the start of the first aeration period and was completed after 20 min. Throughout the cycle, the average P uptake rate was 0.54 mg P/(L.min). CODs decreased during the fill phase and the 1st non-aeration period, and it leveled to around 100 mg/L until the end of the cycle.

At the 1.2 L air/min aeration rate, during the first aeration period, the DO concentration increased dramatically from near zero to around 2.2 mg/L within 20 minutes, and then remained higher than 2.6 mg/L from the 1st aeration period. During this period, most of the NO_x^--N produced was NO_2^--N. NO_2^--N was around 6 mg/L (60% of NO_x^--N) (Fig. 5.4b). Post-denitrification during 100–150 min was not as effective as in the same period in the aeration rate of 0.8 L air/min case. During the 2nd aeration period of 150–200 min, NO_2^--N and NO_3^--N concentrations increased. From Minute 160, NH_4^+-N was less than 5 mg/L. From 200–400 min, NO_2^--N decreased from 12 mg/L to 2.5 mg/L and NO_3^--N increased to 16 mg/L. NO_3^--N was the main form of NO_x^--N present in the effluent.

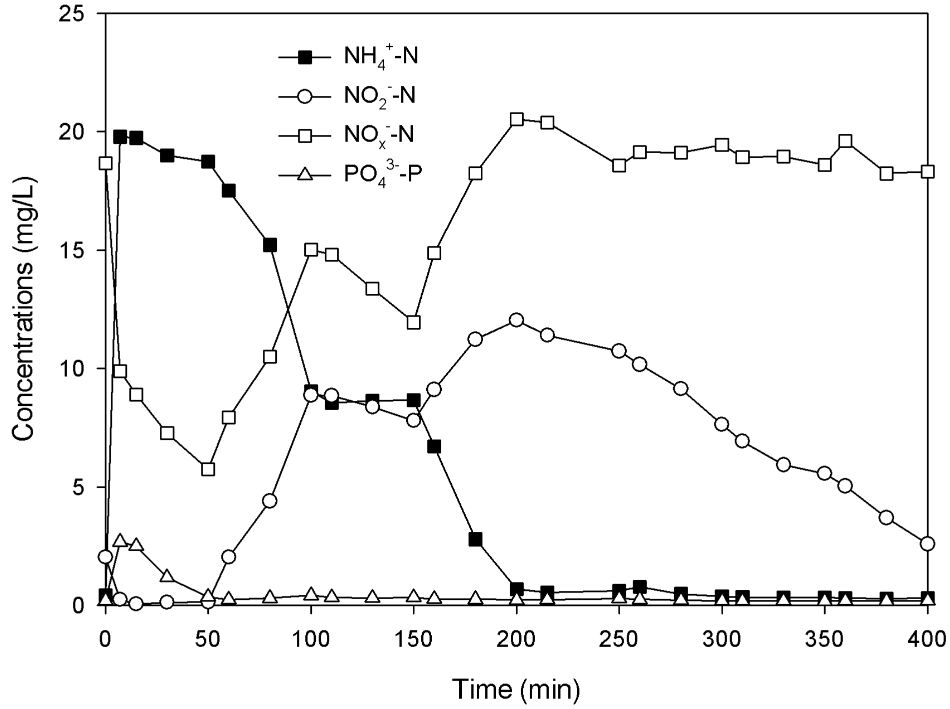
Because high NO_3^--N remained from the previous cycle, the pre-denitrification occurring in the first 50 minutes was not complete. During the fill period, 2 mg/L of NO_2^--N and 17 mg/L of NO_3^--N rapidly decreased to 0 and 9 mg/L, respectively. The highest PO_4^{3-}-P concentration of 2.7 mg/L appeared at the end of the fill period. From 8–50 min, around 4.0 mg/L NO_3^--N was depleted by means of denitrification, while 2.4 mg/L of PO_4^{3-}-P was simultaneously removed. Anoxic P uptake occurred during this period. The phenomenon of simultaneous denitrification and P uptake has also been found by other researchers (Kuba et al., 1997; Meinhold et al., 1999). The P uptake rate under anoxic conditions was 0.054 mg P/(L.min), which was only 10% of the P uptake rate during the same period at an aeration rate of 0.8 L air/min.

The profiles of the ratio of NO_2^--N to TON are given in Fig. 5.5. At the aeration rate of 0.8 L air/min, NO_2^--N/TON was over 80% during most of the operational cycle. When the aeration rate was 1.2 L air/min, the highest ratio was around 65% and took place during the period of 100–250 min. The ratio decreased in the subsequent periods and was equal to 15% at the end of the operational cycle. This was because the NO_2^--N production rate was lower than the NO_2^--N consumption rate.

At the aeration rate of 0.8 L air/min, in the 1st and 2nd non-aeration periods (0–50 min and 100–150 min, respectively), NO_2^--N/TON was reduced to 8% and 26%, respectively, indicating that removal of NO_2^--N by means of denitrification was faster than the removal of NO_3^--N in the anoxic condition. This phenomenon also appeared during the 1st non-aeration period (0–50 min) at the aeration rate of 1.2 L air/min.



(a) Cycle profiles of N and P at the aeration rate of 0.8 L air/min



(b) Cycle profiles of N and P at the aeration rate of 1.2 L air/min

Figure 5.4: Cycle profiles of N and P at the aeration rates of 0.8 and 1.2 L air/min

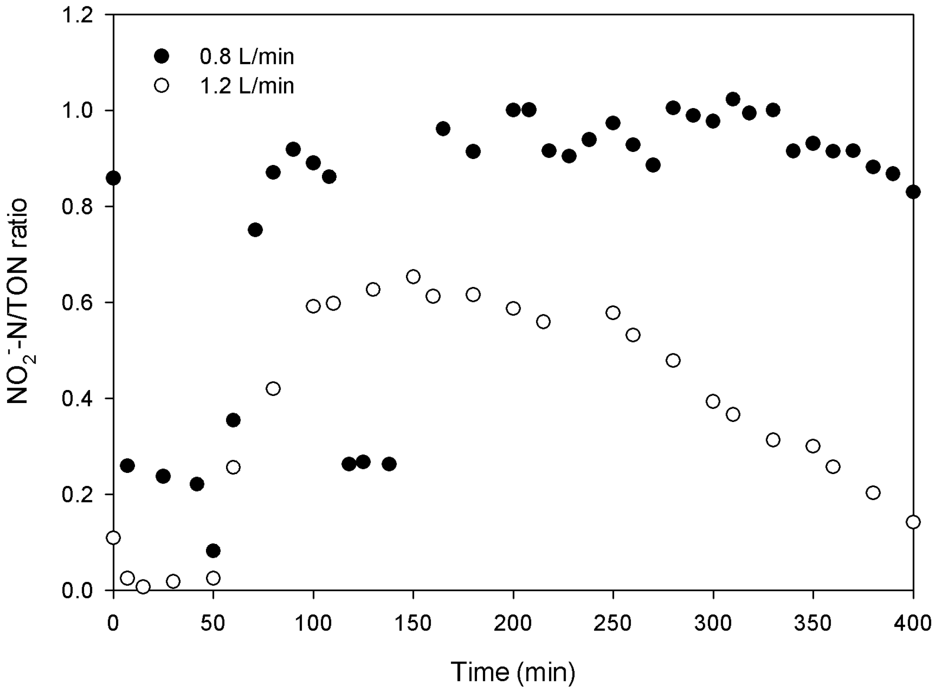


Figure 5.5: Variations of NO_2^--N/TON in a typical cycle at the aeration rate of 0.8 and 1.2 L air/min

The high NO_2^--N/TON ratio in the react phase at the aeration rate of 0.8 and 1.2 L air/min indicates that partial nitrification occurred. However, the 0.8 L air/min aeration rate favored partial nitrification, in comparison with the 1.2 L air/min aeration rate. Low DO concentrations, ranging from 0.2 to 0.7 mg/L, favor partial nitrification (Ruiz et al., 2003; Chuang et al., 2007a). It also has been found that when DO concentrations were in the medium range of 1.4 mg/L, NO_2^--N accumulation can still take place (Ciudad et al., 2005). However, in the present SBR, the average DO levels during the four aeration periods were 0.7, 1.3, 2.6 and 6.0 mg/L. DO concentrations were not consistent with the findings of the other researchers mentioned above. NO_2^--N/TON ratios were 96% and 89% in the 3rd and 4th aeration periods, respectively. These were higher than the ratios during the 1st and 2nd aeration periods — 64% and 72%, respectively.

It is possible that the intermittent aeration pattern applied in the SBR caused partial nitrification (Mota et al., 2005a,b; Li et al., 2008). Further research should be carried out to study the mechanisms triggering NO_2^--N accumulation in the SBR.

5.4.2 Automatic control of SBRs using DO, pH and ORP real-time data

Studies on automatic control of conventional SBRs using DO, pH and ORP real-time data have been carried out to enhance N and P removals and to reduce operational cost (Tilche et al., 1999; Lee et al., 2001; Lee and Oleszkiewicz, 2003; Casellas et al., 2006; Marsili-Libelli, 2006). The application of DO, pH and ORP real-time data to control the operation of intermittently aerated SBRs is now discussed.

DO data are always used to identify the end point of nitrification. The early identification of the end point of nitrification can save the operation cost on aeration. Marsili-Libelli (2006) proposed that DO probe data be used to identify the end of the nitrification phase: when the curve of DO concentrations with respect to time levels off, i.e., $dDO/dt = 0$, nitrification ends. From Fig. 5.1, it is found that at the aeration rate (A) of 0.8 L air/min, an ammonium breakpoint appeared during the period of 250–300 min, since NH_4^+-N was almost completely depleted during this period; at A=1.2 L air/min, an ammonium breakpoint appeared during the aeration period of 150–200 min, corresponding to the almost complete depletion of NH_4^+-N. However, the two ammonium breakpoints were not easily found on the profiles of DO curves (Fig. 5.1). At the two points, DO curve did not level off, which is different to the findings of Marsili-Libelli (2006). It is found that at the two ammonium breakpoints, $d^2DO/dt^2 = 0$. The reason could be due to the difference in wastewater used in the present study and in Marsili-Libelli's study. If using the ammonium breakpoints to identify the end of nitrification points, at A=0.8 L air/min, the 4th aeration period (350–400 min) can be omitted. Hence, the aeration duration ratio (the ratio of the total aeration duration to the total cycle duration) would be reduced from 42% to 31%. At A=1.2 L air/min, the 3rd and 4th aeration periods (250–300 min and 350–400 min) can be saved, which would reduce the aeration ratio from 42% to 21%.

The pH profile can be used to indicate the end point of nitrification Kim and Hao (2001), and end point of denitrification Casellas et al. (2006). A minimum pH variation ("pH valley") indicates the end of nitrification; a maximum pH value indicates the end of denitrification ("nitrate apex").

In Fig. 5.3, there were two bending points on the curves of pH (pH valley) in a complete cycle at aeration rates of 0.8 L air/min (at Minute 280) and 1.2 L air/min (at Minute 180). The explanation of the pH valley is that the nitrification process in the aerobic periods drove pH decreasing and that the air stripped CO_2 from the wastewater, which caused pH to increase.

ORP can be also used as the control parameter for wastewater treatment. It can provide more flexibility than the DO profile, since it provides a much wider monitoring range than DO, using both positive and negative data to represent the changes in aerobic, anaerobic and anoxic conditions. Comparatively, DO only gives positive data corresponding to aerobic conditions. Bending points found on the real-time ORP curves can reveal the beginning of denitrification, end of denitrification and end of nitrification (Shimabukuro et al., 2004). The absolute values of ORP depend on the characteristics of wastewater, DO concentrations and the biomass concentration, but the relative values of ORP can show the reduction and oxidation conditions in wastewater. Generally, in aerobic conditions, ORP values are positive, showing oxidation conditions; in anaerobic and anoxic conditions, ORP values are negative, showing reduction conditions.

The profiles of ORP at the four aeration rates are given in Fig. 5.2. In the wastewater, there were three typical oxidation-reduction states. At $A = 0.2$ L air/min, in the bulk wastewater, the ORP value was close to -12 mV during the entire operational cycle. The reason was that the DO concentrations were nearly zero, showing that anaerobic conditions existed during all the phases. The concentrations of NO_x^--N were low, showing that nitrification did not occur at $A = 0.2$ L air/min.

There was an interesting finding concerning ORP in the present study. At aeration rates of 0.2, 0.4, 0.8 and 1.2 L air/min, when the wastewater was anaerobic and DO and NO_x^--N were close to zero, ORP was -12 mV. At an aeration rate of 0.4, 0.8, 1.2 L air/min, when the wastewater was anoxic, i.e., DO was near zero and NO_x^--N was present, ORP was close to -26 mV. In the aerobic phase, ORP increased from negative values to positive values from the onset of aeration. When NO_x^--N was removed by means of denitrification during non-aeration periods, ORP values rose from -26 mV to -12 mV. This was found at aeration rates of 0.4 and 0.8 L air/min during the periods of 0–50 min and 100–150 min. This finding indicates that the end point of denitrification can be detected by measuring an ORP of -12 mV during non-aeration periods. While the reactor was operated at $A = 1.2$ L air/min, the bending point of complete denitrification was not found in ORP profiles during the non-aeration periods (Fig. 5.2). This could be due to the short anoxic time and incomplete denitrification prior to the onset of aerobic conditions.

5.5 Summary

This study examined the effects of aeration rates on the performance of IA-SBRs and investigated the profiles of real-time DO, pH and ORP data. The following results were obtained:

1. An optimum aeration of 0.8 L air/min produced the best system performance. Removals of COD, TN and TP were up to 97%, 94% and 97%, respectively.

2. Partial nitrification followed by denitrification occurred in the IA-SBRs at aeration rates of 0.8 and 1.2 L air /min. At an aeration rate of 0.8 L air/minute, the NO_2^--N/TON ratio during most of the operational cycle was over 80%. When the aeration rate was 1.2 L air/min, the NO_2^--N/TON ratio ranged from 15% to 60%. The cycle operation could be the cause of partial nitrification.

3. The end of nitrification can be identified from DO and pH real-time profile using $d^2DO/dt^2 = 0$ and $dpH/dt = 0$.

4. There were three stages of ORP variation in IA-SBRs treating slaughterhouse wastewater: ORP was around −26 mV in anoxic conditions, around −12 mV in anaerobic conditions, and was positive in aerobic conditions. The end point of denitrification in non-aeration periods can be set at an ORP value of −12 mV.

Chapter 6

Nitrogen Removal from Slaughterhouse Wastewater in a SBR under Oxygen-Limited, Low DO Conditions

6.1 Overview

The chapter describes the effects of aeration control on nutrient removal from slaughterhouse wastewater in a SBR operated with an intermittent aeration (IA) strategy and a continuous aeration (CA) strategy.

6.2 Introduction

As described in Chapter 3, in a 10-L bench-scale IA-SBR operated at an aeration rate of 0.8 L air/min, after the depletion of NH_4^+-N, the DO in the reactor during the aeration periods reached 70% of DO saturation. If the DO concentration is kept low, the operational costs associated with aeration will be reduced.

The common practice in conventional activated sludge processes is to maintain a DO in excess of 2 mg/L for complete nitrification (US EPA, 1993). The aeration cost may be up to 70% of the total electricity cost of wastewater treatment plants for C and N removals. After the depletion of rbCOD, efficient nitrification can be achieved under DO concentrations of 0.5 mg/L in laboratory-scale SBRs treating slaughterhouse wastewater (Chapter 5). In addition, N removal from slaughterhouse wastewater can be enhanced via simultaneous nitrification and denitrification (SND) by maintaining low DO levels in SBRs.

If DO in the reactor is controlled properly by means of intermittent aeration, NH_4^+-N will be partially oxidized to NO_2^--N and then, in the anoxic mixing period, NO_2^--N will be reduced to N_2 gas. Partial nitrification can reduce the operational cost by reducing oxygen supply and reducing the demands for rbCOD in the denitrification process (Khin and Annachhatre, 2004; Ahn, 2006; Paredes et al., 2007).

In this study, a laboratory-scale SBR was operated at low DO concentrations under two aeration strategies: IA and CA. The characteristics of slaughterhouse wastewater treatment in this SBR at low DO concentrations were investigated.

6.3 Materials and methods

6.3.1 Slaughterhouse wastewater

The wastewater was collected from the same place as described in Section 3.3.2.

6.3.2 Laboratory-scale SBR system

The laboratory-scale SBR is illustrated in Fig. 6.1. The reactor was made from transparent Plexiglas and had an effective volume of 10 L, with an inner diameter of 240 mm and a height of 250 mm. Three MasterFlex L/S peristaltic pumps (Cole-Parmer, US) were used to fill wastewater into the reactor during the fill phase, withdraw treated wastewater during the draw phase, and desludge immediately prior to the commencement of the settle phase. In the fill and react phases, the reactor was continuously stirred using an overhead mechanical mixer with a rectangular paddle (dimensions: 80 × 100 mm). An aquarium air pump supplied air through a porous stone diffuser that was located at the bottom of the reactor. A submerged aquarium pump was used to stir the wastewater in the influent tank during the fill phase. The operational sequence of the SBR was controlled by a PLC (S7-222, Siemens, Germany).

DO, pH and ORP in the reactor were real-time monitored using electrodes. The set-up of these probes and data collection was the same as that in the IA-SBR system (described in Chapter 3).

