ประสิทธิภาพของการบำบัดสารประกอบไนโตรเจนด้วยถังปฏิกรณ์ชีวภาพแบบอากาศยกสำหรับถังเลี้ยงกุ้ง

นาย นฤภู จูประจักษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
สาขาวิชา วิศวกรรมเคมี ภาควิชา วิศวกรรมเคมี
คณะ วิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN 974-53-1421-8

EFFICIENCY OF NITROGEN COMPOUND TREATMENT USING AIRLIFT BIOREACTOR FOR SHRIMP CULTURE TANK

Mr. Naruephu Juprajak

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering in Chemical Engineering

Department of Chemical Engineering
Faculty of Engineering
Chulalongkorn University

Academic Year 2004
ISBN 974-53-1421-8

Thesis Title	EFFICIENCY OF NITROGEN COMPOUND TREATMENT USING AIRLIFT BIOREACTOR FOR SHRIMP CULTURE TANK
By	Mr. Naruephu Juprajak
Field of Study	Chemical Engineering
Thesis Advisor	Associate Professor Prasert Pavasant, Ph.D.

Accepted by the Faculty of Engineering, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……Dean of the Faculty of Engineering
(Professor Direk Lavansiri, Ph.D.)

THESIS COMMITTEE

…….. Chairman
(Assistant Professor Seeroong Prichanont, Ph.D.)

…… Thesis Advisor
(Associate Professor Prasert Pavasant, Ph.D.)

…….. Member
(Sorada Kanokpanont, Ph.D.)

…….. Member
(Sorawit Powtongsook, Ph.D.)

นฤภู จูประจักษ์ : ประสิทธิภาพของการบำบัดสารประกอบไนโตรเจนด้วยถังปฏิกรณ์ชีวภาพแบบอากาศยกสำหรับถังเลี้ยงกุ้ง (Efficiency of nitrogen compound treatment using airlift bioreactor for shrimp culture tank) อาจารย์ที่ปรึกษาวิทยานิพนธ์: รองศาสตราจารย์ ดร. ประเสริฐ ภวสันต์, 92หน้า. ISBN 974-53-1421-8.

ถังปฏิกรณ์ทางชีวภาพแบบอากาศยกแบบเบดนิ่งที่มีการไหลวนแบบภายนอกมีความเหมาะสมต่อการบำบัดน้ำเสียจากบ่อเลี้ยงกุ้งซึ่งมีปริมาณสารพิษแอมโมเนียและไนเตรทอยู่ โดยถังปฏิกรณ์จะประกอบด้วยส่วนที่มีสภาวะมีอากาศ และส่วนที่มีสภาวะไร้อากาศ ทั้งสองส่วนบรรจุด้วยวัสดุตรึงแบคทีเรียชนิดพลาสติกไบโอบอล และสามารถรองรับปริมาณน้ำเสียได้ 60 ลิตร ในการทดลองได้แยกออกเป็นสามการทดลอง โดยการทดลองที่หนึ่งจะศึกษาการบำบัดน้ำเสียสังเคราะห์โดยในระบบยังไม่มีกุ้ง จากการทดลองพบการบำบัดสารประกอบไนโตรเจนได้ โดยเกิดปฏิกิริยา ไนตริฟิเคชั่นและดีไนตริฟิเคชั่นพร้อมกัน และสามารถควบคุมปริมาณแอมโมเนีย และไนเตรทในบ่อให้อยู่ในปริมาณต่ำได้ อัตราการเกิดไนตริฟิเคชั่นอยู่ในช่วง 0.563-3.971 มิลลิกรัมแอมโมเนีย-ไนโตรเจน/ลิตร-วัน และอัตราเกิดดีไนตริฟิเคชั่น 2.290-18.913 มิลลิกรัมไนเตรท-ไนโตรเจน/ลิตร-วัน ในการทดลองที่สองในการเลี้ยงกุ้งทดลองในปริมาณน้อย พบว่าในบ่อที่ได้รับการบำบัดน้ำเมื่อเทียบกับระบบที่ไม่มีการบำบัดจะมีอัตราการรอดชีวิตและการเจริญเติบโตของกุ้งที่สูงกว่าเล็กน้อย และสามารถควบคุมสารประกอบไนโตรเจนได้ในปริมานที่ไม่เป็นอันตรายโดยไม่มีการเติมเมททานอล ในการทดลองที่สามนั้นได้เพิ่มปริมาณกุ้ง ก็พบว่าในระบบบำบัดนั้นมีอตราการรอดชีวิตของกุ้งและขนาดกุ้งมากกว่า สภาวะที่ใช้สำหรับระบบการบำบัดน้ำเสียในบ่อเลี้ยงกุ้งนี้คือ ค่าออกซิเจนในน้ำของส่วนสภาวะมีอากาศเป็น 3-5 มิลลิกรัมต่อลิตร และในส่วนสภาวะไม่ให้อากาศเป็น 0-2 มิลลิกรัมต่อลิตร สภาพด่างมากกว่า 100 มิลลิกรัมแคลเซียมคาร์บอเนตต่อลิตร ค่าออกซิเดชั่นรีดักชั่นโพเทนเชี่ยลในสภาวะไม่มีอากาศอยู่ระหว่าง -400 - +100 มิลลิโวลต์ ปริมาณน้ำเสียเข้าออกเครื่องปฏิกรณ์ 24 ลิตรต่อชั่วโมง ปริมาณการใส่เมททานอล (5%v/v) 20.83 มิลลิลิตรต่อชั่วโมง

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต.....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2547

4670484221 : MAJOR CHEMICAL ENGINEERING
KEY WORD: PACKBED EXTERNAL LOOP AIRLIFT BIOREACTOR/ NITROGEN COMPOUNDS REMOVAL/ NITRIFICATION/ DENITRIFICATION/ CLOSED RECIRCULATING SEAWATER SYSTEM/ WASTE WATER TREATMENT/ CLOSED SYSTEM SHRIMP CULTURE

NARUEPHU JUPRAJAK: EFFICIENCY OF NITROGEN COMPOUND TREATMENT USING AIRLIFT BIOREACTOR FOR SHRIMP CULTURE TANK.
THESIS ADVISOR: ASSOCIATE PROFESSOR PRASERT PAVASANT, Ph.D.
92 PP. ISBN 974-53-1421-8.

Packed bed external loop airlift bioreactor (PBABR) was proven to have capacity in treating wastewater containing nitrogen compounds such as ammonia and nitrate. The 60L PBABR comprised aerobic and anaerobic zones for the nitrification and denitrification, respectively. The plastic bioball packing was packed in both aeration and non-aeration zones to allow the attachment of the targeted microorganisms. Three sets of experiment were carried out. The first experiment was performed with synthetic wastewater without the actual shrimp culture, and the system was illustrated to be able to control the level of ammonia and nitrate. The nitrification and denitrification rates in this experiment were 0.563-3.971 and 2.290-18.931 mgN/L/d, respectively. The second experiment was performed with a small number of actual shrimp culture. The treatment system was proven to have a slightly better performance than the control pond without the PBABR. Nitrogen compounds could be controlled satisfactorily without the addition of methanol. Experiment III was conducted with a larger number of shrimp and the treatment pond was found to be superior to the control pond in terms of the survival rate and the final shrimp weight.

Department Chemical Engineering Student's signature…….....
Field of study Chemical Engineering Advisor's signature…….....
Academic year 2004

ACKNOWLEDGEMENTS

This thesis had not been completed without the help and supporting from many persons. Firstly, I would like to express my sincere gratitude to Assistant Professor Dr. Prasert Pavasant, my advisor, for his valuable suggestions, guidance, warm encouragement and generous supervision throughout my master program. In addition, I am also appreciated to Assistant Professor Dr. Seeroog Prechanont, chairman of the committee, Dr. Sorada Kanokpanont, and Dr. Sorawit Powtongsook, member of thesis committee, for many valuable comments and suggestions.

Moreover, I would like to special thank Miss Porntip who always instructs me during my master program. Many thanks for P'Kark, P'Gib, Tic, P'Dew, P'Nuch, P'Nui for having dinner and sharing the time at night. I would like to thanks P'Son, P' Bee, P' Kob for good supporting all time. Sincere thanks to all members of the biochemical engineering, Marine Laboratory and environmental engineering laboratories for their warm and friendly support.

Most of all, I would like to express my utmost gratefulness to my parents and everyone in my family for their inspiration and invaluable supports at all times.

CONTENTS

Page

ABSTRACT (IN THAI)……iv
ABSTRACT (IN ENGLISH)……v
ACKNOWLEDGEMENTS……vi
CONTENTS……vii
LIST OF TABLES……ix
LIST OF FIGURES…… x

CHAPTER I Introduction

1.1 Motivation……1
1.2 Objectives……2
1.3 Expected benefits……3
1.4 Working scopes…… 3

CHAPTER II Backgrounds and Literature Reviews

2.1 Background: Nitrification process……5
2.2 Background: Denitrification process……8
2.3 Literature Reviews: Novel nitrogen removal systems…… .10
2.3.1 Single-reactor-process……10
2.3.2 Novel-process……13

CHAPTER III Materials and Methods

3.1 Experimental setup……39
3.1.1 A packed bed external loop airlift bioreactor (PBABR)……39
3.1.2 Shrimp ponds……40
3.2 Experimental procedures……40
3.2.1 Preparation ……40
3.2.2 Nitrification/Denitrification of Experiment I……40
3.2.3 Nitrification/Denitrification of Experiment II……41
3.2.4 Nitrification/Denitrification of Experiment III……41
3.3 Water quality analysis……42

CONTENTS (Cont.)

Page

CHAPTER IV Results and Discussion

4.1 Synthetic waste water in airlift bioreactor system (Experiment I)..…48

4.1.1 Nitrification……..……..……49

4.1.2 Denitrification……..……..…50

4.1.3 Simultaneous nitrification/denitrification in PBABR……..51

4.1.4 Performance of PBABR…… …53

4.2 Shrimp waste water in airlift bioreactor system (Experimen II)…….54

4.2.1 Nitrification……55

4.2.2 Denitrification…….. 56

4.3 Dense-shrimp waste water in airlift bioreactor system (Experiment III)……..…57

4.3.1 Nitrification…… ……..57

4.3.2 Denitrification……..59

4.4 Overall performance of PBABR……60

CHAPTER V Conclusions and recommendations

5.1 Conclusions..……..……..……82

5.2 Contributions…… ……..……..……..82

5.3 Recommendations……..……..……..83

REFERENCES……..……..……..……84

APPENDICES..……..……..……...88

BIOGRAPHY…….. 92

LIST OF TABLES

Table Page

2.1 Compounds giving substantial inhibition of ammonium oxidation by activated sludge in batch tests……20
2.2 Essential requirements for proper growth of *Nitrobacter* and *Nitrosomonas*……...21
2.3 A comparison of the new processes of nitrogen removal and conventional nitrification/denitrification……..... 22
2.4 Details on the operation of various types of nitrification process…….. 23
2.5 Details on the operation of various types of denitrification process……25
2.6 Details on the operation of novel microbial nitrogen removal process……. 27
4.1 Feeding sehedule summary during synthetic waste water experiment (Experiment I)……...61
4.2 Nitrification/Denitrification rates during synthetic waste water experiment (Experiment I)……...62
4.3 Amount of shrimps during shrimp waste water experiment (Experiment II)…… 63
4.4 Amount of shrimps during Experiment III…… 64
4.5 Operating condition in Experimental III…… 65

LIST OF FIGURES

Figure Page

1.1 Schematic diagram of the pack bed airlift bioreactor …….. 4
2.1 Effect of pH on nitrification rate at 20^{o}C …….. 28
2.2 Effect of temperature on nitrification rate at 30 ^{o}C……29
2.3 Effect of dissolved oxygen on nitrification rate at 30 ^{o}C …….. 30
2.4 Effect of pH on denitrification rate…….. 31
2.5 Schematic diagram of nanofiltration membrane bioreactor ……... 32
2.6 Schematic diagram of membrane-aerated biofilm reactor …… 33
2.7 Schematic diagram of double-biofilm reactor …… 34
2.8 Schematic diagram of shell-and-tube co-immobilized cell bioreactor…... 35
2.9 Schematic diagram of membrane aerated biofilm reactor …… 36
2.10 Possible metabolic pathways for Nitrification and Denitrification …….. 37
2.11 Possible metabolic pathways for anaerobic ammonium oxidation 38
3.1 Schematic diagram of the system……...47
4.1 Nitrogen compound concentration profile in the shrimp pond during Experiment I ……...66
4.2 Nitrogen compound concentration profile at the reactor exit during Experiment I …….. 67
4.3 ORP & nitrogen compound concentration profile in the shrimp pond during Experiment I …….. 68
4.4 Ammonium-nitrogen concentration profile during Experiment II……69
4.5 Nitrite-nitrogen concentration profile in during Experiment II……..70
4.6 Nitrate-nitrogen concentration profile during Experiment II…… 71
4.7 Schematic of Experiment pond…….... 72
4.8 Nitrate-nitrogen concentration at reactor exit and dissolved oxygen profile during Experiment II…… ….. 73
4.9 Nitrate-nitrogen concentration at reactor exit and oxidation-reduction potential profile during Experiment II ……..…… 74
4.10 Ammonium-nitrogen concentration profile during Experiment III ……. 75
4.11 Nitrite-nitrogen concentration profile during Experiment III ……..…76

LIST OF FIGURES (Cont.)

Page

4.12 Nitrate-nitrogen concentration profile during Experiment III ……77
4.13 Nitrate-nitrogen concentration in PBABR ……78
4.14 Oxygen concentration profile in PBABR……79
4.15 Nitrate-nitrogen concentration at reactor exit and dissolved oxygen profile during Experiment III……...80
4.16 Nitrate-nitrogen concentration at reactor exit and oxidation-reduction potential profile during Experiment III……...81

Chapter I

Introduction

1.1 Motivations

Thailand is the world's largest black tiger shrimp exporter, but due to the residues of some chemicals in shrimp products as stated by the EU, major importing countries, such as the USA, European countries, and Japan, became less confident in shrimp products exported from Asia. This significantly affected our local shrimp industry because majority of our production (about 80%) was for export, i.e. the year 2002 alone saw as much as 15.1% decrease in the volume of shrimp demand which was equivalent to a 30.8% decrease in the value compared to that of the year 2001 [Department of fisheries; Marine Shrimp Culture Research Institute]. One of the foreseeable solutions to this is to increase the confidence in the quality of shrimp products by using minimum amount of chemicals during shrimp culture. A close culture system where the culture sea water is being reused is considered appropriate for this situation as the open system often leads to an infection of the shrimp culture resulting in a need for high chemical dosages to control the propagation of the infection.

The seawater in shrimp pond accumulates several unwanted components such as uneaten feed, feces and metabolic wastes. Therefore the reuse of the culture sea water necessitates the application of some form of treatment to prevent the accumulation of metabolic wastes, particularly nitrogen compounds, e.g. ammonia, nitrite, and nitrate in seawater. Ammonia (>0.3mg/L) can be toxic to the shrimp [Malone and Reyes,1997] and may also cause stress and mortality through disease and oxygen depletion. A complete remediation of wastewater containing ammonia can be accomplished through processes of nitrification and denitrification. Nitrification is the oxidation of ammonia to nitrate by nitrifying bacteria whilst denitrification is to reduce the associated nitrate to nitrogen gas by an anoxic reduction process. Nitrates are not generally of great concern to the aquaculturist. Studies have shown that aquatic species can tolerate extremely high levels (greater than 100 mg/l) of nitrate-nitrogen in production systems.

Several nitrification and denitrification processes are available in literature as reviewed by Silapakul (2002). The differences in the nature of these two reactions often render the separate, individual design for each process. The separation of nitrification and denitrification units requires that large area must be available for the installation of the treatment system and this leads to extra needs of other facilities such as energy and maintenance.

Airlift bioreactors are attractive treatment techniques for the nitrification-denitrification process. The airlift system provides both aerated and unaerated compartments which serve as the nitrification and denitrification chambers in the same unit. Hence, in terms of facilities requirement, the airlift reactor is highly attractive.

Recently, a packed bed external loop airlift bioreactor (PBABR) was developed as a complete treatment system for wastewater containing ammonia (Silapakul, 2002). The 60L reactor was designed as one riser (aerated section) interconnected with two downcomers (unaerated section). The ratio between riser and downcomer was selected to ensure that the slower denitrification reaction could occur at a rate comparable with the nitrification. The system was successfully operated in a semi-continuous mode with synthetic wastewater where the initial NH_3 concentration of 10 mg-N/L could be completely removed within 1-14 days. However, the performance of the system with actual shrimp culture system has not been tested and, hence, there might still be hidden problems that need to be considered before this system can be applied. This becomes the main objective of this work.

1.2 Objectives

As stated earlier, the main focus of this work is on the application of the PBABR system to the actual lab scale shrimp culture pond. This is to evaluate the performance of the system in the removal of nitrogen compounds and to investigate whether there are other operational factors that need to be dealt with in the design of the actual PBABR system.

1.3 Expected benefits

The results from this work will be useful for the development of sustainable close system shrimp culture. This will facilitate the production of high quality shrimp and minimize the trade barriers for shrimp export.

1.4 Working scopes

1. PBABR packed with immobilized bioballs will be used in this study where the dimensions of system are shown in Figure 1.1
2. PBABR will be connected to the actual lab scale shrimp pond (1 m^3 in size). The performance of the shrimp culture with the attached PBABR will be compared with the controlled experimental pond of the same size (but without the treatment system).
3. An initial number of shrimp in both experimental and controlled ponds are 7 and will be maintained until the end of experiment.
4. Ammonia, nitrate, nitrite and the survival of shrimp are the monitoring parameters used to indicate the performance of system.
5. All experiments are performed at room temperature.
6. Methanol was used as a carbon source for the denitrifying bacteria.

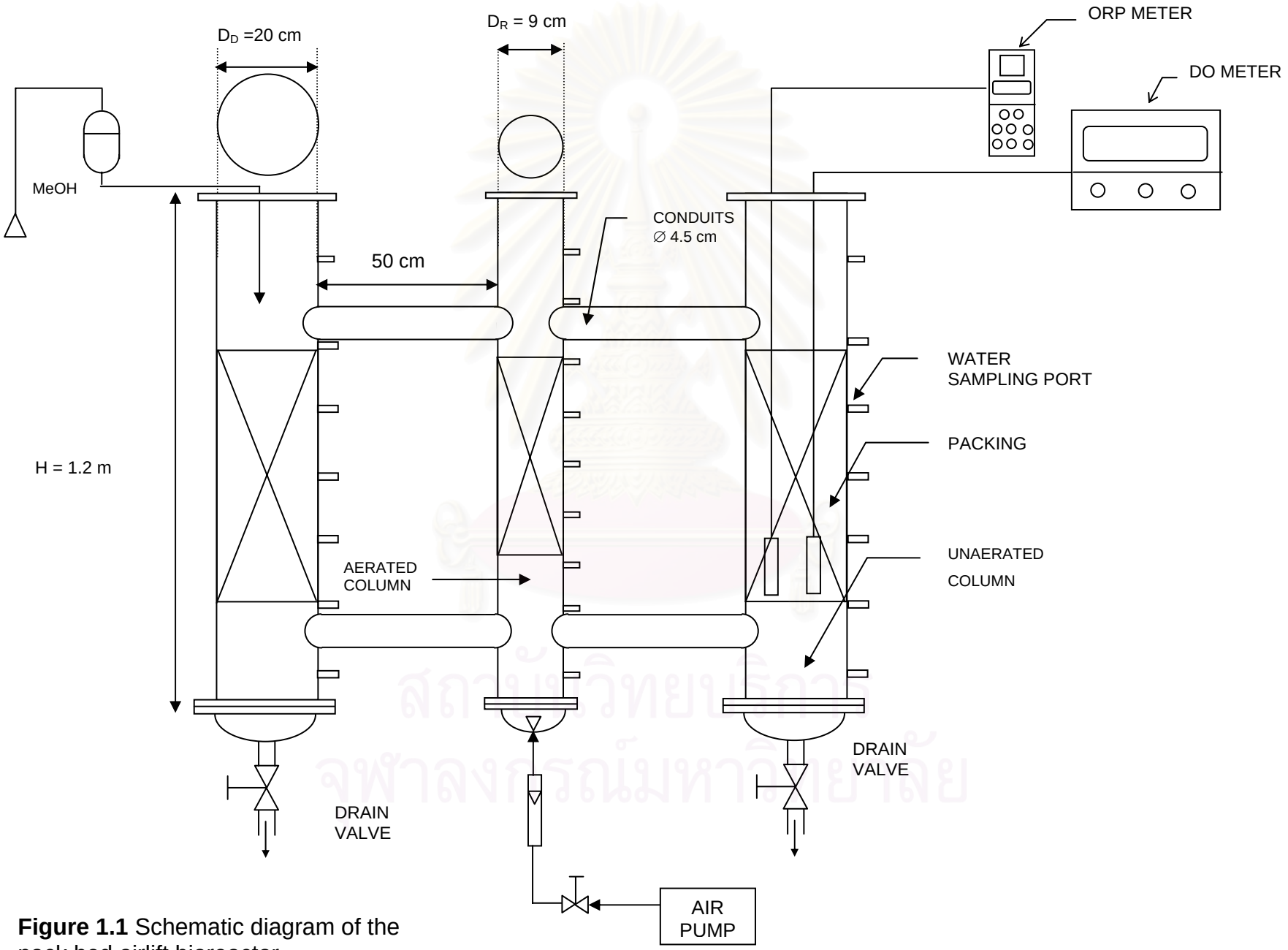


Figure 1.1 Schematic diagram of the pack bed airlift bioreactor

Chapter II

Backgrounds and Literature review

Ammonia found in agricultural wastes can be oxidized to nitrite and nitrate by using the well known aerobic environment, biological treatment process. Nitrate can then be used as electron acceptors by anaerobic bacteria in denitrification mechanism which leads to a reduction in the nitrogen content of the wastewater as all nitrogen escapes from solution in gaseous form. The fact that no liquid or solid waste byproducts are obtained from the combination of these two processes has led to a considerable attention and application of such treatment systems.

2.1 Nitrification processes

Nitrification is a sequential aerobic two-step oxidation of ammonia to nitrate. The oxidation of ammonia to nitrite is mediated by *Nitrosomonas*, and the oxidation of nitrite to nitrate is by *Nitrobacter*, and the two are often known as nitrifying bacteria. The nitrifying bacteria utilize carbon dioxide as a carbon source for biosynthetic process and oxidation of reduced nitrogen compounds as an energy source (Strotmann and Windecker, 1997). The stoichiometric reaction of bacteria (*Nitrosomonas*) in the oxidization of ammonium to nitrite can be simplified as:

$$NH_4^+ + 1.5O_2 \longrightarrow 2H^+ + H_2O + NO_2^- \quad (2.1)$$

Nitrite is then converted to nitrate through the following simplified stoichiometric reaction:

$$NO_2^- + 0.5O_2 \longrightarrow NO_3^- \quad (2.2)$$

The overall nitrification which is the combination of the above two equations can then be expressed by:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O \qquad (2.3)$$

The overall oxygen requirement for the oxidation of ammonia to nitrate is $4.57gO_2/gNH_4^+$-N, which consists of 3.43 and $1.14gO_2$ for the oxidations of ammonium and nitrite, respectively. In fact, when cell synthesis is taken into consideration, approximately the same quantity of O_2 is needed as:

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \longrightarrow 0.98NO_3^- + 0.021cell + 1.88H_2CO_3 + 1.041H_2O \qquad (2.4)$$

This illustrates that the oxygen requirement is 4.2 gO_2/gNH_4^+-N. It is then concluded that this cell synthesis only has slight effect on the overall oxygen requirement for the nitrification and can be safely neglected in the determination of theoretical O_2 demand.

The summary of the conditions controlling nitrification process are briefly described here. (See our previous works for more details: Silapakul, 2002 and Rasrikrangkrai, 2003)

A. pH:

Figure 2.1 shows that the optimal pH is approximately 7-8. The nitrification process itself can inhibit the reaction as HCO_3^- is consumed as carbon source and produces H_2CO_3 which lowers down the level of pH in the system. Thus, in actual applications, calcium carbonate buffer is required to control pH and to be a carbon source.

