ผลของตัวกรองชีวภาพต่อคุณภาพน้ำในบ่อเลี้ยงกุ้งกุลาดำระบบปิด

นางสาว สุทธิกาญจน์ สุทธิ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาวิทยาศาสตร์ทางทะเล ภาควิชาวิทยาศาสตร์ทางทะเล
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547
ISBN 974-53-1723-3

EFFECTS OF BIOFILTER ON WATER QUALITY IN CLOSED RECIRCULATING SYSTEM FOR BLACK TIGER SHRIMP

Miss Suttikarn Sutti

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Marine Science

Department of Marine Science

Faculty of Science

Chulalongkorn University

Academic year 2004

ISBN 974-53-1723-3

Thesis Title	Effects of biofilter on water quality in closed recirculating system for black tiger shrimp
By	Miss Suttikarn Sutti
Field of Study	Marine Science
Thesis Advisor	Professor Piamsak Menasveta, Ph.D.
Thesis Co-advisor	Sorawit Powtongsook, Ph.D.

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

…….... Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTIEE

…….... Chairman
(Assistant Professor Charoen Nitithamyong, Ph.D.)

…… Thesis Advisor
(Professor Piamsak Menasveta, Ph.D.)

…… Thesis Co-advisor
(Sorawit Powtongsook, Ph.D.)

…… Member
(Associate Professor Somkiat Piyatiratitivorakul, Ph.D.)

สุทธิกาญจน์ สุทธิ : ผลของตัวกรองชีวภาพต่อคุณภาพน้ำในบ่อเลี้ยงกุ้งกุลาดำระบบปิด. (EFFECTS OF BIOFILTER ON WATER QUALITY IN CLOSED RECIRCULATING SYSTEM FOR BLACK TIGER SHRIMP) อ. ที่ปรึกษา : ศ.ดร. เปี่ยมศักดิ์ เมนะเศวต, อ.ที่ปรึกษาร่วม : ดร.สรวิศ เผ่าทองศุข 101 หน้า. ISBN 974-53-1723-3

การประเมินประสิทธิภาพของการใช้ตัวกรองชีวภาพเพื่อบำบัดแอมโมเนียในบ่อเลี้ยงกุ้งในวิทยานิพนธ์นี้แบ่งออกเป็นสองส่วน ส่วนแรกเป็นการประเมินการบำบัดแอมโมเนียโดยตัวกรองชีวภาพภายใต้สภาวะของห้องปฏิบัติการและในภาคสนาม ส่วนที่สองเป็นการศึกษาการใช้ตัวกรองชีวภาพในบ่อเลี้ยงกุ้ง ผลการศึกษาในส่วนแรกแสดงให้เห็นว่าตัวกรองชีวภาพที่ผ่านการบ่มตัวในบ่อเลี้ยงกุ้งเป็นเวลา 1 เดือน มีประสิทธิภาพในการบำบัดแอมโมเนียได้ดีกว่าชุดควบคุมซึ่งเป็นตัวกรองชีวภาพใหม่ที่ยังไม่ผ่านการใช้งาน โดยมีอัตราการบำบัดแอมโมเนียเฉลี่ยเท่ากับ 447.59 $\pm$ 205 มิลลิกรัมแอมโมเนียมไนโตรเจน/พื้นที่ผิว 1 ตารางเมตร/วัน ส่วนผลการทดลองในภาคสนามโดยใช้ถังขนาด 77.7L ติดตั้งในบ่อเลี้ยงกุ้งแสดงให้เห็นว่าถังที่มีตัวกรองชีวภาพจะมีประสิทธิภาพในการบำบัดแอมโมเนียดีกว่า และความเข้มข้นของไนไตรต์และไนเตรตที่เพิ่มขึ้นในขณะเดียวกันกับที่พบการลดลงของแอมโมเนียแสดงว่าเกิดปฏิกริยาไนตริฟิเคชันขึ้น แสดงว่าการใช้ตัวกรองชีวภาพในบ่อเลี้ยงกุ้งมีความเป็นไปได้

ในส่วนที่สอง ได้ทำการเลี้ยงกุ้งกุลาดำระยะ Postlarva 17 ความหนาแน่น 60 ตัวต่อตารางเมตร ในบ่อขนาด 29x29 เมตร (0.5 ไร่) ความลึก 1.2 เมตร จำนวน 4 บ่อ โดยใช้น้ำความเค็ม 6 psu แต่ไม่มีการเปลี่ยนถ่ายน้ำในระหว่างการเลี้ยง การเลี้ยงกุ้งรอบแรกใช้เวลา 113 วัน และในรอบที่สองใช้เวลา 114 วัน ผลการทดลองในรอบแรกพบว่าในบ่อชุดทดลองที่มีตัวกรองชีวภาพติดตั้งอยู่จำนวน 5 ชุด และมีหัวพ่นอากาศ มีความเข้มข้นของแอมโมเนียใกล้เคียงกับบ่อเลี้ยงกุ้งชุดควบคุมที่มีเฉพาะหัวพ่นอากาศ โดยมีปริมาณแอมโมเนียต่ำกว่า 0.5 มิลลิกรัมแอมโมเนียม/ลิตร อย่างไรก็ตามพบว่าผลผลิตเฉลี่ยของกุ้งในบ่อชุดทดลองมีค่าสูงกว่าบ่อควบคุม (120.8 และ 57.6 กิโลกรัม/บ่อ ตามลำดับ) หลังจากการเลี้ยงในรอบแรก ได้ทำการเปลี่ยนถ่ายน้ำออกจากบ่อและเติมน้ำใหม่เข้าบ่อทุกบ่อโดยไม่มีการทำความสะอาดพื้นบ่อ การเลี้ยงกุ้งในรอบที่สองได้ทำการติดตั้งตัวกรองชีวภาพและหัวพ่นอากาศในบ่อทั้งสี่เหมือนกันทั้งหมด ผลการทดลองพบว่าปริมาณแอมโมเนียในบ่อทุกบ่อมีค่าต่ำกว่า 0.5 มิลลิกรัมแอมโมเนียม/ลิตร แม้ว่าจะพบการลูมของแพลงก์ตอนพืชอย่างหนาแน่นโดยตลอดระยะเวลาการเลี้ยง แต่ผลผลิตของกุ้งในทุกบ่อมีค่าต่ำกว่าผลผลิตของกุ้งในรอบแรก

ภาควิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อนิสิต .....
สาขาวิชา วิทยาศาสตร์ทางทะเล ลายมือชื่ออาจารย์ที่ปรึกษา.....
ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4572539423 : MAJOR MARINE SCIENCE
KEY WORD: *Penaeus monodon* / BIOFILTER / AMMONIA / NITRIFICATION
SUTTIKARN SUTTI : EFFECTS OF BIOFILTER ON WATER QUALITY IN CLOSED RECIRCULATING SYSTEM FOR BLACK TIGER SHRIMP. THESIS ADVISOR : PROF. PIAMSAK MENASVETA, Ph.D., THESIS COADVISOR : SORAWIT POWTONGSOOK, Ph.D., 101 pp. ISBN 974-53-1723-3

This study investigated the efficiency of using biofilter for ammonia removal in the outdoor shrimp ponds. The experiment was divided in to two parts. The first part was the evaluation of ammonia removal by biofilter under both laboratory and field conditions. The second part was the study of using biofilter in the outdoor shrimp ponds. The results from the first part showed that, under laboratory condition, active biofilter (incubated in shrimp pond for one month) had higher ammonia removal efficiency than control (unused biofilter). The average ammonia removal rate was 447.59 ± 205 mgNH_4-N/m^2 surface area/day. The results from field study using 77.7L chamber placed in shrimp pond also showed that significantly higher ammonia removal was found in the chamber containing biofilter. Increased in nitrite and nitrate in all chambers together with the decreased of ammonia indicated that nitrification process appear in the chamber and the attempt of using biofilter in shrimp pond is possible.

In the second part, postlarva 17 of black tiger shrimp (*Penaeus monodon)* were cultured in four 29x29 m^2 shrimp ponds (0.5 Rai) with 1.2 m depth at the density 60 postlarva/ m^2. Shrimp were grown in 6 psu salinity without water exchanged for 113 and 114 days for the first and second trials, respectively. The results from the first trial showed that ammonia concentration in both treatment ponds (containing 5 sets of biofilter with the aerators) and control ponds (containing only the aerators) was not different (less than 0.5 mgNH_4-N/L). However, the average shrimp production in treatment ponds was higher than control ponds (120.8 and 57.6 kg/pond, respectively). After the first trial, water in all ponds was drained out and soon refilled with the water from reservoir without pond bottom cleaning. With the second trial, all four ponds were installed with biofilter and aerators. The results showed that ammonia concentration in all ponds was less than 0.5 mgNH_4-N/L although dense blooming of phytoplankton was found throughout the culture period. However, shrimp production in all ponds was lower than the previous trial.

Department MARINE SCIENCE Student's signature.....
Field of study MARINE SCIENCE Advisor's signature.....
Academic year 2004 Co-advisor's signature.....

ACKNOWLEDGEMENTS

This thesis had not been completed without help and support from many people and organizations who are gratefully acknowledged here. I would like to express my deepest gratitude to my advisors Professor Piamsak Menasveta and Dr. Sorawit Powtongsook for their advices and supports throughout this research. Associate Professor Somkiat Piyatiratitivorakul and Assistant Professor Charoen Nitithamyong kindly serve as the thesis committee.

My acknowledgement is also expressed to Miss Chansawang Ngampongsai, Miss Chompunut Chairattana, Mrs. Pariya Nupasan, Mr. Seri Donnuea, Miss Tipawan Hongjed and my friends in Department of Marine Science and Marine Biotechnology Research Unit at Chulalongkorn University for their help in laboratory work.

Of course, I would like to express my sincere to my family; father, mother, brother and sister for their inspiration and cheerful support throughout my Master study.

This research was supported by National Research Council of Thailand and I would like to thank the Thailand Graduated Institute of Science and Technology (TGIST) for supporting the scholarship for my study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER I

INTRODUCTION

Shrimp culture is the world's most rapidly expanding aquaculture sector. Shrimp biomass harvested from the oceans has remained constant while production of farmed shrimp increased. Moreover, no significant decrease in the price of shrimp has occurred, indicating continued high demand. In Thailand, the marine shrimp culture species is mainly *Penaeus monodon* which more than 90% of culture production has been exported frozen, both headless and with shell on, to the importing countries as Japan, United States, European countries, the People' s Republic of China, Canada and other countries.

In response to the increasing world of market demand, shrimp farming in Thailand has been developed especially over the last decade. Shrimp culture in Thailand can be classified by density of shrimp into extensive, semi-intensive, intensive and super-intensive depending on cultured area, stocking density, pond preparation and pond management.

Under normal practice of intensive shrimp culture, water exchange is needed in order to remove nitrogenous waste and other potentially toxic metabolic waste products. In the intensive shrimp culture, shrimp production can be increased to 4,000-10,000 kg/ha/yr depending on the density of shrimp. Because of high stocking density, the intensive shrimp culture requires artificial high protein feed supplied to satisfy shrimp nutritional need. However, feeding is known as the major source of pollutants in aquaculture system. Montoya *et al* (1999) concluded that overfeeding is the most common problems because the feed consumption rate of shrimp can not accurately estimated while shrimp farmers prefer to provide excess feed to ensure growth. In the closed recirculating system with zero water exchange, nitrogenous waste from feeding and shrimp excretion is mostly accumulated in the pond and causes the deleterious impacts on water quality and shrimp growth. As a result, water exchanged and effluent from shrimp ponds therefore has high impact on the surrounding environment. Water exchange may also deliver shrimp pathogen that can

directly harmful to the farmed shrimps. So, during the last 15 years, closed or semi-closed recirculation system have been applied to the commercial marine shrimp grow-out systems at much larger scale than previously envisioned. This made shrimp culture more "green" or an environmental friendly agro-industry.

It is well known that one of the most important limiting factors in intensive culture system is the build-up of toxic nitrogenous waste. Accumulation of ammonia or its intermediate product, nitrite, causes mortality and affects growth of cultured animals (Colt and Armstrong, 1981 cited in Chen, 1992). According to nitrogen biogeochemistry of aquaculture pond, ammonia which is the predominant form of inorganic nitrogen was excreted by aquatic animal is oxidized to nitrite and finally to nitrate by the autotrophic aerobic bacteria so called nitrifying bacteria

Total ammonia nitrogen (TAN), the most critical water parameters, consists of two fractions, un-ionized ammonia (NH_3) and ionized ammonia (NH_4^+). Higher pH and high temperature cause the higher percentage of toxic un-ionized ammonia. In general, ammonia can be removed from the pond by natural processes such as evaporation, absorption in pond soil, assimilation by phytoplankton, and nitrification. Although the main process that remove dissolved ammonia in an outdoor aquaculture ponds is phytoplankton uptake, but in the intensive shrimp culture which ammonia increases exponentially overtime, only natural ammonia removal processes may not sufficient.

Nitrification is a biological process mostly used in water treatment for many advantages such as high potential removal efficiency, process stability and reliability, easy process control, less land requirement and moderate cost. Nitrification consists of the sequential two-step oxidation mediated by different microorganisms including the oxidation of ammonia and the oxidation of nitrite, respectively. Biofilter, based on the use of supporting media with a high surface/volume ratio pre-colonized by microorganism, is a common technique used for nitrification treatment in biological nitrogen removal process. A number of techniques using nitrification biofilter such as trickling filters, submerged filters, and rotating biological contactor are carried out and well studied in the indoor closed aquaculture systems.

The present study is one of an effort to develop the use of biofilter in the outdoor closed recirculating system for an intensive culture of black tiger shrimp, *Penaeus monodon*. The study includes the evaluation of nitrification biofilter under laboratory condition and the use of biofilter in the outdoor shrimp ponds.

Objectives

1. To study ammonia removal rate of the nitrification biofilters under both laboratory and field conditions.
2. To evaluate the efficiency of using biofiltrer for ammonia treatment in the outdoor shrimp ponds.

CHAPTER II

LITERATURE REVIEW

2.1 Shrimp Farming in Thailand

In Thailand, black tiger shrimp, *Penaeus monodon*, is the most important aquaculture species in both production and market value. Production of shrimp farming increased very rapidly after the expansion of intensive shrimp farming. Tookwinas (2001) reported that the cultured area and number of farms increased from 40,769 ha and 4,939 farms in 1985 to 71,887 ha and 20,027 farms in 1993. This made Thailand the leading country for shrimp production since 1991.

Shrimp culture in Thailand has been developed for many years, especially over the last two decade, in response to the increasing world market demand. The production system evolved from extensive toward intensive with increasing input of high quality feed and water supply (Thakur and Lin 2003). The management used in marine shrimp farming can be separated in to four types: extensive, semi- intensive, intensive and super-intensive shrimp farming.

The extensive shrimp culture or traditional shrimp farming is characterized by large ponds (5-10 ha or 31.25-62.5 Rai) with irregular shape. Juveniles from wild population were usually brought into the ponds with the inlet water. This type of farm has low stocking density of 0.5-5 shrimp/m^2, and therefore low productivity. No chemical are used but fertilizer may be added to promote the growth as the natural food.

The semi-intensive shrimp farming, on the other hand, was introduced into several countries in Southeast Asia. It is a modification of existing extensive pond with better pond management. This include pond bottom cleansing and leveling, shrimp stocking density control, fertilization and feeding supplement. In this semi-intensive farm, ponds are normally rectangular in shape with and area of about 1-6 ha (6.25-37.5 Rai). The stocking density is about 5-10 shrimp/m^2.

The intensive shrimp farming requires high investment and technical input. Mechanical aeration, mostly by electrical or engine powered paddlewheels, is used to supply dissolved oxygen into the pond. Shrimp are fed with a nutritionally complete, artificial diet. Pond size varies from 0.16-1 ha (1-6.25 Rai) and pond depth is around 1.5-2.0 m. The pond shape can be either rectangular or round. Shrimp stocking density ranges from 20-50 shrimp/m^2. The number of shrimp production crops is possibly 2-2.5 per year. The average production is around 3,750 kg/ha/yr (23,437.5 kg/Rai/yr), but higher yield is common. However, although the intensive shrimp farming has high shrimp yield, wastewater released into the nearby natural water resources can cause the environmental problem. This leads to the development of closed-system shrimp farm with the good aquaculture practice manner, as promoted by the government sector.

Tookwinas (2001) reported that the super-intensive shrimp farming system is the most advance system in which only a few farms in Thailand can operate or convert the intensive farm into a super-intensive farm. This is because of the very high investment and technical inputs required. Pond construction is the same as that of intensive farming but the seed stocking density is higher than 80 shrimps/m^2. The average production is around 6,000-10,000 kg/ha/yr.

