

Nitrous Oxide Emission and Nitrogen Transformation Dynamics in a Hybrid Simultaneous Nitrification, Denitrification and Phosphorus Removal System

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

**THE FACULTY OF GRADUATE STUDIES
(Civil Engineering)**

**THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)**

October 2008

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Abstract

Nitrous oxide is a greenhouse gas with a global warming potential 310 times that of the contribution of CO₂. It is now recognized that the potential exists for N₂O emission to be significant from some biological nutrient removal (BNR) processes. The control of N₂O off-gas must therefore be considered when operating a BNR system.

The main objectives of this research were to investigate the mechanisms involved in the production and reduction of nitrous oxide in biological wastewater treatment systems, and to use N₂O off-gas as a real-time control parameter to assess ammonia oxidation in a simultaneous nitrification, denitrification and phosphorus removal (SNDPR) system. Strategies to diminish the emission of nitrous oxide from the treatment process were also studied.

The data support the conclusion that a hybrid system, including suspended sludge and biofilm in the same reactor of a sequencing batch reactor (SBR) was a more effective system than a conventional suspended growth system, in terms of overall effluent quality, SND efficiency and lower emission of N₂O.

In the hybrid system, nitrification occurred mostly in the suspended sludge; the biofilm played the major role in denitrification. It was also determined that N₂O off-gas from the hybrid system was mainly a result of heterotrophic denitrification, rather than nitrification.

N₂O reduction rates were found to be higher with the existence of an external carbon source and the absence of DO. It was also observed that N₂O reduction rates were higher for acetate, than for lactose, in the presence and absence of DO. Denitrification using

stored carbon resulted in the production of more N_2O off-gas than denitrification using an external carbon source. The largest production of N_2O off-gas occurred when the internal carbon source was PHA during aerobic conditions.

Based on the results of the investigation into the factors affecting N_2O emissions, operating strategies for N_2O off-gas control were suggested. These strategies were the use of lower aeration rates, continuous feeding and higher pH.

The emission of N_2O was found to have a close correlation with ammonia removal, which can be a tool for real-time assessment of ammonia oxidation. Therefore, it can be considered as a potential real-time control parameter for ammonia oxidation in a SNDPR hybrid SBR system.

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Nomenclature

θ	temperature correction coefficient
A	Bunsen adsorption coefficient
A2O	anaerobic-aerobic-anaerobic process
AI	artificial intelligence
ADP	adenosine diphosphate
Anammox	anaerobic ammonia oxidation
ANN	artificial neuron network
ATP	adenosine triphosphate
AOB	ammonia oxidizing bacteria
AOR	ammonia oxidation rate
BNR	biological nutrient removal
BOD	biochemical oxygen demand
CANON	complete autotrophic nitrogen removal over nitrite
CH ₄	methane
C	concentration in the solution
°C	degree Celsius
C _e	concentration of the effluent
C _g	N ₂ O concentration in the off-gas
C _i	concentration of the influent
C _s	saturation concentration of N ₂ O in liquid
CO ₂	carbon dioxide
COD	chemical oxygen demand
D	day
DEPHANOX	denitrification under anoxic conditions

DO	dissolved oxygen
DGAO	denitrifying glycogen accumulating organism
DPAO	denitrifying phosphorus accumulating organism
E	electrode potential
EBPR	enhanced biological phosphorus removal
ECD	electrochemical detector
EDTA	ethylenediamine tetraacetic acid
EPA	U.S. Environmental Protection Agency
F	Faraday factor
FADH ₂	reduced flavin adenine dinucleotide
FISH	fluorescence in situ hybridization
G	Gibb free energy
g	gram
GAO	glycogen accumulating organism
GC	gas chromatography
GWP	global warming potential
H	enthalpy
<i>H</i>	Henry's law coefficient, dimensionless
hr	hour
HB	DL-3-hydroxybutric acid
HRT	hydraulic retention time
IC	inorganic carbon
IR	infrared
K_{La, O_2}	overall oxygen transfer coefficient
K_{La, N_2O}	overall nitrous oxide transfer coefficient
K_s	half-saturation constant

L	litre
M	total mass of N_2O
mg	milligram
min	minute
mL	millilitre
MLSS	mixed liquor suspended solids
mm	millimeter
MW	molecular weight of N_2O
N_2	nitrogen gas
NADH	reduced nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NDIR	non-dispersive infrared absorption
NH_2OH	hydroxylamine
NH_3	ammonia
NH_4^+	ammonium
nm	nanometer
N_2O	nitrous oxide
NO_x	nitrite plus nitrate
NO_2^-	nitrite
NO_3^-	nitrate
NOB	nitrite oxidizing bacteria
NSERC	Natural Science and Engineering Research Council of Canada
OLAND	oxidation-limited autotrophic nitrification
ORP	oxidation reduction potential
OUR	oxygen uptake rate
P	phosphate

p	off-gas pressure
PAO	phosphorous accumulating organism
PHA	poly- β -hydroxyalkanoate
PHB	poly- β -hydroxybutyrate
ppm	parts per million
Q	flow rate of influent
Q_g	air stripping rate
R	carbon containing matter
R^2	correlation coefficient
r_d	denitrification rate
r_i	rate of reaction i
r_{N_2O}	rate of N_2O mass transfer
r_r	N_2O reduction rate
S	entropy
SBR	sequencing batch reactors
SHARON	single-reactor high-activity ammonia over nitrite
SND	simultaneous nitrification denitrification
SNDPR	simultaneous nitrification denitrification and phosphorus removal
SRT	sludge retention time
T	temperature
TKN	Total Kjeldahl Nitrogen
TN	total nitrogen
TOC	total organic carbon
TP	total phosphate
TS	total solid
VFA	volatile fatty acid

V_g	plastic syringe headspace volume
V_l	mixed liquid sample volume in plastic syringe
VSS	volatile suspended solids
UCT	University of Cape Town process
UGC	universal gas constant

Acknowledgments

The achievement of this study is not the work of an individual. It could not have been accomplished without the help and support of many others. First, I'd like to offer my special thanks to my supervisor, Dr. Victor Lo, who gave me a lot of space for doing research. He is not only a great academic and research leader, but also a wonderful spiritual instructor in my life. I am grateful to my co-supervisor, Dr. Don Mavinic, and to committee members, Dr. William Ramey and Dr. Dean Shiskowski, who sacrificed their time for meetings and gave precious comments and suggestions concerning the study.

Dr. Ping-Haung Liao, an experienced researcher and a good partner in brain storming, helped me to generate many ideas.

Laboratory manager Susan Harper and technician Paula Parkinson provided technical support for all lab work. All the data involved their contributions. In addition, my colleagues, especially Parvez, Kathy, Colleen, Winnie, Zaki and Nandini, accompanied me at various times in the lab, giving me a hand when I was in need of one.

I would like to acknowledge the Natural Science and Engineering Research Council of Canada (NSERC), which provided financial support for my doctoral program.

Thanks be given to all the brothers and sisters, (Chenidum/Towela, Daniel/Emily, Oliver/Lanna, Andrea, Dr. Ko, and so on), from the different nations involved in the International Bible Study Fellowship, who kindly support each other and faithfully pray for one another.

To this end, I want to thank my parents, (Mrs. Cheng Tseng and Mr. Wen-song Lo, although he passed away 20 years ago), family (Michael/Elisa, Pedro and Roger) and

friends in Taiwan (Mr. Richard Chang, Mr. Zheng-yi Chen, Nicole...) for all the support, encouragement and comfort provided during my long journey of studying abroad.

Finally, I give thanks to the Lord. For~

He is the image of the invisible God, the firstborn over all creation

For by Him all things were created

things in heaven and on earth, visible and invisible

whether thrones or powers or rulers or authorities

all things were created by Him and for Him

He is before all things, and in Him all things hold together

~The Bible, Colossians 1:15~17~

Co-Authorship Statement

Collaborations and linkage to several parties have provided valuable support to this research. My contributions to this research included the design of experimental approaches such as:

- by separating the suspended sludge with the biofilm from the hybrid system to identify their individual contributions to the N_2O off-gas and nitrogen transformation (Chapter 3),
- by adding different types of carbon sources under different oxygen states to identify the difference of nitrous oxide production rate and nitrous oxide reduction rates for interpreting the production of nitrous oxide (Chapter 4),
- by changing the operation conditions of aeration rate, feed pattern and pH to control the emission of N_2O from the wastewater treatment (Chapter 5).

Through the data analyses, a polynomial function with R^2 larger than 0.97 was found to describe the close correlations of N_2O emission and ammonia oxidation. The idea of using the relationship as a potential tool for ammonia oxidation assessment and real-time control was proposed (Chapter 6). The results of this research were prepared as manuscripts for publication.

From the proposal of this PhD research to the final dissertation, the committee members Dr. Victor Lo, Dr. Don Mavinic, Dr. Dean Shiskowski and Dr. Ramey William provide their valuable academic and technical support of the experiment design, data interpretation and idea inputs. The thesis and the preparation of manuscripts also have been strengthened by all committee members.

Chapter 1 Introduction

1.1 Background

Carbon removal has been a major focus in wastewater treatment. Increases in urban centralization and industrial development have also led to concerns about nitrogen and phosphorus compound concentrations in the wastewater effluent which is discharged into receiving waters. It is known that high concentrations of ammonia are toxic to aquatic life and may lead to public health problems. Excess ammonia consumes dissolved oxygen (DO) and results in insufficient DO in bodies of water. The extent of toxicity depends on temperature and pH (Sawyer *et al*, 2003). High nitrate content in drinking water has been found to cause methemoglobinemia in infants, which is a result of the interaction of nitrite with hemoglobin (Sawyer *et al.*, 2003). Excess nitrogen and phosphorus cause eutrophication in receiving waters. The discharge requirement for phosphorus is normally stricter than for nitrogen. In order to reduce the concentrations of nitrogen and phosphorus in the effluent, biological nutrient removal (BNR) processes for the treatment of wastewaters have been developed.

In wastewater systems, organic nitrogen is first converted to ammonia. Ammonia is oxidized by autotrophic organisms to nitrite and then to nitrate under aerobic conditions. This process is called nitrification. In the denitrification process, in which nitrate and nitrite are removed, heterotrophic organisms are involved. Nitrate and nitrite are used as electron acceptors and eventually reduced to the final products of nitrous oxide (N_2O) gas and nitrogen gas (N_2). Researchers and engineers have applied low DO levels to biological systems and successfully achieved simultaneous nitrification and denitrification

(SND) in bench-scale reactors and in full scale plants (Bertanza, 1997; Pochana and Keller, 1999; Zeng, 2003).

In biological phosphorus removal, heterotrophic organisms take up carbon sources in the anaerobic phase and carbon is stored in the cells. The energy for the stored carbon formation is provided by the breakdown of phosphate. Orthophosphate is then released to the bulk solution. Under aerobic conditions, the stored carbon is consumed by organisms. The energy generated by oxidation of the stored carbon is used for phosphorus uptake to form phosphate in the cells. By alternating the anaerobic and aerobic states, phosphorus is removed from the wastewater and accumulated in the biomass (Comeau, 1986). The microorganisms involved in phosphorus removal are called phosphorus accumulating organisms (PAOs). Some PAOs, denitrifying phosphorus accumulating organisms (DPAOs), can also use nitrate/nitrite as the electron acceptor for phosphate uptake (Murnleitner, 1997). This means that simultaneous removal of phosphorus, nitrification and denitrification (SNDPR) is possible. The results obtained from the application of the SNDPR process in a single sequencing batch reactor (SBR) are very promising (Kuba *et al.*, 1993; Zeng *et al.*, 2003).

In wastewater treatment processes, greenhouse gases such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide are major emissions (IPCC, 2006). For biological waste treatment in Canada and the United States, CO₂ emissions need not be reported for greenhouse gas inventories. However, CH₄ and N₂O have to be included. CH₄ is not significant in BNR systems as the anaerobic phase is relatively short. However, the potential exists for N₂O emissions to be significant when treatment plants apply biological nutrient removal processes (Shiskowski, 2004).

The global warming potential (GWP) over 100 years of N_2O is 310 times that of the contribution of carbon dioxide (IPCC, 2006). In 2005, N_2O emission from wastewater handling in Canada was estimated at 2.3% of total N_2O emissions, which was 0.14% of total greenhouse gas emissions as CO_2 (Environment Canada, 2007). In the same year, the US EPA (2007) reported it was 1.7% of the total N_2O emissions (0.11% of total greenhouse gas emission as CO_2). However, the method of estimating N_2O emissions is highly uncertain. For instance, nitrogen gas (N_2) has traditionally been considered the major end product of nitrogen removal. Lately, however, research has shown that nitrous oxide emissions from wastewater treatment processes are not negligible (Schulthess and Gujer, 1996; Park *et al.*, 2000). Research has found that nitrous oxide is generated from both the processes of ammonia oxidation (autotrophic) and denitrification (heterophic) (Anderson *et al.*, 1993). The emission of N_2O must therefore be a consideration in wastewater treatment processes. A conventional activated sludge system, without ammonia oxidation, has very low or no N_2O emissions, compared with a treatment process with nitrification. When both nitrification and denitrification processes take place, even higher N_2O emissions can be expected than with nitrification only. Further, in nitrification and denitrification processes, a post-denitrification process leads to higher nitrous oxide emissions than a pre-denitrification process (Tallec *et al.*, 2006).

To date, the significance of N_2O emissions from wastewater treatment processes has not been extensively investigated. The volume of nitrous oxide emissions from full scale wastewater treatment plants has been reported (Kimochi *et al.*, 1998; Barton and Atwater, 2002). The time profile of nitrous oxide off-gas and factors affecting the emissions have also been discussed (Park *et al.*, 2001; Zeng *et al.*, 2003; Hwang *et al.*, 2006). However, the mechanisms of N_2O production have not been fully studied. To fully control

N₂O emissions, further study of these mechanisms is required.

Energy conservation in wastewater treatment facilities is also an issue in the design and operation of a plant. Nitrogen removal systems require more energy than conventional carbon conversion processes, due to the air requirements of ammonia oxidation. Oxidizing 1 g ammonia-N requires 4.6 g of oxygen to convert to nitrate compared with 1 g of biochemical oxygen demand (BOD) requiring 1.4 g of oxygen. The development of on-line instrumentation, coupled with real-time control strategies, not only enhances the performance of the treatment system, but also reduces the operating costs. Many approaches have been developed for the real-time control of ammonia oxidation. Shiskowski (2004) investigated the potential for using nitrous oxide as the parameter for nitrification monitoring and system aeration control.

Given these considerations, the ideal biological wastewater treatment system is one with a simple configuration, easy operation, low energy consumption and low greenhouse gas emissions.

1.2 Research Objectives

The main objective of this research has been to investigate the mechanisms involved in the production and reduction of nitrous oxide in a biological wastewater treatment system. The goals were to diminish the emission of nitrous oxide and to apply nitrous oxide off-gas as a real-time control parameter for ammonia oxidation in an SNDPR system in order to assess the oxidation of ammonium.

The specific objectives for this research include:

- Identification of a biological treatment system for SNDPR by using a hybrid SBR.
- Distinguish N_2O production from autotrophic and heterotrophic denitrification in the hybrid SBR system.
- Determination of the denitrification rates and N_2O reduction rates under different carbon and oxygen states and their relationships with N_2O accumulation and emission.
- Exploration of approaches for reducing N_2O off-gas from the BNR system.
- Exploration of the relationship between ammonia oxidation and N_2O production, using N_2O off-gas for assessing ammonia oxidation, and its potential as a real-time control parameter for the SNDPR system.

1.3 Structure of the thesis

The structure of this research is presented as Figure 1.1. The thesis is comprised of nine chapters:

Chapter 1 is the introduction. It reviews the background of the biological removal of nitrogen and phosphorus, nitrous oxide production and the real-time control approaches for an SND system. This chapter also describes sequencing batch reactor designs (the suspended growth system and the hybrid system) and their monitoring systems, including pH/ORP/DO and nitrous oxide off-gas. Chemical and physical analyses for this study are also listed.

Chapter 2 outlines the SNDPR process. This includes the schedule of each cycling and SRT, which were first simulated by computer software and then tested by experiment. The treatment performances and N₂O emissions of the suspended growth system and the hybrid system were compared. Due to superior treatment performance and lower nitrous oxide emissions, the hybrid system was selected and studied as reported in the following chapters.

Chapter 3 reports on individual investigations into the suspended sludge and the biofilm aimed at improving knowledge of their roles in the hybrid system. The term “suspended sludge” in this thesis represents the portion of biomass which is suspended in the hybrid system, while the term “suspended growth system” means the system as found in Reactor A. The studies undertaken into nitrogen- balances for both the suspended sludge and the biofilm were important in enhancing our understanding of the characteristics in the hybrid system. The variations over time, of stored carbons in the biomass, were also examined.

Chapter 4 reports on the identification of N_2O emissions from autotrophic denitrification and heterotrophic denitrification. In this research, N_2O production from the autotrophic process was not found to be significant. In the heterotrophic process, N_2O production was found to be affected by the nitrogen reduction rate and the nitrous oxide reduction rate. Denitrification rates were tested under various conditions, including oxygen states (with and without DO) and carbon states (external and stored carbon) by feeding acetate and lactose. Under the same conditions, N_2O reductions were also tested. Two factors, air stripping and the diffusion caused by mechanical mixing, were taken into consideration when calculating N_2O emissions from the aerobic and anoxic stages.

Chapter 5 reports on investigations into the approaches to reducing N_2O emissions derived from the results reported in Chapter 6. These investigations examined the effects of different aeration rates, pH values and feeding patterns.

Chapter 6 reports on the studies undertaken into the relationship of N_2O off-gas and ammonia oxidation. The application of N_2O off-gas as a tool to assess the performance of ammonia oxidation and a real-time control parameter for a SNDPR system are discussed.

Chapter 7 reports conclusions from this research and suggests further studies.

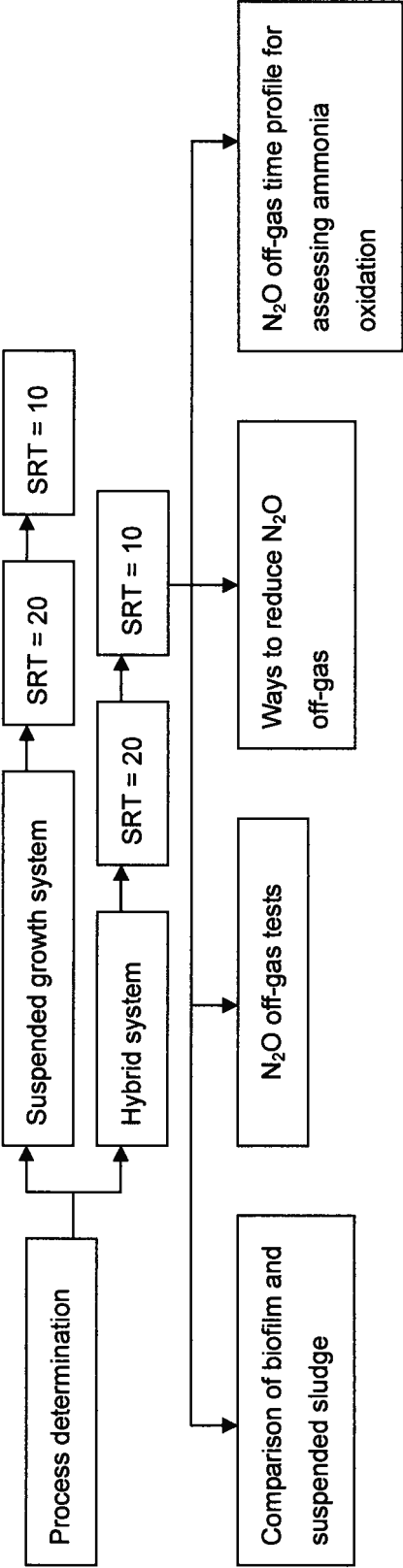


Fig 1.1 Structure of the research

1.4 Literature review

Nitrogen and phosphorus compounds are important nutrients for organisms. A typical formulation $C_{60}H_{87}O_{23}N_{12}P$ can be used to present the organic fraction of prokaryote cells. Nitrogen and phosphorus are 12% and 2% of the mass, respectively (Metcalf & Eddy, 2003). Hence, nitrogen and phosphorus compounds in microbes are significant constituents in wastewater. For instance, the total Kjeldahl Nitrogen (TKN) in municipal wastewater ranges from 10 to 60 mg/L (Proefschrift, 2003), and for swine wastewater it is around 780 mg/L, after solid-liquid separation (Obaja *et al.*, 2003). The concentration of phosphorus is 4~16 mg/L for municipal wastewater (Metcalf & Eddy, 2003) and 120 mg/L for swine wastewater (Obaja *et al.*, 2003).

Many countries have set discharge requirements at less than 10 mg N/L for ammonia plus nitrite and nitrate, and 1 mg/L for total phosphorus. Biological nutrient removal and enhanced biological phosphorus removal (EBPR) processes have been widely applied in wastewater treatment. Recently, the process of simultaneous nitrification and denitrification in a single reactor has been proposed. Phosphorus removal via denitrification has also been studied. The development of a process that combines SND and EBPR in one reactor, namely SNDPR, is of interest to scientists and engineers.

It has been reported that significant amounts of nitrous oxide are emitted during nitrogen removal processes, including both SND and SNDPR. This research aims to investigate N_2O emissions from a SNDPR process in an SBR system, and to develop the strategies for reducing the N_2O emissions. The potential of using N_2O off-gas as a real-time control parameter of ammonia oxidation is also explored.

This literature review includes:

- Biological nitrogen removal
- Biological phosphorus removal
- Simultaneous nitrification, denitrification and phosphorus removal
- Nitrous oxide production

1.4.1 Nitrogen removal

Human activities increase reactive nitrogen compounds into the environment, resulting in various levels of pollution. Reducing the level of the nitrogen compounds released into the environment would have a considerable beneficial impact. One of the most direct ways to remove nitrogen compounds from wastewaters is through wastewater treatment. Important nitrogen conversions present in the environment include nitrification, denitrification, anaerobic ammonia oxidation (Anammox) and nitrogen fixation. Figure 1.2 demonstrates the microbial transformations that occur in the N-cycle. Sections 1.4.1.1 to 1.4.1.3 describe nitrification, denitrification and Anammox. Beginning with Section 1.4.1.4, the concept of simultaneous nitrification/denitrification and its applications are reviewed.

1.4.1.1 Nitrification

Nitrification is the conversion of ammonia to nitrate by microbial activities. It can be classified into three groups based on metabolism: aerobic autotrophic nitrification, anaerobic autotrophic nitrification, and heterotrophic nitrification.

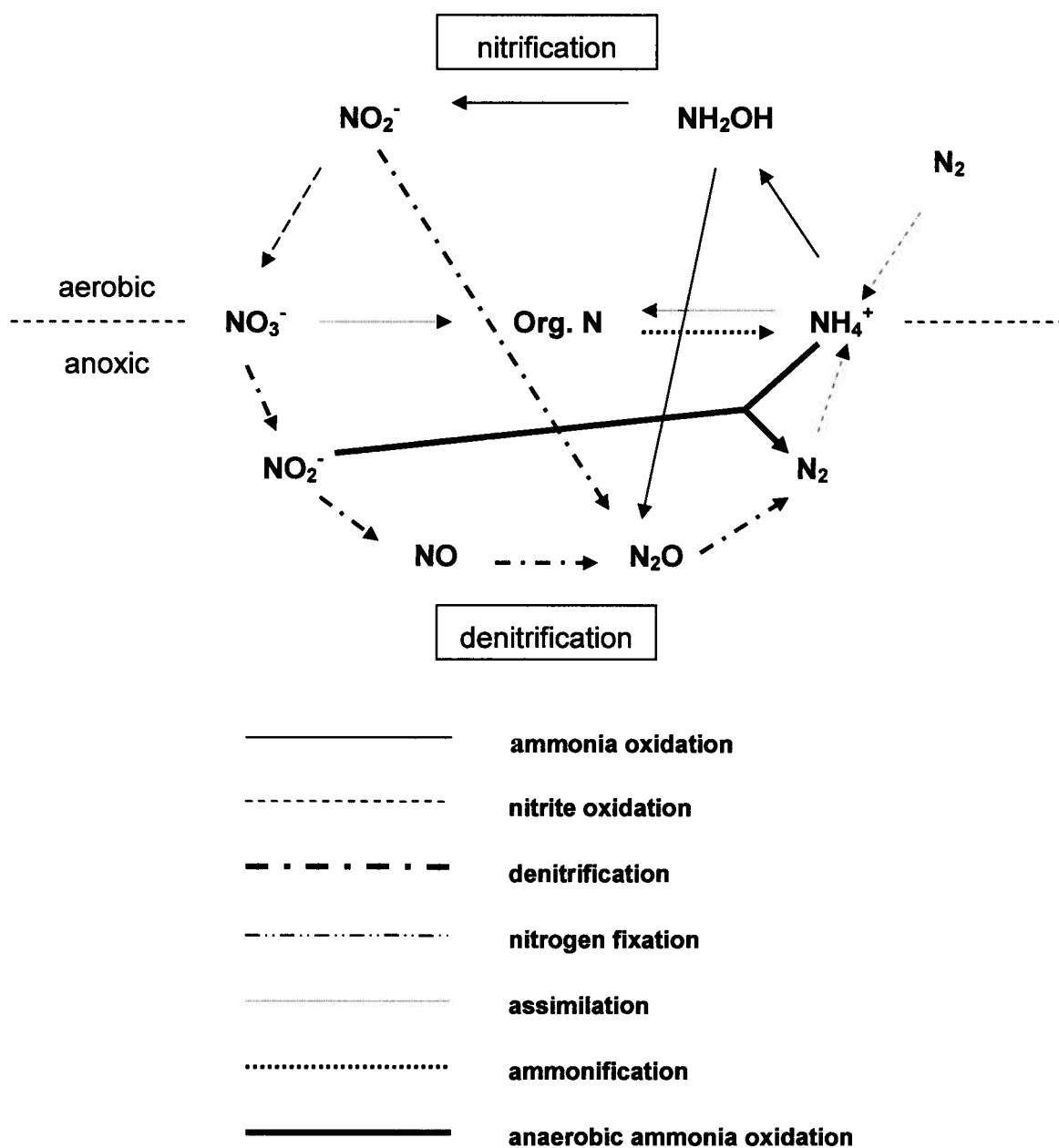
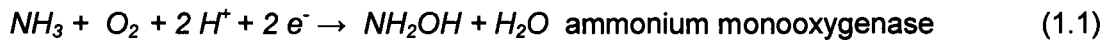


Fig 1.2 Microbial transformation in the nitrogen cycle (adapted from Schramm, 2003)

■ Aerobic autotrophic nitrification

This process makes significant contributions to nitrification. It is carried out by two categories of microorganisms described in section 1 and 2, below. Overall, 4.6 g O₂ is required for converting 1g NH₄⁺ - N to NO₃⁻. Aerobic autotrophic nitrification is pH sensitive. The optimum pH is 7.5~8.5 for this type of nitrification. The process also consumes alkalinity, where 7.17 g CaCO₃ is required to convert 1g NH₄⁺ - N to NO₃⁻. Increasing the C/N ratio decreases nitrification. A BOD/TKN ratio lower than 3 was found in a BNR activated sludge plant (Metcalf and Eddy, 2003). Generally, a longer sludge retention time of around 7 days (from 3 to 15 days) is required for nitrification in a conventional activated sludge system.

1. Conversion of NH₄⁺ to NO₂⁻. *Nitrosomonas* oxidizes ammonium to nitrite through hydroxylamine (NH₂OH). Other ammonium oxidizers include *Nitrosospira*, *Nitrosococcus* and *Nitrosolobus*. The schematic reactions are as follows:



2. Conversion of NO₂⁻ to NO₃⁻. Although traditional engineering texts refer to *Nitrobacter* as the most important nitrite oxidizers for oxidizing nitrite to nitrate, *Nitrospira* has been identified as the main organism in nitrite oxidation in a wastewater treatment system, according to a recent study (Wagner *et al.* 1999).





■ Heterotrophic nitrification

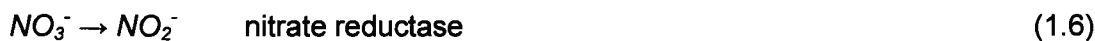
Fungi are considered to be the most common heterotrophic nitrifying organisms (Grady *et al.*, 1999). Heterotrophic nitrification involves co-oxidation of ammonia and organic carbon. Acidic conditions, high oxygen concentrations and available carbon supplies are the main environmental factors which favor heterotrophic nitrification (Wrage *et al.*, 2001).

■ Anaerobic autotrophic nitrification

Ammonia can be anaerobically oxidized by autotrophic microorganisms by using nitrite as the electron acceptor. This process is named anaerobic ammonia oxidation (Anammox). More detail is presented in Section 1.4.1.3.

1.4.1.2 Denitrification

Denitrification is the process of reducing nitrite or nitrate to N_2 , N_2O , or NO . It is carried out in the following sequence:



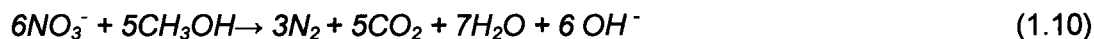
Drysdale *et al.* (2001) found that some bacteria are able to reduce NO_3^- to N_2 via NO_2^- ,

while others are only able to reduce NO_3^- to NO_2^- or only NO_2^- to N_2 .

The heterotrophic process is the main process by which denitrification occurs in wastewater treatment. However, denitrification also happens under autotrophic, and autotrophic and heterotrophic mixed conditions.

■ Heterotrophic denitrification

Heterotrophic denitrification can be accomplished by several genera of heterotrophic bacteria that require organic carbon as the carbon source for cell synthesis and an electron donor for energy production. The example of using methanol as energy and carbon source is shown in Equation (1.10). Since these bacteria are facultative, they have the ability to use oxygen or nitrite and nitrate as electron acceptors. Energy production is most efficient when oxygen is the electron acceptor. The bacteria will prefer oxygen over nitrite or nitrate, which means that the absence of oxygen is an important factor in the reduction of nitrite and nitrate. The most widespread genera of heterotrophic bacteria are *Pseudomonas* and *Alcaligenes*, which are frequently found in soil, water and wastewater (Bitton, 1999).



■ Autotrophic denitrification

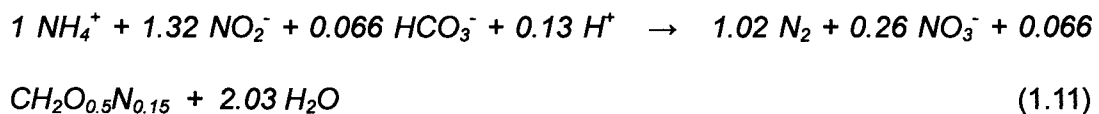
A number of different autotrophic processes can perform denitrification functions. These processes use nitrite or nitrate as an electron acceptor, and reduce them to N_2 , N_2O , or NO . Autotrophic *Nitrosomonas* functions as an ammonia oxidizer in an aerobic environment, but it can also reduce nitrite or nitrate in anoxic situations (Anderson *et al.*,

1993).

1.4.1.3 Anaerobic ammonia oxidation

Anammox is a process in which nitrite is converted to dinitrogen gas by autotrophic Anammox genera, with ammonium functioning as an electron donor. Nitrite acts as the final electron acceptor in this process. This means that nitrite can be converted to dinitrogen gas without the use of COD or the addition of external methanol (Jetten *et al.*, 1998). *Brocadia anammoxidans* appears to be the main organism responsible for the Anammox process. Others are *Kuenenia stuttgartiensis* and *Nitrosomonas eutropha*. The energy produced by *anammox* is only enough for cell survival and not sufficient for growth (Jetten *et al.*, 1999).

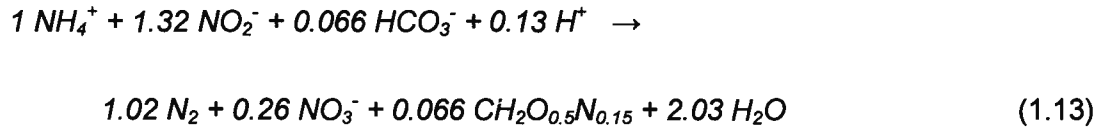
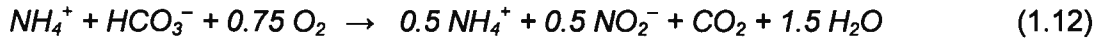
The overall reaction of Anammox is as follows (Strous, 1998):



Some applications of the Anammox process are listed as follows.

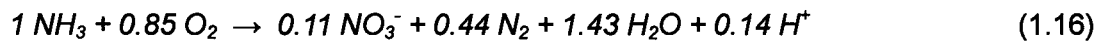
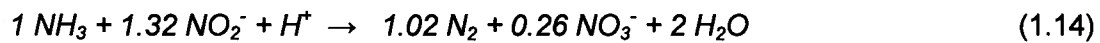
■ Single-reactor high-activity ammonia over nitrite (SHARON)

The SHARON process is operated without any biomass retention. This means that the sludge age (SRT) equals the hydraulic retention time (HRT) (1-2 days). In such a system, the effluent concentration is only dependent on the growth rate (1/SRT) of the bacteria involved. Fast growing ammonium oxidizers are selected by operation of the SHARON process (van Dongen, 2001).



■ Complete autotrophic nitrogen removal over nitrite (CANON)

Completely Autotrophic Nitrogen removal Over Nitrite (CANON) is an advanced application of the Anammox process. This process appears to be particularly suitable for the removal of ammonia from wastewater that does not contain enough organic material to support the conventional nitrification/denitrification process. Ammonia is partly oxidized to nitrite by oxygen-limited aerobic ammonia oxidizers. The nitrite produced, together with a part of the remaining ammonia, is converted to dinitrogen gas by Anammox bacteria according to reaction 1.14 described below, leading to the overall reaction 1.15 described below. It is thought that *Nitrosomonas* may be the organisms involved in this process (Ahn, 2006; Slieker *et al.*, 2003).



■ Oxidation-limited autotrophic nitrification (OLAND)

The conventional process for ammonium removal using two steps, aerobic nitrification and anaerobic denitrification, could be improved by a one-step process in which ammonium is oxidized directly to N_2 . The latter is an autotrophic process which

consumes 63% less oxygen and 100% less reducing agent (Kuai, 1998). The responsible microorganisms were reported as nitrifiers that were able to denitrify under conditions of O_2 limitation. It has been confirmed that pure and mixed cultures of *Nitrosomonas eutropha* are able to denitrify nitrite by using hydrogen and ammonium as electron donors (Bock *et al.*, 1995). However, application of this one-step process is still severely limited in practice, due to the extremely low specific capacity of N removal which is less than 2 mg N per g of volatile suspended solids (VSS) $^{-1}$ day $^{-1}$ (Schmidt and Bock, 1997). Moreover, the operational conditions for control of the process have yet to be identified.

1.4.1.4 Simultaneous nitrification denitrification

Because the free energy and the electric potential of using oxygen and nitrate/nitrate as the electron acceptors are close, the process of simultaneous nitrification and denitrification becomes possible. SND occurs as a consequence of DO concentration gradients formed within microbial flocs or biofilms due to diffusion limitations of the oxygen. That is, the nitrifiers exist in regions with high dissolved oxygen concentrations, while the denitrifiers are preferentially active in internal zones of the floc with very low OR NO dissolved oxygen concentrations. A DO around 0.5 mg/L was reported by Elisabeth (1996) as the point at which the nitrification rate would equal the denitrification rate, leading to complete SND. The BOD/TKN ratio for SND is around 5 (Metcalf and Eddy, 2003).

In another SND process, SND via nitrite, nitrogen removal was found to use nitrite rather than nitrate. Here ammonium was oxidized to nitrite, which was directly denitrified. Zeng *et al.* (2003) found a SND efficiency of 98%, at DO 0.5 mg/L in a SBR system. N_2O , rather than N_2 , was the major denitrification end-product, which is a significant

environmental concern. *Brocadia anammoxidans* and *Nitrosomonas* were identified as the most significant microorganisms in SND via nitrite, nitrogen removal.

Generally, the substrate utilization rate of heterotrophic carbon removal is around 5 g COD / g VSS*d. For nitrification, the nitrogen conversion rate is around 0.5 g N / g VSS*d. Heterotrophic denitrification has a higher utilization rate than autotrophic denitrification, about 0.2 and 0.02 NO₃⁻N mg/g VSS*d, respectively. The nitrite consumption rate in the SND process is also around 0.02 g NO₂⁻-N mg/ g VSS.*d (Zeng *et al.*, 2003).

Denitrifiers are not all absolutely heterotrophic or autotrophic. Facultative chemolithoautotrophic denitrifiers, such as *Paracoccus versutus* (previously called *Thiobacillus versutus*) and *Thiomicrospira thyasiris* (previously called *Thiobacillus thyasiris*), are able to grow autotrophically by oxidizing sulfur compounds as an energy source, but are also capable of heterotrophic growth. Hence, these bacteria can apparently adapt to different environments (i.e. autotrophic, heterotrophic, or mixotrophic conditions) (Matin, 1978). Oh *et al.* (2001) suggested that “mixotrophic conditions, that allow simultaneous reactions of autotrophic and heterotrophic denitrification, provide significant advantages in terms of nitrate and COD removal, decreased sulfate production, and decreased alkalinity consumption, over obligate autotrophic or heterotrophic denitrification.”

1.4.2 Phosphorus removal

Conventional biological phosphorus removal is achieved by recycling the sludge in alternating anaerobic/aerobic conditions. Under anaerobic conditions, phosphorus accumulating organisms (PAOs) take up volatile fatty acids, while releasing phosphorus.

Under aerobic conditions, PAOs take up phosphorus present in the bulk liquid as an energy source for cell growth (Fig 1.3). Many processes have been proposed (Metcalf & Eddy, 2003) for enhanced biological phosphorus removal (EBPR) in a revised conventional activated sludge system. Generally, these processes utilize free oxygen as an electron acceptor when PAOs take up phosphorus during aeration. However, some PAOs can use nitrite/nitrate as an electron acceptor, thereby making possible the process of simultaneous nitrification/denitrification and phosphorus removal.

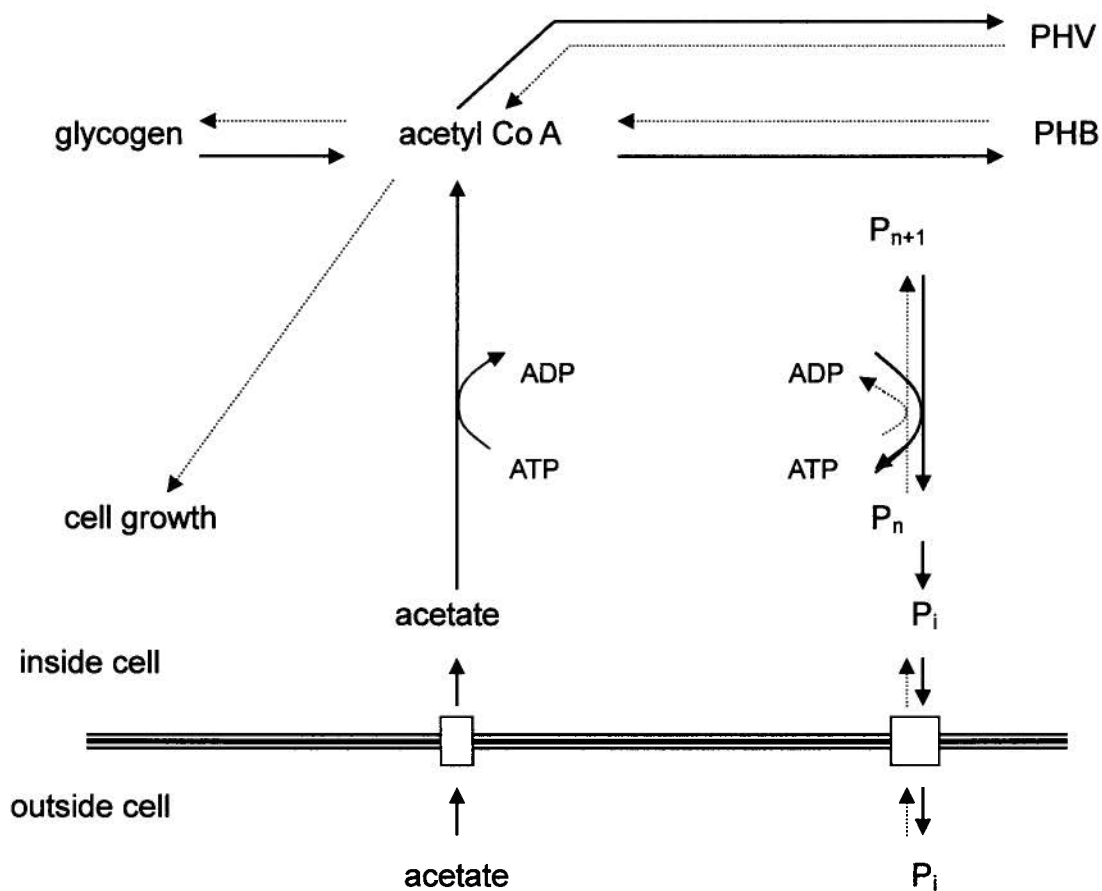


Fig 1.3 Simplified diagram of EBPR metabolism. Gray lines indicate aerobic metabolism and black lines indicate anaerobic metabolism. (adapted from Pramanik *et al.*, 1999)

1.4.2.1 Phosphorus removal and carbon source

- Phosphorus release under anaerobic conditions

Biological phosphorus removal occurs when the mixed flow liquor is subjected to alternate anaerobic and aerobic conditions. In the anaerobic stage, fermentation products are produced from biochemical oxygen demand (BOD) in the wastewater by the action of facultative organisms. The phosphorus storage microorganisms are able to assimilate the fermentation products, such as volatile fatty acids (VFA). By using energy, VFA is stored inside PAO cells as poly- β -hydroxybutyrate (PHB) and orthophosphate is released under anaerobic conditions. Since many of the competing microorganisms cannot function in this manner, the phosphorus microorganisms have a distinct advantage over other organisms in the activated sludge system. During the aerobic phase, the stored substrate products are depleted, and the soluble phosphorus is taken up by the microorganisms in quantities greater than what is needed to function (Metcalf and Eddy, 2003).

- Phosphorus uptake under aerobic conditions

Under aerobic conditions, the stored PHB in PAOs is metabolized to produce energy for new cell growth. The PAOs use oxygen as an electron acceptor and the energy released from PHB oxidation is used to form phosphate bonds in cell storage, so that soluble orthophosphate is taken up from solution to form phosphates within cells. Thus, phosphorus is removed from wastewater.

The uptake of phosphorus is maximized at dissolved oxygen concentrations greater than 2 mg/L. At lower DO concentrations, the excess phosphorus is released from the microorganisms. For biological phosphorus removal to occur, an anaerobic stage is

required for the production of fermentation products. If nitrification occurs, it is therefore necessary for denitrification to take place before enhanced biological phosphorus removal can occur. If this does not happen, and nitrite or nitrate is present, the system is anoxic rather than anaerobic. For this reason, a low dissolved oxygen concentration must be maintained for a longer period when biological phosphorus removal, rather than denitrification, is required (Surampalli *et al.*, 1997).

To have an effluent *P* less than 1 mg/L, a BOD: *P* ratio > 20 was suggested by Gray (2004). Chuang *et al.* (1996) concluded that the phosphorus uptake and PHA utilizing ratio is 0.1~1.0 mg-P/mg-PHA. Jeon *et al.* (2003) identified the microbial communities in a *SBR* by using fluorescence in situ hybridization (FISH) technology and concluded that the *Rhodocyclus*-like group within the *Proteobacteria* subclass was the dominant species responsible for biological phosphorus removal. Chen *et al.* (2005) reported that the ratio of phosphate uptake to phosphate release in an acclimated sludge is 1.28~1.38.

Ndegwa *et al.* (2001) investigated the effects of phosphorus removal at different pH and temperature levels. It was concluded that pH was the dominant factor influencing the levels of soluble ortho-P in manure. Temperature affected soluble ortho-P status only in extended aeration. High manure temperatures reduced aeration efficiency but promoted production of VFAs, which led to the release of ortho-P.

1.4.2.2 Denitrifying phosphorus removal

Nitrification and denitrification can occur concurrently in one reactor under aerobic conditions with low DO, through the so-called simultaneous nitrification and denitrification process described previously. In addition, it has been reported that N removal can be

achieved by partial oxidation of ammonium to nitrite, which is then directly reduced to N_2 gas. This process, termed SND via nitrite, saves the COD requirement, compared with conventional denitrification via nitrate. It has also shown higher denitrification rates and a lower biomass yield during aerobic growth (Turk and Mavinic, 1986).

Furthermore, it has been found that denitrification can be accomplished by the so-called denitrifying PAOs (DPAOs) in anaerobic–anoxic EBPR systems, allowing simultaneous nitrate/nitrite reduction and P uptake using the same COD. Jorgensen *et al.* (1995) concluded that the phosphate accumulation and denitrification in activated sludge can be carried out by the same organisms, such as *Pseudomonas*. This simultaneous enhanced biological phosphorus uptake and denitrification under anoxic conditions is also named the DEPHANOX process (Bortone *et al.*, 1996). In this process, nitrate and/or nitrite, rather than oxygen, act as the electron acceptor. That is, phosphorus uptake can happen without an oxygen supply (Lee *et al.*, 2001). In addition, compared with PAOs, DPAOs are 40% less efficient in generating energy, and thus have a 20% to 30% lower cell yield (Kuba *et al.*, 1994; Murnleitner *et al.*, 1997). The use of DPAOs in BNR systems is therefore highly beneficial in terms of lower COD demand, resulting in reduced aeration costs and less sludge production. Ideally, if SND via nitrite could be accomplished with the DPAOs, even more COD could be saved, because the soluble COD in domestic wastewater is typically a limiting factor (Zeng *et al.*, 2003).

Hu *et al.* (2002) concluded that the denitrifying phosphate uptake rate and biomass yield rate of the growth of DPAOs were lower than PAOs, under aerobic conditions. The phosphate uptake per PHB COD of DPAOs should decrease to about 80% as PAOs under aerobic conditions and the growth yield of DPAOs should be 30% lower than PAOs. The

anoxic phosphate uptake rate varied from 0.3 to 98 mg P/g dry sludge*h (Jorgensen *et al.*, 1995). Lee *et al.* (2001) reported 11~64% phosphorus removal took place under anoxic conditions in a SBR system.

Pala and Bolukbas (2005) investigated the kinetic parameters for biological carbon, nitrogen and phosphorus removal in a municipal wastewater treatment system. The average phosphorus release rate was 1.1 mg P/g VSS*h for the addition of glucose and the maximal anaerobic P release rate was 98 mg P/g VSS*h on average for the addition of acetate. The average P uptake rates for aerobic and anoxic conditions were 0.28 and 0.19 mg P/g VSS*h, respectively. For nitrification, the average NO_x-N production rate was determined as 2.0 mg N/g VSS*h. For denitrification with the addition of acetate, the average NO_x-N consumption rate was 1.6 mg N/g VSS*h. It was 1.2 mg N/g VSS*h with the addition of glucose. In the simultaneous nitrification, denitrification and phosphorus removal (SNDPR) system, the growth yield and the decay coefficients were found to be 0.70 mg VSS/mg COD and 0.053 day⁻¹, respectively. The specific nitrification rate and the specific denitrification rate were determined as 6.9×10⁻⁴ mg NO_x-N/mg VSS*h and 3.4×10⁻⁴ mg NO_x-N/mg VSS*h.

1.4.3 Simultaneous nitrification denitrification and phosphorus removal

Simultaneous nitrification/denitrification and phosphorus removal could occur in the same reactor as described in the previous section. There are many factors affecting the performance of SNDPR, including the concentration of DO, and the available carbon source, nitrite and sulfide. Other physical factors which affect the performance include pH and temperature. Sludge retention time and the competition between denitrifying

phosphorus-accumulating organisms and denitrifying glycogen-accumulating organisms (DGAOs) also affect the efficiency of SND and phosphorus removal.

1.4.3.1 DO concentration

As mentioned in Section 1.4.1.4, the DO concentration has been recognized as one of the key factors for the SND. A higher DO enhances the rate of nitrification but slows down the rate of denitrification. In the study by Zeng *et al.* (2003), the nitrification rate and denitrification rate under DO 0.5 mg/L were similar, around 4 mg N/L*h. That resulted in the SND efficiency of 98%. Zeng *et al.* also indicated that the SND efficiencies decreased from 98 to 51%, with an increase of DO from 0.5 to 2.5 mg/L.

1.4.3.2 Available carbon sources and the competition of DGPOs vs DGAOs

Glycogen-accumulating organisms (GAOs) compete with phosphorus-accumulating organisms. Both PAOs and GAOs are able to take up volatile fatty acids under anaerobic conditions. The reduction capacity and energy are provided from the glycolysis of internal glycogen reserves. Thus, the GAOs do not take up phosphorus and are in competition with PAOs for the same external COD source (Zeng *et al.*, 2003). Pramanik *et al.* (1999) proposed a scheme of EBPR metabolism as shown in Fig 1.3.

Some GAOs, namely denitrifying glycogen-accumulating organisms (DGAOs), can use nitrite/nitrate as the electron acceptor under anoxic conditions. Zeng *et al.* (2003) operated a lab-scale *SBR* in an alternating anaerobic–aerobic mode with a low DO concentration (0.5 mg/L) during the aerobic period, and demonstrated simultaneous nitrification, denitrification, and phosphorus removal. Although the effluent phosphorus

level was less than 0.5 mg/L, their experiments indicated that DGAOs, rather than DPAOs, were responsible for the denitrification activity. In other words, although anoxic phosphorus uptake occurred, the phosphorus removal was dominated by PAOs which utilized oxygen as the electron acceptor, instead of DPAOs during aerobic/anoxic conditions.

1.4.3.3 Nitrite

The major factor influencing the occurrence of DPAOs and the associated anoxic P uptake is the nitrite/nitrate load in the anoxic phase. For anoxic phosphorus removal, the nitrite/nitrate loading rate should be sufficiently large, or exceed the denitrification rate of non-phosphorus accumulating bacteria, since the denitrifying rate of non-phosphorus accumulating bacteria is significantly higher than for DPAOs (Hu *et al.*, 2002). Research (Saito *et al.*, 2004) has proven that the phosphorus removal rate with NO_2^- as the electron acceptor is faster than with NO_3^- .