6.3.3 Operation strategies

The reactor was seeded with sludge taken from an laboratory-scale IA-SBR treating slaughterhouse wastewater with partial nitrification/denitrification. The initial MLSS was around 5,000 mg/L. The SBR was operated under two aeration strategies at ambient temperatures (15–22 °C): IA strategy during the first 74 days and then CA strategy for

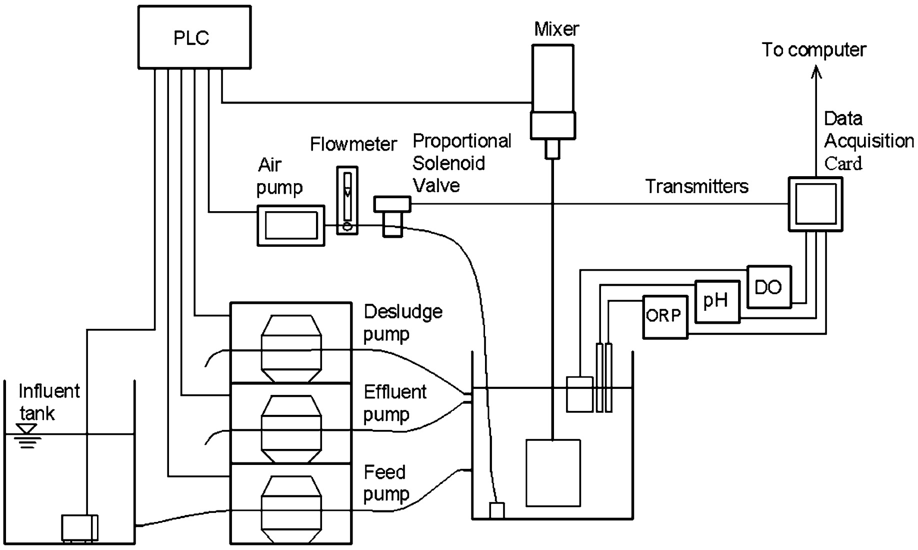


Figure 6.1: Schematic diagram of the laboratory SBR system

21 days.

The sequencing operation of the SBR under the two aeration strategies (IA and CA) is shown in Fig. 6.2. The total cycle duration was 8 hours, giving three cycles per day. The influent slaughterhouse wastewater was fed to the reactor in the first 10 minutes of each cycle. The duration of the react phase was 6 and 7 hours under IA and CA strategies, respectively. During the react phase, the mixer rotated continuously at 110 rpm. Under both operational strategies, the first hour was set as a non-aeration period for pre-denitrification, and then the reactor was either intermittently or continuously aerated at low DO levels. Under the IA aeration strategy, the period of 360–420 min was the settle phase and, in the last 10 minutes of the settle phase, the supernatant was decanted.

6.3.3.1 Intermittent aeration strategy

During the 2nd, 4th and 5th hour of the operational cycle, the air pump was working and the maximum DO was controlled at 1.0 mg/L, with the aeration rate regulated at 1.0 L air/min via the solenoid valve; when the DO was equal to or higher than 1.0 mg/L, the aeration rate was regulated at 0.2 L air/min.

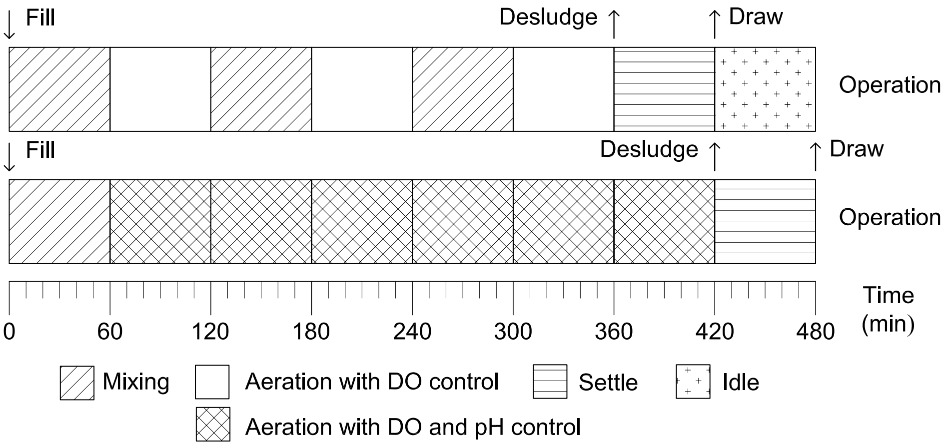


Figure 6.2: Operation strategies of the SBR: intermittent aeration and continuous aeration

6.3.3.2 Continuous aeration strategy

From 60 to 120 min in a cycle, aeration rates of 0.4–1.2 L air/min were applied to keep the DO concentration at around 1.0 mg/L. This was to remove most of the rbCOD in the wastewater so as to avoid the inhibition of nitrification caused by organic C during the subsequent lower DO stage. From 120 to 420 min, the reactor was aerated at aeration rates ranging from 0.1 to 0.4 L air/min. In this study, the DO of 1.0 mg/L and the deviation of pH in the reactor (dpH/dt) of 0.005 unit/min were the criteria for determining the end point of nitrification. When this point was reached, aeration was terminated until end of the cycle.

6.3.4 Online oxygen utilization rate (OUR) test

In several operational cycles after the SBR was at steady state, the OUR — a measure of the deviation of DO (i.e. dDO/dt) as it decreases during non-aeration periods — was monitored *in situ* every 10 minutes. A Clark-type mini-DO sensor, which can detect DO as low as 0.02 mg/L and has a response time of about 4 seconds from 0 to 90% of DO saturation, was used to measure DO in the reactor. A pico-ammeter (PA2000, Unisense, Denmark) received signals from the mini-DO sensor and sent 0–2.5 V analogue signals to a data acquisition card. Then, the OUR values were calculated. The OUR test was carried out for 1 minute (in the initial 20 seconds, the reactor was aerated at an aeration rate of 1.2 L air/min; in the remaining 40 seconds, it was not aerated but stirred with the mechanical mixer) in 10-min intervals. The decrease of DO was recorded during the 40-second non-aeration period and the dDO/dt was calculated.

6.3.5 Analytical methods

These are described in Section 3.3.4.

6.4 Results

6.4.1 Overall performance of the SBR under the IA and CA aeration strategies

During the 74-day operation with the IA aeration strategy, the average influent COD and TN concentrations were 2,800 and 220 mg/L, respectively. 810 mL of wastewater was fed into the reactor during the fill phase, giving an average HRT of 3.63 days. The average OLR and NLR were 0.77 kg COD/(m^3.d) and 0.06 kg N/(m^3.d), respectively.

Following the IA operation, the SBR was operated with the CA aeration strategy for 21 days. During this period, the average influent COD and TN concentrations were 3,500 and 350 mg/L, respectively. Therefore, the influent feed volume was reduced to 680 mL, giving an average HRT of 4.32 days, and an average OLR and NLR of 0.81 kg COD/(m^3.d) and 0.08 kg N/(m^3.d), respectively.

The SRT of the reactor was kept at 19–22 days. The concentration of SS contained in the raw slaughterhouse wastewater had a significant effect on the MLSS levels in the reactor. During the operation under the IA aeration strategy, the average influent SS was 1,650 mg/L and the MLSS in the reactor was around 6,000 mg/L. During the operation under the CA strategy, the average influent SS was 1,150 mg/L and the MLSS in the reactor was around 3,600 mg/L. The SVI of activated sludge throughout the study ranged from 100 to 140 mL/g, indicating that the sludge had satisfactory settling properties.

Good effluent quality and stable performance was achieved within 4 days after the commencement of the respective operational strategies. SS and COD removals of 99% and 98%, respectively, were achieved (Table 6.1). The average TN removals were 95% (IA) and 91% (CA), and the average effluent NH_4^+-N concentrations were 1.1 mg/L (IA) and < 0.5 mg/L (CA). This shows that complete nitrification was achieved under a maximum DO of 1.0 mg/L. NO_3^--N was the main N component in the effluent (Fig. 6.3). Because the NLR was higher when the reactor was operated with the CA strategy and aeration was terminated just after the depletion of NH_4^+-N, NO_2^--N in the effluent was higher than during the IA strategy, but was mainly below 5 mg/L. Under both operational strategies, the final SS, COD, TN and NH_4^+-N concentrations met the standards for discharging slaughterhouse wastewater to surface waters.

Table 6.1: Performance of the SBR operated at low DO levels under the IA and CA operational strategies

Items	IA strategy			CA strategy		
	Influent mg/L	Effluent mg/L	Removal percentage	Influent mg/L	Effluent mg/L	Removal percentage
SS	1,650	10	99%	1,150	9	99%
COD	2,800	52	98%	3,500	50	99%
TN	200	12	95%	350	30	91%
NH_4^+-N		1.1			<0.5	

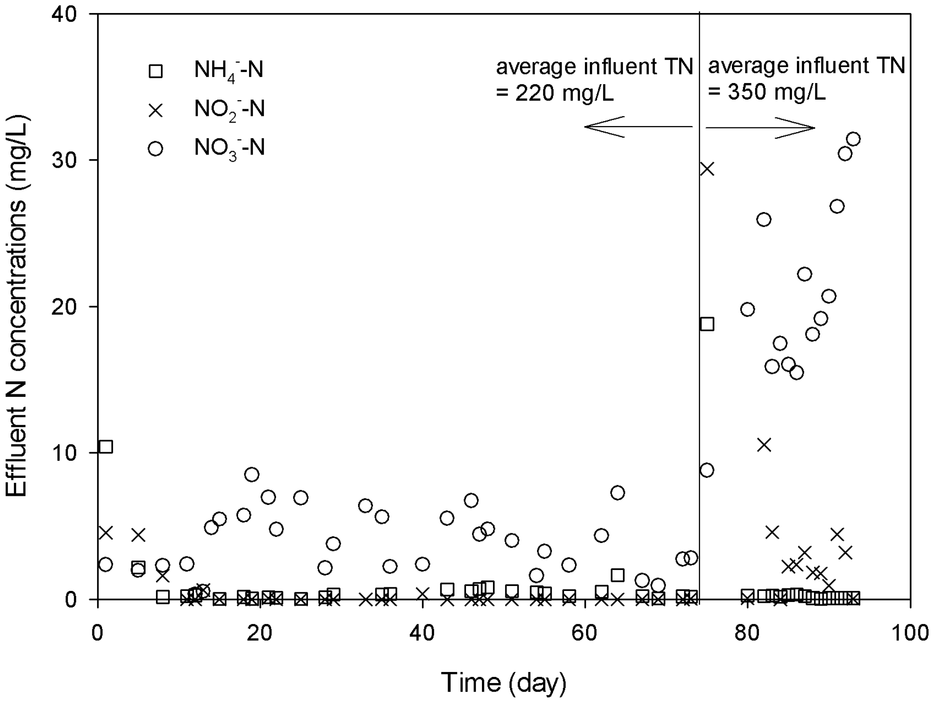


Figure 6.3: Effluent N concentrations under the CA (0–74 days) and IA (75–95 days) operational strategies

6.4.2 Cycle performance of the SBR at low DO levels under the IA aeration strategy

A cycle study was carried out on Day 70. The influent SS, COD and TN concentrations were 1,600, 2,380 and 240 mg/L, respectively, and the MLSS in the reactor was 5,680 mg/L. During the whole cycle, pH ranged from 7.37 to 7.70. In the fill period, pH increased from 7.5 to 7.7—the highest pH value measured in the cycle. During the 1st and 2nd non-aeration periods, the ORP was −25 mV, but rose during the aeration periods.

At Minute 325 when NH_4^+-N was depleted and DO was at 1.0 mg/L, the ORP rose to 45 mV at the end of the final aeration period.

The profiles of N and DO in a typical cycle are shown in Fig. 6.4 and Fig. 6.5. During the 10-min fill period, oxidized nitrogen (NO_2^--N and NO_3^--N) left in the reactor from the preceding operational cycle was rapidly reduced by means of denitrification. After the fill period, NH_4^+-N increased to 14 mg/L.

When aeration started, pH decreased and DO was around 0.1–1.25 mg/L. Nitrification commended at the beginning of the aeration periods. In the 1st aeration period (60–120 min), NH_4^+-N decreased almost linearly from 14.0 to 6.8 mg/L at a rate of 7.16 mg/(L.h). The production of oxidized nitrogen was low and the highest NO_2^--N and NO_3^--N concentrations were around 1.0 mg/L. During the second aeration period (180–240 min), NH_4^+-N was higher than 4 mg/L and DO in the bulk fluid was less than 0.5 mg/L. NH_4^+-N decreased linearly at a rate of 7.76 mg/(L.h) and NO_3^--N production rose significantly in comparison with in the 1st aeration period; from 221 to 240 min, NH_4^+-N in the bulk liquid was less than 4 mg/L and DO rose from 0.4 mg/L to 1.0 mg/L. Hence, NH_4^+-N became the limiting substrate for nitrification. The regulation of the aeration rate started at Minute 232 in order to keep DO at 1.0 mg/L. DO of 1.0 mg/L was maintained during the aeration periods of 232–240 min and 300–360 min (68 minutes out of a total aeration time of 300 minutes); this can save up to 65% of the air supply compared to a constant aeration rate of 1.0 L air/min.

6.4.3 Cycle performance of the SBR at low DO levels under the CA aeration strategy

A cycle study was carried out 15 days after the commencement of CA. The influent SS, COD and TN concentrations were 1,150, 3,400 and 348 mg/L, respectively. The MLSS and MLVSS concentrations in the reactor were 3,600 and 3,420 mg/L, respectively.

When the cycle commenced, the NO_2^--N and NO_3^--N concentrations in the reactor were 1.7 and 16.7 mg/L, respectively (Fig. 6.6). During 0–20 min, they were completely reduced with a maximum denitrification rate of 85 mg N/(L.h). At the same time, pH rose from 7.5 to 8.13. From 20 to 60 min, the bulk fluid in the reactor was anaerobic and the ORP was −25 mV.

From 60 to 120 min, the aeration rate was set at 1.2 L air/min and DO was kept at 1.0 mg/L to remove C from the bulk fluid to avoid inhibition of nitrification at low DO concentrations. During 80–120 min, NH_4^+-N was reduced by 11.0 mg/L at Minute 120,