2.2 Water quality in an intensive shrimp culture pond

Because of several factors in the intensive shrimp farming management as mentioned earlier, the environment in shrimp pond has been altered. Common problems encounter water quality in shrimp ponds include diminishing light penetration by suspended solids, and a hypernutrification that turns into changes of macrofauna and eutrophication of water bodies (Funge-Smith and Briggs, 1998).

Dissolved oxygen (DO) is one of the most important environmental factors in aquaculture. Changed of DO in shrimp pond is related with many environmental conditions, such as the sudden death of phytoplankton, large reproduction of zooplankton, and decomposition of accumulated organic matter. These factors lead to sharp decrease in DO which is a common hazard in shrimp pond. Jiang *et al*. (2004) studied the effect of dissolved oxygen on white shrimp and concluded that hypoxia

can affect the survival, growth, respiration, hemolymph osmotic pressure and therefore cause of mortality of shrimp.

Ammonia is the principal end-product of protein catabolism in crustaceans. It is known as the most common toxic substance that excreted from the animals or decomposed from organic detritus like unconsumed feed and faeces (Chin and Chen, 1987 cited in Ostrensky and Wasielsky Jr., 1995). Cowey and Cho (1991) and Goddard (1996) cited in Montoya *et al.* (1999) suggested that an increase in protein content in feed, in order to meet shrimp nutritional requirement, have been identified as the major source of pollutants in aquaculture. Decomposition of uneaten feed, together with animal excretion, produced high concentration of toxic nitrogen wastes especially ammonia and nitrite. Total ammonia nitrogen (TAN) is the most critical water quality parameter in the recirculating aquaculture system. TAN is a combination of two fractions, un-ionized ammonia (NH_3) and ionized ammonia (NH_4^+), of which the former is extremely toxic to fish. The proportion of the un-ionized form in TAN is dependent upon the pH and temperature of the water and the higher the pH and temperature of the water induces the higher the percentage of toxic unionized ammonia. TAN may be transformed via a number of pathways including assimilated by phytoplankton, volatilized as gaseous ammonia, converted to nitrate nitrite via nitrification processes or discharged during water exchange (Figure 2-1).

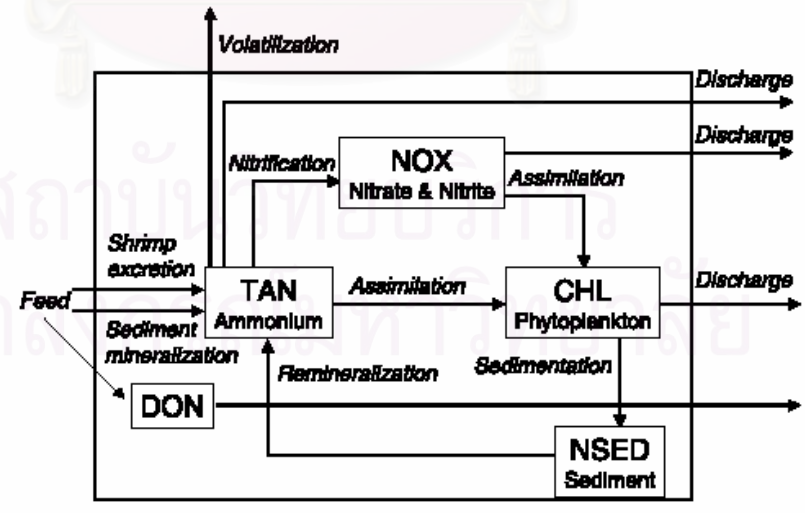


Figure 2-1: Conceptual model of N input, transformation and removal in intensive shrimp pond: TAN= total ammonia N; NOX= nitrate plus nitrite; CHL= chlorophyll_*a* as a measure of phytoplankton; DON; dissolved organic N; NSED; N buried in the sludge (Burford, 2004)

In intensive shrimp culture, accumulation of ammonia deteriorates water quality, increases oxygen consumption, increases ammonia excretion, inhibits shrimp growth, and may even cause high mortalities (Chen and Lin, 1992). The 24-h LC_{50} values of NH_3-N of several penaeid-shrimp species are showed in Table2-1.

Table 2-1: The 24-h LC_{50} values of NH_3-N of several penaeids species (Ostrensky and Wasielsky Jr., 1995).

Species	Stage (mg NH_3-N/L)					References
	Nauplius	Zoea	Mysis	Postlarva	Juvenile	
Penaeus. indicus	0.29	0.95	3.17	-	-	Jayasankar and Muthu (1983)
Penaeus monodon	0.54	0.76	2.17	4.70	-	Chin and Chen (1987)
Penaeus japonicus	1.31	0.97	1.08	1.98	-	Chen et al. (1989)
Penaeus. chinensis	0.25	0.34	1.08	1.85	-	Chen and Lin (1991b)
Metapenaeus ensis	0.65	0.30	2.25	1.90	-	Chen et al. (1991)
Penaeus paulensis	4.25	1.79	2.91	1.40	1.47	Ostrensky et al. (1995)
Penaeus monodon	-	-	-	-	2.68	Chen and Lei (1990)
Metapenaeus ensis	-	-	-	-	2.19	Nan and Chen (1991)
Penaeus chinensis	-	-	-	-	3.88	Chen and Lin (1992)
Penaeus penicillatus	-	-	-	-	3.25	Chen and Lin (1991a)

Nitrite is another potential toxic nitrogenous compound that may accumulate in aquaculture ponds. Nitrite is released as an intermediate product during nitrification and denitrification. The toxicity of nitrite is expressed through the competitive binding of nitrite to haemoglobin forming methemoglobin, which does not have the capacity to carry oxygen (Hargreaves, 1998). But in crustaceans, which have haemocyanin, nitrite is less toxic (Songsangjinda, 2002). Nitrate, on the other hand, is not generally a great concern to aquaculturist and it has been showed that aquatic animals can tolerate extremely high concentration of NO_3^-N, greater than 100 ppm (Ebeling, 1993 cited in Al-hafedh, 2003).

The lack of water for water exchange during phytoplankton bloom in shrimp pond is one of the significant pond management problems. This has always been the problem since the phytoplankton population eventually crash and caused severe stress to the shrimp (Funge-Smith and Briggs, 1998). Besides of phytoplankton bloom that can cause oxygen depletion, some toxic phytoplankton species can directly harm to

shrimp. Smith (1996) reported that bloom of Oscillatoriales, *Oscillatoria corakiana* which is the dominant species in shrimp (*Penaeus monodon* and *Penaeus japonicus*) ponds could result the consequent of high ammonia concentration and following by bacterial disease infection in shrimp.

2.3 Development of closed recirculating system for aquaculture

Recirculation systems have been applied commercially to marine shrimp culture pond in much larger scale than previously envisioned. The aim of using recirculation system is to control of diseases and contamination of unwanted organisms from source water, control of water quality, improve growth performance due to greater control over water quality parameters, and the concern in environmental problems caused by shrimp pond effluent (Menasveta, 2002).

For more detail, transmission of shrimp diseases through water exchanged can be greatly reduced by limiting the use of source water, pre-treating the water, and recondition the effluent for recycling. These precautions, combined with the specific pathogen-free (SPF) or specific pathogen-resistant (SPR) shrimp larvae, can possibly prevent disease incidence during shrimp cultivation (Pruder, 2004). Recirculating pond systems also reduce sediment in the culture pond and decrease the amount of discharged water to natural waters. A wide variety of shrimp pond recirculation schemes have been proposed and used. These share many characteristics with conventional intensive culture systems, but differ in some respects. Although many intensive shrimp farms in the recent year has been modified to closed system with little or zero water discharge, the closed recirculating system itself still encounter with several problems. The major problem is the bloom of phytoplankton or eutrophication in the pond, which is the result from an increase of nutrients and organic matters over the culture period. The super-eutrophic pond water can lead to the flash point of pond carrying capacity by adverse pond environment.

In general, Tookwinas (2000) concluded that the common problems of the closed recirculating system for aquaculture are:

- Growth of shrimp is lower than typical system
- FCR (Feed conversion ratio) value is higher than 1.5
- Unequal growth of shrimp within the pond
- Dirty body surface and abnormal behavior symptom
- Decrease in feed consumption during fluctuation of the environment or according to the molting period
- Problems in water quality such as:
    - low oxygen concentration
    - pH and alkalinity are lower or higher than the optimal level
    - high transparency
    - ammonia and nitrite concentrations are too high
    - biochemical oxygen demand (BOD) is too high
    - bacteria and *Vibrio* spp. is very high

In Thailand, the concept of water recirculating system for shrimp farm has been proposed by the Department of Fisheries (Tookwinas, 2000) as shown in Figure 2-2.

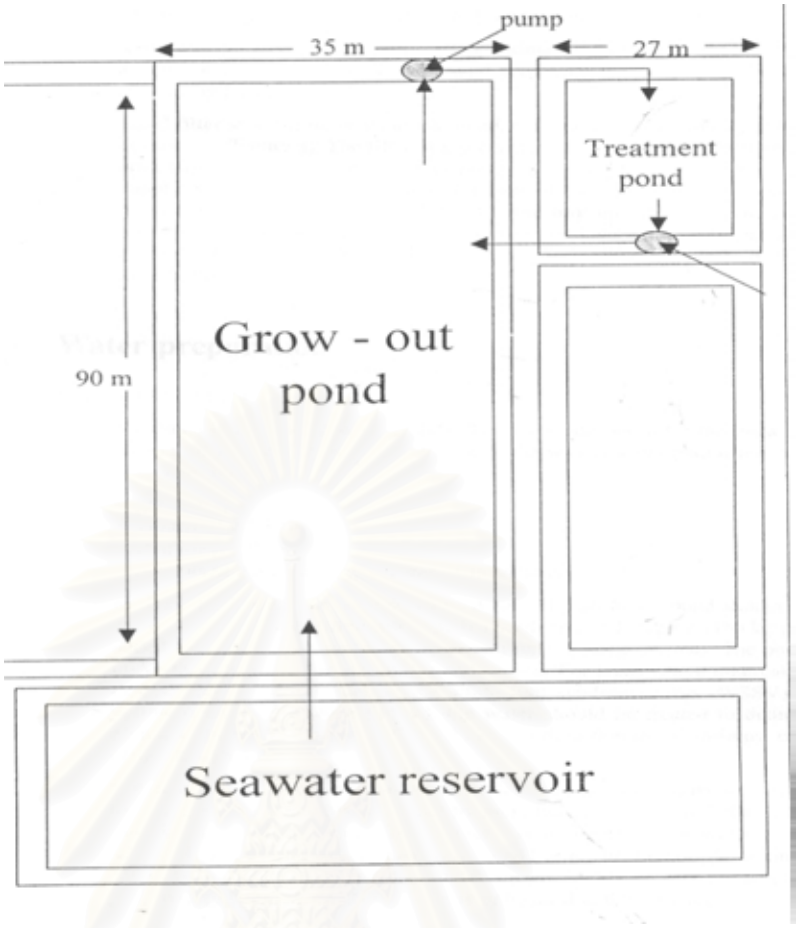


Figure 2-2: Model of closed system shrimp pond in Thailand (Tookwinas, 2000)

The model shrimp farm in Thailand upon as recommended by Tookwinas (2000) consists of 2 rai (35x90m) grow-out pond and a 0.5 rai (30x27m) treatment pond (Figure 2-2). Another pond is required to serve as reservoir for new water for the initial filling as well as to compensate for loss due to evaporation and possible seepage.

The treatment pond which was installed with filter box (Figure 2-3) with a depth of 150 cm is filled with water from a reservoir. During operation, water was recirculated between grow-out pond and treatment pond.

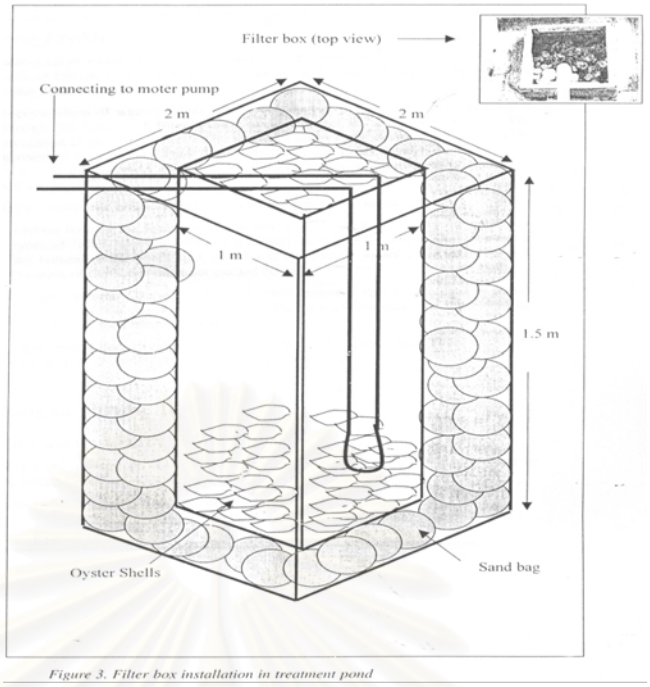


Figure 3. Filter box installation in treatment pond

Figure 2-3: Diagram of the filter box installed in the closed system shrimp pond (Tookwinas, 2000)

2.4 Water treatment processes in aquaculture

The main objectives of conventional wastewater treatment processes are the reduction of biochemical oxygen demand, suspended solids, and pathogenic organism. In addition, it may be necessary to remove nutrients, toxic compounds, non-biodegradable compounds, and dissolved solids. Many of common wastewater treatment operations are show in Table 2-2.

Table 2-2: Wastewater treatment processes and major purposes (modified from Sundstorm and Klei, 1979).

Operation or process	Function
Bar screens and racks	Coarse solids removal
Comminutor	Grinding up of coarse solids
Grit chamber	Grit and sand removal
Skimmer and grease trap	Floating liquid and solid removal

Equalization tank	Damping of flow rate variations
Neutralization	Neutralizing acids and bases
Sedimentation and flotation	Suspended solids removal
Activated sludge reactor, tricking filter, aerated lagoon	Biological removal of soluble organics
Activated carbon adsorber	Removal of soluble non-biodegradable organic compounds
Chemical coagulation	Precipitation of phosphates
Nitrification-denitrification	Biological removal of nitrate
Air stripping	Ammonia removal
Ion exchange	Charged species removal
Bed filtration	Fine solids removal
Reverse osmosis and electrodialysis	Dissolved solids removal
Chlorination and ozonation	Pathogenic organism destruction

Water treatment systems for aquaculture have been developed on the basis of industrial wastewater treatment. However, various modifications are generally needed in order to treat large volume of wastewater from aquaculture pond that contains low concentration of wastes. Selection of the proper technology with low cost of construction and simple in operation are among the most important factor to the success of the system.

One of the most important limiting factors in intensive culture systems is the accumulation of toxic nitrogenous waste, such as ammonia, nitrite and urea (Ostrensky and Wasielsky Jr., 1995). Biological processes, so called biological nitrogen removal, are generally used for nitrogen treatment in aquaculture systems (Sundstorm, 1979). Chow (1972) concluded that the specific biological processes can be divided in three categories as follows:

2.4.1 Bacterial Assimilation: The bacterial assimilation method is based on the fact that the growth of microorganisms requires the availability of certain elements

and nutrients. With proper growth condition, bacteria can convert soluble forms of nitrogen and phosphorus into organic biomass. Regularly, carbohydrates or other organic compounds are being added as a source of carbon to enhance bacterial growth.

2.4.2 Harvesting of Algae: The use of aquatic plants and macroalgae for wastewater treatment has been widely recognized. With this mode of treatment, nitrogen in wastewater can be removed by harvesting algal biomass from the system. The disadvantages of this process are the large land-area requirements and the problems and costs associated with the harvesting and disposal of the algae.

2.4.3 Nitrification-Denitrification: The nitrification-denitrification process appears to be the most promising for many reasons such as high potential removal efficiency, process stability and reliability, easy process control, less land-area requirements and moderate cost. The removal of nitrogen with this process is carried out in one or two steps, depending on the nature of the wastewater. In the first step (nitrification), ammonia (NH_3 or NH_4^+) is aerobically converted to nitrate (NO_3^-). In the second step (denitrification), nitrate is anaerobically converted to nitrogen gas (N_2).