Although nitrite acts as an electron acceptor under anoxic conditions, at high levels, it inhibits the biological reaction which results in phosphorus removal. Meinhold *et al.* (1999) investigated the effect of nitrite on anoxic phosphorus uptake. He concluded that the nitrite at 4~5 mg N/L is not detrimental to anoxic phosphorus removal. However, when the NO_2^- concentration is at 8 mg N/L or larger, it completely inhibited anoxic phosphorus removal. When sludge was acclimated to the nitrite concentration before an anoxic period was added into the aeration period, a concentration of NO_2^- up to 10 mg/L was not detrimental to phosphorus uptake (Lee *et al.*, 2001). Nitrite is an inhibitor to both PAOs and DPAOs. The effect of nitrite on aerobic PAOs is more evident than it is on anoxic DPAOs

(Saito *et al.*, 2004; Lee *et al.*, 2001). These authors also noted that nitrite and/or nitrate could be one of the factors enhancing the number of glycogen-accumulating organisms that compete with the PAOs for substrate in the anaerobic phase with nitrogen removal.

Chaung *et al.* (1996) reported that, under anaerobic/anoxic conditions, the presence of carbon and nitrite/nitrate affected the rate of phosphorus release. When the carbon source is abundant, the system is phosphorus limited. The phosphorus release rate is slightly inhibited as the concentration of nitrate/nitrite increases. When the available carbon is insufficient, the phosphorus release rate of the DPAOs is inhibited by an increase in nitrate/nitrite. Chaung *et al.* (1996) also noted that the sludge with a high phosphorus content has a higher phosphorus release rate in accordance with a lower denitification rate.

Nitrite becomes a key element in SND and SNDPR systems. Ammonium oxidized to nitrite is expected; however, the subsequent oxidization of nitrite to nitrate is not desired. There are at least four ways to reach this objective (Bernet *et al.*, 2005):

1. *pH control* – Nitrite oxidizing bacteria (NOB) are more sensitive to free ammonia and free nitrous acid than ammonium oxidizing bacteria (AOB) (Bernet *et al.*, 2005). As a consequence, as Simm (2004) demonstrated in his research, increasing free ammonia by raising the pH value and increasing nitrous acid by reducing the pH value, cause the inhibition of NOB. Thus, the major nitrogen compound in the system would be nitrite.
2. *Temperature control* – At temperatures higher than 25°C, AOB have a higher growth rate than NOB (Hellinga, 1998). By controlling HRT at 1 day without sludge recycling, NOB can be flushed out of the reactor before they form large populations.

This principle has been applied as SHARON process to full scale facilities for treating the supernatant of anaerobic digestion, that has a relatively high temperature.

3. *DO control* – AOB have been shown to have a higher affinity for oxygen than NOB. Low DO conditions, therefore, promote the growth of AOB (Jayamohan *et al.*, 1988).
4. *SRT control* – The optimum SRT for the growth of AOB is much shorter than for NOB. A short SRT maintains the dominance of AOB in the microbiological community (van Dongen *et al.*, 2001).

Nitrite is the favored compound in SNDPR; on the other hand, the accumulation of nitrite might inhibit the performance of SNDPR. Once nitrite is generated, denitrification via nitrite has to occur simultaneously. To monitor and control nitrite generation and accumulation in wastewater treatment plants is an important issue for SND and/or SNDPR.

1.4.3.4 SNDPR in Hybrid system and the effects of SRT

Most microorganisms survive better with an immobilized life-cycle, attached to surfaces or to each other (Schramm 2003). Microbes with attached growth generally have a longer average sludge retention time than is the case with suspended growth. A hybrid system has both suspended and attached growth in a single reactor. Generally, a shorter SRT provides a higher COD removal rate. However, nitrification (from ammonia to nitrate) requires a longer SRT for the slower growing nitrifiers. The growth rate of Anammox bacteria is very slow, with a doubling time of around 11 days (Strous, *et al.*, 1997). A hybrid

system would make it possible have a much longer sludge age for attached growth microorganisms than for suspended growth organisms. Such a system would maintain nitrifiers and *Anammox* bacteria on biofilm and the short SRT sludge in suspended growth (Huang and Li, 2004).

Youa *et al.* (2003) investigated nitrification efficiency by comparing the hybrid and the suspended growth systems in an A2O (anaerobic-anoxic-aerobic) system. The authors concluded that the proportion of ammonia oxidizers in the hybrid process was around 3 times greater than in the suspended system. The ammonia oxidization rate for the hybrid system was also around 3 times higher than in the suspended growth system. The results showed that a hybrid system could increase the numbers of nitrifying organisms and enhance the nitrification performance of the system.

Daims *et al.* (1999) investigated nitrification by using FISH (fluorescence in situ hybridization) and merase chain reaction (PCR). They indicated that the AOB occurred in higher cell numbers and occupied a considerably larger share of the total volume than the NOB in a hybrid system.

Huang and Li (2004) used a hybrid reactor to maximize wastewater treatment capacity and nitrification (including nitrite oxidization) during a short sludge age operation. They changed the SRT of the reactor from 0.17 day to 6.5 days and found that a hybrid system increased nitrification compared to a suspended growth system with a short SRT (less than 2 days). However, when the SRT is 3 days or longer, the hybrid reactor is mainly composed of suspended growth. This would greatly reduce the contribution of the biofilm. The maximum benefit of using a hybrid system is gained when the SRT is set at between

1 and 2 days. As stated earlier, this form of hybrid reactor is also beneficial to the growth of AOB and their conversion of ammonia to nitrite.

Falkentoft *et al.* (1999) conducted research into denitrifying phosphorus removal in a biofilter system. The authors summarized that the ratio of anoxic P-release to COD-uptake was 0.25, and it was 0.06 mol P/mol e^- for P-uptake to nitrite/nitrate removal under anoxic conditions.

However, the effects of SRT on a SNDPR system (i.e. denitrifying PAOs) have not been fully studied and require further investigation.

1.4.4 Nitrous oxide production

Nitrous oxide is a clear, colorless gas, with a slightly sweet odor. Its molecular weight is 44.02 g/mole. Compared to oxygen, nitrous oxide has relatively high solubility. At 20 °C, the solubility is 1.2 L/L in pure water. Due to its long atmospheric lifetime (approximately 120 years) and heat trapping effects (about 310 times more powerful than CO₂ based on a mole comparison), N₂O is considered to be an important gas in terms of greenhouse effects on the biosphere.

Nitrous oxide occurs both naturally and as a result of human-related activities. It is removed from the atmosphere mainly by photolysis in the stratosphere (USA EPA, 2007). Natural emissions of N₂O primarily result from the bacterial breakdown of nitrogen in soils and in the earth's oceans. On a global basis, it is estimated that natural sources account for over 60% of the total N₂O emissions (IPCC, 2001). In Canada and the United States, the major human-related sources of N₂O are agricultural soil management, mobile and

stationary combustion of fossil fuels, nitric acid production, livestock manure management and human sewage (Environment Canada, 2007 and USEPA, 2007).

1.4.4.1 Autotrophic nitrous oxide production

In autotrophic processes, ammonia oxidizers, like *Nitrosomonas europaea*, reduce NO_2^- to N_2O and N_2 under oxygen stress conditions (Bock *et al.*, 1995; Itokawa *et al.*, 2001; Zeng, 2003). N_2O would also be produced through the chemical decomposition of intermediates such as: (1) NH_2OH or NO_2^- from the reaction of NH_4^+ oxidation into NO_2^- (Chalk and Smith, 1983), or (2) incomplete oxidation of NH_2OH (Hooper and Terry, 1979). Although Yoshinari (1990) reported that this chemical production of N_2O occurs only in the presence of relatively high NO_2^- concentrations and rarely in activated sludge, some studies (Zeng *et al.*, 2003 and Shiskowski, 2004) concluded that the potential exists that N_2O can be the dominant N end product, rather than N_2 , in the aerobic ammonia removal found in SBR systems.

1.4.4.2 Heterotrophic nitrous oxide production

N_2O is also an intermediate product in denitrification processes. It is released under low oxygen conditions with sufficient NO_3^- or NO_2^- and biodegradable organic carbon (Itokawa *et al.*, 2001; Bonin *et al.*, 2002). Two possible reasons for N_2O generation are (1) a carbon limitation that prevents denitrification from reaching the N_2 endpoint; and (2) selective inhibition of the N_2O reductase enzyme that results in a net accumulation of N_2O . This would occur in such circumstances as the presence of dissolved oxygen (Hwang *et al.*, 2006; Noda *et al.*, 2003; Wicht, 1996), the existence of H_2S (Schonharting *et al.*, 1998;

Sorenson *et al.*, 1980), a short sludge retention time (Noda *et al.*, 2003; Wicht, 1996) and a relatively high salinity (Tsuneda *et al.*, 2005).

1.4.4.3 Nitrous oxide emission from wastewater treatment

Among 26 cases, Barton and Atwater (2002) found highly variable N_2O emissions from wastewater treatment. The emissions varied from negligible to greater than 40% of influent nitrogen. NBR treatment plants with pre-denitrification had negligible N_2O emissions (Schulthess and Gujer 1996; Kimochi *et al.*, 1998; Tallec *et al.*, 2006). On the other hand, lab scale reactors which were used for studying SND usually collected large quantities of N_2O emissions (Spector, 1998; Beline *et al.*, 1999; Lemaire *et al.*, 2006).

In their review, Barton and Atwater (2002) also concluded that certain factors result in higher N_2O emissions. These factors include DO levels less than 0.5 mg/L, short SRT (<5 days), low $\text{COD}/\text{NO}_3^- \text{-N}$ ratio in wastewater, low pH conditions and high H_2S -S concentration.

1.4.5 Real-time control of wastewater treatment system

1.4.5.1 ORP/pH/DO real-time control

To achieve nitrogen and phosphorus removal from wastewaters, the operation of a treatment system must secure and optimize both the aeration and hydraulic retention times of the treatment facility (Plisson-Saune *et al.*, 1996). There are two control models for wastewater treatment, fixed-time control and real-time control. In terms of variations in the wastewater influent, a fixed-time control system, in which the period and rate of aeration are fixed, makes it difficult to treat wastewater efficiently. There is a possibility that

energy might be wasted due to unnecessary aeration. With real-time control, aeration and hydraulic retention time are controlled by the level of treatment. There are several ways to achieve real-time control in wastewater treatment: monitoring ORP, pH, N₂O (Shiskowski, 2004), and NADH (Farabegoli *et al.*, 2003). The ORP and pH have already been sufficiently studied and widely applied in the real-time control of nitrogen removal processes. The NADH monitoring is a registered patent (SymBio) of Enviroquip Inc.

Oxidation-reduction potential (ORP) is used to express the potential of an oxidization-reduction reaction. The ORP of the reaction of a hydrogen ion accepting electrons to form a hydrogen molecule is defined as zero volt. The ORP expressed as E (Volt or mVolt) of other reactions is determined using this base for comparison purposes (Ralph *et al.* 2001).

There is a relationship between Gibb free energy and electrode potential, which can be expressed as Equation (1.17).

$$E^{\circ} = -\Delta G^{\circ} / zF \quad (1.17)$$

where E° (Volt) is standard electrode potential, G° is standard Gibb free energy (Kj), z the number of valency, and F the Faraday factor (96.485 Kj/volt-eq). The free energy G is given in Equation 1.18.

$$G^{\circ} = H^{\circ} - TS \quad (1.18)$$

where H is enthalpy, which is a function of heat, and work, T temperature ($^{\circ}\text{K}$), and S entropy which represents the disorder of a system. When G is negative, the reaction tends to happen spontaneously. In a reaction in which the elements and compounds produced are in their standard states, the enthalpy of formation ΔG° is equal to ΔH°_f , heat of

formation, which symbolizes the change of heat in a formation reaction.

In a wastewater treatment environment, a positive value of ORP represents a system in an oxidation state where the major constituent tends to lose electrons. When the ORP is negative, the system is in a reduction state and the main constituent serves as electron acceptor. The pH value slightly affects the ORP of the reaction. A lower pH, which also means a higher H^+ concentration, tends to have a higher oxidation-reduction potential. The presence of free oxygen accompanies with a higher ORP. The ORP is a little bit lower with the existence of nitrite/nitrate without oxygen. Methane and hydrogen sulfide exist in a relatively low electrode potential (Sawyer *et al.*, 2003; Metcalf & Eddy, 2003). The types of electron donors also affect the ORP of the reactions. Metcalf & Eddy (2003) listed the free energy of reactions with various organic and inorganic materials serving as electron donors. The ORP in a wastewater environment represents a combination of multiple reactions. Generally, the ORP for a wastewater system varies from 300 mV under aerobic condition to -300 mV under anaerobic condition (Hu *et al.*, 2005; Plisson-Saune *et al.*, 1996; Khanal *et al.*, 2003).

The ORP profile has been used to identify different phases of nitrogen removal. There are three bending points on an ORP profile during alternating aeration which can be used to indicate different situations in nitrogen conversion. In a typical ORP profile (Fig 1.4), the point β observed at the end of NH_4^+ consumption is well correlated to increases in DO and is a good indication that nitrification is over. This point is called the ammonia valley or nitrogen breakpoint. The point β enables us to detect the beginning of denitrification. The point γ is the disappearance of nitrite/nitrate. This sudden decrease of ORP, namely the denitrification knee (or nitrate knee, or nitrate breakpoint), signifies the end of

denitrification and the beginning of sulfate reductive activities that produce sulfides (Plisson-Saune *et al.*, 1996).

ORP bending and DO profile can be also used for SND control. The optimal range of ORP was observed from -60 to 200 mV for simultaneous nitrification and denitrification (Sperandio and Queinnec, 2004; Fuerhacker *et al.*, 2000).

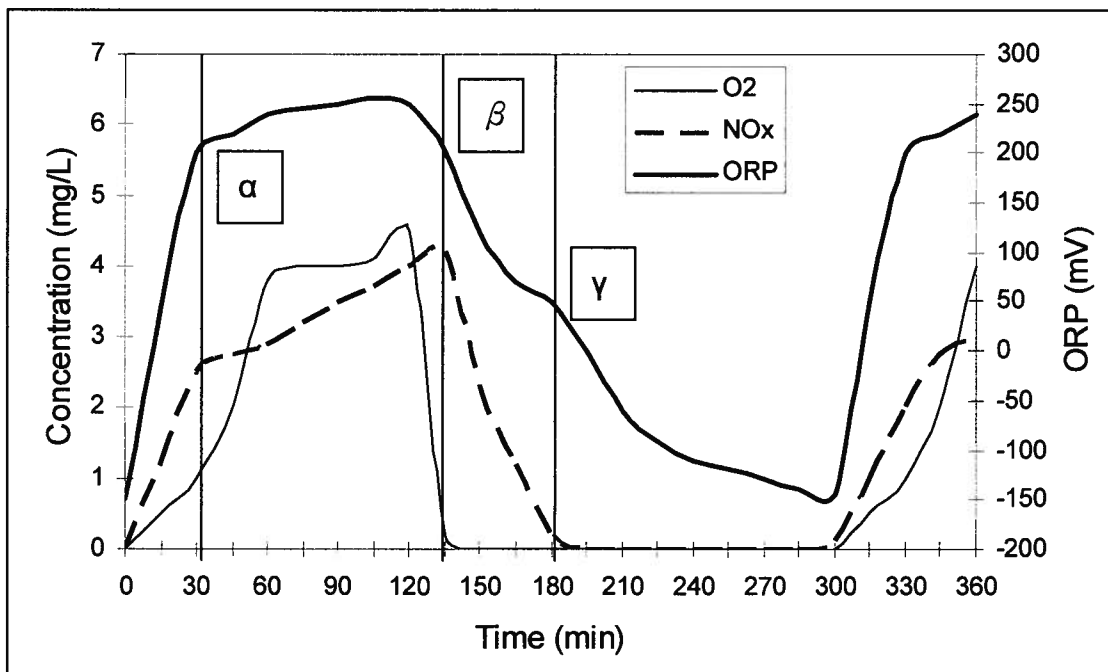


Fig 1.4 Typical ORP profile of nitrification/denitrification cycle (adapted from Plisson-Saune *et al.*, 1996)

pH has also been studied as a parameter to be used in real-time control for nitrogen removal processes. As shown earlier in Equations 1.1 to 5, nitrification consumes alkalinity, thus reducing pH. However, denitrification releases hydroxyl ion (Equation 1.10) which results in an increase of pH. Figure 1.5 shows the relationship of pH and ORP in a nitrogen removal system. The lowest pH in the aerobic phase is the same as the ammonia valley in an ORP profile, which can be used for real-time control of biological treatment processes (Cheng *et al.*, 1999 and 2000; Kim *et al.*, 2004; Spagni *et al.*, 2001). Akin *et al.* (2005) concluded that pH provides good information during the aerobic phase, whereas ORP is more useful in the anoxic phase.

Spagni *et al.* (2001) applied ORP, DO and pH as real-time control parameters to an enhanced biological nutrient removal system. The authors found a significant relationship between pH and P-release/uptake. When phosphate is released under anaerobic conditions, hydrogen ion is also released. This causes pH to decrease (also see Fig 1.3). On the other hand, when phosphorus is taken up, hydrogen ion enters cells from the bulk liquid. The pH in the phosphorus uptake phase is higher. By relating variations in pH and different stages in the EBPR process, pH can be used as a possible control parameter for biological phosphorus removal.

The application of ORP real-time control to SND systems has been reported. An experiment done by Hu *et al.* (2005) found the optimum range to be from -100mV to 100mV for a conventional SBR and from 126 to 249 mV for a biofilm SBR. Saby *et al.* (2003) operated a SND system under ORP between -250mV to 100 mV. The sludge generation was reduced by up to 50%, compared with conventional activated sludge systems.

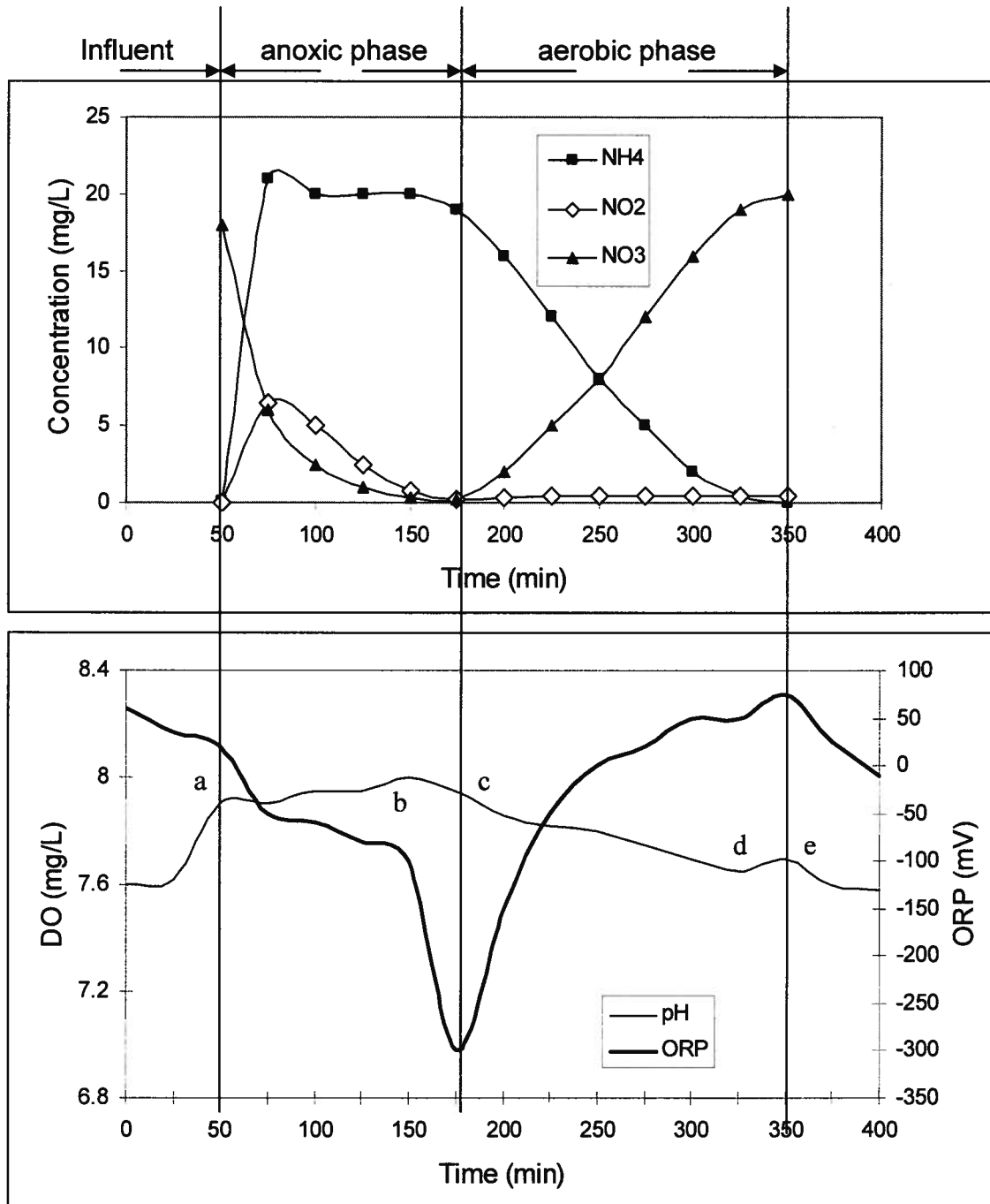


Fig 1.5 Example of real-time control points. a: feeding, b: nitrate knee point, c: beginning of the aerobic phase, d: ammonia valley point, e: end of the aerobic phase. (adapted from Kim *et al.*, 2004)

1.4.5.2 Other real-time control approaches

Some other real-time control approaches including oxygen uptake rate (OUR) and nicotinamide adenine dinucleotide (NADH) have been developed for the real-time control of ammonia oxidation (Battistoni *et al.*, 2003; Kim *et al.*, 2004; Puig *et al.*, 2005; Farabegoli, *et al.*, 2003).

Although N_2O off-gas is an undesired product of wastewater treatment, its high correlation with ammonia oxidation and the potential of being a parameter for the system monitoring has been of interest to researchers. Burgess *et al.* (2002) found the relationship of ammonia oxidation and N_2O concentrations and reported the potential of N_2O detection for the process failure early warning to the system. Shiskowski (2004) investigated the potential for using nitrous oxide as the parameter for nitrification monitoring and system aeration control.

1.4.6 Conclusion of this literature review

In SNDPR treatment, ammonium is first converted to nitrite under limited DO conditions (around 0.5 mg/L). Then, via shortcut denitrification, nitrite plays the role of electron acceptor for denitrification, anammox and phosphorus removal processes. This means that carbon, nitrogen and phosphorus removal can happen in the same reactor.

An abundance of nitrous oxide was found to result from BNR processes as end product of denitrification. N_2O can be produced from both autotrophic and heterotrophic denitrification. Factors affecting N_2O production include DO, available COD, pH, SRT and sulfide. Nitrous oxide is a greenhouse gas. It should be controlled during BNR processes.

A sequencing batch reactor (SBR) provides different operating phases for biological reactions, each requiring specific conditions. It can therefore be used as a system for SNDPR. Controlling aeration by monitoring ORP, DO and pH makes the real-time control of an SBR possible. N_2O off-gas is another potential real-time control parameter, due to the relationship with ammonia oxidation.

Although the emission of N_2O from BNR processes has been identified, the mechanisms of N_2O production have not been fully studied. In order to control N_2O emissions and use N_2O off-gas as a tool for the on-going real-time control of SNDPR, further study into N_2O production is needed.

1.5 Material and methods

1.5.1 Reactor and treatment system

1.5.1.1 SBR system

The sequencing batch reactor (SBR) is a fill-and-draw activated sludge system for wastewater treatment. In this system, wastewater is fed to a single batch reactor and treated to remove undesirable components, then, discharged. Equalization, aeration, and clarification can all be achieved in a single batch reactor. SBR systems have been successfully used to treat both municipal and industrial wastewater. They are uniquely suited for wastewater treatment applications characterized by low or intermittent flow conditions (United States EPA, 1999).

Two bench-scale SBRs were designed for simultaneous nitrification, denitrification and phosphorus removal. One reactor (Reactor A) was operated as a conventional suspended growth system; while the other reactor (Reactor B) was operated as a hybrid. It had suspended growth sludge and biofilm attached to plastic material inside the reactor. The working volume of the reactors was 10.5 L. The decanting volume was 3.5 L, which was a one third of the working volume. Each SBR cycle included six stages: feeding, anaerobic, aerobic, anoxic, post aeration and settling/decanting (Metcalf and Eddy, 2003). The stages of SBR are shown in Figure 1.6.

In Reactor B, 30% of the working volume was filled with plastic bio-carriers (Kaldnes, K3 type, specific surface area = $500 \text{ m}^2/\text{m}^3$, diameter = 20 mm, height = 15 mm; total 450 carriers in Reactor B).

For each reactor, the mixed liquor was stirred by a 7 inch impeller, which was driven

by a motor with a speed set at 60 rpm. This was used to keep the sludge and biofilm carriers suspended and in contact with the substrates. The mixing was stopped at the settling/decanting stage.

The sludge retention time (SRT) was maintained by wasting the mixed liquor at the end of the post aeration period.

Air supply was controlled by flow meters via a pressure regulator. The air was blown into the reactor through a fine porous ceramic diffuser located on the bottom of each reactor. The schedule of air supply was set by an electronic timer. The reactors were operated at an ambient temperature of $22 \pm 2^\circ\text{C}$.

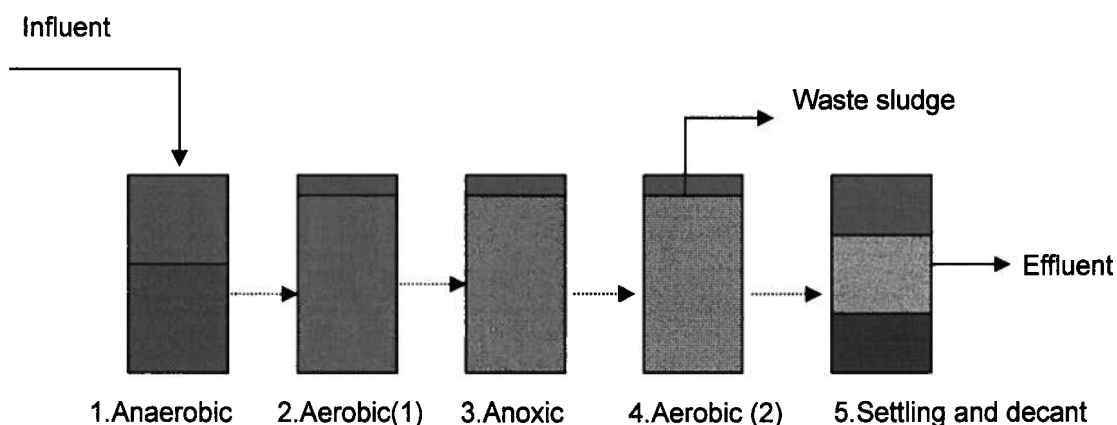


Fig 1.7 SNDPR process of the SBR system

1.5.1.2 Sludge source of SBR

Sludge seed was obtained from the pilot plant at the University of British Columbia in

Vancouver, Canada. This wastewater treatment pilot plant, a modified UCT process, is operated in an enhanced biological phosphorus removal mode.

1.5.1.3 Synthetic Wastewater

Synthetic wastewater (adapted from Zeng *et al.*, 2003) was prepared as the influent for the treatment system. Each 1 L of the synthetic wastewater consisted of 0.3 g of sodium acetate, 0.12 g of lactose, 0.1 g of beef extract (Difco), 0.1 g of NH_4Cl , 0.025 g of KH_2PO_4 , 0.025 g of K_2HPO_4 , 0.05 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.03 g $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.03 g of NaHCO_3 and 3 mL of nutrient solution. One liter of nutrient solution consisted: 1.5 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; 0.15 g H_3BO_3 ; 0.03 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0.18 g KI; 0.12 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 0.06 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 0.12 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 0.15 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; and 10 g ethylenediamine tetraacetic acid (EDTA). The complete influent contained chemical oxygen demand (COD) of 550 mg/L, 35 mg TN-N/L, and 12 mg TP/L. The pH was around 7.

In order to avoid sole C, N and P source in the influent, the carbon source was a mixture of acetate, lactose (splits to yield glucose and galactose) and beef extract; the nitrogen source was ammonium chloride and beef extract; the phosphorus source included KH_2PO_4 , K_2HPO_4 and beef extract. The composition of the beef extract used was TN = 12.4% (amino acid = 2.3%), TP = 2.9%, ash = 9.3% (data provided by Becton), TOC = 26.3%, lipid = 15.4% and carbohydrate = 4.3%.

1.5.2 Nitrous oxide off-gas and pH/ORP/DO monitoring system

The N_2O off-gas was monitored by an infrared N_2O Monitor (Bacharach, N_2O monitor 3010). The off-gas was pumped at a flow rate of 100 mL/min by a rotary pump from the

head space of the reactor to the monitoring system. In order to reduce the interference of CO₂ and moisture, the off-gas first bubbled through 5N KOH contained in a 30 mL impinger flask to remove CO₂. It then went to another ice-chilled impinger flask to condense moisture before it entered the mouth of the monitor (Shiskowski *et al.*, 2005).

DO, pH and ORP probes were installed in each reactor and the monitoring data were recorded in a personal computer. Figure 1.7 illustrates the system of SBR.

1.5.3 Chemical analysis

Concentrations of constituents liquid samples are presented as mg/L and concentrations for gaseous samples were presented as ppm (parts per million).

1.5.3.1 Water sample handling

Water samples were taken from the reactors by a plastic syringe through a tube. Immediately, MLSS was centrifuged at 3,000 rpm for 3 min at room temperature. The supernatant was then removed to test tubes and preserved for purposes of measurement. In order to maintain a constant amount of biomass in the system, the settled sludge was put back into the reactors.

- Ammonia – 5 mL of centrifuged sample was placed into a Lachat tube and acidified by 1 drop of 5% sulfuric acid to obtain a pH below 2. Tubes were stored at 4 °C, prior to being analyzed

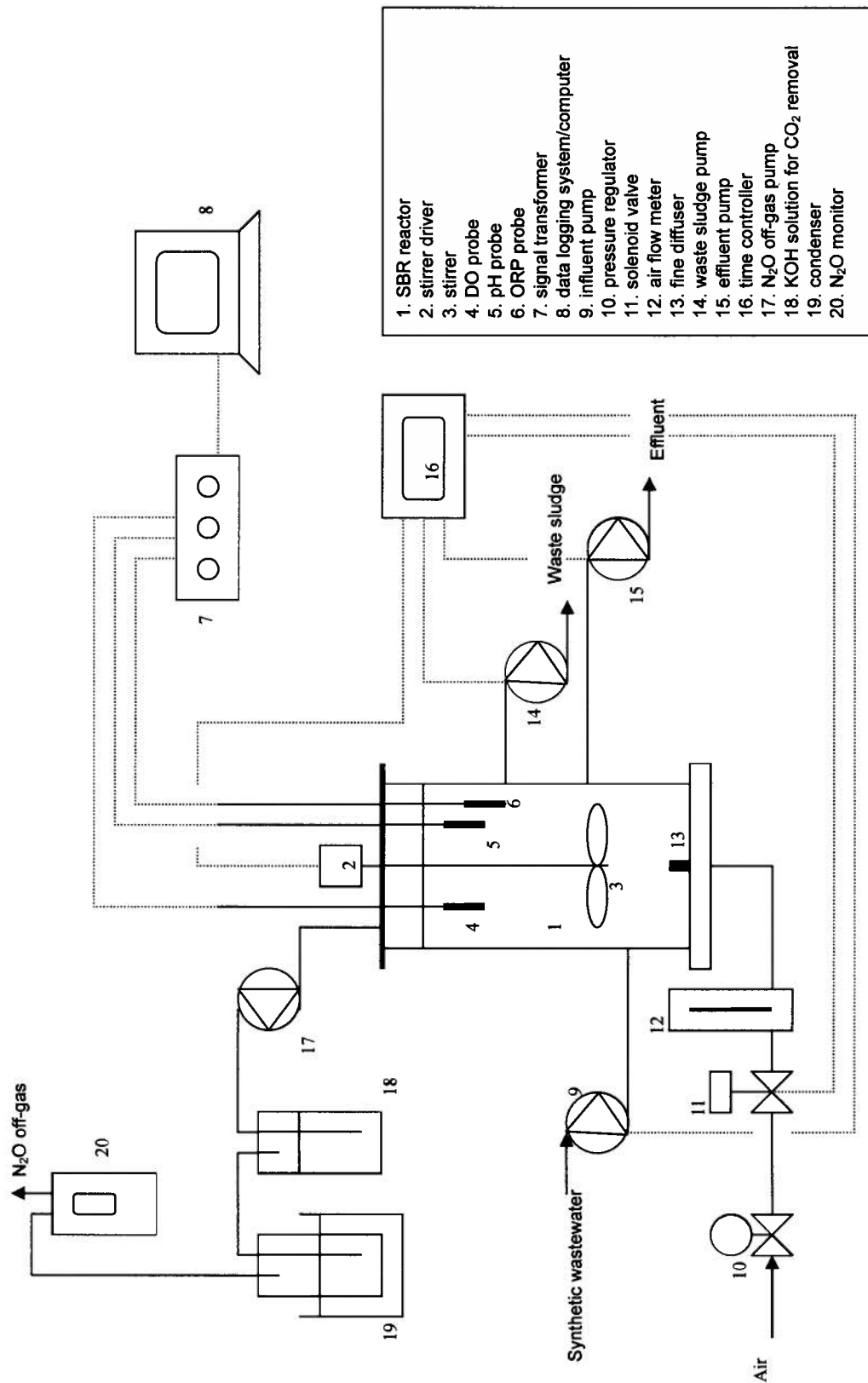


Fig 1.7 Schematic of SBR system

(Two reactors were operated in parallel. The schematic only shows one of two reactors.)

- Nitrate/nitrite and ortho-P – 5 mL of a centrifuged sample was placed into a Lachat tube and preserved by 1 drop of phenyl mercuric acetate (0.1 g phenyl mercuric acetate in 20 mL acetate and 80 mL distilled water). Tubes were stored at 4 °C, prior to being analyzed.
- Total organic carbon (TOC) and total nitrogen (TN) - 5 mL of a centrifuged sample were placed into a Lachat tube and acidified by 1 drop of 5% hydrogen chloride to a pH below 2. Tubes were stored at 4 °C, prior to being analyzed.
- Hydroxylamine - 1 drop of phenyl mercuric acetate was added into the gas chromatography (GC) vial containing 1.5 mL of centrifuged sample. Vials were stored at 4 °C, prior to being analyzed.
- Liquid N₂O – 20 mL of MLSS was taken in a plastic syringe and 3 drops of phenyl mercuric acetate were added to inhibit denitrification. Liquid N₂O concentration was immediately measured (see Section 1.5.3.5).

1.5.3.2 Solid sample handling

- Total phosphate (TP) – for suspended sludge, 2 mL of sample was preserved by 1 drop of 5% sulfuric acid to a pH lower than 2 in a COD vial and stored at 4 °C prior to being processed. For biofilm, the biomass was washed off the carrier using distilled water from carriers. 2 mL of this biofilm biomass was preserved and stored in the same way as the suspended sludge. The total biomass of the biofilm was measured in order to determine the mass of the sample.
- Poly- β -hydroxyalkanoate (PHA) and glycogen – please see Section 1.5.3.3 and

1.5.3.4.

1.5.3.3 Poly- β -hydroxyalkanoate (PHA)

PHA was determined by GC (Comeau *et al.*, 1987). 2 mL MLSS sample was centrifuged at 3,000 rpm (1,000 x g, Thermo IEC Multi rotor) for 5 min at room temp, in a COD vial and the supernatant decanted. The pellet was freeze dried. For biofilm samples, biomass was flushed down from the carrier by pipetting. Dry biomass was calculated by measuring the weights of the dried vials with and without sludge.

To extract the PHA, 2 mL chloroform containing 1.5 mg benzoic acid and 2 mL acidified methanol (3% H₂SO₄) were added into each sample vial, sealed with Teflon caps and incubated in a HACH Block digester at 100°C for 3.5 hours. The vial was shaken vigorously every 20 min for complete mixing. 2 mL of distilled water was added after cooling and was shaken forcefully for 30 sec and then centrifuged at 3,000 rpm for 3 min. 1.5 mL of the denser chloroform layer was transferred to another vial containing 0.5 mL of distilled water. It was then shaken vigorously for 1 min, and centrifuged at 3,000 rpm for 3 min. The chloroform phase (lower phase) containing PHA was transferred to a GC vial for a split injection of 1 μ L of each sample into a HP-6890 GC/5973 mass selective detector (column length-30 m, diameter-25 μ m, coating-0.25 μ m of HP 19091s-433; carrier gas-He, 6.3 psi, 0.9 mL/min; temp profile: increase rate-8 °C/min from 40 °C to 180 °C). DL-3-hydroxybutric acid (HB) sodium salt was prepared as the standard for determining poly-3- β -hydroxyalbutyrate (PHB). PHA was presented as PHB % of dry biomass.

1.5.3.4 Carbohydrate and glycogen

Carbohydrate was determined by the anthrone color development method (Frolund

et al., 1996).

For measuring the glycogen of the biomass, a 1 mL sample of sludge in a 1.5mL micro tube was centrifuged at 10,000 rpm (11,050 x g, Thermo IEC Multi rotor) for 10 minutes. Biofilm samples were prepared as stated in the previous section. The supernatant was removed, and the remaining solid was dried at 103°C over night.

0.5 mL of 30% KOH (wt/v) was added into each tube to lyse cells. The tubes were sealed and heated at 100°C for 3 hours. After cooling to room temperature, 1 mL of ice-cold ethanol was added to precipitate glycogen, and then was centrifuged at 10,000 rpm for 15 minutes. The tube was then left to dry at 60°C over night. The dry precipitation was then dissolved in 1 mL distilled water, and the solution was diluted 5 times by distilled water. 1 mL of diluted sample was mixed with 2 mL of anthrone reagent (0.125% (w/v), anthrone in 94.5% (v/v) H₂SO₄) in COD vial with Teflon caps and incubated in 100°C for 15 min, then cooled at 4 °C for 5 min. The absorbance was measured at 625 nm. Glucose was used as the standard for this determination.

1.5.3.5 Nitrous oxide determination

■ Gaseous N₂O

In addition to the N₂O monitoring, gaseous nitrous oxide was also determined by GC. A 100 µL headspace sample was collected in a gas-tight syringe for GC analysis. The operating conditions for the GC (HP-6890 GC ECD) were: column length-30 m, diameter-0.32 mm, coating - 3 µm of J&W carbonplate; carrier gas-He, 12.2 psi, 20 mL/min; oven temp: 45°C.

■ Dissolved N₂O

Dissolved nitrous oxide concentrations were measured using a modified version of the procedure described by Shiskowski and Mavinic (2005). 20 mL of mixed liquor were collected, and preserved (Section 1.5.3.1) in a 60 mL plastic syringe containing 20 mL of air (for the biofilm tests, a carrier with biofilm was put into a 60 mL plastic syringe with 20 mL effluent and 20 mL air). Immediately, the syringe was vigorously shaken by hand for 30 sec, to partition the nitrous oxide between the mixed liquor and the syringe headspace. After a waiting period of 5 min, a 100 µL headspace sample was collected in a gas-tight syringe for GC injection using the same method as for gaseous N₂O determination. Mixed liquor nitrous oxide concentrations were then estimated using the Bunsen adsorption coefficient (Tiedje, 1982).

$$M = C_g [V_g + (V_l \times \alpha)] \quad (1.19)$$

where M = total mass of N₂O (µg N₂O)

C_g = plastic syringe headspace N₂O concentration (µg N₂O/L)

V_g = plastic syringe headspace volume (L)

V_l = mixed liquid sample volume in plastic syringe (L)

α = Bunsen adsorption coefficient @ T (°C)

$$\text{N}_2\text{O } (\mu\text{g N}_2\text{O/L}) = \{[\text{N}_2\text{O (ppm)} \times p \times \text{MW}] / [\text{UGC} \times T]\} / 1000 \quad (1.20)$$

where p = off-gas pressure (atm)

MW = molecular weight of N_2O (44.02 g/mole)

UGC = universal gas constant (0.082 atm·L/mole·°K)

T = temperature (°K)

1.5.3.6 Ammonium, nitrite, nitrate, ortho-P

$\text{NH}_3/\text{NH}_4^+$, NO_2^- , NO_3^- and ortho-P were determined by flow injection analysis of spectrophotometry (Quikchem 8000, Lachat). The method used for ammonia was 10-107-06-1 (sodium salicylate method); for nitrate and nitrite, it was 10-107-04-1 (sulfanilamide method); for ortho-P it was 10-115-01-1 (ammonium molybdate method).

1.5.3.7 TOC, inorganic carbon (IC) and TN

TOC, IC and TN were measured by a TOC/TN analyzer (IL TOC-TN, Lachat). Samples were acidified with HCl to a pH lower than 2 and purged with nitrogen gas to remove dissolved carbon dioxide. The purged sample was injected into the analyzer and digested by means of thermocatalytic high temperature oxidation in the presence of special catalysts and oxidized by oxygen at a high temperature.



where R = carbon-containing matter

To measure TOC, carbon dioxide generation was detected by a non-dispersive infrared absorption (NDIR) detector. For TN, nitric oxide generation was detected by an electrochemical detector (ECD).

1.5.3.8 Total Kjeldahl Nitrogen

Total Kjeldahl Nitrogen (TKN) was digested according to Standard Methods for Examination of Water and Wastewater (Clescerl *et al.*, 2005, Section 4500N) and measured by flow injection analysis of spectrophotometry (Quikchem 8000, Lachat, method # 10-107-06-2).

1.5.3.9 Total phosphate

Total phosphate (TP) was digested according to the Standard Methods for Examination of Water and Wastewater (Clescerl *et al.*, 2005, Section 4500P) and measured by flow injection analysis of spectrophotometry (Quikchem 8000, Lachat, method # 10-115-01-1).

1.5.3.10 Hydroxylamine

The concentrations of NH_2OH were analyzed by GC as described by Simm (2005). The solution was preserved by means of acetone and transferred to GC vials. GC for this measurement was HP 5890 GC – FID (Column: DB-Wax, 15 m long, 0.53 mm I.D. 0.5 μm film thickness, carrier gas – helium, 20 mL/min, initial purge – 0.25 min. Temperature: injection port – 120°C, Program: 45°C, 0.5 min; 90°C at 5°C /min; 200°C at 40°C /min. Injection depth – 2 mm). Hydroxylamine hydrochloride was used as the standard.

1.5.3.11 Standard Methods

- COD - Chemical oxygen demand was determined according to Standard

Methods for Examination of Water and Wastewater (Clescerl *et al.*, 2005, Section 5220).

- *MLSS* and *TS* - *MLSS* and *TS* were determined according to Standard Methods for Examination of Water and Wastewater (Clescerl *et al.*, 2005, Section 2540).

1.5.3.12 Determination of the size of flocs and the thickness of the biofilm

The size of the sludge floc was analyzed by a specific analyzer - Hydro 2000S (Malvern). The particle sizes were determined by using the scattered pattern of the light when particles passed through the laser beam in accordance with Mie theory (Bohren and Huffman, 1983).

The thickness of the biofilm was measured using a stereo zoom microscope (SMZ-168, Motic) and computer photographc software - Motic Images plus 2.

The biomass of the biofilm carrier was obtained by measuring the difference between new carriers dried at 105 °C and biofilm grown carriers also dried at 105°C.

1.5.3.13 Microbial observation

Sludge floc and the biofilm washed off the carrier were observed using a Nikon microscope (x 100), to observe the protozoa populations in the biomass.

1.5.4 Calculations

1.5.4.1 SND

Simultaneous nitrification denitrification in this study is defined as the loss of nitrogen during the aeration period.

$$SND (\%) = \{1 - [(NH_4^+_{,e} + NO_2^-_{,e} + NO_3^-_{,e}) / (NH_4^+_i - NH_4^+_{,e})]\} \times 100\% \quad (1.23)$$

Where $NH_4^+_{,e}$ = nitrogen in ammonium and ammonia at the end of the aeration (N-mg/L)

$NO_2^-_{,e}$ and $NO_3^-_{,e}$ = nitrogen and nitrate at the end of the aeration (N-mg/L)

$NH_4^+_i$ = nitrogen in ammonium and ammonia at the beginning of the aeration

Organic nitrogen from beef extract was easily taken up by biomass during the anaerobic phase and the early stage of aeration of this study. The amount of the organic nitrogen was about the same amount of N assimilation of waste sludge, and the “ammonia/ammonium” value reasonably represents the nitrogen available for conversion to other nitrogen compounds (Section 2.3.3.1).

1.5.4.2 N₂O off-gas

N₂O off-gas in this study was presented as the time integration of N₂O concentration, divided by the total nitrogen of the influent.

$$N_2O \text{ off-gas } (\%) = N - N_2O / TN \times 100\% \quad (1.24)$$

1.6 References

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Chapter 2 Determination of SNDPR process in an SBR hybrid system

2.1 Introduction

Excess nitrogen and phosphorus in wastewater effluent can have a serious environmental impact receiving waters. To lessen these effects, various biological nutrient removal (BNR) processes have been applied to wastewaters. The drawbacks of the conventional BNR process are that it requires complicated process configurations and skilled operators, and also has relatively high energy consumption. The process of simultaneous nitrification and denitrification provides a relatively simple, compact configuration to achieve nitrogen removal. Furthermore, some microorganisms, namely denitrifying PAOs, are able to use nitrate and/or nitrite as an electron acceptor, for phosphate up-take. This makes it possible to have simultaneous nitrification, denitrification and phosphorus removal from wastewaters (Lee *et al.*, 2001; Turk and Mavinic, 1986).

The sequencing batch biological reactor has been intensively studied for SND and SNDPR processes. Here, the conditions can be provided for both heterotrophic and autotrophic growth in a single reactor (Lemaire *et al.*, 2006; Meyer *et al.*, 2005; Zeng *et al.*, 2003). In comparison with a suspended activated sludge system, a biofilm based system has a number of advantages: its stability by containing a relatively high biomass; its compactness; a higher efficiency of SND and a longer SRT. A sequencing-batch, biofilm

*A version of this chapter has been submitted for publication:

Lo, I., Lo, K.V., Mavinic, D.S., Shiskowski, D. and Remay, W. Comparison of a simultaneous nitrification, denitrification and phosphorus removal process in a suspended-growth and a hybrid sequencing-batch reactors.

reactor has been previously developed for SNDPR (Kumar and Chaudhari, 2003; Gieseke, 2002; Morgenroth and Wilderer, 1998). However, Falkentoft *et al.* (2001) reported that the diffusion limitations for biofilm hinder biological phosphorus removal in the system. Hence, biofilm treatment might have an advantage in removing nitrogen, but might have limitations in terms of phosphorus removal (Kumar and Chaudhari, 2003). A hybrid system which combines suspended sludge and biofilm in a single SBR, might, therefore, be advantageous for the simultaneous removal of nitrogen and phosphorus. The results reported in this chapter are for the performance of both a suspended growth SBR and a hybrid SBR system in terms of nitrogen and phosphorus removal. The emission of nitrous oxide from these two systems was also examined.

2.2 Experimental design

2.2.1 Computer simulation for SNDPR

The simulation work was conducted using the software BioWin 2.1. The configuration of the SNDPR process of the SBR was described in Section 1.5.1.1. The combinations of variables of the simulation are listed in the row shown in Table 2.1. A cycling time of 10 hrs (anaerobic - aerobic - anoxic - post aeration – settling and decanting = 2 - 4 - 3 - 0.5 - 0.5 hr), 8 hrs (anaerobic - aerobic - anoxic - post aeration – settling & decanting = 1 - 4 - 2 - 0.5 - 0.5 hr) and 6 hrs (anaerobic - aerobic - anoxic - post aeration – settling & decanting = 0.5 - 3 - 1.5 - 0.5 - 0.5 hr) were each simulated by using influent of high strength (COD - TN - TP = 600 - 60 - 15 mg/L), medium strength (COD - TN - TP = 450 - 40 - 15 mg/L) and low strength (COD - TN - TP = 300 - 25 - 10 mg/L). The DO was set at 1.0 mg/L. As SRT = 10 and 20 days were controlled by wasting sludge from the reactor during the last 2 min of the post aeration period. The parameters used for simulation were default values, except the rapid COD and total COD ratio was 0.9 for the synthetic wastewater; the later contained a high proportion of rapidly biologically degradable substrates, such as acetate, lactose and beef extract.

2.2.2 Operation of SNDPR

The sludge seed from the UBC pilot plant was added into both reactors to establish an initial concentration of MLSS of 500 mg/L. The SRT was initially maintained at 20 days. Due to slow growth on the biofilm, the start-up period lasted for three months (Oct to Dec, 2005) after seeding. Following the start-up, an acceptable steady-state was achieved for both systems. Effluent ammonia, nitrate, nitrite, ortho-P and MLSS were measured in

order to compare the treatment performance of both systems. After 60 days (Jan to Feb, 2006), the SRT was reduced to 10 days until the end of the experiment (Apr, 2006 to Sep, 2007). Table 2.2 lists the schedule of the SNDPR system operation.

Table 2.1 Variables of simulation

SBR schedule (hr)					
Total cycling time	Anaerobic	Aerobic	anoxic	Post aeration	Settling and decant
10	2	4	3	0.5	0.5
8	1	4	2	0.5	0.5
6	0.5	3	1.5	0.5	0.5
Strength of the influent for each cycling time (mg/L)					
	COD	TN	TP		
High	600	60	20		
Medium	450	40	15		
Low	300	25	10		
SRT = 20 and 10 days were simulated for each test above					

Table 2.2 Schedule of SNDPR operation

Test stage	Date	Day
Start-up (SRT 20 days)	Oct to Dec, 2005	
SRT 20 days	Jan to Feb, 2006	1 - 60
SRT 10 days	Apr, 2006 to Sep, 2007	60 - 620

2.2.3 Comparison of two systems

Track studies of Reactor A (the suspended growth system) and B (the hybrid system) were conducted. Samples were taken every 10 to 60 min for the entire cycle. Nitrogen compounds (ammonia, nitrate and nitrite), TOC, ortho-P, N₂O off-gas, pH/ORP and DO were analyzed and monitored as described in Section 1.5.2.