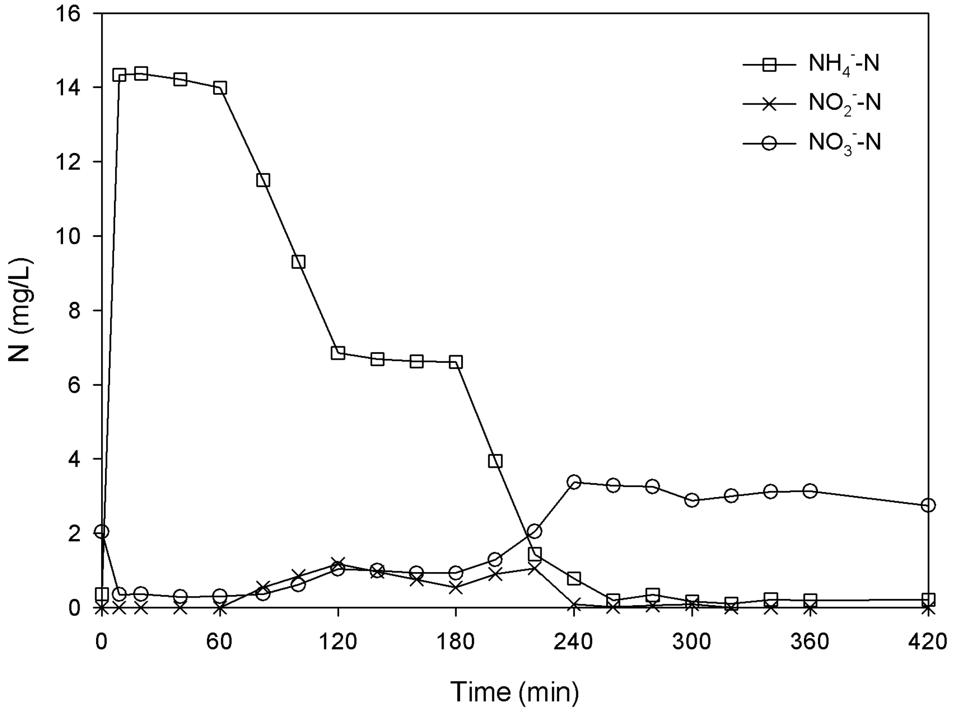


Figure 6.4: N profiles in a typical cycle under the IA aeration strategy

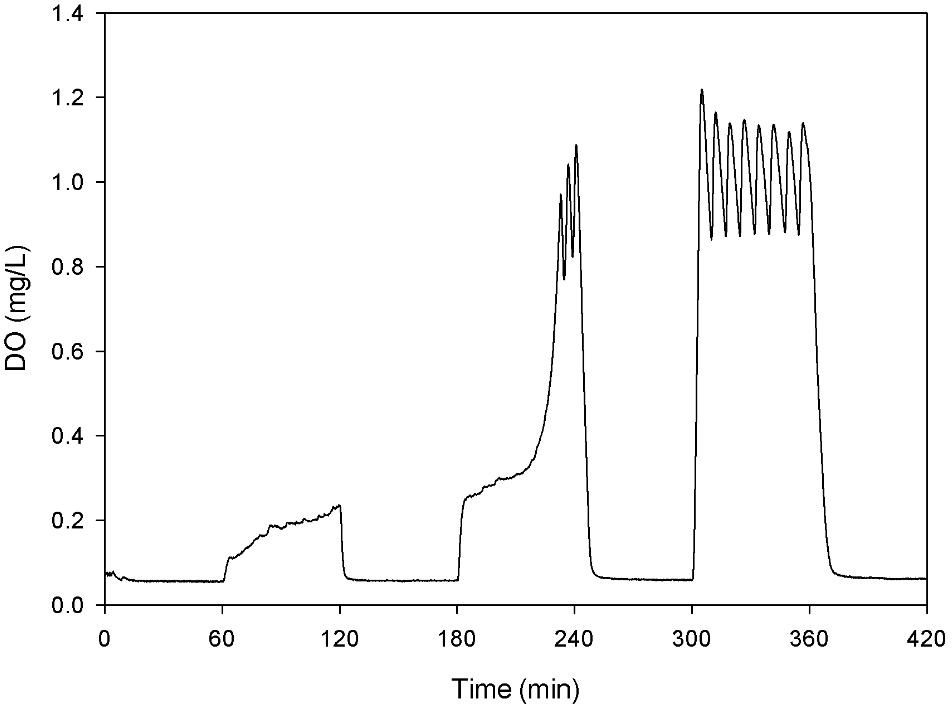


Figure 6.5: DO profile in a typical cycle under the IA aeration strategy

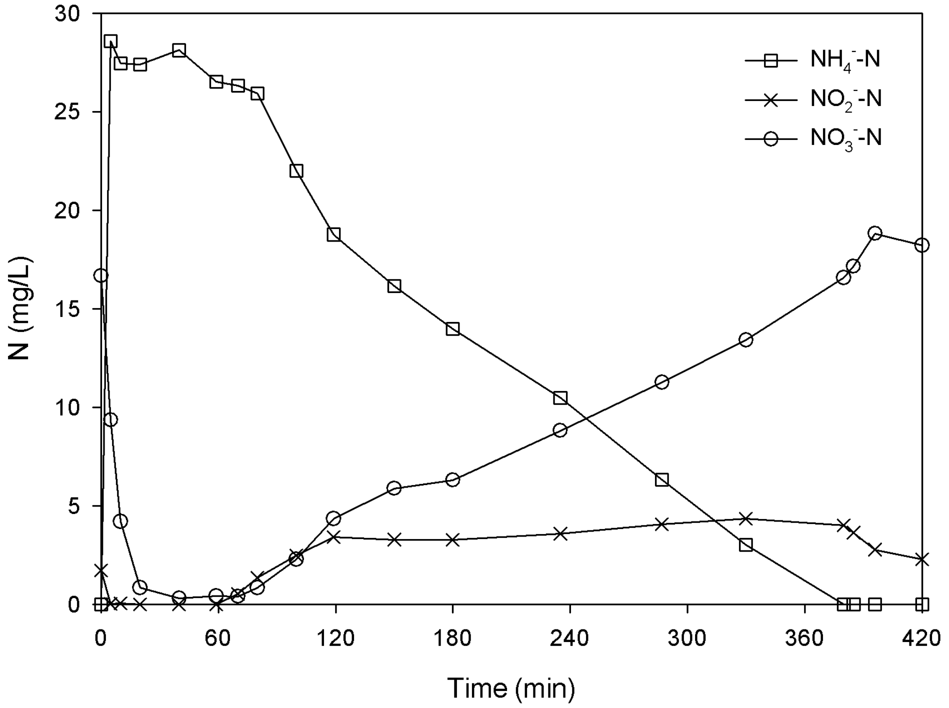


Figure 6.6: N profile in a typical cycle under the CA aeration strategy

the NO_2^--N and NO_3^--N concentrations were 3.4 and 4.3 mg/L, respectively. From 120 to 370 min, DO in the reactor was at 0.15–0.35 mg/L. During this period, NH_4^+-N was reduced by 4.28 mg/(L.h). During 370–397 min, DO was kept at 1.0 mg/L. NH_4^+-N was near zero and the OUR value decreased to -0.25 mg O_2/(L.min). NO_2^--N was oxidized into NO_3^--N; however, the oxidation rate of NO_2^--N to NO_3^--N was as low as 4.6 mg/(L.h). The rate of TON production was 3.9 mg/(L.h). From 394 to 420 min, aeration ceased and DO in the bulk fluid was near zero. Consequently, denitrification occurred, but was not significant; the reduction rate of TON was 2.7 mg/(L.h). The highest OUR (-1.04 mg O_2/(L.h)) was measured at Minute 91.

6.5 Discussion

6.5.1 Nitrogen removal under low DO levels

Hanaki et al. (1990) reported that low DO had no significant effects on NH_4^+-N oxidation in suspended-growth, activated sludge systems, but NO_2^--N oxidation was inhibited at a DO of 0.5 mg/L. As described in Chapter 3, in an intermittently aerated SBR treating slaughterhouse wastewater with a DO at 2.0 mg/L, the measured NH_4^+-N oxidation rate was 3.01 mg NH_4^+-N/(g SS.h). In the present study, the nitrification rate, expressed in terms of NH_4^+-N depletion, was in the range of 1.19–1.37 mg NH_4^+-N/(g SS.h) when the DO was maintained at 0.2–0.3 mg/L, which was around 41.5% of that measured in Chapter 3. However, it was still high enough to achieve complete nitrification in the SBR system operated with an 8-hour cycle duration.

One advantage in operating a SBR at low DO levels is that the operational cost associated with aeration can be reduced. The overall oxygen transfer rate, A, during the aeration periods can be described as:

$$A = -K_L a(S_{Osat} - S_O) \tag{6.1}$$

where, $K_L a$ is the volumetric global mass transfer coefficient (1/min); S_{Osat} is the saturation concentration of oxygen in wastewater (mg/L); and S_O is the actual oxygen concentration in the bulk fluid (mg/L).

From Eq. 6.1, it can be deducted that when S_O is low, the driving force $(S_{Osat} - S_O)$ for oxygen transfer will be increased. For a defined oxygen demand, A, the $K_L a$ becomes smaller, indicating that less aeration energy is needed.

Another benefit of maintaining low DO levels is to enhance SND (Pochana and Keller, 1999). Efficient SND has been reported in oxidation ditch systems operated at DO concentrations of 0.1–0.4 mg/L. For conventional SBRs treating slaughterhouse wastewater with a typical BOD_5/TN-ratio of (7–9)/1, N removal is 60%–80% (European Commission, 2005). Because of the slow nature of poly-β-hydroxybutyrate (PHB) degradation, N removal can be improved by using stored PHB for SND (Third et al., 2003). Third et al. (2005) found that by maintaining low DO levels during the react phase and by terminating aeration upon NH_4^+-N depletion, a large amount of N was removed in SBRs via SND. In the present study, the SBR was operated at low DO levels of less than 1.0 mg/L under two aeration strategies (IA and CA), the N removals were up to 95% and 91%, respectively. From the phase study conducted during the CA regime, SND, in addition to biomass synthesis, accounted for 29% of the total amount of N removed.

6.5.2 Application of OUR as a parameter to control the operation of the SBR system

The identified end point of nitrification can be used to terminate aeration and save aeration energy consumption. Marsili-Libelli (2006) proposed that the level-off point on the real-time DO profile, at which $dDO/dt = 0$, could be used to identify the end of the nitrification phase. As found in Chapter 3, $d^2DO/dt^2 = 0$ could more obviously indicate the end point of nitrification, at which NH_4^+-N concentration was nearly depleted. While due to the variation of the real-time measured DO profile, the d^2DO/dt^2 profile was not stable enough.

In the present study, a mini-DO sensor with quick response was used to measure the real-time DO profile in the reactor, and then the *in situ* OUR was calculated. When COD removal and nitrification simultaneously occurred:

$$OUR_T = OUR_M = OUR_C + OUR_N + OUR_{ER} \tag{6.2}$$

where OUR_T is the total OUR, also can be considered as equal to the measured *in situ* OUR, OUR_M; OUR_C is the oxygen utilization rate for heterotrophic carbon removal; OUR_N is the OUR for nitrification; OUR_{ER} is for endogenous respiration.

After COD removal was nearly complete and nitrification was the dominant bioprocess, then:

$$OUR_T = OUR_N + OUR_{ER} \tag{6.3}$$

When nitrification was complete, then:

$$OUR_T = OUR_{ER} \tag{6.4}$$

When the SBR was operated under the CA aeration strategy, *in situ* OUR was measured and calculated (Fig. 6.7). From 60 to 120 min when DO was maintained at 1.0 mg/L, OUR slightly varied around -0.97 mg O_2/(L.min). From 120 to 360 min, when DO was maintained at 0.2–0.3 mg/L, OUR was around -0.55 mg O_2/(L.min). At the end of the react phase when NH_4^+-N was depleted and DO was at 10% saturation, OUR was -0.25 mg O_2/(L.min). If OUR_{ER} was assumed constant in the whole react phase (the effect of DO on OUR_{ER} can be ignored because of the low DO levels), then OUR_{ER} was estimated as -0.25 mg O_2/(L.min). Therefore, the nitrification OUR_N was estimated as -0.30 mg O_2/(L.min). This gives a NH_4^+-N reduction of 4.0 mg NH_4^+-N/(L.h) $(0.3 \times 60 \div 4.57)$, which was quite close to the measured value of 4.28 mg NH_4^+-N/(L.h).

Therefore, it is feasible to use *in situ* OUR as a parameter to determine the end point of nitrification and aeration. After the SBR reaches steady-state conditions, the change of *in situ* OUR from $(OUR_N + OUR_{ER})$ to OUR_{ER} indicates that nitrification is complete and, as a result, aeration should be ceased.

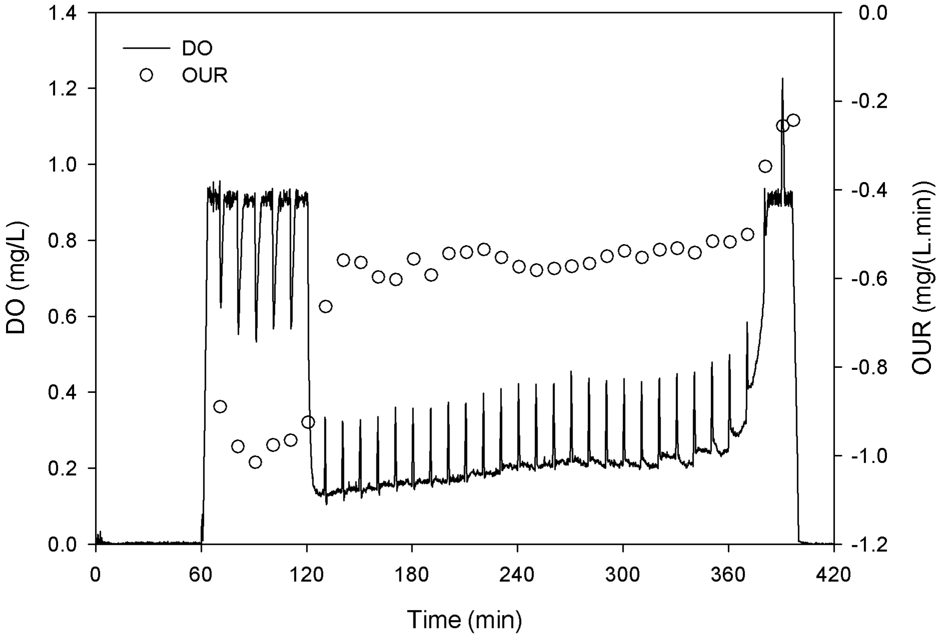


Figure 6.7: Profiles of DO and OUR in a typical cycle under the CA aeration strategy

6.6 Summary

When applying two operational strategies — intermittent aeration (IA) and continuous aeration (CA) — to operate a sequencing batch reactor treating real slaughterhouse wastewater at low DO levels, the following results were obtained:

1. Good effluent quality was obtained in the SBR operated at low DO levels under the two aeration strategies (CA and IA); TN removals were up to 94% and 91%, respectively.

2. Under the IA strategy, by maintaining low DO levels, electricity consumption associated with air supply can be reduced by up to 65%.

3. For the CA strategy, pre-denitrification and the application of low DO levels to encourage the occurrence of SND benefited N removal from slaughterhouse wastewater.

4. *In situ* OUR measurement using mini-DO sensors can be used to control the SBR operation.

Chapter 7

Partial Nitrification under Oxygen-Limited Conditions

7.1 Overview

Partial nitrification for low COD/N-ratio wastewaters in SBRs operated at moderately low temperature has great economical advantages due to the saving of the energy and treatment of a wide range of ammonium-rich wastewaters. An oxygen-limited partial nitrification process was studied in a laboratory-scale SBR treating synthetic wastewater containing rbCOD of 100–300 mg/L and NH_4^+-N of 300 mg/L.

7.2 Introduction

Conventional BNR comprises two successive steps: autotrophic nitrification in aerobic conditions and heterotrophic denitrification in anoxic conditions. The COD/N ratio of wastewater is of great importance to the overall N removal performance. A low COD/N ratio is usually unfavorable due to limited organic C availability for heterotrophic denitrification. For ammonium-rich wastewater with low COD/N ratios, e.g. COD/N ratio < 2.5, the addition of external carbon may lead to a significant increase in operational cost.

The application of the recently developed anammox process is a cost-effective way to treat ammonium-rich wastewater with low COD/N ratios (Jetten et al., 1997). In comparison with conventional BNR processes, the anammox process can save up to 90%

of the operational costs for N removal (Jetten et al., 2005).

$$\begin{aligned}&NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \\ &\rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O\end{aligned} \tag{7.1}$$

The SHARON process has been successfully coupled with the anammox process to treat reject water at full scale (van der Star et al., 2007). In the SHARON process with a SRT of 1–2 days, NOB are completely washed out of the reactor, while sufficient AOB are retained. High temperatures (30–35 °C) and high DO (3–5 mg/L) are the two essential factors in the SHARON process to encourage a faster growth of AOB than NOB.

Many municipal and industrial wastewaters are discharged at ambient temperature, e.g. with a representative value of 15.6 °C (Tchobanoglous et al., 2003). The anammox process has been proven to be efficient with high N removals at 18–20 °C (Isaka et al., 2007a; Dosta et al., 2008). To date, little research on partial nitrification at 15–20 °C to produce high nitrite effluent for the subsequent anammox process has been conducted. Therefore, it is desirable to explore a feasible partial nitrification process that will be efficient and cost-effective at moderately low temperatures.

To achieve stable partial nitrification at temperatures of 15–20 °C, a long SRT should be sustained so that a high biomass concentration is retained in the reactor. Applying high NLR to cause high concentrations of FA or FNA is a common method applied to achieve NO_2^- accumulation in nitrification reactors (Ciudad et al., 2005; Pambrun et al., 2008). However, even under high pH and high FA conditions, long-term partial nitrification cannot be sustained if the oxygen supply is sufficient for nitrifiers (Turk and Mavinic, 1989; Ruiz et al., 2003; Wyffels et al., 2003; Simm et al., 2006). Low DO under limited oxygen supply may be an essential factor to achieve partial nitrification in bioreactors operated with long SRTs. Under low oxygen conditions, stable partial nitrification has been achieved in some SBRs (Wyffels et al., 2003; Gustavsson et al., 2008; Lemaire et al., 2008), but, in these reactors, there exists other factors which are assumed to cause nitrite accumulation, such as NLR, temperature, FA and FNA inhibitions.

The aim of this study was to examine the long-term stability of partial nitrification using oxygen limitation as the major selection factor to treat low COD/N-ratio, ammonium-rich wastewater in a SBR operated at a temperature of around 20 °C.

7.3 Materials and methods

7.3.1 Partial nitrification reactor

A SBR with a working volume of 2.0 L, made from a 2.5 L glass cylinder with a diameter of 120 mm, was used in this study (Fig. 7.1). Two peristaltic pumps (323S, Walson-Marlow, UK) were used to feed the wastewater to the reactor and discharge the effluent. Another peristaltic pump was used to supply a constant airflow for aeration through an air diffuser located at the bottom of the reactor. A mechanical mixer (Yellow-Line, IKA, Germany) with a standard four-blade shaft paddle was used to stir the liquid in the reactor at 150 rpm. The sequential operation of the SBR was controlled by a PLC (S7-CPU-224, Siemens, Germany).

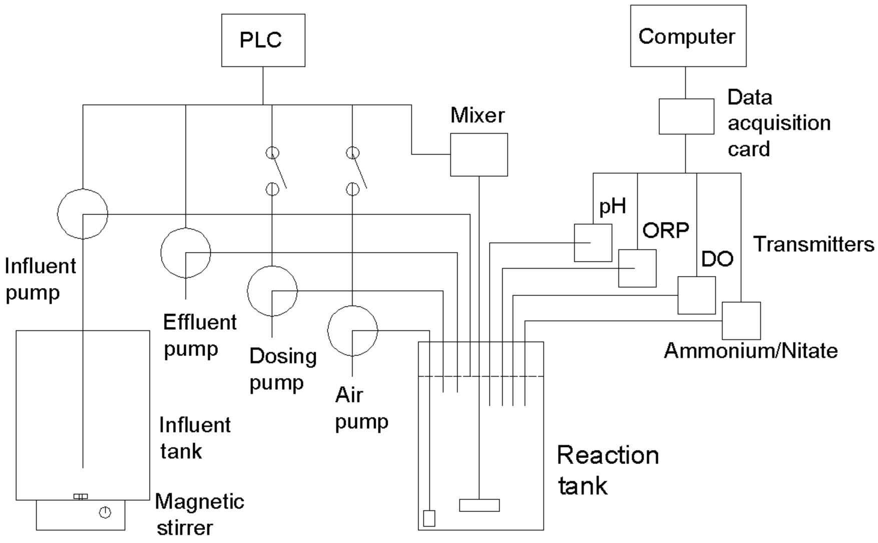


Figure 7.1: Oxygen-limited partial nitrification SBR system

Temperature, pH, ORP, DO, NH_4^+ and NO_3^- concentrations in the reactor were monitored in real-time using individual probes. The pH and ORP probes (Polyplast Pro, Hamilton, Switzerland) were connected to corresponding transmitters (Eutech, Singapore) that transformed the signals from the two electrodes into 4–20 mA analog signals. A peristaltic pump was controlled by a pH controller to dose 0.5 M Na_2CO_3 solution into the reactor, if needed. A highly sensitive Clark-type mini-DO sensor connected to a pico-ammeter (PA2000, Unisense, Denmark) was used to monitor DO in the reactor. The mini-DO sensor had a detection limit of 0.01 mg/L and a response time of about

4 seconds. Ion-selective membrane electrodes (VARiON, WTW, Germany) were used to online monitor NH_4^+ and NO_3^- concentrations in the reactor.

A data acquisition card (USB-6009, National Instruments, USA) and LabView software (V7.1, National Instruments, USA), installed on a desktop computer, were used to collect and process all online signals from different transmitters. The online data were recorded every 5 seconds.