B. Temperature:

Nitrification can occur in a wide range of temperature, i.e. from 4 to 45^oC. Fundamentally, the maximum specific growth rate of bacteria, $\mu_{max}(d^{-1})$, depends directly on the temperature. This relation can be expressed by Arrhenius equation. However, high temperature can damage the cells. Therefore an optimum temperature for the reaction exists, e.g. the optimum temperatures for *Nitrosomonas* and *Nitrobacter* were reported to be 35^oC and 35-42^oC, respectively. (Wild et al.,1971; US.EPA,1975) (see Figure 2.2)

C. Dissolved Oxygen:

From Equation 2.3, oxygen has a significant effect on the nitrification reaction where a higher nitrification rate could be obtained at higher dissolved oxygen (DO) levels (Nagle and Haworth, 1969). However, Figure 2.3 shows that nitrification could be achieved even at a very low DO levels, e.g. 0.3 mgO_2/L. (Painter et al., 1977)

D. Ammonia concentration:

Both ammonium-nitrogen and dissolved oxygen are essential substrates, and the rate of oxidation depends strongly on substrate concentration.

E. Organic carbon:

There was a report saying that the addition of carbon source with a carbon/nitrogen (C/N) ratio of 1 or 2 reduced the total ammonia nitrogen removal rate by almost 70% compared with a pure nitrification process (C/N=0).(Zhu and Chen,2001)

F. Toxics:

Table 2.1 shows concentration level of various compounds which could be toxic to nitrifying bacteria.

G. Salinity:

Usually the nitrification rate drops with an increase in salt concentration. (Dincer et al., 2001)

H. Other essential requirements:

For growth of nitrifying bacteria, many compounds and elements include carbon dioxide, carbonate or bicarbonate, and ammonia or nitrite, phosphate, magnesium, iron, and copper in small quantity are also essential for growth (Painter, 1997). Other essential requirements for *Nitrobacter* and *Nitrosomonas* are summarized in Table 2.2.

2.2 Denitrification Process

Denitrification is the reduction of oxidized nitrogen compounds like nitrite or nitrate to gaseous nitrogen compounds. This process is performed by various chemoorganotrophic, lithoautotrophic, and phototrophic bacteria and some fungi (Shoun and Tanimoto, 1991), especially under oxygen-reduced or anoxic conditions (Focht, 1975). As opposed to nitrification, a relatively broad range of bacteria can accomplish denitrification, e.g. *Pseudomonas*, *Micrococcus*, *Achromobacter* and *Bacillus* (US.EPA, 1975). Many of denitrifying bacteria can shift between oxygen and nitrogen respiration. Denitrification is accomplished with a variety of electron donors, including methanol, acetate, ethanol, lactate and glucose (Grabinska, 1991; Tam et al.,1992; Akunna et al.,1993). The denitrification involves the following reactions:

Step one:

$$NO_3^- + 1/3CH_3OH \longrightarrow NO_2^- + 1/3CO_2 + 2/3H_2O \quad (2.5)$$

Step two:

$$NO_2^- + 1/2CH_3OH \longrightarrow 1/2N_2 + 1/2CO_2 + 1/2H_2O + OH^- \quad (2.6)$$

Overall denitrification:

$$NO_3^- + 5/6CH_3OH \longrightarrow 1/2N_2 + 5/6CO_2 + 7/6H_2O + OH^- \quad (2.7)$$

In brief, the conditions controlling denitrification process are: (More details, please see Silapakul, 2002)

A. pH:

US.EPA (1975) reported that acceptable denitrification rates occurred between pH of 6.0-8.0. Figure 2.4 presents the effects of pH on denitrification rate.

B. Temperature:

Denitrification was found to take place in wide range of temperature, e.g. 10-35^{o}C.

C. Dissolved oxygen:

Denitrifiers have ability of using both oxygen and nitrate as electron accepter in their metabolic processes. Oxygen is a preferred electron accepter as bacteria obtain high energy per mole of oxygen consumed. The energy generated from utilizing oxygen and nitrate are 686 and 649 kcal/mole, respectively (US.EPA, 1975).

However, at anoxic condition (0-1.5mgO_2/L), denitrifying bacteria switch from using oxygen to nitrate as electron acceptor (Painter, 1977).

D. Nitrogen loading:

Nitrate is an essential substrate for denitrification and the reaction rate usually depends on the concentration of nitrate in the form of Monod kinetics:

$$\mu = \mu_M \frac{D}{(K_D+D)} \frac{S}{(K_s+S)} \quad (2.8)$$

μ = Specific growth rate of denitrifying bacteria [d^{-1}]

μ_M= Maximum specific growth rate [d^{-1}]

D = Nitrate concentration [mgNO_3-N/L]

S = Organic carbon concentration [mg/L]

K_D= Saturated constant of nitrate [mgNO_3-N/L]

K_s = Saturated constant of orgnic carbon [mg/L]

E. Organic carbon:

Organic carbon, such as methanol, acetate, ethanol, lactate and glucose, is a substrate suitable for denitrifying bacteria. The reaction rate thus depends on the concentration of substrate in the forms of Monod kinetics.

F. Oxidation reduction potential:

Several ranges of ORP were used in literatures. Denitrification process was achieved by maintaining ORP between 0 and 150mV which was the range where nitrate removal from wastewater could take place without generating toxic byproducts such as H_2S. However, various investigators reported different ORP level for denitrification, e.g. Lee (2000) suggested ORP -200 to -400 mV, Balderston and Sieburth (1976) 0 to 200 mV, Turk (1996) -50 to 200 mV, etc.

Thus far, several reactor systems for the removal of nitrogen compounds are visited and the delineated review for conventional nitrogen removal processes can be found from Silapakul (2002) or summarized here in Tables 2.4 and 2.5.

The next section will focus on the novel microbial nitrogen removal processes.

2.3 Literature reviews on novel nitrogen removal systems

New processes for nitrogen removal are often based on a one-reactor philosophy with both nitrification and denitrification taken place simultaneously. Many other types of reactor are based on partial nitrification of ammonium to nitrite combined with anaerobic ammonium oxidation. Literature involved these new systems are briefly summarized as follows.

2.3.1 Single-reactor-processes

In the present day, several new removal processes have been developed. Specific focus is often on the removal of ammonium because ammonia is considered several times more toxic to aquatic species than nitrate (Castens and Rozich, 1986). As nitrification and denitrification are carried out under different conditions and by different microorganisms, these processes are often separated in time or space to function effectively. However, the two processes are complementary in many ways, i.e. (1) nitrification produces nitrite or nitrate, which is a reactant in denitrification, (2) nitrification reduces the pH that is raised in denitrification, and (3) denitrification generates the alkalinity that is required in nitrification (Menoud et al., 1999; Chen et al., 1998). Therefore, there exist obvious advantages in performing simultaneous nitrification and denitrification in a single reactor. Examples of this class follow.

Rodgers and Zhan (2003) used a vertically moving biofilm system to treat synthetic wastewater. The process consisted of two pre-denitrification units, and three nitrification units. The idea was to employ the biofilm grew on the surface of a plastic module in treating the nitrogen compounds in the wastewater. In anoxic units, the modules were vertically moved, while always submerged in the bulk fluid. In aerobic units, they were also moved vertically, but up into the air and down into the wastewater. In this system, the total nitrogen removal efficiency was 77–82%.

Bodik et al. (2002) used a system called ‘an anaerobic baffled filter reactor with aerobic post-treatment’. The system consisted of an anaerobic baffled filter

reactor and the subsequent aerobic post-treatment. The average removal of the NH_4-N varied during the year from 46.4 to 87.3%.

Nakhla and Farooq (2002) introduced a 'simultaneous nitrification–denitrification in slow sand filters' which could achieve the effluent nitrogen (TN) concentrations at as low as 1.5mg/L. The filtration rates in the range of 0.15–0.38 m/h, filter depth of 0.5–1.5m, and sand size 0.3–0.5mm on nitrogen removal processes at temperatures of 10–39^{o}C were assessed. Nitrification and denitrification efficiencies, along with the total nitrogen removal efficiency correlated well with filtration rate and sand size only, with all three parameters inversely proportional to the square root of the aforementioned two process variables. Nitrification exhibited the most sensitivity to filtration rate and sand size. The rate of nitrification and denitrification were about 2.232mgN/L/d.

Chen et al. (2001) used immobilized-cell reactor and investigated ORP level under real-time control of oxygen supply. At the beginning, the reactor was conducted by cyclic fixed-time aeration–nonaeration operation, followed by real-time control technology using ORP set point, nitrate break point. The presence of nitrate break point signified the termination of anoxic condition and the start of anaerobic condition, which corresponded to transformation from the respiration of oxygen or nitrates to the nonrespiration (strictly anaerobic fermentation) process. The duration of aeration period was found to be optimum at 3 h under the consideration of the removal efficiencies of COD and total nitrogen. The real-time control system not only exhibited better nitrogen removal efficiency than the fixed-time control operation, but it also showed stable effluent quality. Total nitrogen removal rate was about 1.58mgN/L/d.

Membrane technologies are also involved in nitrogen removal process. Simultaneous nitrification and denitrification could be achieved by designing a proper membrane module configuration. For instance, Choi et al. (2002) employed submerged nanofiltration membrane bioreactor (NF MBR) which made use of the cellulose acetate NF membrane in treating synthetic wastewater (Figure 2.5). The 12L bioreactor was filled with activated sludge in which the NF membrane module was directly submerged. The membrane did not allow the passage of oxygen and hence the conditions in the membrane was suitable for the denitrfication whilst the nitrification occurred in the tank (outside the membrane) where aeration was supplied. A hollow-fiber-type cellulose acetate membrane was chosen because of its

large surface area and as a result, the system could be operated for 60 days without fatal fouling and membrane cleaning. Terada et al. (2002) used a membrane-aerated biofilm reactor for the removal of nitrogen from high strength nitrogenous wastewater. Removal percentages of total organic carbon (TOC) and nitrogen were 96% and 83% at removal rates of 5.76g-C m^{-2} d^{-l} and 4.48 g-N m^{-2} d^{-l}, respectively. See the schematic diagram of the membrane-aerated biofilm reactor below (Figure 2.6). Note that the authors did not report the results on the time profiles of oxygen concentration inside the system, only the total removal of nitrogen was reported. Another experiment, Hsieh et al. (2002) used a double-biofilm reactor with a continuous-flow method. The two biofilm modules, termed the permeable support bioreactor (PSB) and the membrane feeding substrate bioreactor (MFSB), were employed as a module system. PSB and MFSB were combined in a single tank to develop a double-biofilm reactor, which was used to treat nitrogen contaminants in wastewater. The part of the reactor that conducted nitrification, PSB, was wrapped in PVA-immobilized nitrifying biofilm. Another part of the reactor, MFSB, was conducting the denitrification. With a membrane supplement of substrates (O_2 for nitrifying bacteria in PSB and CH_3OH for denitrifying bacteria in MFSB), the DO and COD levels were at a low value in the bulk solution thus inhibitive effects between nitrification and denitrification were minimized. The double-biofilm reactor achieved high nitrification and denitrification efficiencies of 96.5% and 82%, respectively or equivalent to removal rates of approx. 5gN/m^2d and 3.7gN/m^2d for nitrification and denitrification respectively. Figure 2.7 illustrates the schematic diagram of the double-biofilm reactor (combined-mode).

Cao et al. (2003) used shell-and-tube co-immobilized cell bioreactor. The configuration of this bioreactor was similar to a shell-and-tube heat exchanger, and consisted of a bundle of parallel tubes made of polyvinyl alcohol (PVA) gel, containing nitrifying and denitrifying bacteria fixed onto the tube sheets. The tube bundle was contained in a cylindrical shell which was provided with two channels, one at each end. Ammonia nitrogen wastewater was introduced into the shell-side space surrounding the tubes. At the same time, air was pumped into wastewater for nitrification. Ethanol solution was pumped into one channel. It flowed through the tubes into the other channel and was withdrawn into the ethanol solution tank for recycling. Only a small amount of ethanol diffused into the wastewater from the recycling ethanol solution, and the BOD value in the effluent was lower than 30

mg/L. The total inorganic nitrogen (TIN) concentration was below 3 mg/L. The schematic diagram of shell-and-tube co-immobilized cell bioreactor in continuous system is shown below (Figure 2.8).

Erler et al. (2003) studied the effects of effluent nitrogen from shrimp farm settlement ponds on bacterial growth and nitrogen removal. Bacterial volumetric growth rates were significantly increased in the presence of shrimp when compared to the control pond (45.2 mgC/m^2d and 22 mgC/m^2d, respectively). The increased in bacterial growth rate was reported to enhance nitrogen uptake and the rate of denitrification was significantly lower in the treatment with shrimp than in the no-shrimp treatment (51±12 mgNm^{-2} per day and 82±16 mgNm^{-2} per day, respectively). Nitrification rate was similar between treatments (79.20 mgNm^{-2} per day and 75.17 mgNm^{-2} per day for Shrimp and No-shrimp treatments).

2.2.2 Novel-processes

Biological nitrogen removal proceeds slowly as the microorganisms responsible for the elimination reactions grow slowly. In addition, the operational control of aerobic and anaerobic conditions needed for nitrification and denitrification can be difficult. Several novel nitrogen removal processes have therefore been developed lately. One important character shared by these new methods is that they can be operated without the requirement of additional organic carbon which otherwise makes full-scale denitrification quite expensive.

Hibiya et al. (2002) used a ‘Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membrane aerated biofilm reactor’. Biofilm was fixed on a hollow-fiber membrane. It was found that ammonium nitrogen was gradually removed whilst the amount of nitrate nitrogen was negligible throughout the reactor. The nitrification rate at the lower part of the reactor was 7 to 125 times higher than those at the central and upper points, respectively. The system is schematically depicted in the figure below (Figure 2.9). The ammonia-oxidizing bacteria were mainly distributed inside the biofilm, whereas other bacteria, including denitrifying bacteria, were mainly distributed over the suspended sludge. Nitrification rates at lower, central and upper points were 3.0, 0.41 and 2.3x10^{-2} gN (m^2 day)$^{-1}$, respectively.

Sliekers et al. (2001) presented a ‘Completely autotrophic nitrogen removal over nitrite in one single reactor’. The reactor was started anoxically to induce anaerobic ammonia oxidation (Anammox) by sparging with helium. Then, oxygen was supplied to the reactor and a nitrifying population developed. Oxygen was kept as the limiting factor. During steady state, anaerobic ammonium-oxidizing bacteria remained present and active. In the reactor, no aerobic nitrite-oxidizers were detected. The denitrifying potential of the biomass was below the detection limit. Ammonia was mainly converted to N_2 (85%) and the remainder (15%) was recovered as NO_3^-.

Some of the new born technologies in this area include the units such as SHARON, ANAMMOX, and CANON processes and they are described below.

Novel biological technologies for nitrogen removal processes

At present, wastewater treatment practices can be significantly improved through the introduction of new microbial treatment technologies. The new processes often involve one step technique to minimize operational requirement. Most new processes are based on partial nitrification of ammonium to nitrite (Eq. 2.9) combined with anaerobic ammonium oxidation (Eq. 2.10). These processes include (i) the single reactor system for high ammonia removal over nitrite (SHARON) process, which involves part conversion of ammonium to nitrite; (ii) the anaerobic ammonium oxidation (ANAMMOX) process, which involves anaerobic ammonium oxidation; and (iii) the completely autographic nitrogen removal over nitrite (CANNON) process, which involves nitrogen removal within one reactor under oxygen-limited conditions.

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + 2H^+ + H_2O \quad (2.9)\ [\Delta G^o\ -275\ kJmol^{-1}]$$

$$NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O \quad (2.10)\ [\Delta G^o\ -357\ kJmol^{-1}]$$

SHARON process

The SHARON process (single reactor system for high ammonia removal over nitrite process) is a new process for biological nitrification. This process is operated without any biomass retention in a single aerated reactor at a relatively high temperature (35^{o}C) and high pH (above 7) (Brouwer et al., 1996; Hellinga et al., 1997). The process involves partial nitrification of ammonium to nitrite, but not to nitrate, and this greatly reduces the expense of aeration. SHARON is the first successful process in which nitrification/denitrification with nitrite as an intermediate has been achieved under stable conditions (van Kempen et al., 2001). The possible metabolic pathways for nitrification and denitrification are shown in Figure below:

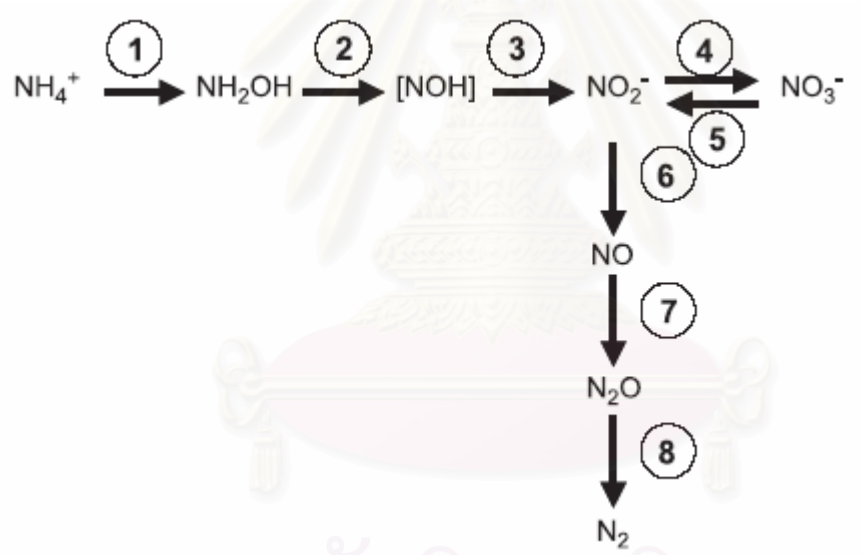


Figure 2.10 Possible metabolic pathways for Nitrification and Denitrification

The operating variables (temperature, pH, hydraulic retention time, substrate concentration, dissolved oxygen) are controlled in a chemostat operation (Beccari et al., 1979; Hellinga et al., 1998). The process requires relatively little oxygen because the oxidation is stopped at the nitrite stage, and this saves on energy and the added carbon source.

To achieve partial nitrification, the oxidation of nitrite to nitrate must be prevented. The oxidation can be prevented in at least two ways. The first is through the use of the difference in activation energy between ammonia and nitrite oxidation (68 kJ mol^{-1} and 44 kJ mol^{-1}, respectively).The high activation energy of ammonia oxidation makes the rate of this process more dependent on temperature, thus this process needs sufficiently high temperatures (more than 26^{o}C). Secondly, further oxidation is prevented by maintaining the system at low oxygen concentration (less than 0.4 mg/L or 5% air saturation) and with a surplus in ammonia, nitrite oxidizers are unable to grow.

ANAMMOX process

Schmidt and Bock (1997) reported that ammonium was able to be oxidized by ammonium oxidizers under anoxic conditions when gaseous nitrogen dioxide (NO_2) was present. The anammox process is the denitrification of nitrite with ammonia as the electron donor. Anammox needs a preceding partial nitrification step that converts half of the wastewater ammonium to nitrite. The relevant reactions are as follows:

$$5NH_4^+ + 3NO_3^- \longrightarrow 4N_2 + 9H_2O + 2H^+ \qquad (2.11)$$

$$NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O \qquad (2.12)$$

The main product of anaerobic ammonium oxidation was N_2, but about 10% of the N-feed (nitrite and ammonium) was converted to NO_3^-.

Strous et al. (1998) estimated the ANAMMOX stoichiometry based on mass balance over ANAMMOX enrichment cultures, as presented in Eq. 2.13:

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \longrightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \qquad (2.13)$$

The ANAMMOX process is based on energy conservation from anaerobic ammonium oxidation with nitrite as electron acceptor without requirement for

external carbon source (Jetten et al., 1999). Hydrazine and hydroxylamine are known to be some intermediates of the process (van de Graaf et al., 1997; Schalk et al., 1998; Jetten et al., 1999). Carbon dioxide is the main carbon source for the growth of ANAMMOX bacteria (van de Graaf et al., 1996). The possible metabolic pathways for anaerobic ammonium oxidation are shown in figure below:

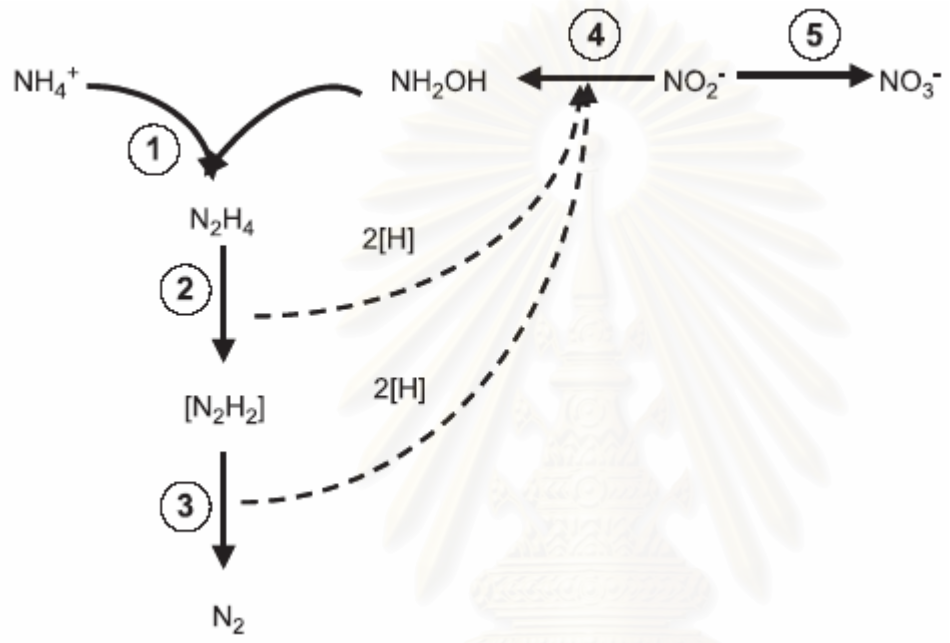


Figure 2.11 Possible metabolic pathways for anaerobic ammonium oxidation

Bacteria capable of oxidizing ammonium were discovered and identified as the new autotrophic members of the order of *Planctomycete*, one of the major distinct divisions of bacteria (Strous et al., 1999). The anaerobic ammonium oxidation reaction is carried out by two ANAMMOX bacteria that have been tentatively named as ‘‘Brocadia anammoxidans’’ (Strous et al., 1999) and ‘‘Kuenenia stuttgartiensis’’ (Schmid et al., 2000). The high ANAMMOX activity is detectable for both bacteria in a pH range between 6.4 and 8.3 and a temperature between 20^{o}C and 43^{o}C (Strous et al., 1999; Egli et al., 2001).

Canon process

Canon is an acronym for ‘completely autotrophic nitrogen removal over nitrite’. This concept is the combination of partial nitrification and anammox in a single, aerated reactor (Strous,1997; Koch,2000; Third K.A. 2002). The name ‘Canon’ also refers to the way the two groups of bacteria cooperate. Under oxygen-limited conditions (< 0.5% air saturation) a coculture of aerobic and anaerobic ammonium-oxidizing bacteria can be established (Strous, 2000), and this system is responsible for the CANON activity. The process relies on a stable interaction between the two groups of autotrophic microorganism populations: Nitrosomonas-like aerobic bacteria and Planctomycete-like anaerobic ammonium-oxidizing bacteria, under oxygenlimited conditions (Third et al., 2001). These autotrophic cultures convert ammonia directly to dinitrogen gas with nitrite as an intermediate.

Under oxygen-limited condition, ammonium is oxidized to nitrite by aerobic nitrifiers, such as *Nitrosomonas* and *Nitrososira* (Eq.2.16)

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + 2H^+ + H_2O \qquad (2.14)$$

Subsequently, anaerobic ammonium oxidizers Planctomycete-like ANAMMOX bacteria convert ammonium with the produced nitrite to dinitrogen gas and trace amounts of nitrate.

$$NH_4^+ + 1.3NO_2^- \longrightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \qquad (2.15)$$

The combination of the above two reactions result in nitrogen removal as follows.

$$NH_4^+ + 0.85O_2 \longrightarrow 0.435N_2 + 0.13NO_3^- + 1.3H_2O + 1.4H^+ \qquad (2.16)$$

A comparison of the new processes of nitrogen removal and conventional nitrification/denitrification are in Table 2.3 .

Table 2.6 summarizes the condition and ammonia/nitrite removal efficiencies of novel nitrogen removal process.