In detail, nitrification is the sequential, two-steps oxidation of ammonia to nitrate. The process is relative with two predominant bacteria genera. The oxidation of ammonia is mediated by *Nitrosomonas* and the oxidation of nitrite is mediate by *Nitrobactor* (Focht and Verstraete, 1977 cited in Hargreaves, 1998). Both organisms are chemoautotrophic, gram-negative, motile rods with long generation times. The reactions proceed are as follows:

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

Environmental factors that affect nitrification process include:

- Temperature: In general, most nitrifying bacteria can growth in wide range of temperature, from 8 to 30°C. But the optimum temperature is about 30 °C.
- Dissolved oxygen: Dissolved oxygen (DO) plays an important role in nitrification process. To achieve nitrification, DO must be higher than 1 mgO_2/L.
- pH: An optimum pH for nitrifying bacteria is approximately 7.8 (Hapogian and Riley, 1998).
- Light: Activity of nitrifying bacteria is inhibited by light (Johnstone and Jones, 1988; Diab and Shilo, 1988 cited in Hagopian and Riley, 1998)
- Surface attachment: In natural water, nitrifying bacteria are always found associated with suspended and settled particles. Therefore, the proper surface area for bacterial attachment must be provided in the treatment system.
- Inhibitors: Several compounds can directly or indirectly affect nitrifying bacteria. With direct effect, some compounds such as cyanide, thiourea, phenol, and anilines can inhibit the enzymes in nitrification process (Bitton (1994) cited in Wutikumpoln (2003). On the other hand, he suggested that many compounds could indirect inhibit nitrification process by reducing dissolved oxygen.

Denitrification is a biological process that can completely remove nitrogen from the system since nitrate-nitrogen is converted into nitrogen gas and therefore released to the atmosphere. With denitrification process, nitrate is a terminal electron acceptor during the oxidation of organic matter which therefore supplying energy for microbial growth. At least 14 genera bacteria can reduce nitrate, however, *Pseudumonas, Bacillus* and *Alcaligines* are among the most prominent denitrifying bacteria (Focht and Verstraete, 1977 cited in Hargreaves, 1998). The denitrification process can be shown as:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

2.5 Biofilter used in aquaculture system

Biological filter (biofilter) is a common technique used in biological water treatment. Biofilter is based on the use of supporting media with a high surface/volume ratio, pre-colonized with microorganism, to treat excess nutrients in the water. This similar to a process occurs in nature, where a microbial consortium associated with a matrix of extracellular polymeric substance bound to any submerged surface (so called biofilm) are responsible for many biogeochemical cycles in aquatic ecosystem (Deco, 1990: Meyer-Rail, 1994 cited in Thompson *et al*, 2002).

In aquaculture system, several types of biofilter have been commonly used (Silapakul, 2002). Most of them are nitrification biofilter. The detail of each biofilter type widely used in the closed-recirculating aquaculture systems are as follows:

2.5.1 Trickling filters

Trickling filters is a simple unit for nitrification process. The operation of trickling filter is performed simply by spraying wastewater at the top of the filter tank and let it flow down through the immobilized nitrifying bacteria on the packing material by gravity. The heat of reaction, despite only a slight quantity, is enough to force the fresh air to flow up counter-currently with the water flow. Oxygen required for microbial growth is directly transferred from the air to the biofilm attached to the surface of packing material. Trickling filter is one of a preferable method of nitrification biofilter used in aquaculture tank. Past works indicated that trickling filters had good performance for nitrification process. The advantage of trickling filter includes low maintenance, cheap installation and great tolerance to the differences in hydraulic and organic loads. However, the primary disadvantage of the trickling filter is the use of low surface area media that require large volumes and floor space (Wheaton, 1994 cited in Greiner, 1998).

2.5.2 Fixed-film biological filters/ Submerged filters

Fixed film biofilter or submerged filter is a conventional nitrification process in recirculating seawater systems. The column is packed with filter media (usually

coarse sand, gravel media, crushed rock media, plastic media or oyster shell) to provide surface area for nitrifying bacteria. During operation, the wastewater is passed through an aerated column. This type of biofilter was successfully used to treat ammonia in shrimp (*Penaeus monodon*) culture tank (Tseng, 1994 cited in Silapakul, 2002).

2.5.3 Rotating biological contactor and Biodrum

Rotating biological contactor (RBC) is consist of a series of disks made of lightweight plastic, which are mounted on the horizontal shaft and rotated while about one-half of their surface is immersed in wastewater tank. During operation, the rotated disks allow a film of wastewater to absorb oxygen directly from the air and, at the same time, trickling down in the biofilm layer. The rotation speed is adjustable to obtain the optimum desiccation time. The growth of attached bacteria is similar in concept to that of the trickling filter, except the microbes are relocated pass through the wastewater rather than gravity flowing of water pass through the fixed biofilm. Biodrum is similar process to the RBC but the series of disks are replaced with a drum packed with biofilter media.

2.5.4 Floating immobilized carriers

Floating immobilized system offers the advantage of using bacterial cells fixed with the small media with high specific surface area. With this technique, the carriers with attached biofilm are left floating in the nitrifying column. As the attached bacterial biofilm on the surface of the carriers are in responsible for the nitrification reaction, these carriers might be either moved within the airlifted reactor column or just left floating only at the top of the column.

A number of techniques have been developed in recently years for the control of total ammonia nitrogen (TAN) concentration in aquaculture systems. Several systems had been evaluated in pilot scale with varying degrees of success. Since nitrification biofilter has been intensively studied and being used in aquaculture systems, list of literatures concerning with these systems are shown in Table 2-3.

Table 2-3: Various types and operating results of nitrification biofilter used in aquaculture system.

References	Type of biofilter	Culture species	Rate
Fdz-Polanco *et al.* (1995)	Submerged biofilter	Not specify	Not specify
Hargrave *et al.* (1996)	Floating bead filter	hybrid tilapia (*Oreochromis niloticus*X*O. Aureus*)	54 mg/m^2/d (series filter) 81 mg/m^2/d (solitary filter)
Greiner and Timmons (1998)	Microbead VS trickling filter	hybrid tilapia (*Oreochromis niloticus*X*O. Aureus*)	0.13-0.57 g/m^2/d microbead filter 0.94-3.94 g/m^2/d trickling filter
Twarowska *et al.* (1997)	Rotating drum filter	Fingerling tilapia	0.33 gTAN/m^2/d
Kamstra *et al.* (1998)	Trickling filter	Eels	Not specify
Tseng *et al.* (1998)	Submerged biofilter	*Penaeus monodon*	Not specify
Sastry *et al.*(1999)	Bubble-washed bead filter	*Oreochromis niloticus*	0.451 g/m^2/d
Zhu and Chen (1999)	Series reactor system	Not specific	1.859 g/m^2/d
Ridha and Cruz (2001)	Polypropylene plastic chip VS poly ethylene block	*Oreochromis niloticus*	9.3 gN/m^3 media/d (chip) 8.9 gN/m^3 media/d (block)
Shan and Obbard (2001)	Immobilized pellet biofilter	Prawn culture	3.2 mgTAN/l/d
Grommen *et al.* (2002)	filter modules containing a layer of gravel	Not specify	0.3-0.5 gTAN/g of volatile suspended solids/d
Lekang and Kleppe (2002)	trickling filter with 2 types of plastic media	Not specify	0.2 gTAN/m^2/day (Norton rings) 0.1 gTAN/m^2/day (crushed leca)
Sandu *et al.* (2002)	fluidized bed biofilter	Not specify	0.225-0.27 gTAN/m^2/d

Thompson *et al.* (2002)	biofilter flexible PVC tube	shrimp (*Farfantepenaeus paulensis*)	Not specify
Zhu and Chen (2002)	fixed film biofilter	Not specific	Not specify
Al-hafedh *et al.* (2003)	Submerged biofilter	*Oreochromis niloticus*	3.46 gTAN/m^3/d (the hightest removal rate)
Gross *et al.* (2003)	plastic filter filled with plastic media	*Litopenaeus vannamei*	Not specify
Suzuki *et al.* (2003)	a cylindrical polyethylene filled in the nitrification tank	eel (*Anguila japonica*)	Not specify
Franco-Nava *et al.* (2004)	Submerged biofilter	European seabass (*Dicentrachus labrax*)	Not specify
Hwang *et al.* (2004)	Continous fixed slab reactor	Not specify	0.1-0.2 gTAN/m^2/d
Summerfelt and Sharrer (2004)	Fluidized sand filter	Salmonoid culture	0.51mgTAN/l
Tseng and Wu (2004)	Submerged biofilter	Eel culture	0.057 gTAN/m^2/d (field test1) 0.076 gTAN/m^2/d (field test2)
Xie *et al.* (2004)	Floating filter	Not specify	0.131 kg/m^3/d
Ling and Chen (2005)	Floating bead biofilter Fluidized sand filter Submerged bio-cube filter	Not specify	not specify but suggested that filter type and C/N ratio had highly effects on nitrification

Most studies on nitrification biofilter used in aquaculture system were performed in tanks under laboratory condition like described in Table 2-3. Only few studied were done in the outdoor earthen pond. Otoshi *et al.* (2003) compared growth and reproductive performance of broodstock shrimp reared in a recirculating aquaculture system (RAS: concrete pond) with a flow-through earthen pond (EP). Although results on this study indicated that overall growth rate was higher in the EP than in the RAS, but broodstock shrimp cultured in a biosecure RAS also had

acceptable growth and high survival rate. Burford *et al*. (2003) studied the effect of microorganisms and phytoplankton on water quality in the zero-exchanged shrimp pond in Balize Aquaculture Ltd. (BAL), Central America in comparison with conventional shrimp pond (earthen floor, low stocking density and periodic water exchange). The closed system pond consisted of shrimp culture ponds, sedimentation ponds and reservoir ponds which all ponds had plastic lining and high aeration rate. The results showed that nitrification did not significantly take place in the conventional shrimp pond. This was possibly due to a number of factors including water exchange rate in conventional pond prevented the slow-growing nitrifying bacteria becoming established and the high sludge loading in conventional ponds produced hydrogen sulfide which inhibit the nitrifying bacteria. On the other hand, the presence of flocculated matter in RAS performed as a substrate for nitrifying bacteria.

Apart of the biological filtration by nitrification process, Burford and Lorezen (2004) applying plastic-carpet material (Aquamats^{TM}) hanging in the shrimp (*Penaeus esculentus*) pond to be a substrate for the periphyton and bacterial biofilm. The result showed that the benefits in providing substrates for shrimp pond is to provide additional food source for shrimps. Other periphyton studies in aquaculture ponds were mainly related with fish culture. Most of them suggested that periphyton could enhance nitrogen retention, reduce toxic nitrogenous wastes and improve fish production (Azim *et al*, 2002; Huchette and Beveridge, 2003; Azim *et al*, 2003; Keshawanath *et al*, 2004).

In Thailand, nitrification biofilter used in an outdoor recirculating shrimp pond was rarely studied. One of the examples was reported by Tookwinas *et al*. (1998) that used aerobic and anaerobic treatment for the closed-system intensive tiger shrimp pond. The result illustrated the success of using aerobic biofilter via nitrification process since 78% of nitrogen compound was converted to nitrate while the anaerobic treatment using denitrification was not feasible. Songsangjinda *et al*. (1999) used sand filter as the physical treatment and macroalgae for biological treatment in shrimp pond. Post-larva of black tiger shrimp were grown in 3,150 m^2 pond at the density 100,000 shrimp/Rai for 147 days. The results showed that shrimp production was 1.2 ton/Rai and survival rate was 73.5 %. Na-anan *et al*. (2000) studied on the closed

recirculating shrimp pond using the same treatment system of Songsangjinda *et al.* (1999). The results were similarly to Songsangjinda *et al.* (1999) with acceptable shrimp production and survival rate and they concluded that the closed recirculating culture system was an alternative way to the sustainable shrimp culture.

The design and construction of biofilter system in this study was improved from the closed-system for tilapia fish culture used in Wutikumpol (2003). The same biofilter material, Hyperdrain™, that had been used by Wutikumpol (2003) was chosen but the system design was improved by better aeration system and biofilter arrangement. The detail of the design and construction is shown in the materials and methods.

CHAPTER III

MATERIALS AND METHODS

This study consisted of two trials of shrimp cultivation in the outdoor ponds and another two additional experiments to evaluated the efficiency of the biofilter under both laboratory and field conditions. The biofilter media used in this study was the Hyper Drain™ which made of a bundle of recycled polypropylene plastic (Figure 3-1) arranged into the cylinder-shape. This biofilter media had 12 cm in diameter and the total length of 4 meters and it was cut to the desired length prior to use. The advantage of this biofilter is that it has high surface to volume ratio. It was the same biofilter media that had been used in Tilapia fish culture pond by Wutikumpoln (2003).

Figure 3-1: The Hyper Drain™, a polypropylene biofilter media used in this study

3.1 Evaluation of the biofilter efficiency in ammonia removal

3.1.1 Efficiency of the biofilter in ammonia treatment under laboratory condition

In general, biofilter media must be incubated for at least 15-20 days before the nitrifying bacteria become active. With this experiment, biofilter was cut into 30 cm

in length and was acclimated in the shrimp pond for more than 20 days to allow the formation of natural microbial biofilm. The acclimation was done at the same time as shrimp culture experiment (Section 3.2) in order to obtained the similar microbial community. As there were two shrimp ponds that contained biofilter units (treatment pond 1 and treatment pond 2, see 3.2), one biofilter was then collected from each pond to be used in this experiment. Thereafter, biofilter was transferred to the laboratory at Chulalongkorn University. The experiment consisted of two trials, both trials were similar in practice except the sampling interval. The experimental unit was a plastic tank filled with 80 liters of 6 psu seawater and continuously aerated using an air-stone. Four tanks were used for each trial and the detail of treatment is shown in Table 3-1 and the photograph of the experiment is shown in Figure 3-2.

Table 3-1: Experimental design for the evaluation of ammonia treatment efficiency by the biofilter under laboratory condition.

Experimental unit	Condition
Control 1	without biofilter
Control 2	with new (unused) biofilter
Treatment 1	with biofilter from shrimp treatment pond 1
Treatment 2	with biofilter from shrimp treatment pond 2

Figure 3-2: The photograph of the experimental units during the experiment.

With the first trial, ammonium chloride (NH_4Cl) solution was added in all tanks to the final concentration of approximately 4 mg NH_4-N/L. Ammonia treatment was investigated by monitoring the decrease in ammonia concentration. In addition, NH_4Cl was added at hour 14 and at hour 17 for the first and the second trial respectively. This prevent the absent of ammonia in treatment tanks that had higher ammonia removal rate than control. With the second trial, an initial ammonium concentration was similar to that of the first trial. Rate of ammonia removal via biofilter per day can be calculated as follow:

$$\text{Rate of ammonia removal} = \left[\frac{N_t - N_0}{t} \right] \times V \times 24 \times L$$

N_t = Ammonia concentration at the time t

N_0 = Ammonia concentration at the initial

t = time (hour)

V = water volume in each tank (Liter)

L = length of the biofilter (m)

3.1.2 Efficiency of the biofilter in ammonia treatment under field condition

The evaluation of biofilter efficiency was performed under natural environment at the closed-recirculating shrimp farm in Patumthani Province located at the north of Bangkok. Before the experiment, biofilter was cut into small pieces, 2 or 4 cm in length, and then immerged in the shrimp pond at approximately 20 cm below the water surface for 30 days. The experiment units were the transparency tube, cylinder-shape made of transparent PVC plastic. The tube was 30 cm in diameter and 150 cm in height. The photograph of the plastic tubes is shown in Figure 3-3.

Figure 3-3: The custom-made clear PVC tubes used in the field experiment.

These PCV tubes were placed vertically in the shrimp pond with one end penetrated in the pond bottom at approximately 15 cm depth while another end was above the water surface. As the pond depth was 110 cm, therefore, the tube contained approximately 77.7 L of water. For the experiment, the total of 6 tubes was divided in to three groups. The experimental plan is shown in Table 3-2 and a diagram of the experiment is shown in Figure 3-4.

Table 3-2: Experimental design for the evaluation of ammonia treatment efficiency by the biofilter under field condition.