2.3 Results and discussion

2.3.1 Computer simulation for SNDPR process

The objectives of the computer simulation were to determine cycling time, to have less waste sludge (MLSS), a shorter aeration time and better effluent quality. Due to limitations of the BioWin 2.1 software, only the suspended growth system was simulated. Operating the system at SRT= 20 days and cycling time = 8 hrs resulted in the lowest amounts of nitrogen and phosphorus in the effluent. The MLSS of the reactor was around 1,600 mg/L. However, the software didn't provide for the upper limitation of P accumulation. For instance, by calculation, the accumulated poly-P of sludge in this study, operated at SRT 20 days, was 15% by mass; this could be in excess of the up-take capacity of PAOs and cause excess P in the effluent. The MLSS was around 1,500 mg/L for the SRT at 10 days. The P content was calculated at around 8% by mass. This number is close to the acceptable value for PAOs.

Another limitation of BioWin 2.1 was that the software did not include nitrite and nitrous oxide simulation. That is, the software assumed all ammonia was converted to nitrate. However, the nitrite concentrations in the bulk solution cannot be ignored. In this study, nitrite concentrates were, at times, found to be higher than nitrate concentrations at the end of the treatment. Various models of 2-stage nitrification have been proposed (Iacopozzi *et al.*, 2007; Chandran *et al.*, 2000). The latest version of BioWin software, BioWin 3.0, which was published after this simulation work was done, covers nitrification, denitrification and partial nitrification. It can, therefore, simulate the concentrations of both nitrate and nitrite. A few papers have proposed mathematical models to predict the production of N_2O from denitrification (Wicht, 1996; van Schulthess and Gujer, 1996).

However, N_2O off-gas cannot be modeled by current, commercially-available simulators. Another disadvantage of BioWin 2.1 was that it doesn't have the capacity to simulate hybrid systems.

Based on the results from the limited simulation, the suspended growth system and the hybrid system were scheduled and operated at the cycling time = 8 hrs (anaerobic - aerobic - anoxic - post aeration – settling & decanting = 1 - 4 - 2 - 0.5 - 0.5 hr). The aeration rate was controlled at 200 mL/min. As SRT of 20 days and 10 days were tested experimentally, in order to understand their effects on nitrogen and phosphorus removal.

2.3.2 Experimental data of the SNDPR process

In accordance with the results from the computer simulation, both systems of SRT 20 days were operated from day 1 to day 60. The operation of SRT 10 days was from day 61 to day 620. In the time between days 180 and 550, the system operating conditions were varied for other purposes (Chapters 3 to 5). The effluent data in that operating period are, therefore, not listed in this section.

The sludge retention time for the hybrid system could not realistically be determined. Theoretically, the SRT for biofilm should be longer than the operational SRT which was maintained by wasting MLSS. Different categories of protozoa can be used as indicators of the mean age of the sludge. Also, rotifers are normally found in a sludge floc with a relatively long SRT (Gray, 2004). From the floc of the suspended growth system, only a few ciliates and rotifers were found. A relatively-high density of rotifers and a few stalked ciliates were found in the biofilm slime. No ciliates or rotifers were found in the suspended sludge of the hybrid system. This indicated that the sludge age of the biofilm was, indeed,

longer than that of the suspended sludge and the suspended growth system.

2.3.2.1 SRT 20 days

The effluent qualities of SRT 20 days are shown in Figure 2.1.

In the suspended growth system (Reactor A), ammonia was, for the most part, fully oxidized to nitrite and nitrate. Denitrification in the suspended growth system was poor. Relatively high nitrate and nitrite were detected in the effluent, and in some cases, the concentration of nitrite was higher than nitrate in the effluent. The concentrations of ortho-P in the effluent were from 0.5 mg/L to 9 mg/L. The MLSS was around 1,400 mg/L (Fig 2.2).

In the hybrid system (Reactor B), ammonia and nitrite were, in most cases, removed from the bulk solution. A nitrate residual in the effluent was usually detected. The ortho-P concentration of the effluent was similar to Reactor A. EBPR removal at SRT 20 days was not very satisfactory (effluent ortho-P was higher than 1 mg/L). The MLSS was around 1,400 mg/L (Fig 2.2). Biofilm mass during this stage was around 45 mg/carrier (Section 2.3.2.3 and Fig 2.4). The total bio mass of the biofilm was approximately 20 g.

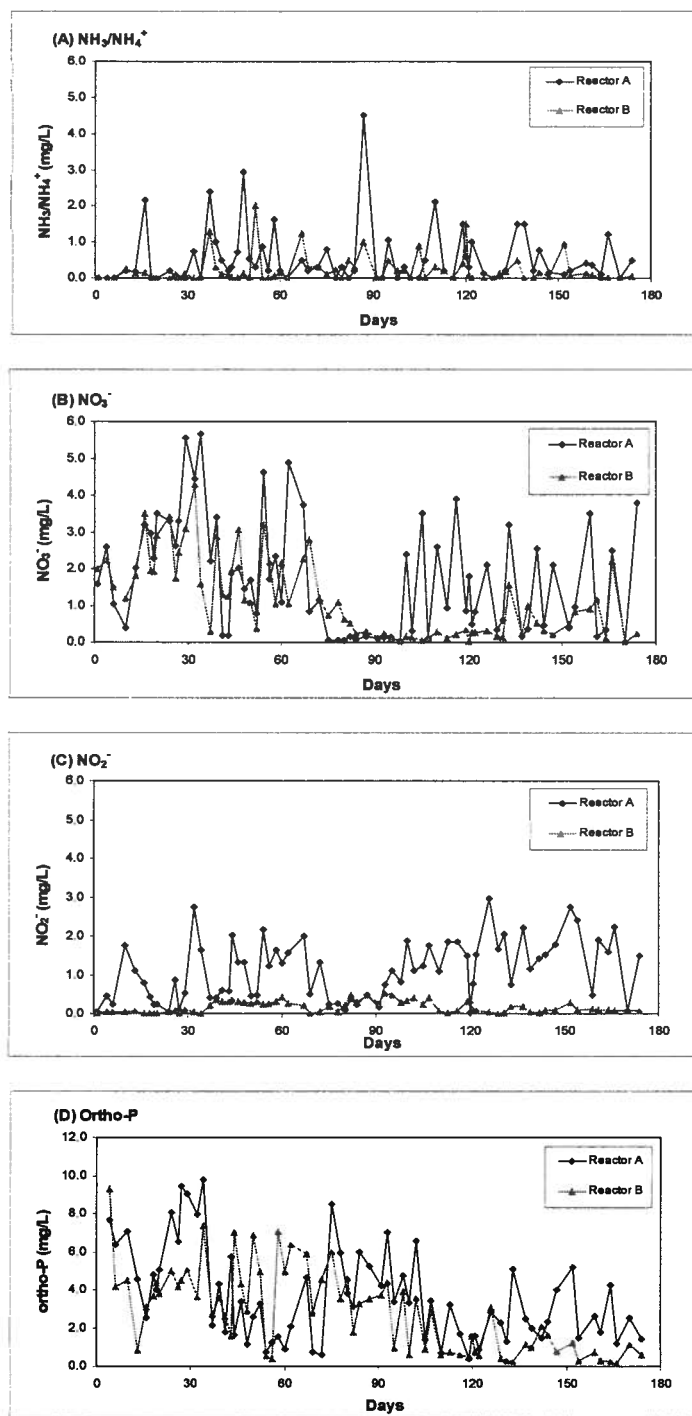


Fig 2.1 Effluent qualities of (A) ammonia/ammonium; (B) nitrate; (C) nitrite; and (D) ortho-P from day 1 to day 180. SRT was 20 days before day 60 and was 10 days after day 60. Reactor A was the suspended growth system and the Reactor B was the hybrid system.

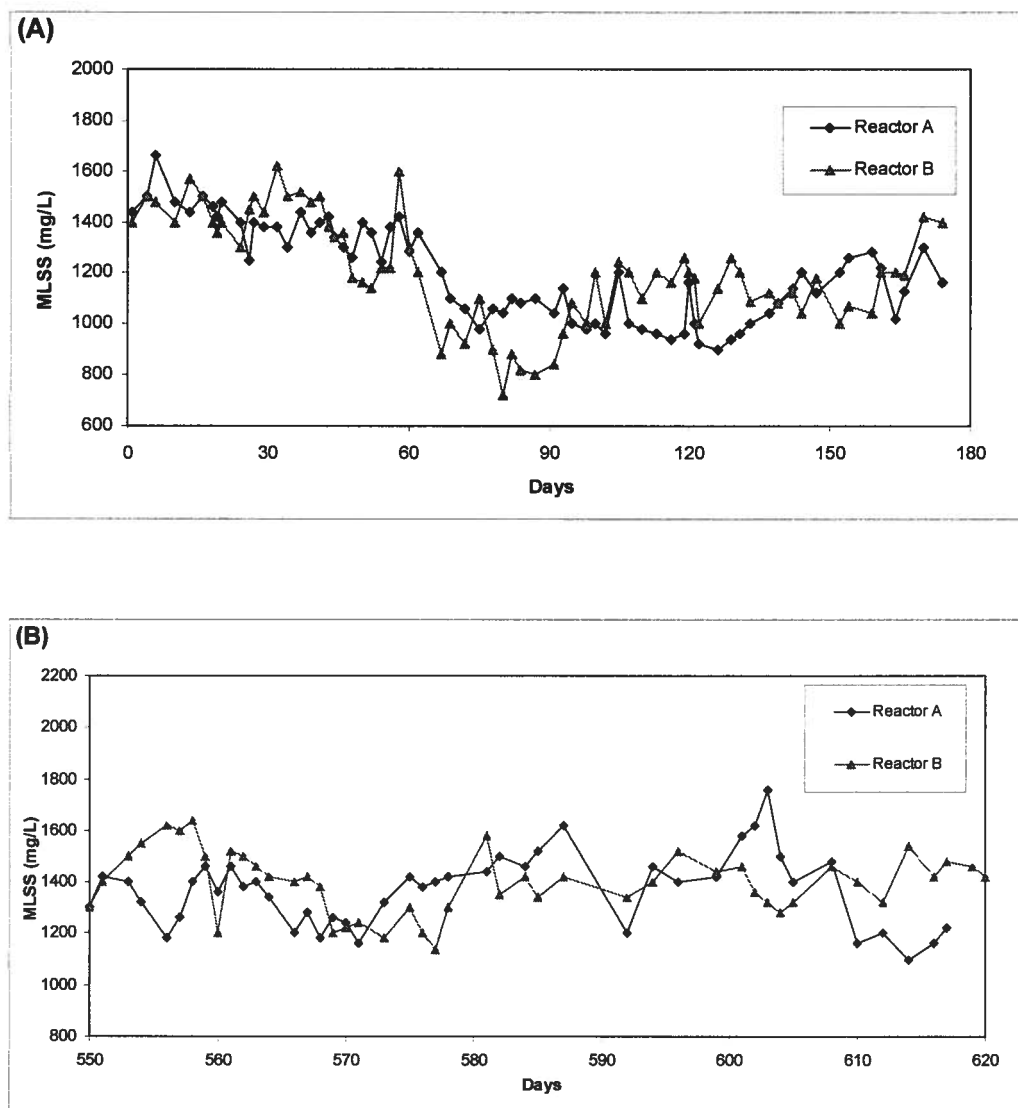


Fig 2.2 MLSS of the suspended growth system and the hybrid system over the start (A) and finish period of the experiment (B). SRT was 20 days before day 60 and was 10 days after day 60.

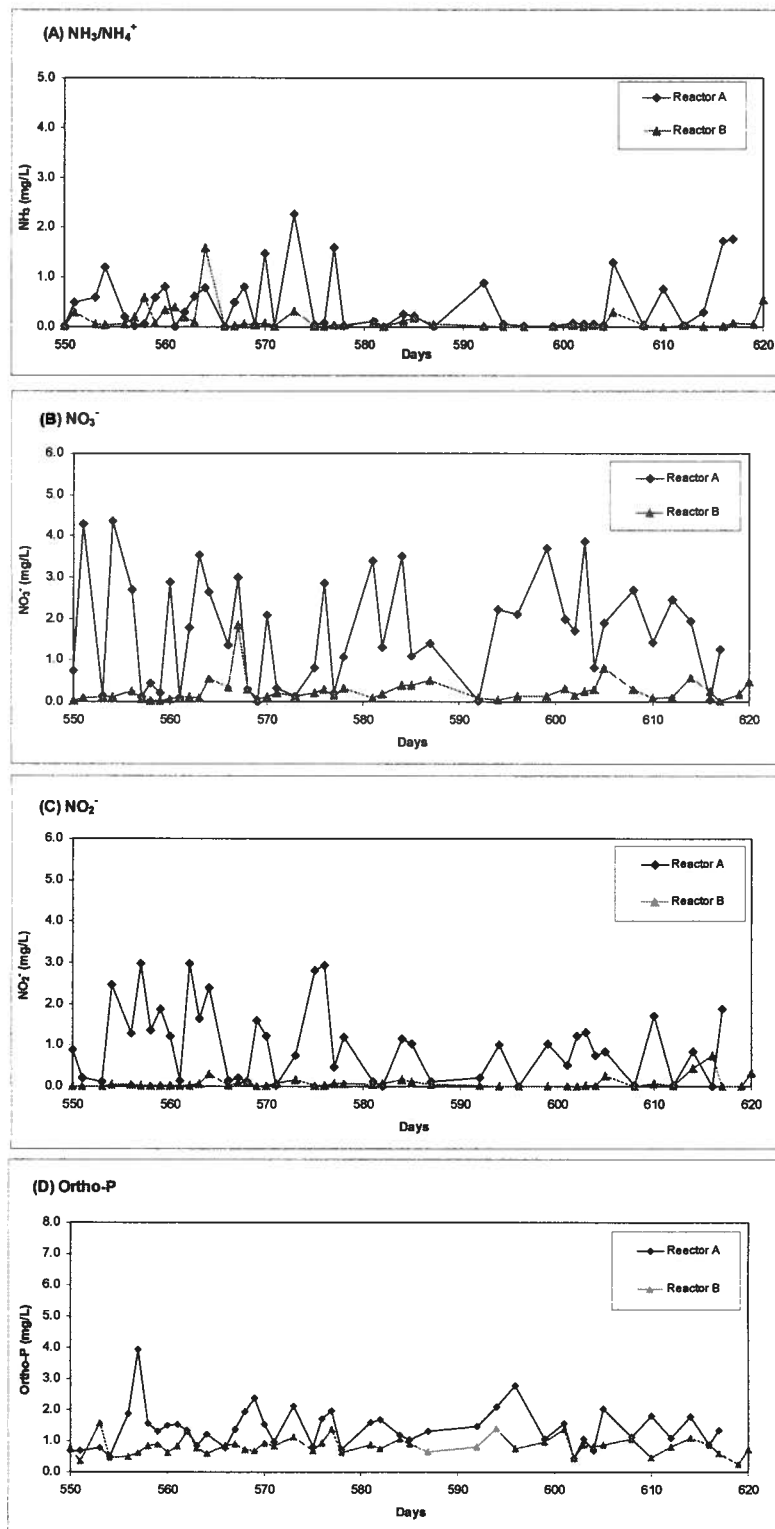


Fig 2.3 Effluent qualities of (A) ammonia/ammonium; (B) nitrate; (C) nitrite; and (D) ortho-P from day 550 to day 620.

Overall, the hybrid system grew relatively abundant biomass and had better denitrification than the suspended growth system.

From the perspective of SNDPR, an SRT of 20 days provided good nitrogen oxidation (Fig 2.1A-C). However, the phosphorus removal performance needed to be improved (Fig 2.2D). A reduction in SRT, achieved by increasing the wasting of sludge, could possibly improve phosphorus removal (Fig 2.3).

2.3.2.2 SRT 10 days

The effluent qualities of day 91 to 180 are shown on Figure 2.1.

For the suspended growth system, in most cases, the removal of ammonium, nitrate and nitrite was similar for an SRT of 20 days. The concentrations of nitrite were still high at the end of the cycle. Effluent ortho-P was reduced to a level lower than 3 mg/L (Fig 2.2). The MLSS decreased to around 1,300 mg/L (Fig 2.3).

For the hybrid system, when the SRT was set at 10 days, the MLSS was also about 1,300 mg/L, and the biofilm kept growing over time (see Section 2.3.2.3). Compared with the results from an SRT of 20 days, the concentrations of nitrate and nitrite at the end of the cycle were reduced. Better denitrification was achieved because of the increase in biofilm containing abundant denitrifiers over time (also see Section 2.3.2.3). The overall SND for Reactor A was $46.1 \pm 7.6\%$ (test number = 4); and for Reactor B, it was $75.1 \pm 5.1\%$ (test number = 5). The bio-P process was significantly improved, as well. Effluent ortho-P was reduced further and reached a level of around 1 mg/L.

Based on these results, the hybrid system operated at an SRT of 10 days had a better overall performance of SNDPR, than the suspended growth system. The hybrid

system was, therefore, selected as the system for the subsequent tests.

The long term (day 550 to day 620) effluent qualities of both reactors, with an SRT of 10 days, are shown in Fig 2.3. The results confirmed that the performance of SNDPR was not changed by varying the operating conditions for other tests, during the period from day 180 to day 550. In addition to monitoring the effluent qualities, several track studies were undertaken every few months to confirm the consistency of SNDPR performance, for long term operation. The data are presented in Section 2.3.4.

SRTs from 1 day to more than 30 days for sewage nitrification-denitrification have been used (Ahn, 2006; Kargi and Uygur, 2002). Nitrite oxidizing bacteria (NOBs) require long SRT minimum, compared with ammonia oxidation bacteria (AOBs) (Hellinga *et al.*, 1998; van Dongen *et al.*, 2001). A shorter SRT would, therefore, reduce the population of NOB. This would slow down the rate of nitrite oxidation, leading to a greater opportunity for nitrite built-up and/or nitrite reduction. However, a microbial analysis is required to prove the change in the NOB population at different SRTs. Kargi and Uygur (2002) reported there was only a slight difference in SNDPR performance between SRTs of 10 and 15 days, when tested in an SBR. However, it did have a better sludge volume index at a SRT of 10 days. If the SRT was longer than 15 days, the result was lower nutrient removal efficiencies. They, therefore, concluded that for an SNDPR system, a sludge age of 10 days would yield the best results.

Research on SNDPR using biofilm (Chuang *et al.*, 1997) found “the SRT should be controlled for longer than 10 days to achieve efficient nitrogen removal. However, an SRT of less than 12 days is deemed necessary to complete the phosphorus removal.” This conclusion is also in agreement with the results of this study.

Nitrite has been proven to act as an inhibitor of P uptake (Meinhold *et al.*, 1999; Saito *et al.*, 2004; Kuba *et al.*, 1996). Comeau *et al.* (1987) found that, with nitrite concentrations higher than 10 mg-N/L, phosphate up-take ceased. This explains the higher P concentrations at the higher nitrite concentrations in the effluent for Reactor A.

2.3.2.3 The growth of biofilm

The biofilm of the hybrid system grew slowly during the experimental period. The mass increased with an increase in the duration of the experimental period (Fig 2.4). At day 1, which was the first day after the startup period, the biomass of the biofilm per carrier was 41 ± 20 mg and had a total mass of 18.5 g. At day 120, there was 59 ± 23 mg biomass per carrier, with a total biofilm of 26.6 g. At day 550, it was 99 ± 17 mg per carrier and 44.6 g in total mass. It was 104 ± 22 mg per one piece of media, and total mass was 46.8 g at day 660 (for all biofilm mass determination, replicates of 3). From day 120 to day 550, the average net growth rate of the biofilm was around 1.2×10^{-3} mass/mass.d⁻¹. The thickness of the biofilm was around 0.9 mm at the beginning of the experiment (day 1) and it grew to a thickness of 2.2 mm by the end of the experiment (day 660). The ratio of VSS/TS of the biomass was measured as 0.83 at the end of the cycle. The reduction of SRT from 20 days to 10 days didn't seem to cause a decrease in the biomass on the biofilm.

Detachment of biofilm can be caused by hydraulic wash out or by the force of aeration bubbles striking the biofilm (Golz *et al.*, 1996). The structure of the biofilm carrier effectively reduces the shear forces of the flow and the bubbles (Fig 2.5). This allows them to provide a suitable environment for the continuous growth of microorganisms. The thickness of the biofilm limits the mass transfer. Hence, the growth of the organisms is

relatively slow and, in the inner part of the biofilm, inert biomass is found (Chang *et al.*, 2003).

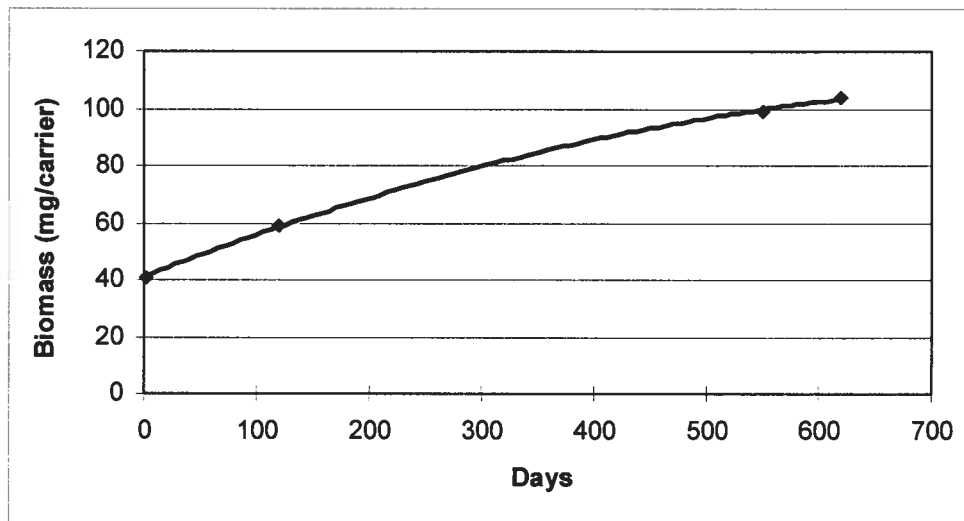


Fig 2.4 The growth of the biofilm in hybrid system

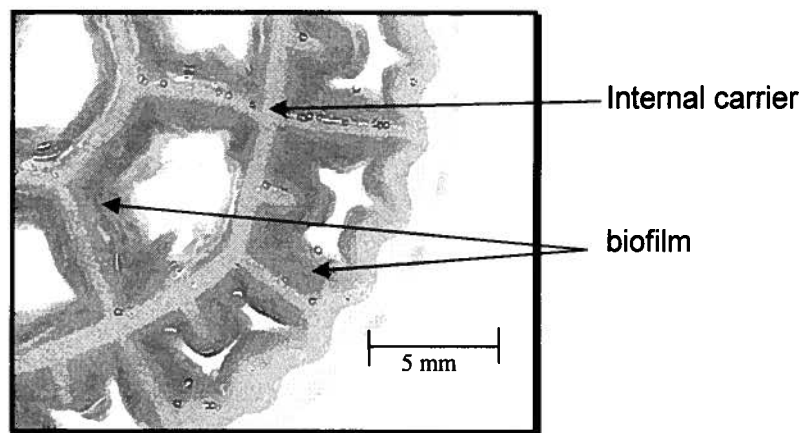


Fig 2.5 Cross-section showing one quarter of the cylindrical structure of the carrier with biofilm grown on the inside surface

2.3.2.4 The size of floc

The size of the floc was analyzed by a particle analyzer (Hydro 200G, Malvern). Sludge samples were taken at day 120. For the suspended growth system, the activated sludge floc was in a range of 7-to-275 μm with a mean value of 70 μm . The size of the suspended sludge of the hybrid system was distributed in a range from 7 to 320 μm , with a mean value of 70 μm . The mean values of the sludge size were the same for the suspended growth system and the hybrid system. Larger particles found in hybrid system might be fallen biomass from the biofilm carriers.

The VSS/TS ratios for the suspended growth system and the suspended sludge of the hybrid system were similar; they were 0.84 and 0.82, respectively.

The relationships of sludge size, DO and SND have been studied (Li and Bishop, 2004a; Li and Bishop, 2004b; Giesecke *et al.*, 2002). Satoh *et al.* (2003) suggested that an operational DO level of 0.3 to 1.1 mg/L should be used for an SND process, with sludge floc of 3 mm in diameter. Li and Bishop (2004a) reported that the aerobic region in the activated sludge floc was limited to the surface layer (100–200 μm) of the sludge aggregate, when DO was 1.2 mg/L. In other words, based on the above information, the suspended sludge in Reactor A (mean size was 70 μm) some could be fully penetrated by oxygen when the DO was around 1 mg/L and thereby lack an anaerobic zone in the sludge floc for denitrification. This could explain why there were relatively high concentrations of nitrite and nitrate in the effluent and the relatively low SND efficiency of Reactor A.

2.3.3 Track analysis

Two track studies of the suspended growth system and the hybrid system were

compared. The sequence of the cycle was: anaerobic stage 1 hr (from min 1 to 60) including 10 min of feeding (min 1-to-10), aeration 4 hrs (min 61-to-300), anoxic stage 2 hrs (min 301-to-420), post aeration 0.5 hr (min 421-to-450), settling 20 min (min 451-to-470) and discharge 10 min (min 471-to-480). The aeration rate was 200 mL/min. The strength of the influent was around 550 mg COD/L, 35 mg TN-N/L, and 12 mg TP/L, as described in Section 1.5.1.3.

2.3.3.1 Nitrogen removal

In the suspended growth system (Fig 2.6A), The MLSS of the suspended growth system was measured at about 1,280 mg/L. TN at the beginning was about 11.1 mg/L. The average ammonia oxidation rate was around 22.4 N-mg/h (the specific rate was around 1.66 mg-N/g biomass*h). Ammonia was nearly completely oxidized. There was 0.8 N-mg/L residual ammonia at the end of aeration. Nitrite accumulation was also observed. The concentration of nitrite was higher than nitrate at any time during the aeration period. This indicates that the ammonia oxidation rate was higher than the nitrite oxidation rate; hence, the AOBs were more active than the NOBs. After aeration stopped at 300 min, the reduction rates of both nitrate and nitrite were relatively low. The overall nitrogen reduction rate was 3.4 N-mg/h (the specific rate was 0.25 mg-N/g biomass*h). In the aerobic stage, nitrite concentrations were higher than nitrate concentrations at any time during the anoxic stage. The nitrite reduction rate was lower than the rate of nitrate reduction. At the end of the cycle, both nitrate and nitrite remained in the effluent.

The factors affecting nitrite buildup are pH, temperature, DO and SRT (Bernet *et al.*, 2005; van Dongen *et al.*, 2001). The effect of DO on nitrite buildup has been broadly

studied. Ruiz *et al.* (2006) reported a 65 % nitrite buildup during nitrification, at a DO around 0.7 mg/L. Bernet *et al* (2005) used a ratio of $O_2/N-NH_4^+$ at 0.1 in the bulk solution to get more than 80% of $N-NH_4^+$ converted to nitrite. In this study, the DO level was surmised to be the key factor in nitrite accumulation in Reactor A. Nevertheless, the biofilm provided greater potential for denitrification, and nitrite build-up was not observed in Reactor B.

In the hybrid system (Fig 2.6B), the MLSS was around 1,320 mg/L and the total biofilm mass was estimated as 44 g. The TN at the beginning of the cycle was around 12.5 mg/L. Ammonia was nearly completely oxidized at the end of the aeration period. The average ammonia oxidation rate was around 28.8 N-mg/h (the specific rate was around 0.48 mg-N/g biomass*h). The profiles of nitrate and nitrite were at a relatively low level and were similar to each other. The overall nitrogen reduction rate was 9.3 N-mg/h (the specific rate was 0.16 mg-N/g biomass*h). Ammonia, nitrate and nitrite were completely removed from the bulk solution, at the end of the batch test.

Although ammonia oxidation in the suspended growth system and the hybrid system were similar during the period of aeration, the hybrid system had better overall SND. SND efficiencies were 38.9 and 72.4% for Reactor A and B, respectively. In the anoxic stage, the hybrid system also had better denitrification than the suspended growth system.

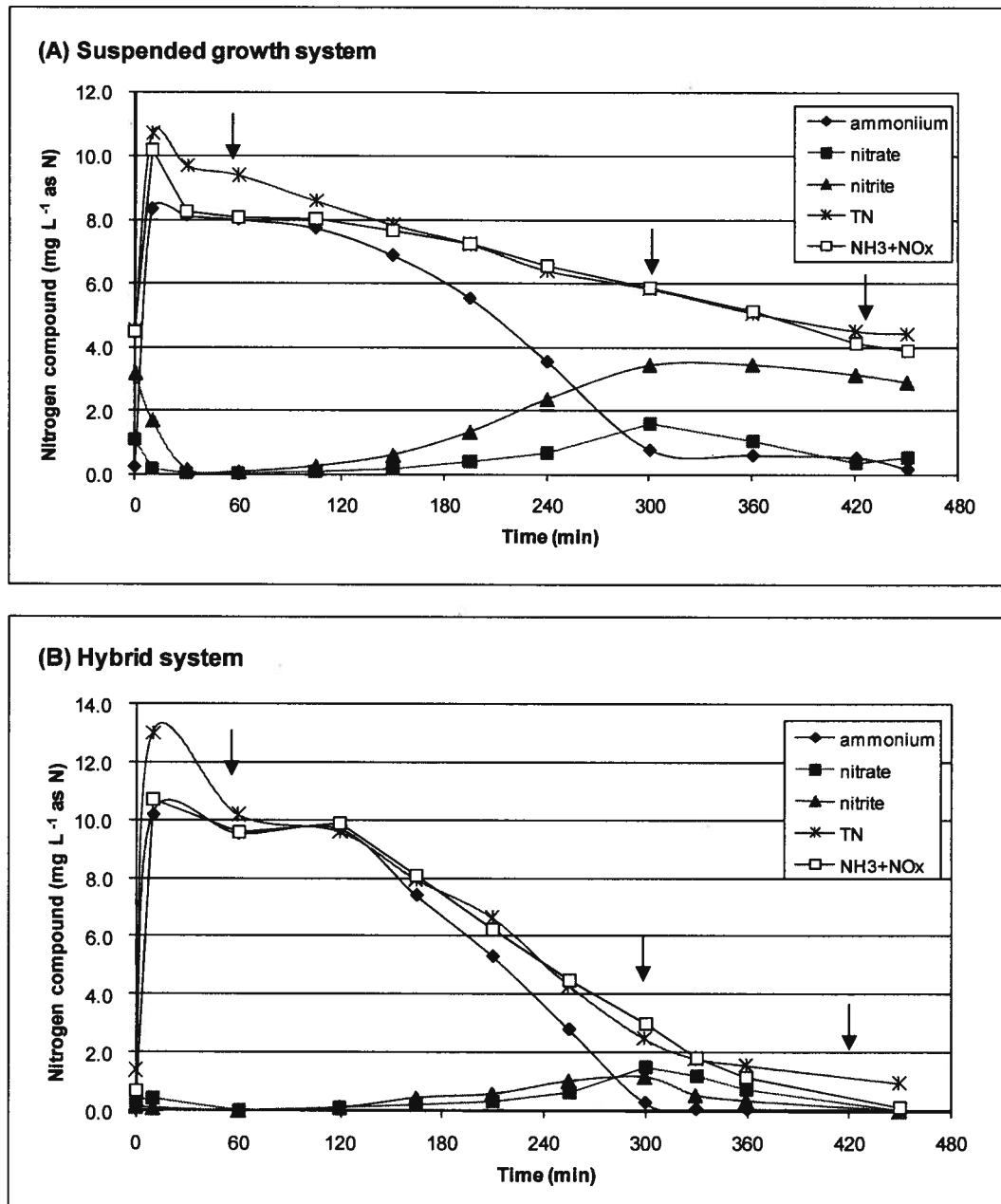


Fig 2.6 Typical time profiles of nitrogen species of (A) suspended growth system; (B) biofilm system. (Feeding min 0-to-10 min, anaerobic stage min 0-to-60, aeration min 61-to-300, anoxic stage min 301-to-420, post aeration min 421-to-450, settling min 451-to-470 and discharge min 471-to-480).

The curve of TN dropped rapidly in anaerobic phase, and it leaned to the curve of NH_3 plus NO_x at the early aeration period (Fig 2.6; also Fig 3.1-3, Fig 5.1-4, and Fig 5.6-8). In other word, the organic N of the influent (in this case, it was about the difference between the concentrations of "TN" and " NH_3+NO_x ") nearly completely diminished during the earlier period of the treatment. Organic nitrogen from beef extract was mainly taken up or trapped by the biomass. The amount of the influent organic nitrogen ($100 \text{ mg/L} \times 12.4\% = 12.4 \text{ mg/L}$) was roughly the same as the organic nitrogen discharged from the system by wasting sludge ($1,400 \text{ mg/L} \times 1/10 \times 8\% = 11.2 \text{ mg/L}$). Hence, to simplify the calculation of SND efficiency, ammonia/ammonium was reasonably considered as the "N source" converting to other N compounds during the aeration period.

2.3.3.2 Carbon and phosphorus removal

Acetate, lactose and beef extract were used as the carbon sources in the synthetic wastewater. The carbon up-take is presented as the decrease of TOC for this study. For both Reactor A and B, most of the carbon was taken up during the anaerobic phase (min 1-to-60 min, Fig 2.7A). Due to more biomass in the hybrid system, it had a more rapid uptake rate than the suspended growth system. In the suspended growth system, a portion of the carbon source was consumed during the aeration period. Final TOC concentrations for the suspended growth system and the hybrid system were 11 and 7 mg/L, respectively. The residual nitrate and nitrite from the previous cycle were rapidly reduced in the anaerobic phase, accompanied by the carbon up-take.

Ortho-P was released while the carbon source was taken up, during the anaerobic period. The highest ortho-P was obtained at the end of the anaerobic period (Fig 2.7B).

They were around 18 and 28 mg/L for Reactor A and B, respectively. When aeration started, the concentration of P decreased. Ortho-P in both Reactor A and B reached a low and stable level below 1.0 mg/L, before the end of aeration. The hybrid system had both higher P release and uptake rates, than did the suspended growth system. The PAOs in the hybrid system were more active than in the suspended growth system. The concentrations of P were not re-released during the remainder of the treatment.

2.3.3.3 Nitrous oxide production

Figure 2.8 presents the time profiles for N_2O off-gas for both the suspended growth system and the hybrid system. The N_2O off-gas production was about 19.3 and 14.9 % as TN of influent, respectively.

During aeration, the suspended growth system had less denitrification (Fig 2.6), leading to a lower N_2O emission than for the hybrid system. On the other hand, Reactor A in the anoxic phase had more nitrate and nitrite in the bulk solution, than did Reactor B. This means that more N_2O off-gas was diffused from the reactor. There were different patterns of N_2O emission from the two systems. N_2O off-gas from the suspended growth system reached its highest point at around 220 minutes. For the hybrid system, the peak emission time occurred later, at around 270 minutes. The most likely reason for the earlier peak in the suspended growth was due to poor denitrification that hindered nitrite reduction to nitrous oxide. The second N_2O off-gas peak of Reactor A shown in Fig 2.8A (from 420 min to 450 min) was caused by air stripping during post-aeration. In Reactor B, N_2O reduction was complete and there was no second peak of N_2O off-gas. Although the SND of the suspended system was not as high as in the hybrid system, there was more

N₂O off-gas produced than was the case with the hybrid system.

The measured N₂O off-gas included the part of being stripped out during the aerobic phase and the part of surface diffusion during the anoxic phase. For Reactor A, the mass of the N₂O emissions during the aeration period (60–300 min), anoxic period (301–420 min) and post aeration (421–450 min) were 10.87, 6.08 and 3.98 mg N (20.93 mg N in total), respectively; when modified by TN in the effluent, the off-gas productions were 10.0%, 5.6% and 3.7% (19.3% in total), respectively. For Reactor B, the mass of the N₂O emissions during the aeration period (60–300min) and anoxic period (301–420) were 16.91 and 4.59 mg N (21.50 mg N in total, there was no N₂O emission from post aeration), respectively; when modified by TN in the effluent, the off-gas productions were 11.7% and 3.2% (14.9% in total), respectively.

During the aerobic phase, the highest concentration of N₂O off-gas from the suspended growth reactor was lower than that from the hybrid reactor. However, the accumulated nitrite reduced to nitrous oxide kept emitting to the air during the anoxic phase plus the large amount of off-gas striped out from post aeration, the total amount of N₂O off-gas of the suspended growth system was larger than that of the hybrid system. Although the number of N₂O emission of reactor A and B were not significantly different, when the relatively high residual nitrite and nitrate in the effluent (Fig 2.6A) reduce in the receiving water body, it is expected that there is extra N₂O emission form the receiving water body. Thus, it indicates that the hybrid system has better potential to reduce the production of nitrous oxide during the SND process.

SRT is a factor affecting N₂O emission. Noda *et al.*, (2003) tested N₂O emission at different SRT from 7 to 20 days and summarized “the results showed that under low SRTs

conditions, nitrification efficiency was reduced and the N_2O emission rate in the oxic reactors was increased". The relatively long SRT of the biofilm might be a benefit in N_2O reduction.

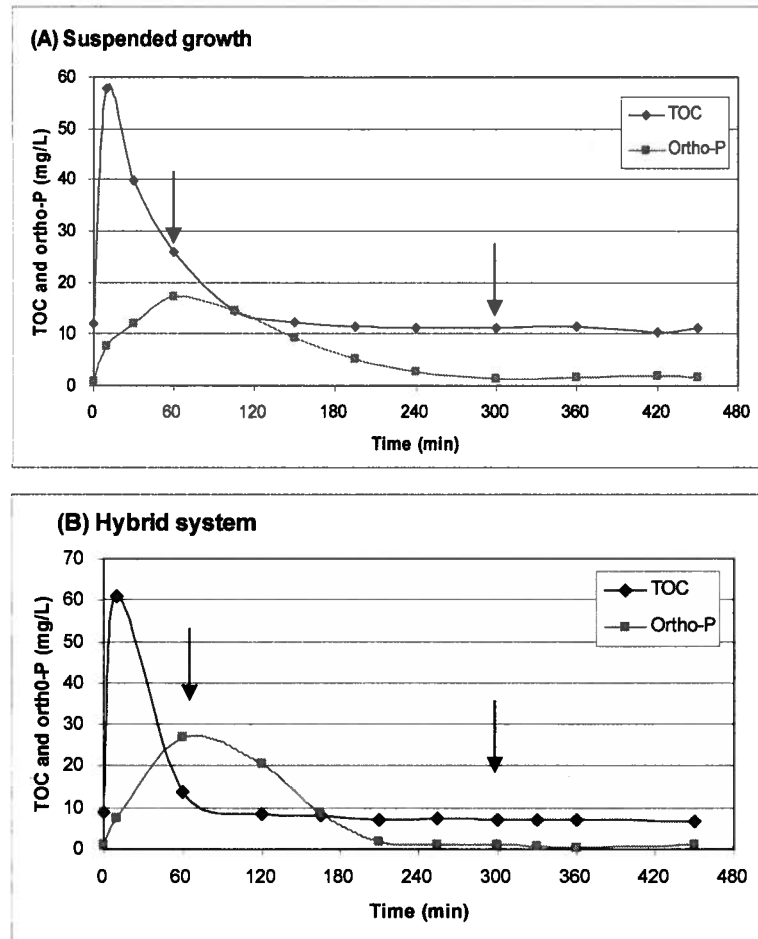


Fig 2.7 Typical time profiles of carbon utilization and ortho-P of (A) suspended growth system; (B) biofilm system. (Feeding min 0-to-10 min, anaerobic stage min 0-to-60, aeration min 61-to-300, anoxic stage min 301-to-420, post aeration min 421-to-450, settling min 451-to-470 and discharge min 471-to-480).

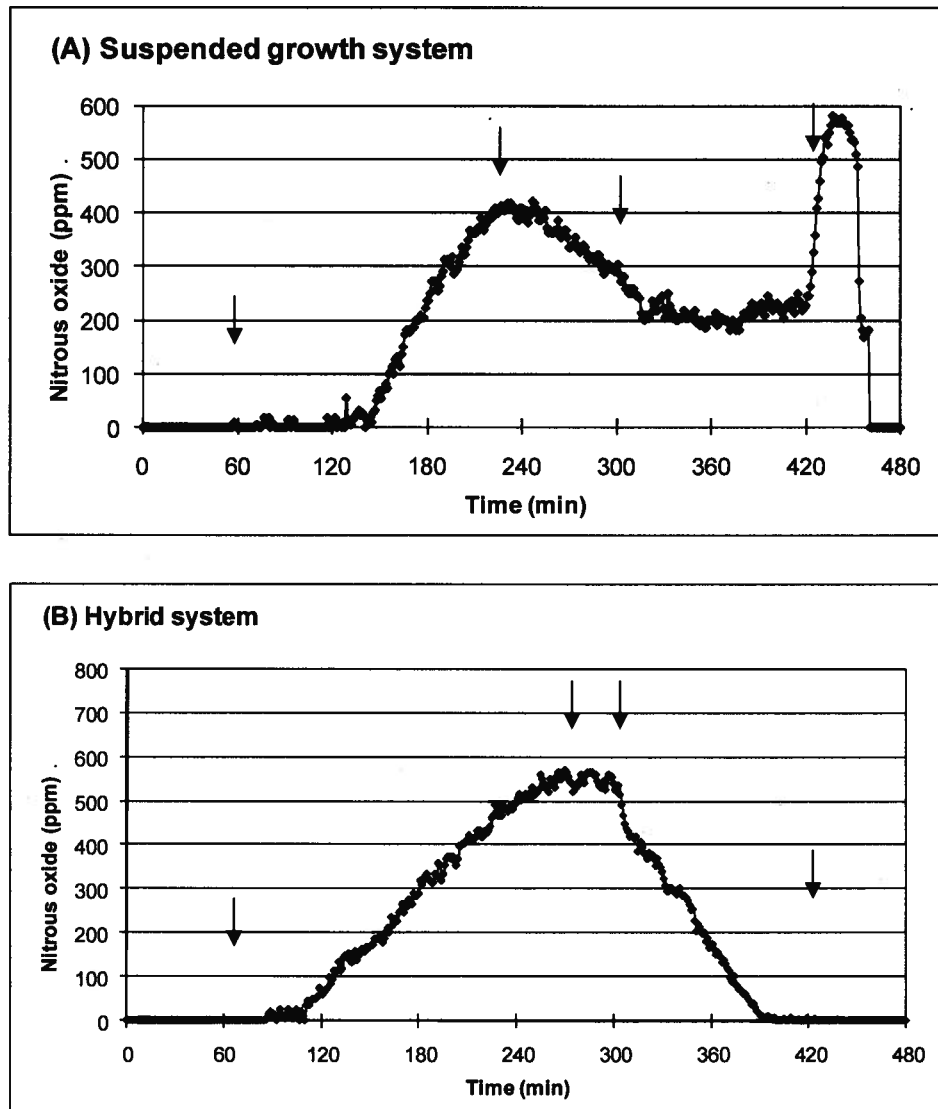


Fig 2.8 Typical time profiles of nitrous oxide off-gas of (A) suspended growth system; (B) biofilm system. (Feeding min 0-to-10, anaerobic stage min 0-to-60, aeration min 61-to-300, anoxic stage min 301-to-420, post aeration min 421-to-450, settling min 451-to-470 and discharge min 471-to-480).

2.3.3.4 pH/ORP/DO profiles

The pH/ORP and DO profiles of the suspended growth system and the hybrid system are shown in Figure 2.9 and 2.10, respectively.

- Anaerobic phase (time 0-to-60 min)

The up-take of carbon sources and the release of phosphate in anaerobic phase caused an increase in the pH value. The existence of organic carbon led to a sudden drop of ORP to lower than -200mV in the first 20 min. The decrease of ORP slowed down when the carbon sources were used up. During the anaerobic phase, Reactor B had greater reduction capacity than Reactor A. At the end of the anaerobic phase, The ORP for Reactor A was around -240 mV, while it was around -290 mV for Reactor B.

- Aerobic phase (time 61-to-300 min)

ORP immediately rose when air was supplied into the reactors. The rise of DO was not evident at the beginning of the aeration period, due to a high oxygen uptake rate for carbon utilization. When the heterotrophic carbon utilization decreased and ammonia oxidation became the dominant reaction, the DO rose and pH declined. The DO of Reactor A increased from 0 to 2 mg/L; the DO of Reactor B remained at a lower level, below 1 mg/L. When ammonia was completely oxidized, a rapid increase in DO was observed. The ORP ranges of ammonia oxidation in both Reactor A and B were between -120 to 70 mV. The ORP profile of Reactor A was slightly higher than for Reactor B, even though the ammonia oxidation was not complete in Reactor A. When ammonia oxidation was completed, there was an increase in pH due to the halt in alkalinity consumption and the stripping out of carbon dioxide from the bulk solution. In Reactor A, ammonia oxidation

was not completed. No pH rise occurred during aeration.

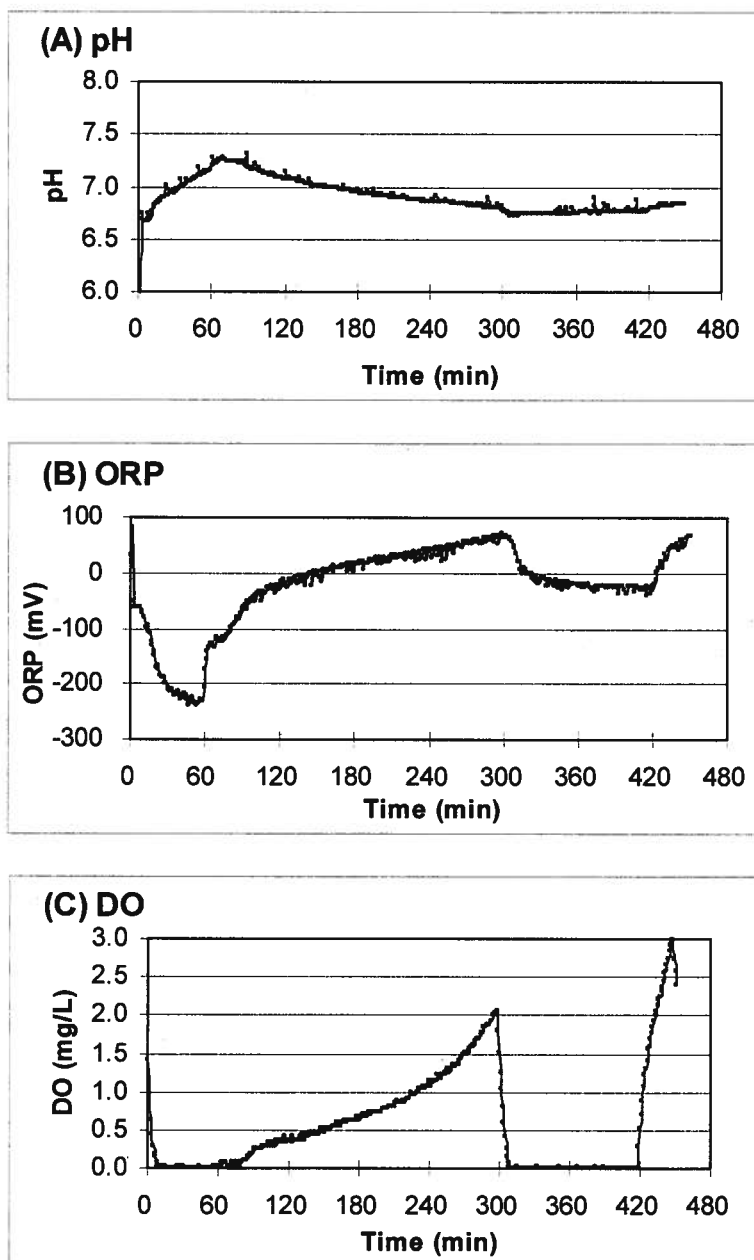


Fig 2.9 Typical time profiles of pH/ORP/DO of the suspended growth system

(Feeding min 1-to-10, anaerobic stage min 1-to-60, aeration min 61-to-300, anoxic stage min 301-to-420, post aeration min 421-to-450, settling min 451-to-470 and discharge min 471-to-480).

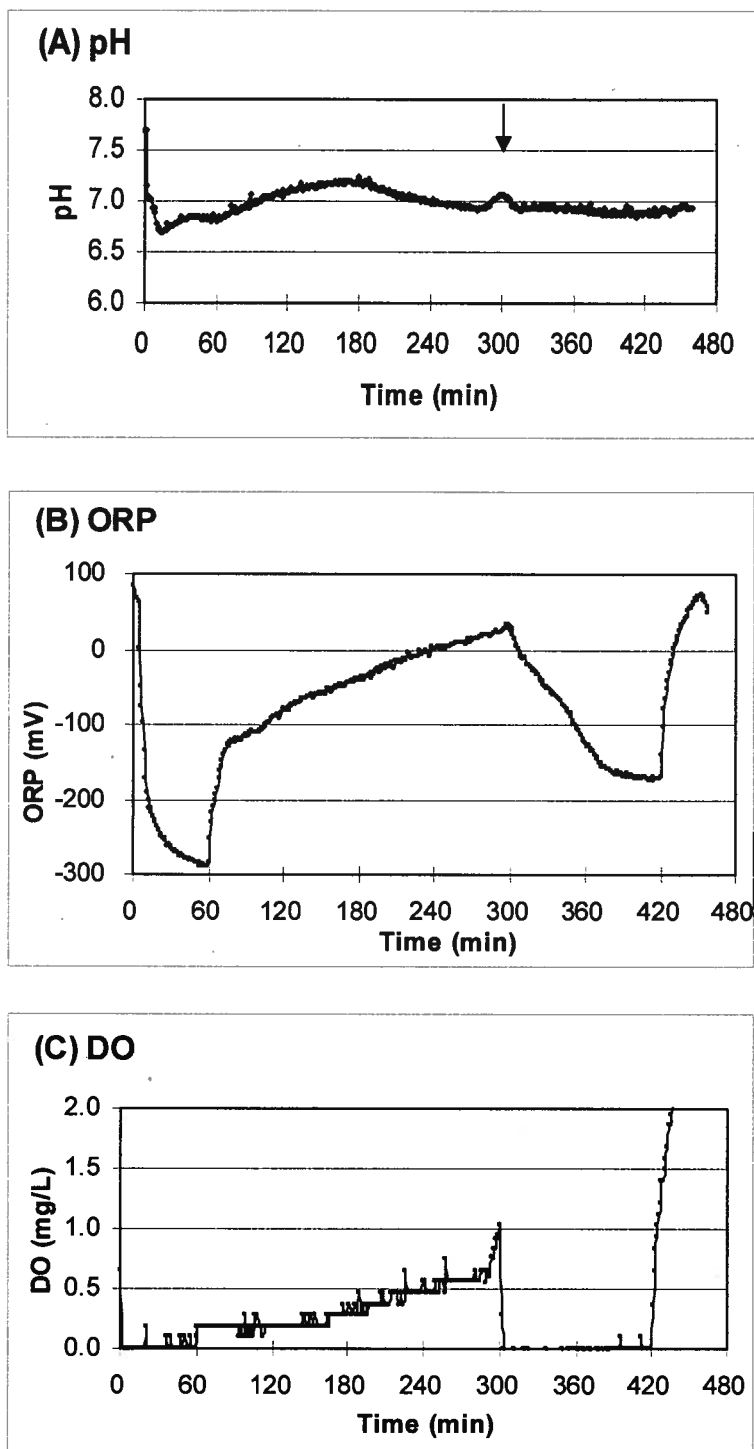


Fig 2.10 Typical time profiles of pH/ORP/DO of the hybrid system (Feeding min 1-to-10, anaerobic stage min 1-to-60, aeration min 61-to-300, anoxic stage min 301-to-420, post aeration min 421-to-450, settling min 451-to-470 and discharge min 471-to-480).