For now, these have not been an installation of such process in a large scale. This is probably due to the difficulties in control and also high investment cost. The difficulties in identification of microorganism also prevents their wider application.

Table 2.1 Compounds giving substantial inhibition of ammonium oxidation by activated sludge in batch tests (Tomlinson et al, 1966 ; Wood et al, 1981; Hockenbury and Grady, 1977; King and Painter, 1985)

Concentration(mg/L)	Toxic compounds
≈1mg/L	Thiourea, Allylthiourea, Thiosemicarbazide, Thioacetamide Na methyldithiocarbamate, Dithio-oxamide, Methyl isothiocyanate Mercaptobenzthiazole, Allyl isothiocyanate, Dodecylamine N-methyl aniline, Na cyanide
≈10mg/L	Methyl thiuronium sulphate, Aniline, 1-Naphthylamine, Ethylene diamine Quinoline, Skatole, Phenol, *P*-Benzoquinone
≈20mg/L	Na dimethyldithiocarbamate, Tetramethylthiuramthiocarbamate Na cyclopentamethylene-thiocarbamate, Na cyclopentamethylene- dithiocarbamate, Guanidine carbonate, Naphthyl ethylenediamine Pyridine, 2,2-Bipyridine, *o-,p-,m*-Cresols, Allyl alcohol, Chloroform, Cetyl trimethyl ammonium
20-100mg/L	Piperidinium cyclopenta, Methylene dithiocarbamate, Benzyl thiouronium chloride, Tetramethylthiuram disulphide, Benzthiazole disulphide, Diguanide, Hydrazine, Hexamethylene diamine, *P*-Nitraniline, *P*-Amino propiophenone, *P*-Phenyl azoaniline, Benzidine dihydrochloride, 2-Chloro-6 (trichloromethyl)pyridine, 8-Hydroxy-quinoline, Cetyl pyridinium chloride, Diallyl ether, Na azide, Carbon disulphide, *P*-Nitrobenzaldehyde, Dichlorophen
>100mg/L	Dicyandiamide, Trimethylamine HCl, Triethylamine, Benzylamine, Ninhydrin, Benzocaine, Strychnine HCl, 2,4,6-Tribromophenol, K thiocyanate[300], Methylene blue[100], EDTA[350], Streptomycin[400], Methylaniline HCl[1550]

Table 2.2 Essential requirements for proper growth of *Nitrobacter* and *Nitrosomonas*. (Painter, 1977)

Requirement	[mg/L]
Phosphate (P)	5
Copper (Cu)	0.03
Sodium (Na)	0.002-0.005
For *Nitrobacter* only	
Zinc	1
Molydenum	0.001
For *Nitrosomonas* only	
EDTA	5

Table 2.3 A comparison of the new processes of nitrogen removal and conventional nitrification/denitrification (Jetten et al., 2002)

System	Conventional nitrification/ denitrification	SHARON	ANAMMOX	CANON
Number of reactors	2	1	1	1
Feed	Wastewater	Wastewater	Ammonium+nitrite	Wastewater
Discharge	NO_2^-,NO_3^-;N_2	NH_4^+, NO_2^-	NO_3^-, N_2	NO_3^-, N_2
Conditions	Oxic; anoxic	Oxic	Anoxic	Oxygen limited
Oxygen requirement	High	Low	None	Low
pH control	Yes	None	None	None
Biomass retention	None	None	Yes	Yes
COD requirement	Yes	None	None	None
Sludge production	High	Low	Low	Low
Bacteria	Nitrifiers+various heterotrophs	Aerobic NH4+ oxidizer	planctomycetes	Aerobic NH4+ oxidizers+planctomycetes

Table 2.4 Details on the operation of various types of nitrification process

Reference	Nitrification rate [gN/m2/d]	Type Volume [m3]	Packing	Flow rate [L/min]	Retention time [min]	Do [mgO2/L]	Specific surface area [m2/m3]	pH	Temperature [oC]	Salinity [%]	NH4-N [mgNH4-N/L]
Activated sludge											
Campos et al(1999)		0.004			78	2-6		9.8	20		7.5
Campos et al(2002)		0.004		0.0007-0.002		>2		7.8	20		
Airlift reactor											
Millamena et al.(1994)						3.6					
Sakairi et al.(1996)	1.3	0.0157	Polyethyleimine	b.		5		8.1-8.4	28		1.3
Benthum et al.(1998)	1.25	0.003	Basalt	0.465L/h	240	>3		7.5	30		5
Benthum et al.(1999)	4.33	1.7	Basalt	7.0-8.0							4.37
Seo and Kim(2001)	2	2.5		0.1vvm.	48h	5.2		7.8-8.2	25		
Batch CSTR											
Kim et al.(2000)	0.82		Ba-algenated Ca-algenated Carageenan Agar bead		18	7.5-7.9			25		20
Sequency batch reactor											
Sliekers et al.(2002)	0.15	2			24h			7.8	30		14
Shrestha et al.(2002)								7.0-8.0	30		
Fluidized-bed filter											
Reyes and Lawson(1996)		170	Polyethylene			5.3	178	7.98	30.4		
Skjolstrup et al.(1998)		53					1000	7	17.6		2.2
Submerged filter											
Bower(1982)	91%	3	Limestone	b.				8.32	25.3	30	6.23
Wickins(1985)	0.43		Plastic	0.083			160-200		28	20-34	0.2
Strotmann and Windecker(1997)	19mg/L h (100%)	1.5	Raschig ring	6.25	240	3		7			
Davis and Arnold(1998)	0.59	0.72	Polypropylene	280	25.714	10.2	223.1				
Tseng(1998)	0.23	0.72	Plastic	b.	20	3.6	150	7-8	32	33ppt	3.64
Menasveta et al.(2001)	0.068	6	Plastic ball	b.				7.5			2

Reference	Nitrification rate [gN/m2-d]	Type Volume [m3]	Packing	Flow rate [L/min]	Retention time [min]	Do [mgO2/L]	Specific surface area [m2/m3]	pH	Temperature [oC]	Salinity [%]	NH4-N [mgNH4-N/L]
Trickling filter											
Otte and Rosenthal(1979)	0.75	1.06	Plastic foil filter	83.33	2.5*10-5	6.0-7.5	480	8.2	20	8	15
Roger(1985)	0.012	0.04	Slag	0.16	0.342	5.0-6.0	18.3	7.1-8.5	25.5-30	20	9.3
Nijhof(1995)	0.22	3	Sieve screen	3458.33	2.49*10-5	7.4-8.2	200	7.0-7.5	25		0.5-5
Kamtra(1998)	0.24-0.55			b.		7.0-8.0	100-150	7	22-24		
Greiner and Timmons(1998)	0.94-3.92	0.06	Commercial	b.		>5.0	164	6.7	26.4		
Lakang and Kleppe(2000)	0.1-0.2		Finturf articial glass Kaldnes rings Plastic rings Leca(Clay)	0.5	2.98	6.7-10.7	248 500 220 500-1000	6-7	14-16		1.5
Rotating biofilter											
Contactor (RBC)											
Reyes and Lawson(1996)	0.257	1.4		73.6-78.2		5.3	246	7.98	30.4		
Schuster and Stelz(1998)		0.12						6-7	15		
Biodrum											
Roger(1985)	0.05	0.04	Slag	0.08		5.0-6.0	18.3	7.8	25-30	20	10
Wortman and Wheaton(1991)	0.4-1.6	0.009	Polypropylene	0.62		4.6-11	278.83	7.5-8.5	25	7-35	8-9
Immobilized in porous											
Carrier											
Sakairi et al(1996)		0.0157	Cellulose carrier	0.052		5		8	28		
Greiner and Timmons(1998)						>5		6-7	26.4		
Kim et al.(2000)	8.2		Alginate		0.3h				55		
Seo et al(2001)	2.63mg/L-h	0.045	PVA gel beads	4.5	60	5.2		7.8-8.2	23-27	0-30	10
Membrane biofilm reactor											
Huang et al.(2001)					5h	4.0-5.0		6.8-7.2			
Delgado et al.(2002)		170						8.5			
Pond											
Gross et al(2000)	0.07										5.9

Table 2.5 Details on the operation of various types of denitrification process

Reference	Denitrification rate [mgN/L/min]	Type Volume [L]	Packing	Flow rate [L/min]	Retention time [min]	Do [mgO2/L]	ORP [mV]	pH	Temperature [oC]	C:N	Salinity [%]	NO3-N [mgNO3-N/L]
Activated sludge tank												
Otte and Rosenthal (1979)	0.008	1060		2		6.0-7.5		7	22-26		8	1208(max)
Fixed film column												
Balderston&Sieburth (1976)	0.007	1.5	Limestone (11mm) plastic Koch Flexiring (25mm)	0.0075	200	<1.2	0-200		20	>1(met)	18	100
Turk(1996)	0.02-0.025	60	Glass beads	0.33	105-1.5	<1.5	-50to200			1.5		80
Abeysinghe et al(1996)			Perspex plastic								1	20
Sauthier et al(1998)	8g/m3h	30	Crush bricks	0.14	200	0.5-1	0to-200		20-25	1-2(met)		60
Shanableh&Hijazi(1998)			Polypropylene pell rings	0.1	135					1-4		8
Nagadomi et al(1999)			Polyvinyl alcohol beads							0		
Lee et al(2000)			Glass beads		200	0.8-1.2	-325to400	7-8		Met		
Boley et al(2000)		82.5	Polymer pellet	0.01				8	27-28	0		40
Menasveta (2001)	0.0124		Plastic ball&crushed oyster shells	0.04-0.1				7-8	25	0.92 (met)	28-32	200
Sinhabhandhu(2001)	0.0124		Plastic bioballs	0.043					29	1-2(met)	30	100
Fluidized bed column												
Van Rijn et al(1990) and Arbiv et al(1995)	0.2	131.5	Sand (0.3-0.9mm)	5-40	12-13	<0.2		7	27			50
Floating immobilized												
Sakairi (1996)	1.44per carrier	9	Cellulose carriers(3mm)	0.022		1		8	30	1.3	7.23	20

Reference	Denitrification rate [mgN/L-min]	Type Volume [L]	Packing	Flow rate [L/min]	Retention time [min]	Do [mgO2/L]	ORP [mV]	pH	Temperature [oC]	C:N	Salinity [%]	NO3-N [mgNO3-N/L]
Boley(2000)	0.02-2.7 0.03-3.84 g/m2-d	82.5	Biodegradable polymer pallet(0.39-0.52m2)	0.003-0.01				6-8	20-25	0		5-40
Pond												
Gross et al(2000)	0.038g/m2d							7-9	21-28	0		

Table 2.6 Details on the operation of novel microbial nitrogen removal process

Reference	Removal rate [gN/m2/d] (Nitri:Denitri)	Type Volume [m3]	Packing	Flow rate [L/min]	Retention time [min]	Do [mgO2/L]	Specific surface area [m2/m3]	pH	Temperature [oC]	Salinity [%]	N-loading [mgN/L]	C:N
One-reactor-two-process												
Rodgers and Zhan(2003)	1.3-1.8 : 2.9-3.8	0.576	Polypropylene				150		11			
Bod ık et al(2002)		0.75			3h:8h			7	4.5-23		38.4-43.9	
Nakhla(2002)				8-20	1.3h	0-6.1		7.5	10-39		3.6-4.6	
Chen et al(2001)		0.016	PVA gel beads			4-6 : 1-2		7.5	25			
Choi et al(2002)		0.012	cellulose acetate	5kg/cm2	24h		11.7m2	7.2	16.4		7.7	
TERADA et al(2002)	4.48	0.00015	polyethylene	5cm/s	15day		50	7.5	25	1.3	3000	ethanol
Hsieh et al.(2002)	5 : 3.7		PVA : silicone membrane		8h			6-8	30		118-707	methanol
Cao et al (2003)			polyvinyl alcohol			3-5			30			ethanol
Erler et al(2003)	75mgN/m2d 51mgN/m2d	10000*4	fiberglass tanks								2.5-5	40mg/l ethanol
Novel-processes												
Ciudad et al(2004)		0.0029			7-10.5h	1.4		7.8	25		15	No carbon
Hibiya et al(2002)	0.77	100cm3	polyethylene	0.4L/day			57		30		25	No carbon
Sliekers et al (2001)	0.064-0.315kg/m3d	2L		1.45mL/min	1day	0.02		7.8	30		0.62-1.2g/L	No carbon

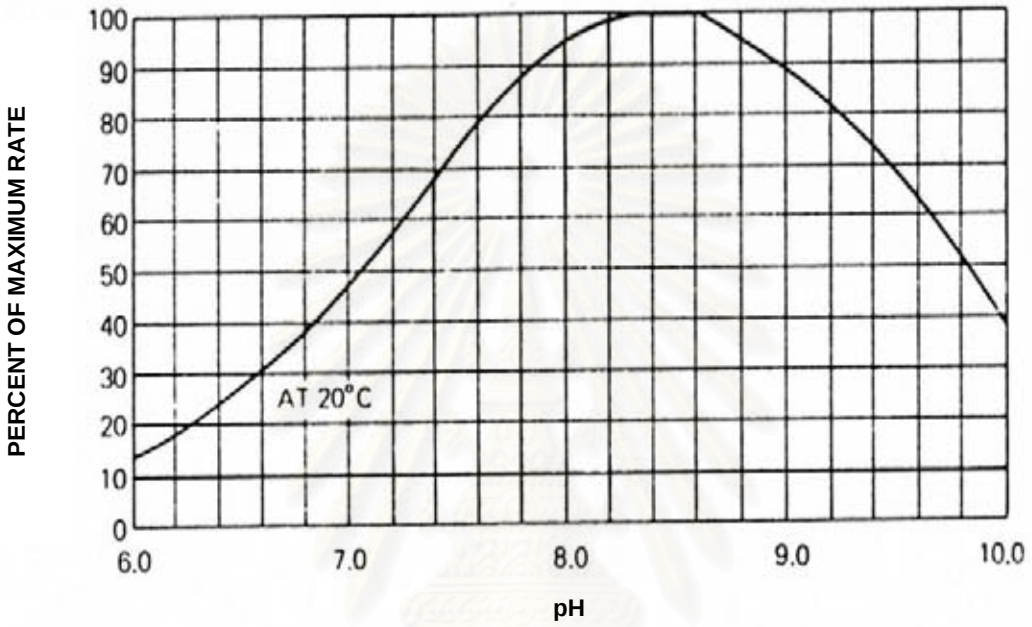


Figure 2.1 Effect of pH on nitrification rate at 20^{o}C (Wild et al., 1971)

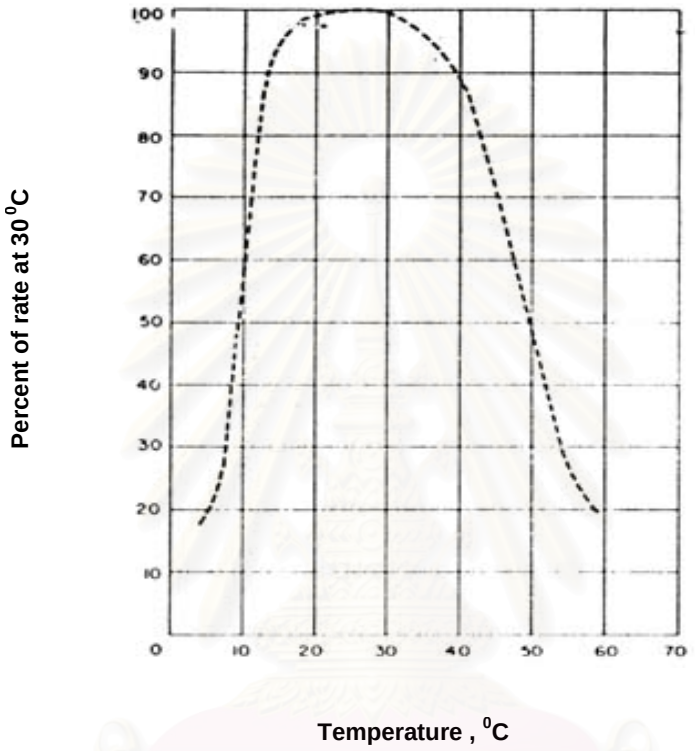


Figure 2.2 Effect of temperature on nitrification rate compared to the rate at 30°C (Wild et al, 1971)

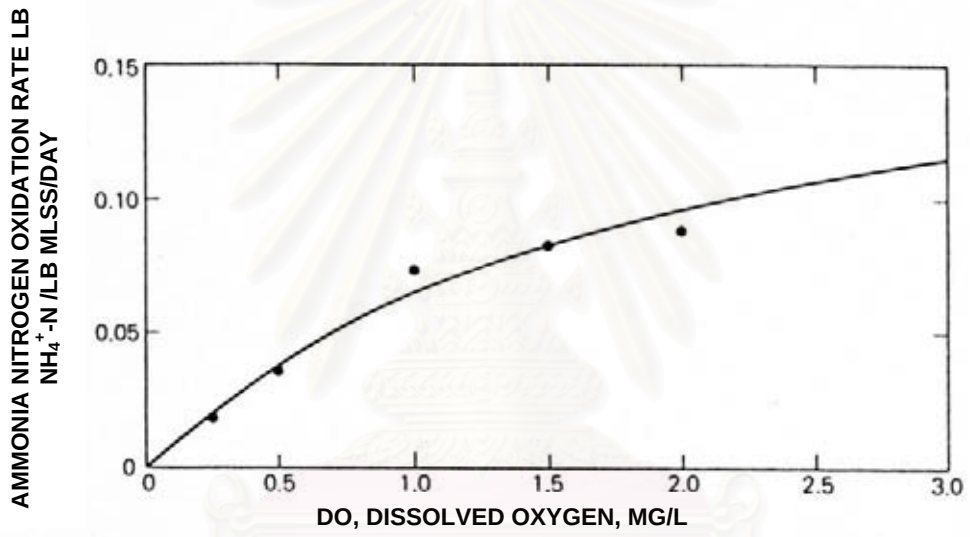


Figure 2.3 Effect of dissolved oxygen on nitrification rate at 30^{o}C (Nagel and Hawort, 1969)

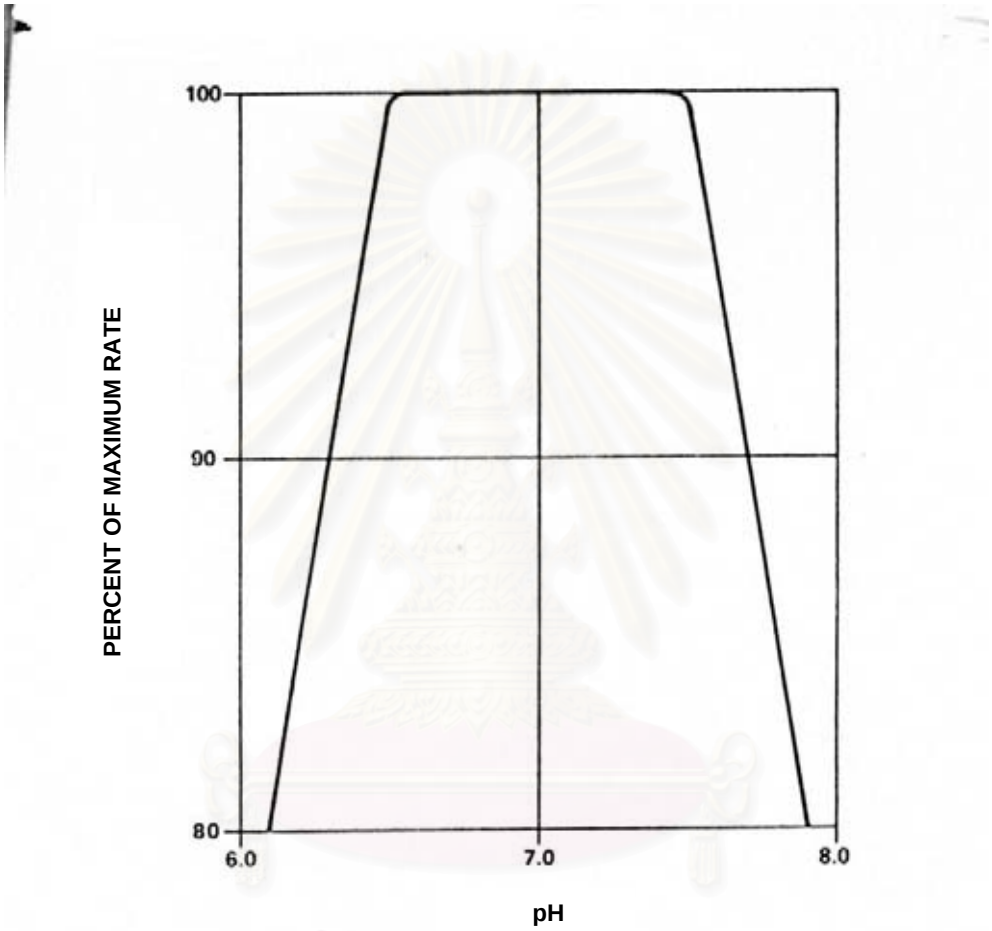


Figure 2.4 Effect of pH on denitrification rate (Sawyer et al, 1973)

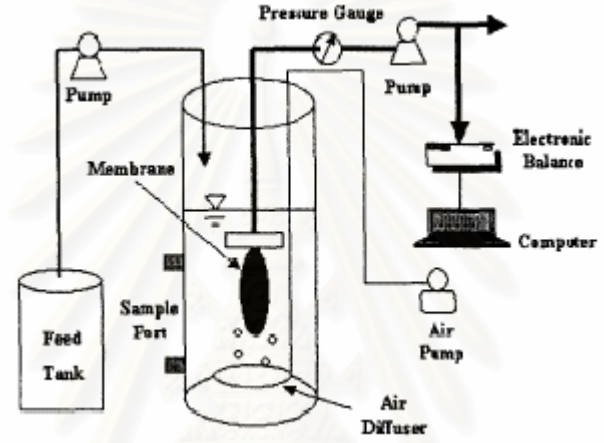


Figure 2.5 Schematic diagram of nanofiltration membrane bioreactor (Choi et al., 2002)

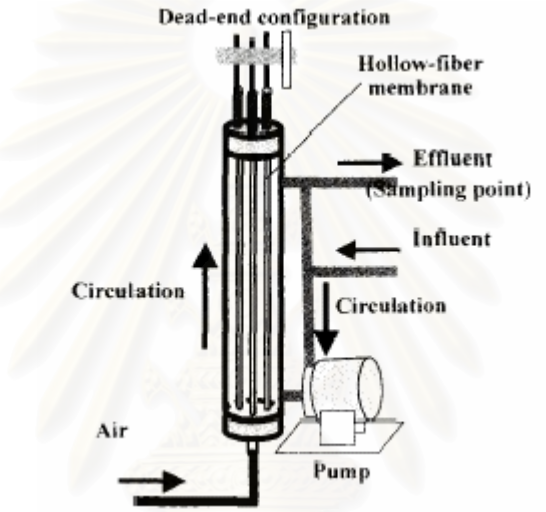


Figure 2.6 Schematic diagram of membrane-aerated biofilm reactor (Terada et al., 2002)

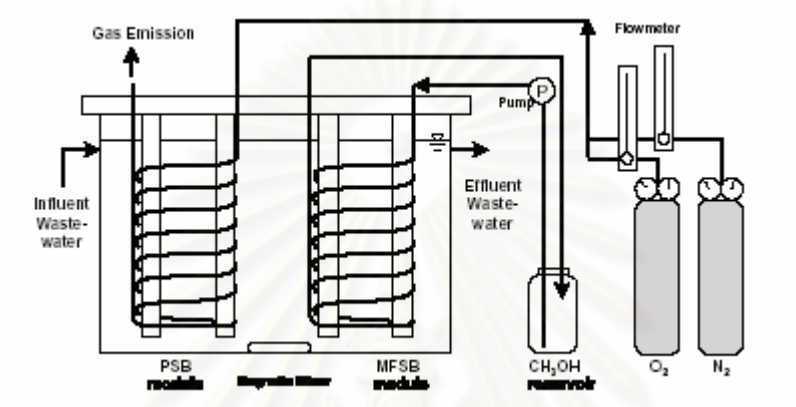


Figure 2.7 Schematic diagram of double-biofilm reactor (Hsieh et al., 2002)