Experimental unit	Condition	Tube no.
Control	no biofilter, aerated with an air-stone	1, 2
Treatment 1	With 2 cm length biofilter, aerated with an air-stone	3, 4
Treatment 2	With 4 cm length biofilter, aerated with an air-stone	5, 6

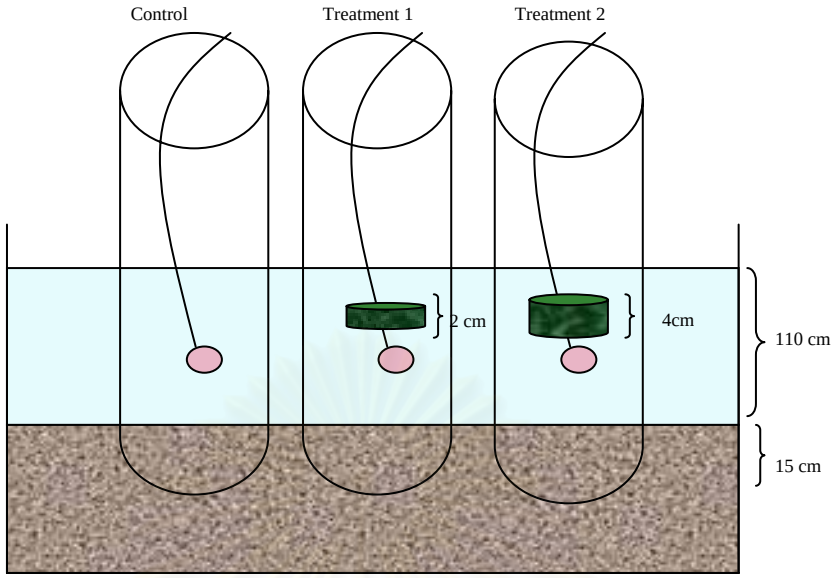


Figure 3-4: The diagram represents the experimental tubes used in the study of biofilter efficiency under field condition.

All experimental tubes were gently aerated with an air-stone and left under natural condition throughout the experiment. At the beginning, ammonium chloride was added into the chamber to the final concentration of approximately 2 mg NH_4-N/L. Thereafter, during the first trial, water was sampled at 0, 15 h, 43 h and 15 day while in the second trial, water was sampled at 0, 2, 5 and 16 days. Concentration of inorganic nitrogen (ammonia, nitrite and nitrate) in the chambers was analyzed using the standard method for water analysis (see 3.3.2-3.3.4).

3.2 Use of biofilter in the outdoor closed-recirculating shrimp pond

3.2.1 Facility

The closed-recirculating shrimp culture systems were set up at the shrimp farm in Patumthani province. The experimental units consisted of four 29x29 m^2 (0.5 Rai) earthen ponds with approximately 1.2 m depth. Since the shrimp farm was in the freshwater area, high salinity seawater (28 psu) was added into the ponds to increase the salinity to 6 psu. During experiment, there was no addition of any chemicals or antibiotics except the use of $CaCO_3$ to maintain alkalinity.

3.2.2 Design and construction of the closed-recirculating shrimp ponds

This experiment was designed for evaluating the efficiency of using nitrification biofilter for ammonia removal and horizontal net as an additional surface for shrimp attachment in the outdoor shrimp pond. For the treatment pond with biofilter, five sets of biofilter unit, each made of 11 cylinder-shape porous plastic tube "Hyperdrain^{TM}" (12.5 cm in diameter and 1.5 m in length), were arranged in parallel like a raft and fixed over the aerator disc (Figure 3-5, 3-6). The specification of biofilter is shown in appendix. In addition, to increase substrate for shrimp, eight pieces of 5x2.5 m^2 (2.5 cm mesh) nylon net were arranged in horizontal position by fasten with series of bamboo poles. The net's plane was 20 cm above the bottom of the pond (Figure 3-7, 3-8).

Figure 3-5: Biofilter (cylinder-shape porous plastic tubes) and aerator disc before being installed in the pond.

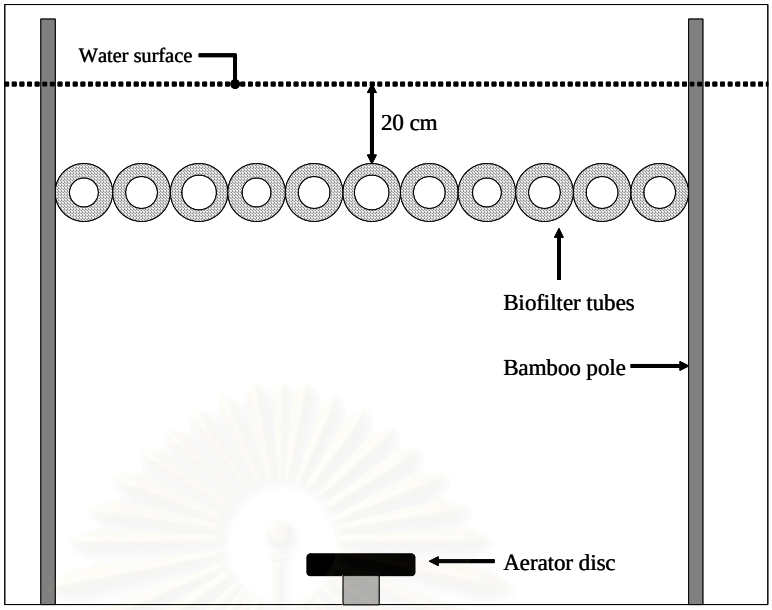


Figure 3-6: Diagram of a set of biofilter in the treatment ponds

Figure 3-7: Installation of the net in treatment ponds. This net was an additional substrate for shrimp attachment

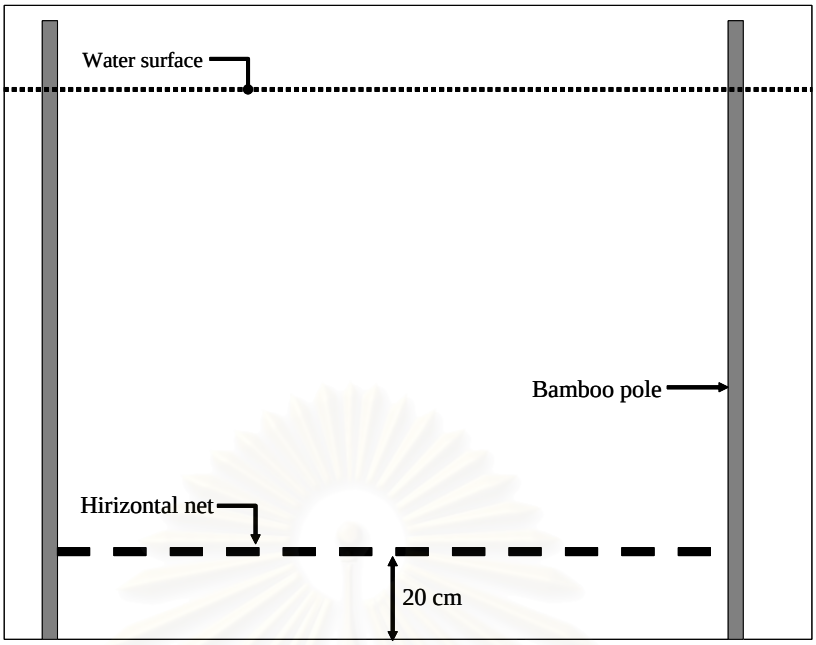


Figure 3-8: Diagram of the net installment in treatment ponds. The net's plane was 20 cm above the pond bottom.

3.2.3 Experimental design

The study was carried out in private shrimp farm in Patumthani province. Four shrimp ponds were used and the detail of pond assignment is shown in Table 3-3. The experiment consisted of two trials (crops) of shrimp culture. With the first trial, the experimental ponds were divided in to control ponds (without biofilter) and treatment ponds (with biofilter and net). With the second trial, all four ponds were installed with biofilter and net. The assignment of shrimp ponds in this study is in Table 3-3.

Table 3-3: Assignment of shrimp ponds used in this study.

Pond number, as assigned by the farm	Trial I of this study	Trial II of this study
Pond no. 2	Control pond 1 (A)*	Pond no. 1 (A, BF, N)
Pond no. 3	Control pond 2 (A)	Pond no. 2 (A, BF, N)
Pond no. 4	Treatment pond 1 (A, BF, N)	Pond no. 3 (A, BF, N)
Pond no. 5	Treatment pond 2 (A, BF, N)	Pond no. 4 (A, BF, N)

*Remark: A=aerator, BF=biofilter, N=horizontal net

In detail, all experimental ponds were installed with five aerators. Four aerator discs were placed at the corner sites and one aerator was at the center of the pond. Biofilters and horizontal net were set up only in the treatment ponds of trial I and in all ponds of trial II (Figure 3-9, 3-10 and 3-11). Stocking density of the P15 shrimps were 50,000 larva/pond (60 shrimp/m^2) and 40,000 larva/pond (48 shrimp/m^2) for the first and second trials respectively.

In trial I, 50,000 of post-larva black tiger shrimps were grown for 113 days (from April 4th, 2003 to August 14th, 2003). In trial II, post-larva black tiger shrimps were cultured at 40,000 post-larva/pond for 114 days (from December 29th, 2003 to April 20th, 2004). There was no water exchanged or discharged during the experiment.

Figure 3-9: The control pond containing only five aerators.

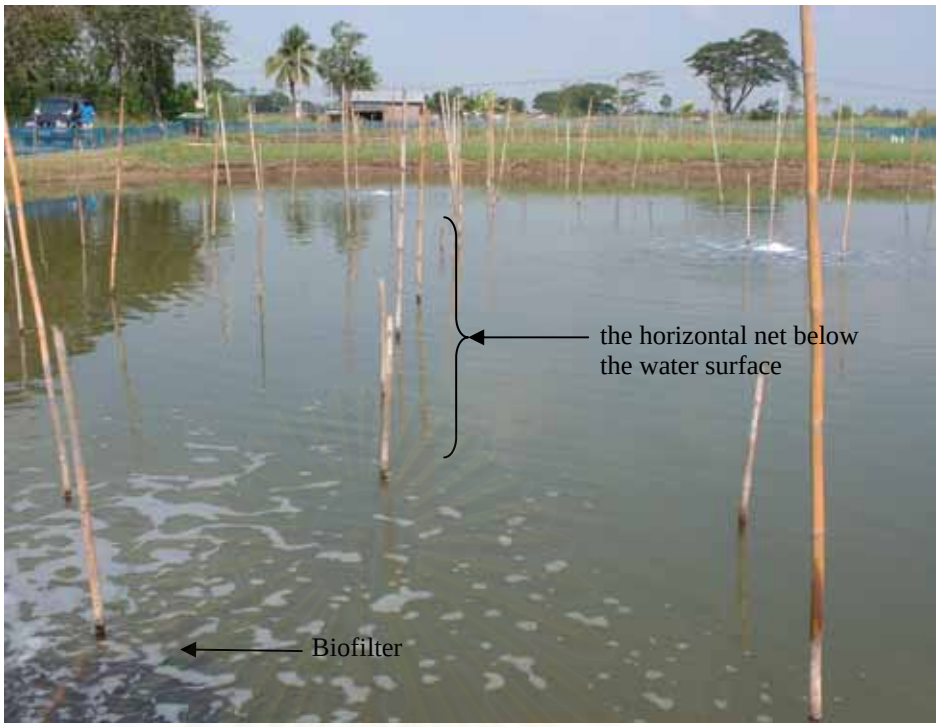


Figure 3-10: The treatment pond containing biofilter sets and horizontal net

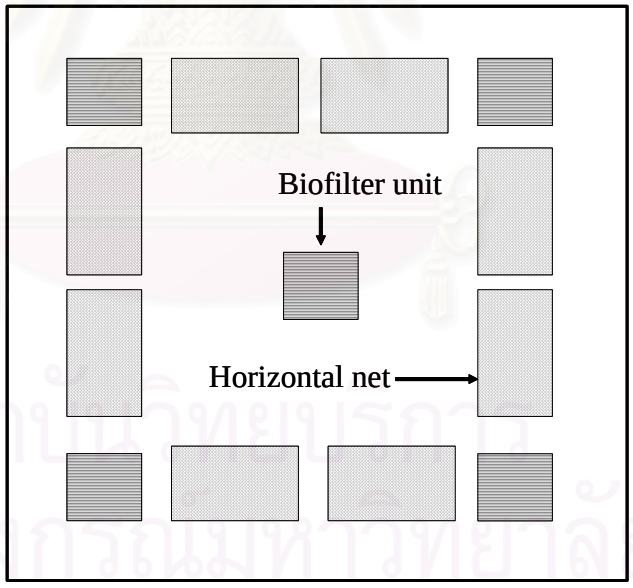


Figure 3-11. Diagram illustrated biofilter units and horizontal nets installed in treatment ponds of trial I and in all ponds of trial II.

During experiment, shrimps were fed with commercial shrimp feed (STAR FEED®). Feeding amount and frequency was adjusted according to the regular feeding program. During the first month, shrimps in each pond were fed 200-300

grams/meal, 3 times/day at 06.00, 12.00, and 17.00 hr. During the second month, shrimps were fed at 400-800 grams/meal, three times/day at 06.00, 12.00, and17.00 hr. Finally, during the third and fourth months, shrimps were fed 800-1,000 grams/meal, four times/day at 06.00, 12.00, 17.00, and 22.00 hr. However, feeding amount was also adjusted to the shrimp consumption as observed with feeding pens.

3.2.4 Data acquisition

Water quality, shrimp growth and shrimp production were regularly monitored throughout the experiment. In each pond, water sample which was a mixture of the water from five sampling points around the pond was collected with 1 L plastic bottle. Water samples were then kept in the ice box and immediately transferred to the laboratory. The samples were analyzed within 24h or kept in -20°C refrigerator if necessary. The detail of sampling program and analytical methods are shown in Table 3-4.

Table 3-4: Monitoring program of environmental parameters and shrimp growth during the experiment.

Parameter	Method/instrument/detail	Sampling interval
Temperature	YSI D.O. meter	Every 2 weeks
Dissolved oxygen	YSI D.O. meter	Every 2 weeks
pH	pH meter (HANNA instrument)	Every 2 weeks
Salinity	Hand refractometer	Every 2 weeks
Transparency	Secchi disc	Every 2 weeks
Alkalinity	Titration (Strickland and Parsons, 1972)	Every 2 weeks
Biochemical oxygen demand	Titration (Strickland and Parsons, 1972)	Every 2 weeks
Ammonia	Colorimetric (Strickland and Parsons, 1972)	Every 2 weeks
Nitrite	Colorimetric (Strickland and Parsons, 1972)	Every 2 weeks
Nitrate	Colorimetric (Strickland and Parsons, 1972)	Every 2 weeks
Phosphate	Colorimetric (Strickland and Parsons, 1972)	Every 2 weeks
Chlorophyll_*a*	Extracted using 90% acetone (Strickland and Parsons, 1972)	Every 2 weeks

Plankton	Sedgewick-Rafter counter	Every 2 weeks
Organic matter in soil	Burned in 700°C oven	Every 4 weeks
Ammonia, Nitrite and Nitrate in soil	Colorimetric (Strickland and Parsons, 1972)	Every 4 weeks
Shrimp weight	Weight with 2 decimals electrical balance	Every 4 weeks
Shrimp length	Measure the total length (from rostum to tail) with ruler	Every 4 weeks
FCR (feed conversion ratio)	Comparing between shrimp weight gain and total feed used	At the end of the trial
Shrimp production	Total weight of shrimp	At the end of the trial
Survival rate	Comparing between total shrimp at the first and the last day	At the end of the trial

3.3 Water quality analysis

3.3.1 Alkalinity

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

Alkalinity (mg/L) = (H_2SO_4 used during titration x 1000)/Volume of seawater sample

3.3.2 Ammonium (NH_4^+-N)

Ammonium was analyzed by phenol-hypochloride reaction method as modified from Strickland and Parson (1972). Five milliliters of filtered water sample diluted with deionized water was mixed with 0.2 ml of phenol solution (dissolve 20 g

of crystalline analytical grade phenol in 200 ml of 95% (v/v) ethyl alcohol), 0.2 ml of sodium nitroprusside solution (1 g of $Na_2Fe(CN)5NO.2H_2O$ in 200 ml of deionized water), and 0.5 ml of freshly prepared oxidizing reagent (100 g of sodium citrate and 5 g of sodium hydroxide in 500 ml of deionized water mixed with sodium hypochloride 4:1 (v/v)) respectively. After mixing and stand for 1 hour, absorbance of the solution was measured at 640 nm using spectrophotometer (GENESIS®; model 10 UV scanning) against the blank (deionized water). Concentration of ammonium was calculated using the standard curve of ammonium solution (0.01-1.00 mg NH_4^+-N/L)

3.3.3 Nitrite (NO_2^--N)

Nitrite was analyzed by sulfanilamide reaction as modified from Strickland and Parson (1972). Five milliliters of diluted water sample was mixed with 0.1 ml of sulfanilamide solution (dissolve 5 g of sulfanilamide in a mixture of 50 ml of concentrate hydrochloric acid and about 300 ml of distilled water) and 0.1 ml of (1-Naphthyl)-ethylenediamine dihydrochloride solution (0.5 g of N-(1-Naphthyl)-ethylenediamine dihydrochloride in 500 ml of distilled water). After mixing and stand for 1 hour, absorbance of the solution was measured at 543 nm using spectrophotometer (GENESIS®; model 10 UV scanning) against distilled water blank. Concentration of nitrite was calculated using the standard curve of nitrite solution (0.007-0.224 mg NO_2^-N/L)

3.3.4 Nitrate (NO_3^--N)

Nitrate was analyzed by cadmium-conversion of nitrate to nitrite following by nitrite analysis using sulfanilamide reaction as described in nitrite analysis (3.3.3). Before analysis, 1 ml of concentrate ammonium chloride solution was added in 50 ml of filtered water sample. This mixture was poured in the glass column containing cadmium granules coated with copper sulfate. Flow rate of the cadmium column was set up at 50 ml per 5 minutes. With cadmium column, nitrate in water sample was converted to nitrite. Therefore, the correct nitrate-N concentration must be subtracted with nitrite-N concentration in the sample.