7.3.2 Synthetic wastewater

Synthetic wastewater, made from tap water, was used in this study. Glucose and $(NH_4)_2SO_4$ were the main organic C source and ammonium source, respectively. The NH_4^+-N concentration in the synthetic wastewater was 300 mg/L. The COD/N ratio was set at $1:1$ or $1:3$ by changing influent the COD concentration to 300 mg/L or 100 mg/L. The other main components in the synthetic wastewater were: NaH_2PO_4 (31 mg P/L), yeast extract (50 mg/L), and $NaHCO_3$ (1800 mg/L). The supplement of mineral elements was after van de Graaf et al. (1996). The mole ratio of NH_4^+/HCO_3^- was 1/1. The synthetic wastewater was prepared once every week and stored at 4 °C in a refrigerator. The influent for the reactor was replaced daily by using the stored feed in the refrigerator.

7.3.3 Seed sludge

The reactor was seeded with return sludge of a local municipal WWTP in western Ireland. Initially, 1.0 L of the seed sludge and 1.0 L of the synthetic wastewater was added into the SBR, giving a MLSS concentration in the reactor of about 3,000 mg/L and a VSS to SS ratio of 82%.

7.3.4 Operation conditions of the SBR

The sequencing operation of the SBR is shown in Fig. 7.2. The cycle duration was 6 hours. During the first 5 minutes of the cycle, on average, 250 mL of the influent was delivered into the reactor, giving a HRT of 2.0 days and NLR of 0.15 g N/(L.d).

The SBR was operated under two operational conditions: CA in the start-up period and IA in the stable partial nitrification period.

The purpose of the start-up period was to apply a high temperature and a high DO level to enhance the growth of AOB, and to use high FA concentrations to temporarily inhibit the growth of NOB. A water bath was used to maintain 25 °C in the reactor. The CA strategy was applied at an aeration rate of 200 mL air/min. The NH_4^+-N concentration

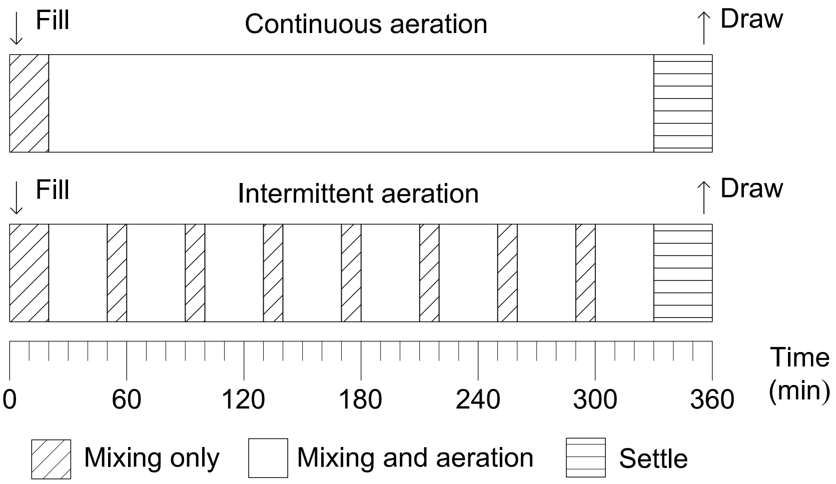


Figure 7.2: Sequential operation of the SBR

in the reactor was kept above 100 mg/L by manually replacing a certain amount of the supernatant with the feed during the settle phase. pH in the reactor was automatically controlled at 8.2–8.5. Thus, the FA concentration in the reactor was kept above 8.5 mg N/L during the start-up period.

The IA pattern was applied to sustain partial nitrification after the 7-day start-up period. The reactor was operated at an ambient temperature of around 20 °C. The maximum designed aeration time in a complete cycle was 240 minutes (Fig. 7.2) and the aeration rate was set at 150 mL air/min. The aeration was automatically terminated when the online measurement of NH_4^+-N reached 20 mg/L. The pH controller maintained a pH of 7.1–7.3 by dosing Na_2CO_3 solution into the reactor.

7.3.5 *In situ* measurement of OUR

In typical cycles, an external aeration pump with an aeration rate of about 2 L air/min was used to increase the DO concentration in the reactor to more than 3.0 mg/L. Subsequently, the aeration stopped and the reactor was continuously stirred at 150 rpm. DO and NH_4^+-N were online monitored using a mini-DO sensor and an ammonium probe, respectively. OUR was calculated by averaging the decreasing rate of DO with respect to time. Before and after each OUR measurement, 2.0 mL of the mixed liquor was sampled from the reactor for chemical measurement of the NH_4^+-N concentration.

7.3.6 Batch experiments testing the biomass activity

Batch experiments were carried out to test the activity of NOB in the biomass under oxygen-sufficient conditions, where NH_4^+-N was the limiting substrate.

Initially, 100 mL of the mixed liquor was taken out of the reactor and washed with a fresh medium 3 times to minimize NO_3^--N present in the sludge. The medium contained $(NH_4)_2SO_4$ (30 mg N/L), Na_2SO_4 (1000 mg/L), $NaHCO_3$ (756 mg/L) and NaH_2PO_4 (0.31 mg P/L), and was supplemented with mineral elements as mentioned above. The washed sludge was added into a 500-mL glass jar and the medium was added to reach a working volume of 450 mL. The mixture was stirred with a mechanical mixer at 20 rpm and the temperature was kept at 20 °C using a water bath. It was aerated at an airflow rate of 10 mL air/min by an air diffuser. A mini-DO sensor was used to monitor the DO concentrations in the bulk liquid. Liquid samples were taken from the jar (2 mL each time) for analysis of the soluble N components.

7.3.7 Analytical methods

These are described in Section 3.3.4.

7.3.8 Molecular analysis of the nitrifiers in the biomass

The real-time polymerase chain reaction (PCR) technique was used to assess relative population sizes of AOB and NOB in the reactor. Sludge samples were pelleted by centrifugation and DNA was extracted from sample pellets using an UltraClean Soil DNA extraction kit (Mobio). DNA extractions were quantified using a Quant-iT ds DNA BR assay kit (Invitrogen), and standardised to 2 μg/mL before use in real-time PCR assays. Real-time PCR was carried out in duplicate for quantification of gene targets using a Lightcycler-480 instrument (Roche) with SYBR Green I master mix (Roche) according to the manufacturer's instructions. Primers described by Geets et al. (2007) were used to detect ammonia monooxygenase gene (primers amoA1F/amoA2R; Rotthauwe et al., 1997), nitrite oxidoreductase gene (primers nxrB1F/nxrB1R; Vanparys et al., 2006), and *Nitrospira* species 16S rRNA gene (primers NSR1113F/NSR1264R; Dionisi et al., 2002a). In addition, primers 338f and 519r (Muyzer et al., 1993) were used to target the 16S rRNA gene of all Eubacteria, allowing the other targets to be expressed as a fraction of the total number of Eubacterial 16S rRNA genes.

The thermal cycling program was modified from the PCR program described by Geets et al. (2007) for the same primers, using the following optimised annealing temperatures in independent assays: 338f/519r 55 °C, amoA1F/amoA2R 60 °C, nxrB1F/nxrB1R 65 °C, NSR1113F/NSR1264R 65 °C. A 94 °C initial denaturation for 4 min was followed by 40 cycles 94 °C denaturation for 1 min; optimised annealing temperature for 1 min; 72 °C elongation for 1 minute, and a final elongation step at 72 °C for 10 min.

Standard curves for gene quantification were prepared from suitable PCR products originating from reactor samples and cloned into pCR4-TOPO plasmids using a TOPO TA cloning kit (Invitrogen). For each standard, multiple clones were screened by sequencing and, in all cases, phylogenetic analyses showed that the optimised PCR had amplified the correct target. Overnight *E. coli* clone cultures were grown in LB broth with 50 μg/mL kanamycin, and then plasmids were harvested and purified using Wizard Plus SV minipreps (Promega). The concentration of DNA in the plasmid preparations was determined, and, therefore, the concentrations of plasmids and cloned DNA were calculated. Standards for real-time PCR were prepared by adjusting the plasmid extractions to 2×10^8 copies per μl then performing 10-fold serial dilutions in 10 mM Tris buffer at pH 8.0.

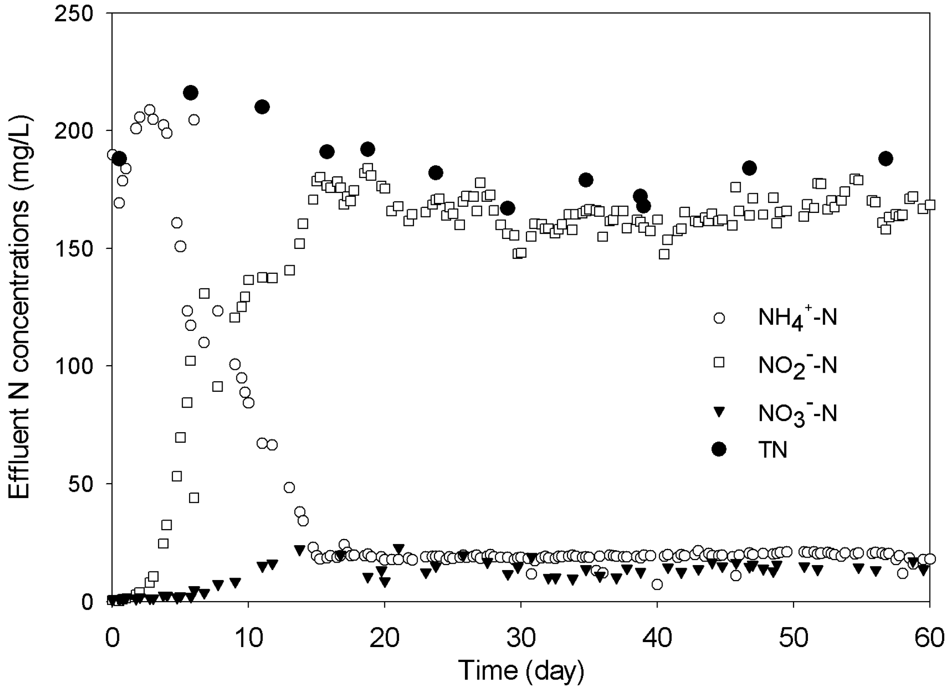
7.4 Results

7.4.1 Overall performance of the partial nitrification SBR

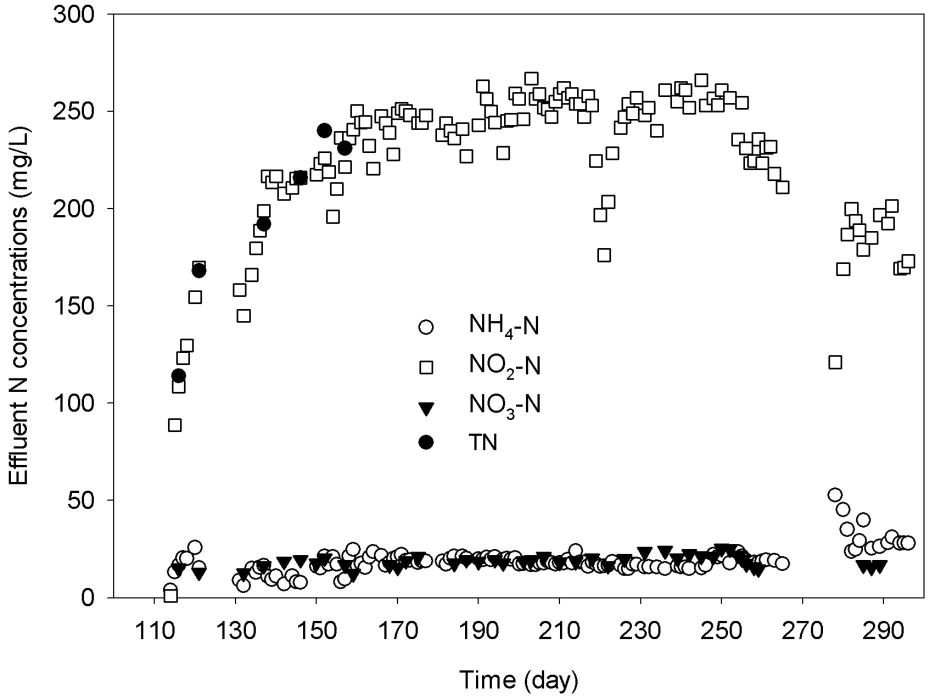
The operation of the partial nitrification SBR comprised four stages: start-up (7 days; Day 1–7); stable partial nitrification with the synthetic wastewater with a COD/N ratio of 1/1 (55 days; Day 8–62) ; static storage (47 days; Day 63–109) and re-start period (10 days; Day 110–119); and stable partial nitrification with the synthetic wastewater with a COD/N ratio of 1/3 (120 days; Day 131–250) (Fig. 7.3).

On Day 1, a high DO of over 4.5 mg/L was maintained in the reactor at a continuous aeration rate of 200 mL air/min. With sufficient oxygen supply and at an elevated temperature of 25 °C, NO_2^--N in the reactor quickly rose from less than 3 mg/L to 130 mg/L in 6 days. On Day 7, the *in situ* NH_4^+-N utilization rates at temperatures of 25 °C and 20 °C were 21.5 mg/(L.h) and 17.2 mg/(L.h), respectively. The NO_3^--N concentration in the effluent was less than 10 mg/L during the start-up period. The high FA concentration ($>$ 8.2 mg N/L) may have effectively suppressed the activity of NOB in the 7 days.

From Day 8, the reactor started to run under oxygen-limited conditions at ambient temperature of 18–21 °C. The aeration pattern was changed to IA and the aeration rate was 150 mL air/min. DO in the reactor was less than 0.2 mg/L in the aeration periods. The period from Day 8 to Day 16 was a transition period and the effluent NH_4^+-N concen-



(a) Start-up and stable partial nitrification at a COD/N-ratio of 1/1



(b) Resumption and stable partial nitrification at a COD/N-ratio of 3/1

Figure 7.3: Start-up, stable performance and resumption of the partial nitrification process

tration gradually decreased to 20 mg/L. From Day 17 to Day 62, the aeration duration was controlled by the NH_4^+-N concentration and the aeration was terminated when the online NH_4^+-N measurement reached 20 mg/L. During the stable operation period of 45 days (Day 17–62), the average NH_4^+-N and NO_2^--N concentrations in the effluent were 19 mg/L and 170 mg/L, respectively. The effluent NO_3^--N concentration was mostly less than 20 mg/L.

The operation of the reactor ceased for 47 days (Day 63–109) and then resumed under the oxygen-limited conditions. During the 47-day non-operation (open air static storage) period, NH_4^+-N and NO_2^--N in the liquid was oxidized to NO_3^--N. On Day 110, when the operation was resumed, the supernatant was replaced with distilled water to minimize the NO_3^--N concentration, and the operation commenced under oxygen-limited conditions at an ambient temperature of around 20 °C. After 10 days (Day 110–119), stable partial nitrification was achieved in the reactor, and the effluent quality was similar to that in the previous steady-state partial nitrification period.

From Day 120 to Day 130 (11 days), the reactor operation was stopped due to laboratory construction work. From Day 131 to Day 250 (120 days), synthetic wastewater with a low COD/N ratio of 1/3 was fed to the reactor. Efficient partial nitrification was maintained for 120 days with average NH_4^+-N and NO_3^--N concentrations of about 20 mg/L each. The NO_2^--N concentration in the effluent increased gradually and then stabilized at 250 mg/L (Fig. 7.3b). The NO_2^--N concentration dropped on Day 215 due to a temporary increase of the influent COD to 500 mg/L implemented during a 3-day case study.

The results show that after a 7-day start-up period, stable partial nitrification was achieved under oxygen-limited conditions for a period of up to 180 days. The partial nitrification process can quickly recover after non-operation (static storage) for 11 days (Day 120–130) or 47 days (Day 63–109). Over the entire operation period, MLVSS in the reactor was stable at 2,500 mg/L. The settling property of the activated sludge flocs was satisfactory, with a typical SVI value of 110 mL/g SS. The effluent SS was between 20–30 mg/L. Except for sludge sampling for analysis, sludge wasting was not conducted during the whole operation period. It is estimated that the average SRT was over 100 days.

7.4.2 Cycle performance of the SBR

A typical cycle performance, carried out on Day 40, is presented in Fig. 7.4 and 7.5. The NH_4^+-N and NO_3^--N online measurement profiles were close to the values obtained using chemical analysis methods (Fig. 7.4). After the 5-min fill period, the NH_4^+-N concentration increased to its highest level in the cycle, 51.8 mg/L. Although pH in the influent was as low as 6.5, the occurrence of denitrification during the fill period significantly increased the actual pH in the reactor. The highest pH level of 7.42 was found at the end of the fill period (Fig. 7.5a). The sharp increase of pH indicates that the influent rbCOD was rapidly used up for denitrification during the 5-min fill period. The rapid depletion of rbCOD during the fill period created a better condition for the nitrifiers to compete for oxygen from heterotrophs in successive aeration periods. The average DO concentration during the aeration periods was 0.08 mg/L, and DO during the non-aeration periods was less than 0.02 mg/L. An almost constant ORP value of −26 mV was sustained during the whole cycle, which indicates that the bulk liquid in the SBR was under anoxic conditions.