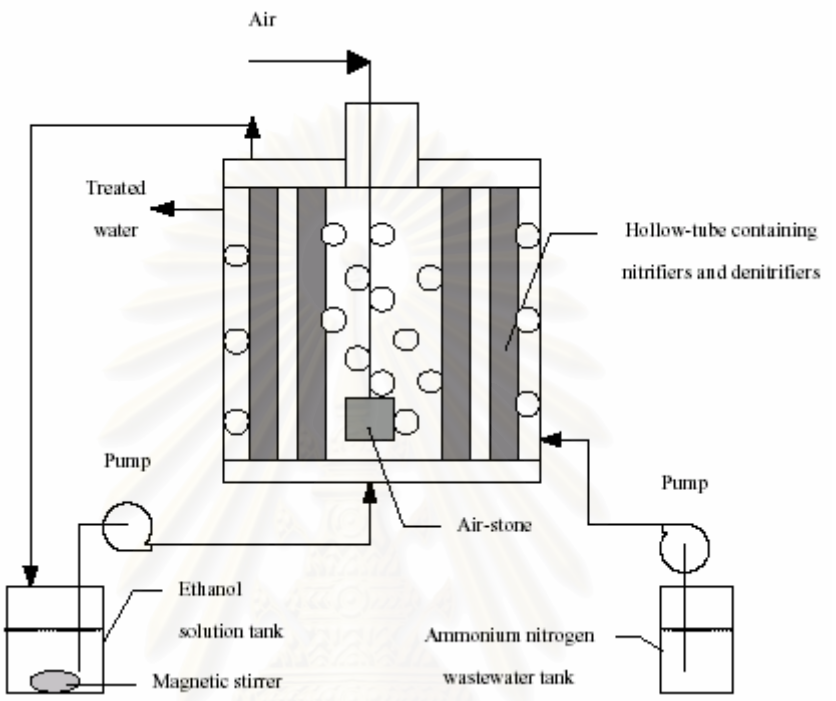


Figure 2.8 Schematic diagram of shell-and-tube co-immobilized cell bioreactor (Cao et al., 2003)

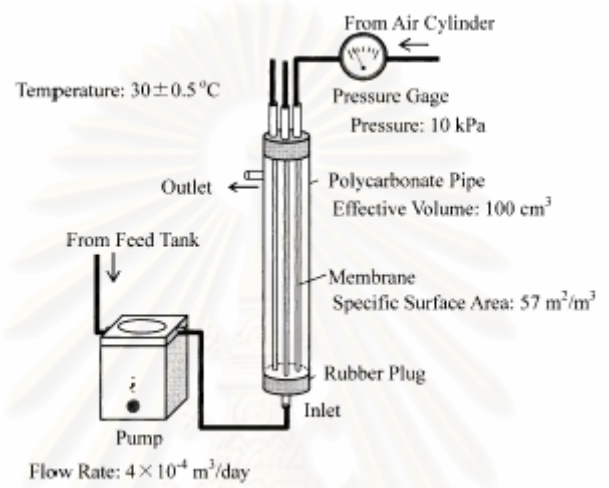


Figure 2.9 Schematic diagram of membrane aerated biofilm reactor (Hibiya et al., 2002)

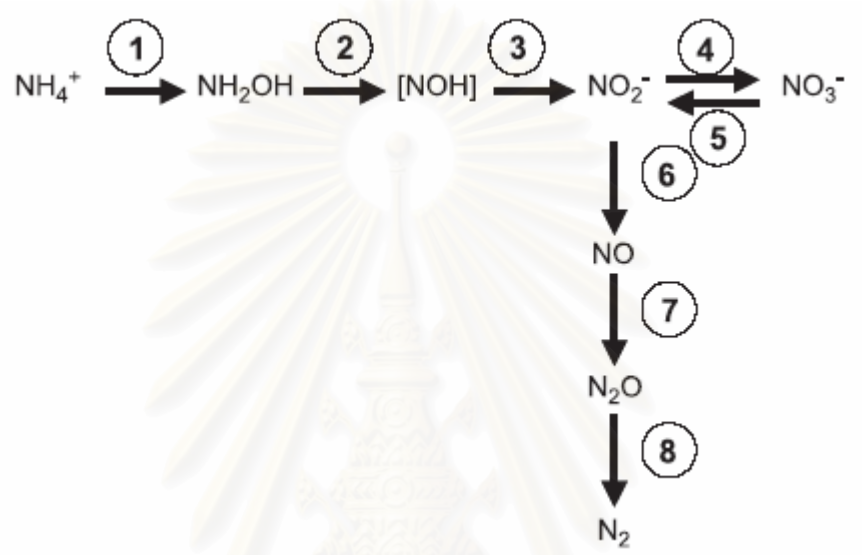


Figure 2.10 Possible metabolic pathways for Nitrification and Denitrification

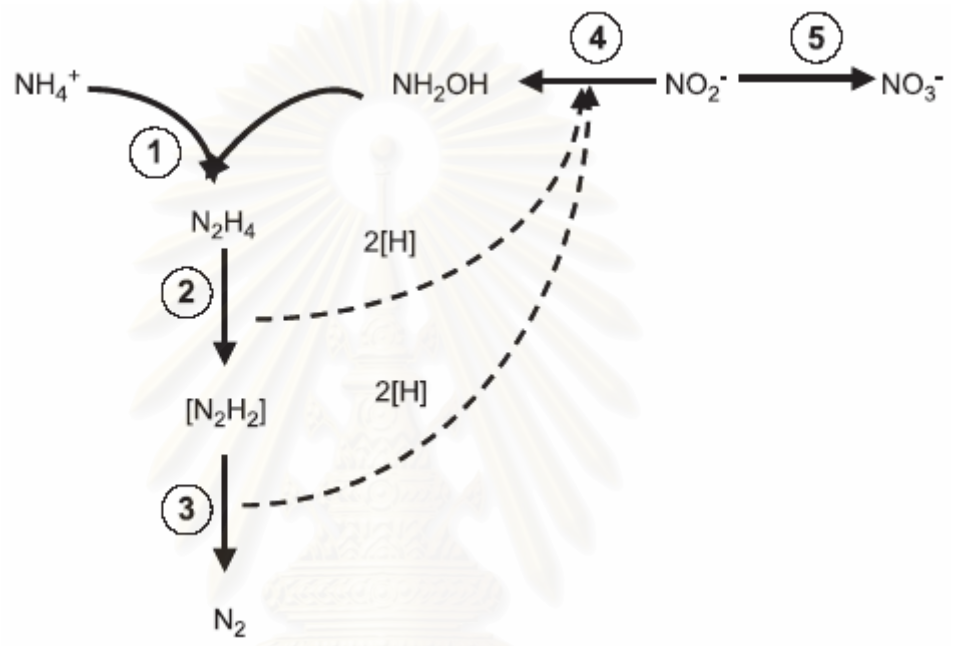


Figure 2.11 Possible metabolic pathways for anaerobic ammonium oxidation

Chapter III

Materials and Methods

3.1 Experimental setup

3.1.1 A packed bed external loop airlift bioreactor (PBABR)

The experiment is performed in a 60 L external loop airlift reactor packed with immobilized nitrified and denitrified plastic bioballs. The plastic bioball has a diameter of 2.5 cm with a surface area of 32 cm^2. The packed bed and the PBABR are the same as designed in our previous work, [Silapakul, 2002] . The reactor is designed as one riser (aerated section) interconnected with two downcomer (unaerated section). The riser is a cylindrical column with a volume of 8 L and 9 cm. in diameter and each downcommer volume is 37 L and 20 cm. in diameter. The heights of both aerated/unaerated columns are 1.2 m and the cross-sectional area of the unaerated column (A_D) is larger than the aerated (A_R) with a ratio A_D/A_R of 9.87. This is to ensure that the slower denitrification reaction can occur at a rate comparable with the nitrification. Ammonia is converted to nitrate (nitrification reaction) in the aerated section and nitrate is then converted to nitrogen gas (denitrification reaction) in unaerated section .The packing in aerated section is packed with 200 bioballs whereas each unaerated column is packed with 2000 bioballs. This packing is supported by an aluminium perforated plate installed at the bottom of the column. The water form shrimp pond is pumped into the riser section which is then overflowed at the top of the downcomer. A recirculating conduit was attached to the bottom of the downcomer which allows the medium to flow back to the pond (see Figure 3.1 for the schematic diagram of the system). The air flow rate was variable to maintain the dissolved oxygen level in the aerating section. The dissolved oxygen (DO) was measured by Hanna HI 964400 and the oxidation/reduction potential (ORP) was measured by Hanna HI 98240

3.1.2 Shrimp ponds

Two shrimp ponds were compared in this study. The controlled pond was the normal 1 m^3 pond with a packing section as a conventional treatment system for the culture. The treatment pond was also 1 m^3 in size and equipped with the PBABR system. The salinity in both ponds were controlled at 30 ppt.

3.2 Experimental procedures

3.2.1 Preparation of immobilized nitrifiers/denitrifiers

The plastic bioballs was immerged in an ammonia laden solution for about 2 weeks. Nitrifying bacteria will be naturally immobilized onto the surface of these plastic bioballs. The bioballs will be then ready for the operation of PBABR. Similarly, the denitrifying bioballs are obtained from the inoculation of the nitrate laden sea water in the O_2 limited container for about 3 weeks, or until the nitrate disappeared. As the nitrate starts to disappear, the bioballs are ready to use. In this inoculation, methanol is added to ensure the C/N ratio of approx. unity.

3.2.2 Nitrification/Denitrification of synthetic waste water (Experiment I)

1) Prepare seawater with salinity of about 30 ppt in the shrimp pond.
2) Mix ammonia and/or potassium nitrate with seawater at the required concentration.
3) Pump seawater from the pond into the PBABR with the overflow back into the pond. The flowrate can be adjusted by varying the by-pass valve. (See table 4.1)
4) Add methanol into the downcomer to serve as a carbon source for denitrifying bacteria. (See table 4.1)
5) Supply nitrogen gas in downcomer of the PBABR (to help remove oxygen).
6) Take 30 mL sample from a sampling port to measure concentrations of ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) by the Strickland and Parson method,1972 (See Section 3.3)
7) Monitor DO (by Hanna HI 964400), and ORP (by Hanna HI 98240) at downcomer.

3.2.3 Nitrification/Denitrification of shrimp waste water (Experiment II)

1) Prepare seawater with salinity of about 30 ppt in two shrimp ponds. One pond is connected to the PBABR and is referred to as treatment pond. The other pond is not connected to the PBABR, and is referred to as control pond.
2) Take 30 mL sample from a sampling port to measure initial concentrations of ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) by the Strickland and Parson method,1972 (see Section 3.3).
3) Liberate 7 black tiger shrimp in both ponds and culture the shrimp. Note that the initial average weights of shrimp are 14.07and14.14 g for the control pond and treatment pond, respectively.
4) Pump seawater from the treatment pond in to the PBABR with the overflow back to the pond. The flowrate can be adjusted by varying the size of by-pass valve.
5) Monitor DO (by Hanna HI 964400), and ORP (by Hanna HI 98240) at downcomer.
6) Take sample to measure NH_4-N,NO_2-N and NO_3-N daily.
7) Check the survival of both shrimp culture, measure the weight of the initial and final shrimp culture.

3.2.4 Nitrification/Denitrification of dense-shrimp waste water (Experiment III)

1) Prepare seawater with salinity of about 30 ppt in two shrimp ponds, i.e. control and treatment ponds.
2) Take 30 mL sample from a sampling port to measure initial concentrations of ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) by the Strickland and Parson method,1972 (see Section 3.3).
3) Liberate 18 black tiger shrimps in both ponds and culture the shrimp with the initial average weight of 20.11 and 19.26 g for the control and treatment ponds, respectively.
4) Pump seawater from the treatment pond in to the PBABR with the overflow back to the pond. The flowrate can be adjusted by varying the size of the by-pass valve. (See table 4.5)
5) Add methanol into the downcomer to serve as a carbon source for denitrifying bacteria. (See table 4.5)

6) Monitor DO (by Hanna HI 964400), and ORP (by Hanna HI 98240) at downcomer.
7) Take sample to measure NH_4-N,NO_2-N and NO_3-N daily.
8) Check the survival of both shrimp culture, measure the weight of the initial and final shrimp culture

During the experiment, make-up seawater is added to replace the volume lost in sampling procedure. Evaporation loss is replaced by distilled water.

3.3 Water quality analysis

3.3.1 Analytical methods for measuring nitrogen compounds (Strickland and Parson,1972)

A. Ammonium-nitrogen concentration measurement

Reagents

1) De-ionized water
2) Phenol solution
   - Dissolve 20 g of crystalline phenol in 200 mL of 95% v/v ethyl alcohol.
3) Sodium nitroprusside solution
   - Dissolve 1.0 g of sodium nitroprusside ($Na_2Fe(CN)_5NO.2H_2O$) in 200 mL of deionized water. Store in an amber bottle. The solution is stable for at least 1 month.
4) Alkaline reagent
   - Dissolve 100 g of sodium citrate and 5 g of sodium hydroxide (analytical reagent grade) in 500 mL of de-ionized water. This solution is stable indefinitely.
5) Sodium hypochlorite solution (1.5N)
6) Oxidizing solution
   - Mix 100 mL of reagent 4 and 25 mL. Of reagent 5. Keep this solution stoppered while it is not in use. Prepare fresh everyday.

Procedure

1) Add 5 mL of sample to a tube from a 5 mL pipette.
2) Add 0.2 mL of phenol solution, from a pipette, swirl the solution.

3) Add 0.2 mL of sodium nitroprusside solution and 0.5 mL of oxidizing solution.
4) Mixing after each addition.
5) Allow the tube to stand at temperature between 20-27 ^{o}C for 1 h. The top of the tube should be covered with aluminum foil at this storage to lessen the contamination by atmospheric ammonia.
6) Read the absorbance at 640 nm in a spectrophotometer against distilled water using 10-cm cells.
7) Correct the measured absorbance by that of a reagent blank and read the ammonia-nitrogen concentration from the standard calibration curve.

Blank

- Carry out the method exactly as described in 1) to 6) above using freshly deionized water. Blank absorbance using a 10-cm cell should not exceed 0.075.

Calibration

Standard ammonia solution

- Dissolve 0.1 g of ammonia sulfate in 1000 mL of de-ionized water.
- Add 1 mL of chloroform
- Store this solution in refrigerant (This solution is stable).
- The amount of ammonia in 1 mL of the prepare solution is equal to 0.021 mg NH_3-N.

B. Nitrite-nitrogen concentration measurement

Reagents

1) Sulphanilamide solution
   - Dissolve 5 g of sulphanilamide in a mixture of 50 mL of concentrated hydrochloric acid and 300 mL of distilled water.
   - Dilute to 500 mL with water (the solution is stable for many months).
2) N-(1-Naphthyl)-ethylenediamine dihydrochloride solution (NNED solution)
   - Dissolve 0.5 g of dihydrochloride in 500 mL of distilled water.
   - Store the solution in a dark bottle. (The solution should be renewed once a month).

Procedure

1) Add 5 mL of sample to the tube with a 5mL pipette.

2) Add 0.1 mL of sulphanilamide solution, from automatic pipette to each sample, mix, and allow the reagent to react for between 2 and 8 min.
3) Add 0.1 mL of naphthylenediamine solution and mix immediately. Between 10 min and 1 h afterwards measure the absorbance at 543 nm in a spectrophotometer.
4) Correct the measured absorbance by subtracting reagent blank and read the nitrite-nitrogen concentration from a standard calibration curve.

Blank

Synthetic seawater solution

- Dissolve 310 g of sodium chloride (NaCl), 100g of magnesium sulphate ($MgSO_4.7H_2O$) and 0.5 g of sodium bicarbonate ($NaHCO_3.H_2O$) in 10 L of distilled water.

Calibration

Standard nitrite solution

- Dry anhydrous sodium nitrite ($NaNO_2$) at 110^oC for 1 h.
- Dissolve 0.345 g of anhydrous sodium nitrite in 1000 mL of distilled water
- Add 1 mL of chloroform as a preservative.
- Store the solution in dark bottle (the solution is stable for at least 1-2 months).
- The amount of nitrite in 1 mL of the prepare solution is equal to 0.005 mg NO_2-N.

C. Nitrate-nitrogen concentration measurement

Reagents

Use redistilled or distilled, deionized water of highest purity to prepare all solution and dilutions.

Procedure

1) Measure sample absorbance at wavelength of 220 nm to obtain NO_3^- and dissolved organic matter absorbance.
2) Measure sample absorbance again at 275 nm to determine the absorbance due to dissolved organic matter only

3) Use absorbance difference in 1)-2) to determine the absorbance only due to NO_3^- and correct the measured absorbance by a reagent blank and read the Nitrate-nitrogen concentration from the standard calibration curve.

Blank

Use redistilled distilled or deionized water to set the zero absorbance of absorbance difference (220nm and 275nm)

Calibration

Standard nitrate solution

- Dissolve 1.02 g of analytical reagent quality potassium nitrate (KNO_3) in 1000 mL of distilled water (the solution is stable indefinitely in the absence of evaporation).
- The amount of nitrate in 1 mL of the prepare solution is equal to 0.01 mg NO_3-N.

3.3.2 Analytical methods for measuring alkalinity and dissolved oxygen by titration method (Strickland and Parson,1972)

1. Alkalinity: Alkalinity analysis was modified from the titration method described in Strickland and Parsons (1972), however, pH meter was used instead of observing colour changed of the methyl orange indicator. Hundred milliliter (100mL) of filtered seawater sample in Erlenmeyer flask, continuously stirred with magnetic bar, was titrate with 0.01 M H_2SO_4 (0.01 H_2SO_4 in boiled distilled water) until the pH of the solution reached pH 4.40. Alkalinity was calculated as the following:

$$\text{Alkalinity} = (H_2SO_4 \text{ titrated} \times 1000)/\text{Volume of seawater sample}$$

2. Dissolved oxygen: Dissolved oxygen analysis was modified from the titration method described in Strickland and Parsons (1972).

Take sample from six point of reactor into six 60 mL BOD bottles and adding 0.2mL of manganous sulphate reagent ($MnSO_4.H_2O$ 365 g/L) then adding 0.2mL of alkaline iodide solution (mixed of 500 g NaOH in 500mL distillated water and 300 g KI in 450mL distillated water). Then, these contents were mixed thoroughly by shaking until the precipitated manganous-manganic hydroxide were dispersed. When the precipitate had settled slightly (in 2-3 minutes), shaking the bottle again, finally, 0.2 mL of concentrated sulfuric acid were added for dissolving the precipitate.

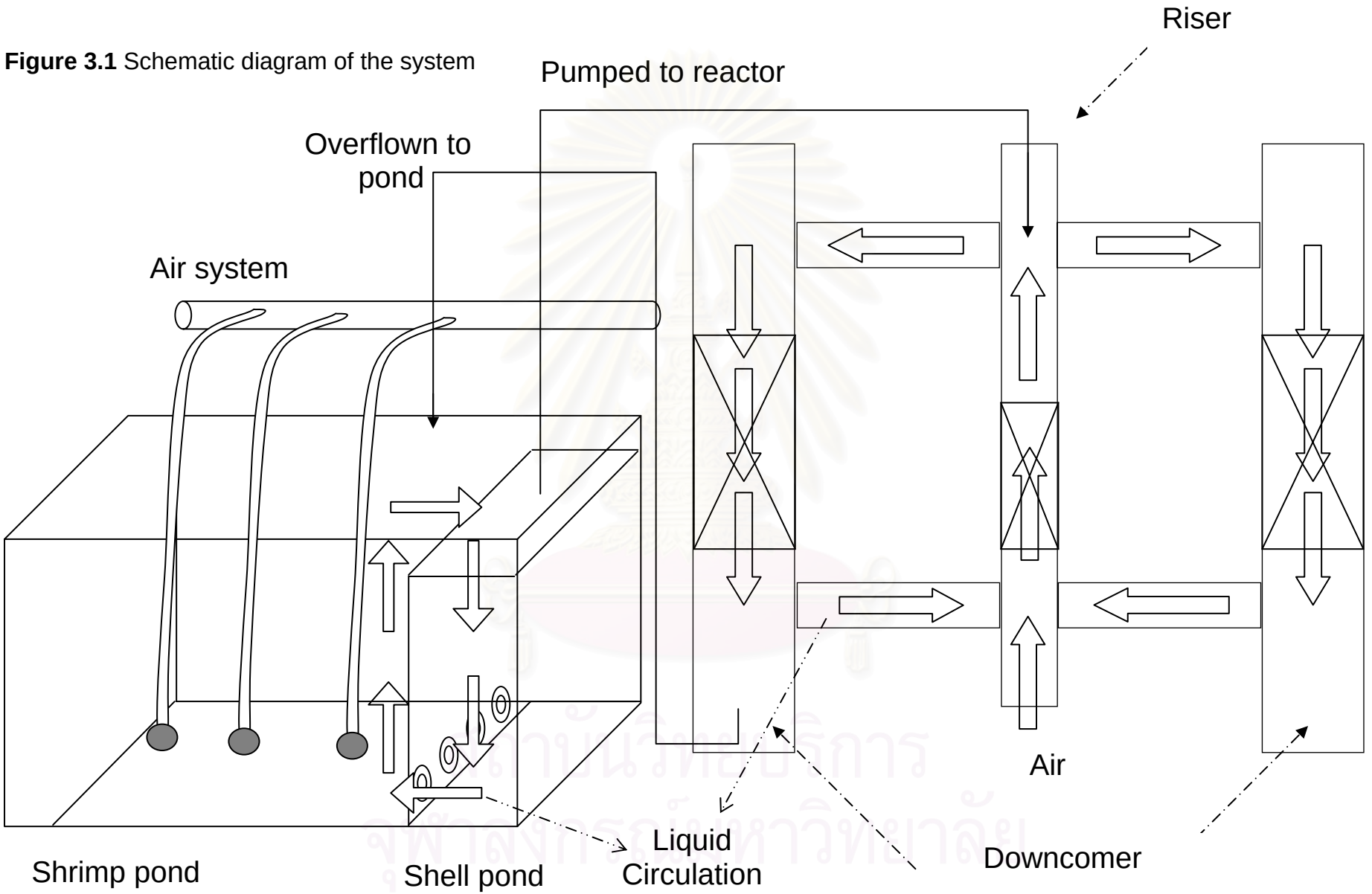
Ten milliliter of the solution were transferred into a flask and titrated with standard 0.01 N thiosulphate solution ($Na_2S_2O_3.5H_2O$ 2.9g and Na_2CO_3 0.1g mix with distillated water 1L and adding CS_2 1mL) until a very pale straw color remained. Five milliliter of starch indicator were added and titrate until see the no color solution. Dissolved oxygen was calculated as the following:

Molecule of thiosulphate = volume thiosulphate used x 0.01 / 1000

Molecule of O_2 = Molecule of thiosulphate x 100 / 4

Dissolved oxygen (mgO_2/L) = Molecule of O_2 x 32

Figure 3.1 Schematic diagram of the system



Chapter IV

Results and discussion

4.1 Nitrification/Denitrification of synthetic waste water in airlift bioreactor system (Experiment I)

Experiment I was designed as an initial experiment for the proper inoculation of the nitrifying and denitrifying bacteria required for the operation of the packed bed airlift bioreactor (PBABR). The experiment was performed with the actual pond system with the total volume of 500L (plus 60L of the PBABR) and with the seawater at the normal salinity level (30 ppt) but with synthetic N-laden waste seawater. No shrimp was liberated into the pond in this experiment. The synthetic N-laden waste was prepared by mixing ammonia or potassium nitrate with seawater at the required concentration as indicated in Table 4.1. In reading this table, the first column represents the day of the experiment which covered the range of 68 days. The second and third rows are the amount of NH_4Cl and KNO_3, respectively, that were added into the pond in grams where the forth and fifth rows are the targeted concentration levels of the two chemicals in mg-N/L. The sixth row is the information on the addition of methanol, either in grams or in flow rate into the system whereas the next row provides the information on the supply of nitrogen gas in the downcomer of the PBABR (to help remove oxygen). The last row is the circulation flow rate of the seawater between the pond and the PBABR. The objective of this preliminary experiment was to acclimatize the system to the N-laden condition to ensure that PBABR had the capability in treating such nitrogen compounds.

Results of this part are shown in Figures 4.1 and 4.2 for the concentration profiles of NH_4-N NO_2^--N and NO_3^-N in the pond and at the PBABR exit. Figure 4.3 shows the concentrations of all three nitrogen compounds and total nitrogen along with the time variation of the oxidation-reduction potential (ORP).