- Anoxic phase (time 301-to-420 min)

The ORP profile during the anoxic phase provided information on the denitrification process. Fig 2.6 shows that denitrification in Reactor A was relatively slow and was incomplete during the anoxic phase. For Reactor B, denitrification was relatively rapid and was completed at around time 390 minutes. The ORP profile of Reactor A in the anoxic phase remained at around -20 mV; for Reactor B, it continued dropping and flattened out at around -180 mV. Fig 2.8B shows that no N_2O off-gas was detected at the end of the anoxic phase (around 420 minutes). It also indicates the full reduction of nitrite and nitrate. Summarizing the ORP profiles from this and other track studies (data not shown); a value of -180 mV could be applied as an indicator of the end of denitrification.

In this case, a pH increase indicated the completion of ammonia oxidation. It can be applied to the control point of ammonia oxidation. The ORP trend could indicate the oxygen state in the bioreactor and could be applied to control the end point of denitrification. A sudden rise in DO also shows that ammonia oxidation in the bulk solution was complete.

2.3.4 Long term performance

In order to confirm the performance of SNDPR for both Reactor A and B for a long term period of operation (635 days in total), track studies were conducted every couple of months. Table 2.3 lists the results at the end of the aeration. In the operation period with an SRT = 10 days, the SND of Reactor A ranged from 27 to 40 % (30.50 ± 5.63), and N_2O off-gas was from 18 to 27 % (22.25 ± 4.19). The SND of Reactor B ranged from 64 to 75 % (69.54 ± 3.88) and N_2O from 15 to 22 % (18.72 ± 3.09). In this experimental period, Reactor

A usually had relatively high nitrite in the bulk solution. The results indicate that (1) there was no significant shift of microbial activities, in terms of SNDPR, for the long term operation, and (2) the hybrid system had better overall SNDPR performance than the suspended growth system.

Table 2.3 Track studies for assessing consistency of long term performance^a

Reactor	Day of Track	NH ₄ ⁺ (mg/L)	NO ₃ ⁻ (mg/L)	NO ₂ ⁻ (mg/L)	N ₂ O (%)	SND (%)	Ortho-P (mg/L)
A	35 ^b	0.12	2.49	2.02	--	23.8	3.12
	230	0.13	2.39	4.65	24.2	28.3	1.94
	420	0.03	2.71	4.35	27.2	27.9	0.38
	470	0.19	3.85	2.87	18.3	26.9	0.10
	555	0.55	1.58	3.45	19.3	38.9	1.02
	Ave ^c	0.23	2.36	3.83	22.25	30.5	0.86
	Stdev ^c	0.22	0.94	0.82	4.19	5.63	0.82
B	35 ¹	0.21	2.40	1.10	--	48.1	3.39
	240	0.05	2.19	0.32	22.1	67.6	0.20
	380	0.45	1.78	0.41	22.0	68.4	0.31
	470	0.04	1.56	0.84	17.2	64.8	0.10
	560	0.25	1.52	1.20	15.6	72.4	0.25
	595	0.02	2.10	0.55	16.7	74.5	0.29
	Ave ^c	0.16	1.83	0.66	18.27	69.54	0.23
	Stdev ^c	0.19	0.31	0.36	3.09	3.88	0.08

Note : a. The listed data were at the end of the aeration.

b. SRT = 20 days, the other tracks were SRT 10 days.

c. Average and standard deviation do not including the track of SRT 20 days.

2.4 Conclusions

The experiments using the suspended growth system and the hybrid system in a SBR for simultaneous nitrification, denitrification and phosphorous removal showed that:

- The operation of an SBR including feeding, anaerobic, aeration, anoxic, post-aeration, and settling/discharge stages can be applied to SNDPR.
- Data indicate that the hybrid system had better overall effluent qualities, SND efficiencies and lower emissions of N_2O , than the suspended growth system.
- Phosphorus removal was more reliable at an SRT of 10 days than at SRT of 20 days. An SRT of 10 days was therefore selected for the remainder of the study.
- The relatively small size of the suspended sludge made it difficult to maintain an anaerobic zone. This resulted in poor denitrification. The intentional maintenance of low DO for denitrification purposes led to nitrite accumulation. The hybrid system, using biofilm, provided a solution to the above problems and therefore proved to be superior to the conventional suspended growth system, in terms of SNDPR.

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Chapter 3 Contributions of biofilm and suspended sludge in the hybrid system to SNDPR

3.1 Introduction

The following three microbial populations are included in an SNDPR system: ammonia oxidation bacteria (AOBs), nitrite oxidation bacteria (NOBs), and phosphorous accumulating organisms (PAOs, including denitrifying PAOs). NOBs need a longer SRT than AOBs (van Dongen, 2001). On the other hand, to maintain good P-removal, sludge containing stored poly-P needs to be withdrawn from the reactor and new cells made available to take up phosphorus from the bulk solution. Therefore, the SRT for PAOs has to be maintained below a certain level (Chapter 2; Chuang *et al.*, 1997). The hybrid system integrates biofilm within a suspended-growth system to maximize the hold-up and the SRT of sludge in the biofilm, which has the potential of operating with a relatively short SRT. Another advantage of a hybrid system in the shortcut denitrification process is to encourage rapid denitrification of nitrite, when it is produced by the ammonium oxidizers (Chung *et al.*, 2006). In addition, generally, a hybrid system has the advantage of a short hydraulic retention time (HRT) and a small system volume, due to the accumulation of

*Two papers from this chapter will be submitted for publication:

Lo, I., Lo, K.V., Mavinic, D.S., Shiskowski, D. and Remay, W. Contributions of Biofilm and Suspended Sludge to Carbon Conversion and Phosphorus Removal in a Hybrid System for Simultaneous Nitrification, Denitrification and Phosphorous Removal.

Lo, I., Lo, K.V., Mavinic, D.S., Shiskowski, D. and Remay, W. Contributions of Biofilm and Suspended Sludge to Nitrogen Transformation and Nitrous Oxide Emission in a Hybrid Sequencing Batch System for Simultaneous Nitrification, Denitrification and Phosphorous Removal.

biofilm biomass along with suspended biomass. The accumulation of biomass also provides stability to the system.

In a hybrid system, the biofilm and the suspended sludge may function differently, in terms of nitrogen and phosphorus removal. Falkentoft *et al.* (2001) has mentioned that diffusion limitations hindered biological phosphorus removal in the biofilm. Kumar and Chaudhari (2003) also found that both the suspended sludge and the hybrid system achieved greater than 90% ammonia oxidation. However, the nitrogen removal efficiency in a suspended growth SBR was only 48%, where as nitrogen removal was in excess of 62% in biofilm SBRs. As reported in Section 2.3.2.2 of this thesis, SND efficiencies for the hybrid system and the suspended growth system were on average, 75.1 ± 5.1 % and 46.1 ± 7.6 %, respectively. It was also reported that the emissions of nitrous oxide were also different; these averaged 15 and 19% for the hybrid system and the suspended growth system, respectively. The different N_2O emissions may be due to the microbial communities of the suspended sludge and the biofilm systems.

To date, few studies have examined the effects of the biofilm and the suspended sludge on removal efficiency in a hybrid SNDPR system. The effects of sludge makeup on nitrogen, phosphate removal and N_2O emissions were therefore investigated.

3.2 Experimental design

3.2.1 Track studies of the biofilm, the suspended sludge and hybrid system

Only the hybrid system (Reactor B), which contained the suspended sludge and the biofilm carriers, was chosen for this study. All biofilm carriers in Reactor B were split from the mixed liquor and transferred to another reactor, with the effluent of treated synthetic wastewater. Thus, one reactor had suspended sludge only and another reactor had biofilm only. The wastewater strength of the influent for the tests was 65% of the normal synthetic wastewater as presented in Section 1.5.1.3. In order to compare N_2O emission, the aeration rate was controlled instead of maintaining a constant DO level. Two reactors were run in parallel with an aeration rate of 200 mL/min. Samples were taken every 10 to 30 min in order to determine ammonia/ammonium, nitrite, nitrate, N_2O (off-gas and in liquid), NO (off-gas and in liquid), NH_2OH , TOC and ortho-P. MLSS, TN and TP were measured at the end of the cycle.

3.2.2 Heterotrophic denitrification tests

Heterotrophic denitrification is defined here as denitrification which occurs when organisms use organic carbon as an energy source. Heterotrophic denitrification of the biofilm, the suspended sludge and the hybrid system were tested under conditions with internal carbon substrates, but without DO and ammonia. In these tests, 0.4 g of NaNO_3 (initial concentration of the reactor was approximately 7 mg N/L) was spiked into the reactors instead of feeding influent. Tests were conducted anaerobically for 2 hours. Samples were taken every 20 min for nitrite and nitrate analysis.

3.2.3 Carbon conversion and P removal tests

Different carbon sources form different types of stored carbon. For instance, acetate forms PHA and glucose forms glycogen (Oehmen *et al.*, 2007). Moreover, different carbon sources might have different utilization rates by different organisms. Instead of sole carbon source, the influent contained acetate, lactose (hydrolysis to yield two simple sugars – glucose and galactose) and beef extract (Section 1.5.1.3). Thus, the conversion of (1) acetate (spiked as sodium acetate 2 g; initial concentration was approximately 55 mg/L as TOC), (2) lactose (1.5 g; initial concentration was approximately 55 mg/L as TOC) and (3) the mixture of acetate, lactose and beef extract (1.2, 0.4 and 0.2 g, respectively; initial concentration was approximately 55 mg/L as TOC) in the biofilm and the suspended sludge were investigated. TOC was measured to indicate the amount of external carbon. Samples were taken for TOC and ortho-P analyses. Sludge samples were also taken for determining internal storage carbons of PHA and glycogen. The sampling procedure and analyses were described in Chapter 1.

3.2.4 Denitrifying phosphorus removal test of the hybrid system

Denitrifying phosphorus removal is a microbial mechanism for phosphorus removal, which uses nitrate and/or nitrite as an electron acceptor instead of oxygen. Denitrifying phosphorus removal was tested in the hybrid system. Around 55 mg C/L (as TOC) of initial concentration of acetate and lactose and the mixture of acetate and lactose were added in the anaerobic stage as carbon sources. After 60 min of carbon up-take, approximately 7 mg N/L of nitrate and nitrite were spiked into separate reactors as the electron acceptors. Samples were taken for TOC, soluble ortho-P, nitrate and nitrite analyses.

3.3 Results and discussion

3.3.1 Nitrogen removal

The tests of the biofilm, the suspended sludge and the hybrid system were carried out in parallel under the same operational conditions. Given that the biomass in the suspended sludge reactor and the biofilm reactor was less than in the hybrid system, the strength of the influent for the biofilm and the suspended sludge was reduced to 65% of the influent of the hybrid system.

The aeration time lasted until the ammonium was fully oxidized. The results indicated that the ammonia oxidation rates (AOR) for the three tests were different. The aeration time for the suspended sludge and hybrid system tests were 240 and 270 min, respectively, while it was 330 min for the biofilm test, due to its relatively low ammonia oxidation rate (AOR). Time profiles for nitrogen compounds, TOC, ortho-P, DO, ORP and N₂O off-gas of the biofilm, the suspended sludge and the hybrid system are shown in Figures 3.1-3.3, respectively.

In these tests, the MLSS of the suspended sludge was measured as 1,200 mg/L (12.6 g in the reactor) and the total biomass of the biofilm was 44.6 g. The ratio of biomass in suspended sludge reactor and the biofilm was 1 to 3.5. In the hybrid reactor, the total biomass was 57.2 g. The VSS/TS was around 0.8 for both the suspended sludge and the biofilm. TKN was $7.2 \pm 3.4\%$ and $8.4 \pm 2.9\%$ (test number = 3) as dry mass for the biofilm and the suspended sludge, respectively. Using the formula for cells ($C_{60}H_{87}O_{23}N_{12}P$; Metcalf & Eddy, 2003), a nitrogen level of 12.2% of dry mass can be expected. The reason for the lower TKN content in this study was due to the characteristics of the BNR organisms which contain stored phosphate and carbon inside the cells and bias the total

mass away from nitrogen.

The total nitrogen removal of the biofilm, the suspended sludge and the hybrid system around 49.5, 26.7 and 71.5%, respectively.

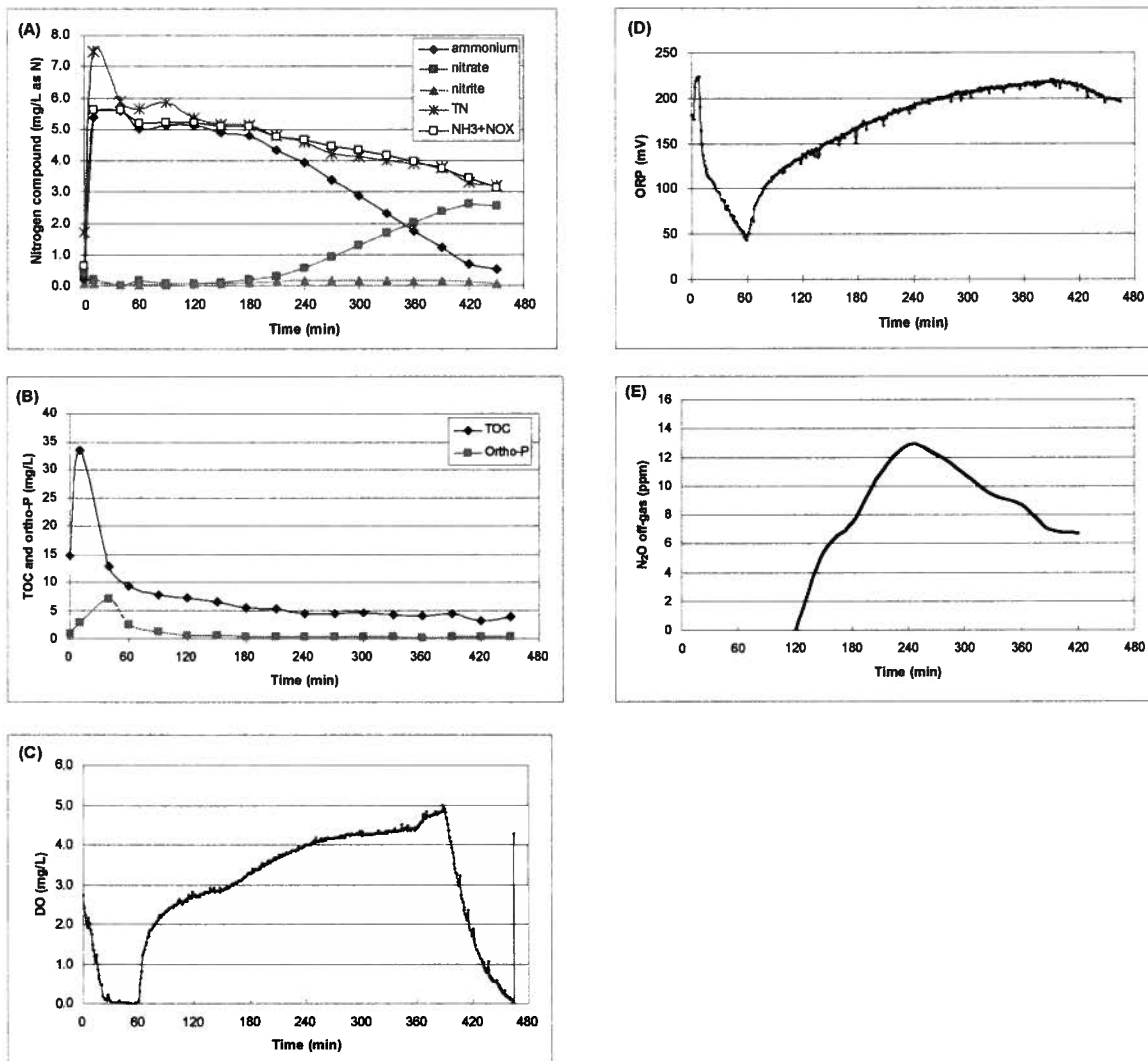


Fig 3.1 Time profiles of the biofilm (A) nitrogen; (B) TOC and ortho-P; (C) DO; (D) ORP; (E) N₂O. (The aeration was from time 60 min to 390 min)

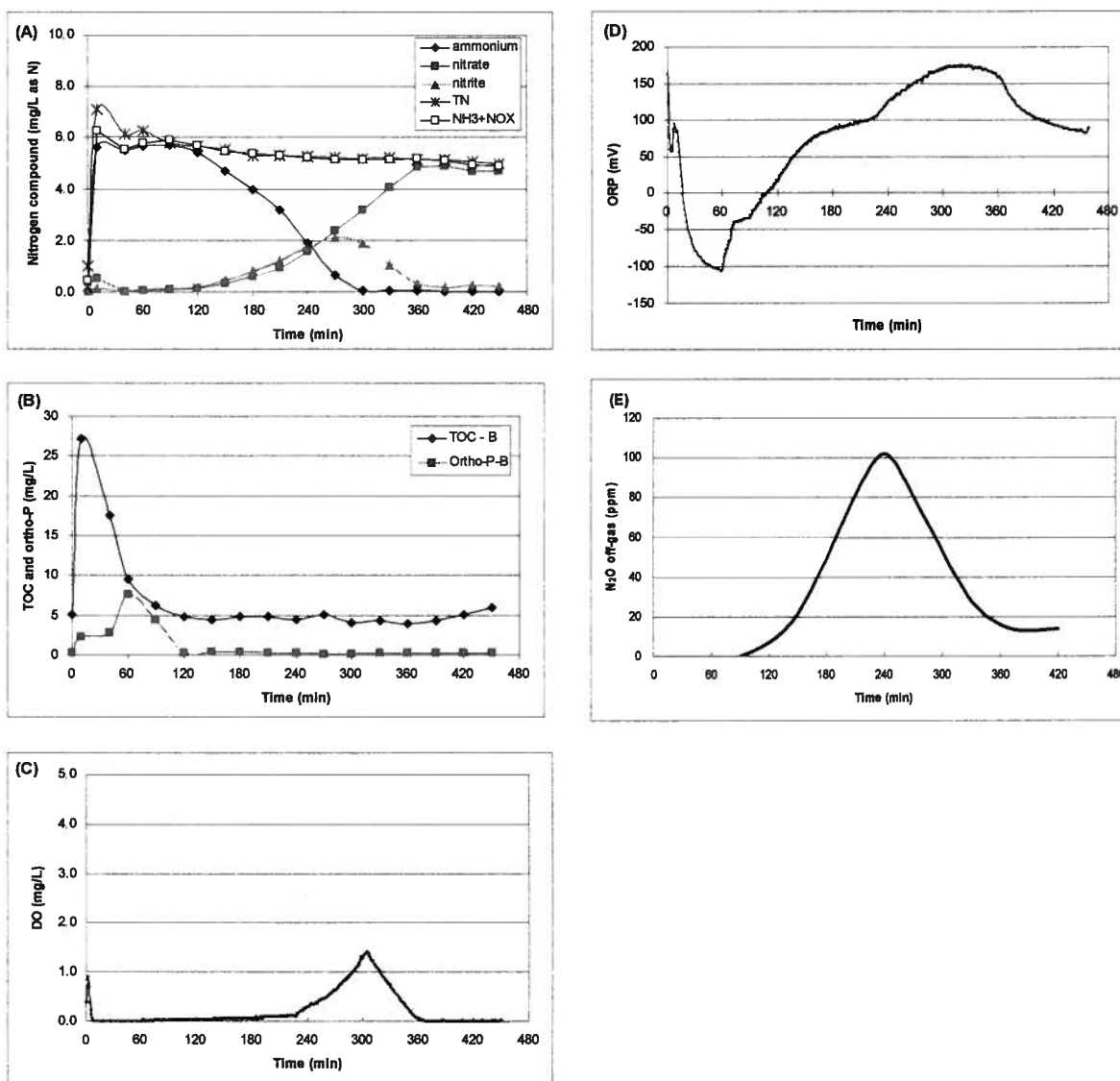


Fig 3.2 Time profiles of the suspended sludge (A) nitrogen; (B) TOC and ortho-P; (C) DO; (D) ORP; (E) N_2O . (The aeration was from time 60 min to 300 min)

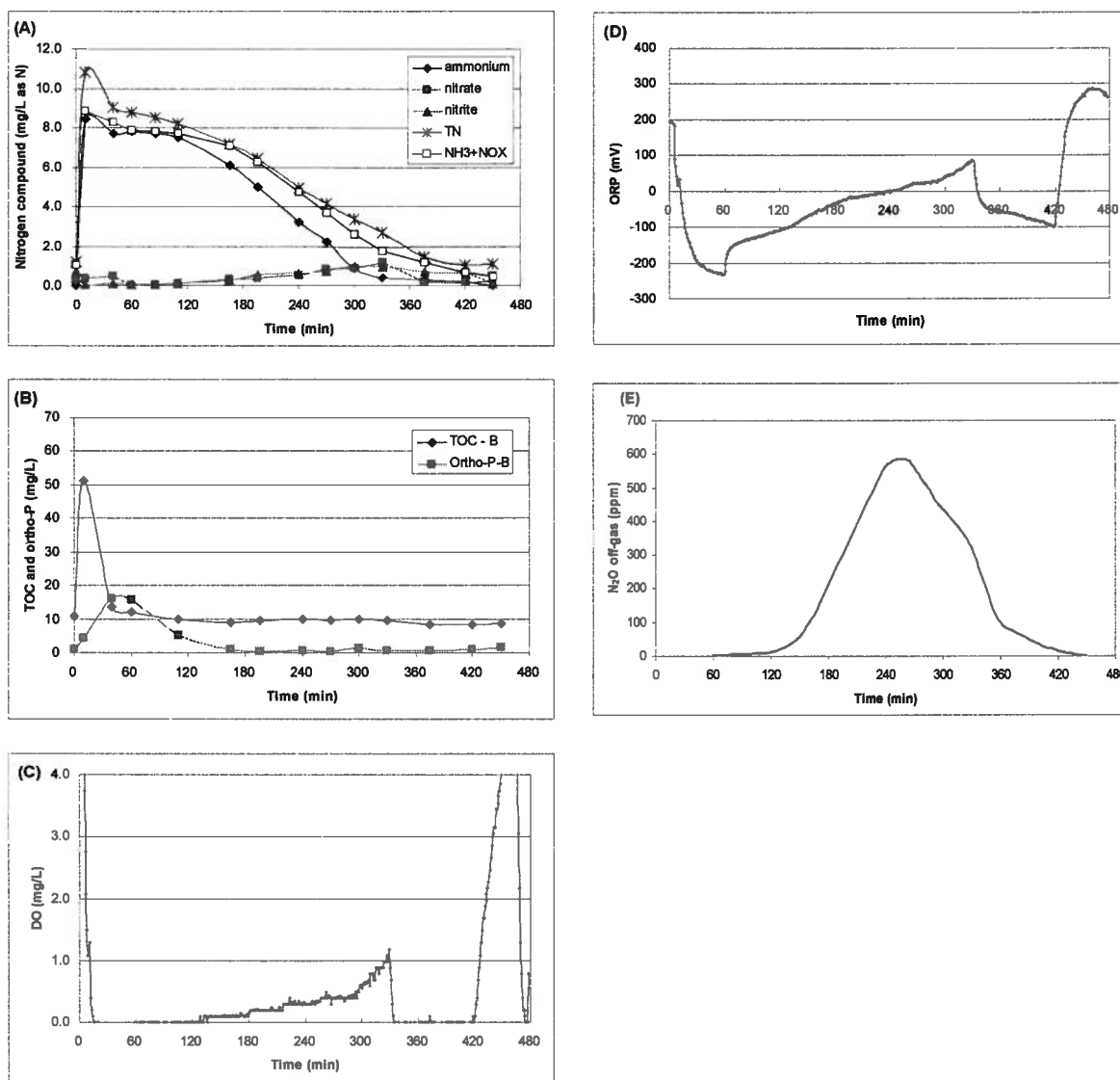


Fig 3.3 Time profiles of the hybrid system (A) nitrogen; (B) TOC and ortho-P; (C) DO; (D) ORP; (E) N₂O. (The aeration was from time 60 min to 330 min)

3.3.1.1 Nitrification

In the biofilm test, ammonia oxidation was relatively slow at a rate of 0.24 mg N/g biomass^{*h}. The lower the AOR, the slower the oxygen up-take was. It also resulted in a relatively higher DO level in the reactor. Comparing the DO of the biofilm system with the suspended sludge and the hybrid systems, the biofilm system had a higher DO level during the aeration time (Fig 3.1C). This higher DO facilitated nitrite conversion to nitrate (Fig 3.1A). Nitrite build-up did not happen in the biofilm system. Nitrite concentrations at anytime during the test were below than 0.2 mg/L.

The slow AOR led to a low nitrite production rate. Once the rate of nitrite conversion was greater or equal to the nitrite production rate, nitrite concentrations in the solution could be very low or even zero. The diminishment of nitrite might be caused by nitrite oxidation, heterotrophic denitrification, and/or autotrophic denitrification. The emission of N₂O from the biofilm was only 0.5% of TN (see Section 3.3.1.4). The thickness of the biofilm can hinder the transfer of oxygen. As a result, the inner layer of the biofilm is in an anaerobic condition (Chang *et al.*, 2003; Stewart, 2003; Jun *et al.*, 2003). Some published materials reported on a limited penetration depth of biofilm (e.g. 0.5 mm) under low DO conditions (e.g. 1 mg/L) in the bulk solution. Li and Bishop (2004) used micro DO sensor to measure the DO gradient in a biofilm and found the penetration limitation of 1 mg/L DO to 0.5 mm biofilm thickness. However, the relatively high DO of 2 to 5 mg/L used in the present study could partially penetrate the biofilm (2.2 mm at the end of the research, Section 2.3.2.3), and thereby inhibit the reaction of nitrite reductase and result in a high level of nitrate (von Schulthess *et al.*, 1994). It has also been proven that nitrite could coexist with oxygen as an electron acceptor, and be denitrified at a relatively slow rate

(Bell and Ferguson, 1991; Henze, 2000).

For the suspended sludge system, the specific ammonia oxidation rate averaged 1.54 $\text{NH}_3\text{-N}$ mg/g biomass*h, which was 6.4 times higher than the AOR of the biofilm system. Since the biomass of the biofilm was 3.5 times the biomass of the suspended sludge, the contribution of the suspended sludge to ammonia oxidation was 1.8 times higher in the hybrid reactor than that of the biofilm. However, the DO level of the biofilm test was higher than the test of suspended sludge which resulted in a higher AOR. That is, if DO conditions were the same, the contribution of the suspended sludge to ammonia oxidation would be larger than 1.8 times comparing with the biofilm in the hybrid system. Thus, the suspended sludge played the major role in ammonia oxidation in the hybrid system. Nitrite accumulation was also found in the suspended sludge (Fig 3.2A). The highest nitrite concentration accumulated in the bulk solution was around one third of the total nitrogen in the feed. The accumulation of nitrite implies that the performance of NOBs is lower than that of AOBs.

After 240 min, the AOR slowed down because the ammonia concentration decreased. From Fig 3.2C, it can be seen that there was a rapid rise in DO near the end of the aeration period. Thus, the rate of nitrite oxidation was enhanced and led to a breakthrough in the nitrite built-up. A rapid rise also occurred in the nitrate profile. The TN profile (Fig 3.2A) of the suspended sludge was flat and decreased relatively slowly, which pointed to inefficient nitrogen removal. Nitrate was the major end product of ammonia oxidation in this test.

In the hybrid system, the average AOR was 0.38 $\text{NH}_3\text{-N}$ mg/g biomass*h. AORs of the three tests indicate that AOBs were more abundant in the suspended sludge, and the

suspended sludge contributed most to the ammonia oxidation process in the hybrid system. The concentrations of nitrite and nitrate were relatively low during the test. From the TN profile, the hybrid system had better overall nitrogen removal than did the biofilm and the suspended sludge, when they were tested individually.

Simm *et al.* (2005) applied the technology of fluorescence in-situ hybridization (FISH) to identify the spatial distribution of AOBs and NOBs in sludge floc and concluded that AOBs tend to grow in the inner part of the floc and NOBs in the outer part of the sludge floc. This may be the result of competition for substrates by different microbial populations. Schramm *et al.* (2000) suggests the oxygen half saturation coefficient for AOBs may be significantly lower than for NOBs. Other research reports that NOBs were out-competed at low DO levels and this was beneficial for the accumulation of nitrite for the processes of autotrophic nitrogen removal over nitrite (Ciudad *et al.*, 2007; Gali *et al.*, 2007). The DO of the hybrid system was usually maintained at between 0 to 1.5 mg/L. The low DO and the denitrifying competition for utilization of nitrite limited the growth of NOBs. This may be the reason for the nitrite accumulation in the suspended sludge test. In this case, there was insufficient evidence to suggest that the better NOB performances in the hybrid system and biofilm system were due to low levels of nitrite. Nitrite might be diminished by heterotrophic denitrification.

Theoretically, denitrification of the biofilm system should be more efficient than is the case with the suspended sludge and the hybrid systems. However, as mentioned previously, a low degree of denitrification in the biofilm test was observed due to the higher DO in the bulk solution. A further test of heterotrophic denitrification under conditions of no DO therefore had to be investigated, in order to better understand the reactors

performance (Section 3.3.1.2).

The ORP profiles at the end of aeration of the biofilm and the suspended sludge tests were relatively high (up to more than 150 mV; Fig 3.1D and Fig 3.2D) compared with the hybrid system (below 100 mV; Fig 3.3E). A greater abundance of nitrate in the bulk solution of the biofilm and the suspended systems than in the hybrid system indicated that a higher range of ORP was favorable to nitrite oxidation rather than its reduction.

Another observation from the biofilm test was that the DO profile (Fig 3.1C) increased immediately after aeration started at the same aeration rate as in the other two tests (Fig 3.2C and Fig 3.3C). That is, there was a relatively low oxygen up-take rate in the case of the biofilm. This indicated either that the activity of heterotrophic aerobic organisms in the biofilm was low, or the population of heterotrophic aerobic organisms was small, or the thickness of the biofilm slowed down the transfer rate of oxygen. The use of the micro-sensor technology has shown the limitations of oxygen transfer and nitrogen compounds, carbon substrates to the biofilm (Gieseke *et al.*, 2005; Li and Bishop, 2004).

3.3.1.2 Heterotrophic denitrification

Under zero DO, 0.57, 0.14 and 0.51 N mg/g biomss*h were the specific heterotrophic denitrification rates of the biofilm, the suspended sludge and the hybrid system, respectively, when adding 0.4 g of sodium nitrate. The specific denitrification rate for the biofilm was 4.1 times more than the rate of the suspended sludge. This is attributed, in large part, to the quantity of biofilm. The total biomass of the biofilm was 3.5 times that of the suspended sludge. That is, the contribution to denitrification from the biofilm was 14.3 times greater than for the suspended sludge in the hybrid system. Thus, in the hybrid

system contribution to denitrification was mainly from the biofilm.

Nitrogen removal can also be accomplished by autotrophic denitrification, which happens during the process of autotrophic ammonia oxidation (Chapter 1). Although N_2O emissions from the biofilm and the suspended sludge systems were very low (0.5 and 4.2%, respectively; see Section 3.3.1.4), there was insufficient evidence to prove that there was no or little autotrophic denitrification happening in the systems. N_2O generated from autotrophic denitrification could be reduced by heterotrophic denitrifiers. Chapter 4 reports on an experiment designed to identify autotrophic denitrification, using the hybrid system.

3.3.1.3 Simultaneous nitrification denitrification (SND)

There were 41.0 and 16.7% SND efficiencies for the biofilm and the suspended sludge at the end of aeration, while the hybrid system had an SND efficiency of about 75.9%. The unexpected low SND achieved in the biofilm test was probably due to the inhibition of denitrification by high DO in the bulk solution, as discussed in the previous section.

The efficiency of SND is dependent on a low DO (Pochana and Keller, 1999). The SND efficiency of the suspended sludge, however, was also relatively low, even though the level of DO usually remained below 1 mg/L. Most of the nitrogen was converted to nitrate at the end of the aeration in the suspended sludge test. As proven in the previous section, a poor denitrification rate was achieved for the suspended sludge. However, the hybrid system facilitated the SND process. It had a high oxygen uptake rate, OUR, in the suspended sludge, resulting in a low DO, and subsequent denitrification of the biofilm.

3.3.1.4 N₂O off-gas

Nitrogen removal might also occur via dinitrogen gas (N₂) or gaseous nitrous oxide. During the aeration period, the biofilm reactor had the lowest N₂O emission of 0.5% as TN, in the influent. It was 4.2% for the suspended sludge system. The hybrid system had the highest N₂O off-gas, at about 21.2%.

The production of nitrous oxide is affected by TN, carbon source, DO, pH, temperature, salt (Wicht, 1996; Tsuneda *et al.*, 2005) and by the population of microorganisms. It was ascertained that N₂O emissions were affected mostly by DO level and microbial populations in this work. The following reasons may explain the low N₂O emissions from the biofilm test. First, heterotrophic microbes were the majority of the microorganisms in the biofilm and the inner part of the biofilm provided anaerobic conditions suitable for reducing N₂O. Second, the AOR by AOBs was relatively low and resulted in a relatively slow N₂O production. This gave sufficient time for the N₂O gas to be reduced. Third, previous research has reported that abundant N₂O is produced at low DO levels (e.g. 0.3 mg/L) (Tallec *et al.*, 2007). This suggests that the relatively high DO (around 5 mg/L) in the biofilm test enhanced the rate of nitrite oxidation and diminished the opportunity for nitrite reduction to nitrous oxide gas.

For the suspended sludge reactor, there was no or little nitrous oxide emission. As discussed previously, most of the nitrogen was oxidized to nitrate, and little denitrification occurred. From the N₂O off-gas profiles shown in Figure 3.2E, N₂O was still being emitted from the reactor at the end of the cycle, due to the carry-over denitrification. This suggests that although the N₂O off-gas was not abundant during the treatment, N₂O off-gas might still be emitted to the air when denitrification occurs in the receiving waters.

The hybrid system facilitated SND. Low concentrations of both nitrite and nitrate were observed in the hybrid system. This implied the shortcut of denitrification via nitrite occurred in the reactor. However, N_2O off-gas was more than 20% of the TN in the influent. High N_2O emissions from a BNR system have been observed (Zeng, 2003a; Hwang *et al.*, 2006). The amount of N_2O emissions exceeded 50% of TN (Itokawa *et al.*, 1996). If the production rate of N_2O is higher than its reduction rate, N_2O emission occurs. In this hybrid system, it seems likely that the N_2O production rate (which can also be expressed by “ NO_x (nitrate plus nitrite) denitrification rate”) was higher than the N_2O reduction rate. Hence, the accumulated N_2O was stripped out by aeration. Further studies into the relationship between N_2O production and reduction rates will be presented in the next chapter.

3.3.1.5 Conclusions on the removal of nitrogen

The results of the nitrogen removal test are summarized in Table 3.1. In the hybrid system, the main contributions from the suspended sludge were ammonia oxidation and nitrite oxidation. The specific AOR of the suspended sludge was about 6.4 times higher than was the case with the biofilm. Denitrification was the major contribution of the biofilm. The specific denitrification rate of the biofilm was about 4.1 times the rate of the suspended sludge. A good performance in simultaneous nitrification and denitrification was achieved when the biofilm and the suspended sludge grew together in the same reactor. N_2O emissions from the hybrid system were higher than in the individual tests of the biofilm and the suspended sludge reactors.

Table 3.1 Summary of the nitrogen removal of the biofilm, the suspended sludge and the hybrid system

	Biofilm	Suspended sludge	Hybrid system
AOR (N mg/g biomass*h)	0.24	1.54	0.38
DNR (N mg/g biomass*h)	0.57	0.14	0.51
SND (%)	41.0	16.7	75.9

Note: aeration rate = 200 mL/min

3.3.2 Nitrogen balance

TN, NH_4^+ , NO_2^- , NO_3^- , N_2O (both gas and in liquid) and NH_2OH were measured for the nitrogen balance. The results of the change in nitrogen balance over time are summarized in Table 3.2. Major items including ammonia/ammonium, nitrite, nitrate, nitrous oxide, nitrogen gas and total nitrogen are presented in Figure 3.4 to 3.6.

Soluble organic N was determined by TN minus NH_4^+ , NO_2^- , NO_3^- , and NH_2OH . Nitrogen used for assimilation was estimated by the TKN percentage of the biomass and the growth of the biomass, which is also the waste sludge in this stable system. The determination of nitrogen gas (N_2) was calculated, rather than measured. It was determined as the remainder of TN minus measured N species, minus soluble organic N, and minus assimilation of N. In the aeration phase in both the suspended sludge and the hybrid system, nitrogen gas was not the main end product of denitrification. It was only in the biofilm test that nitrogen gas was the main product of denitrification.

TKNs were determined to be 7.4 and 8.2% for the biofilm and the suspended sludge, respectively (Section 3.3.1.). When assume the steady state of the system was reached, the MLSS remained more or less the same. This suspended sludge was actually a mixture of the “sloughed biofilm” from carriers and the portion of the “original suspended sludge”. From the difference in the TKN of the biofilm and the suspended sludge, it was possible to estimate the percentages of the “sloughed biofilm” and the “original suspended sludge” in the waste sludge.

If the waste sludge of every cycle had more than 50% of the sloughed biofilm, the suspended sludge would finally be fully replaced by departed biofilm, after a certain number of cycles. The difference between Figure 3.1 and 3.2 indicate that the suspended

sludge contained not only the fallen biofilm biomass, but also the “original suspended sludge”. This had to be higher than 50% in the suspended sludge. However, based on the calculation of the nitrogen-balance, the percentage of the “original suspended sludge” could not be sufficiently high to cause a negative nitrogen gas production. In this nitrogen-balance calculation, if the “original suspended sludge” was 56%, it would result in a negative nitrogen gas production. Therefore, the medium value of 50% and 56%, so a medium of 53% of the “original suspended sludge”, was assumed. Thus, the “sloughed biofilm” was 47% of the suspended sludge. According to the above assumption, the waste biofilm mass was about 0.59 g per day ($1,200 \text{ MLSS-mg/L} \times 1.05 \text{ L} \times 47\%$). The SRT of the biofilm at the time of the test was estimated at 76 days ($44.6 \text{ g} / 0.59 \text{ g} \cdot \text{d}$).

Table 3.2 Summary of nitrogen balance at the end of aeration

% of TN	NH_4^+	NO_3^-	NO_2^-	N_2O^1	N_2^2	Soluble org-N	new cell-N ³
Biofilm	18.1	27.7	2.5	0.5 (0)	29.1	5.5	16.8
Suspended sludge	0.6	51.9	23.6	4.2 (0.2)	1.9	0.7	17.4
Hybrid system	6.4	8.8	9.5	21.2 (1.5)	14.9	1.3	33.5

1. Data outside the parenthesis were N_2O off-gas; in parenthesis were N_2O in liquid
2. N_2 was determined by calculation.
3. New cell-N was calculated by assuming that the suspended sludge contained 47% of biomass sloughed from biofilm carriers.

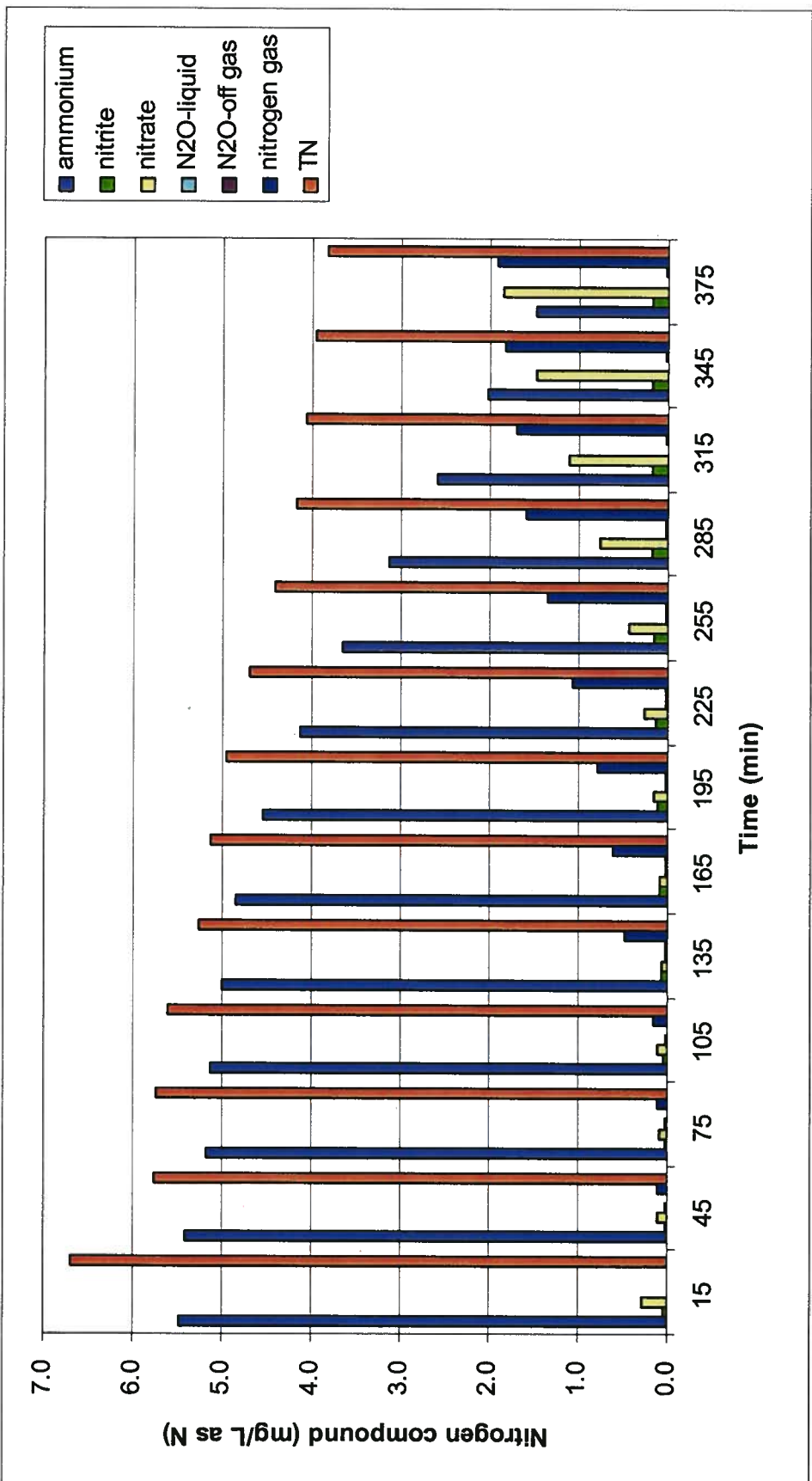


Fig 3.4 Nitrogen balance of the biofilm

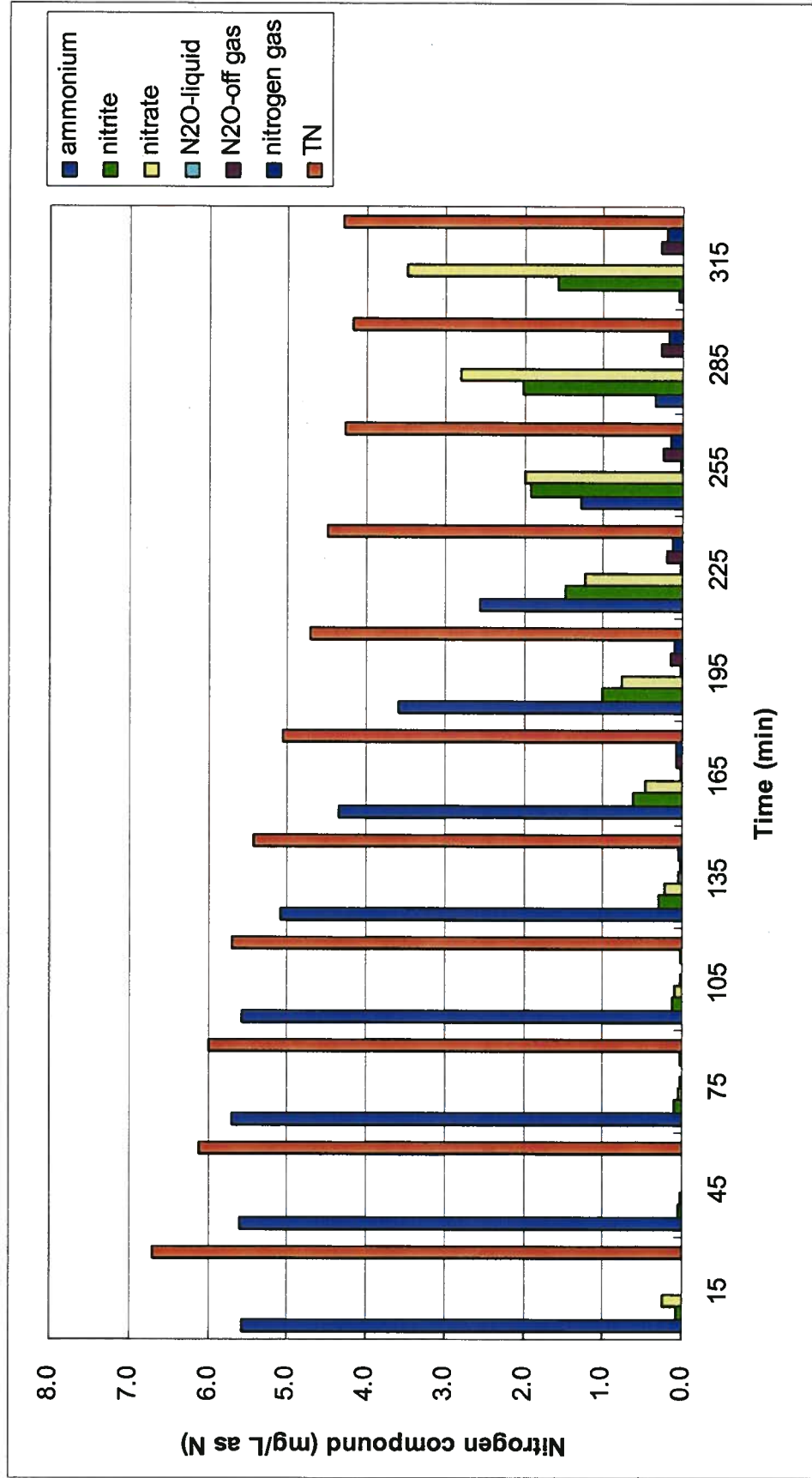


Fig 3.5 Nitrogen balance of the suspended sludge

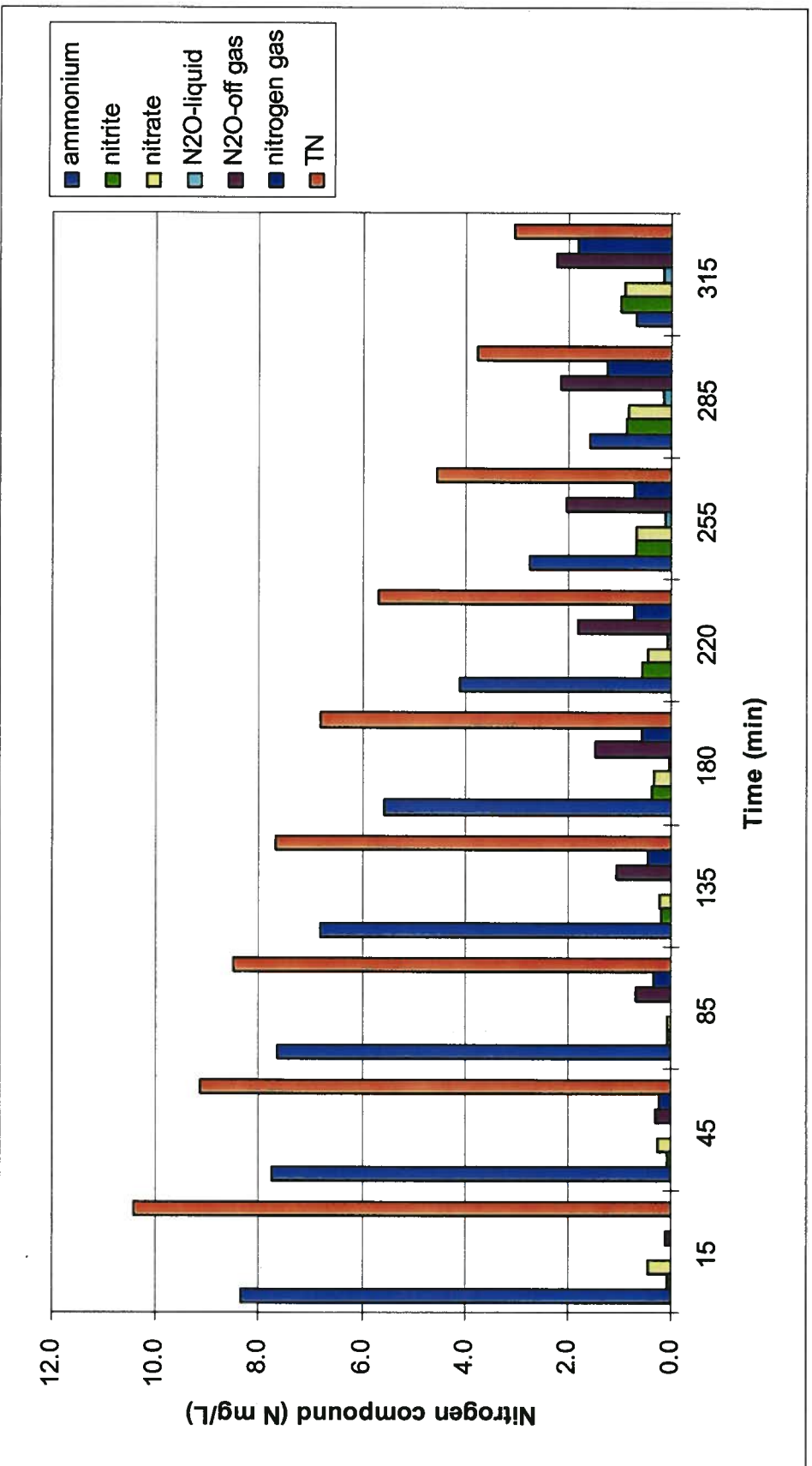


Fig 3.6 Nitrogen balance of the hybrid system

Nitrogen gas released from the suspended sludge system was calculated as only 1.9% of TN. This low N_2 emission was in agreement with the previous research done by Shiskowski (2004). The calculated results obtained from the above assumption were 16.8%, 17.4% and 33.5% of TN for conversion to new cells for the biofilm, the suspended sludge and the hybrid system, respectively. The fact that the sum of the first two numbers (the biofilm and the suspended sludge) was close to the percentage of TN converted to new cells in the hybrid system, also supports the assumption of the relative percentage of the biofilm and the suspended sludge in MLSS.