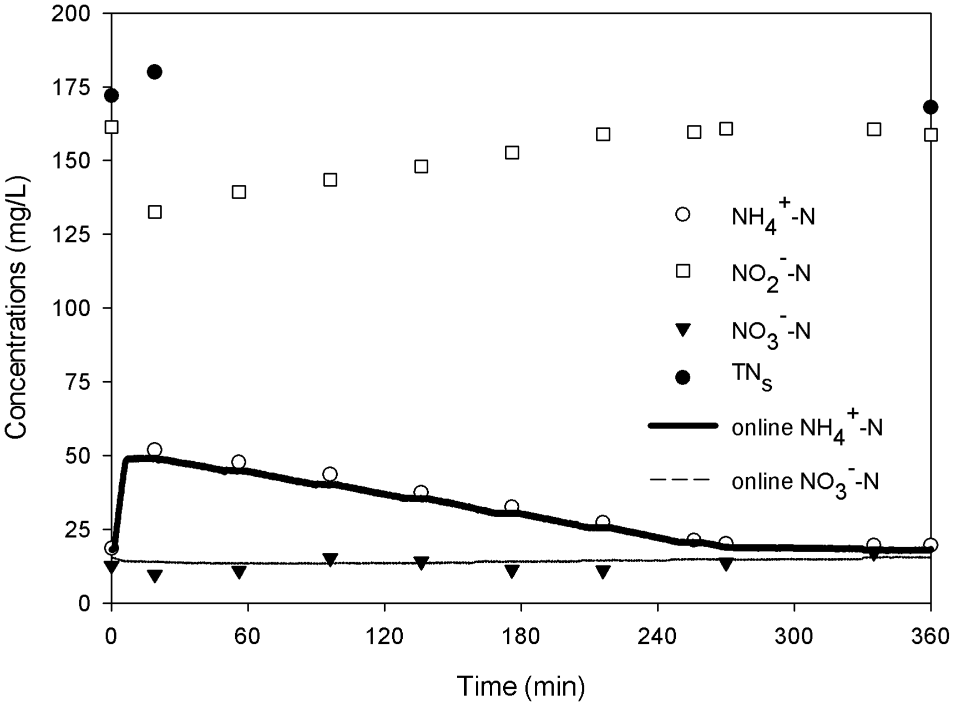
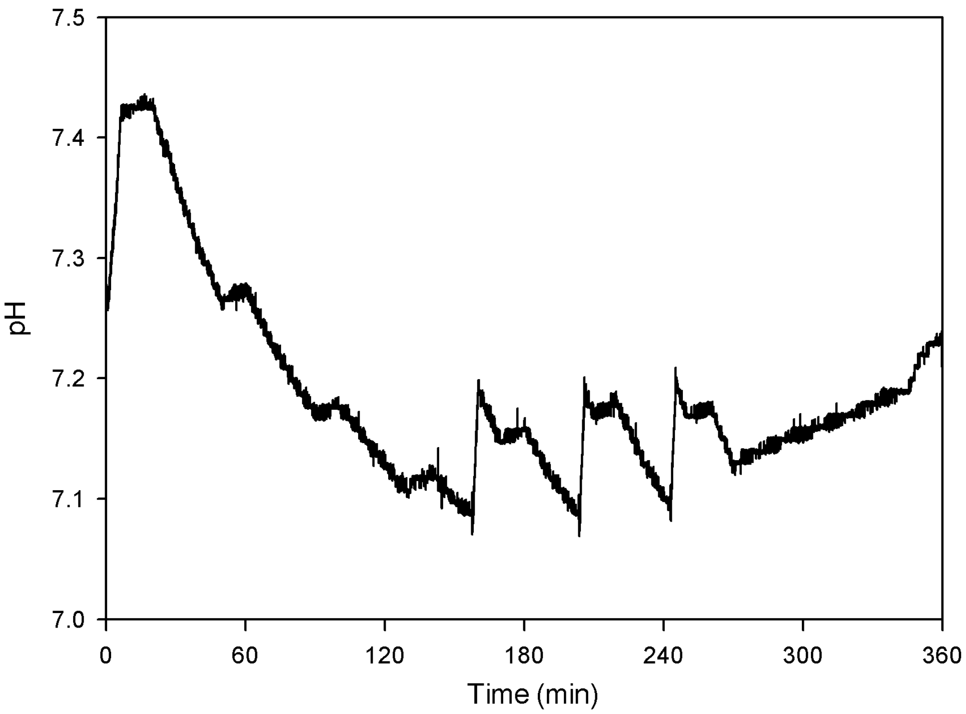


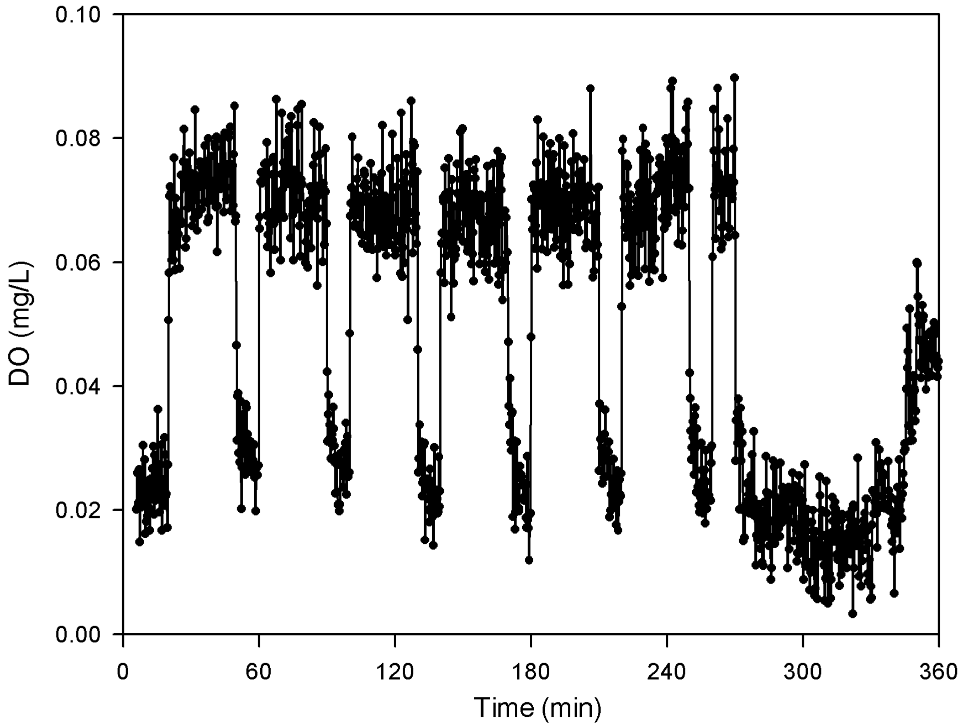
Figure 7.4: Cycle performance of the partial nitrification process: N profiles

The NH_4^+-N oxidation rate was almost constant during the aeration periods with the average value of 9.84 mg/(L.h). It was observed that the NH_4^+-N concentration decreased linearly and the NO_2^--N concentration rose linearly. The NO_3^--N concentration in the reactor was almost constant at 14 mg/L during the whole cycle.

The aeration was automatically terminated by the online NH_4^+-N controller at Minute 270 when the online measurement of NH_4^+-N was equal to 20 mg/L. During the non-



(a) pH



(b) DO

Figure 7.5: Cycle performance of the partial nitrification process: pH and DO profiles

aeration period, NH_4^+-N and NO_2^--N concentrations were stable. The TNs after the fill period and at the end of the cycle was 180 mg/L and 168 mg/L, respectively.

An extended aeration period was applied to a cycle on Day 41 to examine the DO and pH variations under NH_4^+-limiting conditions. Just after the aeration in the reactor was terminated when the online measurement of NH_4^+-N reached 20 mg/L, a continuous aeration mode with an aeration rate of 150 mL air/min was applied for 130 min, while NH_4^+-N in the reactor decreased from around 15 mg/L to near zero. The online-monitored profiles of DO, pH and NH_4^+-N are shown in Fig. 7.6. Before NH_4^+-N was decreased to less than 5–10 mg/L, oxygen was the limiting factor for nitrification. DO in the reactor was at 0.05 mg/L and the NH_4^+-N oxidation rate was 8.54 mg/(L.h). When NH_4^+-N was below 5 mg/L, NH_4^+-N became the limiting substrate for nitrification, and DO gradually increased in the reactor. When NH_4^+-N decreased to less than 3–2.5 mg/L, the DO concentration rose sharply and a "DO elbow" occurred on the DO profile (Fig. 7.6). Simultaneously, the pH value in the bulk fluid increased slowly due to the limited NH_4^+ oxidation activity and air stripping of CO_2 out of the reactor. A "pH valley" occurred on the pH profile after the appearance of the "DO elbow" (Fig. 7.6). When NH_4^+-N was almost completely depleted, sufficient DO (2.5 mg/L) was present in the reactor for a period of 150 min, NO_2^--N remained almost constant, and there was no observable increase of NO_3^--N in the reactor.

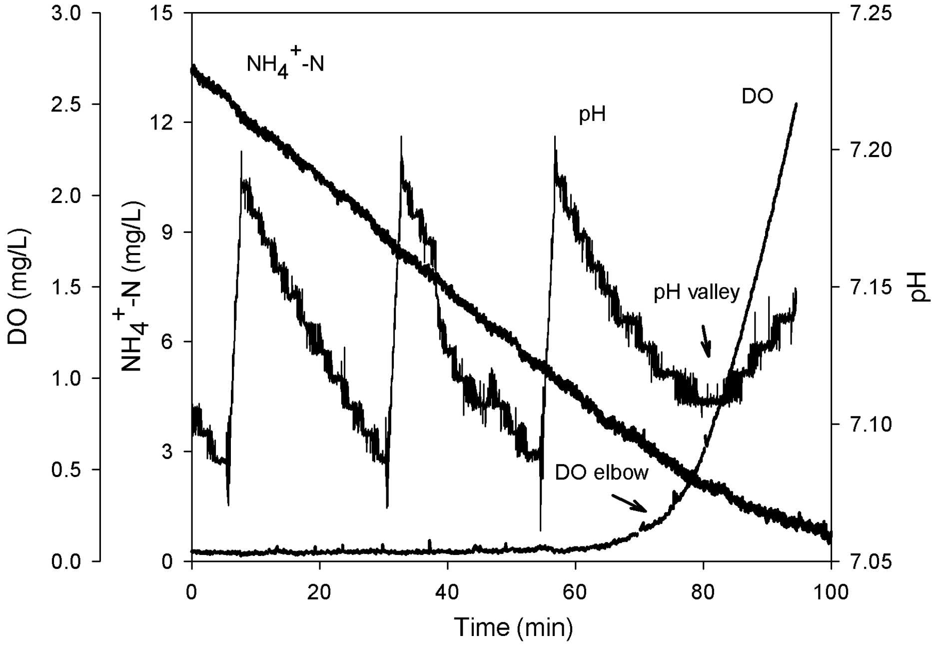


Figure 7.6: Extended aeration on cycle performance of the partial nitrification process

7.4.3 *In situ* OUR test

An *in situ* OUR test was carried out in the reactor on Day 41 at NH_4^+-N concentrations of 22, 17, 0.5 and <0.1 mg/L (Fig. 7.7). While NH_4^+-N was not limiting in the reactor (NH_4^+-N $=$ 17 or 22 mg/L), OUR leveled off when DO >1.0 mg/L. If OUR at a DO of 2.5 mg/L was assumed as the maximum OUR, the estimated Ko for AOB was 0.20 mg/L. In the reactor, the average DO during the aeration periods was in the range of 0.05–0.15 mg/L. Thus, the NH_4^+-N oxidation rate at a DO of 0.10 mg/L represented about 34% of the maximum NH_4^+-N oxidation rate. When NH_4^+-N was completely depleted (NH_4^+-N <0.1 mg/L) and became the limiting substrate, the apparent OUR was only about 0.05 mg DO/min, which was only 3% of the maximum OUR when NH_4^+-N and DO were not limiting.

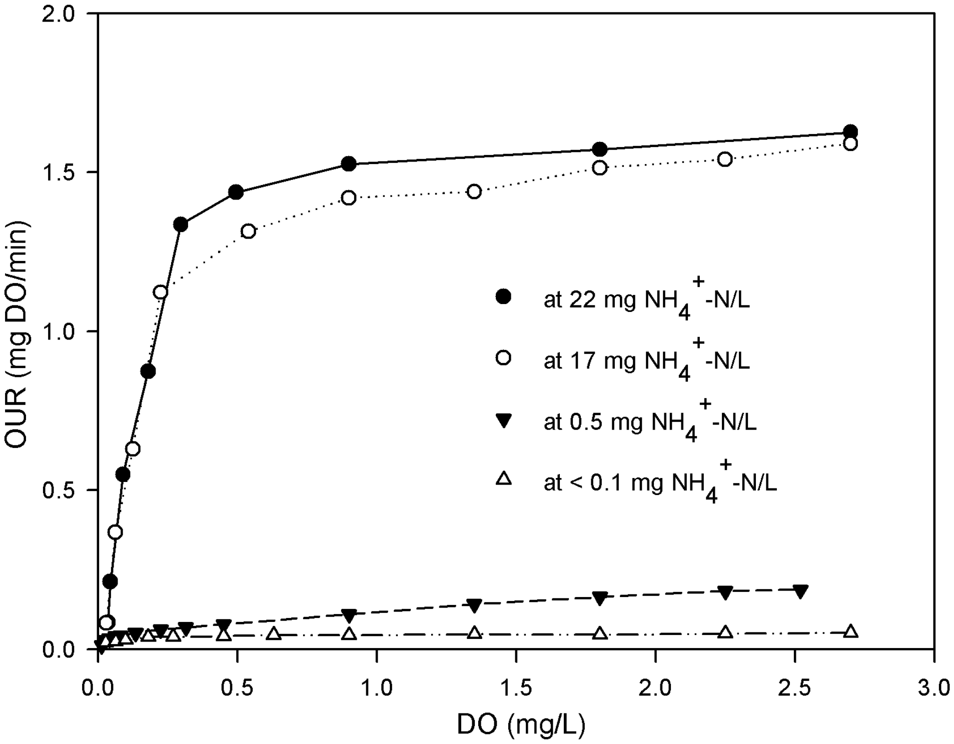


Figure 7.7: OUR profiles in the partial nitrification process

7.4.4 Batch test on extended aeration

To study the recovery of the NOB activity in the biomass under NH_4^+-N limitation and high DO conditions, a batch test was conducted using a mixture of the sludge taken from the reactor on Day 58. The MLSS and MLVSS concentrations in the batch test were 695 mg/L and 605 mg/L, respectively.

In the first 5 hours, when NH_4^+-N in the batch vessel decreased from 30 mg/L to 2 mg/L and DO was 0.1–0.2 mg/L, the NH_4^+-N oxidation rate and the NO_2^--N production rate were 5.86 mg N/(g VSS.h) and 5.75 mg N/(g VSS.h), respectively.

From Hour 6, NH_4^+-N became the limiting substrate for nitrification and DO rose to over 5 mg/L thereafter. Even with sufficient DO (> 5 mg/L) and sufficient NO_2^--N (about 26 mg/L), the NO_3^--N production rate was less than 0.1 mg N/(g VSS.h) in a 30-hour period (Fig. 7.8). A significant increase of the NO_3^--N production rate was observed after Hour 60. The NO_3^--N production rate reached about 0.6 mg N/(g VSS.h) at Hour 80, which was about 6% of the maximum NO_2^--N production rate of the biomass under optimized conditions.

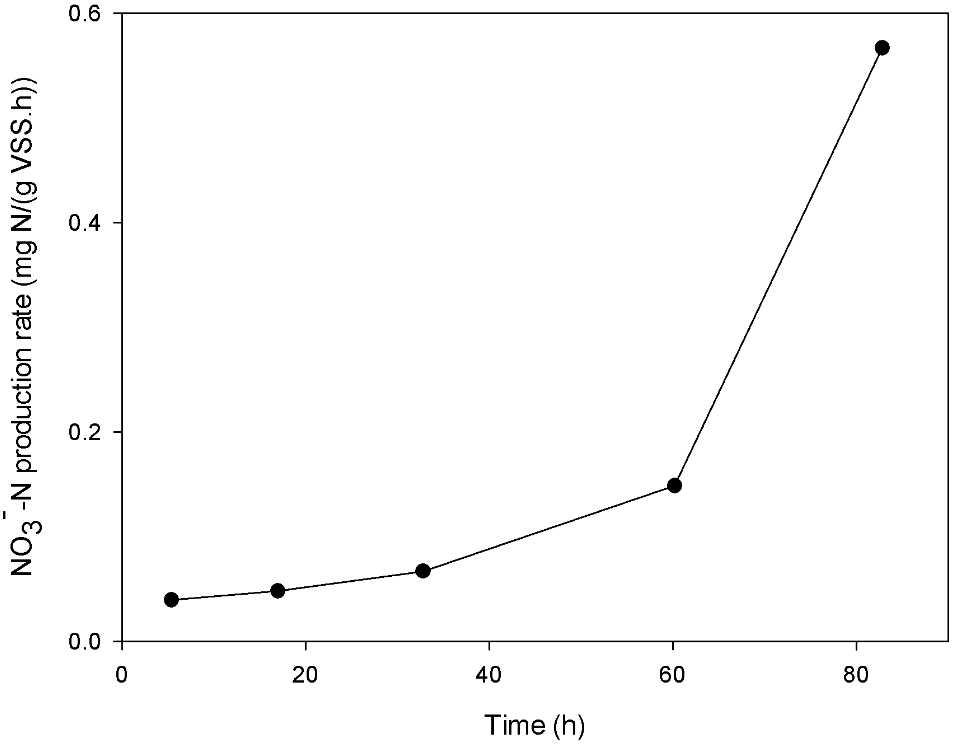


Figure 7.8: Batch test result of extended aeration

7.4.5 Microbial community analysis of the biomass in the reactor

Real-time PCR was used to measure population shifts of the main nitrifiers in the reactor, and to help assess whether the oxygen limitation regime of the SBR maintained a selective advantage for AOB over NOB. For the purposes of this study, the most important measures were the relative amount of AOB compared to NOB in the reactor, and shifts in population with respect to time or reactor performance. Since DNA extraction is unlikely to be quantitatively reproducible, we controlled the amount of DNA used in the assays, and standardized results with respect to the eubacterial 16S rRNA gene, to give a measure of the amount of each target gene compared to the bacterial population as a whole. Due to known and potential differences in the gene copy number between species for the gene targets used in this study, the ratios presented should not be regarded as an exact proxy for cell numbers.