3.3.5 Phosphate (PO_4^--P)

Phosphate was analyzed by ammonium molybdate reaction (Strickland and Parson, 1972). Prior to analysis, 5 ml of water sample was mixed with 0.5 ml of mixed reagent (mixture of 100 ml ammonium molybdate, 250 ml sulphuric acid, 100 ascorbic acid and 50 ml potassium antimonyl-tartrate solutions) was added in each sample tube. After mixing and stand for 1 hour, absorbance of the solution was measured at 885 nm using spectrophotometer (GENESIS®; model 10 UV scanning) against distilled water blank. Concentration of phosphate was calculated using the standard curve of phosphate solution (0.01-1.00 mg PO_4^--P/L).

3.3.6 Biological Oxygen Demand (BOD)

The determination of biological oxygen demand (BOD) was modified from Strickland and Parson (1972).

Water samples were aerated to saturation concentration of oxygen and then transferred into two 300 ml BOD bottles. Oxygen concentration in the first BOD bottle was analyzed immediately with chemical dissolved oxygen analysis. Oxygen concentration in the second BOD bottle was analyzed after incubated the BOD bottle at 20°C for 5 days. The correct biological oxygen demand was calculated by the subtraction between the dissolved oxygen in the first day and the remaining oxygen in the later 5 days.

To measure dissolved oxygen, water in the BOD bottle was mixed with 1.0 ml of manganous sulphate reagent (365 g of manganous sulphate monohydrate in 1 L of distilled water) and 1.0 ml of alkaline iodide solution (500 g of sodium hydroxide in 500 ml of distilled water mixed with 300 g of potassium iodide in 450 ml of distilled water). Then, these contents were mixed thoroughly by shaking until the precipitated manganous-manganic hydroxide was dispersed. Before analysis, 0.1 ml of concentrated sulfuric acid was added in the BOD bottle to dissolve the precipitate. Fifty milliliter of the solution were transferred into a flask and titrated with standard

0.01 N thiosulphate solution until a very pale straw color remained. Five milliliter of starch indicator was added and concluded the titration.

3.3.7 Chlorophyll_*a*

The chlorophyll_*a* analysis method used in this study was according to Strickland and Parson (1972). Phytoplankton cells in approximately 100 ml of the water sample were filtered with a 47-mm Whatman GF/C filter paper. Chlorophyll-a pigment was then extracted using 90% acetone in cool and complete darkness for 20 hours. After centrifugation, absorbance of acetone supernatant was measured at 630, 645 and 665 nm using spectrophotometer against 90% acetone blank.

The concentration of pigments was calculated following this equation:

$$\text{mg (or m-SPU) pigment/m}^3 = C/V$$

where C was a value obtained from the following equation

$$C \text{ (chlorophyll\_}a\text{)} = 11.6_{665nm} - 1.31_{645nm} - 0.14_{630nm}$$

and V was the volume of water filtered in liters.

3.3.8 Plankton

Phytoplankton and zooplankton in shrimp pond was collected by filtering 24 L of water from shrimp pond with 43 microns plankton net. The samples were fixed with formaldehide solution to the final concentration of 2% v/v. Finally, the sample was classified and counted using a Sedgewick-Rafter counter under light microscope according to Paphawasit *et al* (2003).

3.3.9 Organic matter in soil

Soil sample in shrimp pond was collected using grab sampler. The sample was air-dried at room temperature and following by oven-dried at 110°C for 2 hours. Dried soil sample was further burned at 700°C for 2 hours in exactly known weight ceramic crucible and cool down in desiccator. Finally, the organic matter can be calculated as the following:

$$\%\text{organic matter} = \frac{\text{the subtraction between the initiate and the later soil weight} \times 100}{\text{the initiate soil weight}}$$

3.3.10 Nutrient analysis in soil

One grams of dried soil sample (in powder) was re-dissolved with 10 ml of deionized water. After vigorous shaking, the supernatant was collected. The supernatant liquid should be processed with in 24 hr or kept in the refrigerator if necessary. Nutrients *i.e.* ammonium, nitrite and nitrate was then analyzed using the method previously described (see 3.3.2-3.3.4).

CHAPTER IV

RESULTS

4.1 Evaluation of the biofilter efficiency in ammonia removal

4.1.1 Efficiency of the biofilter in ammonia removal under laboratory condition

The results from laboratory condition showed that, after adding NH_4Cl solution to each tank, ammonium concentrations were rapidly decreased in treatment tanks containing biofilter from shrimp pond (Figure 4-1 and 4-2). With the first trial, ammonia concentration in treatment 1 reduced from 2.51 to 0.83 mg NH_4-N/L and slightly lower reduction from 1.91 to 1.70 mg NH_4-N/L was found in treatment 2. On the other hand, ammonia concentrations were almost constant in control 1 and even increased in control 2. After 14 hours, ammonia concentration in treatment tanks was close to zero. Hence, NH_4Cl was added to prevent the absent of ammonia in treatment tanks. After checking ammonia removal every 4 hours, the results confirmed that ammonia removal in treatment tanks were faster than control tanks. Within 50 hours, ammonia concentration was reduced from 3.82 to 0.01 mg NH_4-N/L and from 3.64 to 0.91 mg NH_4-N/L in treatment 1 and treatment 2, respectively. In contrast, both control tanks had significantly lower ammonia reduction rate. The calculation results showed that the nitrification rate of 1 month-old biofilter from shrimp ponds as determined under laboratory condition were 693 mg-N/m/day for biofilter from treatment pond 1 (pond no.4) and 230 mg-N/m/day in biofilter from treatment pond 2 (pond no.5), respectively.

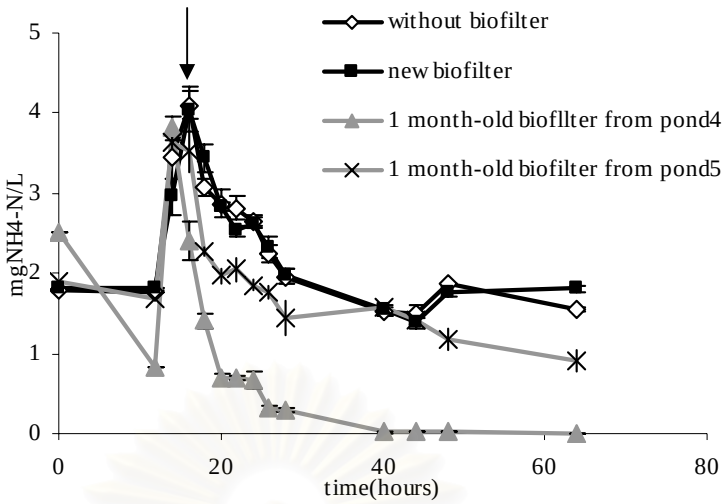


Figure 4-1: Biofilter efficiency in ammonia treatment under laboratory condition (first trial)

In the second trial, reduction of ammonia in treatment tanks was faster than in control tanks. For more detail, within 17 hours, ammonia concentration rapidly decreased from 2.73 to 0.77 mg NH_4-N/L and from 2.75 to 1.93 mg NH_4-N/L in treatment 1 and 2, respectively. On the other hand, ammonia concentration in control tanks during the first 17 hours was slightly reduced from 2.71 to 2.17 mg NH_4-N/L. Reduction of ammonia after an addition of NH_4Cl at 17th hour clearly showed that treatment tanks containing active biofilters from shrimp pond had higher ammonia removal than in controls. It was found that biofilters removed more than 70% of ammonia within 24 hours. The similar results from both trials confirmed that biofilter had a capability in ammonia removal. Calculation of ammonia removal rate of both trials is showed in Table 4-1.

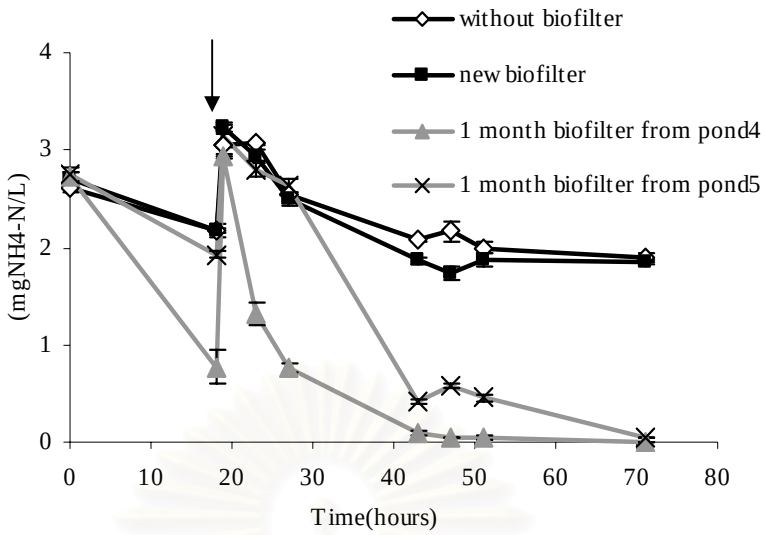


Figure 4-2: Biofilter efficiency in ammonia treatment under laboratory condition (second trial).

Table 4-1: Calculation of ammonia removal rate of biofilters from shrimp ponds under laboratory condition.

Source of biofilters	Ammonia removal rate (mg NH^4-N/m/day and mgNH4-N/m^2 surface area/day)*
From pond 4, Trial I	693.92
From pond 5, Trial I	230.71
From pond 4, Trial II	529.39
From pond 5, Trial II	336.34
Average (±SD)	447.59± 205.58

* Hyper drain media surface area is 1.0 m^2/m

Observation of the biofilters from shrimp ponds (Figure 4-3) using light microscope showed that biofilm attached with the biofilter was predominated with bacteria and microorganisms. Several species of phytoplankton, both benthic and planktonic species (Figure 4-4), and zooplankton (Figure 4-5) were also found.

Figure 4-3: One month-old biofilter coated with sludge and biofilm

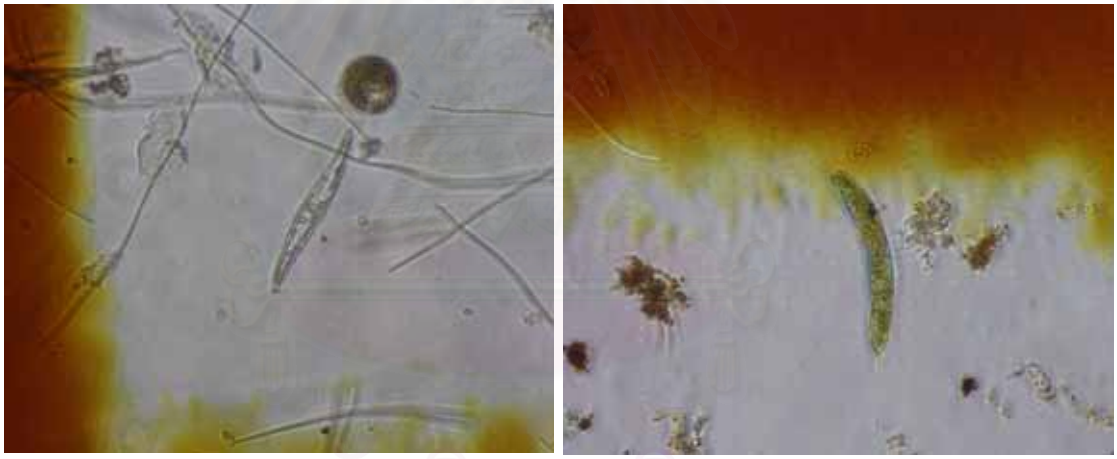


Figure 4-4: Phytoplankton found in biofilm taken from 1 month-old biofilter

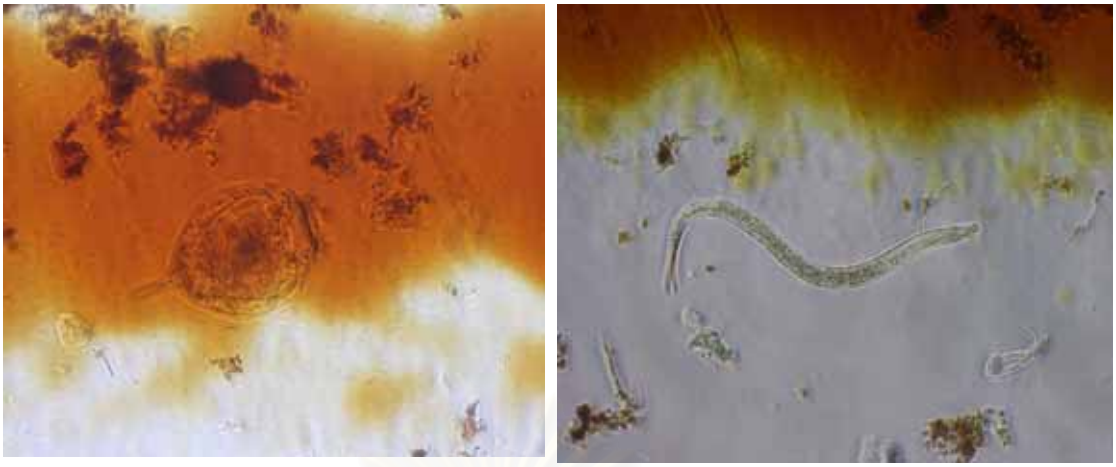


Figure 4-5: Zooplankton and nematode found in biofilm taken from 1 month-old biofilter

4.1.2 Efficiency of the biofilter in ammonia treatment under field condition

The efficiency of ammonia removal by nitrification biofilter was investigated in shrimp pond using transparence chambers containing 2 or 4 cm length of biofilter. The photograph of this experiment is showed in Figure 4-6.

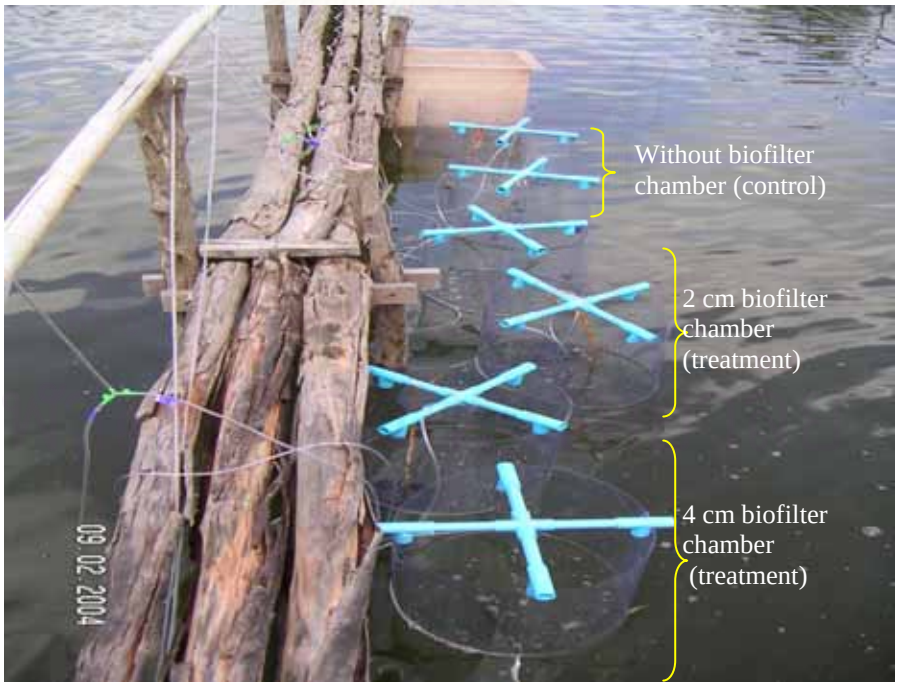


Figure 4-6: Experimental unit used during the evaluation of nitrification of the biofilter under field condition

After adding ammonium chloride at the final concentration approximately 2 mg NH_4-N/L to each chamber, concentrations of inorganic nitrogen (ammonia, nitrite and nitrate) in all chambers were monitored and the results are showed in Figure 4-7, 4-8, and 4-9. In the first trial, concentration of ammonia in all chambers during the first 43 hours was slightly reduced from 3.09 to 2.50 mg NH_4-N/L in the chamber without biofilter, 2.67 to 2.44 mg NH_4-N/L in the chamber containing 2 cm biofilter, and 3.63 to 2.49 mg NH_4-N/L in the chamber containing 4 cm biofilter. However, significant difference in ammonia concentration was found at day 15 in which chambers contained biofilter had lower ammonia than in control (Figure 4-7A). The increasing of nitrate concentration were found in all chambers, rising from undetected concentration to 0.05, 0.03 and 0.06 mg NO_3-N/L in the chamber without biofilter, the 2 cm biofilter chamber and the 4 cm biofilter chamber, respectively (Figure 4-7B). These data indicated that there was the nitrification process occurred in the chambers.