It was estimated that there was 29.1% of TN converting to N_2 gas at the end of the aeration phase, in the biofilm test. There was 0.5% N_2O off-gas and nitrate plus nitrite was 30.2% of TN. Although the DO was relatively high in the biofilm test, the production of N_2 illustrated the potential for the reduction from NO_2^- to NO to N_2O and finally, to N_2 . Although DO inhibits N_2O reductase, nitric oxide reductase and nitrous oxide reductase are active under aerobic conditions (von Schulthess *et al.*, 1994; Bell and Ferguson, 1991).

For the suspended sludge reactor, the concentration of nitrate plus nitrite was as high as 75.5%, at the end of aeration. Gaseous end products were 1.9% for N_2 and 4.2% for N_2O . There was little N_2O produced, due to low rate of denitrification.

For the hybrid system, about one-third of TN of the influent was utilized in the formation of new cells. The amount of nitrate plus nitrite was 17.3% at the end of aeration. The gaseous end product of N_2O was 21.2% and N_2 of 14.9% of TN.

Free ammonia (NH_3) was calculated as 0.04 mg/L at the beginning of the treatment (temp 22°C and pH around 7; the acid dissociation constant pK_a of NH_3 is 9.23). Thus, the nitrogen lost by ammonia stripping in this case was negligible. Hydroxylamine is an

intermediate product of the ammonia oxidation process. The concentrations of NH_2OH were below the detection limit (0.05 mg/L) for both systems. This implied that the reaction rate of hydroxylamine was very fast.

3.3.3 Conversion of carbon

External carbon from acetate, lactose and synthetic wastewater, including acetate, lactose and beef extract, were added to the biofilm and the suspended sludge reactor. Similar trends for carbon conversion in both systems were observed. External carbon was taken up and converted to stored carbon as PHA and glycogen. Glycogen was found in each test carried out during this study. It was found that poly-hydroxybutyrate (PHB) was the only species in the poly-hydroxyalkanoates (PHA) group: species of poly-hydroxybutyrate, poly-hydroxyvalerate (PHV), poly-hydroxy-2-methylbutyrate (PH2MB) and poly-hydroxy-2-methylvalerate (PH2MV) (Oehmen et al., 2007).

When acetate was the only carbon source in the feed, the rate of uptake for the suspended sludge was faster than for the biofilm (Fig 3.7A). In the anaerobic period (Fig 3.7B), there was no PHA accumulation for either the suspended sludge and the biofilm. Glycogen was the major stored carbon in cells under anaerobic conditions. When aeration started, the amount of stored glycogen decreased and the amount of PHA increased. PHA was depleted under aeration and glycogen increased again at the end of aeration.

When lactose was the sole carbon source, it was taken up relatively easily by the suspended sludge. However, a low up-take rate of lactose was observed in the biofilm. PHA was not found at anytime with a lactose feed (Fig 3.8B). A similar trend was observed for glycogen, which decreased when aeration started, then increased again at the end of

the aeration period.

Fig 3.7 to 3.9 show that glycogen decreased in the beginning of the anaerobic phase. There was, however, little release of phosphorus. It was most likely the degradation of glycogen, rather than the breakdown of poly-P, that provided the required energy for external carbon up-take. This agrees with the results of the study by Wang and Park (1998).

The carbon conversion found in this study was different from the carbon conversion in conventional EBPR processes. Further study is needed on the biological mechanisms involved.

The effects of various carbon sources on denitrification and nitrous oxide production will be presented in the next chapter.

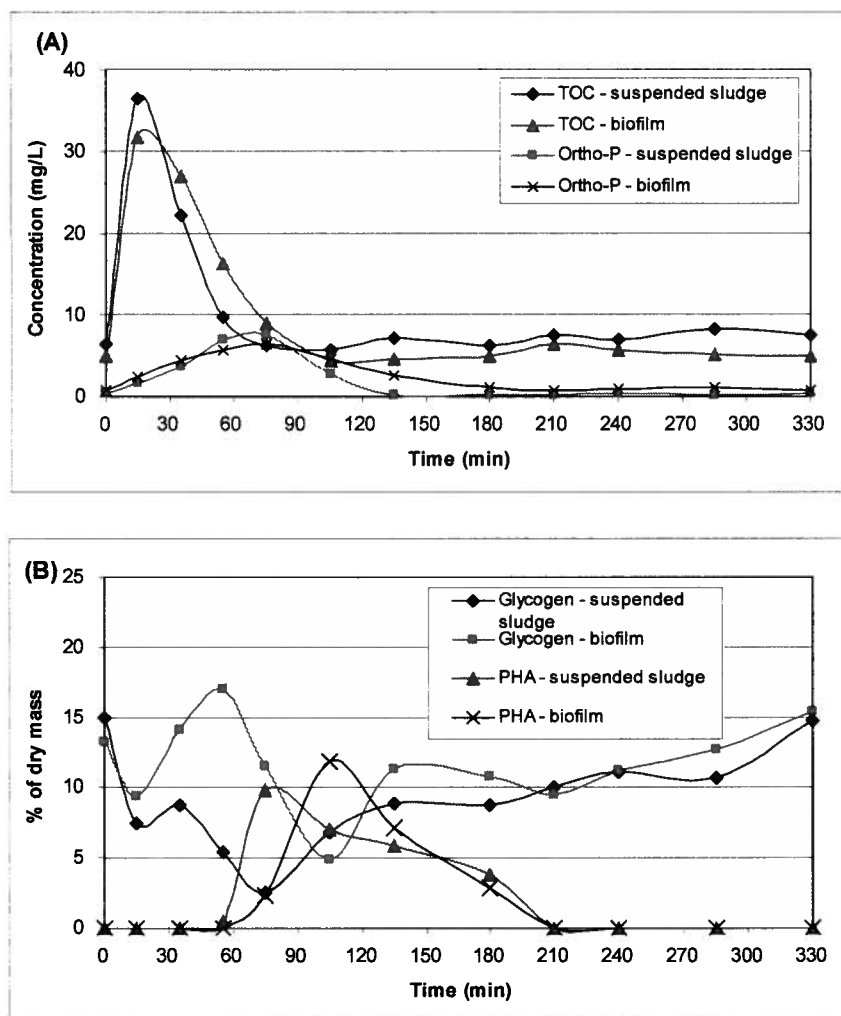


Fig 3.7 Carbon profiles following acetate addition (time 0~60 min and 240~300 were anaerobic, time 60~240 min was aerobic) (a) External carbon (presented by TOC); (b) Internal carbon (PHA and glycogen)

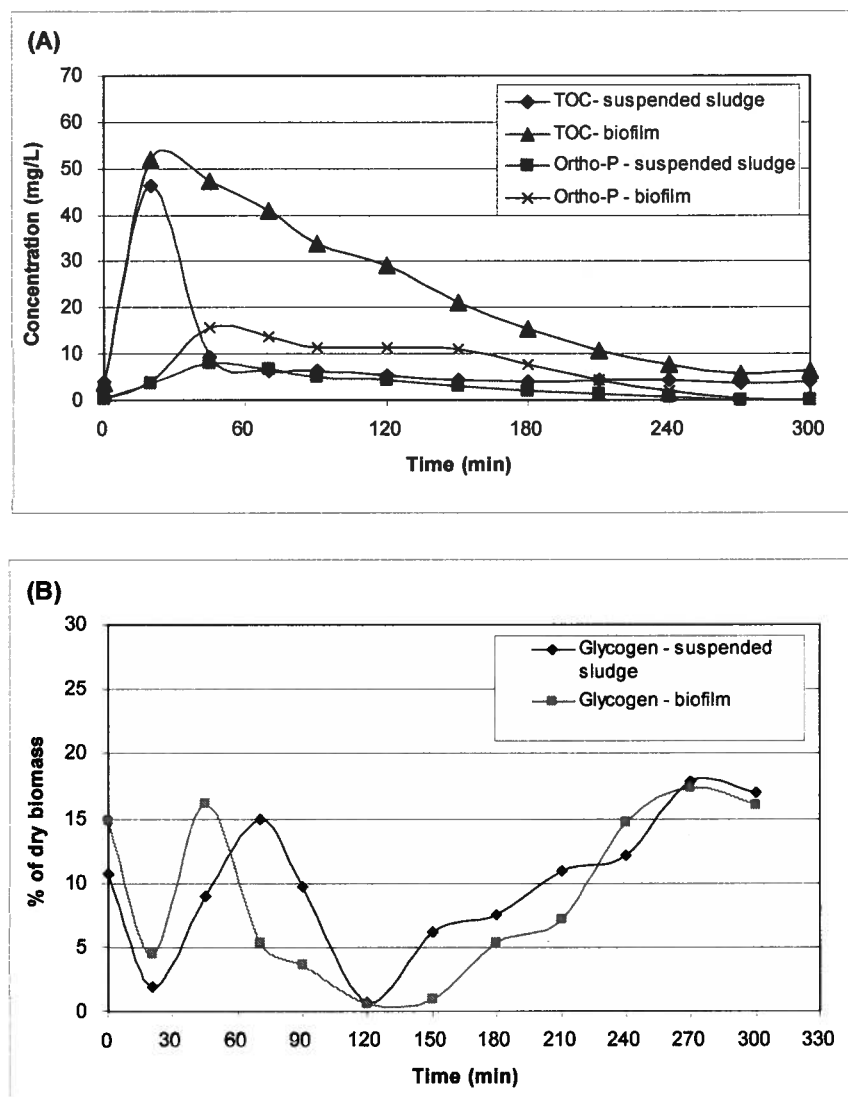


Fig 3.8 Carbon profiles following lactose addition (time 0~60 min and 240~300 were anaerobic, time 60~240 min was aerobic) (a) External carbon (presented by TOC); (b) Internal carbon (PHA and glycogen)

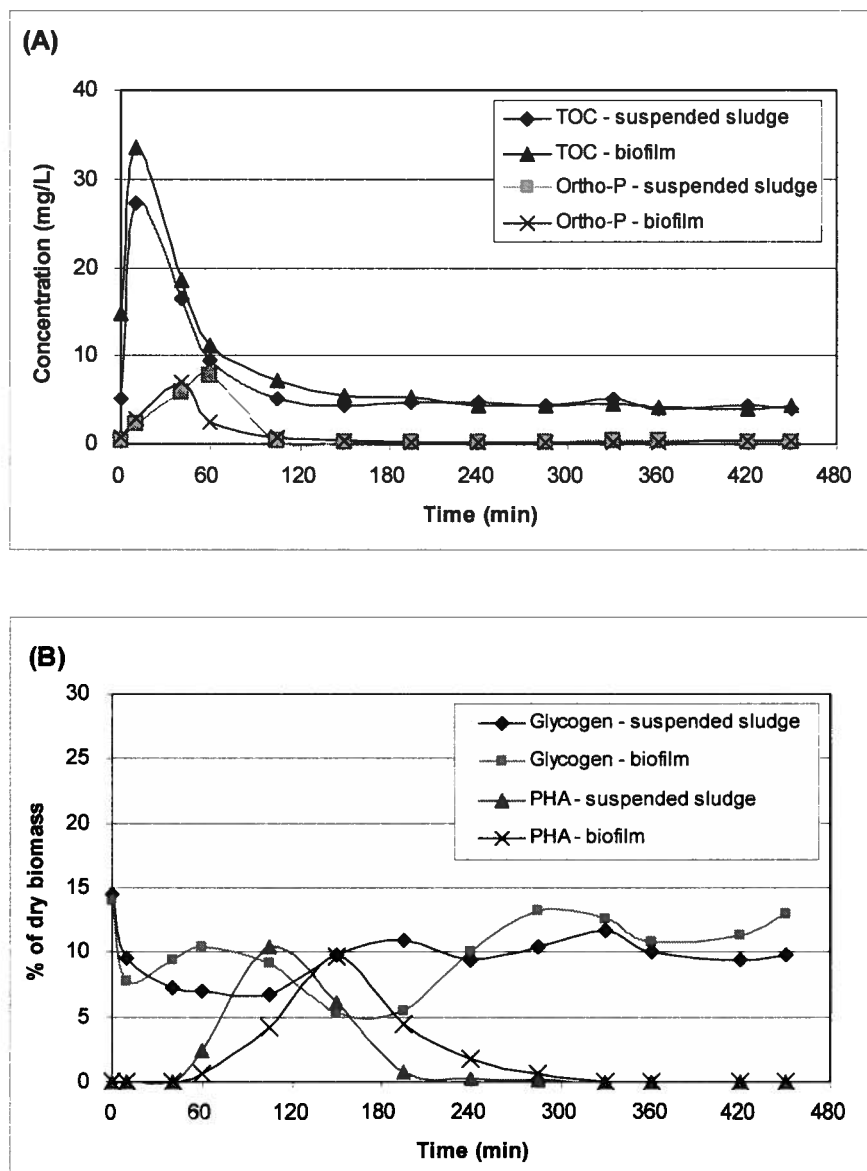


Fig 3.9 Carbon profiles following synthetic wastewater (time 0~60 min and 240~300 were anaerobic, time 60~240 min was aerobic) (a) External carbon (presented by TOC); (b) Internal carbon (PHA and glycogen)

3.3.4 Phosphorus removal

Phosphorus removal occurred in both the biofilm and the suspended sludge reactor, regardless of which carbon materials were fed into the reactors. Phosphorous was reduced from 12 mg/L to less than 1 mg/L. TP of the biofilm was measured as 2.6% of dry mass and it was 6.5% for the suspended sludge (it is around 2.2% of dry mass for non-bio-P cells by the formula given in Section 3.3.1). Phosphorus removal was more effective in the case of suspended sludge system than it was in the biofilm system; the diffusion limitations of biofilm might have hindered carbon up-take (Falkentoft *et al.*, 2001).

There was not much P release in the anaerobic phase during carbon was taken up (Fig 5.7-5.9A). Oehmen *et al.* (2007) shows that there are other metabolism pathways for phosphorus removal beyond the utilization of stored PHA. Kumar and Chaudhari (2003) reported a more than 80% P removal in a glucose fed biofilm SBR system. Moreover, Wang and Park (1998) reported that, when using glucose as sole carbon source, good P removal was obtained. In their study, there was no PHA in the EBPR sludge. PHA was not detected in the anaerobic stage in this study. P removal without the use of PHA was observed. Hence, further study on the metabolism pathway is needed.

3.3.5 Denitrifying phosphorus removal of the hybrid system

Since there was little or no denitrification occurring in the suspended sludge (the biofilm contributed 14.3 times more of what the suspended sludge in the hybrid system; Section 3.3.1), denitrifying phosphorus removal was examined only in the hybrid system.

Denitrifying phosphorus removal occurred in the hybrid system. When the system was fed with acetate as a carbon source, phosphate up-take was better when nitrate was

used as an electron acceptor, rather than nitrite (Fig 3.10).

The system had a higher degree of P-release and P up-take with lactose than with acetate (Fig 3.11). For the feeding with the mixture of acetate and lactose (2:1 by weight) as carbon sources (Fig 3.12). When nitrite served as the electron acceptor, P removal hardly happened.

Nitrite has been proven to be an inhibitor of P up-take (Meinhold *et al.*, 1999; Comeau *et al.*, 1987; Kuba *et al.*, 1996). Comeau *et al.* (1987) found that, in an anaerobic condition, when nitrite concentrations were higher than 10 mg-N/L, phosphate up-take stopped. When it was lower than 5 mg-N/L, nitrite could be used as an electron acceptor (Meinhold *et al.*, 1999).

Denitrifying P removal can also occur under aerobic conditions (Lee *et al.*, 2001; Zeng *et al.*, 2003b). The Activated Sludge Model # 3 (AMS3) shows the function of simulating denitrifying phosphorus removal. The defined saturation/inhibition coefficient for oxygen of AMS3 is 0.2 mg/L, and it is 0.5 mg/L for nitrate (Henze, *et al.*, 2000). Saito *et al.* (2004) found aerobic phosphorus uptake was more effective than anoxic phosphorus uptake. Nevertheless, how much DPAOs contributed to P-removal in the hybrid system depended on the fraction of DPAO in the sludge and the concentration of nitrate/nitrite in the bulk solution.

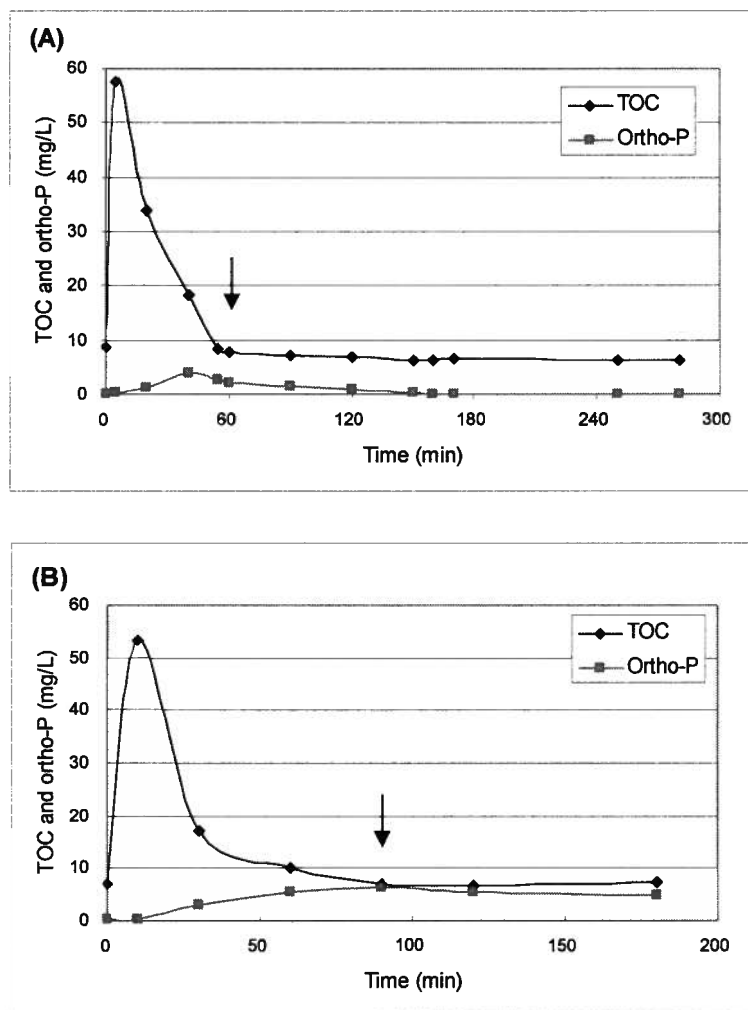


Fig 3.10 Phosphorus removal with acetate following addition of electron acceptor (A) nitrate spiked at time = 60 min and (B) nitrite spiked at time = 90 min.

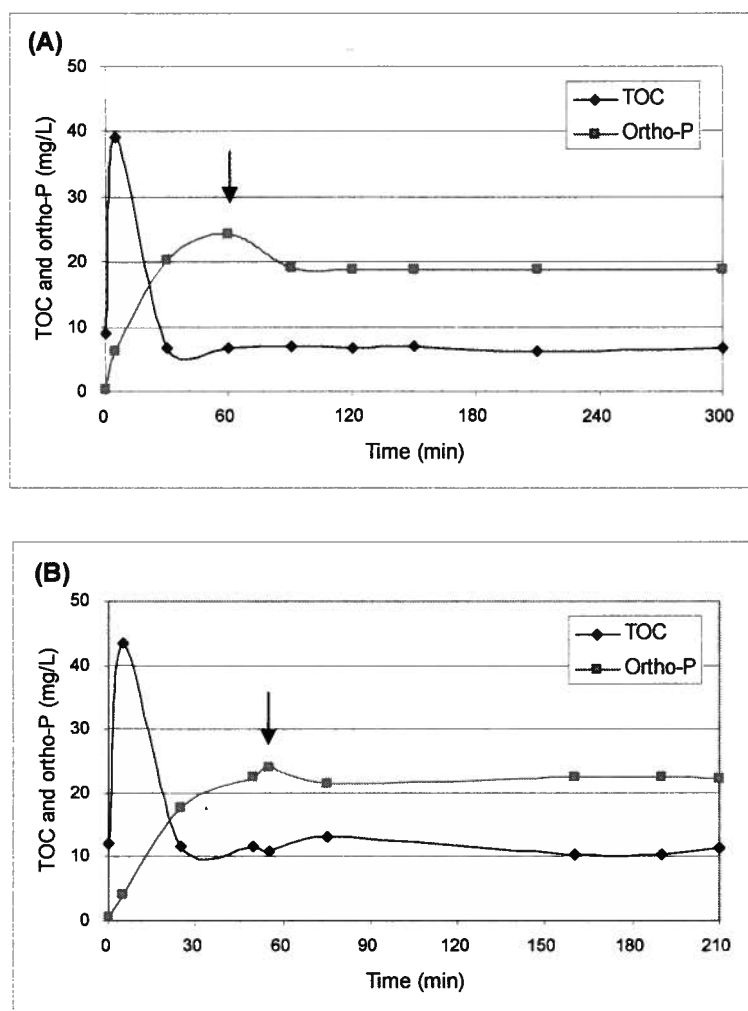


Fig 3.11 Phosphorus removal with lactose following addition of electron acceptor (A)nitrate and (B)nitrite spiked at time = 60 min.

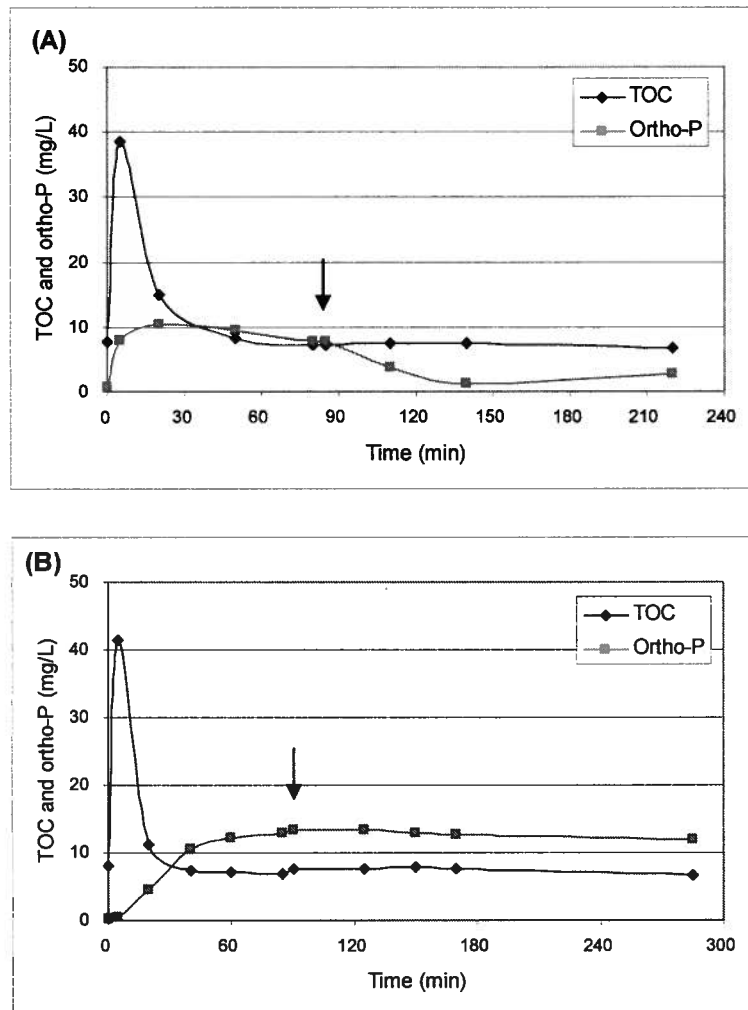


Fig 3.12 Phosphorus removal with the combination of acetate plus lactose following addition of electron acceptor (A)nitrate and (B)nitrite spiked at time = 80 min.

3.4 Conclusions

The following conclusions result from the individual studies done on biofilm and suspended sludge in terms of nitrogen removal, carbon conversion and P removal efficiencies in a hybrid system:

- For the hybrid system, nitrification occurred mostly in the suspended sludge, while the biofilm played the major role in denitrification. The interaction between the biofilm and the suspended sludge in a hybrid reactor resulted in better, overall nitrogen removal performance.
- Under aeration, nitrous oxide was the main end product of denitrification of the suspended sludge and the hybrid system. For the biofilm, more N_2 was produced than N_2O , as the end product of denitrification.
- Glycogen was the major stored carbon in the process. PHA was not formed during the anaerobic period in any of the cases.
- The suspended sludge contributed more to phosphorus removal than did the biofilm.
- Denitrifying phosphorus removal happened in the hybrid system. Nitrate was more efficient for phosphate up-take than was nitrite as an electron acceptor.

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Chapter 4 Investigation of nitrous oxide production

4.1 Introduction

Nitrous oxide emissions (which occur when wastewater is treated by means of some biological nutrient removal processes) are known to contribute to the greenhouse gas (Tallec *et al.*, 2007; Hwang *et al.*, 2006). Among the various BNR processes, the simultaneous nitrification, denitrification and phosphorus removal (SNDPR) process seems to result in the greatest N₂O emissions. It has been reported that more than half of the nitrogen in the influent was converted to nitrous oxide as an end product of denitrification, rather than to nitrogen gas (Lemaire *et al.*, 2006; Shiskowski, 2004).

Previous research has shown that N₂O off-gas was emitted during the aerobic stage of BNR treatment (Lemaire *et al.*, 2006; Meyer *et al.*, 2005; Shiskowski, 2004; Zeng *et al.*, 2003). A low DO level is a key operational factor for a successful SND, and yet low DO conditions also favour the accumulation of nitrite. It has been suggested that the inhibition of nitrous oxide reductase, under aerobic conditions, is a significant factor in N₂O emissions. And, as stated previously, the existence of nitrite is the main factor in the emission of N₂O (Lemaire *et al.*, 2006).

Carbon is another factor affecting N₂O emissions. N₂O emissions can be reduced by the addition of external carbon sources (Park *et al.*, 2000; Chung and Chung, 2000).

*A version of this chapter will be submitted for publication:

Lo, I., Lo, K.V., Mavinic, D.S., Shiskowski, D. and Remay, W. Investigation of Nitrous Oxide Emission form a Hybrid System for Simultaneous Nitrification, Denitrification and Phosphorous Removal.

Methanol has been used for this purpose. A full-scale, nitrogen removal plant has applied methanol additions, to reduce the emission of N₂O (Tallec *et al.*, 2006).

On another hand, mathematical models of N₂O production, during heterotrophic denitrification, have been proposed by several researchers (van Schulthess *et al.*, 1994; van Schulthess and Gujer, 1996; Wicht, 1996). However, nitrous oxide reduction, using stored carbon, has not been fully studied.

In an EBPR system, external carbon is taken up to form stored carbon under anaerobic conditions. The stored carbon is then is used for aerobic growth and phosphate up-take. PHA and glycogen are two major carbons stored in the cells (Oehmen *et al.*, 2007). In a SNDPR system, denitrification and nitrous oxide reduction occur after most of the external carbon has been converted to stored carbon. Meyer *et al.* (2005) summarized that, “nitrous oxide was identified as a product of denitrification, when based on stored PHA as the carbon source. This observation is of critical importance to the application of PHA-driven denitrification in activated sludge processes.”

In this chapter, results are reported from the investigation into the effects of the carbon source on nitrous oxide emissions during the SNDPR process. Denitrification and N₂O reduction for different carbon types (including acetate and lactose), carbon states (including external and internal carbon sources) and oxygen states (with DO and no DO) were investigated.

4.2 Experimental design

4.2.1 Determination of N₂O off-gas from autotrophic phase

Two sets of experiments were conducted to identify nitrous oxide production from autotrophic phase. In the first experiment, carbon sources without beef extract were used as normal feed (presented in chapter 1). Two track analyses were conducted side by side. The purpose of this was to examine N₂O production via heterotrophic denitrification. In the first track, around 7 mg N/L initial concentration of ammonia was spiked into the reactor, as the sole nitrogen source. In the second track analysis, the same amount of nitrite N as in the previous track was continuously and evenly added into the reactor, to simulate the nitrite oxidized from ammonia. This was to ensure that N₂O production came from heterotrophic denitrification. The difference in N₂O production was then attributed to autotrophic ammonia oxidation.

The second experiment was to examine the effects of the carbon source on the process. The reactor was aerated for 4 hours before the track study, in order to reduce the stored carbon in the cells. In this way, heterotrophic denitrification was minimized. In the batch test, ammonia was spiked as the only nitrogen source. Inorganic carbon was measured to ensure enough carbon sources for autotrophs.

The aeration rate was 200 mL/min for all tests. Samples were taken for nitrate, nitrite and TOC determination. N₂O off-gas was monitored for each test.

4.2.2 Determination of denitrification rates and N₂O off-gas from heterotrophic denitrification

For the tests of denitrification via an external carbon source, around 55 mg C/L as

TOC initial concentration of carbon substrates of sodium acetate and lactose were added into the reactor. Immediately, sodium nitrate or sodium nitrite, as electron acceptors, were spiked into the reactor in an amount sufficient to reach 7 mg N/L. Heterotrophic denitrification, using external carbon substrates, was tested under both aerobic and anoxic conditions. The aeration rate was controlled at 200 mL/min of air or nitrogen gas.

A 60 min anaerobic period was provided for denitrification, using external carbon substrates, before nitrite or nitrate were spiked so as to allow the carbon substrates to be taken up. Denitrification, using external carbon substrates, was tested under both aerobic and anoxic conditions.

The duration of each test was 3 hours. Samples were taken for nitrate, nitrite and TOC determination. N₂O off-gas was monitored for all tests.

4.2.3 N₂O reduction rate determination

N₂O reduction was examined using external carbon or stored carbon under DO or no DO conditions. The biofilm and the suspended sludge were examined separately.

For external carbon with DO, carbon sources (acetate or lactose) were added into the reactor to make an initial concentration of about 55 mg/L as TOC. 20 mL of MLSS was taken from the reactor and transferred into a 60 mL gas tight plastic syringe for the suspended sludge test. Another 20 mL of N₂O gas, with a concentration of 2,000 ppm (diluted by air), was injected into the syringe via the rubber stopper. The syringe was incubated and shaken vigorously in a shaker (Burrell). The N₂O of the headspace was measured by the method described in Chapter 1. N₂O reduction was calculated by the decrease of the amount of total nitrous oxide, including gaseous and soluble N₂O.

When N₂O reduction of the biofilm was tested, a carrier of biofilm was removed from the reactor and put into a syringe. The mixed liquor was centrifuged at 3,000 x g for 3 min at room temperature immediately after the addition of carbon. 20 mL of centrifuged mixed liquor (without sludge) was then transferred into a syringe, by following the same procedure used in the test of the suspended sludge.

For the tests of external carbon without DO, the DO level of the reactor was measured as zero. Sample was sucked into the syringe through a tube. Standard N₂O gas was diluted to 2,000 ppm with N₂ gas, instead of air. Then, 20 mL of the diluted N₂O was injected into the syringe for the reduction tests.

For the stored carbon tests, the carbon sources were added into the reactor under anaerobic conditions, for a period of 1 hour. This was to allow both the suspended sludge and the biofilm to take up the carbon substrates. The remaining procedures were the same as for the tests with external carbon.

The tests were also conducted without external and stored carbon. To reduce the level of internal stored carbon, the reactor was aerated for 4 hours. Before the actual tests began, the air supply was turned off. DO was measured as zero before the sample was sucked through a tube to the syringe.

4.3 Results and discussion

In this report, the “production of N₂O” includes both N₂O off-gas and soluble N₂O in the bulk liquid. “N₂O off-ga” and “N₂O emission” includes two sources: air stripping and mechanical mixing. The soluble N₂O fraction was not, however, included. The “nitrogen reduction rate” here is defined as the reduction of nitrate plus nitrite nitrogen in the bulk solution. It doesn’t include nitrogen lost from nitrous oxide to nitrogen gas. Since the oxidation rate of nitric oxide to nitrous oxide (NO) was relatively fast and there was very little NO off-gas (Chapter 3), the “nitrogen reduction rate” is also used to express N₂O production rate.

4.3.1 Nitrous oxide from autotrophic phase

In the first experimental set (Fig 4.1), nitrous oxide off-gas for the first track averaged 10.3% of the nitrogen of the ammonia; it was 9.9% for the second track, during which nitrite was continuously and evenly added into the reactor to simulate the nitrite produced from ammonia oxidation. Under the same aeration rate, the DO for the first test was from 0 to around 2 mg/L, and pH ranged from 7.1 to 7.5. For the second track, both DO and pH levels were slightly higher than the first track because there was no ammonia oxidation in the system. The DO level was from 0 to about 3 mg/L, and pH was from 7.3 to 7.6. A higher DO and/or pH level decreased the emission of N₂O (Section 5.3.1.1 and 5.3.3). That is, if the DO and pH conditions for the second track were the same as the first track, N₂O emission might be more and the difference between two tracks would be less.

Nitrous oxide, via autotrophic denitrification, occurred when ammonia was oxidized to nitrite. This means that, by spiking nitrite, nitrous oxide from the second track was

produced mostly from heterotrophic denitrification. The small difference in N₂O off-gas (0.4%) suggested that autotrophic nitrous oxide production in this reactor might be insignificant. However, any N₂O produced from autotrophic denitrification might be quickly reduced by the relatively strong reduction potential of the existing carbon source. Another experiment therefore needed to be conducted, in order to eliminate the effect of the carbon source on N₂O emissions.

The second experiment was conducted in the same week of the previous experiment to diminish the potential microbial population change of the system. There was no external carbon source or stored carbon in the system. Ammonia was the sole nitrogen source. IC was not a limited factor. It was measured approximately 30 mg/L. The DO of the track ranged from 0 to about 3 mg/L, and pH was from 7.2 to 7.4. The measured N₂O off-gas was only 1.0% as N of the spiked ammonia (Fig 4.2). The results show that nitrous oxide production from autotrophic phase was not significant in the hybrid system.

Large amounts of N₂O emitted from autotrophic denitrification have been reported (Lemaire *et al.*, 2006; Shiskowski, 2004; Zeng *et al.*, 2003). However, low N₂O emission from autotrophic denitrification has also been reported (Takaya *et al.*, 2003; Inamori *et al.*, 1997). A possible reason for low autotrophic N₂O emission from the hybrid reactor could be a result of the fact that the oxidation of hydroxylamine to nitrite occurred relatively quick. There was, thus, little possibility that N₂O will remain in the bulk solution. Another possible reason is that the N₂O reduction rate of the hybrid system was faster than the production rate of autotrophic N₂O. In other words, any N₂O produced from autotrophs was quickly reduced by the N₂O reduction function of the hybrid system. The factors affecting the reaction rates of N₂O production are discussed in the following sections.

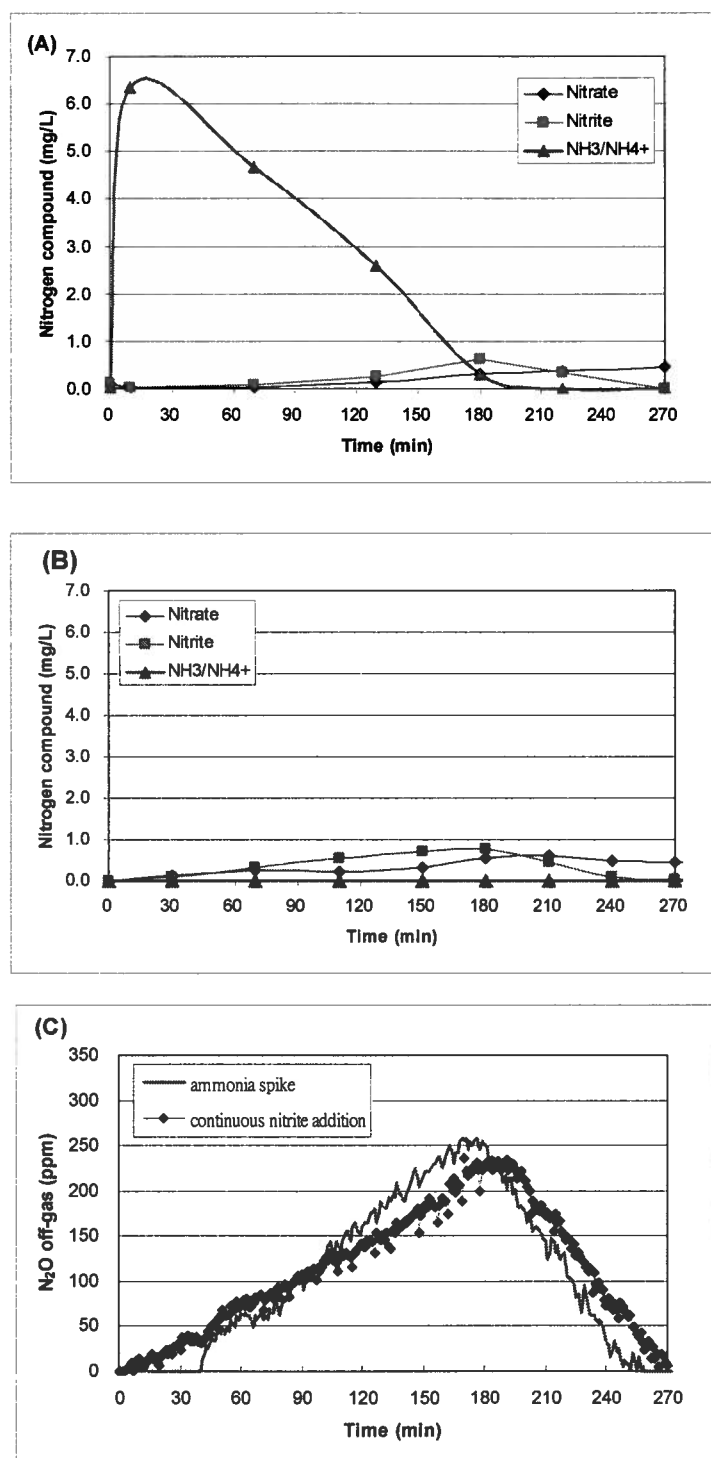


Fig 4.1 Nitrogen compounds of N₂O production tests in aerobic denitrification with carbon sources (A) ammonium as only nitrogen source, (B) continuous nitrite addition, and (C) the N₂O profiles following the ammonia spike and the nitrite addition (N₂O was 10.3 % for (A) and 9.9% for (B))

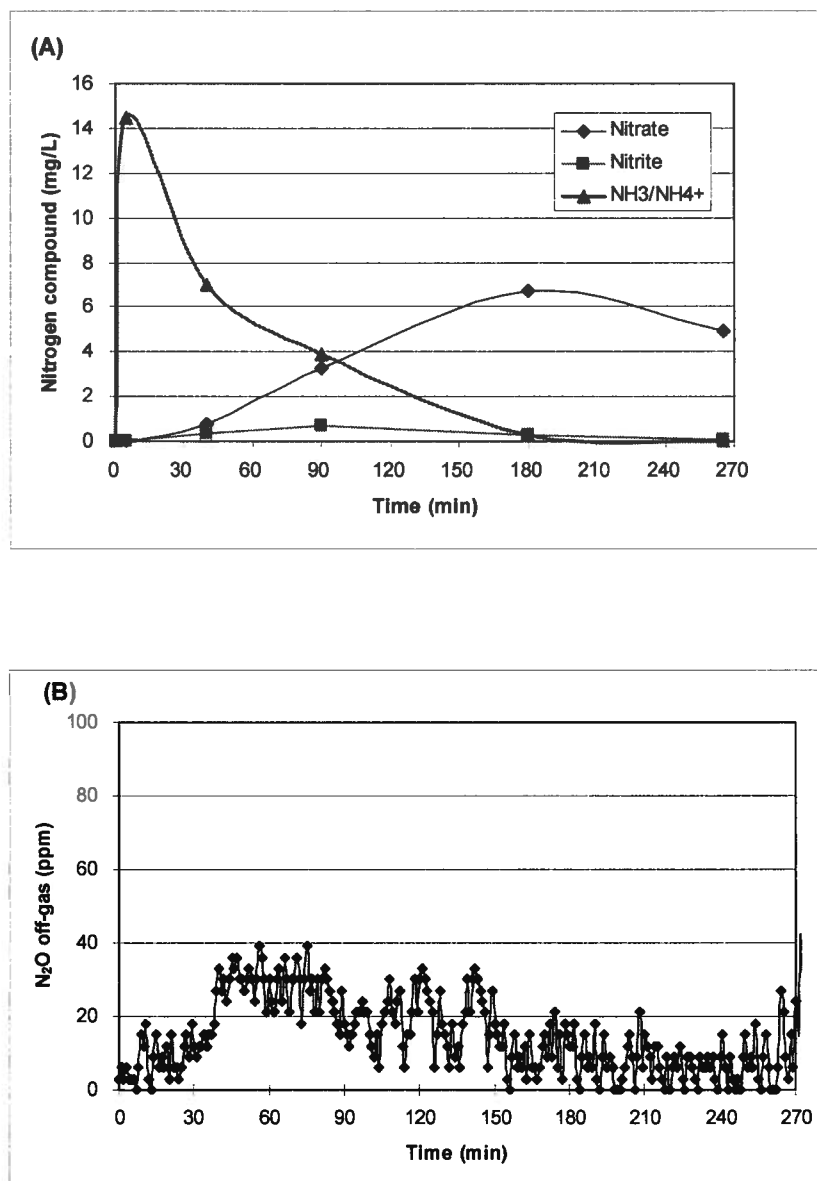


Fig 4.2 Nitrogen compounds of N_2O production tests in aerobic denitrification without carbon source (A) nitrogen compound profiles, (B) the N_2O profile following the ammonia spike (N_2O was 1.0 %)

4.3.2 N₂O production from heterotrophic denitrification

4.3.2.1 Heterotrophic denitrification and N₂O off-gas using an external carbon source

- Aerobic

Carbon, in terms of COD, was taken up within 45 min. The DO of the bulk solution remained at a low level of less than 0.5 mg/L (data not shown).

When nitrate was used as an electron acceptor and acetate as an external carbon source, the overall nitrogen reduction rate was 1.23 mg N/g biomass*h. At the end of the test (45 min after the carbon addition), about 57% of nitrate remained in the bulk solution. N₂O emissions were only about 1.0% of the total nitrogen added into the test.

When lactose was used as an external carbon source, little nitrate reduction occurred. In this case, the nitrogen reduction was only 0.06 mg N/g biomass*h. The residual nitrate was about 95% after 45 min and N₂O off-gas was about 1.8 % of the total nitrogen.

The nitrogen reduction rates were faster when nitrite was used as an electron acceptor than they were when nitrate was used. They were 1.49 and 1.01 mg N/g-biomass*h for acetate and lactose, respectively. About 15% of nitrite was converted to nitrate using acetate, while it averaged 24% for lactose. The N₂O-N off-gas was about 2.3 and 6.8% for acetate and lactose, respectively. The results are summarized in Table 4.1.

It therefore appears that acetate had a stronger nitrogen reduction potential than lactose. Nitrogen reduction rates were faster and there was less NO₃⁻ in the bulk solution at the end of the tests.

Table 4.1 Summary of N₂O off-gas, denitrification and N₂O reduction rates under different carbon and oxygen conditions

Carbon type	Stripping gas	Carbon source	Nitrogen type	Nitrogen reduction rate*	NO ₃ ⁻ (%)	N ₂ O (%)
<i>External</i>	air (aerobic)	acetate	NO ₃ ⁻	1.23 ± 0.36	57.1 ± 8.6	1.0 ± 0.4
			NO ₂ ⁻	1.49 ± 0.08	14.7 ± 1.5	2.3 ± 0.2
		lactose	NO ₃ ⁻	0.06 ± 0.05	94.6 ± 3.3	1.8 ± 1.2
			NO ₂ ⁻	1.01 ± 0.19	24.0 ± 10.8	6.8 ± 2.4
	N ₂ (anoxic)	acetate	NO ₃ ⁻	1.91 ± 0.20	0	0.4 ± 0.3
			NO ₂ ⁻	1.52 ± 0.20	0	1.6 ± 1.1
		lactose	NO ₃ ⁻	1.22 ± 0.08	0	1.6 ± 0.9
			NO ₂ ⁻	1.70 ± 0.20	0	5.3 ± 0.7
<i>stored</i>	air (aerobic)	acetate	NO ₃ ⁻	0.96 ± 0.22	8.9 ± 3.8	50.2 ± 2.3
			NO ₂ ⁻	0.79 ± 0.06	24.4 ± 11.4	66.3 ± 4.6
		lactose	NO ₃ ⁻	0.14 ± 0.11	71.2 ± 21.6	16.6 ± 7.1
			NO ₂ ⁻	0.52 ± 0.03	57.4 ± 12.9	41.7 ± 6.2
	N ₂ (anoxic)	acetate	NO ₃ ⁻	1.16 ± 0.19	0	27.1 ± 3.4
			NO ₂ ⁻	1.29 ± 0.20	0	18.4 ± 4.3
		lactose	NO ₃ ⁻	1.31 ± 0.35	0	46.4 ± 6.2
			NO ₂ ⁻	1.31 ± 0.45	0	57.2 ± 9.4
<i>No carbon</i>	air (aerobic)	--	NO ₃ ⁻	0.03 ± 0.02	93.8 ± 5.5	1.3 ± 0.4
			NO ₂ ⁻	0.39 ± 0.07	88.7 ± 5.2	13.8 ± 2.4
	N ₂ (anoxic)	--	NO ₃ ⁻	0.24 ± 0.05	36.6 ± 11.4	0.6 ± 0.6
			NO ₂ ⁻	0.49 ± 0.11	0	22.7 ± 4.8

*: unit = mg N/g biomass*h

- Anoxic

Both nitrate and nitrite were completely reduced without dissolved oxygen during carbon up-take. There was also relatively high reduction rate with a relatively low N₂O production. Nitrate reduction rates for acetate and lactose were approximately 1.91 and 1.70 mg N/g biomass*h; N₂O off-gases were about 0.4 and 1.6 %, respectively. Nitrite reduction rates were 1.52 and 1.22 mg N/g biomass*h for acetate and lactose, and their N₂O emission were 1.6 and 5.3 %, respectively.

Dionisi *et al.* (2004) reported the use of acetate, glucose, glutamic acid and ethanol as carbon sources in tests of anoxic substrate removal in an SBR. Their results showed that specific nitrate reduction rates could vary widely and a difference of up to 5 times was reported. The relative rates, ranked from high to low, were acetate, glucose, glutamic acid and ethanol. The results obtained in this study agree with their findings for the most part. The differing reduction potential of various carbon sources and their different half-saturation coefficients resulted in a range of nitrogen reduction rates.

4.3.2.2 Heterotrophic denitrification by using stored carbon

- Aerobic

Under aerobic conditions, N₂O generation was relatively high when denitrification occurred, using internal carbon sources. Tests were carried out for 3 hours. For the nitrate spike, acetate was converted to the form of PHA as the dominant stored carbon in cells (Section 3.3.3). The production of nitrous oxide off-gas was about 50.2%. N₂O off-gas was about 16.6% when lactose was used as an external carbon, and then, glycogen became the dominant stored carbon. Denitrification rates for the existence of PHA and Glycogen

were approximately 0.96 and 0.14 mg N/g biomass*h, respectively.

When nitrite was tested, nitrogen reduction rates were about 0.79 and 0.52 mg N/g-biomass*h over the presence of PHA and glycogen; N₂O off-gases were approximately 66.3 and 41.7%, respectively.

Under aerobic conditions, both nitrate and nitrite were not completely consumed. After three hours testing time, some nitrate still remained in the bulk solution for both tests.

- Anoxic

Only glycogen was detected as stored carbon for both acetate and lactose additions under anaerobic conditions (Fig 3.7 and 3.8). This implies that their denitrification rates were similar. The denitrification rates for the nitrate spike were approximately 1.16 and 1.31 mg N/g biomass*h, while they were 1.29 and 1.31 mg N/g biomass*hof nitrite spike for the acetate and lactose additions, respectively. The measured N₂O emissions were different. For NO₃⁻ tests, they were about 27.1 and 46.4% for acetate and lactose additions. While they were approximately 18.4 and 57.2% for NO₂⁻ tests, respectively. A higher amount of N₂O off-gas was produced for an internal carbon source than for an external carbon source.

4.3.2.3 Denitrification without carbon source

Nitrate and nitrite reduced slowly without a carbon addition. There was a higher reduction rate, (0.39 mg N/g biomass*h), and higher N₂O off-gas (13.8%) for a nitrite addition than for nitrate, (0.03 mg N/g biomass*hand 1.3% for denitrification and N₂O emission, respectively) under aerobic conditions. For anoxic conditions, there was a

nitrogen reduction rate of about 0.49 mg N/g biomass*hand N₂O emission averaged 22.7% for nitrite. A nitrogen reduction rate of 0.24 mg N/g biomass*hand a N₂O off-gas of only 0.6% were obtained for NO₃⁻. When the system was aerated without a carbon source, nitrate reduction and N₂O production nearly ceased.

N₂O off-gases were also detected without carbon addition. When nitrite serves as an electron acceptor without DO, nitrous oxide off-gas could be as high as 20.7%.