The target gene measurements are expressed as ratios to the eubacterial population (16S rRNA gene of all bacteria; EUB) in Fig. 7.9. It was found that the 16S copy number per unit DNA was constant, indicating that the bacterial population fraction did not change in comparison to non-bacterial sources of DNA such as archaea and eukaryotes. In the seed sludge (Day 1 sample), the population of all nitrifiers was only 10^{-5} of EUB. On Day 6, the AOB/EUB ratio increased to 1% but the NOB/EUB ratio remained less than 10^{-4}. In the subsequent stable partial nitrification period (until Day 253), the AOB/EUB ratio stabilized at 10% but the NOB/EUB ratio was always at least 10-fold less.

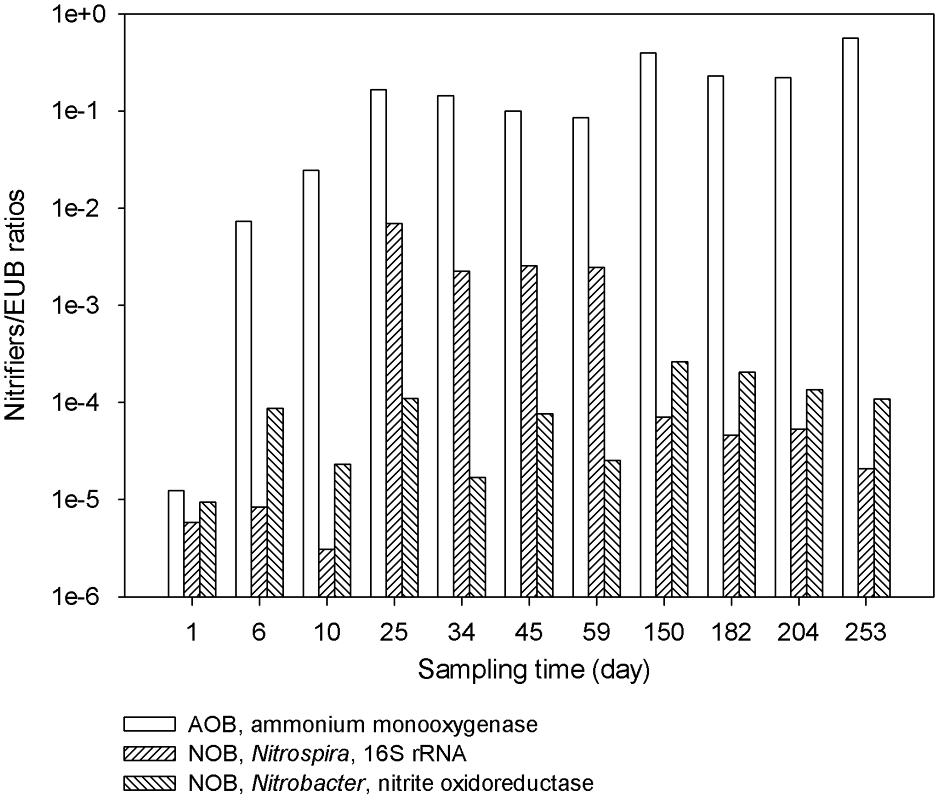


Figure 7.9: Population variations of AOB and NOB in the sludge

7.5 Discussion

The accumulation of nitrite in the nitrifying system is due to the unbalanced overall activity of AOB and NOB. If the NO_2^--N production rate by AOB is higher than the NO_2^--N oxidation rate of NOB, nitrite accumulation occurs. To achieve efficient partial nitrification, a larger AOB population should be maintained in the reactor, while the NOB population size should be kept at a minimum level. This means that in long-term operation of partial nitrification processes, the overall growth rate of NOB must be less than that of AOB (Blackburne et al., 2008b). In stable partial nitrification systems, especially in oxygen-limited suspended-growth activated sludge partial nitrification systems, the dominant AOB are *Nitrosomonas* (Sliekers et al., 2002; Wyffels et al., 2004; Sliekers et al., 2005). Both NOB-*Nitrobacter* and NOB-*Nitrospira* can survive at low concentrations, or even at undetectable levels, in the partial nitrification biomass. These findings are similar to the molecular analysis results of the sludge samples obtained in this study. If sufficient oxygen is supplied, complete nitrification could recover from oxygen-limited partial nitrification systems in a short period of time, e.g. 80 hours (obtained in this study), or 11 days (Wyffels et al., 2003).

7.5.1 Factors used to achieve long-term stable partial nitrification

The NLR is important to the partial nitrification process. Partial nitrification normally takes place when NH_4^+-N supply is sufficient for AOB. While at low NLR, NH_4^+ becomes the limiting substrate for AOB and NO_2^- also becomes limiting for NOB. Thereafter, even under low DO conditions, partial nitrification can not be achieved and NH_4^+ is completely oxidized to NO_3^-, because with a limiting NH_4^+ supply, NOB-*Nitrospira* can be a better O_2 competitor than AOB, and can become the main NOB in the biomass (Sliekers et al., 2005). *Nitrospira* species have a very low threshold of FA and FNA, and their growth can be easily inhibited at high NLR. In the present study, the NLR was 0.15 g N/(L.d), which is higher than NLR in most municipal wastewater treatment plants.

High temperature (equal to or higher than 25 °C) can elevate the growth of AOB-*Nitrosomonas* over NOB-*Nitrobacter* when NH_4^+ and O_2 are not limiting (Hellinga et al., 1998; Philips et al., 2002). The SHARON process applies a SRT of 1–2 days to wash out NOB, while keeping sufficient AOB in the reactor. A high temperature of 30–35 °C is a critical factor for the SHARON process (Pollice et al., 2002). In the present study, the nitrifying reactor was intended to operate under a temperature of about 20 °C, so a long SRT was necessary to keep a sufficient amount of AOB in the reactor.

While under high DO conditions, partial nitrification is not achievable. NOB-*Nitrobacter* has a higher specific growth rate than AOB at temperatures below 25 °C (Hellinga et al., 1998). In high NLR nitrification systems, high FA or FNA levels have a greater inhibition effect on the growth of NOB than AOB. However, *Nitrobacter* can be acclimated to high FA or high FNA conditions, so the use of FA or FNA as the main selection pressure cannot sustain long-term partial nitrification, especially in high DO conditions (Turk and Mavinic, 1989; Wyffels et al., 2003; Simm et al., 2006).

Thus, oxygen-limited conditions are essential to achieve partial nitrification in long SRT activated sludge systems. A mass ratio of DO/NH_3-N of less than 5 can be used to define the oxygen limited conditions in the nitrification reactors (Cecen and Gonenc, 1995). In this study, with a pH of 7.1–7.4, NH_4^+-N > 20 mg/L and DO < 0.3 mg/L, the FA level in the reactor was less than 0.2 mg N/L and the actual mass ratio of DO/NH_3-N was less than 1.5. Under the oxygen-limited conditions, the growth of NOB can be suppressed by AOB. AOB-*Nitrosomonas* is regarded as a better oxygen competitor than NOB-*Nitrobacter* (Laanbroek and Gerards, 1993; Wiesmann, 1994) and *Nitrospira* (Sliekers et al., 2005), provided that NH_4^+-N is sufficient. Pollice et al. (2002) observed that, under a limited-oxygen supply, stable partial nitrification was achieved independent of the sludge age.

Moreover, the initial NOB-*Nitrobacter* size has an important effect on successful partial nitrification. In this study, after the start-up period, a high AOB/NOB ratio was established (ie, AOB/NOB > 100). This study shows that, even without significant inhibition by FA, FNA or COD, the NOB did not grow to become the dominant nitrifiers under sustained oxygen-limited conditions. Once a high NOB-*Nitrobacter* population was established in the system (ie, *Nitrobacter*/EUB $> 5\%$), a significant *Nitrobacter* activity could survive under low DO for a long period (for example, over 30 days in a control reactor in this study; data not shown). Using oxygen limitation as the only selection factor, partial nitrification is not reversible at least over a 30-day period. Thus, a re-start-up could be a better solution by completely replacing the sludge containing high NOB-*Nitrobacter* with typical activated sludge from MWWTPs, in which the population ratio of *Nitrobacter*/EUB is very low (ie, about 10^{-5} in the seed sludge of this study).

7.5.2 Quick recovery of the partial nitrification performance after long-term biomass storage

This study shows that the *in situ* long-term (47-day) non-operation (static storage of biomass) did not cause any problems in the recovery of the partial nitrification process.

Since air diffusion into the liquid from the atmosphere through the liquid-atmosphere interface was negligible, the settled sludge layer was mainly under anoxic or anaerobic conditions. The population size of NOB-*Nitrospira* decreased significantly during the 47-day storage period (Fig. 7.9), indicating that NOB-*Nitrospira* had a higher decay rate than AOB-*Nitrosomonas* during the long-term non-operation period. Salem et al. (2006) have found that under anoxic or anaerobic conditions, the decay rates for nitrifiers are significantly lower than under aerobic conditions, and AOB has an even lower decay rate than NOB under anoxic conditions. Tappe et al. (1999) observed that AOB were more readily recovered from starvation than NOB when ammonium became available. Their findings can also help to explain how the partial nitrification performance was completely resumed within 10 days in this study.

7.5.3 Control strategy for the oxygen-limited partial nitrification process

The above analysis shows that after the start-up period, oxygen-limited conditions, in combination with sufficient NH_4^+-N supply, can be used successfully to sustain long-term partial nitrification. The failure of the oxygen-limited partial nitrification process is due to growth and accumulation of NOB-*Nitrobacter* in the biomass to a high level. Reduced NLR and excessive oxygen supply could be the two main factors stimulating the growth of NOB-*Nitrobactor*. When NLR is high, it is easier to maintain oxygen-limited conditions for partial nitrification. For example, Gustavsson et al. (2008) sustained partial nitrification for over a year in a SBR treating high-strength ammonium wastewater (NH_4^+-N of 910 mg/L) under a high NLR (0.7 g N/(L.d)) with a long SRT (29 days). The DO in the reactor was below 0.6 mg/L and the effluent NH_4^+-N concentration was 50–150 mg/L.

However, when treating wastewater containing moderate ammonium concentrations (e.g. 100–500 mg N/L) under lower NLR (e.g. <0.25 g N/(L.d)), it is difficult to sustain oxygen-limited conditions. In SBRs, a reliable way to achieve oxygen-limited conditions is to terminate the aeration process before the depletion of NH_4^+-N in the reactor (Sliekers et al., 2005).

The use of online monitoring of pH and DO is a practical way to control aeration in laboratory- and pilot-scale partial nitrification reactors (Yang et al., 2007; Blackburne et al., 2008b). The dDO/dt ("DO elbow") and dpH/dt ("pH valley") are two control parameters to terminate aeration to avoid further oxidation of NO_2^- to NO_3^- by NOB. In this study, the batch experiment results show that the "DO elbow" and the "pH valley" appeared after NH_4^+-N became limiting, e.g. NH_4^+-N <5 mg/L. While the "pH valley" or the “DO elbow” was detectable, the DO concentration in the reactor increased to 0.3–0.5 mg/L, even to 1.0 mg/L. The increase of DO could cause an increased population of NOB-*Nitrobacter* and disturb the partial nitrification process. In addition, the calculation of dpH/dt would be hampered by the dosing of external chemicals when pH control is in operation.

Online NH_4^+-N monitoring can provide a direct parameter. The pH variation does not greatly affect the signal of the NH_4^+-N probe at low NH_4^+-N concentrations. Online NH_4^+-N control can be used to terminate aeration when the effluent NH_4^+-N concentration is as low as 5–10 mg/L. The combination of online NH_4^+-N monitoring and a low DO level is a feasible and effective approach to avoid extended oxidation and sustain the oxygen-limited partial nitrification conditions.

7.6 Summary

The results of this study show that:

1) After the establishment of a high AOB/NOB population ratio during a 7-day start-up period, partial nitrification was sustained for a period of up to 180 days under oxygen-limited conditions.

2) Oxygen limitation can be used as a main selection factor to sustain partial nitrification at ambient temperatures and to produce a high NO_2^--N concentration effluent for the successive anammox process.

3) The molecular analysis shows that AOB represented up to 10% of the total bacteria population and NOB (both *Nitrobacter* and *Nitrospira*) were out-competed by AOB during the entire operation period.

4) The partial nitrification biomass can be kept in long-term static storage without adverse impacts on its performance in partial nitrification.

Chapter 8

Nitrous Oxide Emission from the Oxygen-Limited Partial Nitrification Reactor

8.1 Overview

Nitrous oxide (N_2O) emission from wastewater treatment facilities is increasingly being recognized as a concern in global warming. N_2O emissions vary significantly in different wastewater treatment processes. In this study, N_2O emissions from the oxygen-limited partial nitrification process treating ammonium-rich wastewater at ambient temperature, described in Chapter 7, was examined, and the effects of the influent rbCOD on the N_2O emissions were studied.

8.2 Introduction

N_2O is a greenhouse gas (GHG) with a steady-state lifetime of 114 years in the atmosphere (IPCC, 2001).The global warming effect of N_2O is 300 times higher than that of carbon dioxide (CO_2) over a period of 100 years. The anthropogenic contribution to the total emission of N_2O into the atmosphere is estimated at 40% (Vitousek et al., 1997). The increasing use of fertilizer in agriculture is considered to be the main factor in the increasing N_2O levels in the atmosphere, with an annual rate of increase of 0.2–0.3% in recent years (Vitousek et al., 1997; Prather, 2007).

In wastewater treatment processes, N_2O emission is primarily linked to heterotrophic denitrification and nitrifier denitrification (Khalil and Rasmussen, 1992). Heterotrophic denitrification is the stepwise reduction of NO_3^- to N_2 by heterotrophic denitrifiers with internal and external C sources as electron donors, and N_2O is an intermediate product in the process (Skiba et al., 2008):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{8.1}$$

In the autotrophic nitrification process, some members of AOB have active NO_2^- reductase enzyme systems that allow the use of hydroxylamine (NH_2OH) as the electron donor and NO_2^- as the electron acceptor to generate N_2O. This route, termed nitrifier denitrification, has the potential to produce substantial amounts of N_2O (Wrage et al., 2001).

The average N_2O emission from municipal WWTPs is estimated to be 0.5% of the total influent NLR (IPCC, 2006). However, due to variety of the wastewater composition and operation conditions, N_2O emissions from wastewater treatment facilities vary significantly. It can be up to 15% of the NLR in full-scale reactors, and up to 95% of the influent NLR in laboratory-scale bioreactors (Kampschreur et al., 2009). A range of operational parameters affecting N_2O emissions, such as DO, NH_4^+, NO_2^-, pH and COD/N ratio, have been identified (Itokawa et al., 2001; Zeng et al., 2003; Kampschreur et al., 2008a; Zhou et al., 2008). The potential release of high levels of N_2O from wastewater treatment facilities remains a concern, especially when treating ammonium-rich wastewater (Kampschreur et al., 2009).

N_2O emissions from anammox reactors are negligible compared to SHARON reactors. The total N_2O emission from this two-reactor system is estimated at 2.3% of the influent NLR, and is comparable to emissions from conventional activated sludge processes (Kampschreur et al., 2008b).

In order to achieve partial nitrification for ammonium-rich wastewater at moderately low temperatures of 15–20 °C, an oxygen-limited partial nitrification reactor was developed, which has been described in Chapter 7. The studied reactor was characterized by both high NO_2^- concentrations (>150 mg/L) and low DO levels (<0.2 mg/L) that are known to stimulate N_2O emissions (Kampschreur et al., 2009). The N_2O dynamics in the reactor was therefore a concern, as too high levels of N_2O emission in the oxygen-limited partial nitrification process would limit its potential for full-scale operation. The aim of this study was to examine the N_2O dynamics in the oxygen-limited partial nitrification process, and determine the controlling factors.

8.3 Materials and methods

8.3.1 Oxygen-limited partial nitrification reactor

The setting up and operation of the oxygen-limited SBR system were described in Sections 7.3.1 to 7.3.4.

8.3.2 N_2O online measurement in the partial nitrification reactor

The emission of N_2O from the partial nitrification reactor was through diffusion across the water-air interface and air stripping of dissolved N_2O from the liquid phase into the atmosphere. Off-gas detection is a direct measurement of N_2O emission, but due to the low aeration rate of the laboratory-scale bioreactor, online off-gas detection was not applicable. Alternatively, direct online measurement of dissolved N_2O in the water phase was applied, which has been used to study N_2O emissions from bioreactors by other researchers (Kampschreur et al., 2008a). A sensitive N_2O micro-sensor (Andersen et al., 2001) was used to measure the dissolved N_2O concentration in the water phase. The N_2O sensor tip was immersed 2–3 cm below the liquid surface in the reactor and the online data was automatically recorded every 3 seconds. The standard N_2O solution for calibrating the N_2O sensor had a salinity of 0.01%, and was diluted from a N_2O-saturated water solution, which was prepared by flushing distilled water with technical-grade pressurized N_2O gas.

8.3.3 Measurement of N_2O diffusion and air stripping rates

The diffusion and air stripping rates of N_2O from the liquid to the atmosphere were estimated using 0.01% salinity water medium without microbial activity. The tests were carried out under similar conditions (such as shape of the container, temperature, mechanical mixing rate, and aeration rate) to the operational conditions in the partial nitrification reactor.

The two-film theory gas-liquid mass transfer equation can describe the diffusion of dissolved N_2O to the atmosphere (Tchobanoglous et al., 2003):

$$\frac{dC}{dt} = -K_L a(C - C_s) \tag{8.2}$$

where C is N_2O concentration in the bulk liquid phase, mg N/L; $K_L a$ is overall mass transfer coefficient, 1/h; and C_s (mg N/L) is N_2O concentration in the liquid phase equilibrium with the average fraction of N_2O in the atmosphere given by Henry's law.