With the second trial, significance difference in ammonia removal was found in day 5 and day 16 of the experiment (Figure 4-8A). The results were similar to the first trial that significant difference in ammonia removal was found between control and treatment. Within 16 days, ammonia concentration was reduced from 2.67 to 0.48 mg NH_4-N/L in the without biofilter chamber, reduced from 3.22 to 0.09 mg NH_4-N/L and 2.17 to 0.08 mg NH_4-N/L in the chamber containing 2 cm or 4 cm biofilter, respectively. However, these data showed that biofilter length of 2 or 4 cm provided the same ammonia reduction rate. Nitrate concentration, on the other hand, was found highest in day 5 and declined afterward (Figure 4-8B).

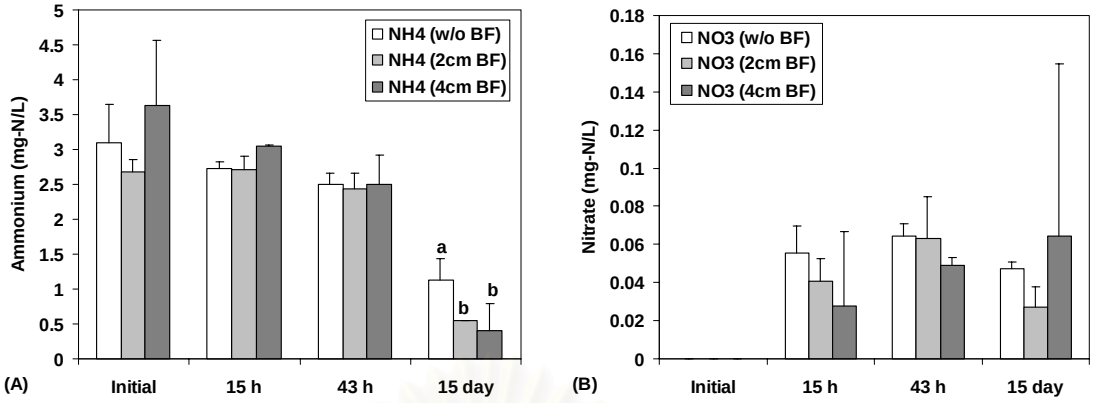


Figure 4-7: Concentration of ammonia (A) and nitrate (B) in the chamber with 2 or 4 cm length biofilter (BF) or without biofilter (w/o BF) during the first trial.

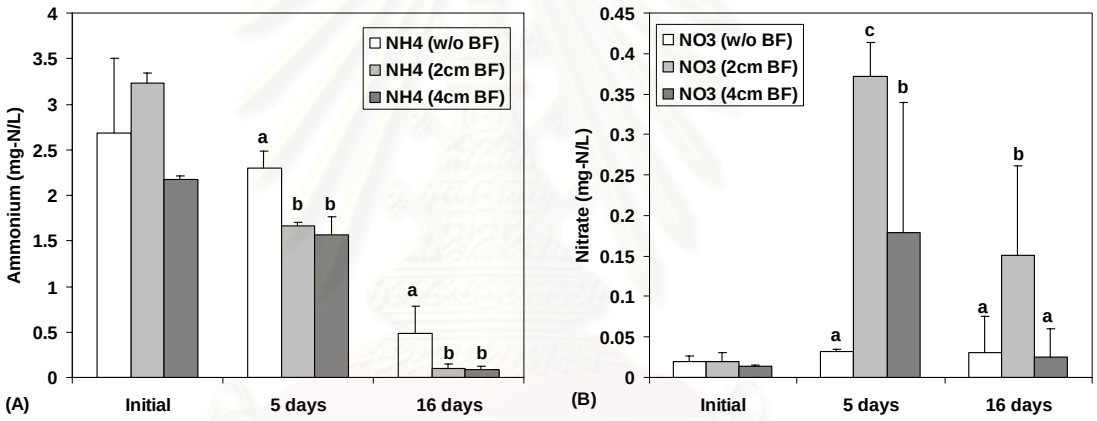


Figure 4-8: Concentration of ammonia (A) and nitrate (B) in the chamber with 2 or 4 cm length biofilter (BF) or without biofilter (w/o BF) during the second trial.

Increase of nitrite concentration, which is the intermediate in nitrification process, indicated that nitrification process occurred in the chambers that containing biofilter. In the first trial, nitrite concentration in all chambers was mostly constant in the first 43 hours (approximately 0.1 mg NO_2-N/L). However, significant difference in nitrite concentration was found at day 15 in which treatment chambers had higher nitrite than in control chambers (Figure 4-9A). With the second trial, significant difference of nitrite concentration was also found in day 5 and day 16 of the experiment (Figure 4-9B). The result was similar to the nitrate result in Figure4-8B that nitrite concentration was found highest in day 5 and declined afterward.

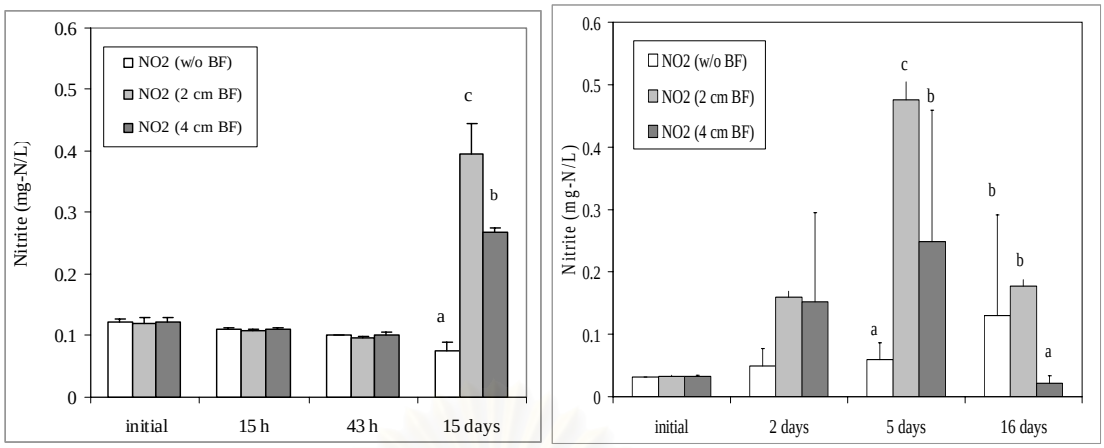


Figure 4-9: Concentration of nitrite in the chamber with 2 or 4 cm length biofilter (BF) or without biofilter (w/o BF) in the first trial (A) and the second trial (B).

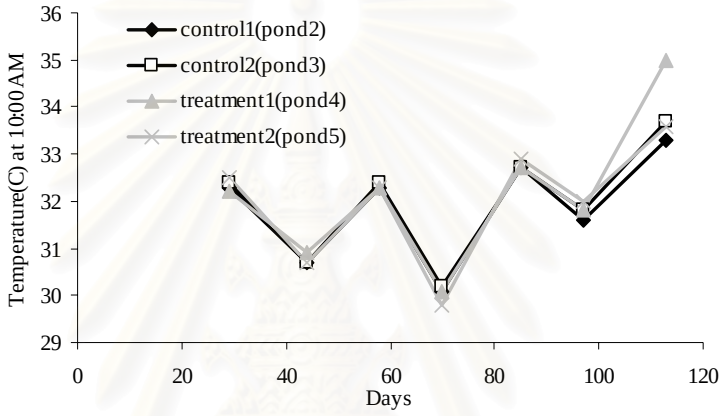
4.2 Efficiency of biofilter in the outdoor closed-recirculating shrimp pond

This experiment was designed for evaluating the efficiency of using nitrification biofilter for ammonia removal and horizontal net as an additional surface for shrimp attachment in the outdoor shrimp pond. The results in this section were divided into five parts including water quality (4.2.1), nutrient dynamics (4.2.2), chlorophyll and plankton dynamics (4.2.3), shrimp growth determination (4.2.4), and soil analysis (4.2.5).

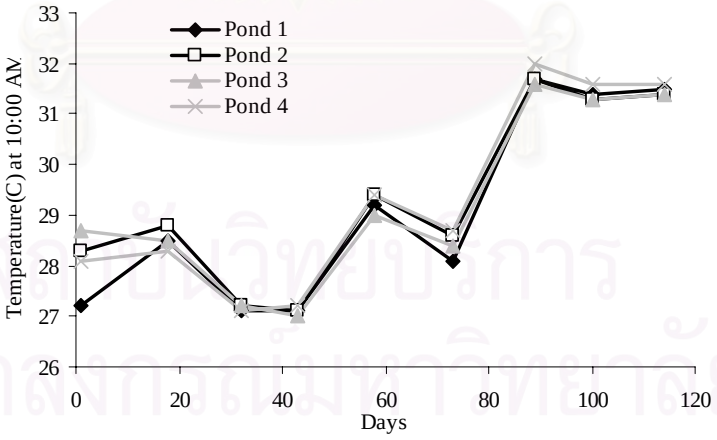
4.2.1 Water quality

4.2.1.1 Temperature

As shown in Figure 4-10, water temperature in all ponds of trial I fluctuated between 29.8-35 °C. In trial II, water temperature was between 27-31.7°C. Increase of temperature in the last month of the experiments was found in both trials. However, there was no significant different between water temperature in control and treatment ponds.



(A)



(B)

Figure 4-10: Water temperature monitored at 10:00 am during (A) trial I (B) trial II

4.2.1.2 pH

The results of pH monitoring at 10:00 am in both trials are shown in Figure 4-11. In trial I, pH fluctuated between 6.22 and 9.02 and pH in treatment ponds was lower than in control ponds throughout the experiment. In trial II, the pH was between 6.82 and 9.43 and the difference in pH was found at the end of the experiment in which the pH in pond 4 was only 6.82 while pH in other ponds was higher than 7.30.

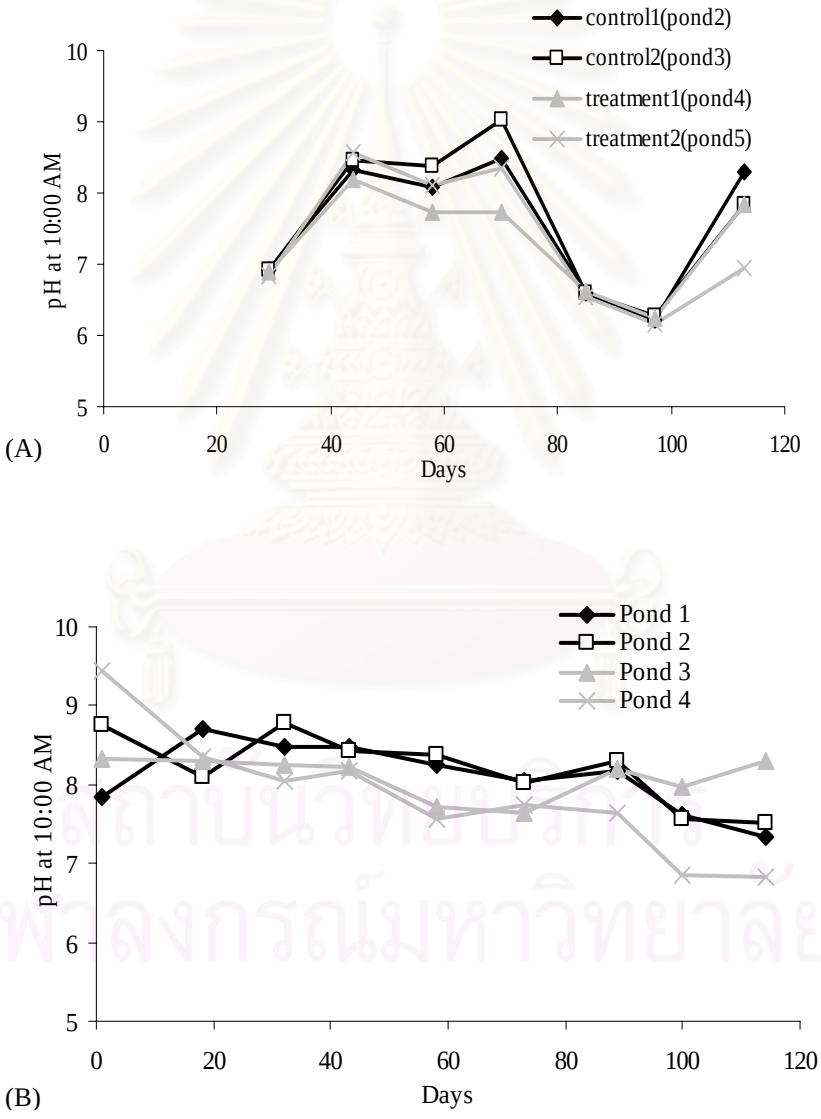
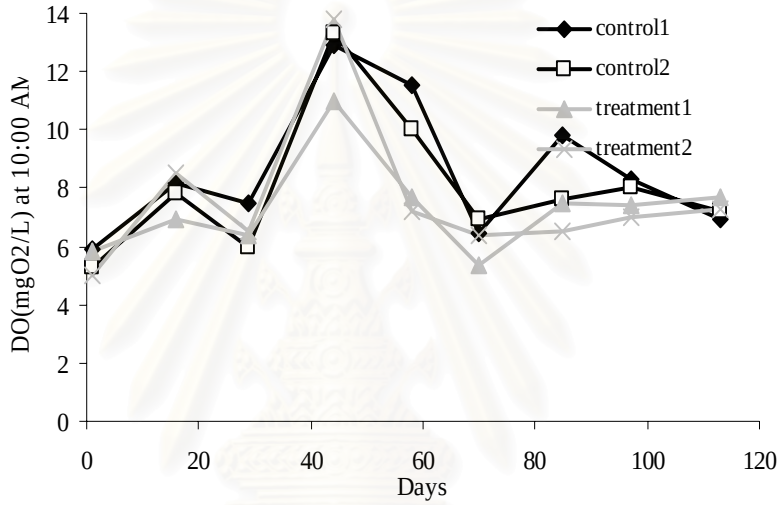


Figure 4-11: Water pH monitored at 10:00 am during (A) trial I (B) trial II

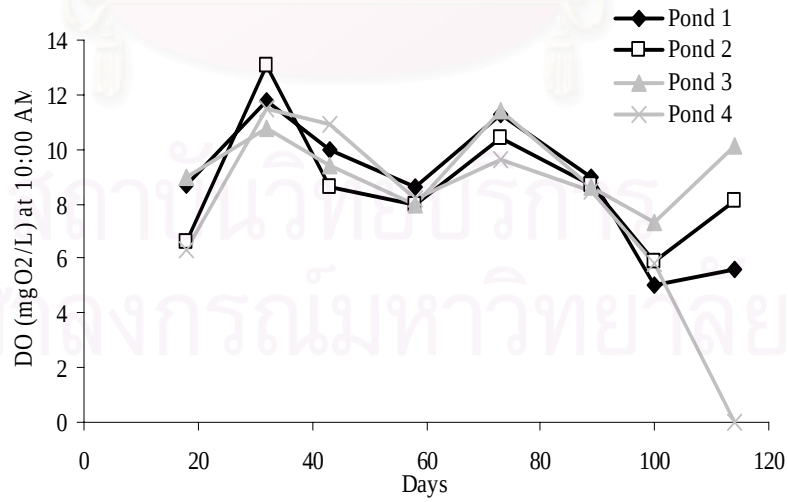
4.2.1.3 Dissolved oxygen

The results in Figure 4-12 indicated that DO in treatment ponds were lower than controls but fluctuation of DO in all ponds was similar in pattern. The highest DO (12.9-13.8 mg/L) was found at day 44. Average DO in all treatments of trial II was higher than that found in trial I. However, some dead shrimps in pond 4 were found since day 100-114 and DO in pond 4 measured in day 114 was dropped to 0 mgO_2/L together with mass mortality of shrimp. At this point, water in pond 4 turned black with strong smell of hydrogen sulfide. (Figure 4-12).



(A)

.