4.3.2.4 Summary of heterotrophic denitrification

Denitrification rates, under aerobic conditions, were lower than for the conditions without DO. Nitrogen reduction rates were faster under these conditions with the addition of external carbons, than with a stored carbon. The lowest rate of nitrogen reduction was obtained with an insufficient amount of carbon source in the system.

Nitrogen reduction rates were found to be dependent on carbon sources. The nitrogen reduction rates, when acetate served as an external carbon source and nitrate as an electron acceptor, were higher than in the case of lactose. In contrast, higher reductions were obtained with lactose and nitrite. Acetate was converted to PHB and glycogen, while only glycogen was formed from lactose. Under conditions of no DO, there was no significant difference in nitrogen reduction, with the various stored carbons.

In the denitrification process, a higher N₂O off-gas was obtained for stored carbon, than for an external carbon source. The largest amount of N₂O off-gas emissions was obtained with PHA and nitrite.

There seemed to be no significant relationship between the nitrogen reduction rate and the emission of N₂O off-gas, under heterotrophic denitrification without carbon.

Although the nitrogen reduction rates obtained from the different experimental sets were similar, different N₂O reduction rates might result in different N₂O off-gas rates. Nitrous oxide reduction rates are reported in the next section.

4.3.3 N₂O reduction

4.3.3.1 Determination of liquid-gas phase diffusion coefficient

The Bunsen adsorption coefficient “ α ” was determined as 0.24 ± 0.01 (8 tests) in completely denitrified effluent at approximately 20 °C and neutral pH (the method and Equation 1.19 in Chapter 1), while the value of “ α ” was 0.62 in pure water at 20 °C (Tiedje, 1982). A higher “ α ” indicates that the solution has a relatively high solubility of N₂O. The lower value of “ α ” in this study might be related to the existence of constituents in the effluent that decreased N₂O solubility. Further study of the effects of such constituents on N₂O solubility is needed.

4.3.3.2 N₂O reduction tests

Higher nitrous reduction rates were obtained under anoxic conditions than under aerobic conditions. Higher rates were also obtained when acetate was used as a carbon source rather than lactose. Without oxygen, the reduction rates were measured as an average of 2.42 and 1.31 mg N/g biomass*h for acetate and lactose. The rates were about 1.01 and 0.83 mg N/g biomass*h with additions of acetate and lactose, for the formation of glycogen (Section 3.3.3).

With oxygen, the reduction rates were approximately 1.19 and 0.53 mg N/g-biomass*h for acetate and lactose as an external carbon source, respectively. They were

about 0.69 and 0.48 mg N/g biomass*h when acetate and lactose were added as a stored carbon production source. The stored carbons were PHA and glycogen dominated, respectively. The N₂O reduction rate was the lowest when there was no carbon addition made. They were approximately 0.47 mg N/g biomass*h for anoxic and 0.41 mg N/g-biomass*h for aerobic conditions. Figure 6.3 shows that nitrous oxide concentrations decreased with time. The rates of decrease also decreased with time and it appears that the reduction rate of N₂O is of the first order. The maximum nitrous oxide reduction rates during the first 30 min are summarized in Table 4.2.

N₂O reduction occurred under both aerobic and anoxic conditions. This is consistent with the results of Bell and Ferguson (1991). Nitric and nitrous oxide reductases were active in a pure culture study done under aerobic conditions. However, N₂O reduction was inhibited by DO; the reduction activities were slower under aerobic conditions, than those under anoxic/anaerobic conditions.

N₂O reduction is also reported to be affected by an external carbon. Chung and Chung (2000) reported that “biological nitrogen potential” could be used to identify the reduction rates of N₂O. They found that a higher C/N ratio resulted in lower N₂O production. The production of N₂O could be increased up to 100 times under conditions of carbon deficiency, as opposed to carbon sufficiency. Wicht (1996) reported the half-saturation constant for N₂O reduction was 1.76 mg COD/L by feeding acetate. Under the same conditions, half-saturation constants were 3.47 and 1.97 for nitrate and nitrite reduction rates, respectively. That is, N₂O reduction was more favorable with a lower carbon concentration, than nitrate and nitrite reduction.

Table 4.2 N₂O reduction rates

N ₂ O reduction Mg N/g biomass*h		Acetate		Lactose		no Carbon	
		DO	no DO	DO	no DO	DO	no DO
External	sus ¹ -1	1.87	6.07	0.43	1.85	0.38	0.51
	sus-2	1.45	5.76	0.55	1.72	0.43	0.55
	sus-3	2.42	5.92	0.47	1.81	0.60	0.43
	sus-ave	1.91	5.92	0.48	1.79	0.47	0.50
	STDV	0.49	0.16	0.06	0.07	0.12	0.06
	bio ¹ -1	0.81	1.42	0.45	0.95	0.38	0.46
	bio-2	1.03	0.91	0.71	1.10	0.42	0.44
	bio-3	1.04	1.60	0.47	1.41	0.37	0.48
	bio-ave	0.96	1.31	0.54	1.15	0.39	0.46
	STDV	0.13	0.36	0.14	0.23	0.03	0.02
Over all		1.19	2.42	0.53	1.31	0.41	0.47
stored	sus-1	0.69	1.94	0.19	0.80		
	sus-2	0.96	2.12	0.32	0.83		
	sus-3	0.78	2.08	0.23	0.99		
	sus-ave	0.81	2.05	0.24	0.87		
	STDV	0.13	0.09	0.07	0.10		
	bio-1	0.54	0.86	0.57	0.81		
	bio-2	0.71	0.48	0.60	0.74		
	bio-3	0.68	0.70	0.51	0.88		
	bio-ave	0.65	0.68	0.56	0.81		
	STDV	0.09	0.19	0.05	0.07		
Over all		0.69	1.01	0.48	0.83		

1. "sus" stands for suspended sludge; "bio" stands for biofilm.

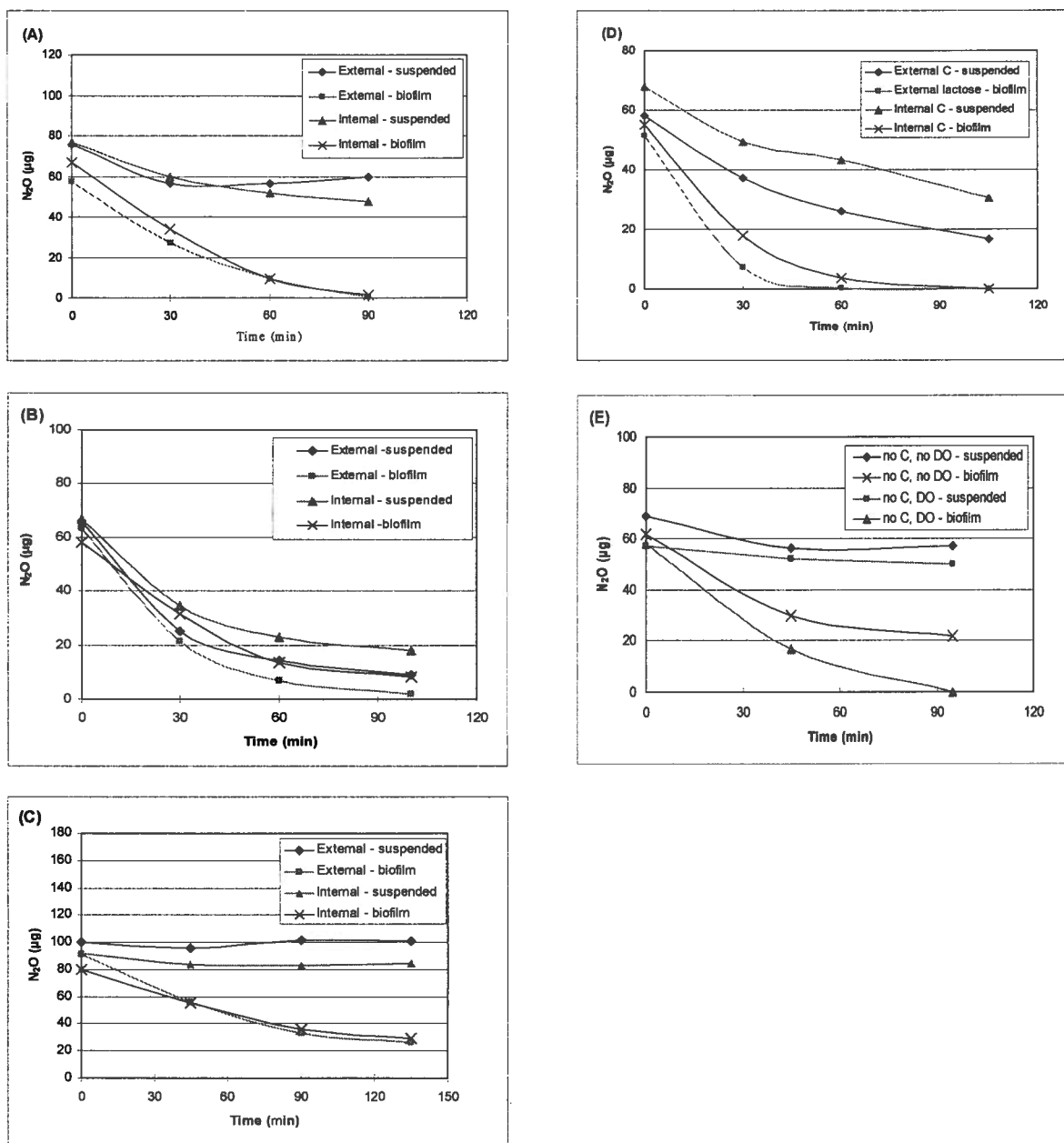


Fig 4.3 Examples of N₂O reduction time profile (A) acetate as carbon source with DO; (B) acetate as carbon source without DO; (C) lactose as carbon source with DO; (D) lactose as carbon source without DO; (E) no carbon source.

Acetate is commonly used as the carbon source in BNR systems. Other carbon sources have also been investigated for their effects on N₂O emissions. Using methanol as an external carbon source in BNR wastewater treatment, Tallec *et al.* (2006) found N₂O off-gas was only 0.2% of the reduced nitrate. Park *et al.* (2000) used methanol as the external carbon to remove 95% of the N₂O in a lab-scale municipal wastewater study. Sanchez-Martin *et al.* (2008) found that 94% of N₂O emissions were reduced with an addition of glucose into soil samples.

4.3.4 N₂O off-gas

Table 4.3 lists the results for the nitrogen reduction tests, the N₂O reduction rates and N₂O off-gas. When the nitrogen reduction rate was higher than the N₂O reduction rate, N₂O accumulated in the solution and could be stripped out or diffused into the air, as an off-gas. A larger amount of N₂O off-gas was due to a large difference in the nitrogen reduction rate and the N₂O reduction rate.

However, the results obtained from this study were not consistent. This inconsistency may be due to the differing experimental conditions applied in different parts of this study. The level of DO was not consistently controlled either. Additional studies are needed to determine the factors affecting the production of N₂O off-gas.

The DO levels were saturated in the initial N₂O analysis. This would inhibit the activities of nitrous oxide reductase. As a result, low N₂O reduction rates were obtained. The tests of external carbon source – aerobic – acetate – nitrate and nitrite shown in Table 4.3 were examples that had lower observed N₂O off-gas production.

The relationship of the production of N₂O to the nitrite concentration has been

summarized by Zeng *et al.* (2002). These authors attributed production of N₂O to the accumulation of nitrite, which inhibits the activity of nitrous oxide reductase. However, in the present study, large amounts of nitrous oxide were emitted from the reactor with a relatively low concentration of nitrite (eg: the test of internal carbon source – anoxic – acetate - nitrate). This suggests that N₂O production may be attributed to having a higher nitrogen reduction rate than a nitrous oxide reduction rate.

Table 4.3 Nitrogen reduction rates, N₂O reduction rates and N₂O off-gas

Carbon type	Stripping gas	Carbon source	Nitrogen type	Nitrogen reduction rate*	N ₂ O reduction rate*	N ₂ O off-gas (%)
<i>External</i>	air (aerobic)	acetate	NO ₃ ⁻	1.23	1.19	1.0
			NO ₂ ⁻	1.49		2.3
		lactose	NO ₃ ⁻	0.06	0.53	1.8
			NO ₂ ⁻	1.01		6.8
	N ₂ (anoxic)	acetate	NO ₃ ⁻	1.91	2.42	0.4
			NO ₂ ⁻	1.52		1.6
		lactose	NO ₃ ⁻	1.22	1.31	1.6
			NO ₂ ⁻	1.70		5.3
<i>stored</i>	air (aerobic)	acetate	NO ₃ ⁻	0.96	0.69	50.2
			NO ₂ ⁻	0.79		66.3
		lactose	NO ₃ ⁻	0.14	0.48	16.6
			NO ₂ ⁻	0.52		41.7
	N ₂ (anoxic)	acetate	NO ₃ ⁻	1.16	1.01	27.1
			NO ₂ ⁻	1.29		18.4
		lactose	NO ₃ ⁻	1.31	0.83	46.4
			NO ₂ ⁻	1.31		57.2
<i>No carbon</i>	air (aerobic)	--	NO ₃ ⁻	0.03	0.41	1.3
			NO ₂ ⁻	0.39		13.8
	N ₂ (anoxic)	--	NO ₃ ⁻	0.24	0.47	0.6
			NO ₂ ⁻	0.49		22.7

*: unit = mg N/g biomass*h

The reduction of nitrous oxide is most likely a 1st order reaction. The reduction rate decreased with a decrease of nitrous oxide concentrations in the solution. When denitrification was slow, the concentration of nitrous oxide was also low. The slow reduction rate of N₂O would give higher N₂O emissions. For instance, the denitrification rate for a set of internal carbon source – air – lactose – nitrate was much slower than the N₂O reduction rate. N₂O off-gas should have been close to zero. However, it produced N₂O off-gas. The maximum N₂O reduction rate of this case was measured as 0.48 mg N/g biomass*h for a relatively high initial N₂O, while the nitrogen reduction rate was only 0.14 mg N/g biomass*h. The N₂O concentration in the solution was expected to remain at a very low level with little nitrous oxide emission. In this case, the N₂O reduction rate for the test should be very slow as well, and therefore allow N₂O to be stripped out from the bulk solution.

The differences in the N₂O reduction rates were due to differences in carbon types, DO and N₂O concentrations over time, in the liquid. The estimated nitrous oxide reduction rates and a track analysis are reported in the next two sections. The air stripping and mechanical surface diffusion are herein reported.

4.3.4.1 Stripping of N₂O - aerobic phase

The mass-balance equation for the stripping of N₂O can be written as:

accumulation = inflow – outflow + reaction

$$\frac{dC}{dt} * V = (QC_i - QC_e) + (r_i * V) \quad (4.1)$$

where C = concentration in the solution (mg/L)

V = volume of the reactor (L)

Q = flow rate of influent (L/h)

C_i = concentration of the influent (mg/L)

C_e = concentration of the effluent (mg/L)

r_i = rate of reaction i (mg N/L/h)

In a batch reactor, for N₂O mass-balance, inflow = 0, outflow = N₂O off-gas

$$\frac{dC}{dt} * V = (-Q_g * C_g) + \sum r_i * V \quad (4.2)$$

where Q_g = air stripping rate (L/h)

C_g = N₂O concentration in the off-gas (mg/L)

r_i = denitrification rate, r_d (+) and nitrous oxide reduction rate, r_r (-) (mg N/L/h)

For stripping (van Schulthess *et al.*, 1994),

$$C_g = H * C_l * \left[1 - \exp\left(-\frac{K_{La, N_2O}}{H} \frac{V}{Q_g}\right) \right] \quad (4.3)$$

where H = Henry's law coefficient (dimensionless), which is 16 for N₂O (Fogg and Sangster, 2003)

K_{La, N_2O} = overall nitrous oxide transfer coefficient at 20°C (h⁻¹)

Combine Equation 4.2 and Equation 4.3

$$\frac{dC}{dt} = -\frac{Q_g}{V} * H * C_l * \left[1 - \exp\left(-\frac{K_{La, N_2O}}{H} \frac{V}{Q_g}\right) \right] + \sum r_i \quad (4.4)$$

K_{La, N_2O} can be calculated by the relation of K_{La, O_2} as Equation 4.5.

$$\frac{K_{La, N_2O}}{K_{La, O_2}} = \left(\frac{M_{w, N_2O}}{M_{w, O_2}} \right)^n \quad (4.5)$$

where K_{La, O_2} = overall oxygen transfer coefficient at 20°C (h⁻¹)

M_{w, N_2O} = molecular weight of N₂O (g) = 44

M_{w, O_2} = molecular weight of O₂ (g) = 32

Considering the effect of temperature, the overall oxygen transfer coefficient was modified by Equation 6.6.

$$K_{La, (T)} = K_{La, (20)} \theta^{T-20} \quad (4.6)$$

where $K_{La, (T)}$ = overall oxygen transfer coefficient at T°C (h⁻¹)

$K_{La, (20)}$ = overall oxygen transfer coefficient at 20°C (h⁻¹)

θ = temperature correction coefficient (1.024)

K_{La, N_2O} determination was conducted in Reactor B by using 450 new biofilm carriers with the decanted effluent, which had biomass less than 10 mg/L. The reactor was aerated by 0.2% N₂O (in air) standard gas at a rate of 200 mL/min. K_{La, O_2} was determined as 2.82 h⁻¹ and K_{La, N_2O} was 2.31 h⁻¹ (22°C). Using Equation 6.5, constant n was calculated as 0.82. Figure 4.4 and 4.5 present the overall oxygen and nitrous oxide transfer at different aeration rates in the reactor. At a higher aeration rate, the overall N₂O transfer is also higher, and more N₂O is stripped out from the bulk solution.

Although the nitrous oxide reduction rate varies with N₂O concentrations in the liquid, it can be determined by calculation. Denitrification rates, nitrous oxide concentration of

off-gas and N₂O concentration in the liquid have been measured; hence, N₂O reduction rates in the aeration period can be calculated from Equation 4.4. Figure 6.6 presents the concentrations of N₂O off-gas and N₂O in the bulk solution of a track study. Figure 4.7 is the time profile of nitrogen compounds for this track. The denitrification rates and calculated N₂O reduction rates are shown in Table 4.3 and Figure 4.8.

4.3.4.2 Diffusion by mechanical stirring – anoxic phase

The nitrous oxide mass-balance for N₂O diffusion can be written as follows (Metcalf and Eddy, 2003).

$$\frac{dC}{dt} * V = (-QC_i - QC_e) + (r_{N_2O} * V) + \sum r_i * V \quad (4.7)$$

where r_{N_2O} = rate of N₂O mass transfer (mg/L*h)

$$r_{N_2O} = K_{La, N_2O} (C - C_g) \quad (4.8)$$

In a batch reactor, inflow = 0. For diffusion of a completely-mixed reactor,

$$\frac{dC}{dt} = (K_{La, N_2O} * (C - C_g) + \sum r_i \quad (4.9)$$

Without aeration, oxygen transfer into the reactor was by a mechanical stirring device only. DO rose from around 1 to 4 mg/L in 2 hours. The DO time profile of the mechanical stirring is shown in Fig 6.8 (tested in the effluent with clean biofilm carriers and 60 rpm stirring). The value of $K_{La, O_2} = 0.23 \text{ (h}^{-1}\text{)}$, $K_{La, N_2O} = 0.82 * K_{La, O_2} = 0.19 \text{ (h}^{-1}\text{)}$ at 22°C. Using Equation 4.9, the nitrous oxide reduction rates were calculated and are shown in

Figure 4.9 and Table 4.3. The value of K_{La,N_2O} was based on the experiment of aeration tests, it is expected to be much higher than the true overall diffusion constant.

During the aeration period, the nitrous oxide reduction rates were slower than the nitrogen reduction rates (nitrous oxide production rate) with only small differences. N_2O off-gas increased over time. When aeration stopped, N_2O reduction rates were slightly higher than the nitrogen reduction rates. The profile of N_2O off-gas also decreased with time.

The DO increase by mechanical stirring implied that there was some oxygen “entrained” into the reactor in the anaerobic and anoxic phases; however, the transfer rate was not fast enough to raise the DO level of the bulk solution in the reactor from much above zero.

The emission of N_2O involved a number of factors, such as: autotrophic denitrification, heterotrophic denitrification, N_2O reduction, DO levels, carbon types, carbon states, aeration, mechanical stirring, pH, temp. Although a few simple models of N_2O off-gas from denitrification have been proposed (Wicht, 1996; van Schulthess and Gujer, 1996), the development of a comprehensive mathematical model is required in the future for modeling and predicting the emission of N_2O from a full-scale wastewater treatment plant.

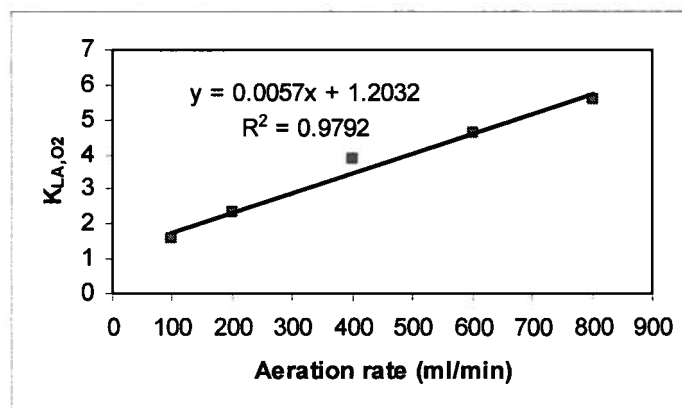


Fig 4.4 K_{LA} of O₂ for hybrid system reactor (22°C)

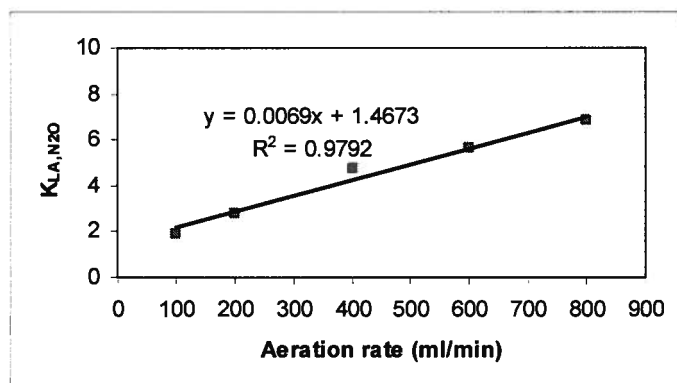


Fig 4.5 K_{LA} of N₂O for hybrid system reactor (22°C)

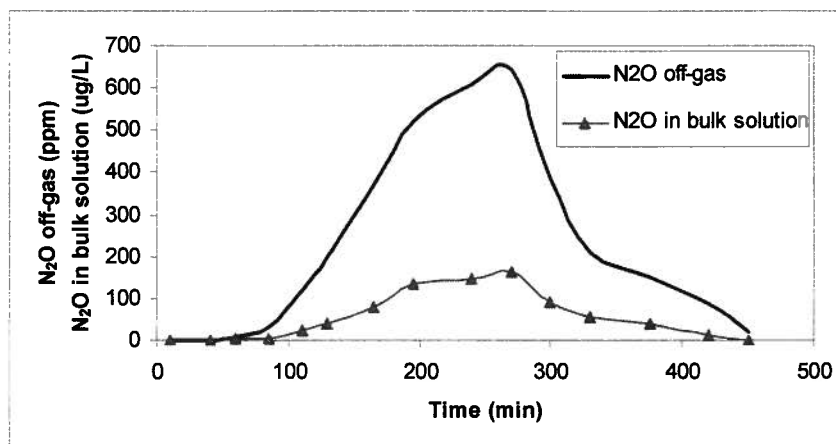


Fig 4.6 Time profiles of N₂O off-gas and N₂O in bulk solution

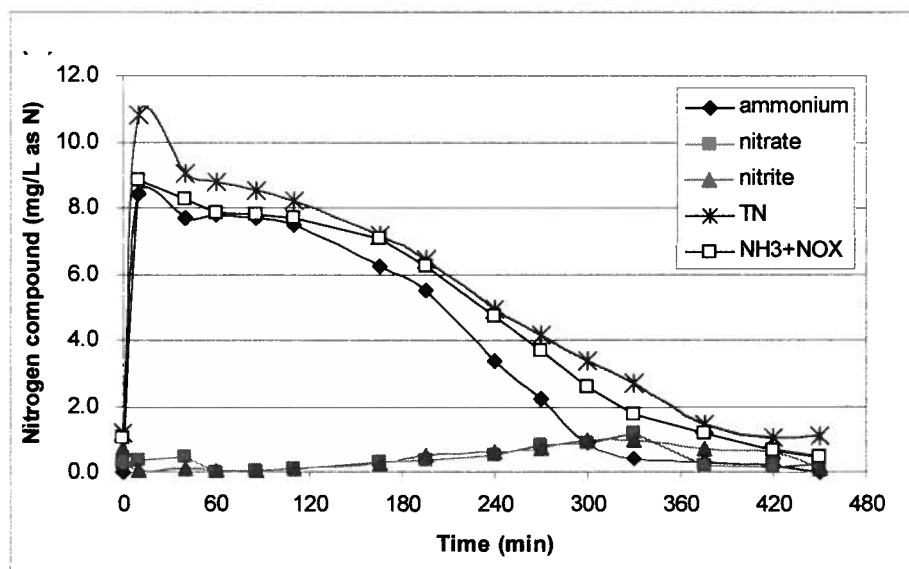


Fig 4.7 Time profiles of nitrogen compounds

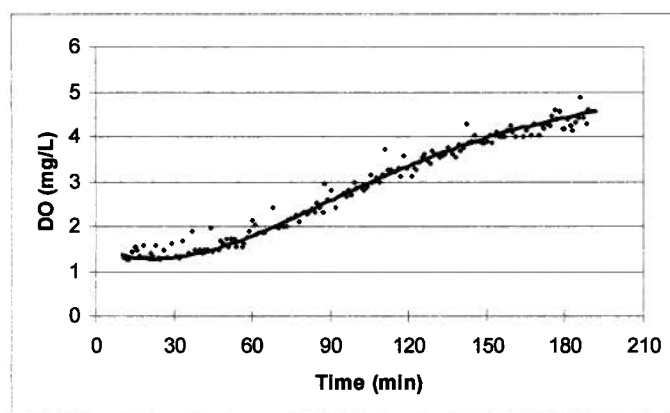


Fig 4.8 DO profile of mechanical stirring in the hybrid reactor

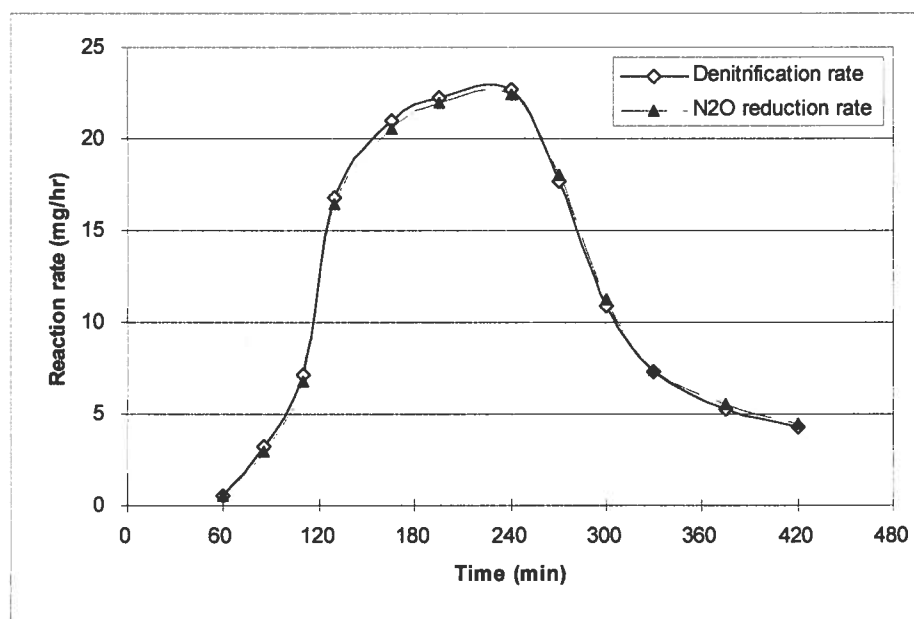


Fig 4.9 Time profiles of denitrification rates and calculated N_2O reduction rates

Table 4.4 Denitrification rates, nitrous oxide off-gas and calculated nitrous oxide reduction rates

Time (min)	C _g (ppm)	C _l (mg/L)	r _d (mg/hr)	r _r (mg/hr)	Note
10	0	0			anaerobic phase
			0	0	
40	0	0			
			0	0	
60	8	0.02			aeration started
			0.58	0.51	
85	82	0.05			
			3.20	2.94	
110	168	0.09			
			7.13	6.82	
130	230	0.17			
			16.80	16.40	
165	371	0.34			
			21.00	20.56	
195	520	0.55			
			22.25	21.95	
240	609	0.61			
			22.68	22.43	
270	644	0.67			
			17.64	18.04	
300	392	0.38			
			10.92	11.23	
330	210	0.25			aeration stopped
			7.28	7.40	
375	151	0.13			
			5.25	5.28	
420	88	0.05			post aeration
			4.29	4.45	
450	20	0.00			Settling

Note:

C_g = N₂O concentration in the off-gasC_l = N₂O concentration in the bulk solutionr_d = denitrification rater_r = nitrous oxide reduction rate

4.4 Conclusions

In this hybrid SBR SNDPR system, major N₂O off-gas was produced via the process of heterotrophic denitrification. N₂O emission from autotrophic denitrification was identified as insignificant.

N₂O off-gas can be expressed by the following two equations:

$$\text{N}_2\text{O off-gas} = \text{N}_2\text{O production (nitrate plus nitrite nitrogen reduction)} - \text{N}_2\text{O reduction} - \text{N}_2\text{O accumulation}$$

$$\text{N}_2\text{O off-gas} = \text{air stripping} + \text{mechanical surface diffusion}$$

The nitrogen reduction rate and N₂O reduction rate were affected by both DO conditions and the types and states of the carbon source, and resulted in various N₂O emissions:

- Denitrification rates under aerobic conditions were lower than for conditions without measurable DO.
- Nitrogen reduction rates were faster with an external carbon source than for conditions with a stored carbon.
- Nitrogen reduction rates varied with the carbon types. As an external carbon source, acetate seemed be favored for nitrate, while lactose had an affinity for nitrite.
- The rates of nitrogen reduction for the existence of PHB, as stored carbon, were higher than for glycogen, as stored carbon.
- N₂O reduction rates were higher when an external carbon source exists.

- N₂O reduction rates were higher under conditions without DO than under conditions with DO.
- N₂O reduction rates were higher for acetate than for lactose under conditions with and without DO.
- Denitrification, using stored carbon, produced more N₂O off-gas than the use of an external carbon source. The largest amount of N₂O off-gas production occurred in the existence of PHA and under aerobic conditions.
- N₂O reduction was higher for attached growth than the suspended growth when DO was present but also without DO.

Most likely, N₂O reduction was a 1-order reaction (although this assumption was not verified in this work). It varied with the concentrations of N₂O in the liquid. The real N₂O reduction rate can be calculated by means of a mass-balance of nitrous oxide. N₂O emission involves many parameters. A comprehensive mathematical model is required for modeling and predicting the emission of N₂O during actual wastewater treatment.

4.5 References

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Chapter 5 Factors affecting nitrous oxide emission

5.1 Introduction

N₂O is considered to be a greenhouse gas with a global warming potential of 310 times that of the contribution of carbon dioxide. Recently, it has been found that significant amounts of N₂O off-gas are emitted from some biological nutrient removal processes, especially the SND process (Meyer *et al.*, 2005; Zheng *et al.*, 2003; Third *et al.*, 2003). Tallec *et al.* (2006) concluded that N₂O production is closely correlated with nitrite accumulation. It has also been established that its production was enhanced by optimal growth conditions of pH (7.2), temperature (26 °C) and most importantly, relatively low oxygenation (Hynes and Knowles, 1984; Kuai and Verstraete, 1998; Jiang and Bakken 1999; Beline *et al.* 2001). Since urban wastewater treatment plants prefer conditions of less oxygenation (as they are more cost effective), the fact that these conditions are also favorable to N₂O production poses a problem.

Some researchers have tried to investigate the mechanisms of N₂O production, in order to reduce the amount of N₂O emissions. In one study, it was been found that maintaining DO levels greater than 0.5 mg/L and having SRTs of greater than five days, would greatly reduce N₂O emissions (Zheng *et al.*, 1994).

*A version of this chapter has been submitted for publication:

Lo, I., Lo, K.V., Mavinic, D.S., Shiskowski, D. and Remay, W. Reduction of Nitrous Oxide Formation from a Biological Nutrient Removal System in Wastewater Treatment – Factors of Aeration, External Carbon and pH, Water Environment Federation's Annual Technical Exhibition and Conference, 2008, Chicago (conference proceeding).

The carbon to nitrogen ratio (C/N) has also been investigated as one of the factors which impact on the production of N₂O. The lower the ratio of COD/NO₃-N, the higher the percentage of N₂O in the gas produced (Hong et al., 1993). "N₂O production can be avoided by achieving complete denitrification by maintaining a high COD/NO₃-N in wastewater, long SRTs and neutral to alkaline pH conditions" (Hanaki *et al.*, 1992).

Torn and Sorensson (1996) found nitrous oxide production was at its maximum at a pH of 5.5 and undetectable above a pH of 6.5. Shiskowski and Mavinic (2006) investigated the effects of pH on nitrite and N₂O generation.

Both the advantages of using a SBR hybrid system for the SNDPR process and the N₂O production mechanisms have been identified in the previous chapters. This chapter reports on investigations into the effects which the aeration rate, the mode of influent feeding and the pH have on N₂O off-gas production with the aim of operation strategies for reducing N₂O emissions from the treatment system.

5.2 Experimental design

5.2.1 *The effect of the aeration rate*

The influent and operating conditions for the tests on aeration were described in Chapter 1, except that, here, the aeration rates were varied. Aeration rates of 100, 200, 400 and 600 mL/min were applied, in order to investigate the effects of aeration on the hybrid system.

5.2.2 The effect of the feed pattern

The effects of the feed pattern were investigated by feeding influent continuously during the aeration period. Four sets of tests were conducted: 100, 90, 80 and 70% influent were fed in the beginning of the anaerobic phase, and the remaining part 0, 10, 20 and 30% of the influent were continuously fed into the reactor during the aerobic phase.

5.2.3 The effect of pH

Low, neutral and high pH at 5.7, 7.0 and 8.2 were maintained, in order to investigate how pH affects nitrogen removal. The actual track study was conducted 2 weeks after the change of pH, in order for the system to be acclimated. N₂O liquid-gas phase diffusion coefficient (Section 1.5.3.5) and N₂O reduction rates (Section 4.2.3) at different pH levels were also examined. Acetate (initial concentration in the reactor was around 55 mg/L as TOC) was used as the carbon source for the tests. Since N₂O production was found to be insignificant when external carbon exists (Chapter 4), only the conditions of stored carbon were tested.

5.3 Results and discussion

5.3.1 The effects of the aeration rate on N₂O off-gas

Aeration rates of 100, 200, 400 and 600 mL/min were applied to investigate N₂O off-gas production from the hybrid system. The time profiles for nitrogen compounds, DO, ORP and N₂O off-gas are shown in Figures 5.1 to 5.4.

Aeration started after 60 min of anaerobic carbon uptake. The time periods for completing ammonia oxidation for each test were 390, 240, 210 and 150 min for aeration rates of 100, 200, 400 and 600 mL/min, respectively (Fig 5.5A). N₂O off-gases produced during aeration were approximately 8.3, 13.5, 28.1 and 21.3% as total nitrogen in the influent (Fig 5.5B). Table 5.1 summarizes the performance, in terms of nitrogen removal, for the different aeration rates.

Ammonia oxidation has been proven to be closely related to DO (Gujer *et al.* 1999). During the aeration period, a higher aeration rate results in a higher ammonia oxidation rate (AOR). The average AORs were from 0.24 mg N/g biomass*h at 100 mL/min to 0.60 mg N/g biomass*h at 600 mL/min. A higher aeration rate accelerated the rate of ammonia oxidation. On the other hand, a lower AOR resulted in slower production of nitrite and nitrate. This limited the rate of denitrification (DNR). The average DNRs ranged from 0.23 mg/g biomass*h at 100 mL/min to 0.42 mg/g biomass*h, at 600 mL/min. The SND efficiency was lower at a high aeration rate, due to the fact that it resulted in a relatively high AOR and nitrite oxidation rate (NOR). These have been higher than the DNR and caused greater accumulation of nitrate and nitrite. A lower aeration rate also resulted in a lower DO and led to a better SND. The SND ranged from 73% at aeration rate 600mL/min to 83% at 100 mL/min.

The range of aeration rates from 100 to 600 mL/min did not seem to greatly affect nitrite oxidation and reduction, The concentrations of nitrite in these track analyses remained at low levels, below 2 mg/L. Nitrite did not built up, nor were high amounts of nitrate found at the end of the aeration period.

Other researchers have studied the effects of DO on nitrous oxide production during wastewater treatment (Tallec *et al.*, 2007; Tallec *et al.*, 2006; Shiskowski, 2004; Park *et al.*, 2000, Noda *et al.*, 2003). Noda *et al.* (2003) found that N₂O emission was enhanced under lower DO conditions, where the available oxygen was insufficient for nitrification. In this case, autotrophic N₂O production was not significant (Chapter 2). It is obvious that air flow rates directly affected the N₂O emission of the overall gas-liquid transfer coefficient, resulting in higher N₂O off-gas production. It is larger when the aeration rate is higher (Section 4.3.4.1). A lower N₂O emission was found because of the higher residual nitrite and nitrate at the end of the aeration period, accompanied by a decrease of SND, at aeration of 600 mL/min (Fig 5.5B and Table 5.1).

Free ammonia (NH₃) was calculated as 0.04 mg/L at the beginning of the treatment (temp 22°C and pH around 7; the acid dissociation constant pK_a of NH₃ is 9.23). Thus, the nitrogen lost by ammonia stripping in this case was negligible. Based on the results, a lower aeration rate led to a lower AOR and DNR. Thus, a lower aeration rate resulted in a longer treatment time for nitrogen removal. However, the SND was higher when the aeration rate was lower. In this study, N₂O off-gas increased with an increase in the aeration rate below 400 mL/min.

Table 5.1 Summary of nitrous oxide off-gas test at different aeration rates

Aeration (mL/min)	AOR ¹	DNR ¹	N ₂ O off-gas (%)	SND (%)
100	0.24	0.23	8.3	83.2
200	0.51	0.40	13.5	82.7
400	0.52	0.38	28.1	81.4
600	0.60	0.42	21.3	73.2

1. Average rates; unit = mg N/g biomass*h

2. Rates were presented as measured average rates of the stage.

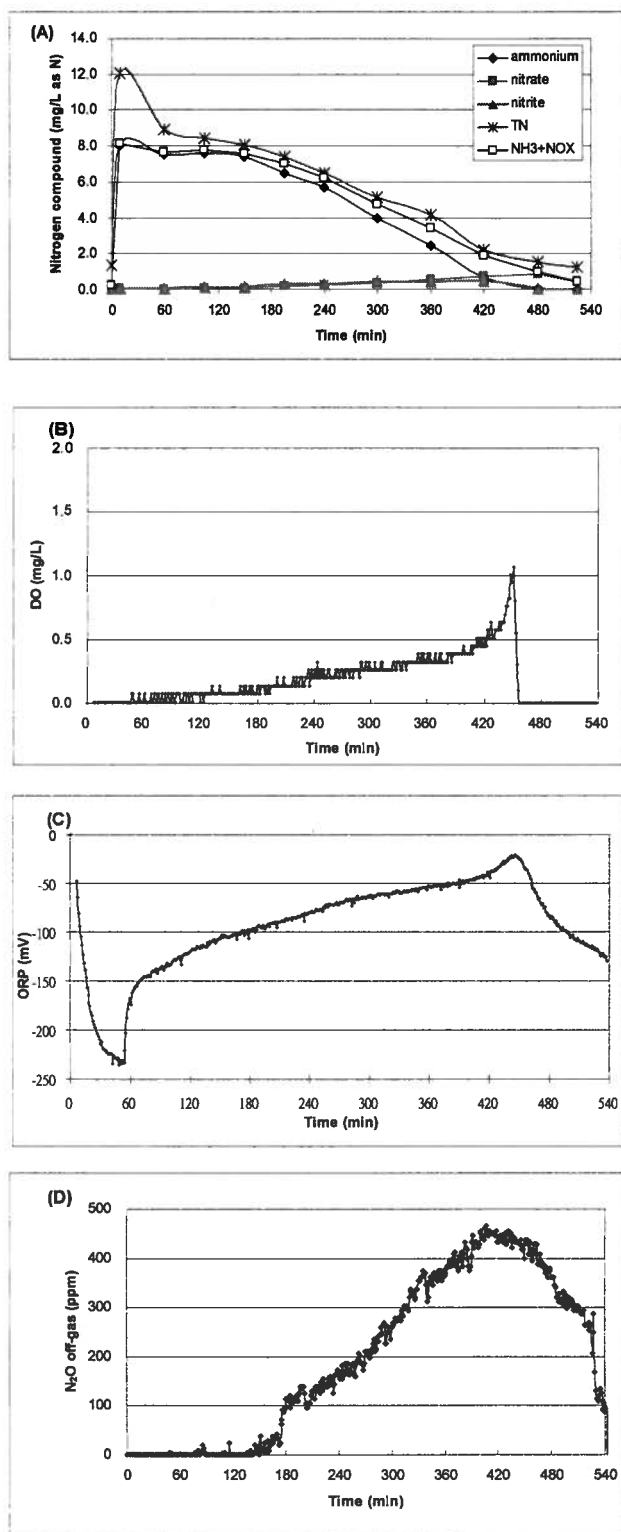


Fig 5.1 Time profiles of aeration rate at 100 mL/min (A) nitrogen species; (B) DO; (C) ORP; (D) N_2O off-gas.

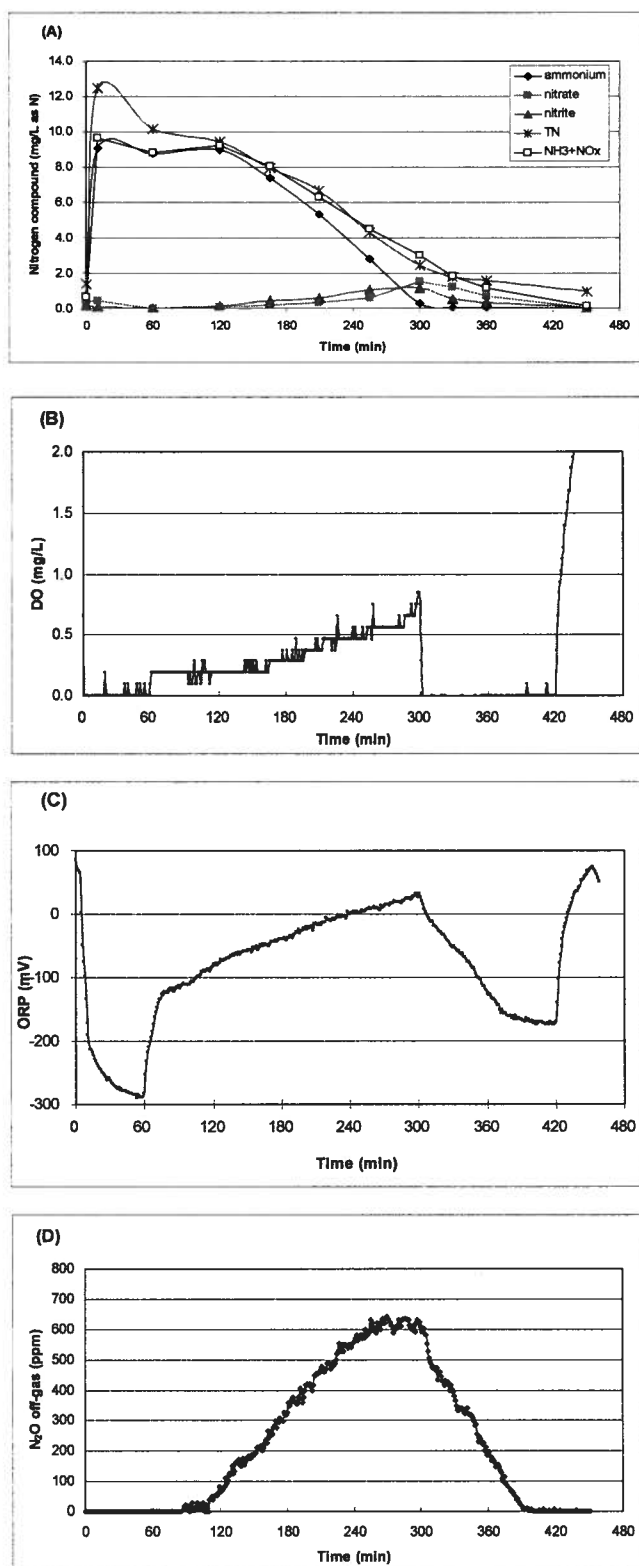


Fig 5.2 Time profiles of aeration rate at 200 mL/min (A) nitrogen species; (B) DO; (C) ORP; (D) N_2O off-gas.

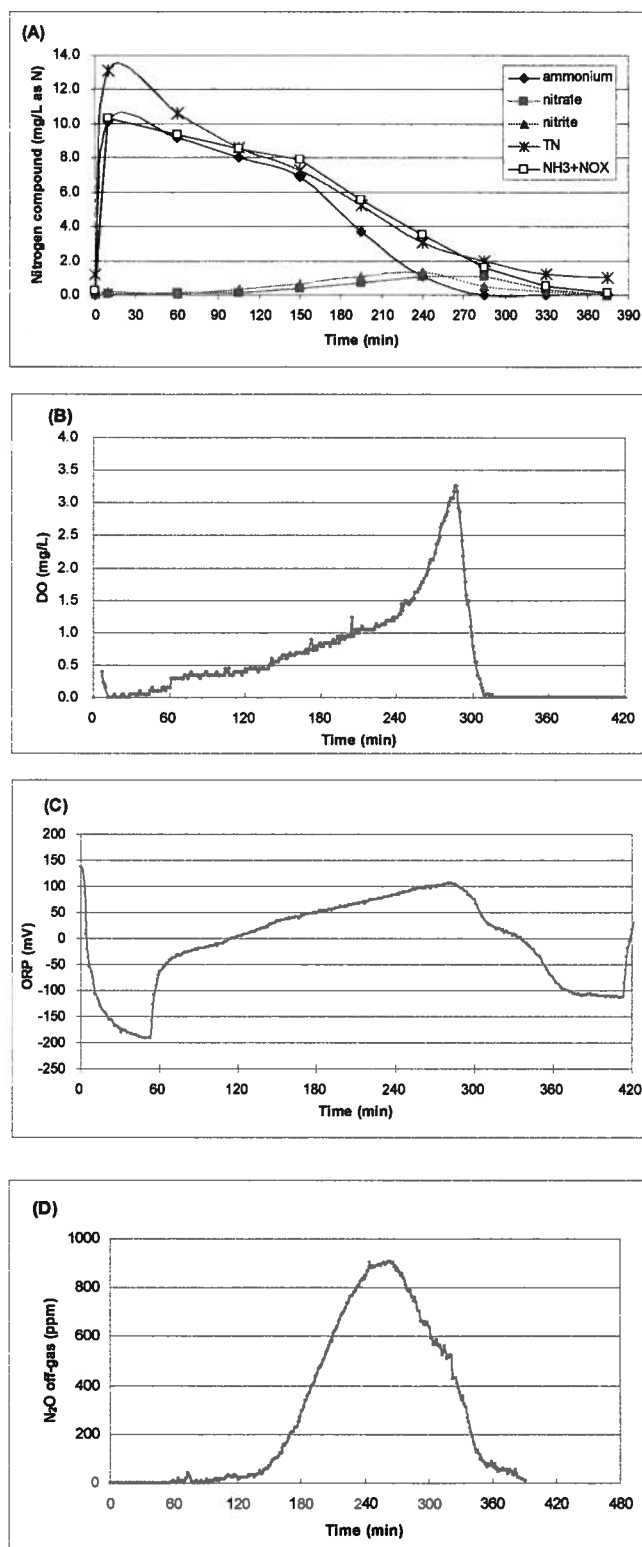


Fig 5.3 Time profiles of aeration rate at 400 mL/min (A) nitrogen species; (B) DO; (C)ORP; (D) N_2O off-gas.

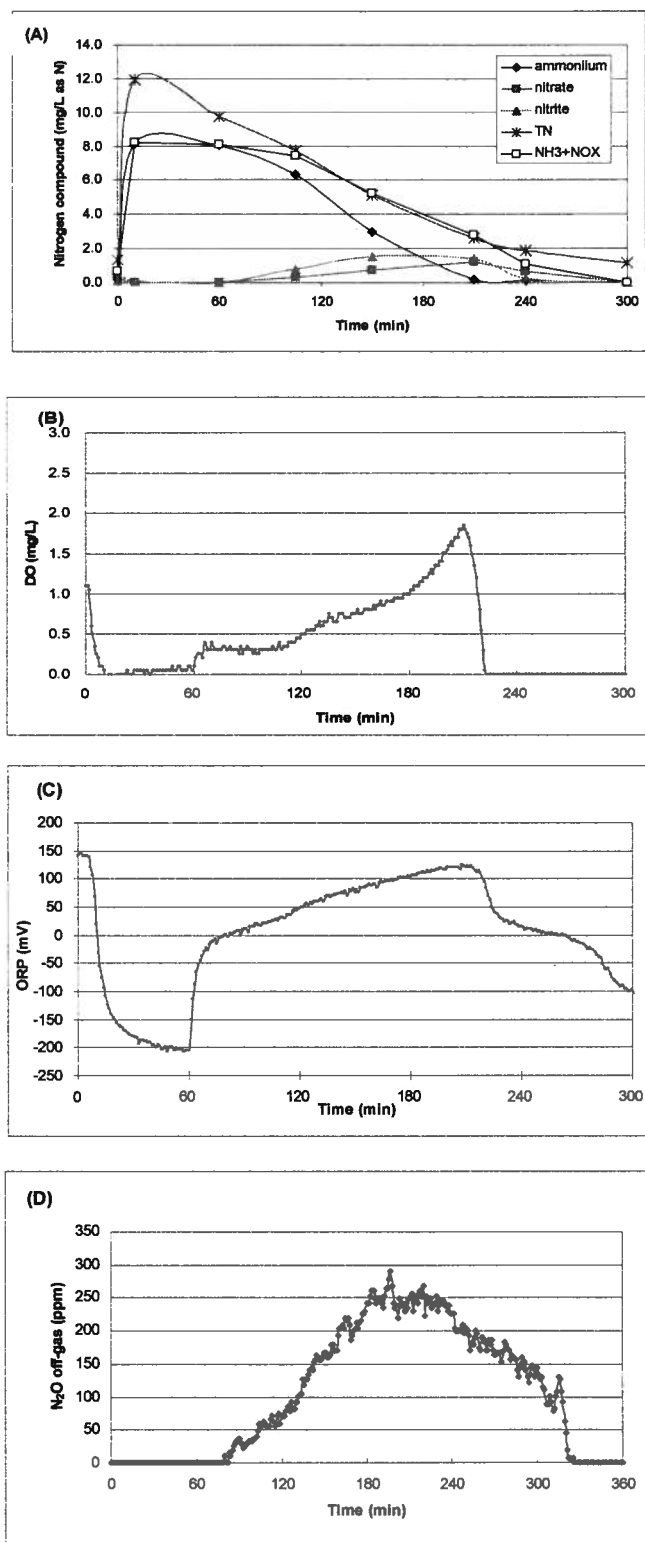


Fig 5.4 Time profiles of aeration rate at 600 mL/min (A) nitrogen species; (B) DO; (C) ORP; (D) N_2O off-gas.