4.1.1 Nitrification (days 1-28)

In the first period during the days 1-28, ammonia was intermittently added into the pond. Figures 4.1 and 4.2 illustrate that the concentrations in the pond and at the reactor exit were not much different. This indicates that the time scale of changes in the concentration of nitrogen compounds was much larger than the time scale of the mixing within the system. Therefore the system could be treated as homogeneous. However, the level of ammonia was not equal to the expected level (as stated in Table 4.1), e.g. the initial ammonia concentration was estimated to be 10 mg-N/L but the actual concentration was only 8.02 mg-N/L in the pond. This might be due to the error in the estimating the amount of seawater in the system as 500L was only from the rough estimation, not from the design data.

Nitrification was proven to be achieved within a few days of operation, and so was the denitrification. Note that the PBABR had been inoculated before the start of this experiment with the synthetic wastewater in a batch experiment but the results were not shown in this work and therefore the bacteria were probably well prepared for the nitrification/denitrification reactions. Also it should be mentioned that during these first few days, no aeration was supplied to the culture pond (500L pond) and therefore the level of dissolved oxygen and ORP were low. This supported the activity of denitrifying bacteria. Hence, in this first instance, a complete reaction took place where ammonia was changed to nitrite and then nitrate, and subsequently nitrate to nitrogen gas (see Chapter 2). As soon as the ammonia was depleted, more ammonia was supplied into the pond to maintain the level of ammonia at about 5-8 mg-N/L.

The nitrification rates in from of the ammonia-nitrogen removal rate was calculated from

$$NR = \frac{NH_4 - N_i - NH_4 - N_f}{t_f - t_i} \quad (4.1)$$

where

NR	=	Ammonia nitrogen removal rate (mg NH_4-N/L/d)
NH_4-N_i	=	Initial concentration of ammonium nitrogen (mg NH_4-N/L)
NH_4-N_f	=	Final concentration of ammonium nitrogen (mg NH_4-N/L)
t_i	=	Initial time (day)
t_f	=	Final time (day)

Nitrification rates during each time period are reported in Table 4.2. Nitrite is the intermediate compound occurred during the ammonia and nitrate decompositions, and in this experiment, no accumulation of nitrite was found.

4.1.2 Denitrification (days 2-45)

Denitrification, which is illustrated by the decrease in nitrate concentration, initially occurred rapidly from the day 2 to day 13, after which nitrate concentration drastically increased and remained at high level for several days. During the first period of experiment, the 500L pond was not aerated and therefore the dissolved oxygen (DO) level in the pond was close to zero. This water was fed into the downcomer section of the PBABR which facilitated the denitrification. However, the aeration in the pond was turned on after the day 13 to simulate the actual treatment condition where aeration was needed for the shrimp culture. The DO level in the pond rose to about 5-6 ppm where the ORP was maintained at high level of 100-300 mV and therefore when this water was fed to the downcomer section of the PBABR, the anoxic denitrification could not take place properly. This is the reason why the nitrate concentration was high after the day 13.

During the days 28-48, no ammonia was added into the pond and therefore only nitrate existed in the pond. Several attempts were carried out to bring the concentration of nitrate down. Table 4.1 shows that 66.5g of methanol was added into the downcomer to be the carbon source for denitrifying bacteria without the attempt to bring the DO level down. This amount of methanol was estimated to give the C:N ratio of 1 which was reported to be the suitable level for denitrification. However, the high ORP condition still prevented the denitrification and nitrate could not be removed. In the day 36, nitrogen gas was supplied to both downcomers to lower the dissolved oxygen. However, due to the equipment limitation, the location where nitrogen was supplied could only be set at the level just above the packing in the downcomer. This very short distance was not adequate to bring the DO level down and therefore high ORP was still observed.

As methanol was not consumed in the downcomer, it could escape to the pond and was being biodegraded. This was reflected by the observation of a large number of small gas bubble formation in the pond. This was considered not suitable for the shrimp culture as methanol can be quite toxic to the culture. In addition, ammonia had not been supplied to the system for more than 10 days which could render the nitrifying bacteria inactive or even dead. Hence, more ammonia was added to the system at the day 49. At the same time, nitrogen was still supplied to the downcomer section. Methanol was, this time, continuously fed into both downcomers at the rate of 3 mL/h (v/v). The discussion follows in the next section.

4.1.3 Simultaneous nitrification/denitrification in PBABR (days 45-68 th)

As stated at the very last part of the previous section, both N_2 and methanol were continuously added into the downcomer of the PBABR at approximately day 49. Note that 10%methanol was employed at the rate of 3 mL/h. The system responded well as the nitrate level dropped drastically. Methanol was only added during the days 45-62 whereas nitrogen gas was only supplied during the days 45-52. During the period where nitrogen was added to the downcomer, the ORP dropped to below zero which was a signal for an effective denitrification. After the day 52, nitrogen was no longer supplied to the downcomer and Figure 4.3 shows that the ORP in this period started to switch

back to the positive region with a value of about 100 mV where denitrification should not take place. However, nitrate still continued to drop to almost zero even without the addition of nitrogen gas. It was possible that the bacteria continued to grow and assimilated the nitrogen (in the form of nitrate) into the new cell through cell division/growth. Methanol was switched off after the day 62 and it was obvious that nitrate was, again, accumulated in the reactor. This emphasized the importance of the carbon source on the nitrate removal mechanisms.

From the experimental finding, two potential mechanisms for the removal of nitrate could be proposed. Firstly nitrate could undergo the normal denitrification process and was converted to nitrogen gas, and the next one was that nitrate was assimilated into the new cell through cell synthesis activity.

It should be mentioned that during the days 49-66 th, ammonia was also added into the pond. During the days 49-58, ammonia was not as degraded as fast as that obtained during the first stage (the nitrification rate in this period was max. at 0.563 mgN/L/d whilst the rate during the initial period could be as high as 3.972 mgN/L/d). This could be due to the previous long period of zero ammonia which caused the bacteria to be inactive. However, after the day 58, nitrification process became active again.

In this part of experiment, the rate of denitrification could be approximated from the rate of nitrate reduction as:

$$DNR = \frac{NO_3 - N_i - NO_3 - N_f}{t_f - t_i} \qquad (4.2)$$

where

DNR = Nitrate nitrogen removal rate (mg NO_3-N/L/d)

NO_3-N_i = Initial concentration of nitrate nitrogen (mg NO_3-N/L)

NO_3-N_f = Final concentration of nitrate nitrogen (mg NO_3-N/L)

t_i = Initial time (day)

t_f = Final time (day)

In this calculation, it was assumed that the rate of nitrate generated from nitrification was small with respect to the rate of nitrate reduction from denitrification. The denitrification rate was summarized in Table 4.2.

4.1.4 Performance of PBABR

Nitrification rate in this experiment over the various conditions in experimental period was found to be in a range between 0.563-3.972 mgN/L/d or 0.023-0.165 mgN/L/h. This was within the same range as what was achieved by Silapakul (2002) at 0.03-0.4 mgN/L/h. However, the rate achieved in this work was based on the volume of the medium both in the PBABR and the pond which was approx. 560L whilst the previous work of Silapakul (2002) was only based on the total volume of the PBABR at 60L. Therefore if the rate was converted to the conventional rate which is based on the surface area of the packing, the nitrification rate obtained in this experiment was found to be extremely high (could be as high as 3.47 $gN/m^2/d$). Note that there might be some nitrification taken place in the pond. However, if the nitrification was carried out by the immobilized microorganism, then the rate in the pond should be very low as the surface area of the pond was negligible when compared with the surface area of the packing, and therefore this was not part of the calculation here. (See Table 4.2 for more information on the nitrification/denitrification rates occurred in Experiment I.) Note that the conversion of the nitrification/denitrification rates from mgN/L/d to $mgN/m^2/d$ is achieved by multiplying the rate in mgN/L/d with the volume of the system (560L) and dividing with the area of the surface area of the packing for nitrification/denitrification. The packing surface area for nitrification is 0.64 m^2 and for denitrification is 6.4 m^2.

The calculation for the rate of denitrification was slightly more complicated as stated earlier in Section 4.1.3. However, with the assumption proposed under Eq. (4.2), a rough estimate of denitrification rate (DNR) in the PBABR could be determined and the results are displayed in Table 4.2. Again, the denitrification was found to be as high as 1.26 $gN/m^2/d$ compared with 0.05 $gN/m^2/d$ obtained from Silapakul (2002).

4.2 Nitrification/denitrification of shrimp waste water in airlift bioreactor system (Experiment II)

As the bacteria were well acclimatized to the condition in the PBABR, the system was applied to the actual shrimp pond straight after Experiment I. Two shrimp ponds were started at the same time, i.e. the control and the actual experiments. The control experiment was operated without the connection to the PBABR whereas the actual experiment was connected to the PBABR with the initial liquid circulation flowrate of 21 L/h. Due to the difficulty in the transfer of living shrimp to the laboratory, the number of shrimp in this experiment was limited at seven (in each pond). No chemical was added to the system, which meant that all nitrogen compounds were released directly from the shrimp. The samples were taken from the control pond, the treatment pond and at the reactor exit for the determination of all nitrogen derivatives and the results are shown in Figures 4.4 - 4.6. Neither methanol nor nitrogen was added into the system in this experiment as the number of shrimp was small and the system was thought to be able to cope with the nitrate naturally.

Note that the shrimp pond was equipped with the side shell section (see Figure 4.7). The culture was internally circulated between the main pond section and the side shell section by a small airlift pipe. This side shell section acted as a natural nitrification treatment unit where the treated water was circulated back to the pond through the interconnected tubes at the bottom of the pond. This side shell section will be referred to as “Shell pond” hereafter in this work.

Details of the average weight and the number of survival of shrimp in both ponds were reported in Table 4.3 which indicates that that the pond with PBABR performed slightly better in the cultivation of shrimp as there were a higher survival number of shrimp and the final specific weight was higher.

The levels of alkalinity and pH were about 121 mg$CaCO_3$/L and 7.93 for control pond, and 123 mg$CaCO_3$/L and 7.98 for the treatment pond. These two parameters were rather stable over the range of experimental period.

4.2.1 Nitrification

Figure 4.4 shows that there was a peak in ammonia concentration in the treatment pond. This was because the PBABR was out of order and the circulation between the shrimp pond and the shell pond was blocked. After a few days, this problem was solved and the system was brought back to normal safely. This figure also shows that there was a continual increase in the ammonia level in both ponds as the shrimp increased in size and the rate of ammonia release was higher. The ammonia was removed well in the reactor and a very low level of ammonia was found at the PBABR exit. However, the circulation rate between the shrimp pond and the PBABR was only 21 L/h which was far less than the total volume of 500 L in the pond. However, the level of ammonia in this case was still lower than the dangerous level and the performance of the system was considered satisfactory in this regards. The concentration of ammonia in the pond was, as a result of having some being removed in the PBABR, slightly lower than that in the control pond.

To investigate the effect of the PBABR, the shell pond was disconnected during the days 40-50. Suddenly, an increase in ammonia was observed in both ponds but more seriously was the increase in the nitrite level in this period particularly in the control pond (see Figure 4.5). This caused death of shrimp in the control pond. When the shell pond was brought back to normal, the shrimp in the control pond did not seem to recover and the death toll started to increase until the end of the experiment.

The decrease in the number of shrimp in the control pond meant that there was less generation of ammonia. This was reflected in the time profile of ammonia as the control pond seemed to possess a smaller level of ammonia at the end of the experiment (about day 50 onwards).

Figure 4.5 demonstrates that the shell pond was quite effective in controlling the level of nitrite. Most of the time, nitrite was controlled below 0.2 mgN/L which should be safe for the shrimp. During the shut down of the shell pond, the PBABR was also shown to be effective in controlling the level of nitrite

even with a very low circulation of shrimp culture (at 3 L/h). In fact, the recirculation rate was reduced from 24 to 3 L/h on the day 28 to see the effect of the flowrate on the overall performance of the system. This flowrate was increased again to 12 L/h on day 55, but little seemed to be influenced by the recirculating flowrate. It was concluded at this point that there was no effect of recirculation flowrate for the cultivation of the small number of shrimp.

An interesting result was illustrated in Figure 4.6 where the nitrate concentration in the control pond was found to be constantly higher than that in the treatment pond. This shows that the shell pond was not effective in carrying out denitrification. In fact, it could not be concluded that denitrification occurred in the treatment pond but the PBABR seemed to be more effective in controlling the level of nitrate. As stated earlier, nitrate could either undergo denitrification or other cell assimilation such as growth and therefore was removed from the culture.

4.2.2 Denitrification

It was noted that this experiment was performed without the addition of methanol or other carbon source for the denitrifying bacteria. From the previous study (Silapakul, 2002), the PBABR was shown to be able to remove nitrate effectively without requiring the addition of methanol or other carbon source. However, in this case, nitrate seemed to accumulate slowly. The lower level of nitrate in the treatment pond compared with that in the control pond indicated that there must be some kind of nitrate removal mechanism taken place in the PBABR. The nitrate concentration in the treatment pond was leveled off during the day 35 which suggested that the generation and the consumption of nitrate were at the same extent. It was observed that the shrimp did not seem to increase in size after this period. This implied no further growth and therefore the activity of the shrimp should be maintained at a constant level, and the reactor seemed to cope well with such condition. At times, a drop in nitrate could be observed which suggested an ability of the system in dealing with nitrate, perhaps through the denitrification.

The time profiles of DO and ORP of this experiment are shown in Figures 4.8 and 4.9. Due to the problems with the measuring equipments, DO and ORP

could only be measured at some time period, and not all the time. However, it could be seen that DO and ORP dropped during the time where denitrification took place. However, as a continuous measurement of these parameters could not be done, it was difficult to discuss the results here.

4.3 Nitrification/denitrification of dense-shrimp waste water in airlift bioreactor (Experimental III)

In this section, the same experiment as Experiment II was repeated with a larger number of shrimp. Each of the two ponds was operated with 18 shrimp. The effect of methanol was investigated in the later days of the experiment. The results of this experiment are shown in Figures 4.10, 4.11, and 4.12 for ammonia, nitrite, and nitrate, respectively. In this experiment, the distributions of nitrate and oxygen in the PBABR were analyzed by measuring their concentrations at six points (locations shown in Figures 4.13 and 4.14). This was to determine whether the anaerobic condition could have been induced in the system. Table 4.4 shows the number of shrimps in both control and treatment ponds whereas Table 4.5 displays the methanol feed rate and operating conditions during the experiment.

4.3.1 Nitrification

Figure 4.10 illustrates that there was a considerable level of oscillation in the concentration of ammonia in both control and treatment ponds. The PBABR was found to be able to remove ammonia quite effectively as the ammonia in the effluent from the reactor was always low. In fact, ammonia in the effluent from PBABR was much lower than that in the pond which indicated that the level of treatment was not in the level comparable to the generation rate from the shrimp culture. However, ammonia in the culture pond was also treated by the nitrification taken place in the shell pond (as stated in the previous section) which prevented the accumulation of the ammonia in the pond. Interestingly, ammonia levels in both ponds were within the same range. This suggested that the rate of ammonia removal in the PBABR did not contribute greatly to the total nitrification rate. Note that the circulation flowrate in the early part of the experiment was set

at a very high level. This was aimed to achieve a rapid overall circulation of the culture medium. However, this circulation flowrate was reduced in the later period on this experiment to see the effect on the dissolved oxygen concentration inside the reactor. In any case, the effect of circulation flowrate was marginal and could not be well observed with a significant meaning.

Figure 4.10 shows that there were peaks in the ammonia concentration particularly in the treatment pond during the first 20-25 days. This was due to the equipment failure. It was found that several equipments such as air pump, liquid pump, were worn out and needed maintenance after many months of running. However, the system could recover rapidly once the spare parts were introduced. The high peak during the day 40 was potentially due to the addition of high dose of methanol with the break in the air pump. Actually, methanol was continually added to the pond since the day 22, but at the day 34, the methanol feed rate was increased drastically to see the effect on the denitrification rate. However, without a proper aeration, the system broke down and the nitrate was consumed quickly due to the lack of oxygen in the medium. After that, methanol might not have been required in such a high dose and could not be consumed properly and some could escape from the PBABR to the nitrification sections both in the riser of the PBABR and in the shell pond. Nitrification was susceptible to the presence of organic carbon source and therefore, due to the shock organic loads, some nitrifying bacteria could break or could be turned into inactive cells. Note also that there was a high accumulation of nitrite during this period, and this could also help worsen the condition in the pond. In fact, during this period, a large amount of foam was observed both in the pond and in the PBABR which was the indicator of the cell lyses. Therefore a drop in nitrification rate became apparent and the rise in ammonia concentration in the treatment pond was obvious. Shrimp death rate also increased as a result of this high methanol dose. However, the system could, again, recover after a few days of operation but the quantity of shrimp became small and the experiment ceased.

Figure 4.11 demonstrates the time profile of nitrite which shows that nitrite was well controlled in both ponds. During the days 12-22, nitrite (and also ammonia) in the treatment pond was found to be significantly lower than the control pond, and this could be the reason of a high death rate in the control pond. In this period, the number of shrimp in the control pond reduced greatly

from 16 to 8, whilst the treatment system only saw the decrease from 16 to 12. After this period, nitrite was well controlled and the death rate became low. Nitrite was accumulated again during the day 44-46 which could be as a result of unconsumed methanol as mentioned earlier. This caused a very high death rate of shrimp in the treatment pond.

4.3.2 Denitrification

The time profile of nitrate is shown in Figure 4.12. It can be seen clearly that nitrate concentrations in both control and treatment ponds continuously increased during the first 40 days of operation. This meant that denitrification could not be achieved. In the control pond, nitrate accumulation rate was a little lower than that in the treatment pond. This was because the number of shrimp in the control pond was always lower and therefore the generation rate of nitrogen waste was lower in this system. For the treatment system, nitrate levels in the pond and in the effluent from the PBABR were almost the same which demonstrated that the PBABR did not help reduce nitrate at all. A number of attempts were conducted in order to bring the nitrate down, for instance, the circulation flowrate of medium was brought down during the course of the experiment, or the increase in the methanol feed rate, but these did not have significant effect on the nitrate removal rate.

During the last period of the experiment (days 41-49), nitrate reduction could be achieved which meant that denitrification finally occurred. This was due to the accidental lack of oxygen as the air pump was broken which created a suitable condition for the denitrification. Nitrate was brought down to a very low level and remained at that level until the end of the experiment. However, nitrite accumulated in day 45.

Figures 4.13 and 4.14 are the nitrate and oxygen concentrations at the various locations inside the PBABR. Figure 4.13 is constructed from the data during the later period where the denitrification took place. It could be seen that nitrate was quite low in all locations. Figure 4.14 is the concentration profiles in the reactor and it shows that during the later period, the oxygen level at locations 4 and 6 were quite low which could be the reason why denitrification occurred. In fact, the oxygen at location 2 should also be low but this was not observed. This

was because the supply of methanol was only done at location 5 and not location 1 which was close to the overflow location of the medium back to the pond. This was to prevent the short-circuit of methanol to the pond. However, by doing so, the oxygen could not be brought down to the level achieved at location 6.

The time profiles of DO and ORP are shown in Figures 4.15 and 4.16, respectively. The location for the DO probe was at the top of the denitrification column. DO was found to decrease at approximately the same time where denitrification took place which supports the discussion above. The ORP profile looked a bit more complicated. During the first 35 days, the ORP probe was installed at the location 2 which was not the downcomer that methanol was supplied and therefore a high level of ORP was detected. This meant that no denitrification occurred in this particular downcomer. The ORP probe was moved to location 6 after the day 35 and a negative measurement was detected. This simply meant that denitrification could take place in the column where methanol was supplied.

4.4 Overall performance of PBABR

Overall, the survival rate in the treatment pond was more satisfactorily higher than that in the control pond. The average shrimp weight and length was also better in the treatment system. This was primarily due to the additional nitrification reaction which resulted in a better control of ammonia and nitrite in the culture system. However, the application of PBABR to the actual shrimp pond did not provide the denitrification rate in the level that it was shown to be able to achieve with the synthetic waste water. This was due to several difficulties in dealing with the actual shrimp culture particularly the methanol feed rate. Until the end of this experiment, we still could not find a proper methanol feed rate that would result in a good control of nitrate.

Table 4.1 Feeding schedule summary during synthetic waste water experiment (Experiment I)

Experimental time (day)	NH_4Cl(g)	KNO_3(g)	NH_4-N (mgN/L)	NO_3-N (mgN/L)	methanol	N_2	water flow rate (L/hr)
1	20	40	10	10			120
8	10	40	5	10			
14	10		5				
17	10		5				
21	20		10		66.5 g		
26	20		10				
36						Y	
45						Y	
49	10		5			Y	
51	10		5			Y	
56					3mL/hr 10%(v/v)	Y	108
58	10		5			Y	
60						Y	27
62	20		10				

Table 4.2 Nitrification/Denitrification rates during synthetic waste water experiment (Experiment I)

Experiment day	NH4-N(mgN/L)		NR		Experiment day	NO_3-N(mgN/L)		DNR	
	initial	final	mgN/L/d	$gN/m^2/d$		initial	final	mgN/L/d	$gN/m^2/d$
1-5	5.245	0	1.31125	1.14734375	2-5	22.288	2.366	6.641	0.58105833
8-13	4.398	0.031	0.8734	0.764225	8-9	13.563	2.114	11.449	1.0017875
14-15	3.398	0.039	3.359	2.939125				-	0
17-19	5.123	0.123	2.5	2.1875				-	0
21-23	6.006	0.193	2.9065	2.5431875	21-22	48.666	34.241	14.425	1.2621875
26-28	8.017	0.074	3.9715	3.4750625				-	0
49-51	3.001	0.704	1.1485	1.0049375	40-41	58.65	48.56	10.090	0.882875
52-58	3.394	0.016	0.563	0.492625	50-52	53.003	26.676	13.164	1.15180625
59-60	2.393	0.651	1.742	1.52425	56-59	21.38	2.467	6.304	0.55162917
63-66	7.314	0.018	2.432	2.128				-	-

Table 4.3 Amount of shrimps during shrimp waste water experiment (Experiment II)

Experimental period (day)	Control pond			Treatment pond		
	amount of shrimp	average weight (g/shrimp)	SD	amount of shrimp	average weight (g/shrimp)	SD
1-12	7	14.071	2.47	7	14.14	4.36
13-21	7			6		
22-27	6			6		
28-45	5	16.02	2.5232915	6	17.48	5.989797
46-51	4			6		
52-63	3			6		
64-66	2	10.7	3.3941125	5	17.2	4.779644
67-74	1			4		

Table 4.4 Amount of shrimp during Experiment III

Experimental period (day)	Control pond					Treatment pond				
	amount of shrimp	average weight (g)	SD weight	average length (cm)	SD length	amount of shrimp	average weight (g)	SD weight	average length (cm)	SD length
1-4	18	20.112	4.110	12.123	0.382	18	19.263	4.020	12.112	0.392
5-6	17					18				
7-9	16					17				
10-12	16					16				
13-14	15					16				
15-15	14					16				
16-16	12					16				
17-19	12					15				
20-21	9					13				
22-23	8	20.133	4.225	12.367	0.404	12	18.433	3.557	12.967	0.896
24-26	7					11				
27-33	6					10				
34-37	5					9				
38-40	5					7				
41-48	4					5				
49-54	4	20.837	4.997	12.533	0.451	4	20.236	4.359	14.467	0.503
55-60	3					4				

Table 4.5 Operating conditions in Experimental III

Experimental period (day)	water flow rate (L/h)	Alkalinity ($mgCaCO_3/L$)	5%(v/v) methanol	pH
1-8	96L/h	>100		7-9
8-22	24L/h	>100		
22-29		>100	5.952mL/h	
29-34		>100	4.167mL/h	
34-36		>100		
36-38		>100		
38-40		>100		
40-42		>100	20.833mL/h	
42-44		>100		
44-46		>100		
46-47		>100		
56	18L/h	>100	-	