(B)

Figure 4-12: Dissolved oxygen monitored at 10:00 am during (A) trial I (B) trial II

4.2.1.4 Salinity

At the beginning of trial I, both control and treatment ponds had the same salinity at 6 psu. Decrease in salinity of all ponds was due to raining especially in the last month. In trial II, trend of salinity was differed from trial I. At the beginning, initial salinity in all ponds was slightly different in which pond 4 had the lowest salinity (3 psu) while the other three ponds had the similar salinity (5-6 psu). During experiment, salinity in all ponds was steadily rose after day 40 due to dry season (Figure 4-13).

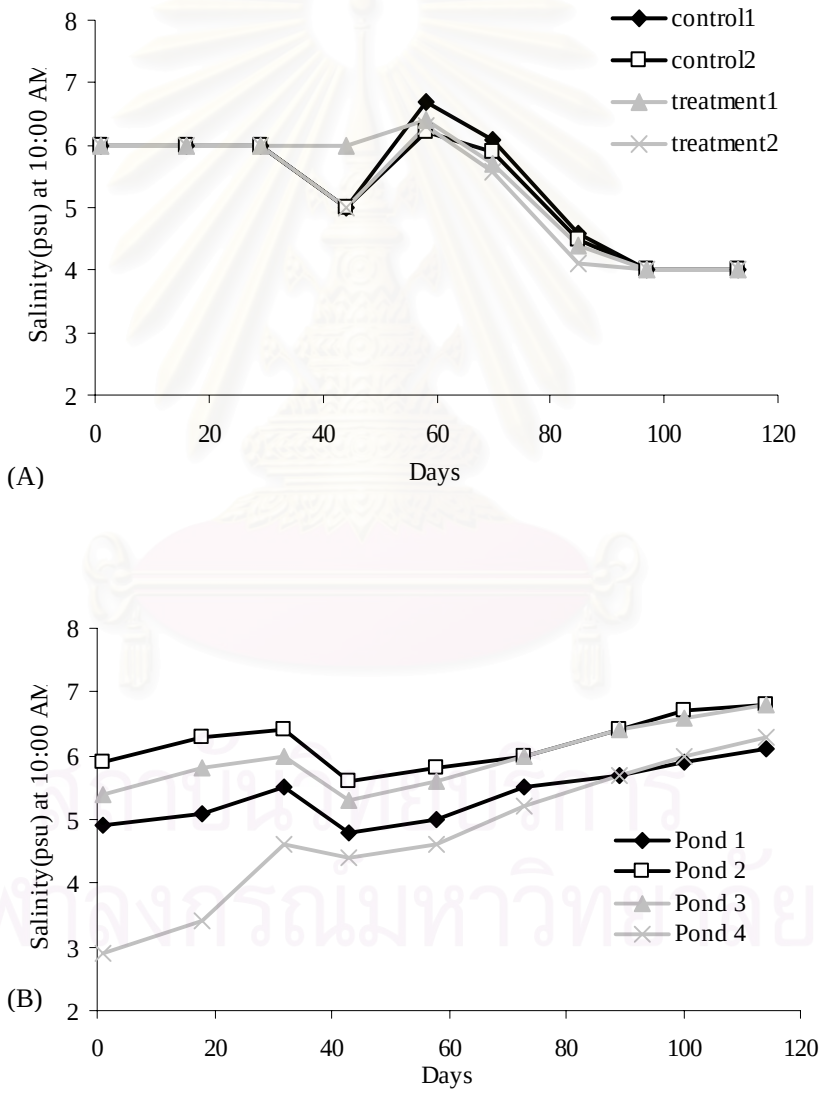
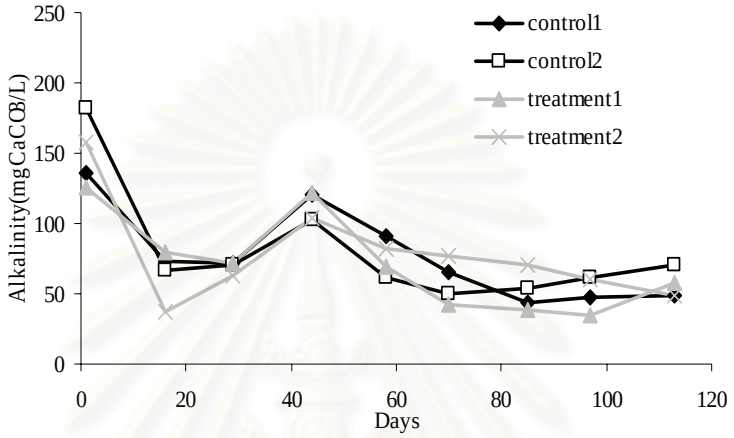


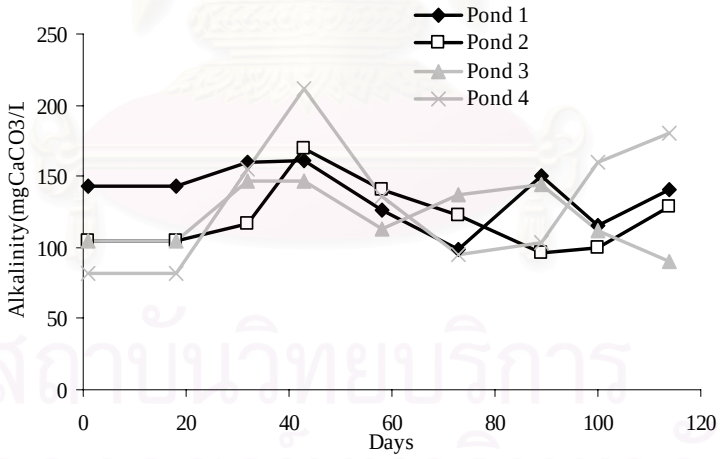
Figure 4-13: Salinity monitored at 10:00 am during (A) trial I (B) trial II

4.2.1.5 Alkalinity

In trial I (Figure 4-14A), alkalinity fluctuated within 40-120 mg $CaCO_3$/L and $CaCO_3$ adding was needed when alkalinity in any ponds had lower than 60 mg $CaCO_3$/L. On the other hand, alkalinity of all ponds in trial II was between 80-200 mg $CaCO_3$/L which was higher than trial I (Figure 4-14B).



(A)

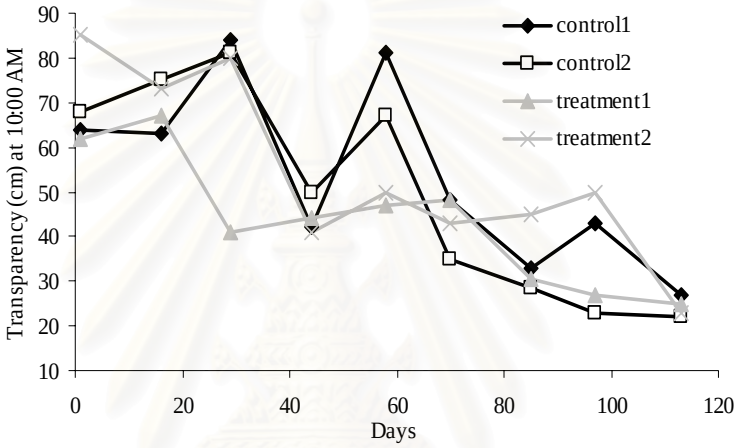


(B)

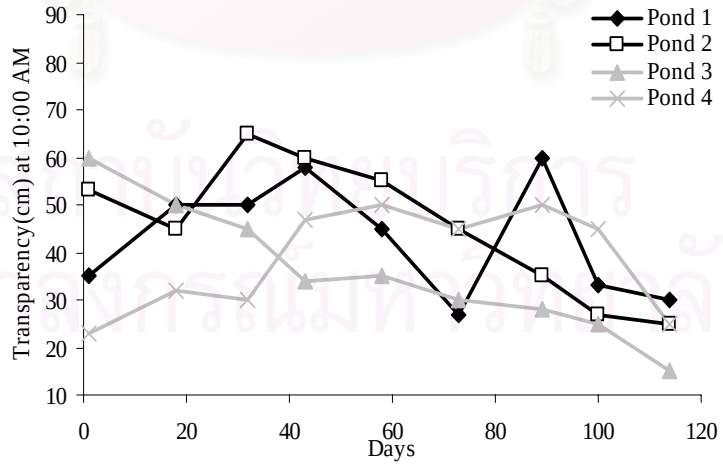
Figure 4-14: Alkalinity monitored in laboratory during (A) trial I (B) trial II

4.2.1.6 Transparency

In trial I, high transparency of more than 60 cm was found at the beginning of the experiment. Thereafter, transparency was found decrease to less than 30 cm at the last day. Average transparency of trial II was apparently lower than trial I. In pond 1, 2 and 4, transparency was rise up at the middle of the experiment and deplete in the last month. Transparency of pond 3, however, linearly decreased from 60 to 15 cm and average transparency was the lowest among all ponds.



(A)

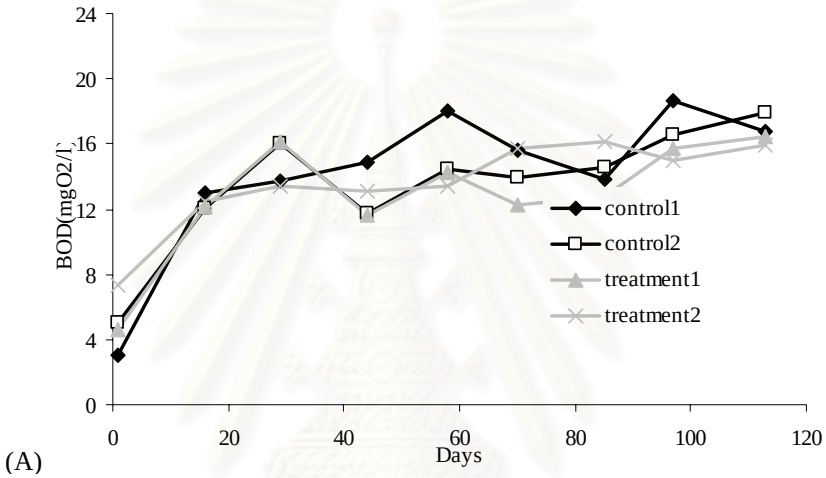


(B)

Figure 4-15: Transparency monitored at 10:00 am during (A) trial I (B) trial II

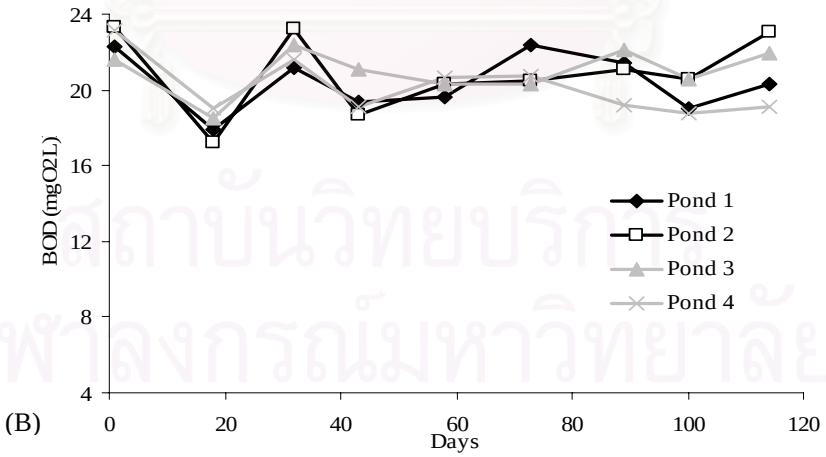
4.2.1.7 Biological Oxygen Demand (BOD)

As shown in Figure 4-16, BOD in both controls and treatments of trial I were low at the beginning (3-7mgO_2/L). Since day 20, BOD in all ponds rose up and then fluctuated between 12 and 18 mgO_2/L. The experiment in trial II that use the same pond of trial I without sediment discharge, high BOD value of more than 20 mg/L was found since the beginning and BOD was constantly high throughout the experiment.



(A)

.



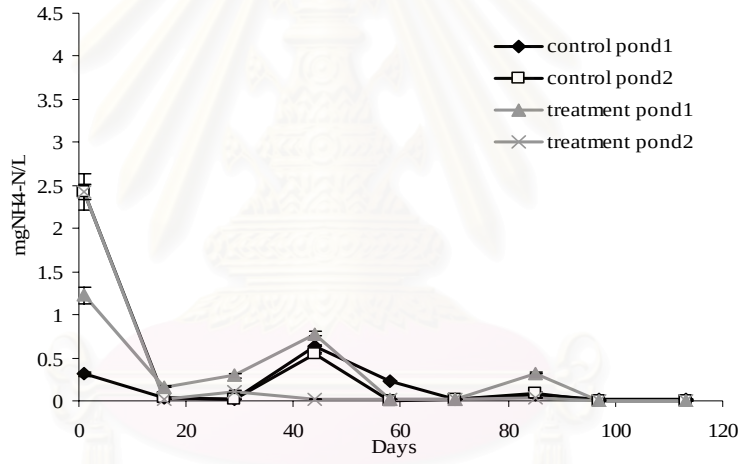
(B)

Figure 4-16: Biochemical oxygen demand (BOD) during (A) trial I (B) trial II

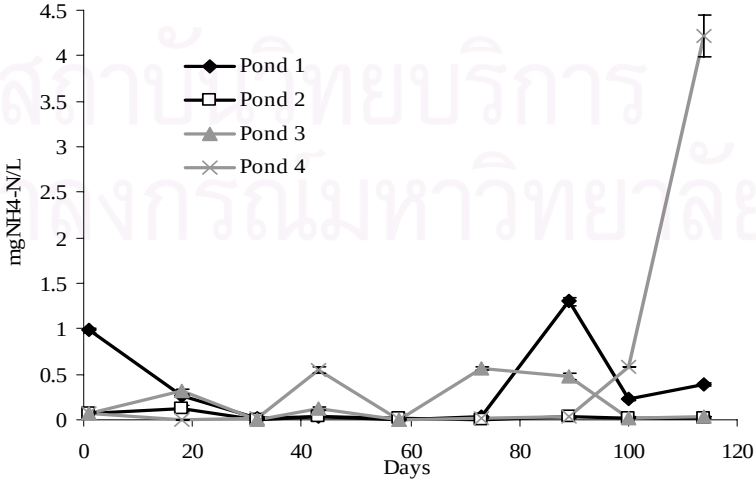
4.2.2 Nutrient dynamics

4.2.2.1 Ammonia

As shown in Figure 4-17A, during 113 days of trial I, ammonia concentration in both control and treatment ponds was higher than 2 mg NH_4-N/L at the initial day then the concentration decreased to below 1 mg NH_4-N/L through out the experiment. Peak of ammonia in control ponds 1, 2 and treatment pond 1 was found at day 44. However, after day 44, ammonia in all ponds was lower than 0.3 mg NH_4-N/L until the end of the experiment. Ammonia concentration of trial II as illustrated in Figure 4-17B was lower than 1 mg NH_4-N/L except during the last day of cultivation that ammonia in pond 4 increased rapidly to 4.21 mg NH_4-N/L together with mass mortality of shrimps.



(A)

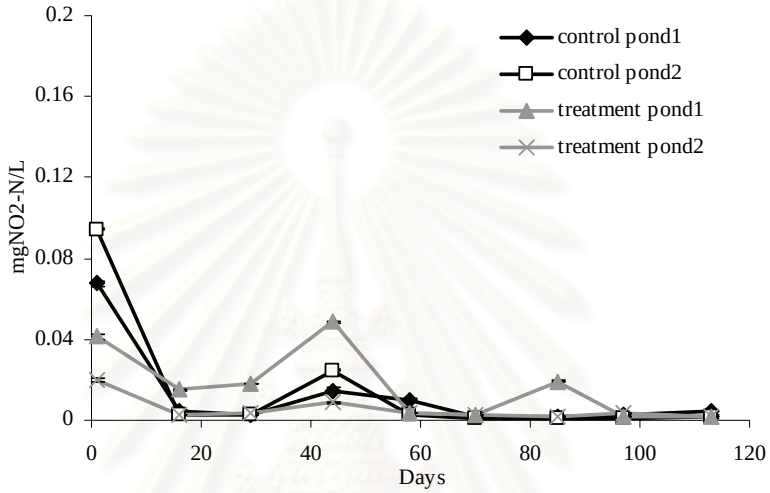


(B)

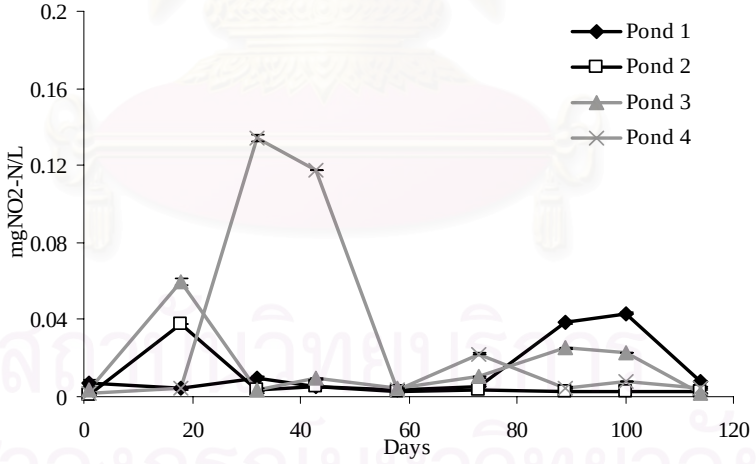
Figure 4-17: Ammonia concentration during (A) trial I (B) trial II

4.2.2.2 Nitrite

Nitrite concentration in all ponds of both trials was lower than 0.14 mg NO_2-N/L through out the experiments. However, peak of nitrite in trial I corresponding with ammonia peak (Figure 4-18A) was found at day 44. Another peak of nitrite was found in pond 4 of trial II (Figure 4-18B) during day 32 and 43 but the highest nitrite concentration was only 0.13 mg NO_2-N/L.