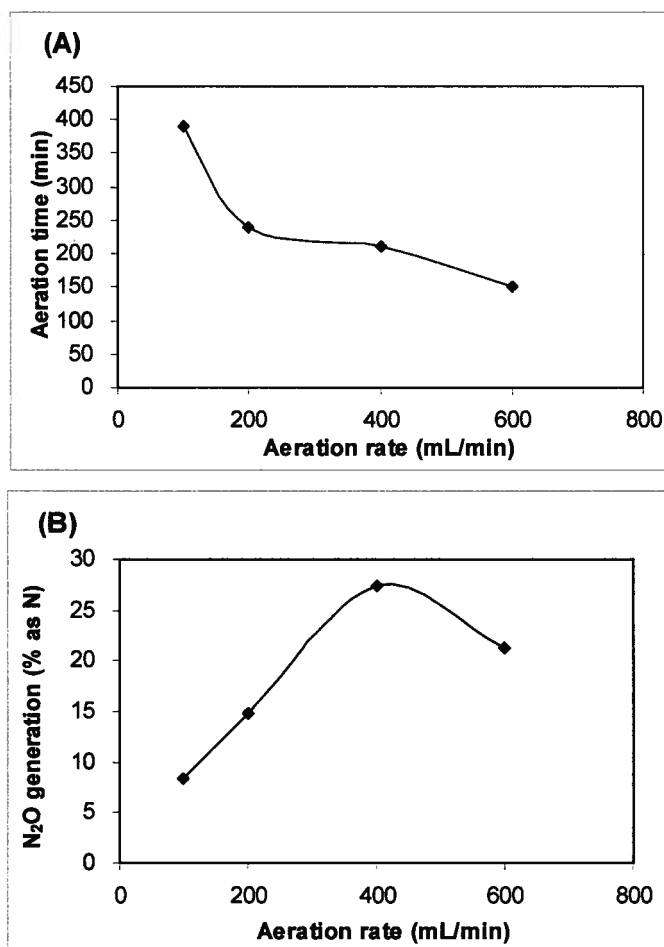


Fig 5.5 Nitrous oxide productions and aeration rates (A) aeration time vs. aeration rate when ammonium was depleted; (B) N_2O production vs. aeration rate

5.3.2 The effect of feed pattern

The conclusions of Chapter 4 state that the existence of an external carbon results in a higher N_2O reduction rate. The addition of an external carbon might, therefore, reduce the production of N_2O off-gas from the treatment process. Four track analyses with 0, 10, 20 and 30% of the influent were reserved from the feed and were fed continuously and evenly into the reactor during the aeration period. The aeration rate was 200 mL/min, and

the aeration time was 240 min for each track. The time profiles of nitrogen compounds, TOC, ortho-P, DO, ORP and N₂O off-gas are shown in Fig 5.6 to 5.9. The results are summarized in Table 5.2.

The production of N₂O off-gas during the aeration time was reduced by the increase in continuous feeding in the aerobic zone. It was reduced from 16% (N₂O-N/TN) at 0% continuous feed in the aeration period to 7%, at 30% continuous feed in the aeration period. Residual nitrite and nitrate were also reduced with the increase in continuous feeding during the aerobic phase. With 30% continuous feed, both nitrite and nitrate were zero at the end of the aeration period. Various continuous feeds did cause an evident change of SND. SND levels at the end of aeration were approximately 83, 75, 78 and 93% for 0, 10, 20 and 30% continuous feed, respectively. The ammonia oxidation rate was reduced by increasing the continuous feeding in the aerobic phase since part of the oxygen was used for carbon oxidation.

When the influent was continuously fed into the reactor, not only carbon but also ammonia and phosphorus were also fed into the treatment system. At the end of aeration, the concentrations of NH₃/NH₄⁺ and ortho-P were higher when the continuous feed was higher. Results from this study suggest that a continuous feed of more than 30% during the aerobic phase, might result in excess residual ammonia and phosphorus in the effluent.

Another study of the effects of fill modes on N₂O emissions at different stages of the process found that significant N₂O conversion occurred when filling the influent at the aerobic stage; however, only a small amount of N₂O conversion was obtained when the influent was filled at the anoxic stage. That research concluded that “for control of N₂O

emissions in the SBR, the aerobic fill mode could be an effective method" (Park *et al.*, 2001). Shiskowski and Mavinic (2005) slowly pumped sodium acetate into an SBR during the aerobic phase, to simulate the existence of slowly degradable carbon in the solution and found significant reduction of N₂O off-gas, via heterotrophic denitrification.

The continuous feeding evidently reduced nitrous oxide off-gas and enhanced SND efficiency, in this SNDPR hybrid SBR. Ammonia, phosphorus and carbon removal still remained at acceptable levels.

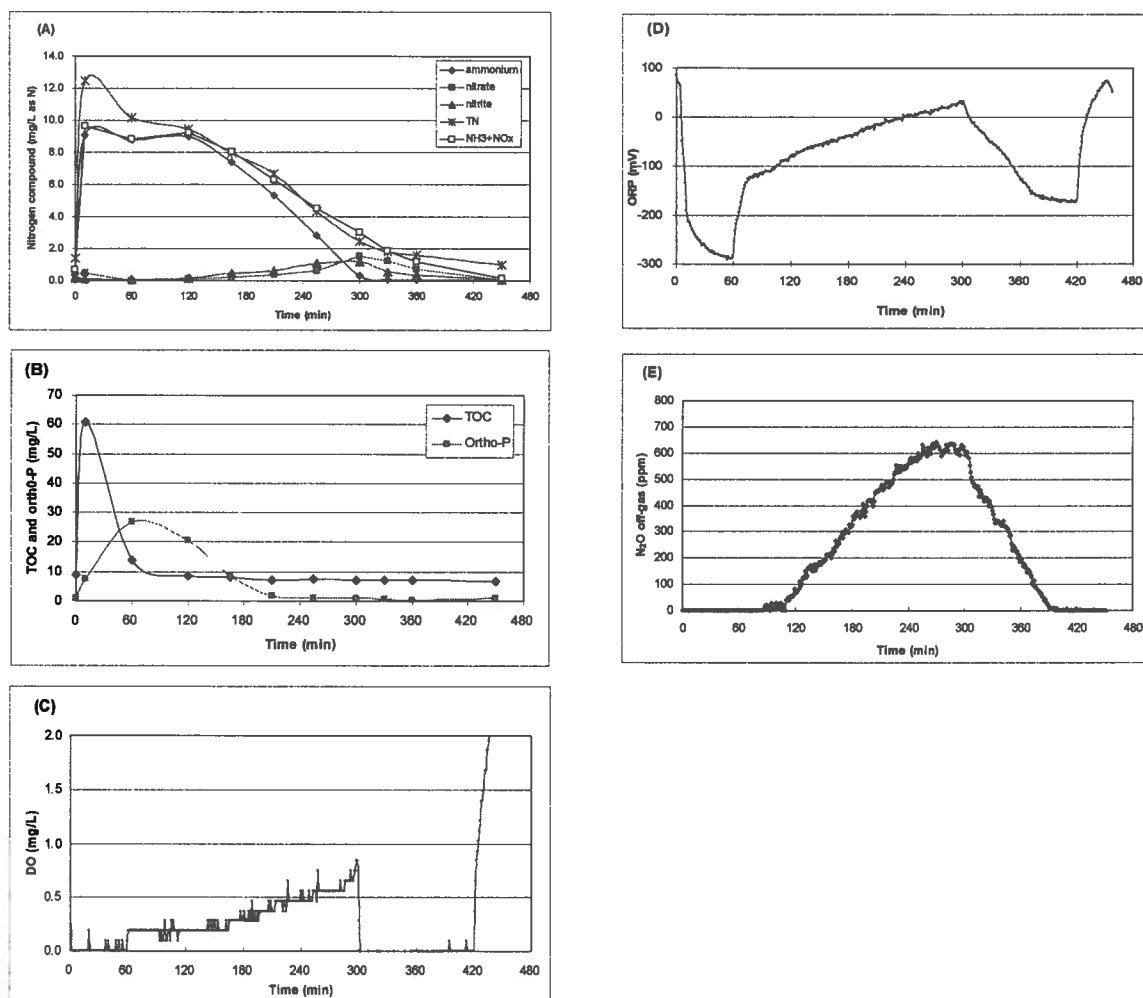


Fig 5.6 Time profiles of 0% second feed (A) nitrogen species; (B) TOC and ortho-P; (C) DO; (D)ORP; (E)N₂O off-gas.

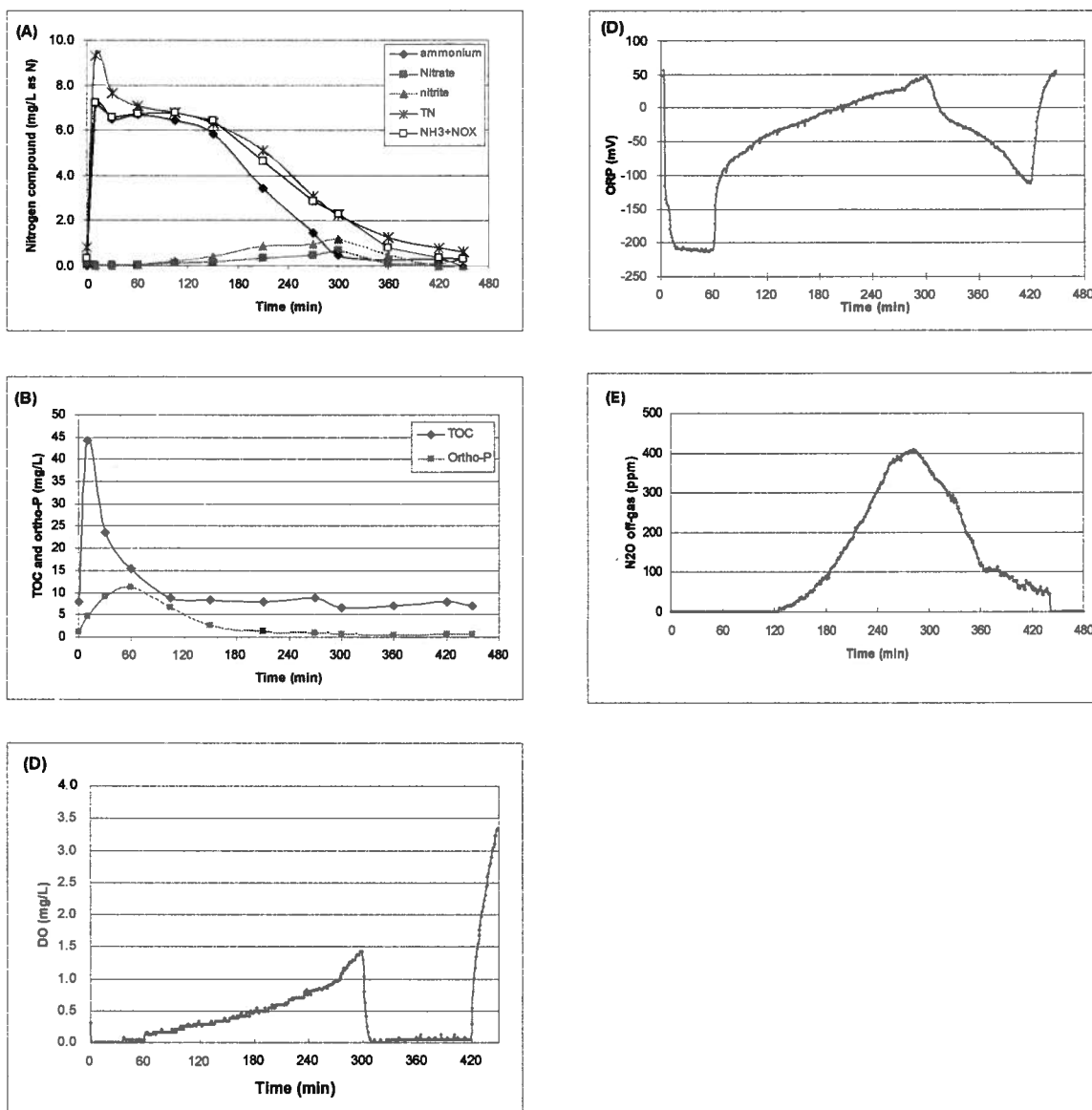


Fig 5.7 Time profiles of 10% second feed (A) nitrogen species; (B) TOC and ortho-P; (C) DO; (D)ORP; (E) N_2O off-gas.

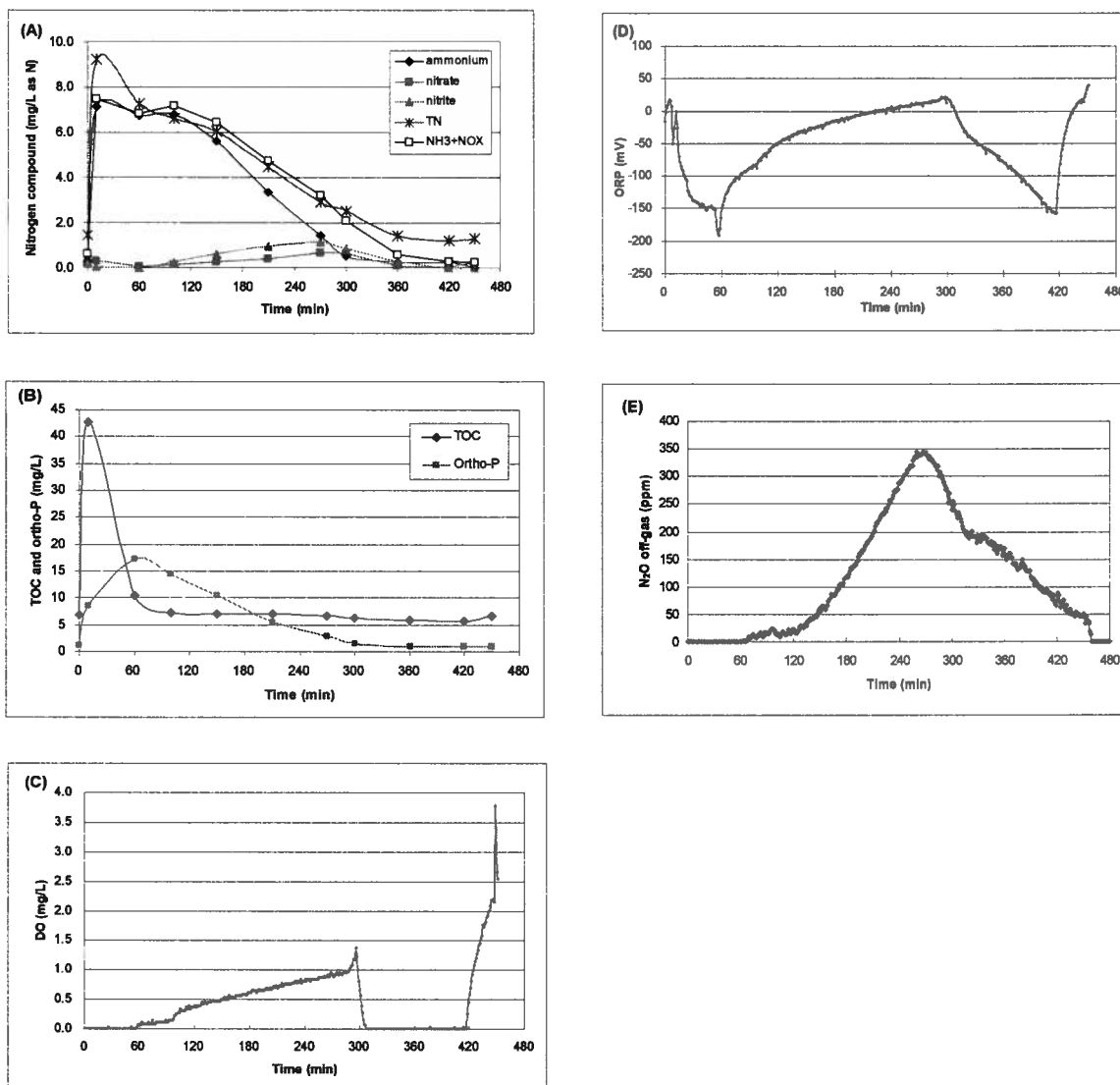


Fig 5.8 Time profiles of 20% second feed (A) nitrogen species; (B) TOC and ortho-P; (C) DO; (D) ORP; (E) N_2O off-gas.

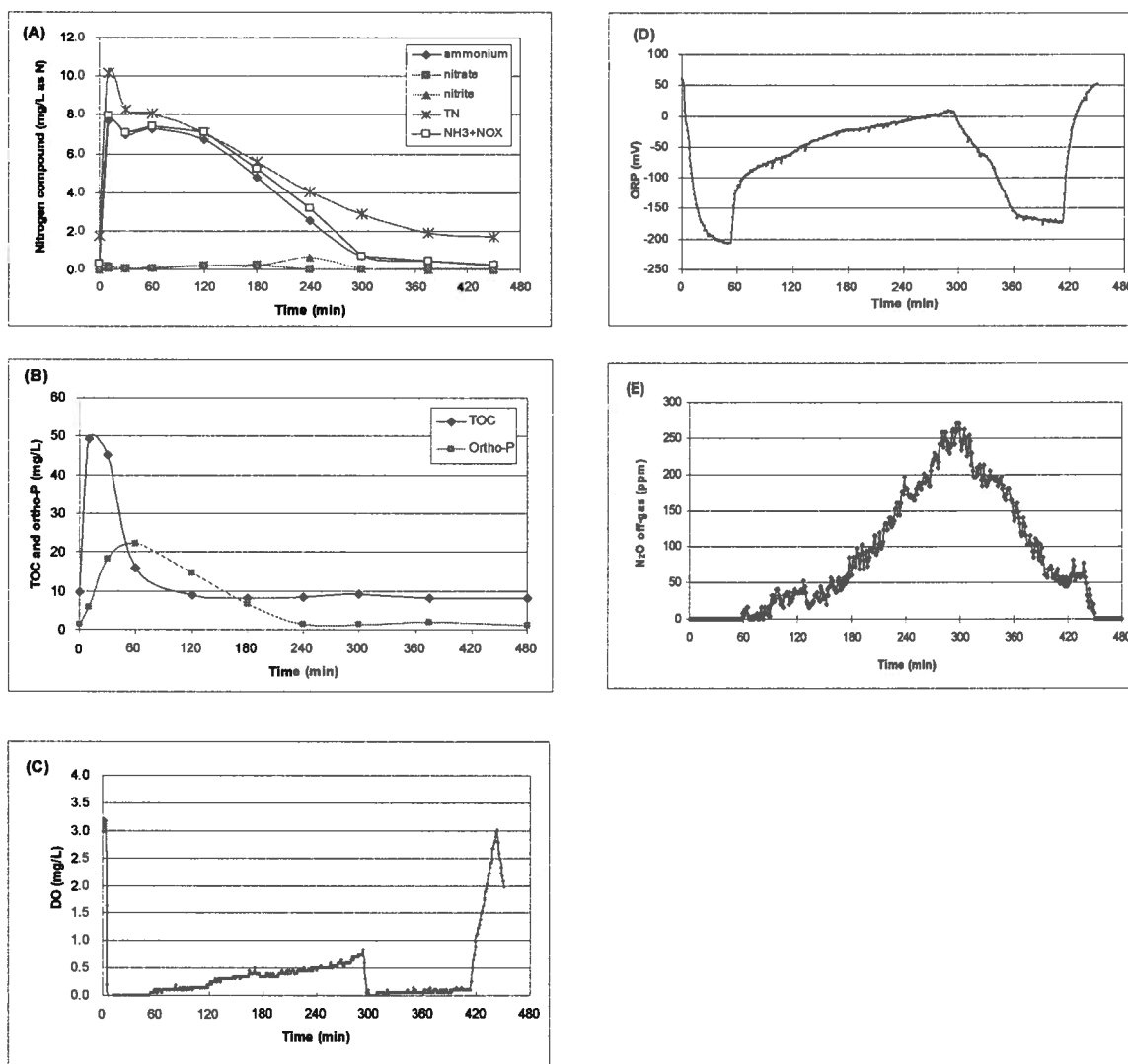


Fig 5.9 Time profiles of 30% second feed (A) nitrogen species; (B) TOC and ortho-P; (C) DO; (D)ORP; (E) N_2O off-gas.

Table 5.2 Summary of nitrous oxide off-gas test at different feed patterns

Second feed	NH ₄ ⁺ (N mg/L)	NO ₃ ⁻ (N mg/L)	NO ₂ ⁻ (N mg/L)	Ortho-P (P mg/L)	AOR ¹	DNR ¹	N ₂ O (%)	SND (%)
0	0.30 (0)	1.52 (0.38)	1.20 (0)	0.69 (0.60)	0.47	0.35	15.6	82.7
10%	0.49 (0)	0.78 (0.33)	1.27 (0)	0.74 (0.68)	0.37	0.30	14.2	75.0
20%	0.52 (0)	0.67 (0.27)	0.88 (0)	1.43 (0.96)	0.35	0.29	9.6	77.6
30%	0.69 (0.2)	0 (0)	0 (0)	1.40 (1.00)	0.34	0.30	6.6	92.9

1. Average rates; unit = mg/g biomass*h as N.
2. Data outside the parentheses were concentrations at the end of aeration; data in parentheses were at the end of the cycle.
3. Rates were presented as measured maximum rates of the stage.

5.3.3 The effect of pH

5.3.3.1 Nitrous oxide off-gas at different pH levels

The production of N₂O off-gas was tested for pHs of 5.7, 7.0 and 8.2. Figure 5.10 shows the nitrogen compound time profiles for these track studies. The summary of the results is shown in Table 5.3.

When pH was at 8.2, AOR was approximately 0.61 mg/g biomass*h as N (the actual AOR would be lower because of the lost of free ammonia caused by stripping at this pH); it was 0.47 mg/hr/g biomass as N at a pH = 5.7. Hence, the AOR at pH = 8.2 was around

1.30 times higher than it was at pH =5.7. That is, it took longer for complete ammonia oxidation at a lower pH.

The highest denitrification rate occurred at neutral pH (0.44 mg/hr/g-biomass as N). A higher pH caused a lower denitrification rate (0.28 mg/hr/g-biomass as N at pH = 8); it was lowest (0.16 mg/hr/g-biomass as N) at a pH = 5.7. Thus the NDR at pH 7.0 was 2.8 times higher than it was at pH 5.7. Nitrite did not accumulate in any case, with the concentrations of nitrite below 1 mg/L.

The N₂O off-gas of the pH 7.0 and 8.2 were about 15.6 and 4.0 % (N₂O-N/TN). For the track analysis of pH =5.7, ammonia could not be fully oxidized during the 3-hour aeration time. Around 30% of ammonia still remained in the solution, and N₂O off-gas was measured at about 19.1% of TN at this time. The emission of N₂O decreased with a increase in pH.

SNDs were approximately 83 and 74% for pH 7.0 and 8.2, respectively. The SND efficiency at pH 5.7 is not given because ammonia oxidation was not complete. SND at pH = 8.2 was lower than it was at pH = 7, probably due to the presence of more nitrite and nitrate in the solution during the aeration phase (as a result of a higher AOR).

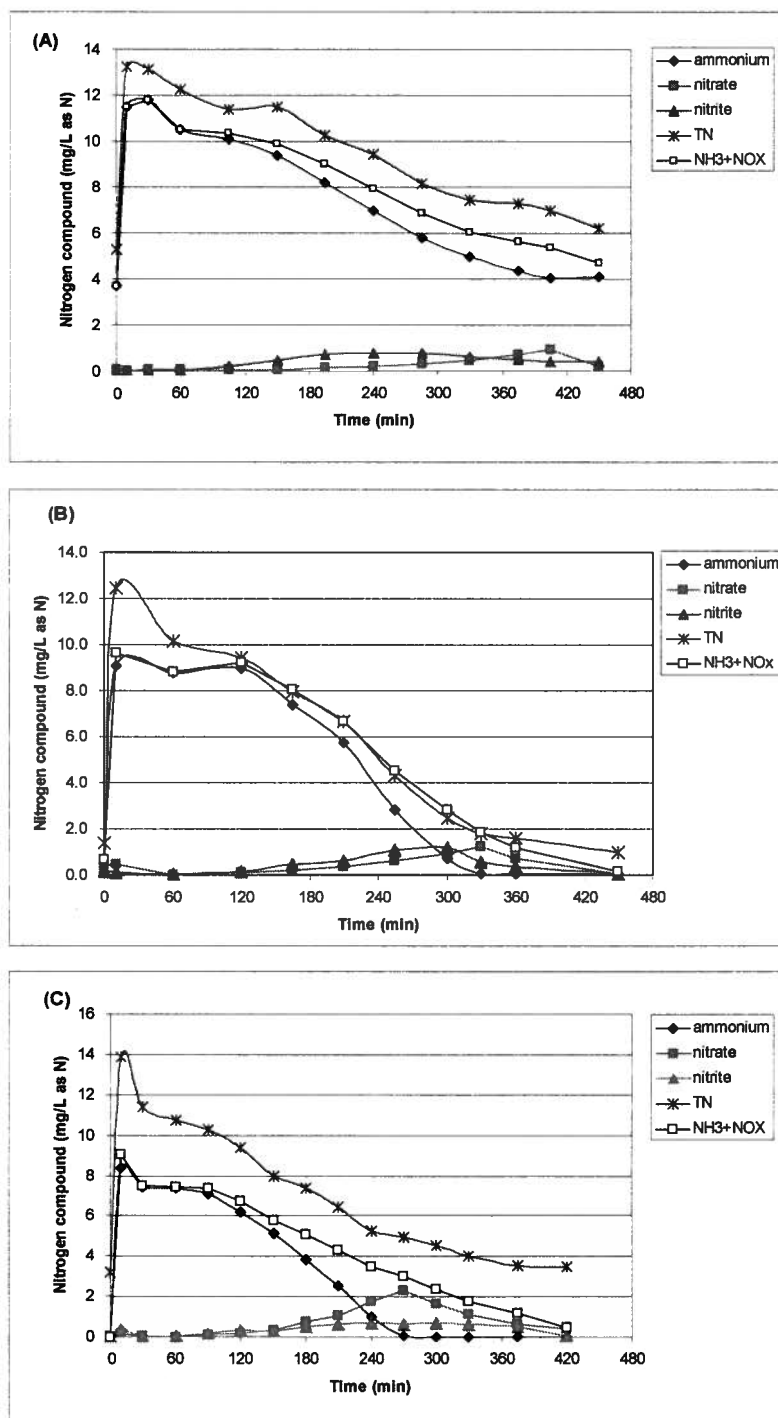


Fig 5.10 Nitrogen time profiles at (A) pH=5.7; (B) pH=7.0; (C) pH=8.2 when fed with synthetic wastewater (aeration stopped at time 390, 300, and 270 min, respectively)

Table 5.3 Summary of nitrous oxide off-gas test at different pH levels

pH	AOR ¹ (mg/hr/g-biomass as N)	DNR ¹ (mg/hr/g-biomass as N)	N ₂ O production (%)	SND (%)
5.7	0.47	0.16	19.1 ²	— ²
7.0	0.53	0.44	15.6	82.7
8.2	0.61	0.28	4.0	73.9

1. Average rates; unit = mg/g biomass*h as N.

2. Ammonia oxidation was not completed at the end of the cycle.

5.3.3.2 Soluble nitrous oxide at different pH levels

The solubility of N₂O varies at different pH levels. The values of the liquid-gas phase diffusion coefficients were 0.58, 0.24 and 0.36 for pH 5.7, 7.0 and 8.2, respectively at 22°C (Table 5.4). It has been found previously to be 0.62 for pure water at pH=7.0 and a temperature of 20°C (Tiedje, 1982). Further study is, therefore, needed to explore the reasons why the liquid-gas phase diffusion coefficient of N₂O varied under such different pH conditions. The concentrations of N₂O in the liquid and off-gas, for each batch, were analyzed and are shown in Figure 5.11.

In the test at pH 5.7, there were relatively high concentrations of N₂O in the liquid. It seemed that, under aerobic conditions, nitrous oxide reductase was less active at low pH levels. When aeration stopped at time 390 min, the N₂O concentrations in the liquid suddenly increased. This sudden accumulation of soluble N₂O indicated that the emission of N₂O was largely due to air stripping and poor N₂O reduction. The time profile of soluble N₂O at pH 7.0 was similar to the profile for off-gas. However, the concentrations of soluble

N₂O were only around half of those in the off-gas. At pH 8.2, the concentration of N₂O in the bulk liquid was close to the concentration of off-gas during aeration. Soluble N₂O increased rapidly when aeration stopped. Possibly because the activity of N₂O reductase was inhibited under conditions without DO, at the higher pH level. When soluble N₂O had accumulated to a certain concentration, it decreased again during the anoxic stage.

Table 5.4 The liquid-gas diffusion coefficient of nitrous oxide (T=22°C)

pH	5.7	7	8.2
A	0.58±0.09	0.24±0.01	0.36±0.06

1. Replicate = 3
2. $\alpha = 0.62$ in pure water at 20°C and pH = 7.0 (Tiedje, 1982)

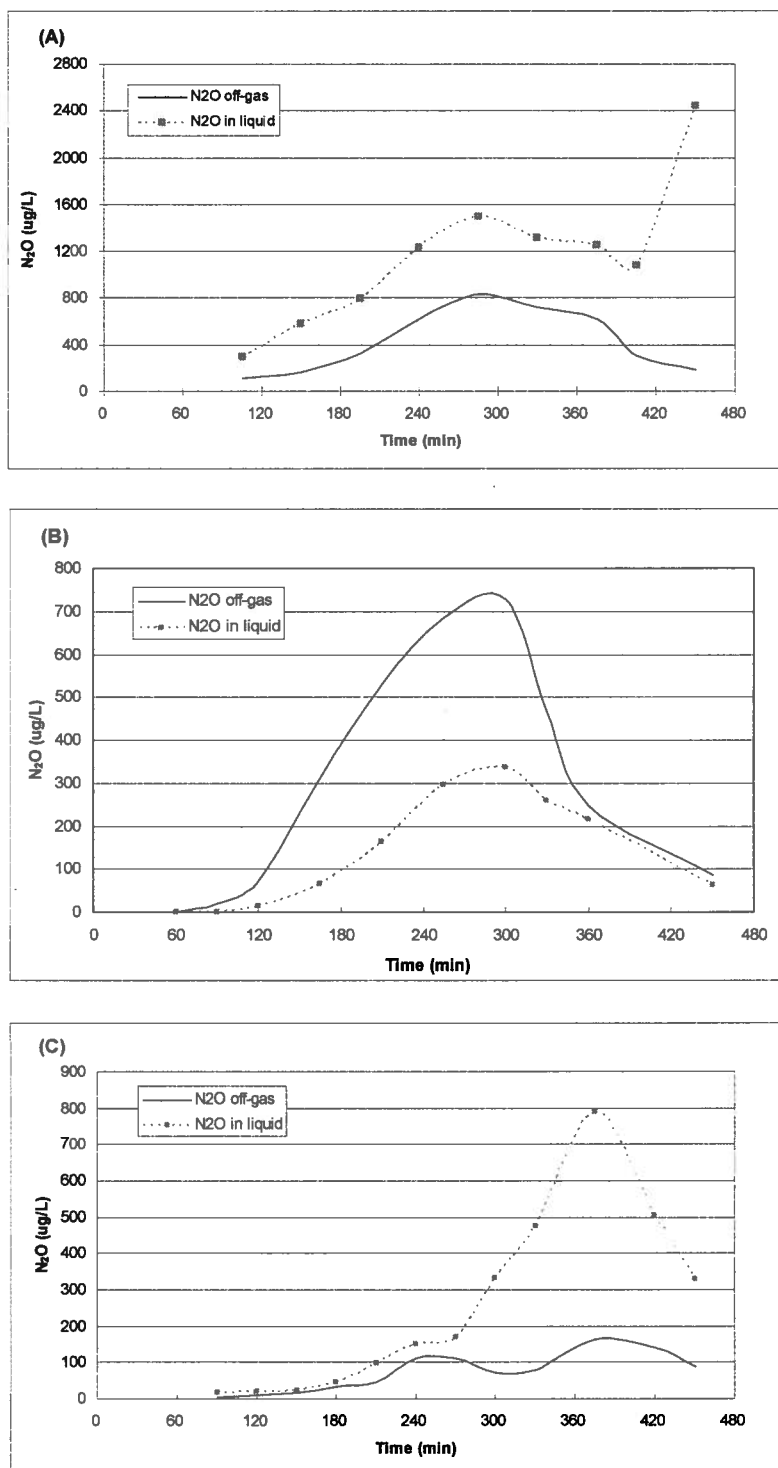


Fig 5.11 N₂O off-gas and N₂O in bulk liquid at different pH level (A) pH=5.7; (B) pH=7.0; (C) pH=8.2 (aeration stopped at time 390, 300, and 270 min, respectively)

5.3.3.3 Nitrous oxide reduction at different pH levels

Nitrous oxide reduction rates for the suspended sludge and the biofilm were determined individually, in order to investigate the effects of pH on N₂O reduction. Given that N₂O emissions were minor when acetate existed as an external carbon, only N₂O reduction using stored carbon was analyzed. The results are shown in Table 5.5 and Figure 5.12. It is interesting that under aerobic conditions, the suspended sludge had a higher N₂O reduction rate at higher pH levels. It was approximately 3.08 mg/hr/g-biomass as N at pH = 8.2 and 0.43 mg/g biomass*h as N at pH 5.7. On the other hand, the reduction rate of the biofilm was not affected by pH as much as the suspended sludge under aerobic conditions. Under aerobic conditions, the overall nitrous oxide reduction rate in the hybrid system was higher at a higher pH.

Under conditions without dissolved oxygen, the biofilm had a higher N₂O reduction rate at a low pH. The rate was about 2.4 mg/hr/g-biomass as N at pH = 5.7 and 0.74 mg/hr/g-biomass as N at pH = 8.2. In contrast, the reduction rate for the suspended sludge varied little with changes in pH. The rates were around 1.7 mg/hr/g-biomass as N. In the hybrid system, the overall nitrous oxide reduction rate was higher at a lower pH, under anoxic/anaerobic conditions.

In Chapter 3, it was reported that the suspended sludge was dominated by AOBs and NOBs, while the biofilm was dominated by denitrifiers. Results from the pH tests reported above suggest that the N₂O reduction enzyme of AOBs is more active at a higher pH level, while the N₂O reduction enzyme of the denitrifiers is more active at a lower pH level.

The accumulation of NO₂⁻ is commonly considered to be the main factor in N₂O

production in a wastewater treatment system (Tallec *et al.*, 2006). However, the results showing different N₂O emissions for low nitrite levels (Section 5.3.3.1) and the variety of N₂O reduction rates at different pH levels suggest, instead, that the N₂O reduction rate is the determining factor for N₂O production in a wastewater treatment system.

The use of stored carbon, under aerobic conditions, comprises the major stage of simultaneous nitrification and denitrification in an SNDPR process. Under these conditions, the N₂O reduction rate was higher at a higher pH. The investigation of N₂O off-gas in this study also suggested that, when the system was operated at a higher pH level, the N₂O emissions were reduced.

Table 5.5 Nitrous oxide reduction at different pH levels under aerobic condition and anoxic condition by using acetate as internal carbon (unit: mg/hr/g-biomass as N)

pH	5.7	7.0	8.2
With DO			
Suspended sludge	0.43 ± 0.12	0.81 ± 0.21	3.08 ± 0.57
Biofilm	0.32 ± 0.08	0.65 ± 0.14	0.73 ± 0.16
Overall	0.34	0.69	1.26
Without DO			
Suspended sludge	1.60 ± 0.27	1.82 ± 0.39	1.74 ± 0.28
Biofilm	2.4 ± 0.54	0.68 ± 0.23	0.74 ± 0.12
Overall	2.22	0.94	0.76

Replicate = 3.

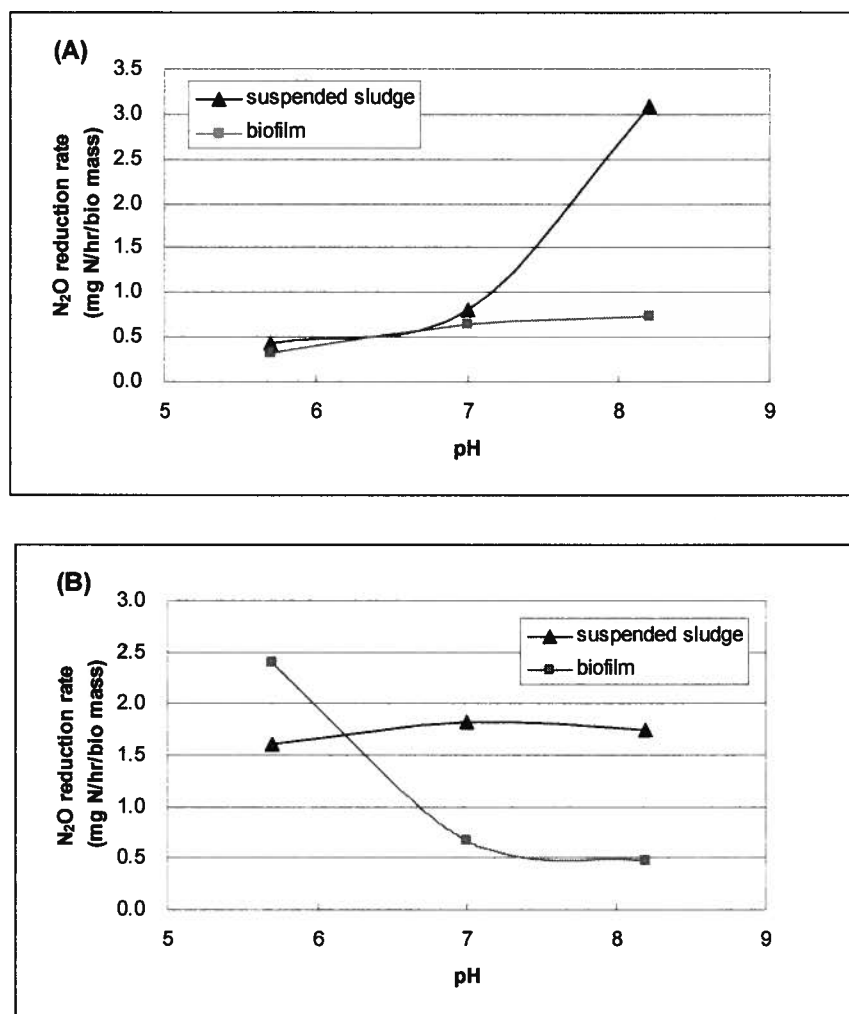


Fig 5.12 Nitrous oxide reduction at different pH levels at (A) aerobic condition and (B) anoxic condition by using acetate as internal carbon stored by previously feeding acetate

5.4 Conclusions

Factors affecting N₂O emissions and investigated included the aeration rate, the feeding pattern and pH. The results suggest strategies for N₂O emission control.

- Aeration rate affects N₂O off-gas, the ammonia oxidation rate, the denitrification rate and the SND efficiency of an SNDPR system. At aeration rates below 400 mL/min, a lower aeration rate produced lower N₂O emissions, and a higher aeration rate led to higher AOR, DNR and N₂O emissions. SND efficiency was lower with a high aeration rate.
- The addition of external carbon sources by the continuous feeding of influent into the reactor during the aeration period enhanced the reduction of N₂O off-gas. In this case, about 30% continuous feeding in the aerobic phase is suggested.
- When the system was operated at a higher pH level, the emission of N₂O was reduced.
- Suspended sludge had higher N₂O reduction rates at a higher pH under aerobic conditions, while the biofilm had a higher N₂O reduction rate at lower pH levels, under conditions without DO.

Using conditions of a lower aeration rate, continuous feeding and higher pH are possible operating strategies which could successfully reduce the emission of nitrous oxide in a hybrid SNDPR process.

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Chapter 6 Using N₂O off-gas as a tool for ammonia oxidation assessment and its potential for the system real-time control

6.1 Introduction

Energy conservation is an issue in the design and operation of advanced wastewater treatment facilities. Nitrogen removal systems require more energy than conventional carbon conversion processes, due to the air requirements for ammonia oxidation. The development of on-line instrumentation, coupled with real-time control strategies, not only enhances the performance of the treatment process, but also reduces the operating costs. Some practical approaches, including pH/ORP, DO, oxygen uptake rate (OUR) and nicotinamide adenine dinucleotide (NADH), have been developed for the real-time control of ammonia oxidation (Battistoni *et al.*, 2003; Kim *et al.*, 2004; Puig *et al.*, 2005; Farabegoli, *et al.*, 2003).

Although N₂O off-gas is an undesired product of wastewater treatment, it has been a focus of interest for researchers because of its high correlation with ammonia oxidation and the potential for it to be used as a parameter for system monitoring. Burgess *et al.* (2002) established a relationship between ammonia oxidation and N₂O concentrations. They also reported the potential for using N₂O detection for early warning of process failure in the system. Shiskowski (2004) also investigated the potential for making nitrous oxide the parameter for nitrification monitoring and system aeration control.

*A version of this chapter will be submitted for publication:

Lo, I., Lo, K.V., Mavinic, D.S., Shiskowski, D. and Remay, W. Using N₂O off-gas as a tool for ammonia oxidation assessment and its potential for the system real-time control.

These real-time control strategies are dependent on condition changes in the bulk solution after the reaction is complete. That is, when ammonia oxidation is finished, a rapid rise in DO and pH are observed. Since regulations do not require ammonia to be fully removed from the effluent, using this point as the control point means that the effluent is over treated and a certain amount of energy is thereby wasted.

N_2O off-gas can result from nitrification and denitrification processes. This research has identified heterotrophic denitrification as the major source of N_2O off-gas. In order to serve as a real-time control assistant for an SNDPR system, analysis of the time profiles of N_2O emissions is required. Various operating conditions including different aeration rates, feed modes, and pH were studied in the hybrid system and are reported here. Tracks of the suspended growth system are also reported in this chapter.

6.2 Experimental design

6.2.1 Relationship between ammonia oxidation and nitrous oxide off-gas

Studies were completed on the profiles of N₂O off-gas and ammonia oxidation under various operating conditions, including aeration rate, pH and feeding pattern for both the hybrid system, and the suspended growth system (Reactor A). The relationship between N₂O off-gas and ammonia oxidation was analyzed. The influent for the tests was described in Chapter 1.

6.2.2 Application of N₂O off-gas to real-time control of ammonia oxidation

A cycle of the hybrid system was completed using N₂O off-gas as a real-time control parameter. Aeration was turned off when emissions of N₂O began to decrease from their highest level. The second aeration period started when N₂O off-gas went down to zero, and it ended when N₂O off-gas began to decrease from the second highest emission level.

6.2.3 Other relationships

Other relationships were also studied, such as the slope of the N₂O off-gas profile vs. ammonia oxidation, N₂O off-gas vs. carbon utilizing, and N₂O off-gas vs. ORP profile.

6.3 Results and discussion

6.3.1 Relationship between ammonia oxidation and nitrous oxide off-gas

A track study was used to illustrate the relationship between N₂O off-gas production and other parameters. Figure 6.1 shows the profiles for this track study, including nitrogen compounds, TOC and ortho-P, pH, ORP, DO and N₂O off-gas plus N₂O in liquid. The anaerobic period was from 1 to 60 min; aeration was from 61 to 300 min; the anoxic phase was from 301 to 420 min; post aeration was from 421 to 450 min and settling and discharge was from 451 to 480 min. Both ammonia oxidation and denitrification were complete in this study. N₂O off-gas averaged 15.1% as N/TN.

In order to compare the correlations between ammonia and N₂O emissions, ammonia oxidation (Fig 6.2A) was presented as ammonia removal efficiency (%) over time, as shown in Fig 6.2B. In the same way, the N₂O off-gas time profile was presented as the N₂O accumulation time profile (%) as shown in Fig 6.2D. Comparing Fig 6.2B and Fig 6.2D, a close relationship of a 2-order polynomial function was found between ammonia removal and the accumulation of N₂O off-gas from the beginning of the aeration period to the point of the highest N₂O emissions (Fig 6.3). The function can be expressed as follows:

$$Y = -aX^2 + bX + c \quad (6.1)$$

Where Y = ammonia removal at time T (%)

X = (N₂O accumulation of time T, %) / (N₂O accumulation of the highest point of N₂O emission, %)

a, b, c = constants

The values of constants “a”, “b” and “c” are -0.0054, 1.2400 and 3.6, respectively. A value of 0.997 of R² indicates that N₂O emission was closely correlated to the oxidation of ammonia.

Constant “a” controls the flexure of the curve. It has a more significant effect on the function at a larger “X”, which happens at a later part of the aeration phase. A larger absolute value of “a” indicates that the system emits more N₂O gas per unit of ammonia removal. In the early part of the aeration period, N₂O emission is relatively small. Thus, the function is dominated by constant “b”. At the same “X”, a larger “b” leads to higher ammonia removal efficiency. Constant “c” indicates ammonia removal at the beginning of N₂O emission. That is, when “c” is larger, more ammonia has been removed without the emission of N₂O. The function was, however, an integration of the effects of “a”, “b” and “c”. The value of a single constant is not sufficient to interpret the relationship between the emission of N₂O and the removal of ammonia.

The time profiles of ammonia oxidation agree with that the ammonia oxidation, which follows a zero-order reaction until the concentration of ammonium went down to a certain level (it was approximately 1 mg/L in this SNDPR system). In the previous chapters (Chapter 1, 2 and 3), the importance of nitrite has been discussed. Nitrite is a product of ammonia oxidation, and, the source of N₂O production. In chapter 4, the results show that the reaction of denitrification is most likely zero-order (data and figures not shown). The reaction rate of nitrite to nitrate is also zero-order (Nogueira and Melo, 2006). However, the results in chapter 6 demonstrated that the reduction of nitrous oxide is, most likely, 1st-order reaction. When more nitrite was accumulated in the bulk solution from ammonia oxidation, there exists the potential of increasing rate of N₂O emission from denitrification.

Finally, it was stripped out and detected as off-gas. The similarity of the profile of dissolved N_2O and the profile of N_2O off-gas (data not shown) agrees the principle of air stripping which follows Henry's law. If the chain reactions and mechanisms of the nitrogen transformation are all zero-order, the curve of the relationship between N_2O off-gas and ammonia removal should be linear. Nevertheless, the difference between zero-order and first order reactions gives the shape of the curve as a polynomial function on Figure 6.3. When the concentration of ammonium became low (e.g. around 1 mg/L in this study), AOR slowed down. The concentration of nitrite also decreased. The change of the concentration multiplies the reaction rate of 1st-order reaction, thus, caused the evident drop of N_2O off-gas time profile from its peak.

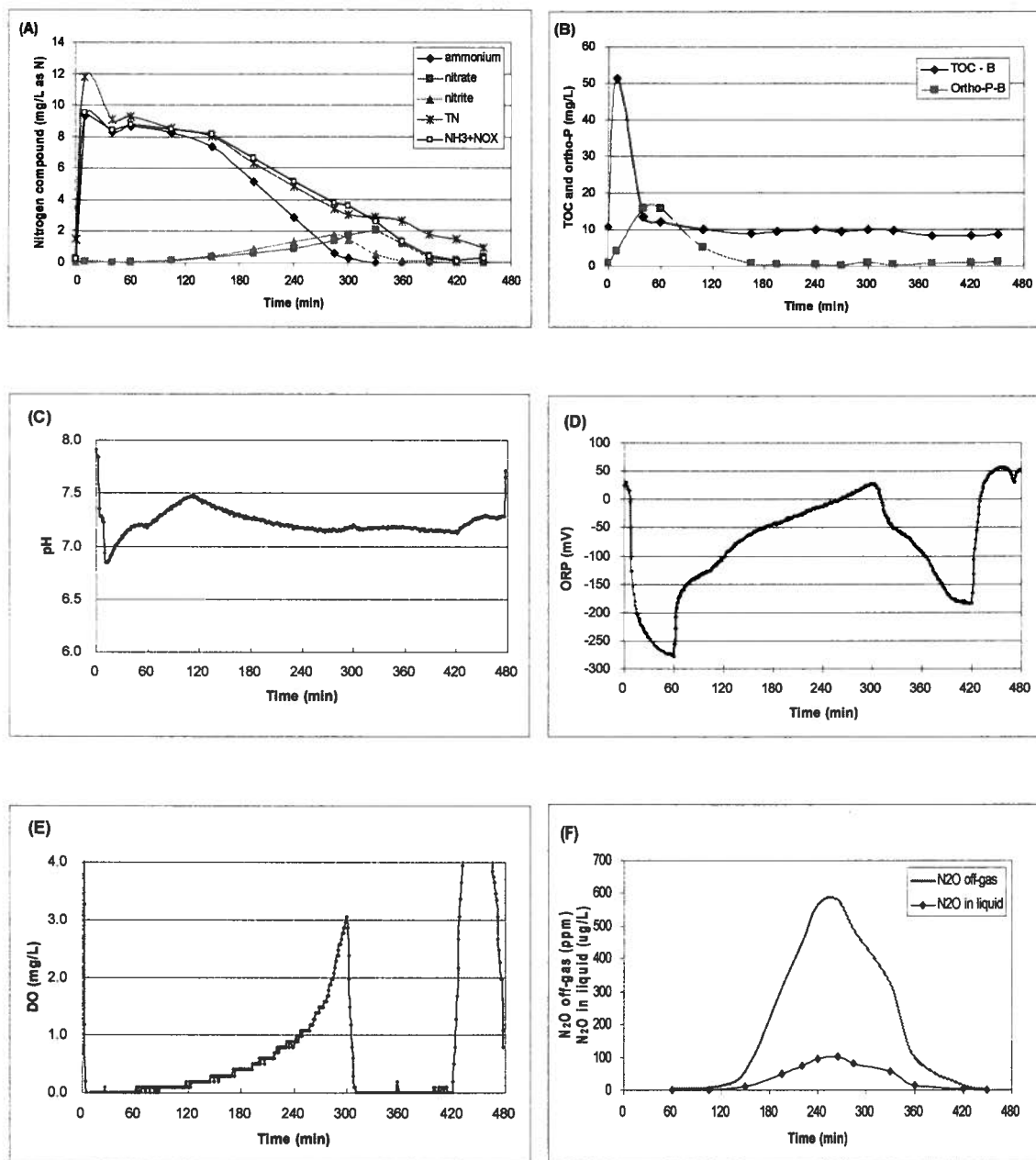


Fig 6.1 Time profiles of a track study (A) nitrogen compounds; (B) TOC and ortho-P; (C) pH; (D) ORP; (E) DO and (F) N_2O off-gas and N_2O in liquid (anaerobic: 0 - 60 min; aeration: 61 to 300 min; anoxic: 301 to 420 min; post aeration: 421 - 450 min; and settling/discharge: 451 - 480 min).