Because the N_2O fraction in the air is only around 320 ppb, C_s is close to zero. Thus, Eq. (8.2) can be simplified to:

$$\frac{dC}{dt} = -K_L a C \tag{8.3}$$

The water medium test was carried out in a 2.5 L jar. The saturated N_2O-N concentration in water at 20 °C and salinity of 0.01% is 804.5 mg N/L. 5.0 mL of N_2O-saturated water solution was added into 2000 mL of distilled water in the jar, resulting in an initial N_2O concentration of 2.0 mg N/L. When the N_2O diffusion rate was tested, the jar was stirred at the same rate (150 rpm) as in the partial nitrification reactor. When the air stripping rate (diffusion was included) was tested, the jar was aerated with the same aeration rate (150 mL air/min) as in the reactor during the aeration periods, and stirred at 150 rpm. A N_2O micro-sensor was used for online monitoring and data were recorded every 3 seconds.

8.3.4 Batch tests of DO effects on the N_2O production rate

In the SBR, a high sludge concentration of about 2,500 mg VSS/L caused a rapid decrease of DO when aeration was stopped. It was difficult to estimate rates of N_2O production at different DO levels using the on-line reactor data. Therefore, the DO effect on the N_2O production rate was tested in separate batch experiments carried out in a 450-mL glass jar. The MLVSS level in the batch test was about 1/5 of the MLVSS level in the reactor.

The settled effluent and the mixed liquor from the reactor was used as the medium in the batch test. A small air pump was used to increase the DO level > 5 mg/L; then, the aeration was stopped and the liquid was stirred at 100 rpm. A Clark-type mini-DO sensor and a N_2O micro-sensor were used for online monitoring of DO and N_2O, respectively. N_2O production rates under different DO levels were then calculated from the online DO and N_2O profiles

8.3.5 Analytical methods

This was described in Section 3.3.4.

8.4 Results

8.4.1 Performance of the partial nitrification reactor

The performance of the partial nitrification reactor was detailed in Chapter 7.

8.4.2 N_2O stripping and diffusion rates obtained in the water medium tests

The rates of N_2O emission from the liquid phase to the atmosphere through either diffusion across the water-air interface or air stripping, were estimated using water medium tests. The results are shown in Figs. 8.1 and 8.2. The profiles of the measured diffusion and striping rates of N_2O can be described using linear equations with respect to the dissolved N_2O concentration.

N_2O emission rates through diffusion:

$$\frac{dC}{dt} = -0.13C \tag{8.4}$$

N_2O emission rates through air stripping:

$$\frac{dC}{dt} = -1.77C \tag{8.5}$$

The measured mass transfer coefficient for the N_2O diffusion rate, K_La, was 0.13/h. Similarly, the mass transfer coefficient describing the N_2O stripping rate, K_La, was 1.77/h.

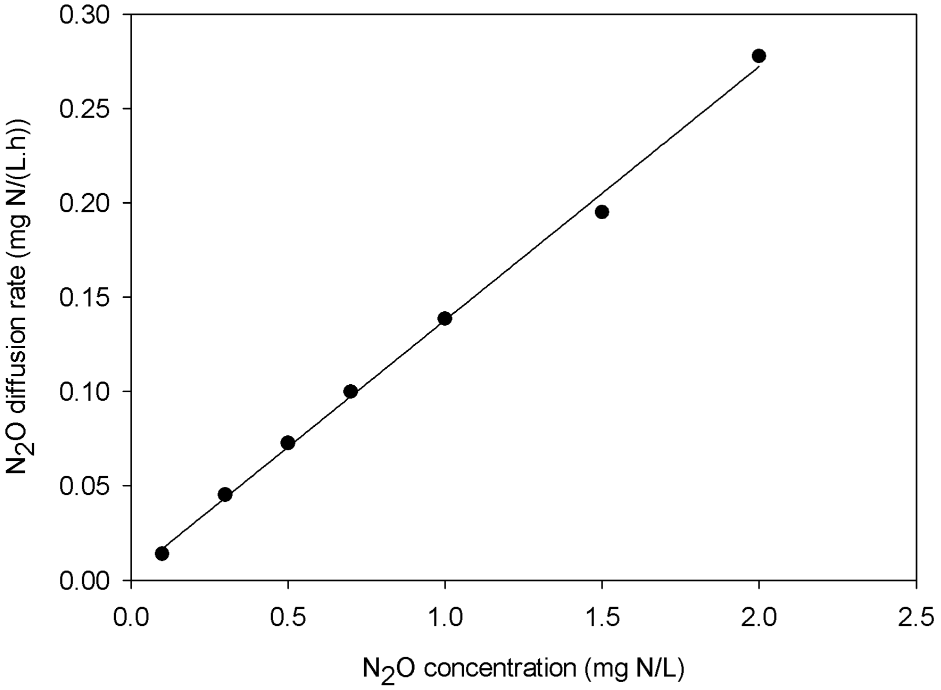


Figure 8.1: Measurement of the N_2O diffusion rate at various N_2O concentrations

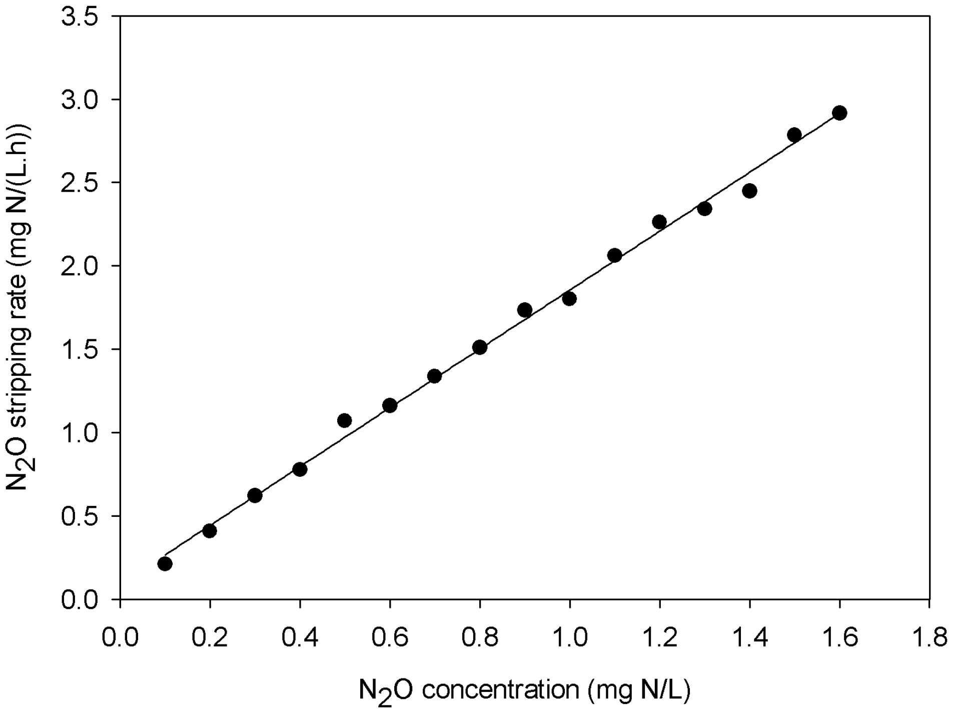


Figure 8.2: Measurement of the N_2O stripping rate at various N_2O concentrations

8.4.3 Overall N_2O emission from the partial nitrification reactor

During stable partial nitrification periods, profiles of the dissolved N_2O concentration in typical cycles were tested under various influent organic C levels (Fig. 8.3). The reactor was fed with three types of synthetic wastewater containing rbCOD of 0, 100 and 500 mg/L each for 2–4 days, and enhanced MWW containing COD of 370 mg/L for 20 days. The profiles of DO and NH_4^+-N removal were similar in these cycle studies.

Similar N_2O dynamic patterns were evident for all tested wastewater streams. Generally, the highest N_2O concentration appeared at the end of the first anoxic (fill + mixing) period. This shows that the anoxic fill period greatly stimulated N_2O production. It is conjectured that the high increase in the N_2O concentration during this period was mainly caused by heterotrophic denitrification. A significant decrease in the N_2O concentration was found in the first 2 hours of the cycle. In the remaining reaction phase, the dissolved N_2O concentration stabilized, with net accumulation during non-aeration periods and net reduction during aeration periods. When treating synthetic wastewater containing rbCOD of 500 mg/L, the stabilized dissolved N_2O level was 0.5–0.8 mg N/L, which was significantly higher than when treating the other three wastewaters. For this wastewater, a significant N_2O increase was also observed during the settling phase.

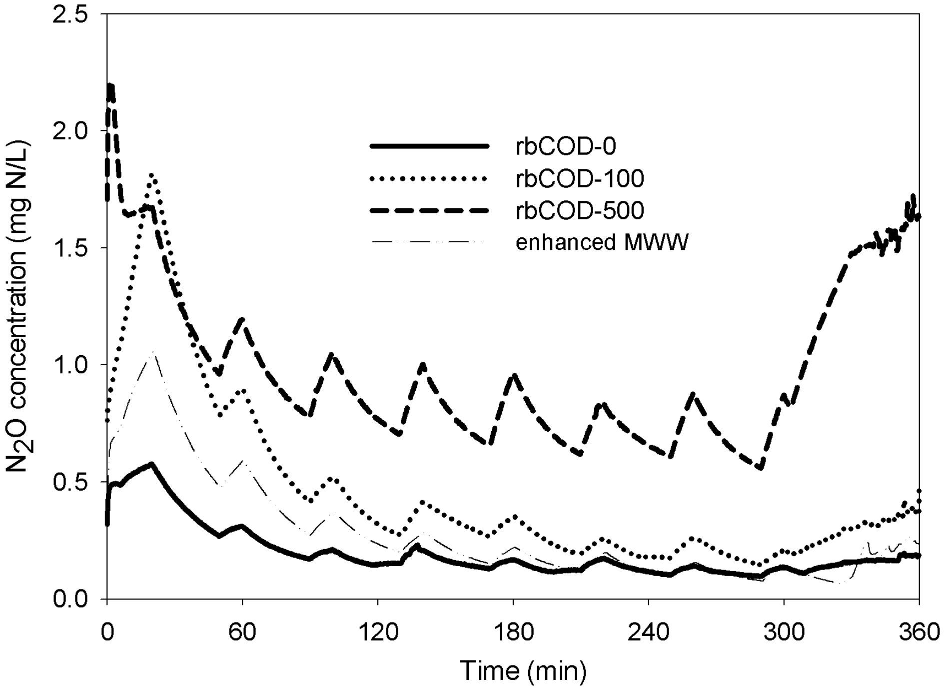


Figure 8.3: Cycly profiles of dissolved N_2O at different influent organic loadings

By applying the air stripping and diffusion rate equations (Eqs. (8.4) and (8.5)), the estimated N_2O emissions from the reactor are calculated (Table 8.1).

Table 8.1: N_2O emission levels at different organic loadings

Wastewater	rbCOD-0	rbCOD-100	rbCOD-500	enhanced MWW
N_2O emission level (percentage of influent NLR)	1.73%	6.23%	12.08%	5.78%

8.4.4 N_2O accumulation during anoxic non-aeration periods

N_2O accumulation rates during the non-aeration periods in typical cycles are shown in Fig. 8.4. Because N_2O emission through diffusion can be largely ignored, as the results from the water medium studies confirm, the N_2O accumulation rate can be considered equal to the apparent N_2O production rate. In general, anoxic N_2O production decreased with the decreasing influent COD level. At a rbCOD level of 0, the N_2O production was 0.14 mg N/(L.h) and the production rate was constant during different non-aeration periods. The increase in the N_2O accumulation rate during the fourth anoxic period

(Fig. 8.4) was mainly caused by lower pH values of about 7.1. Then, the pH values were increased to 7.3 by the pH controller.

When the influent wastewater contained rbCOD of 500 mg/L, a very low N_2O production rate occurred during the initial fill period. The N_2O production rate was largely constant at around 1.2 mg N/(L.h) during the subsequent anoxic periods, which was high in comparison with the other treated waste streams.

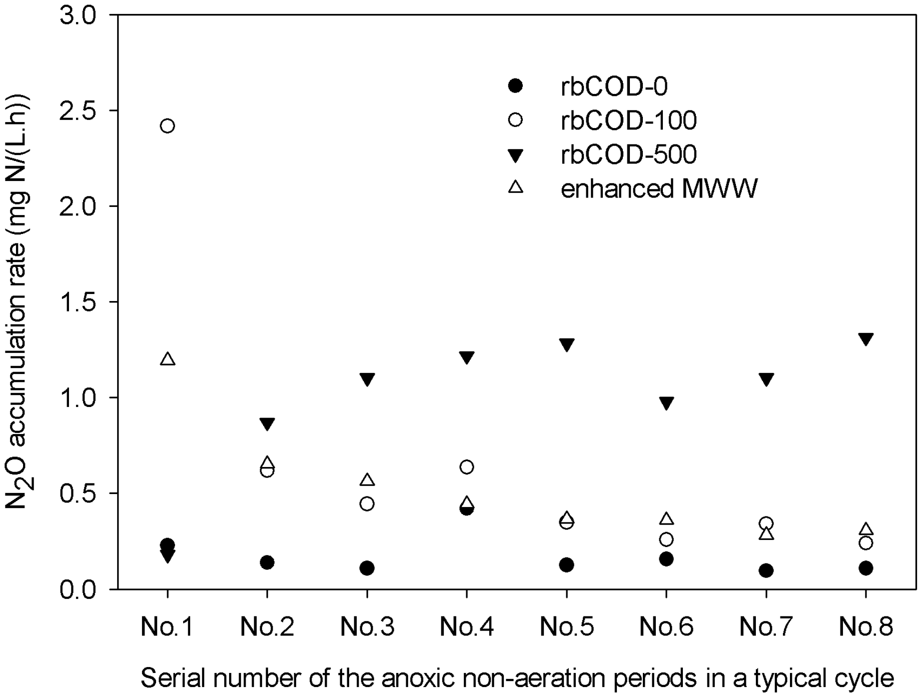


Figure 8.4: N_2O accumulation rate during the anoxic periods in the reactor

8.4.5 N_2O production under the continuous aeration pattern

The profile of the dissolved N_2O concentration in the reactor under the CA strategy was measured and compared with the profile obtained under the IA strategy (Fig. 8.5). When the reactor was operated in the oxygen-limited, continuous aeration pattern, the reactor was continuously aerated in the react phase with a constant aeration rate of 150 mL air/min. During the fill and mixing period, a significant increase of N_2O in the reactor occurred due to incomplete heterotrophic denitrification. Under IA operation, the N_2O concentrations rose during the anoxic, non-aeration periods, decreased, and stabilized within 2 hours.

The N_2O production rate during the CA period was equal to the summation of the

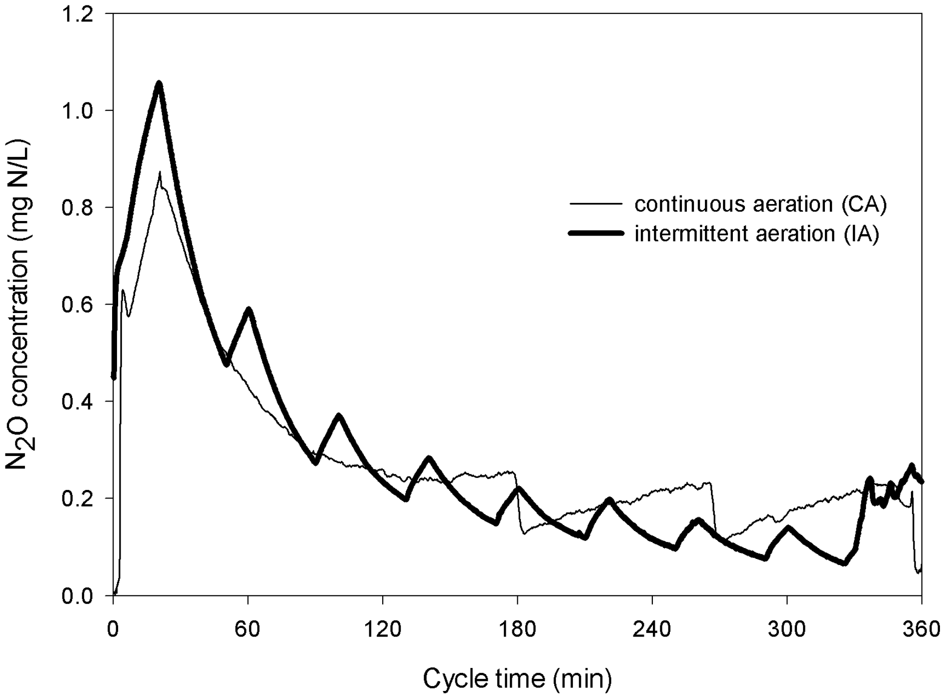


Figure 8.5: Profiles of the dissolved N_2O concentration during the IA and CA operational cycles

N_2O emission rate through air striping, and the changing rate of the dissolved N_2O concentration in the reactor. The net N_2O production during the aeration period (20–120 min) under the CA aeration pattern is shown in Fig. 8.6. Aeration, which started from Minute 20, immediately reduced the N_2O production rate from over 1.2 to 0.6 mg N(/L.h). From 20 to 50 min, the N_2O production rate decreased from 0.6 to 0.42 mg N/(L.h), and from 50-min onwards, the N_2O production rate tended to stabilize at 0.3 mg N/(L.h). This shows that N_2O production could be reduced to a low level after aeration for about 30 minutes under oxygen-limited conditions.

8.4.6 N_2O production under different DO levels

The effect of DO concentrations on the N_2O production was tested in a batch experiment, as described previously. The profiles of DO and N_2O concentrations in the bulk liquid are shown in Fig. 8.7. No significant N_2O production was detected when DO was in the range of 6.5 to 0.3 mg/L. The N_2O production rate under anoxic conditions, measured in the partial nitrification reactor, was comparable to the production rate measured under the low DO conditions (0.05–0.2 mg/L).