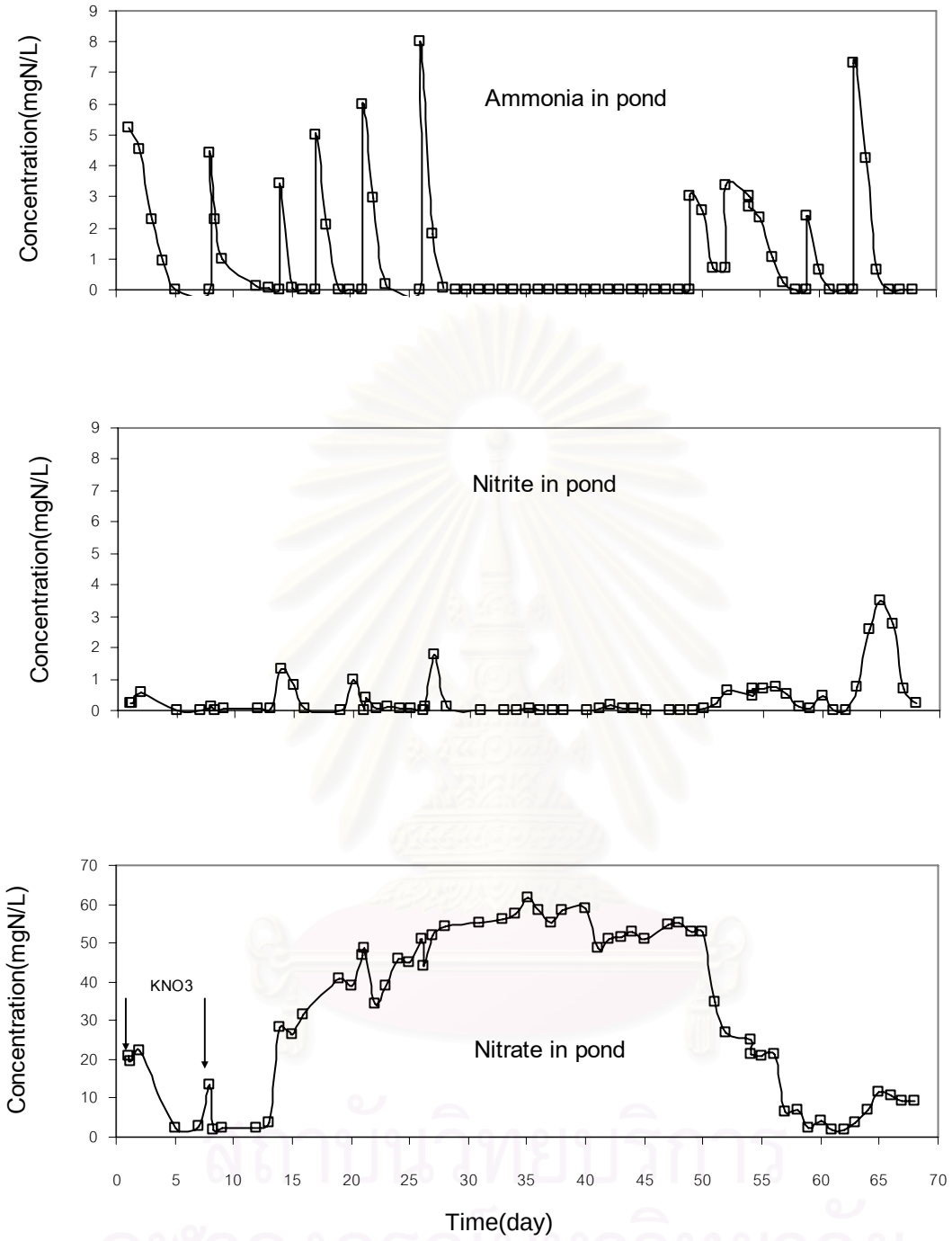


Figure 4.1 Nitrogen compound concentration profile in the shrimp pond during Experiment I

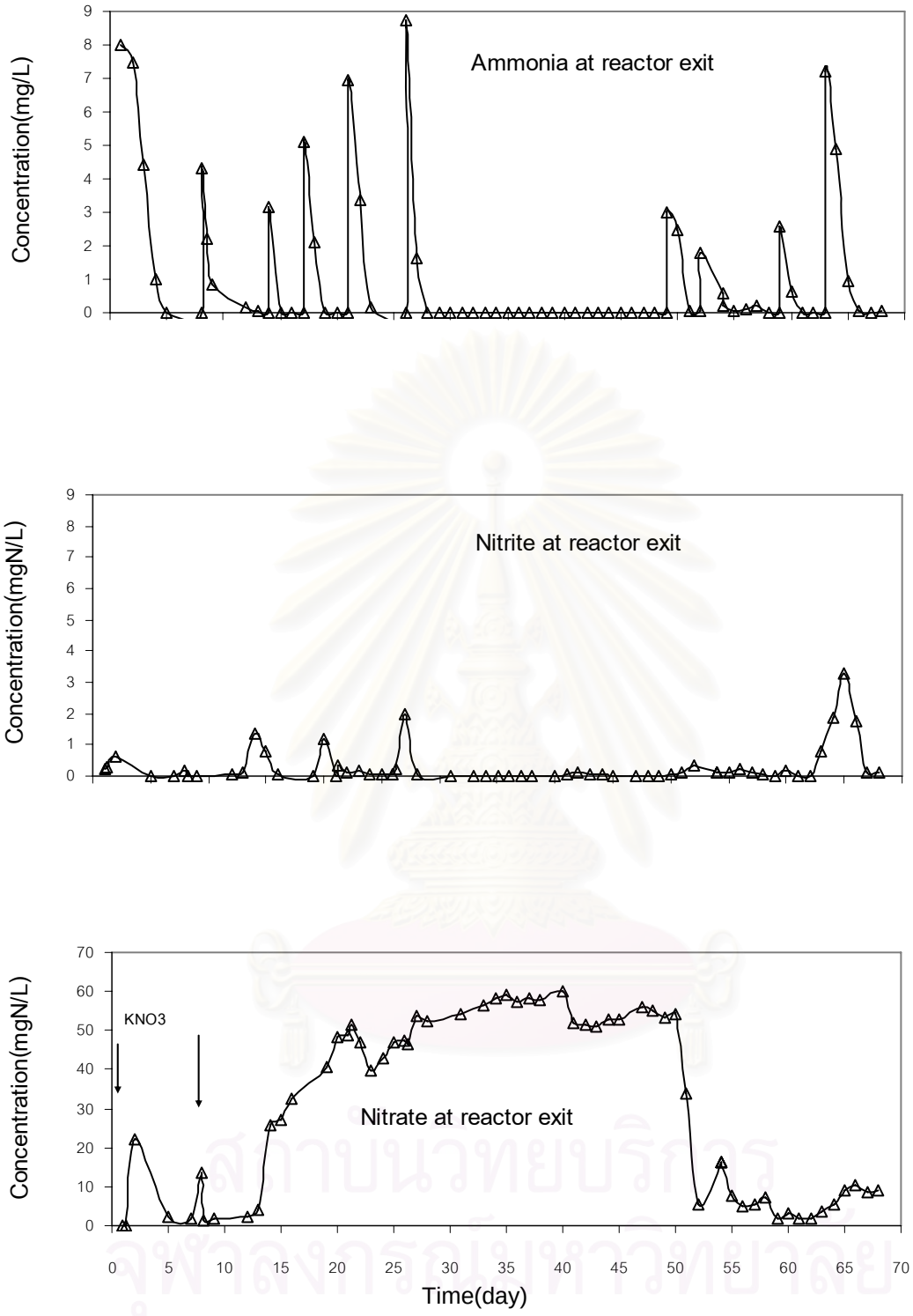
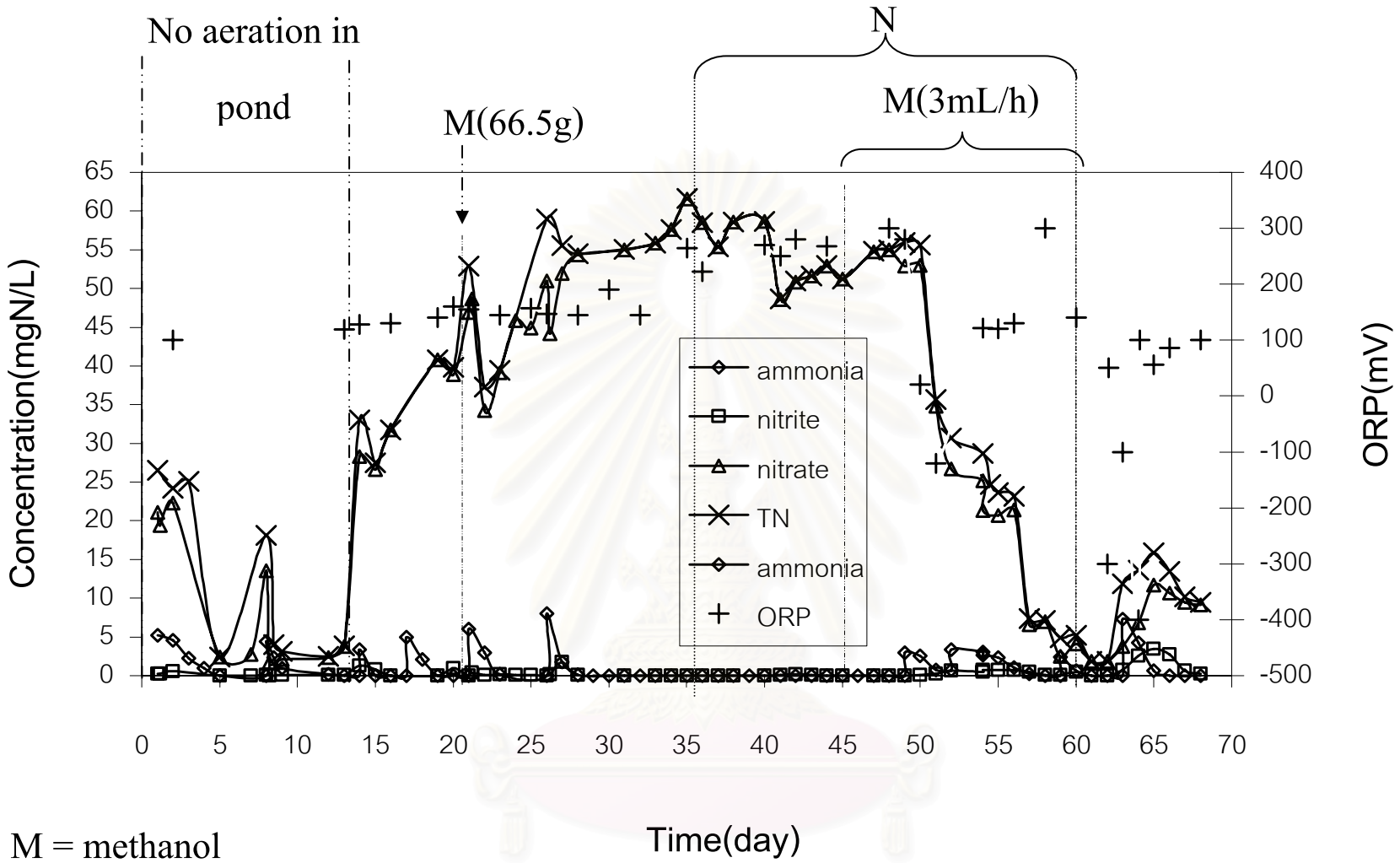


Figure 4.2 Nitrogen compound concentration profile at the reactor exit during Experiment I



M = methanol

N = N_2 gas

Figure 4.3 ORP&Nitrogen compound concentration profile in the shrimp pond during Experiment I

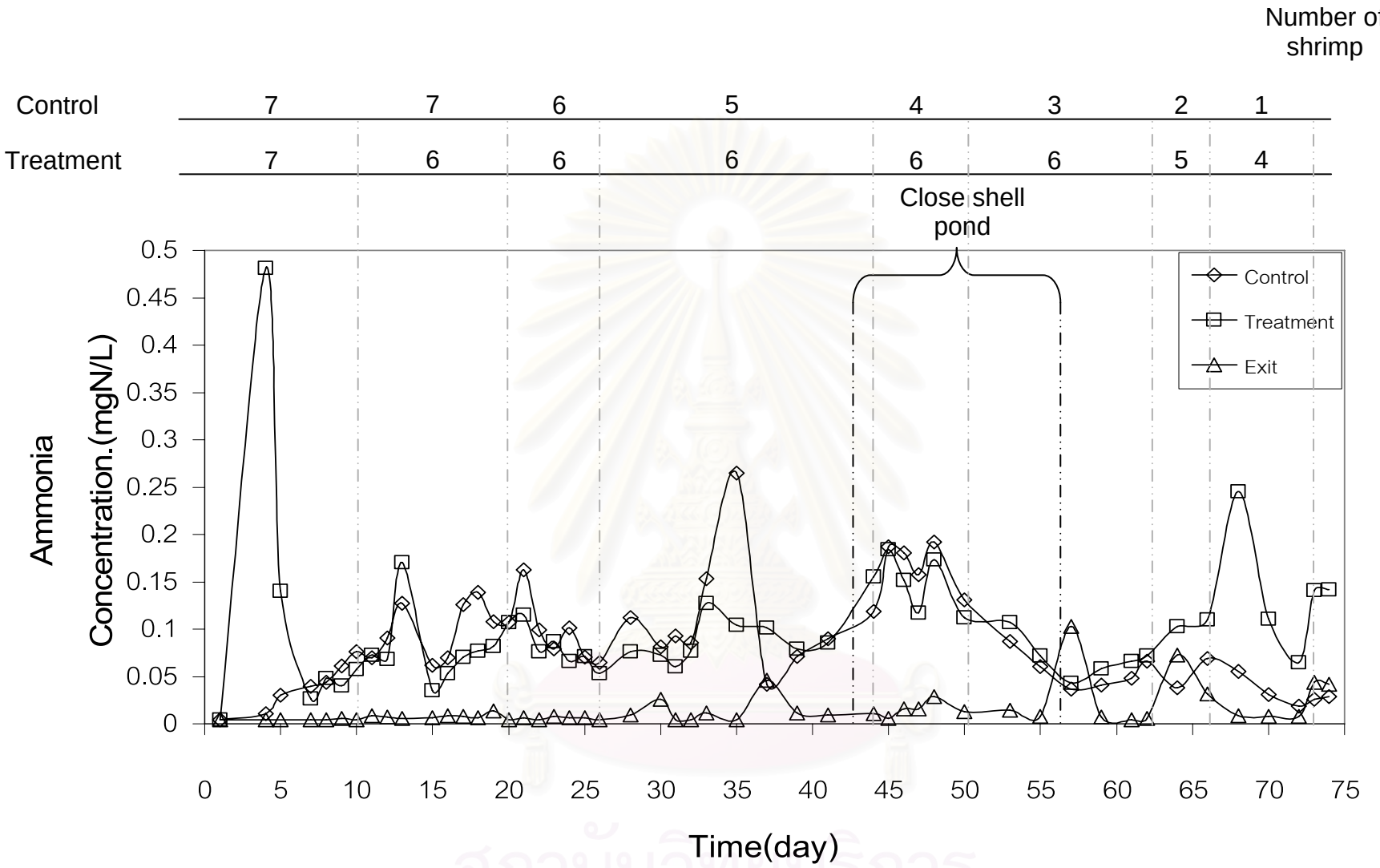


Figure 4.4 Ammonium-nitrogen concentration profile during Experiment II

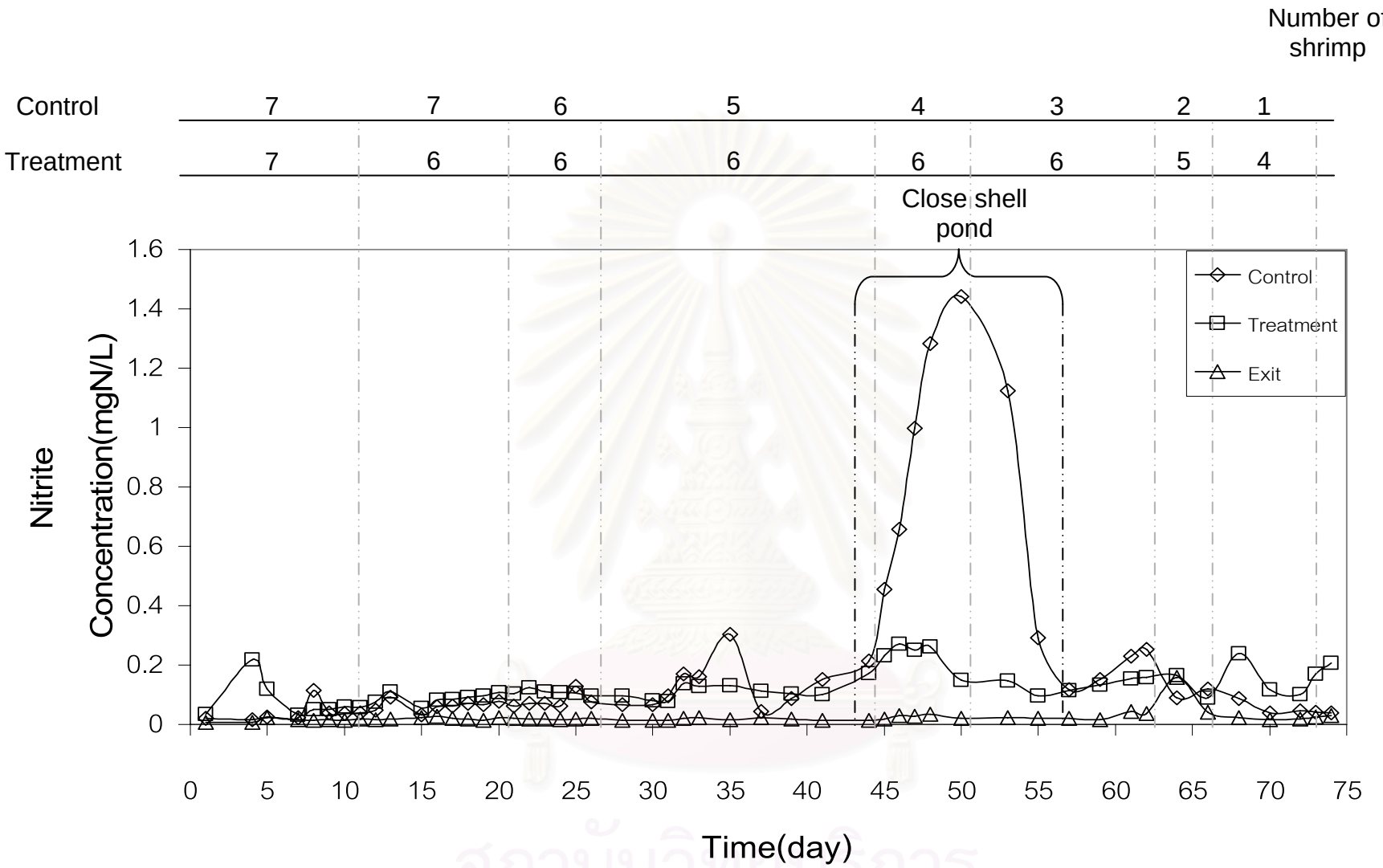


Figure 4.5 Nitrite-nitrogen concentration profile during Experiment II

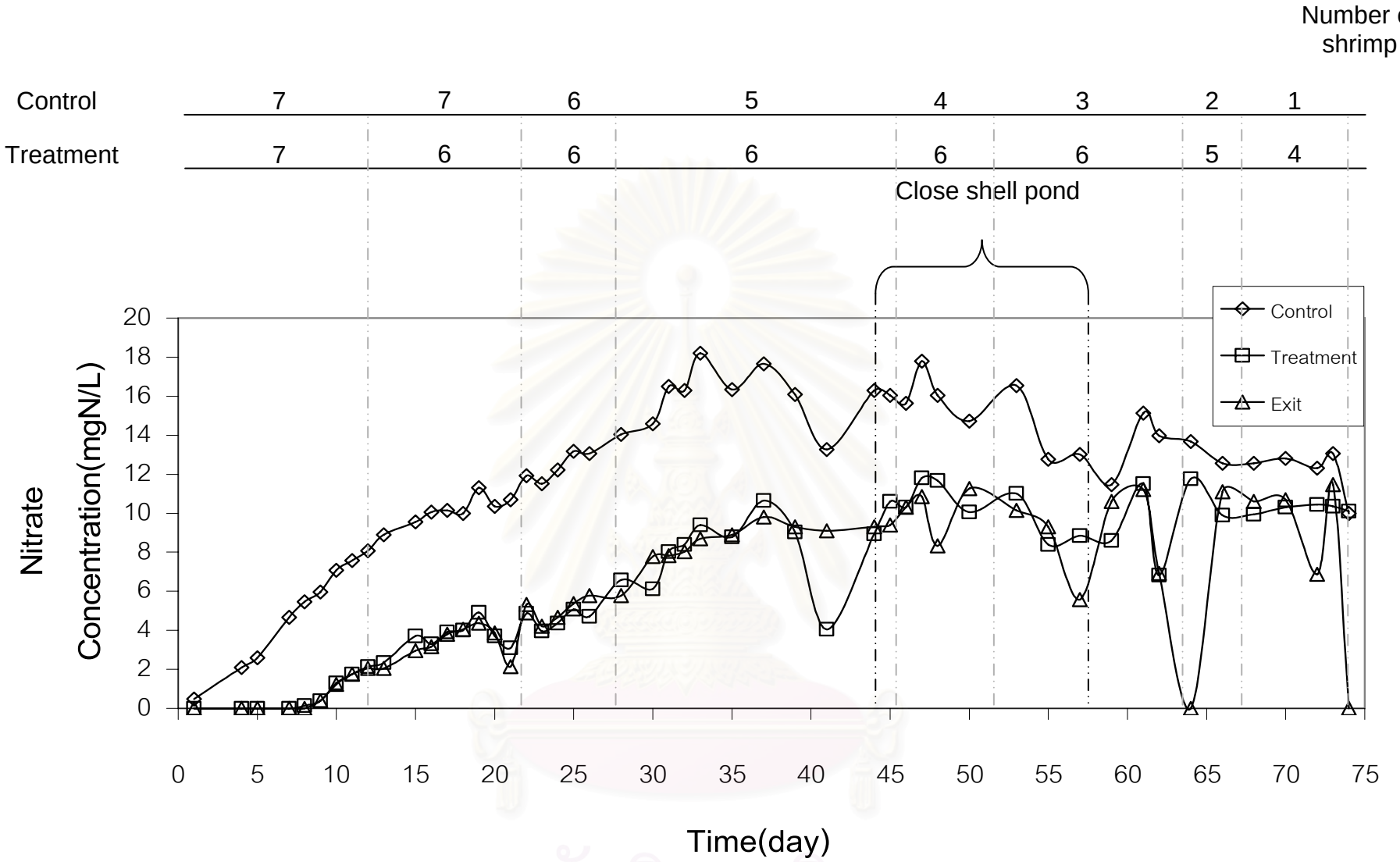


Figure 4.6 Nitrate-nitrogen concentration profile during Experiment II

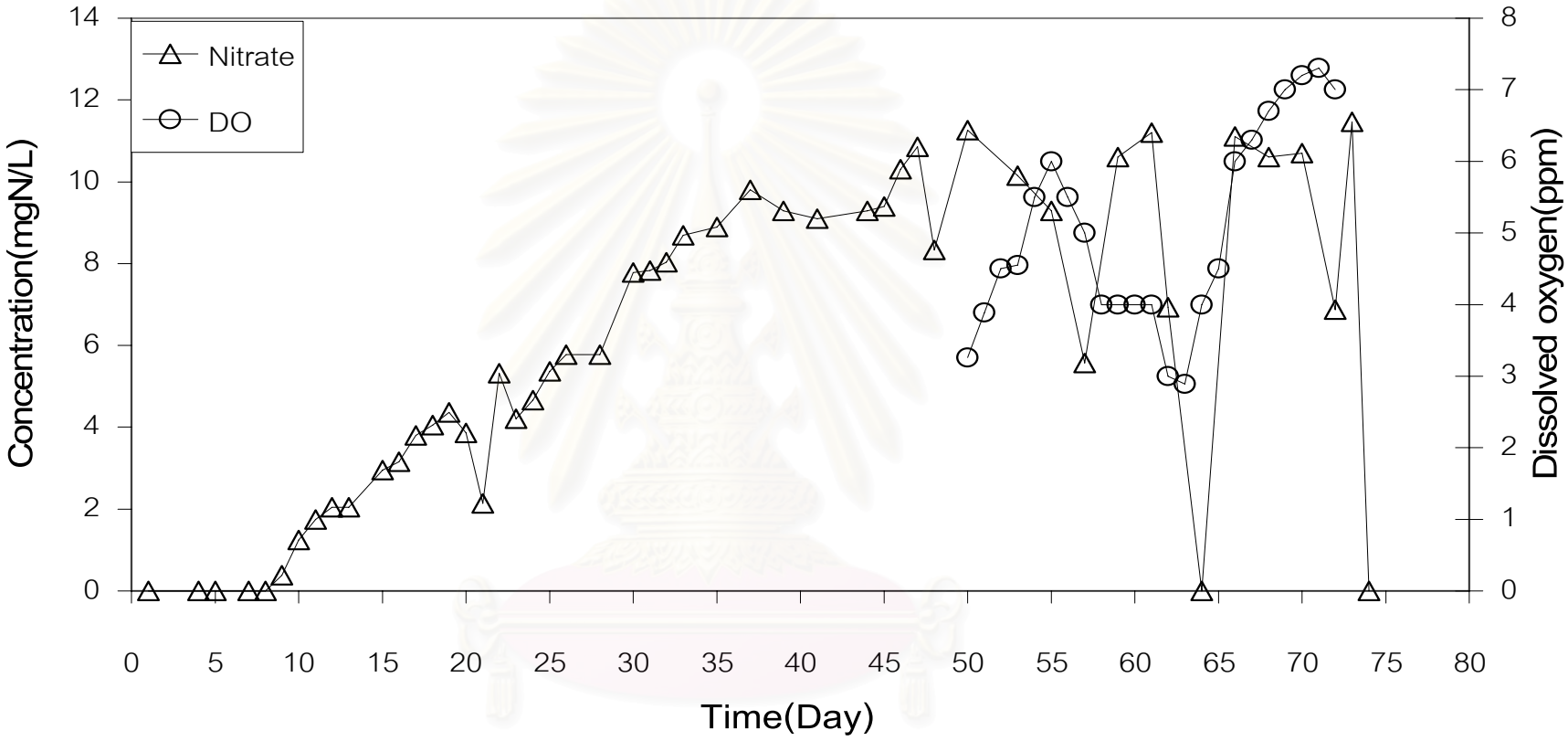


Figure 4.8 Nitrate-nitrogen concentration at reactor exit and dissolved oxygen profile during Experiment II