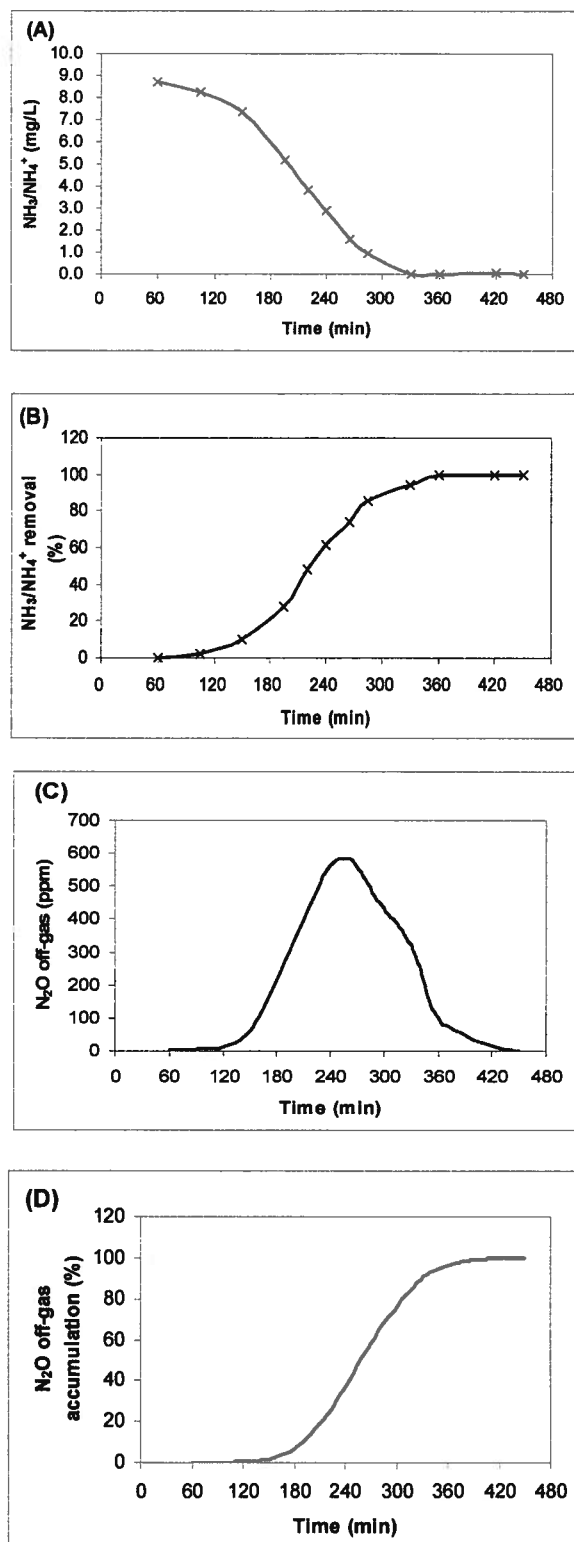


Fig 6.2 Typical time profiles of (A) ammonia (B) ammonia removal (C) N_2O off-gas and (D) accumulation of N_2O off-gas.

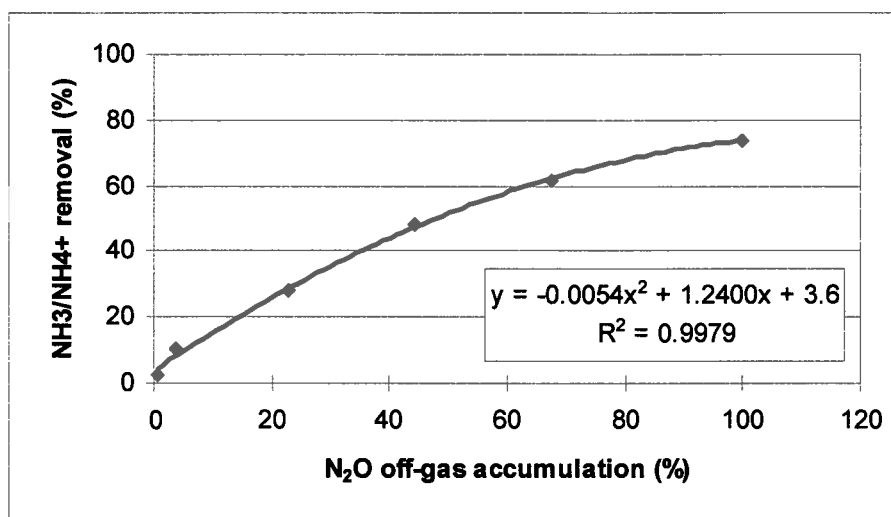


Fig 6.3 Relationship between N₂O off-gas and ammonia removal

6.3.1.1 Ammonia removal versus N₂O off-gas at different aeration rates

The relationship between ammonia removal and N₂O accumulation was examined at different aeration rates (100, 200, 400 and 600 mL/min) and these are shown in Figure 6.4 to 6.7. The results are summarized in Table 6.1.

Aeration rates were found to change the correlation between N₂O emission and ammonia oxidation. No significant rules were found between the change of aeration rates and the value of constant “a”, “b” and “c” for those 4 sets of the experiments. Although the constants of the model (Equation 6.1) were not correlated, ammonia removal and N₂O off-gas were highly correlated for each individual test. The R² value of Equation 6.1 for each test was over 0.97. Ammonia removal at the highest N₂O off-gas levels were 89.2, 81.5, 85.0 and 86.6 % for aeration rates of 100, 200, 400 and 600 mL/min, respectively.

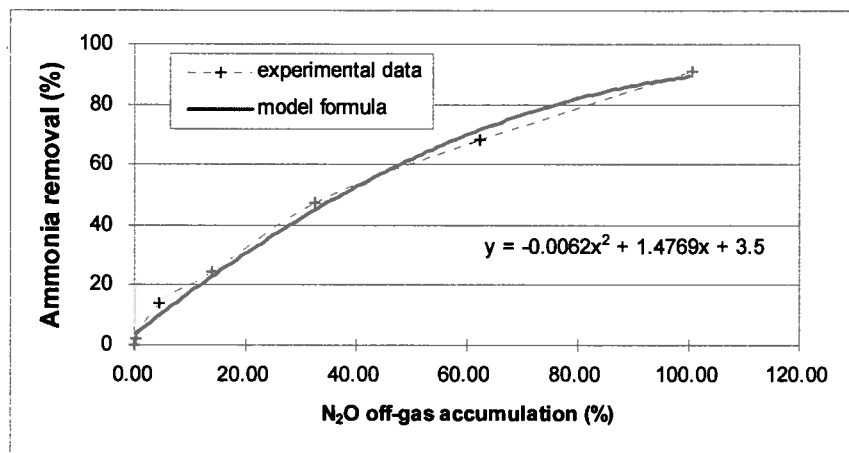


Fig 6.4 Relationship between N₂O off-gas and ammonia removal at aeration rate 100 mL/min

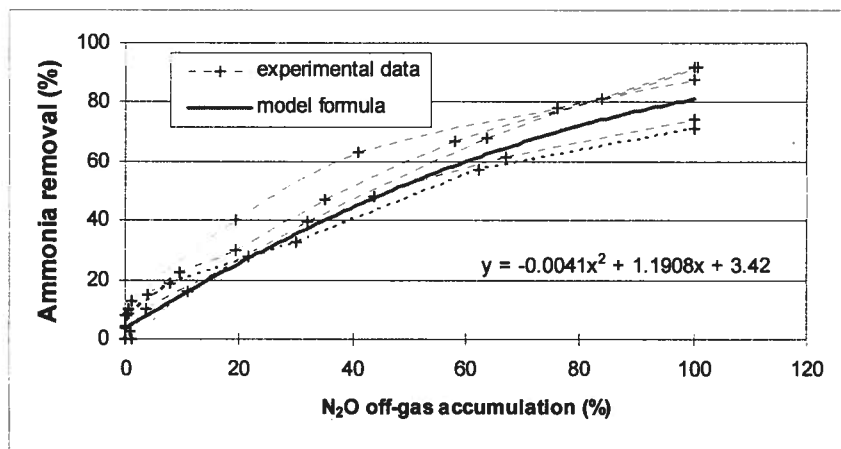


Fig 6.5 Relationship between N₂O off-gas and ammonia removal at aeration rate 200 mL/min

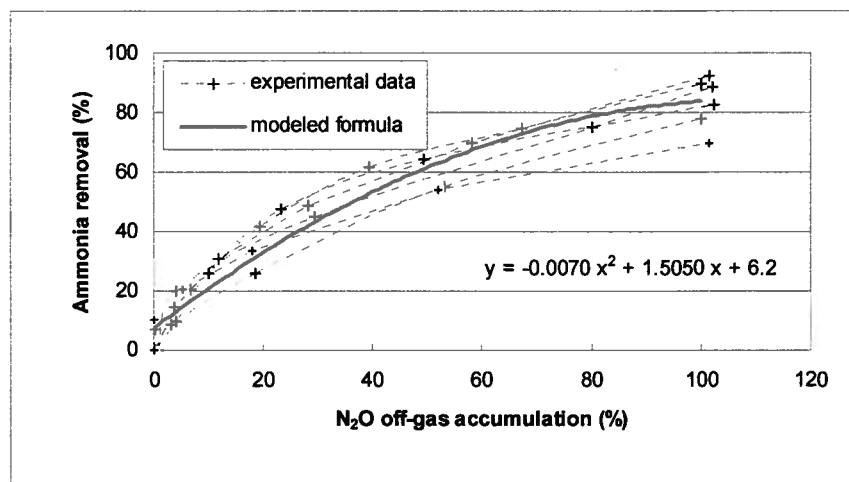


Fig 6.6 Relationship between N₂O off-gas and ammonia removal at aeration rate 400 mL/min

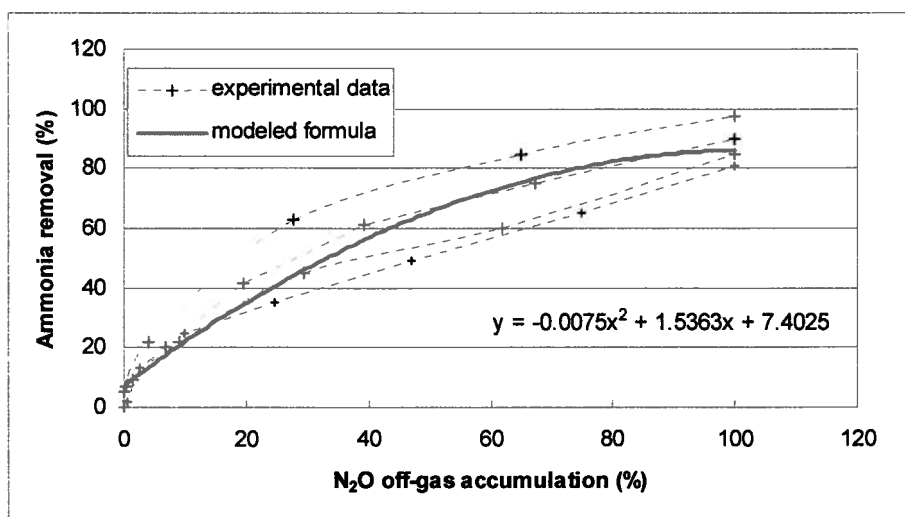


Fig 6.7 Relationship between N₂O off-gas and ammonia removal at aeration rate = 600 mL/min

6.3.1.2 Ammonia removal versus N₂O off-gas at different feeding patterns

The curves for ammonia removal vs. N₂O off-gas accumulation of the different continuous feeds are presented in Figure 6.8 and Table 6.1. Basically, a higher continuous feed had higher “a” and “b” values, but smaller values for “c”. R² for each test was higher than 0.97. Ammonia removal at the highest concentration of N₂O off-gas was 90.6%, on average.

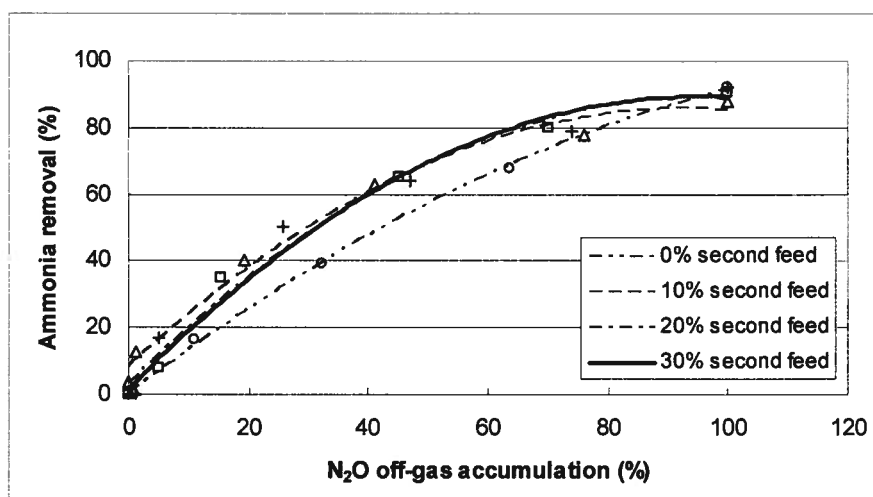


Fig 6.8 Relationship between N₂O off-gas and ammonia removal at different feeding patterns

6.3.1.3 Ammonia removal versus N₂O off-gas at different pH's

Figure 6.9 and Table 6.1 show ammonia oxidation vs. N₂O off-gas accumulation curves at pH 5.7, 7.0 and 8.2. The curve for pH 5.7 departed from those for pH 7.0 and 8.2. From the results reported in the previous chapter, both nitrification and denitrification performance was poor at pH = 5.7, and a relatively high amount of N₂O off-gas was emitted from the system. The curve for pH = 5.7 was, as expected, significantly below the other two curves. The curves of N₂O off-gas for pH from 5.7 to 7.0 might be distributed in the range between the curve of pH = 5.7 and pH=7.0 on Figure 6.9.

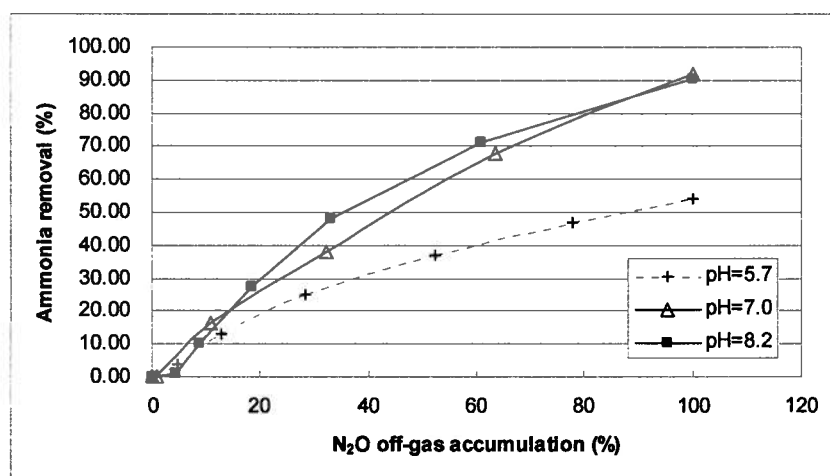


Fig 6.9 Relationship between N₂O off-gas and ammonia removal at different pH values

6.3.1.4 Ammonia removal versus N₂O off-gas of the suspended growth system (Reactor

A)

The data for the suspended growth system (Reactor A) were also checked. Fig 6.10 shows 3 tracks of Reactor A at the aeration rate of 200 mL/min. At the highest concentration of N₂O off-gas, ammonia removal was 67.3%, on average. This value was smaller than the value obtained from the hybrid SBR, which was around 85%. This suggests that, in a system with a better SND efficiency (i.e. the hybrid system), the time of the N₂O off-gas summit might be delayed. The average R² of these three was 0.98. The relationship between ammonia removal and N₂O accumulation could not only be applied to the hybrid system, but also to the conventional suspended growth system.

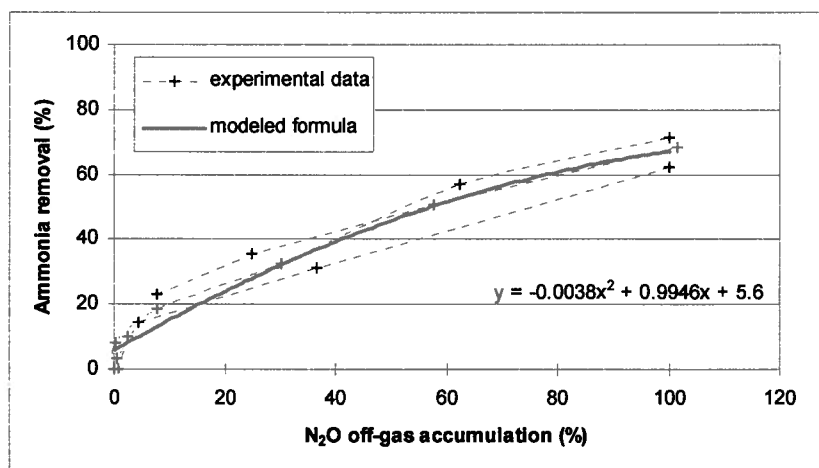


Fig 6.10 Relationship between N₂O off-gas and ammonia removal of suspended growth system (Reactor A)

Table 6.1 Summary of the relationship between ammonia removal and nitrous oxide off-gas

Item	Track	N ₂ O/TN %	SND %	TN mg/L	C/N	slope of N ₂ O off-gas ppm/min	a	b	c	R ²	NH ₃ removal at the summit of N ₂ O off-gas (%)
Aeration rate											
100 mL/min	70710	8.3	83.2	46.5	5.1	1.92	-0.0062	1.4769	3.5	0.999	89.2
200 mL/min	70631	16.6	70.9	37.3	6.8	3.76	-0.0021	0.9961	0.0	0.996	78.4
	70706	15.5	63.3	37.6	6.7	1.95	-0.0044	1.0954	5.4	0.995	71.2
	70713	15.6	82.7	37.3	6.9	4.18	-0.0045	1.3626	0.1	0.999	91.9
	70814	21.2	75.9	33.9	5.2	4.71	-0.0043	1.2600	8.0	0.998	92.1
	70817	15.1	82.7	34.2	5.1	5.97	-0.0054	1.2400	3.6	0.997	74.1
	Average	16.8	75.1				-0.0041	1.1908	3.4	0.997	81.5
	Stdev	2.52	8.3				0.0012	0.1447	3.5	0.002	9.9
400 mL/min	61009	23.4	46.5	31.2	5.4	2.17	-0.0092	1.7307	6.0	0.984	89.9
	61024	27.7	70.4	33.9	4.1	5.90	-0.0077	1.6182	6.5	0.979	92.6
	61026	22.6	58.1	34.8	3.7	9.10	-0.0096	1.7058	7.7	0.979	82.5
	61029	11.3	47.9	31.5	2.7	4.20	-0.0041	0.9629	13.7	0.982	72.0
	70711	27.8	81.4	39.3	5.5	6.00	-0.0049	1.3266	7.3	0.956	90.7
	70818	27.9	63.3	30.9	5.9	4.30	-0.0029	1.0718	3.4	0.994	82.0
	Average	23.5	61.3				-0.0064	1.4027	7.4	0.979	85.0
	Stdev	6.4	13.4				0.0028	0.3330	3.4	0.012	7.7

* : aeration rate was 200 mL/min.

Table 6.1 Summary of the relationship between ammonia removal and nitrous oxide off-gas (continued)

Item	Track	N ₂ O/TN %	SND %	TN mg/L	C/N	slop of N ₂ O off-gas ppm/min	a	b	c	R ²	NH ₃ removal at the summit of N ₂ O off-gas (%)
Aeration rate											
600 mL/min	60926	19.7	51.7	38.4	3.8	2.79	-0.0053	1.2965	6.3	0.975	85.0
	60930	12.6	58.2	56.2	4.5	3.01	-0.0037	1.0761	8.5	0.977	80.7
	61009	23.4	46.5	31.7	5.3	2.17	-0.0092	1.7307	6.0	0.983	89.9
	70714	21.3	76.7	35.7	6.0	3.29	-0.0118	2.0419	8.8	0.972	90.8
	average	19.3	58.3				-0.0075	1.5363	7.4	0.977	86.6
	Stdev	4.7	13.2				0.0037	0.4331	1.5	0.005	4.7
Second feed*											
0%	70713	15.6	82.7	37.3	6.9	4.18	-0.0045	1.3626	0.1	0.999	91.9
10%	70630	14.2	75.0	34.0	5.8	10.90	-0.0077	1.5005	12.3	0.992	87.8
20%	70702	9.6	77.6	33.6	7.5	8.40	-0.0086	1.7082	4.7	0.975	92.2
30%	70705	6.6	92.9	30.3	8.3	5.03	-0.0091	1.7690	3.5	0.986	90.5
	average									0.988	90.6
	Stdev									0.010	2.1

*: aeration rate was 200 mL/min.

Table 6.1 Summary of the relationship between ammonia removal and nitrous oxide off-gas (continued)

Item	Track	N ₂ O/TN %	SND %	TN mg/L	C/N	slope of N ₂ O off-gas ppm/min	a	b	c	R ²	NH ₃ removal at the summit of N ₂ O off-gas (%)
pH*											
5.7	71109	>19	--	29.1	4.5	4.67	-0.0037	0.8852	1.2	0.995	53.8
7.0	70713	15.6	82.7	37.3	6.9	4.18	-0.0045	1.3626	0.1	0.999	91.9
8.2	71026	4.0	73.9	33.1	5.3	1.12	-0.0080	1.7293	-2.9	0.996	90.6
	Average									0.997	
	Stdev									0.002	
Suspended growth system*											
	70225	27.2	34.3	32.5	4.1	0.90	-0.0055	1.1519	6.8	0.957	68.6
	70706	15.5	63.3	52.7	4.3	1.95	-0.0044	1.0954	5.4	0.995	71.2
	70707	19.3	46.9	33.1	6.5	4.83	-0.0015	0.7366	4.6	0.990	62.0
	Average	20.7	48.1				-0.0038	0.9946	5.6	0.980	67.3
	Stdev	6.0	14.5				0.0021	0.2252	1.1	0.021	4.7

* : aeration rate was 200 mL/min.

6.3.2 An example of applying N₂O off-gas to real-time control

Based on the results reported in the previous section, N₂O off-gas was applied to a track of the hybrid system (see Fig 6.11). When the concentration of N₂O off-gas descended from the peak, ammonia decreased from about 47.5 mg/L in the influent to 5.5 mg/L in the bulk solution. The removal efficiency was approximately 88.5%. The second aeration reduced the residual ammonia to a concentration of about 0.7 mg/L. The overall ammonia oxidation efficiency, with both aerations, was 98.5%. Nitrite and nitrate in the effluent were only 0.2 mg/L and 1.8 mg/L, respectively. The overall nitrogen removal efficiency was 94.3%. N₂O emission from the first aeration was about 9.7% as TN in the influent; the overall emission (including the first and the second aeration) totaled about 12.5% as TN.

The anoxic stage (271-360 min) provided the conditions for denitrification and nitrous oxide reduction. In this period, without aeration, N₂O emission was relatively low and there was greater opportunity for reduction to N₂ to occur.

The results suggest that the control strategy can be applied to different ammonia ranges. If the ammonia concentration, after the aeration is still higher than the required discharge criteria, the system might be required to repeat the aerobic and anoxic phases for a few times, until the effluent is below the discharge requirements. The need for this could occur if the ammonia in the influent is relatively high, or the requirements are relatively strict, or the ammonia oxidation in the system was low at the control point.

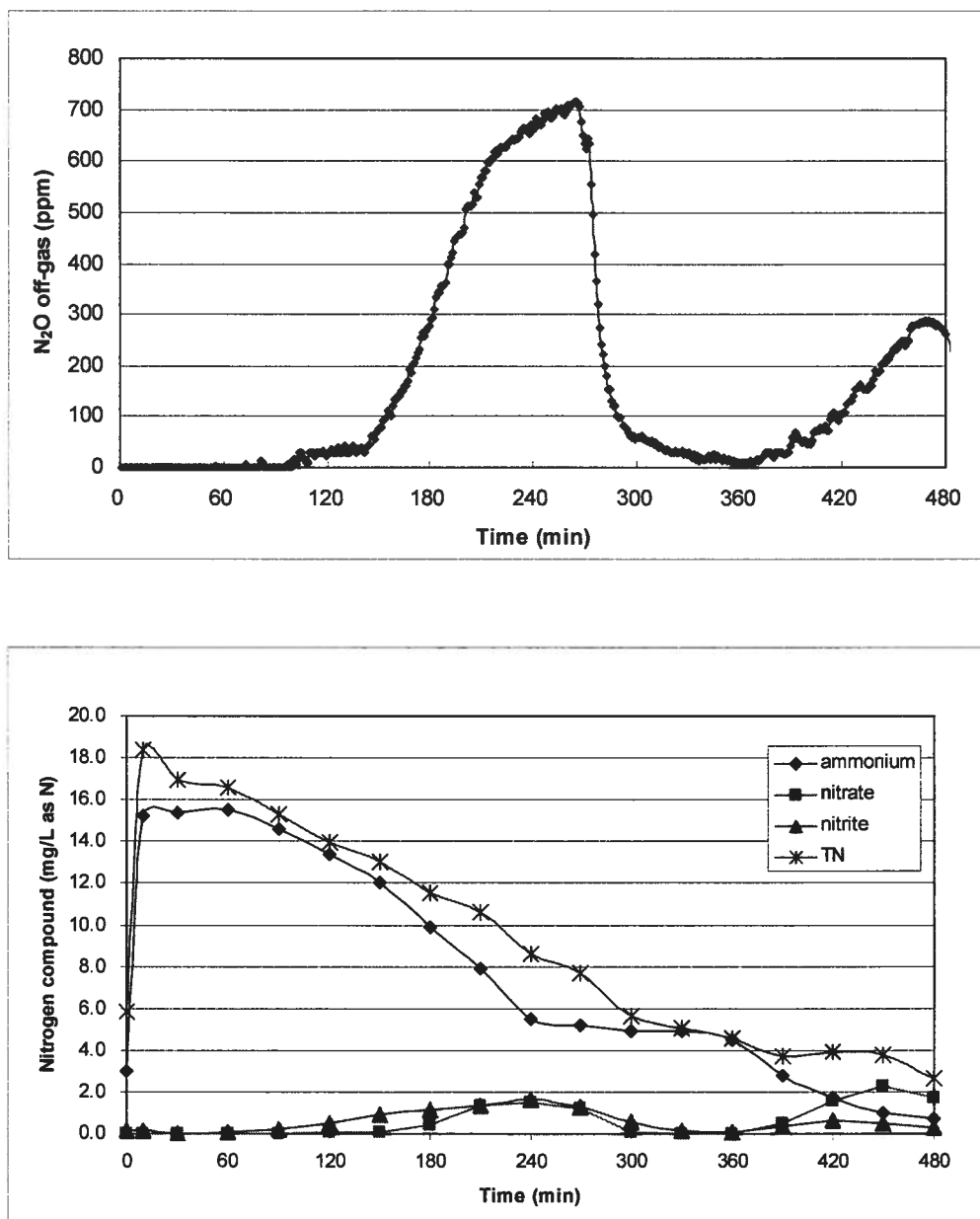


Fig 6.11 An example of applying N_2O off-gas to real-time control of ammonia oxidation (A) the profile of N_2O off-gas; (B) profiles of nitrogen compounds (Anaerobic: 0-60min; the first aeration: 61-270 min; anoxic: 271-360; the second aeration: 361-450)

6.3.3 Other relationships

6.3.3.1 Slope of N₂O off-gas profile versus ammonia oxidation

After a certain period of aeration, the increasing rate of N₂O off-gas reached a peak, then, decreased. Figure 6.12 is an example of the time differential of N₂O off-gas. (The data of Section 6.3.3.1 to 6.3.3.3 are from the same track as Section 6.3.1.) This is also the N₂O off-gas increase rate. The stable N₂O emission rate was about 6 ppm/min in this case. Table 6.1 lists the slope of N₂O profiles for all tests, under different conditions. Shiskowski (2004) has suggested that the slope of N₂O emission might provide information for the real-time control of a NBR SBR system, using an Artificial Neural Network (ANN) approach. Due to insufficient experimental data, this study could not find the evidence necessary to confirm that the slopes were correlated to the system performance in ammonia removal, N₂O emission and SND all of, which occurred as a result of changing the operating variables (such as aeration rate, feed mode, pH, TN and C/N). To effectively apply the slope of N₂O emission in real-time control, more experimental data would be required to confirm this hypothesis.

6.3.3.2 N₂O off-gas versus carbon utilizing

In aerobic metabolism, the oxygen uptake rate of carbon oxidation is more rapid than the OUR for ammonia oxidation (Metcalf and Eddy, 2003). In the early stages of aeration in an SBR system, carbon oxidation is therefore the dominant process. When carbon oxidation is nearly complete, ammonia oxidation becomes dominant. The commencement of emissions of N₂O can therefore be an indicator of the break point of the “carbon utilization dominated stage” (Shiskowski, 2004). In Figure 6.12 and Figure 6.1F, the increase in N₂O emissions began at around time 120 min. Meanwhile, the pH also

dropped due to the onset of ammonia oxidation (Fig 6.1C). In Figure 6.1D, the rapid increase of ORP at around 120 min (also Fig 6.12) indicates that the treatment had shifted from a carbon oxidation-dominated stage to an ammonia oxidation-dominated stage, and the ammonia removal rate increases after this point (Fig 6.1A). The increase in the DO profile shown in Figure 6.1E provides the same information on the break point for carbon utilization (Shiskowski, 2004).

For an actual biological treatment system, focusing only on carbon removal but not on ammonia removal is not desirable; the beginning of the increase in N₂O off-gas can be a useful indicator of a system shift from carbon utilization to ammonia oxidation.

6.3.3.3 N₂O off-gas versus ORP profile

In the aeration period, when ORP was lower than -100 mV (before time 120 min), N₂O off-gas was negligible (Fig 6.1D and 6.1F). When the ORP was higher than -100 mV, a large amount of N₂O off-gas was detected. In the anoxic stage, when the ORP was lower than -180 mV (time 375 min), both curves for ORP (Fig 6.1E and Fig 6.13) and N₂O (Fig 6.1F and 6.12) flattened out, that is, denitrification was nearly complete when the ORP was lower than -180 mV. Nitrate, nitrite and nitrous oxide reduction at this point were almost complete, and N₂O was reduced to dinitrogen gas.

At the end of denitrification, there is a sudden decrease of the ORP. This is the denitrification knee (or nitrate knee, or nitrate breakpoint). This point has been proposed as a control point for complete denitrification (Plisson-Saune *et al.*, 1996). Although the nitrate knee for the ORP time profile, shown in Figure 6.1D, is not very clear, it was obtained from its first derivative. A sudden drop in the profile at around time 370 min indicates the end of denitrification. After this point, the N₂O emission rate decreased and the amount of gas was less. A tail for the N₂O profile was also observed (Fig 6.1F).

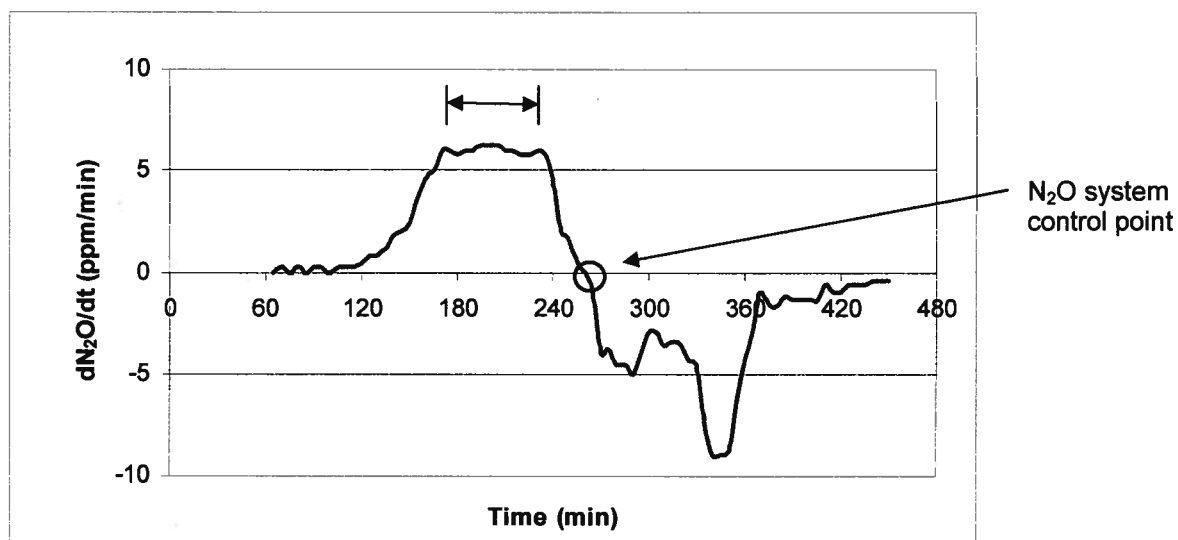


Fig 6.12 Time differential of N₂O off-gas (the range between two arrows represents the highest N₂O emission of the treatment; when $dN_2O/dt = 0$, N₂O off-gas starts decline from its peak)

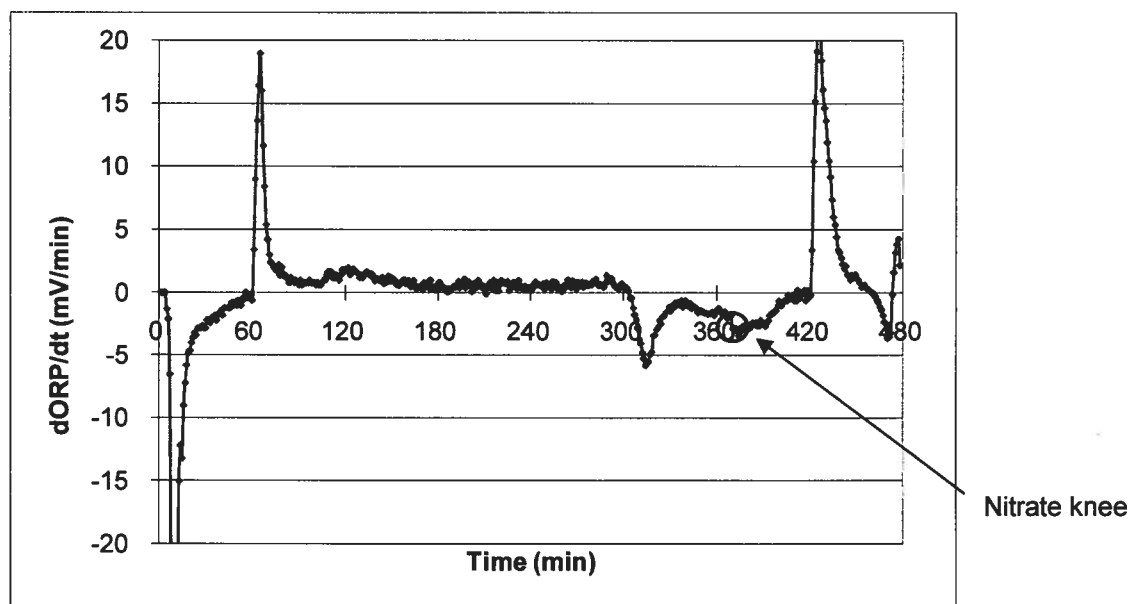


Fig 6.13 Time differential of ORP

6.3.4 System control for SNDPR in the hybrid SBR

6.3.4.1 Control concept

- Carbon conversion and phosphorus removal (anaerobic phase) - The removal efficiency for phosphorus is closely related to carbon uptake. Insufficient carbon uptake leads to poor bio-P performance (Oehmen *et al.*, 2007). The decrease of ORP in the anaerobic stage indicates the end of nitrate/nitrite and carbon uptake. A decrease in the drop in ORP shows that rapidly biodegradable COD (i.e. volatile fatty acids (VFAs) or simple saccharides) has been almost completely taken up (Fig 6.1D). Thus, by setting a value of the first derivative (i.e. $dORP/dt < 1$ mV/min on Fig 6.13 at around time 50 min), this point can possibly be used as a control point for carbon uptake and phosphorus release.
- Nitrification (aerobic phase) – the indicators given by pH, ORP and DO have been applied in the real-time control of complete ammonia oxidation. This study has established the close correlation of N₂O emission and ammonia oxidation. The time at which N₂O off-gas started to decline from its peak (or the start of sudden drop of slope from the plateau, Fig 6.12) was proposed as the control point for ammonia oxidation. At this control point, a certain efficiency of ammonia oxidation is obtained, i.e. about 85% ammonia oxidation.
- Denitrification (anoxic phase) – The ORP time profile serves as an indicator for the completion of denitrification (the nitrate knee). Alternatively, using N₂O off-gas as an indicator, the tail or the end of N₂O emission, also indicates the end of denitrification.
- Settling/discharge – using fix-time control.

6.3.4.2 Integrate application framework

-
- Reduction of N₂O off-gas – apply N₂O emission reduction strategies to the treatment system, such as aeration control, continuous feed control and pH control, to diminish the emission of N₂O (Chapter 5) as much as possible.
 - SNDPR real-time control – apply the control strategies presented in the previous section to the system. If the ammonia concentration, after the first aeration is still higher than the required discharge criteria, the system might be required to repeat the aerobic and anoxic phases a few times, until the effluent is below the discharge requirements (i.e. 1st aeration plus anoxic phase has 85.0% ammonia oxidation; 2nd aeration plus anoxic phase has 93.2%; 3rd has 97.0%...). The need for this could occur if the ammonia in the influent is relatively high, or the requirements are relatively strict, or the ammonia oxidation in the system was low at the control point.

6.3.4.3 Advantages of N₂O off-gas real-time control

Compared to other real-time control approaches, using N₂O emission as a control parameter for ammonia oxidation has additional advantages such as:

- N₂O off-gas is easily measured, the indicators are clear and easily used (i.e. N₂O off-gas from 0 to hundreds ppm compared with DO from 0 to 2 mg/L).
- The control point is before the end point of the reaction. This makes it more energy efficient.
- It has the flexibility of repeating the aerobic and anoxic stages, until the required quality of effluent is achieved.
- It can be used as an indicator of complete denitrification.
- Monitoring N₂O off-gas also gives information on the actual emission of N₂O.

6.3.4.4 Uncertainties and constraints

This study was conducted using synthetic wastewater containing sodium acetate, lactose and beef extract as the carbon sources; ammonium chloride as ammonia nitrogen and beef extract as the organic nitrogen source; K₂HPO₄, KH₂PO₄ as ortho-P sources and beef extract as the organic phosphorus source. However, the composition of “real wastewater” is more complicated than this. For example, the carbon sources used in this study are rapidly bio-degradable COD. This research has shown the importance of the carbon source in the emission of nitrous oxide. The effects that would ensue from the use of carbon and nitrogen compounds, with slow bio-degradability, were not considered in this study. Their use might cause different results in the correlation of ammonia oxidation and N₂O off-gas.

The paradox of this study is that N₂O off-gas is an undesirable product of wastewater treatment, yet it is proposed here as a control parameter. Some cases have been reported in which there was very little or no N₂O off-gas detected from treatment plants (Barton and Atwater, 2002). This was particularly true for those systems where full nitrification and pre-denitrification were achieved by adding external carbon sources, such as methanol or acetate. Little emission of N₂O might make it difficult to find the ammonia oxidation control point, using N₂O off-gas as a control parameter.

The communities of microorganisms involved might also cause uncertainty for the model. Many factors can result in a shift in the microbial communities such as changes in system configurations, operating procedures, influent composition, pH, DO level, temperature etc. Such changes in the microbial communities might lead to differences in terms of nitrification/denitrification and pose limitations for the model in this work.

Inhibition of nitrification and denitrification might also constrain the application of the model. For example, inhibitors to nitrous oxide reductases might result in the accumulation

of N₂O; inhibitors to NO reductases and/or NH₂OH oxidase might hinder the production of N₂O (Murray and Knowles, 2004). Either the accumulation or the inhibition of N₂O might interfere with the correlation of ammonia oxidation vs. nitrous oxide off-gas.

6.3.4.5 Artificial intelligence

Artificial intelligence (AI) control of wastewater treatment processes has brought about a revolution in the field of advanced control. Knowledge-based techniques, which use linguistic rules derived by human experts, are at the core of this system. Fuzzy systems, which allow one to process qualitative knowledge and to project qualitative-reasoning based controllers, and artificial neural networks (ANN) which allow for the building of parametric nonlinear models and controllers in a constructive way, are tools that have already proved their capabilities in wastewater treatment (Pires *et al.*, 2003). ANN, which has the ability to be “trained” by “learning” from historical data, makes it attractive for implementation in this study. The supervised learning function of ANN minimizes the error between the real output of the treatment system and the prediction of the model by the sets of input variables (Baxter *et al.*, 2001). In this case, constants a, b and c of the model can be the output in the first layer of the ANN. By using the model, ammonia oxidation can be predicted. The control parts receive the signal from ANN for aeration, feeding, pH control, etc.

The mechanical training of the ANN control system requires huge data sets. Comprehensive data input enhances its accuracy. Input sets might include influent volume, TN, ammonia, org-N, NO₂⁻, NO₃⁻, COD (rapid and slow degradable), TP, ortho-P, org-P, pH, N₂O, DO, temp...etc. As such, significant additional research and data logging are needed to seriously recommend this approach.

6.4 Conclusions

By studying 26 tracks taken under different operating conditions, a close correlation between N₂O off-gas vs. ammonia removal was found. This could be expressed as a 2-order, polynomial function as:

$$Y = -aX^2 + bX + c$$

Where Y = ammonia removal at time T (%)

X = (N₂O accumulation of time T, %) / (N₂O accumulation of the highest point of N₂O emission, %)

a, b, c = constants

This relationship implied a potential for using N₂O off-gas time profile to assess ammonia oxidation and as a real-time control parameter for ammonia oxidation, in an SBR SNDPR system. Other results obtained were as follows:

- Applying N₂O emission reduction strategies to the treatment system did not change the high correlation between ammonia oxidation and N₂O off-gas (the values of R² were all higher than 0.97). Even though the amount of N₂O emission was small, it could be applied in the real-time control of ammonia oxidation.
- When N₂O off-gas reached its highest concentration in the hybrid system, ammonia removal was around 85%; it was around 67% for the suspended sludge system.
- The beginning of an increase in N₂O emissions indicates the break point for carbon utilization.

- In the anoxic phase, when the ORP is below -180 mV, nitrate, nitrite and nitrous oxide reduction were almost complete.
- N₂O off-gas can be easily measured and used as a clear indicator for system control. The proposed control point for ammonia oxidation is before the end point of the reaction. This makes the treatment more energy efficient. Monitoring N₂O off-gas also gives information on the actual emission of N₂O.

6.5 References

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Chapter 7 Summary conclusions and further studies

7.1 Introduction

In wastewater treatment processes, greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide are major emissions (IPCC, 2006). For biological waste treatment in Canada and the United States, CO_2 emissions need not be reported for greenhouse gas inventories. However, CH_4 and N_2O have to be included. CH_4 is not significant in BNR systems as the anaerobic phase is relatively short. However, the potential exists for N_2O emissions to be significant when treatment plants apply biological nutrient removal processes (Shiskowski, 2004).

The global warming potential (GWP) over 100 years of N_2O is 310 times that of the contribution of carbon dioxide (IPCC, 2006). Lately, research has shown that nitrous oxide emissions from wastewater treatment processes are not negligible (Schulthess and Gujer, 1996; Park *et al.*, 2000). Research has found that nitrous oxide is generated from both the processes of ammonia oxidation (autotrophic) and denitrification (heterophic) (Anderson *et al.*, 1993). When both nitrification and denitrification processes take place, higher N_2O emissions can be expected than with nitrification only. Further, in nitrification and denitrification processes, a post-denitrification process leads to higher nitrous oxide emissions than a pre-denitrification process (Tallec *et al.*, 2006). The emission of N_2O must therefore be a consideration in wastewater treatment processes.

To date, the significance of N_2O emissions from wastewater treatment processes has not been extensively investigated; either the mechanisms of N_2O production have not been fully studied. To fully control N_2O emissions, further study of these mechanisms is required.

7.2 Summary and conclusions

In this study, a hybrid system, that included suspended sludge and biofilm in the same SBR reactor for simultaneous nitrification, denitrification and phosphorous removal, was identified as a more effective system than the conventional suspended growth system. This was assessed in terms of effluent quality, SND efficiency and lower emissions of N_2O . The relatively small size of the suspended sludge made it difficult to maintain an anaerobic zone. This resulted in poor denitrification. The intentional maintenance of low DO for denitrification also led to nitrite accumulation. The addition of the biofilm to the hybrid system provided solutions to the above problems, and proved superior to the conventional suspended growth system, in terms of simultaneous nitrification, denitrification and phosphorus removal, SNDPR.

The individual contributions of the suspended sludge and the biofilm to SNDPR and N_2O emissions were investigated. In the hybrid system, nitrification occurred mainly in the suspended sludge, while the biofilm played the major role in denitrification. The interaction between the biofilm and the suspended sludge in the same reactor resulted in excellent, overall nitrogen removal. The biomass of the suspended sludge contributed more to phosphorus removal than did the biofilm. Nitrous oxide, rather than dinitrogen gas was found to be the main product of denitrification for the hybrid system during aeration. It was also observed that N_2O off-gas from the hybrid system mainly resulted from heterotrophic denitrification, rather than from nitrification.

The emission of N_2O off-gas can be expressed using the following two categories: chemical and physical mechanisms. From the chemical perspective, the nitrogen reduction rate and the N_2O reduction rate were affected by DO conditions, and by the types and states of the carbon source:

- N_2O reduction rates were higher when external carbon exists.
- N_2O reduction rates were higher under conditions without DO than under conditions with DO.
- N_2O reduction rates were higher for acetate than for lactose with and without DO.
- Denitrification, using stored carbon, resulted in more N_2O off-gas than with the use of an external carbon source. The largest amount of N_2O off-gas occurred when the internal carbon was PHA and under aerobic conditions.

It is most likely that N_2O reduction is a 1st-order reaction. It varied with the concentrations of N_2O in the liquid. The real N_2O reduction rate can be calculated using a mass-balance of nitrous oxide. From the physical perspective, N_2O off-gas emission involves both air stripping and mechanical surface diffusion.

Operating strategies for N_2O emission control in a SNDPR system were suggested. These included a lower aeration rate, continuous feeding and a higher pH. These operating strategies could successfully reduce the emission of nitrous oxide in a hybrid SNDPR process. It was also found that the suspended sludge had a higher N_2O reduction rate at higher pH under aerobic conditions, while the biofilm had a higher N_2O reduction rate at a lower pH under conditions without DO.

N_2O emissions were found to be closely correlated with ammonia removal. The R^2 of this relationship was found to be higher than 0.97. When N_2O off-gas reached its highest concentration, ammonia removal was around 85%, on average. This high correlation between N_2O emission and ammonia removal and the peak of N_2O off-gas can potentially provide real-time control for ammonia oxidation, in an SNDPR hybrid SBR system. The point at which N_2O off-gas started descending from its peak is proposed as the control point for ammonia oxidation.

7.3 Further studies

1. This study has proven that the hybrid SBR system can achieve good nitrogen and phosphorus removal efficiencies. The system also has the ability to reduce N_2O off-gas by the abundant biomass found in the biofilm. However, some of the biochemical metabolic reactions require further study, such as those of N_2O reduction using different types and states of carbon sources, and those of bio-P removal with little P release and little or no PHA formation, during the anaerobic phase.

2. Different type of biofilm media may cause the different behavior to the system. The contribution of different type of biofilm carriers to N_2O off-gas and SNDPR by using is therefore worthy to study.

3. N_2O emission has many parameters. A comprehensive mathematical model is required for modeling and predicting the emission of N_2O during wastewater treatment. This should include N_2O emission from autotrophic and heterotrophic denitrification (by external and/or stored carbon sources).

4. Lower solubility of N_2O results in a higher diffusion rate. In this study, nitrous oxide was found to have the lowest solubility at a pH of 7.0. The factors causing a lower solubility need further study.

5. Temperature is also a significant factor in nitrification and denitrification. In this study, the effects of temperature on N_2O emissions were not considered. Further study is needed of temperature vs. N_2O emission and the implications of this for real-time control, using a liquid temperature range of 5 to 30°C.

6. In this study, N_2O emissions from autotrophic denitrification were found to be insignificant. Whether the correlation of N_2O off-gas and ammonia oxidation in an autotrophic denitrification dominated system would follow Equation 6.1, is worthy of further

study.

7. In order to apply the conclusions to full scale treatment plants, a pilot scale test, using real wastewater with various influent flow rates, strength and temperature, is needed, as well as a costs-benefit analysis on the operation strategy.

8. To apply artificial intelligence in N_2O real-time control for ammonia oxidation, intensive track studies aimed at determining the value of constant "a", "b" and "c" in the function of N_2O emission versus ammonia removal, are required.

7.4 References

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