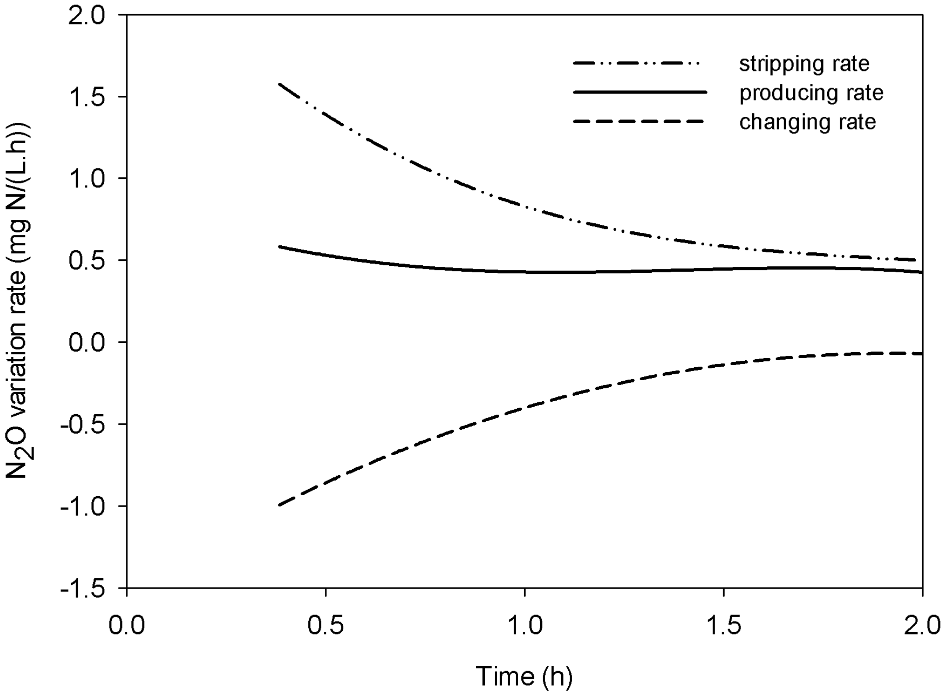


Figure 8.6: N_2O production rate under oxygen-limited, continuously aerated conditions

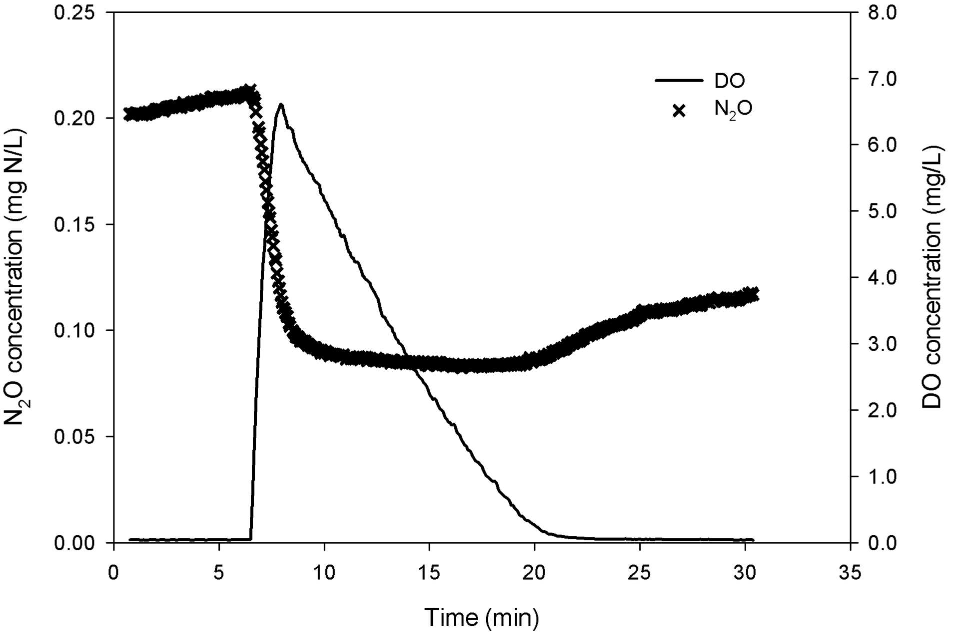


Figure 8.7: Effect of the DO concentration on N_2O production

8.5 Discussion

8.5.1 N_2O emission via nitrifier denitrification under oxygen limited partial nitrification conditions

As shown in Fig. 8.7, there was no significant accumulation of N_2O when DO > 0.3 mg/L for the biomass where AOB accounted for up to 10% of the total bacterial population. In the partial nitrification reactor, when the oxygen-limited conditions were applied, DO in the bulk liquid was 0.05–0.2 mg/L during the aeration periods. Both low DO and high NO_2^--N concentrations can stimulate N_2O emissions through heterotrophic denitrification and nitrifier denitrification (Kampschreur et al., 2009).

Nitrifier denitrification is the main source of N_2O emissions from some nitrification reactors. For typical nitrifying activated sludge taken from MWWTPs, the N_2O emission is up to 0.1–0.4% of oxidized NH_4^+-N, and nitrifier denitrification contributes more than 50% to the total N_2O emissions (Tallec et al., 2006). The addition of 10 mg/L of NO_2^--N can stimulate N_2O emissions by nitrifying biomass by 2–8 times (Tallec et al., 2006). In a laboratory-scale nitrifying SBR with temporary NO_2^--N accumulation in the operational cycles, during the stable NH_4^+-N oxidation period, Kampschreur et al. (2008a) found that 2.8% of NH_4^+-N was removed in the form of N_2O-N, and the nitrifier denitrification pathway was thought to be the main source of the N_2O emissions.

The findings from this study show that even under high NO_2^--N and low DO or anoxic conditions, nitrifier denitrification's contribution to the N_2O emission level was less than 1.73% of the total influent NLR (Table 8.1). By feeding the synthetic wastewater containing no rbCOD to the reactor for a period of 2–3 days, nitrifier denitrification and heterotrophic denitrification through endogenous respiration were the two main sources of N_2O production, which was estimated at 1.73% of the total influent NLR. N_2O emission by AOB under fully-aerated conditions was low, at 0.05–0.5% of the NLR, but it can increase to 0.8–2.5% of the NLR under oxygen-limited conditions (Colliver and Stephenson, 2000), which is comparable with the N_2O emissions obtained in this study when treating synthetic wastewater containing no rbCOD.

8.5.2 N_2O emission stimulated by influent organic carbon

Under similar operation conditions, higher influent rbCOD caused much higher N_2O emissions from the oxygen-limited partial nitrification reactor. In comparison with the emission when rbCOD = 0, when rbCOD in the feed was 100 mg/L or 500 mg/L, N_2O

emissions rose from 1.7% to 6%, or 12% of the influent NLR, respectively. The increase in the N_2O emissions was mainly attributed to the heterotrophic denitrification pathway.

The availability of organic C in the influent has a significant effect on heterotrophic denitrification. When treating high COD/N-ratio wastewater (e.g. COD/N ratio > 4), the rbCOD present in the feed is adequate for heterotrophic denitrifiers to perform complete denitrification, converting NO_3^-/NO_2^- into N_2 under anoxic conditions. When treating low COD/N-ratio wastewater (e.g. COD/N ratio < 2.5), an addition of external C is required to achieve complete heterotrophic denitrification; otherwise, incomplete heterotrophic denitrification may cause N_2O production.

Alinsafi et al. (2008) demonstrated that the N_2O emission rate increased from 1.0% to 5.1% of the influent NLR, while the COD/N ratio decreased from 7 to 3; with a pulse addition of NO_2^-, the N_2O emission was stimulated to 14.4%. In a full-scale plant treating high-strength ammonium wastewater with a COD/N ratio of 1.5, the dissolved N_2O level in the mixed liquor was 6.27 mg N/L and the N_2O emission was up to 55% of the influent NLR (Itokawa et al., 1996). In a nitrifying biofilm reactor with organic matter coming from the influent wastewater or from hydrolysis of biofilms, N_2O was the sole or major product of denitrification, and its production was up to 40% of the influent NLR (Garrido et al., 1997).

When treating low COD/N-ratio wastewater, N_2O as the end product of incomplete denitrification is mainly caused by free nitrous acid (FNA) inhibition (Zhou et al., 2008). The N_2O reductase can be seriously inhibited at a FNA as low as 0.0007–0.001 mg N/L (Zhou et al., 2008). In this study, the FNA level in the reactor was always higher than 0.10 mg N/L, so the reduction of N_2O to N_2 in the heterotrophic denitrification process would be severely inhibited by FNA. Therefore, when rbCOD was present, the N_2O emission was mainly controlled by the N_2O production via heterotrophic denitrification, namely reduction of NO_3^- or NO_2^- to N_2O.

Since rbCOD was completely consumed after 30-min from the commencement of the operation cycle, it may be reasonably assumed that internal polymers, e.g., poly-β-hydroxybutyrate (PHB), were the C source for N_2O production during anoxic endogenous denitrification in the presence of NO_2^- (Itokawa et al., 2001). During the SBR operation, storage of internal C sources, such as PHB, is a mechanism used by microorganisms under dynamic conditions to balance the bacterial growth process, and approximately 90% of the total bacterial growth occurring in the famine period utilizes the stored PHB (Dircks et al., 2001). The stored PHB in the biomass during the fill period is at a relatively constant ratio to the influent rbCOD. Ciggin et al. (2009) found that at a low COD/N-ratio and high NO_3^- and NO_2^- concentrations, the PHB formation from acetate dropped to 0.33 g COD/g COD. The consumption of PHB in the biomass is found to be independent of the type of electron acceptors (O_2, NO_2^- or NO_3^-) (Beun et al., 2000). Schalk-Otte et al. (2000) observed that as soon as C compounds were exhausted and the culture entered the starvation phase, PHB became the growth substrate; N_2O was one of the main products of denitrification and N_2O production was 32–64% of the NLR .

When NO_2^- or NO_3^- as electron acceptors are sufficient, the degradation of PHB as the electron donor is the controlling factor for the N_2O production. Therefore, the N_2O production rate would be shown as the same pattern as the PHB degradation rate in ratio with the time. The PHB degradation rate is described as a function of the fraction of PHB in the biomass. When a high PHB content is in the biomass, e.g. the fraction of PHB in the biomass > 0.05 g COD/g COD, it appears to be zero-order; when the fraction of PHB in the biomass is low in the famine period, it is first-order (Beun et al., 2000; Dircks et al., 2001). This could explain why the anoxic N_2O production rate obeyed zero-order or first-order patterns in this study (Fig. 8.4): when treating synthetic wastewater with a rbCOD of 500 mg/L, a higher PHB fraction in the biomass was expected compared to a rbCOD of 100 mg/L; PHB was sufficient during the whole cycle and its degradation rate appeared to be zero-order. Thus, the N_2O production rate by denitrification was zero-order at a value of 1.2 mg N/(L.h); at an influent rbCOD of 100 mg/L, the PHB degradation rate and the N_2O production rate appeared as first-order, and when PHB was depleted at the end of the operational cycle, the N_2O concentration in the reactor was very close to the case of rbCOD $= 0$ (Fig. 8.4).

8.5.3 Measures to reduce N_2O emission from partial nitrification reactors

The N_2O emission potential of this oxygen-limited partial nitrification process can be decreased by reducing influent rbCOD, reducing the duration of the anoxic period, and reducing the FNA concentration in the reactor.

Prior to the partial nitrification process, pre-treatment of the ammonium-rich wastewater to remove rbCOD from the wastewater could reduce the N_2O emission. Less influent rbCOD can significantly reduce the PHB formation in the biomass and, as a consequence, produce less N_2O via heterotrophic denitrification in the subsequent oxygen-limited partial nitrification process.

Pulse- or short-period feeding stimulates accumulation of PHB in the biomass under anoxic conditions, compared to continuous feeding (Ciggin et al., 2007). The results obtained in this study show that the N_2O production rate under anoxic, non-aeration conditions was significantly higher than under oxygen-limited, aeration conditions. Applying an extended continuous feeding mode and reducing anoxic, non-aeration periods may therefore help to reduce the total N_2O emissions.

The high FNA concentration is a significant factor inhibiting the activity of the N_2O reductase in the heterotrophic denitrification process and causing accumulation of N_2O in the reactor. In this study, the NO_2^--N concentration in the reactor was 200–300 mg/L. To keep FNA below the inhibition threshold (0.001 mg N/L) for the activity of N_2O reductase (Zhou et al., 2008), pH in the reactor should be kept above 8.6. However, this is not always practical in many types of wastewater.

By integrating the partial nitrification process with the anammox process in one reactor, the NO_2^- concentration can be maintained at a low level of less than 5–6 mg/L (Wett, 2006). In the one-reactor system where conditions are maintained at pH above 7.4 and NO_2^--N concentrations are below 10 mg/L, the FNA concentration is less than 0.001 mg N/L. Thus, the N_2O emission can be significantly reduced. With a DO of less than 0.3 mg/L and a pH of 7.04, the N_2O emission from such a one-reactor system was reported to be lower than 1.5% of the influent NLR (Weissenbacher et al., 2010). The emission of N_2O from the integrated one-reactor N removal system is comparable to the conventional activated sludge process.

8.6 Summary

1. The oxygen-limited partial nitrification process treating low COD/N-ratio, ammonium-rich wastewater had an N_2O emission level ranging 1.7–12% of the total influent nitrogen loading rate.

2. The FNA concentration in the reactor was constantly over 0.1 mg N/L and was a factor causing N_2O accumulation. The influent readily biodegradable COD concentration was a key factor stimulating high levels of N_2O emissions via the heterotrophic denitrification pathway.

3. N_2O emissions from the oxygen-limited partial nitrification process could be reduced by minimizing the influent rbCOD concentration, reducing the duration of the anoxic operation, and decreasing FNA concentrations in the reactor.

Chapter 9

Conclusions and Recommendations

9.1 Introduction

This thesis consists of the development of a SBR for nutrient removal from slaughterhouse wastewater and the development of a technology to achieve partial nitrification for ammonium-rich wastewater treatment. The slaughterhouse wastewater was treated in laboratory-scale SBRs, and the aeration pattern and control of the aeration rates were the research focus. Efficient, long-term partial nitrification was achieved under sustained oxygen-limited conditions, and the N_2O emission from this partial nitrification process was also evaluated.

9.2 Main conclusions from the study on slaughterhouse wastewater treatment

The main conclusions from the study on nutrient removal from slaughterhouse wastewater are:

1. In a 10-L intermittently aerated laboratory-scale SBR, at an influent OLR of 1.2 g COD/(L.d), average effluent concentrations of COD, TN and TP were 150 mg/L, 15 mg/L and 0.8 mg/L, respectively. This represented COD, TN and TP removals of 96%, 96% and 99%, respectively. A nitrogen balance was carried out and showed that 66% of N removed was due to denitrification, and 95% of N removal by means of denitrification was via NO_2^--N.

2. Two 10-L intermittently aerated, laboratory-scale SBRs were tested under four aeration rates, 0.2, 0.4, 0.8 and 1.2 L air/min. The optimum system performance was attained at an aeration rate of 0.8 L air/min. Partial nitrification followed by denitrification

occurred in the intermittently aerated SBRs at aeration rates of 0.8 and 1.2 L air /min. At the aeration rate of 0.8 L air/min, NO_2^--N/TON during most of the operational cycle was over 80%. When the aeration rate was 1.2 L air/min, NO_2^--N/TON ranged from 15% to 60%. The end of nitrification can be identified from DO and pH real-time profiles using $d^2DO/dt^2 = 0$ and $dpH/dt = 0$. The end point of denitrification in non-aeration periods can be set at an ORP value of -12 mV.

3. When the laboratory-scale SBR was operated at low DO levels, good effluent quality was obtained under both intermittent aeration and continuous aeration operational strategies, with TN removals of up to 94% and 91%, respectively. Under the intermittent aeration strategy, by maintaining low DO levels, electricity consumption associated with air supply can be reduced by up to 65%. *In situ* OUR measurement using mini-DO sensors can be used to control the SBR operation.

9.3 Main conclusions from the study on oxygen-limited partial nitrification

The main conclusions from the development of the oxygen-limited, partial nitrification process are:

1. In a 2-L intermittently aerated laboratory-scale SBR treating synthetic low COD/N-ratio wastewater, after the establishment of a high AOB/NOB population ratio during a 7-day start-up period, partial nitrification was sustained for a period of up to 180 days under the oxygen-limited conditions. Oxygen limitation can be used as a main selection factor to sustain partial nitrification at ambient temperature and to produce a high NO_2^--N-concentration effluent for the successive anammox process. Molecular analysis shows that AOB represented up to 10% of the total bacteria population and NOB (both *Nitrobacter* and *Nitrospira*) were out-competed by AOB during the entire operation period. The partial nitrification biomass can be kept in long-term static storage without adverse impacts on its performance in partial nitrification.

2. The oxygen-limited partial nitrification process treating low COD/N-ratio wastewater had a high N_2O emission potential, with N_2O emissions ranging from 1.7–12% of total influent NLR. An FNA concentration of over 0.1 mg-N/L was considered to severely inhibit the heterotrophic denitrification step from N_2O to N_2. The influent rbCOD concentration was a key factor to stimulate the N_2O emission via heterotrophic denitrification. The N_2O emission increased with the increase in the rbCOD concentration. The N_2O emissions from the oxygen-limited partial nitrification process could be reduced by

reducing rbCOD from the wastewater, applying an extended fill period, and avoiding the anoxic react phase in a SBR operational cycle. To integrate the oxygen-limited, partial nitrification process and the anammox process in one reactor might greatly reduce N_2O emissions level.

9.4 Recommendations

It is recommended that further studies on slaughterhouse wastewater treatment should be conducted. The research content should include:

1. treatment of the wastewater at low temperature (5–10 °C) using the intermittently aerated SBR.

2. construction of a pilot-scale demonstration system.

3. examination of N_2O emissions from intermittently aerated SBRs treating slaughterhouse wastewater.

For the partial nitrification technology, the following studies should be conducted:

1. treatment of the effluent from oxygen-limited, partial nitrification reactors using the anammox process.

2. evaluation of more operational parameters affecting the partial nitrification efficiency of the developed technology.

3. establishment of mathematical modeling of the oxygen-limited, partial nitrification process.

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