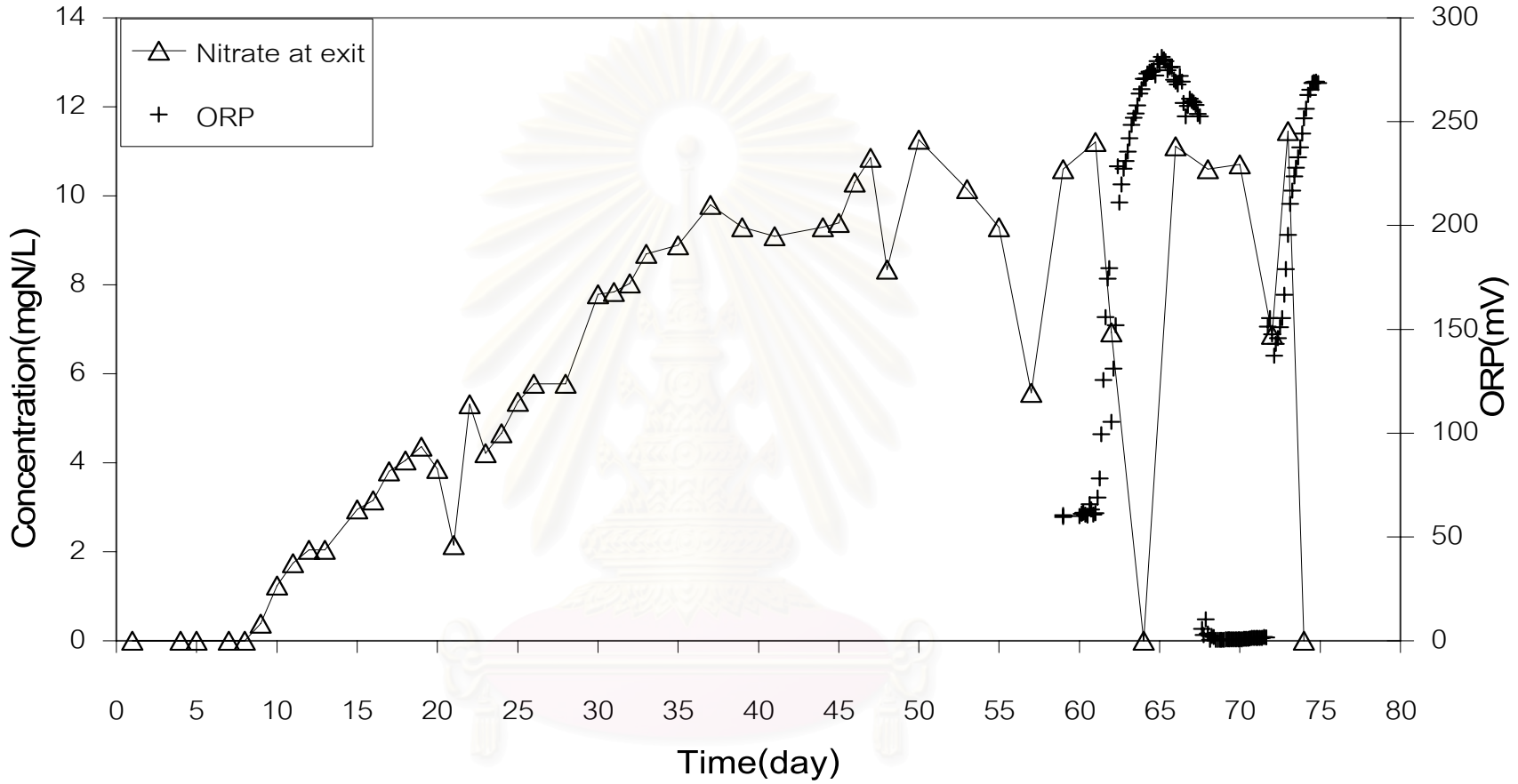


Figure 4.9 Nitrate-nitrogen concentration at reactor exit and oxidation-reduction potential profile during Experiment II

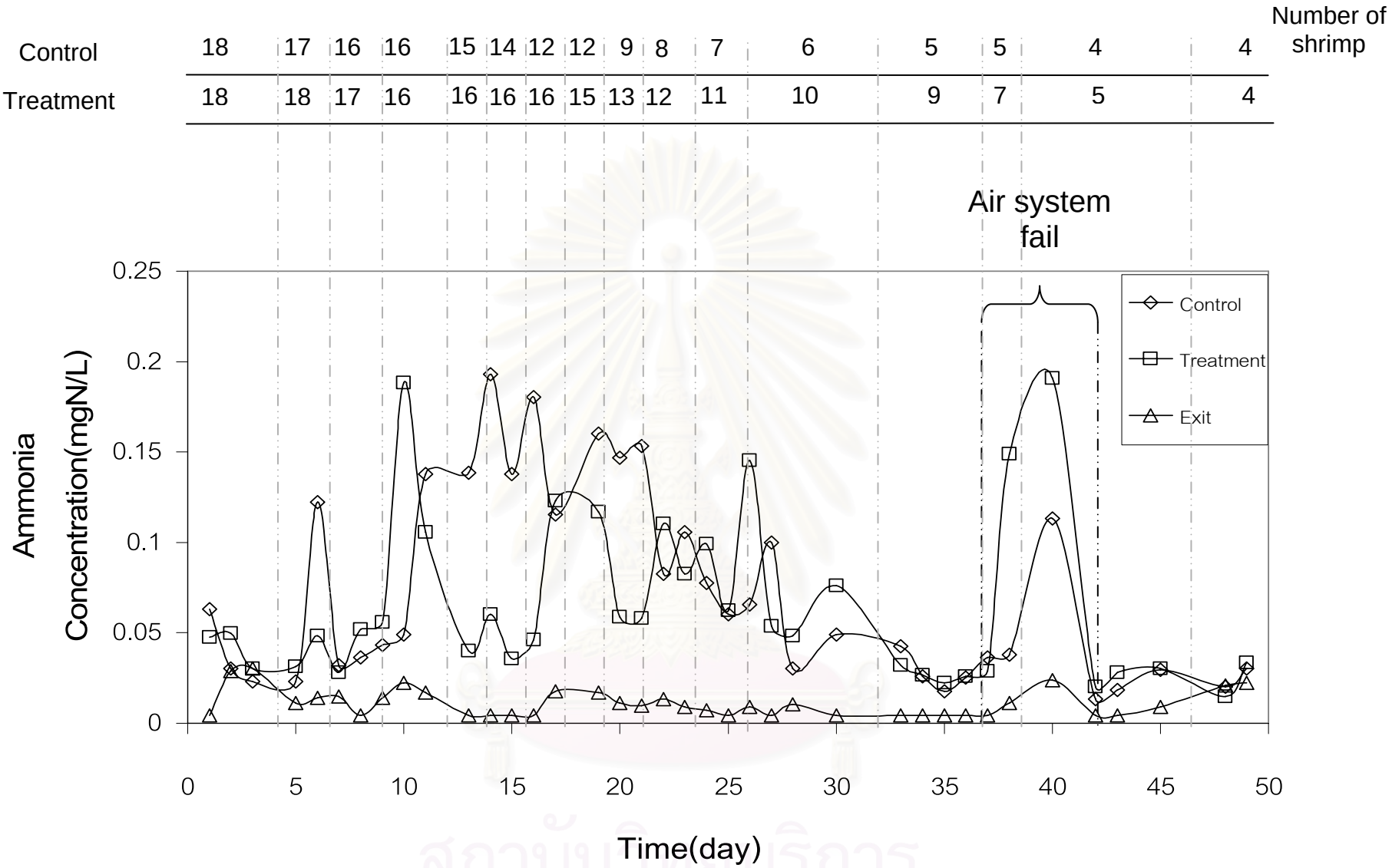


Figure 4.10 Ammonium-nitrogen concentration profile during Experiment III

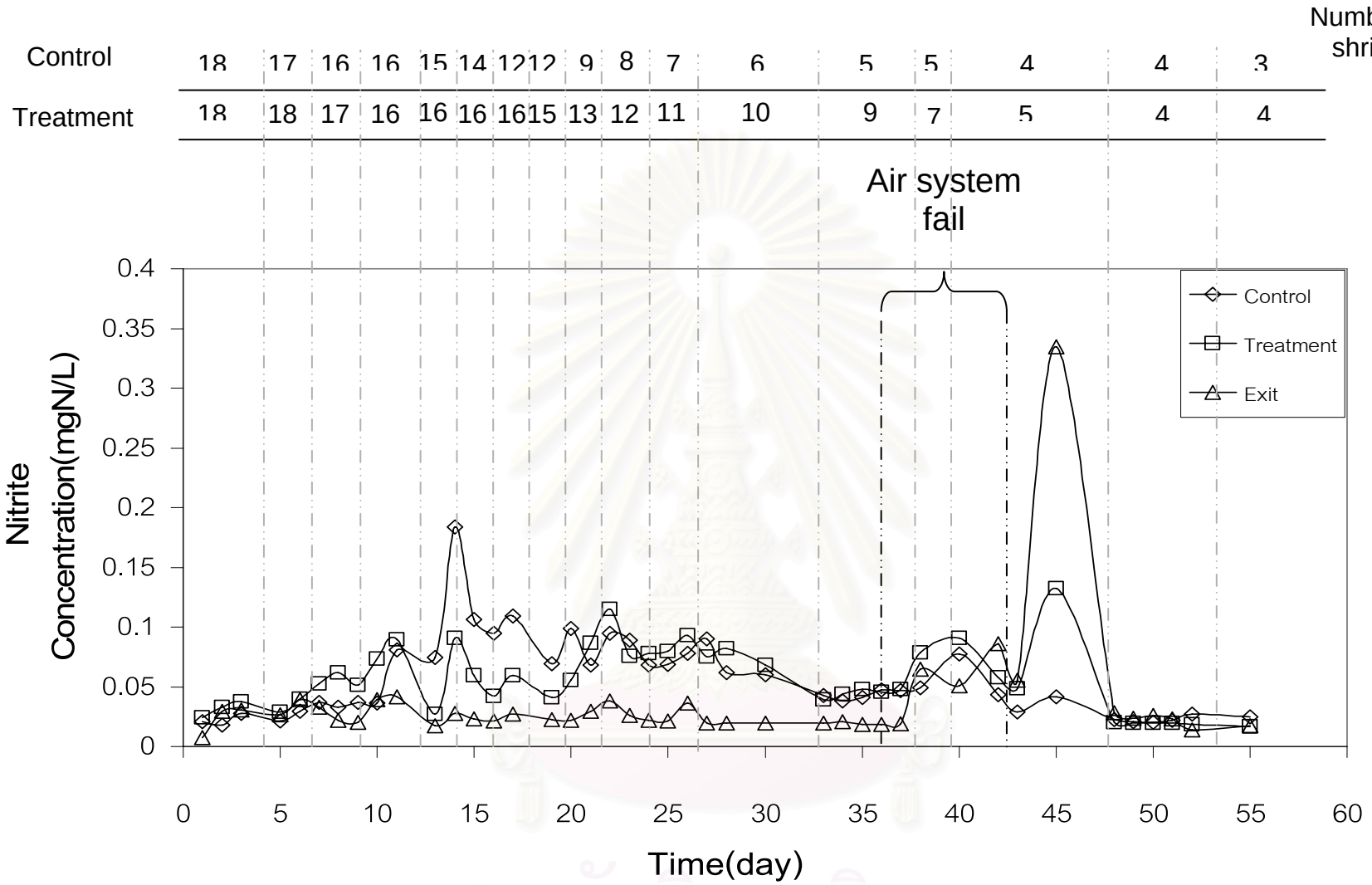


Figure 4.11 Nitrite-nitrogen concentration profile during Experiment III

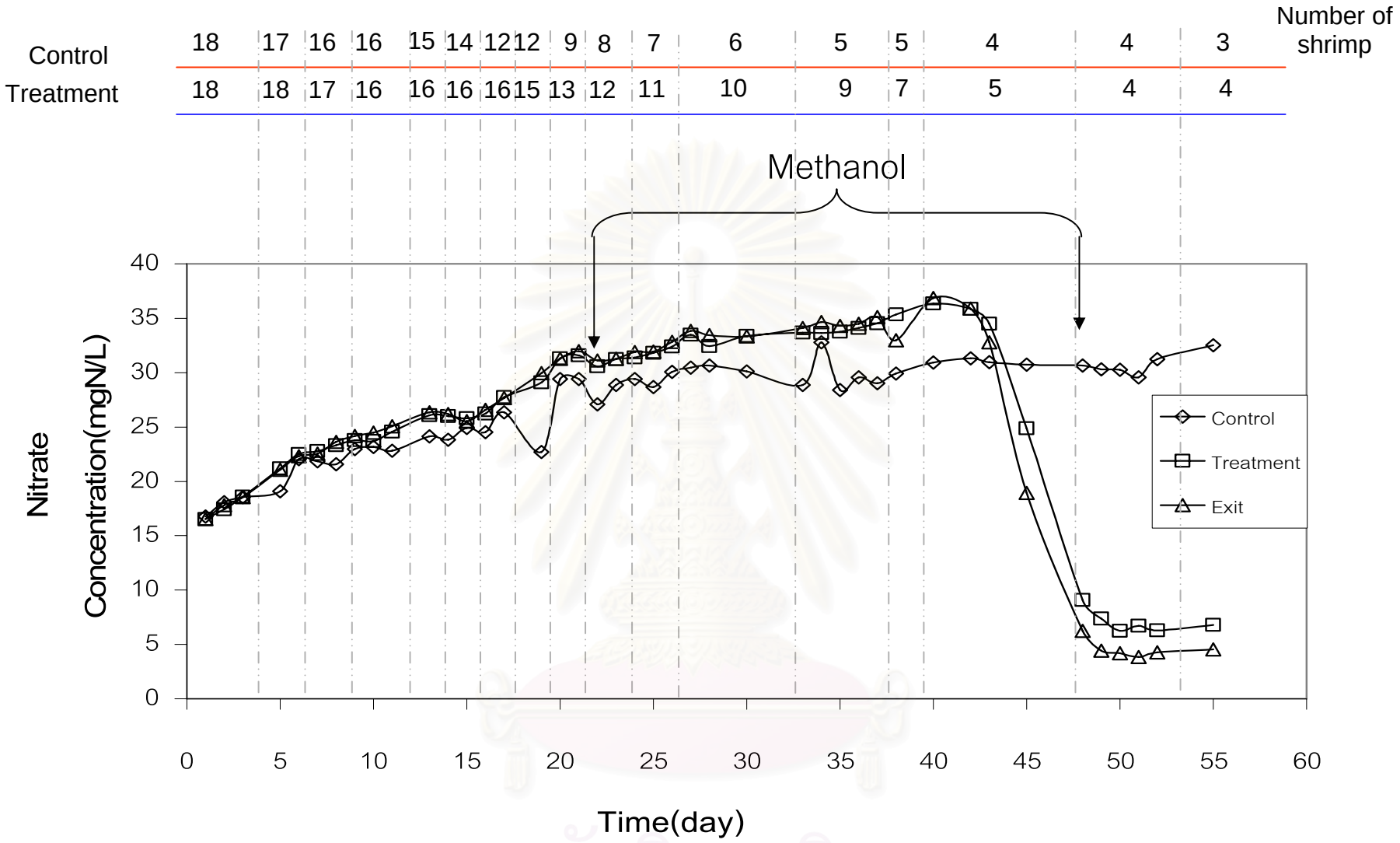


Figure 4.12 Nitrate-nitrogen concentration profile during Experiment III

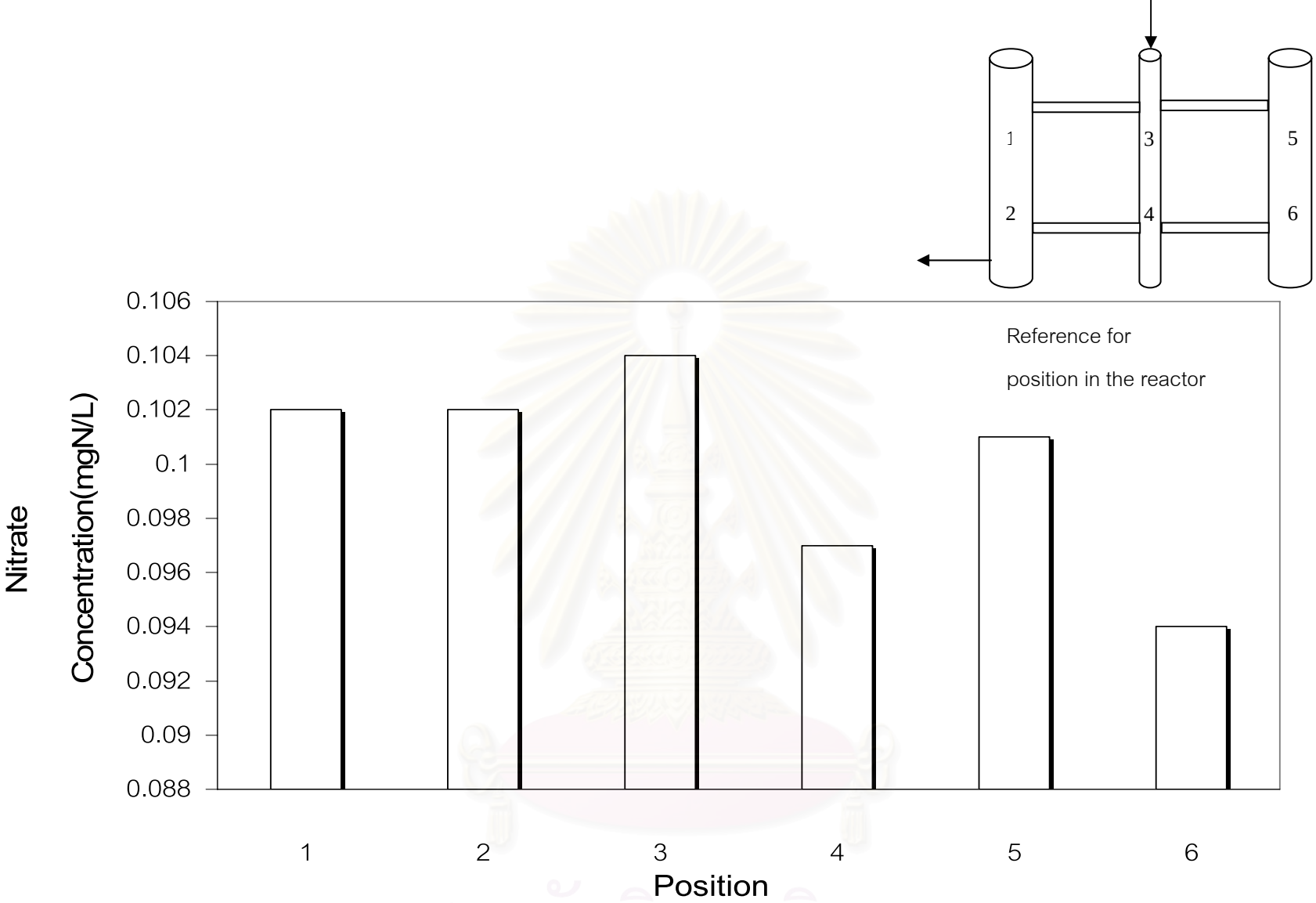


Figure 4.13 Nitrate-nitrogen concentration in PBABR (day 55 th) of Experiment III

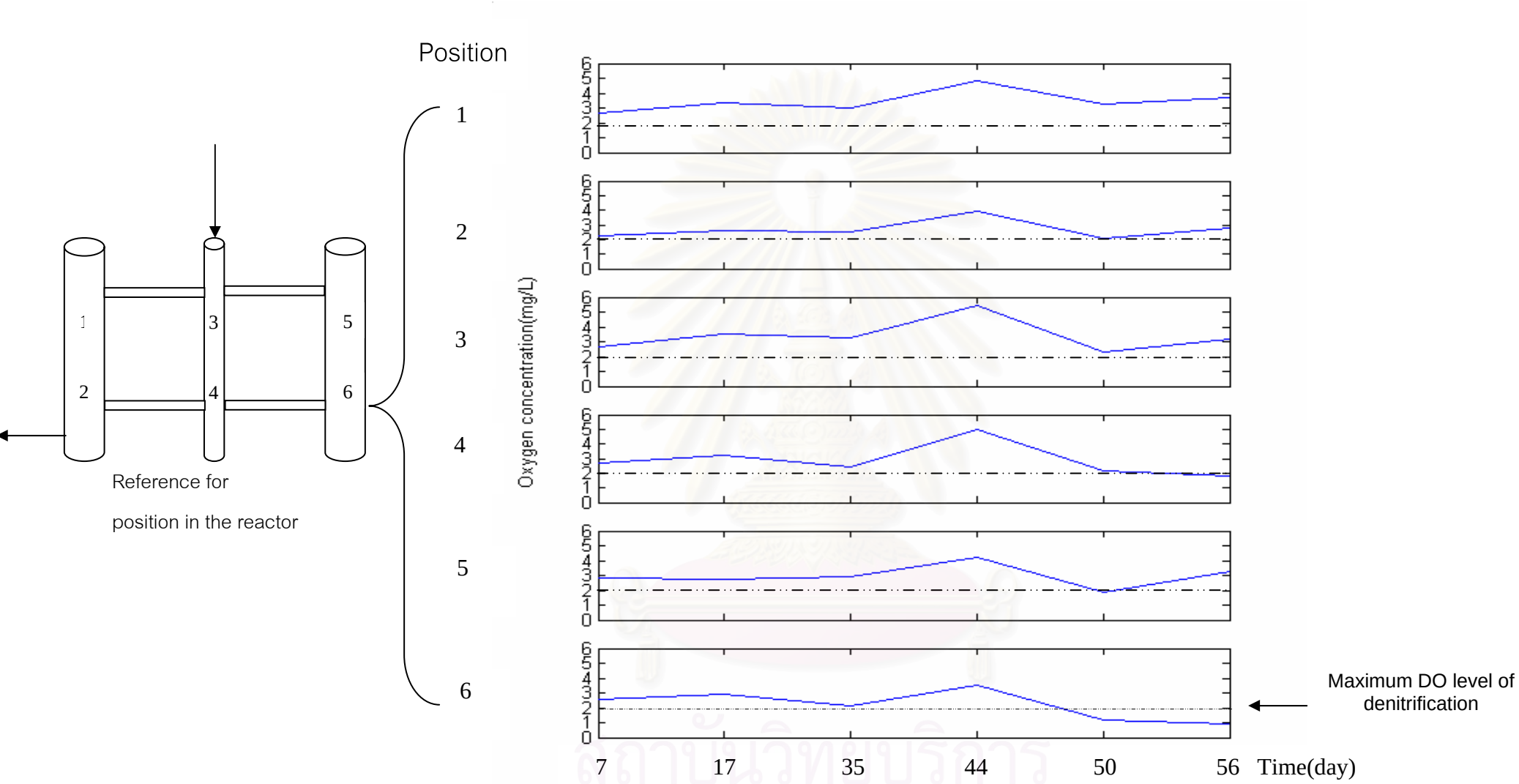


Figure 4.14 Oxygen concentration profile in PBABR of Experiment III

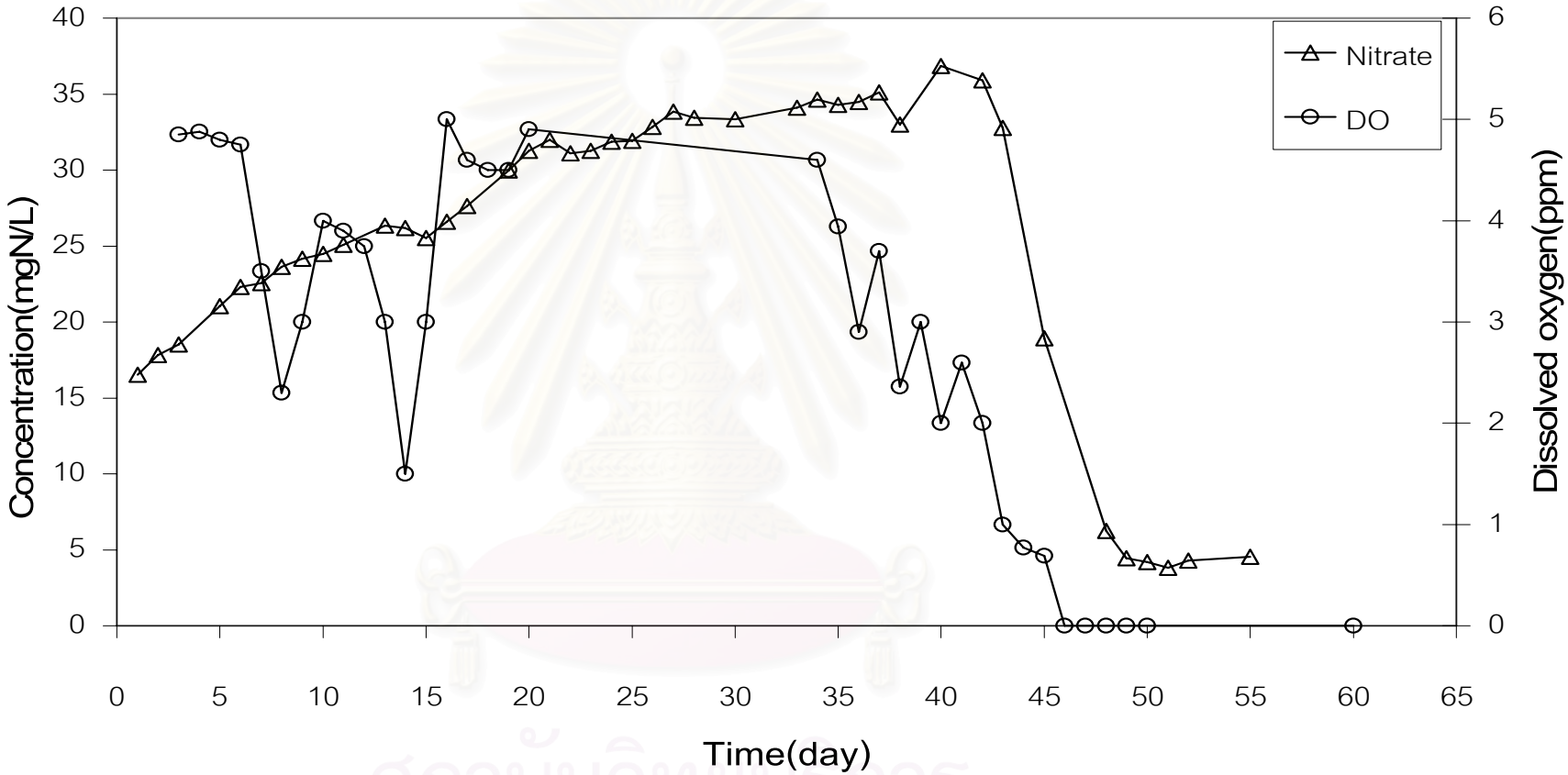


Figure 4.15 Nitrate-nitrogen concentration at reactor exit and dissolved oxygen profile during Experiment III

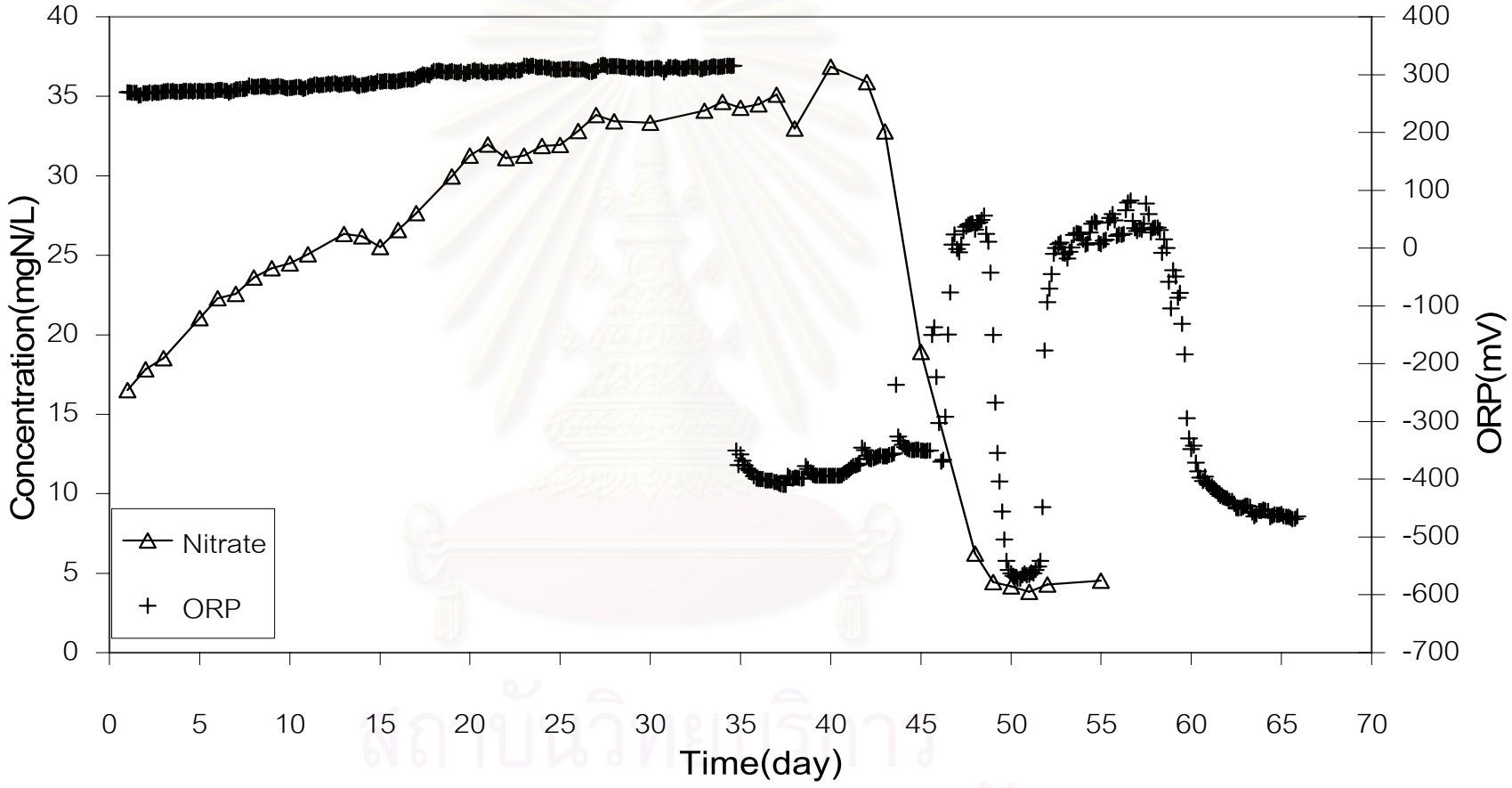


Figure 4.16 Nitrate-nitrogen concentration at reactor exit and oxidation-reduction potential profile during Experiment III (day 0-35 point at left downcomer and day 35-66 point at right downcomer)

Chapter V

Conclusions and recommendations

5.1 Conclusions

1. The ability of the Pack bed external loop airlift bioreactor (PBABR) in carrying out nitrification/denitrification was emphasized in this work. In the experiment without actual shrimp culture, nitrification and denitrification were found to occur at the rate of 0.563-3.971mgN/L/d and 6.304-14.425 mgN/L/d, respectively.
2. Circulation rate of medium between the pond and the PBABR was found to have no significant effect on the performance of the PBABR.
3. Methanol flow rate was found to have direct effects to dissolved oxygen concentration in reactor and could be adjusted during the operation to achieve the required level of treatment. However, the criteria for the addition of methanol still could not be found from this work. Note that methanol should be supplied with great care and at a very slow rate to minimize the escape to the shrimp pond.
4. The experiment with the actual shrimp culture was performed in the controlled and treatment system. Both shrimp ponds were attached with the side shell pond which was found to have adequate capability in controlling ammonia level but not nitrite and nitrate.
5. In the two experiments with actual shrimp culture, the treatment pond with PBABR performed better than the control pond without PBABR in terms of final shrimp weight.

5.2 Contributions

This research was the extent of previous research by Silapakul (2002) where PBABR was firstly introduced. However, in her work, PBABR was only operated with synthetic wastewater and the question of the actual applicability of

the system was still in doubt. In particular, Silapakul (2002) still could not explain the disappearance of nitrate as her experiment was conducted without the measurement of ORP. Moreover, in that experiment, nitrate disappeared even without the addition of methanol. Therefore it could be possible that nitrate was being consumed in the growth reaction instead of denitrification. This work, on the other hand, proved that denitrification could be achieved in the PBABR as indicated by the negative value of ORP. It was also shown that the PBABR could be applied to the actual shrimp pond without causing serious death of shrimp. In fact, it was found that, with the number of shrimp employed in this work, the side shell section of the pond could accommodate the ammonia rather well. The introduction of PBABR to the shrimp pond provided a solution to the remaining nitrite and nitrate and shrimp culture could last longer with higher growth.

5.3 Recommendations

Due to the time limitation of the master course work and also due to the fact that this work required a considerably lengthy experimental time, there are other aspects of work which should be visited to make the work more completed. These points are summarized below:

1. The effect of the circulation flow rate was not clear in this work. As the PBABR was small when compared with the shrimp pond, it was anticipated that a higher circulation would help in a higher level of treatment. The effect of the circulation rate must be looked at more closely in the next experiment.
2. The size of PBABR was arbitrarily at the time of this research. A proper size of PBABR should be investigated.
3. A proper monitoring of the various parameters such as the amount of gas produced from the system together with its composition, the amount of methanol left in the system, etc. should be established to allow the material balances which will in turn help in analyzing the system more clearly.

REFERENCES

AKIHIKO TERADA,' KAZUAKI HIBIYA,' JUN NAGAI,'SATOSHI TSUNEDA,'" AND AKIRA HIRATA'. Nitrogen Removal Characteristics and Biofilm Analysis of a Membrane-Aerated Biofilm Reactor Applicable to High-Strength Nitrogenous Wastewater Treatment. Department of Chemical Engineering, 2002

Balderston W.L., and Sieburth, J.M. Nitrate removal in closed-system aquaculture by columnar denitrification. Applied and Environment Microbiology 32 (1976): 808-818.

Campos, J.L., Mosquera-Corral, A., Sanchez, R., Mendez, R., and Lema, J.M. Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. Water Research 36 (2002): 2555-2560.

Canale, R. P. Biological waste treatment. New York: Interscience Publication, 1971.

Dincer, A.R. and Karg F. Kinetics of sequential nitrification and denitrification processes. Enzyme and Microbial Technology 27 (2000): 37-42.

Dirk Erler a,b, , Peter C. Pollard c, Wayne Knibb. Effects of secondary crops on bacterial growth
and nitrogen removal in shrimp farm effluent treatment systems. Faculty of Science, 2003

Focht, D.D., Verstraete, W. Biochemical ecology of nitrification and denitrification.

G. Ciudada, O. Rubilara, P. Mun˜oza, G. Ruizb, R. Chamyb, C. Vergaraa, D. Jeison. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. Department of Chemical Engineering, 2004

George Nakhla a, , Shaukat Farooq. Simultaneous nitrification–denitrification in slow sand filters. Department of Chemical 2002

Guo-min Cao*, Qing-xiang Zhao, Xian-bo Sun, Tong Zhang. Integrated nitrogen removal in a shell-and-tube
co-immobilized cell bioreactor. Department of Environmental

Engineering, 2003

Igor Bod ık a,*, Karol Kratochv ıl b, Eva Ga sparikov a a, Miroslav Hut nan. Nitrogen removal in an anaerobic baffled filter reactor with aerobic post-treatment. Department of Environmental Engineering,(2002)

Jae-Hoon Choi*, Seok Dockko, Kensuke Fukushi, Kazuo Yamamoto. A novel application of a submerged nanofiltration membrane bioreactor (NF ME3R) for wastewater treatment. Department of Urban Engineering,2002

Kaiser, H., and Schmitz, O. Water quality in a closed recirculating fish culture system influenced by addition of a carbon source in relation to feed uptaked by fish. Aquaculture and Fisheries Management 19 (1988): 265-273.

Kazuaki Hibiya, Akihiko Terada, Satoshi Tsuneda *, Akira Hirata. Simultaneous nitrification and denitrification by controlling vertical and horizontal microenvironment in a membraneaerated biofilm reactor. Department of Chemical Engineering,2002

Kim, S.K., Kong, I., Lee, B.H., Kang, L., Lee, M.G., and Suh, K.H. Removal of ammonium-N from a recirculation aquacultural system using an immobilized nitrifier. Aquacultural Engineering 21 (2000): 139-150.

Kitimasak, N., Aranyakananda, P., and Menasveta, P. Comparison of rotating bio-drum and submerged biofilters in closed, seawater recirculating systems with black tiger shrimp (*Penaeus monodon*) and sea bass (*Lates calcarifer*). Department of Marine Science, Chulalongkorn University, 1997.

Kuo-Cheng Chen,*, Ching-Yen Chena, Juin-Wei Penga, Jer-Yiing Houng. Real-time control of an immobilized-cell reactor for wastewater treatment using ORP. Department of Chemical Engineering,2001

Lee, P.G., Lea, R.N., Dohmann, E., Prebilsky, W., Turk, P.E., and Whitson, J.L. Denitrification in aquaculture systems: an example of a fuzzy logic control problem. Aquacultural Engineering 23 (2000): 37-59.

Lee, P.G., Turk, P.E., and Whitson, J.L. Controlled denitrification process and system. U.S. Patent. No. 5, 556, 630. (Jan 9, 1996).

Michael Rodgers, Xin-Min Zhan. Biological nitrogen removal using a vertically

moving biofilm system. Department of Civil Engineering, National University of Ireland, Galway, Ireland (2003)

Nagel, C.A., and Haworth, J.G. Operational Factors Affecting Nitrification in the Activated Sludge Process. Paper presented at the 42nd Annual Conference of the Water Pollution Control Federation Dallas, Texas, October, 1969. Cited in Culp, R.L., and Culp, G.L. Advanced wastewater treatment. New York : Van Nostrand Reinhold, 1971.

Otte, G., and Rosenthal, H. Management of a closed brackish water system for high-density fish culture by biological and chemical water treatment. Aquaculture 18 (1979): 169-181.

Painter, H.A. and Loveless, J.E. Effect of temperature and pH value on the growth rate constants of nitrifying in activated sludge process. Water Science and Technology. 17 (1983): 237-248.

Painter, H.A. Microbial transformations of inorganic nitrogen. Water Technology. 8 (1977): 3-29.

Sliekers, A.O., Derwort, J.L.., Campos Gomez, J.L., Strous, M., Kuenen, J.G., Jetten, M.S.M. Completely autotrophic nitrogen removal over nitrite in one single reactor. Water Research 36(2002): 2475-2482.

Strickland, J.D.H., and Parson, T.R. A Practice handbook of seawater analysis. 2nd ed. Fisheries Research Board of Canada Bulletin NO.167, Ottawa, 1972.

Strotmann, U.J., and Windecker, G. Kinetics of ammonium removal with suspends and immobilized nitrifying bacteria in different reactor system. Chemosphere 35 (1997): 2939-2952.

Tseng, K.F., Su, H.M., and Su, M.S. Culture of *Penaeus monodon* in a recirculating system. Aquacultural Engineering 17 (1998): 138-147.

Turk, P.L. Bacterial Bed. U.S. Patent. No. 5, 556, 536. (Sep 17, 1996).

van Benthum, W.A.J., Derissen, B.P., van Loosdrecht, M.C.M., and Heijnen, J.J. Nitrogen removal using nitrifying biofilm growth and denitrifying suspended growth in a biofilm airlift suspension reactor coupled with chemostat. Water Research 32 (7, 1998): 2009-2018.

van Benthum, W.A.J., Garrido, J.M., Mathijssen, J.P.M., Sunde, J., van Loosdrecht, M.C.M., and Heijnen, J.J. Nitrogen removal in intermittently

aerated biofilm airlift reactor. Journal of Environmental Engineering (March, 1998): 239-248.

van Benthum, W.A.J., Garrido-Fernandez, J.M., Tijhuis, L., van Loosdrecht, M. C.M., and Heijnen, J.J. Formation and detachment of biofilms and granules in a nitrifying biofilms airlift suspension reactor. Biotechnology Progress 12 (1996): 764-772

van Benthum, W.A.J., van den Hoogen, J.H.A., van der Lans R.G.J.M., van Loosdrecht, M.C.M., and Heijnen, J.J. The biofilm airlift suspension extension reactor. Part I: Design and two-phase hydrodynamics. Chemical Engineering Science 54(1999): 1909-1924.

van Benthum, W.A.J., van der Lans R.G.J.M., van Loosdrecht, M.C.M., and Heijnen, J.J. The biofilm airlift suspension extension reactor. Part II: Three-phase hydrodynamics. Chemical Engineering Science 55(2000): 699-711

van Benthum, W.A.J., van Loosdrecht, M.C.M., and Heijnen, J.J. Control of heterotrophic layer formation on nitrifying biofilms in a biofilm airlift suspension reactor. Biotechnology and Bioengineering 53 (1997): 397-405

Wild, H.E., Sawyer, C.N., and McMahon, T.C. Factors affecting nitrification kinetics. Journal WPCF 43 (1971): 1845-1853.

Yuan-Lynn Hsieh a,*, Szu-Kung Tseng a, Yu-Jie Chang. Yuan-Lynn Hsieh a,*, Szu-Kung Tseng a, Yu-Jie Chang. Department of Environmental Engineering,(2002)

Zhu, S., and Chen, S. An experimental study on nitrification biofilm performances using a series reactor system. Aquacultural Engineering 20 (1999): 245-259.

Zhu, S., and Chen, S. Effects of organic carbon on nitrification rate in fixed film biofilters. Aquacultural Engineering 25 (2001): 1-11.

Appendix A

Figure A-1 Plastic bioballs for immobilized nitrifying/denitrifying bacteria used as packing in PBABR

Figure A-2 Shrimp and Shell ponds

Figure A-3 Packed bed external loop airlift bioreactor (PBABR)

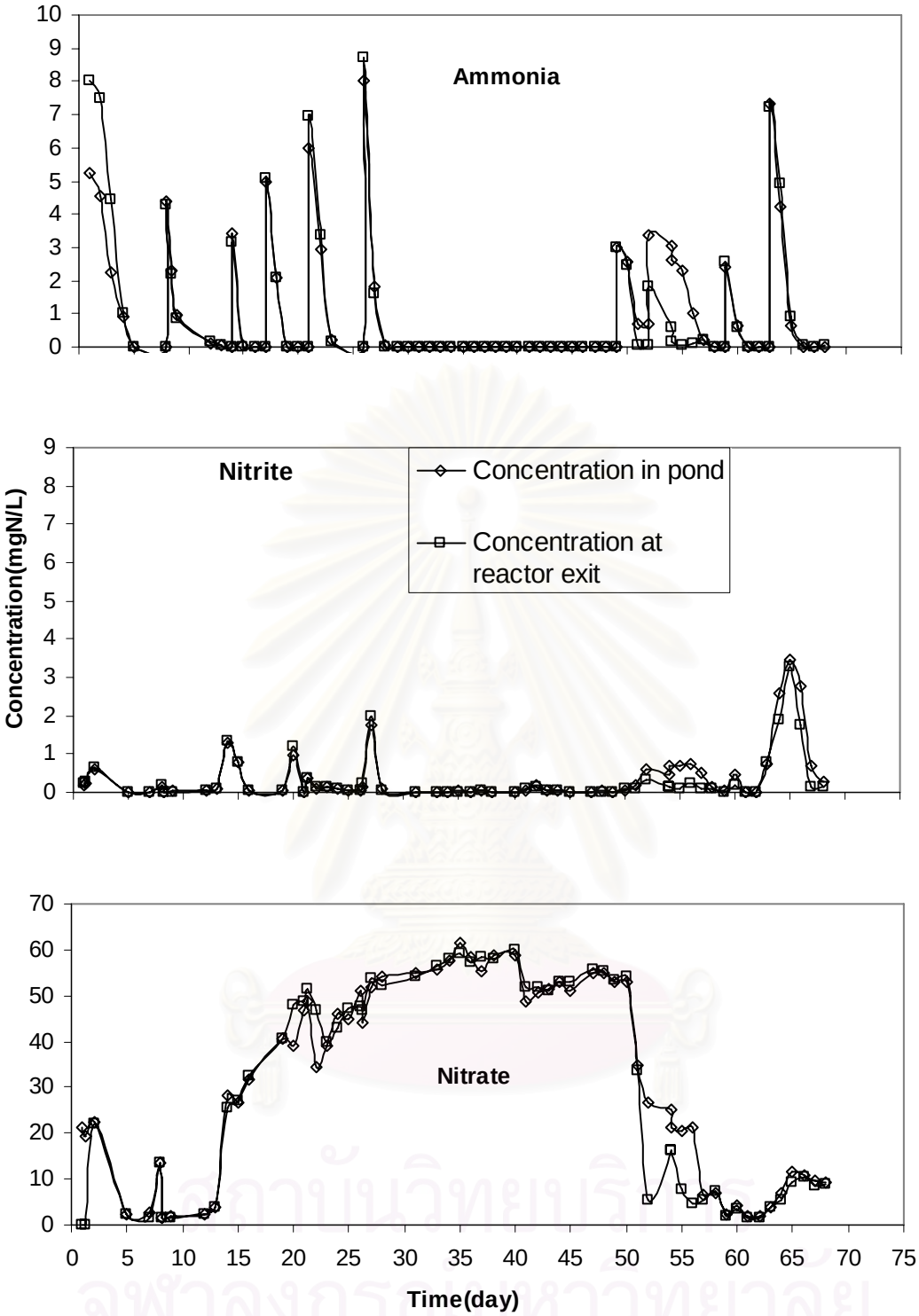


Figure A-4 Nitrogen compound concentration profiles in the shrimp pond and at reactor exit during Experiment I

BIOGRAPHY

Mr. Naruephu Juprajak was born on November 7th, 1981 in Bangkok. He finished his secondary school from Mahidol wittayanusorn School in March, 1998. After that, he studied in the bachelor of chemical engineering in Faculty of Engineer at Chulalongkorn University. He continued his further study in Master's degree in Chemical Engineering at Chulalongkorn University. He participated in the Biochemical Engineering Laboratories and achieved his Master's degree in April, 2005.