(A)



(B)

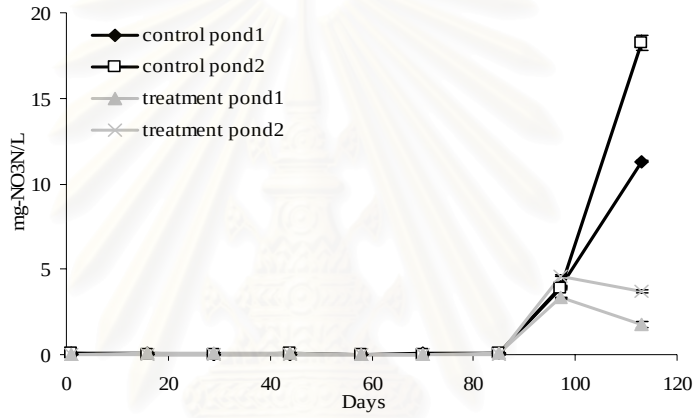
Figure 4-18: Nitrite concentration during (A) trial I (B) trial II

4.2.2.3 Nitrate

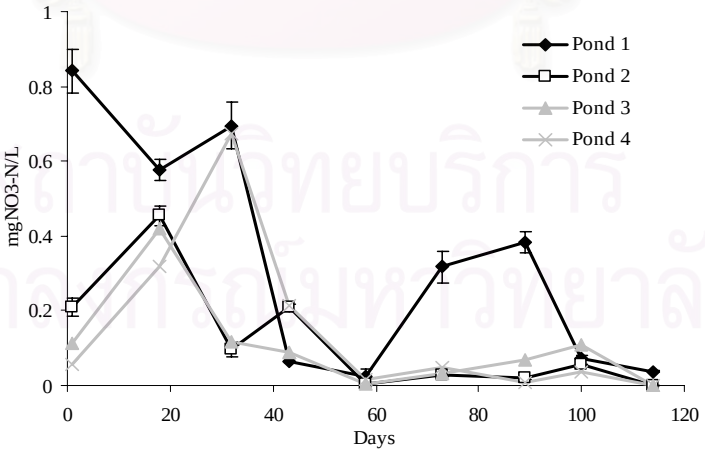
With trial I, nitrate in all ponds during the first 85 days was low with the concentration between 0.02-0.1 mgNO_3N/L. However, very high peak of nitrate was detected since day 97 in which nitrate concentration was risen up to the

approximately 4 mgNO_3-N/L in all ponds. The highest concentration of nitrate was 11.31 and 18.26 mg NO_3-N/L at day 113 of control pond 1 and control pond 2, respectively. Lower concentration of nitrate was found in treatment ponds (1.76 and 3.71 mg NO_3-N/L for treatment 1 and 2, respectively) but the concentration was still remarkable high compare to the ordinary aquaculture ponds.

In trial II, nitrate concentration in all ponds was not as high as in trial I. The highest nitrate concentration was found in pond 1 at the first day (0.84mgNO_3N/L). Nitrate concentration in all ponds fluctuated within 0.003-0.7 mgNO_3N/L through 114 days and no nitrate peak like in trial was found (Figure 4-19B).



(A)

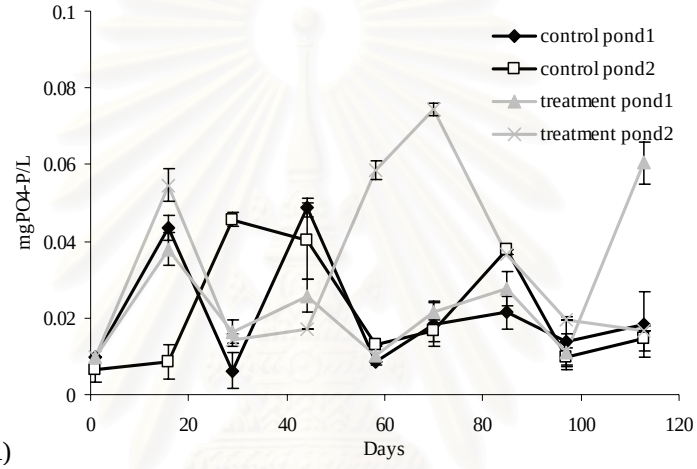


(B)

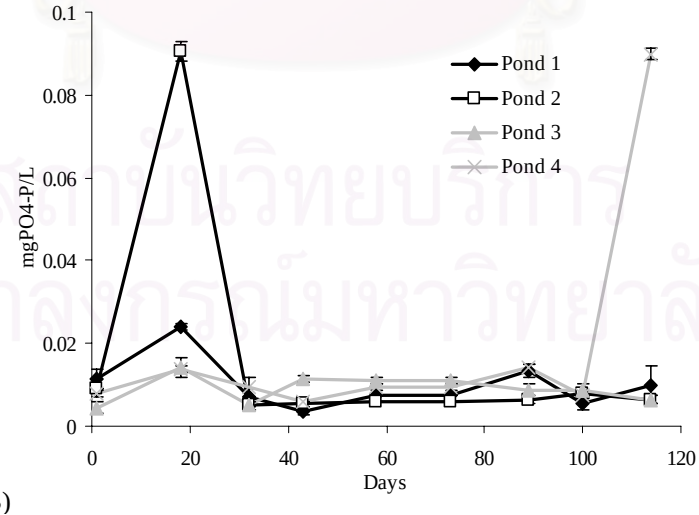
Figure 4-19: Nitrate concentration during (A) trial I (B) trial II

4.2.2.4 Phosphate

In trial I, orthophosphate concentration in both control ponds and treatment ponds fluctuated within 0.01-0.075 mgPO_4-P/L throughout the culture period (Figure 4-20A). Phosphate concentration in all ponds of trial II was lower with less fluctuation than in trial I (less than 0.02 mg PO_4-P/L). However, peaks of phosphate were found in pond 2 at day 18 (0.09 mg PO_4-P/L) and in pond 4 at day 114 (0.09 mgPO_4-P/L).



(A)



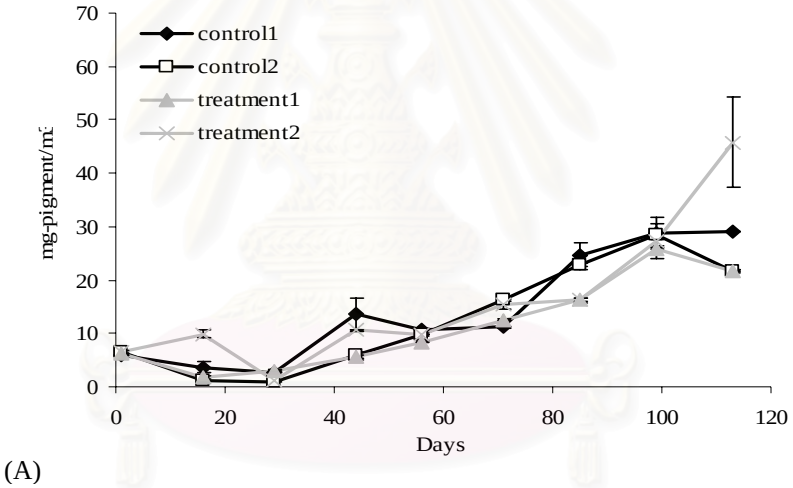
(B)

Figure 4-20: Phosphate concentration during (A) trial I (B) trial II

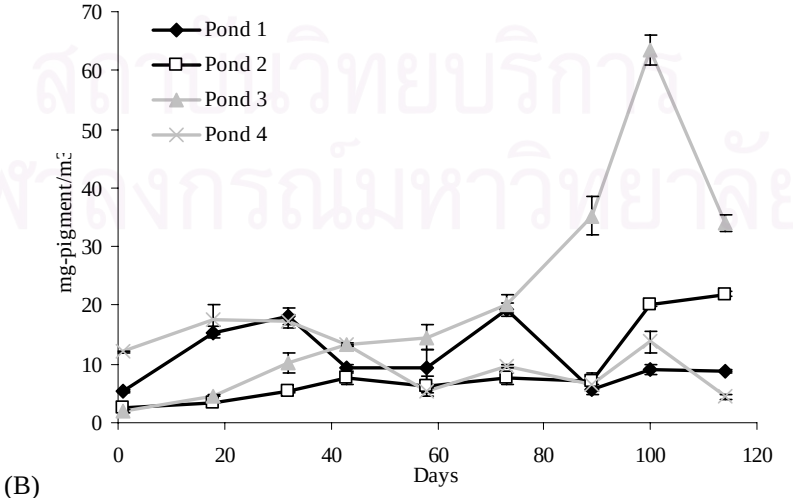
4.2.3 Chlorophyll and plankton dynamics

4.2.3.1 Chlorophyll_*a*

In trial I, both control ponds and treatment ponds had the same initiate chlorophyll_*a* at 5.88-6.45 mg-pigment/m^3. During shrimp culture, chlorophyll_*a* in all ponds was gradually increased. After day 60, chlorophyll_*a* in treatment ponds was slightly lower than in control ponds except in day 113 that chlorophyll_*a* in treatment pond 2 was risen up to 45.70 mg-pigment/m^3 which was the highest concentration (Figure 4-21A). In trial II, trend of chlorophyll_*a* in all ponds were quite different from trial I. It was found fluctuate between 2-20 mg-pigment/m^3 except chlorophyll_*a* in pond 3 that steadily increased to 63.41 mg-pigment/m^3 in day 100 before it was drop down to 34.04 mg-pigment/m^3 in day 114 (Figure 4-21B).



(A)



(B)

Figure 4-21: Chlorophyll_*a* concentration during (A) trial I (B) trial II

4.2.3.2 Plankton dynamics

4.2.3.2.1 Phytoplankton

The results from both trials showed that all ponds had the similar dominant groups of phytoplankton including pennate diatoms, centric diatoms, cyanobacteria (blue-green algae), and green algae. It was found that cyanobacteria, especially *Oscillatoria* sp. (Figure 4-22), was the dominant phytoplankton at most time.

Phytoplankton in trial I was predominated by cyanobacteria especially during the final half of the culture period. As shown in Figure 4-23, peaks of cyanobacteria were found in day 70 and day 114 but the highest cyanobacteria concentration was found in day 114 of the treatment pond 1 (77,189 cyanobacteria/L).

In trial II, pennate diatom, centric diatom, cyanobacteria, and green algae were found in all ponds (Figure 4-24). The initial phytoplankton concentration was low in all ponds except high concentration of cyanobacteria was found in pond 4 (36,079 cyanobacteria/L). In pond 1 which cyanobacteria seemed to be the most abundant over the other phytoplankton groups, peak of centric diatom (50,796 cells/L) was found in day 89 and the concentration was rapidly drop to 975 cells/L in the next two weeks. A bloom of cyanobacteria was found in pond 3 that cyanobacteria increased to 107,646 cyanobacteria/L in day 100 before suddenly rising up to 3,208,488 cyanobacteria/L in the last day of shrimp culture (day 114). It has to be noted that the dominant cyanobacteria during the first bloom in day 100 was *Oscillatoria* sp. but in the second bloom in day 114 the dominant cyanobacteria was changed to *Microcystis* sp. (Figure 4-22). This phenomenon made water color in pond 3 became dark green which was not found in the other ponds.

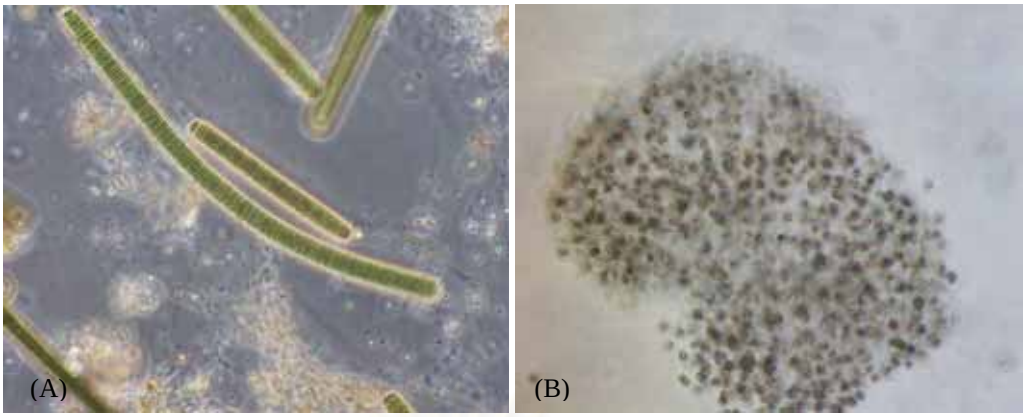


Figure 4-22: Dominant cyanobacteria species found in this experiment (A) *Oscillatoria* sp. and (B) *Microcystis* sp.

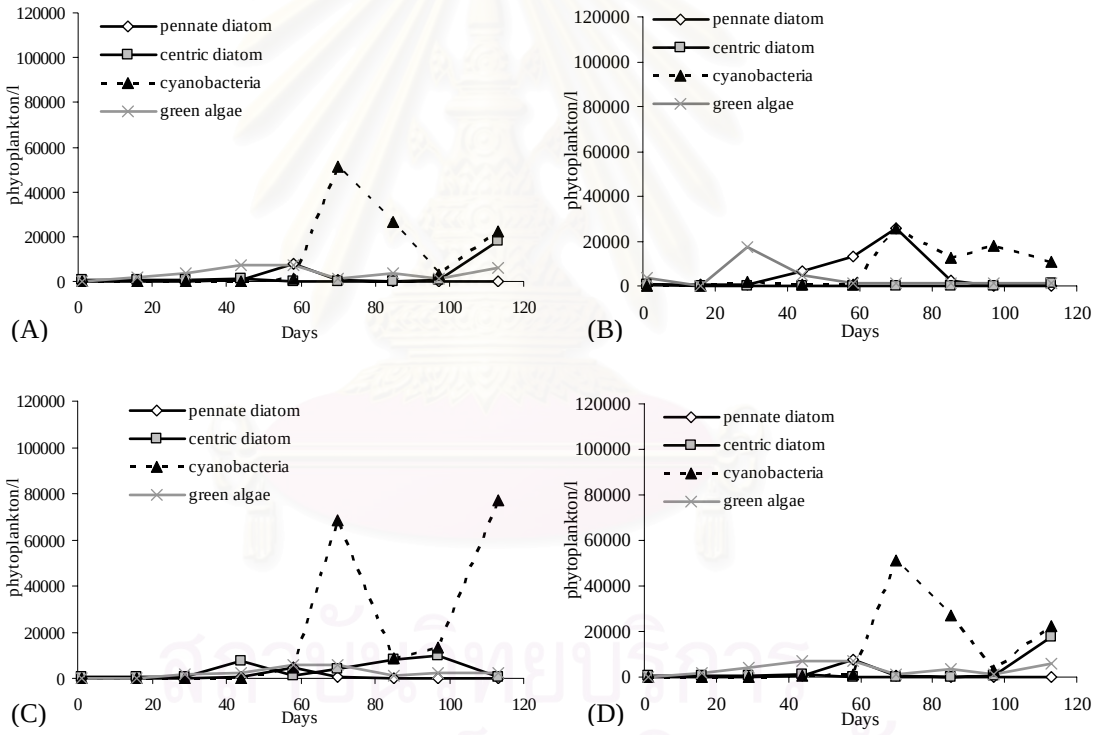


Figure 4-23: Dominant phytoplankton in control ponds, (A) and (B), and treatment ponds, (C) and (D) in trial I

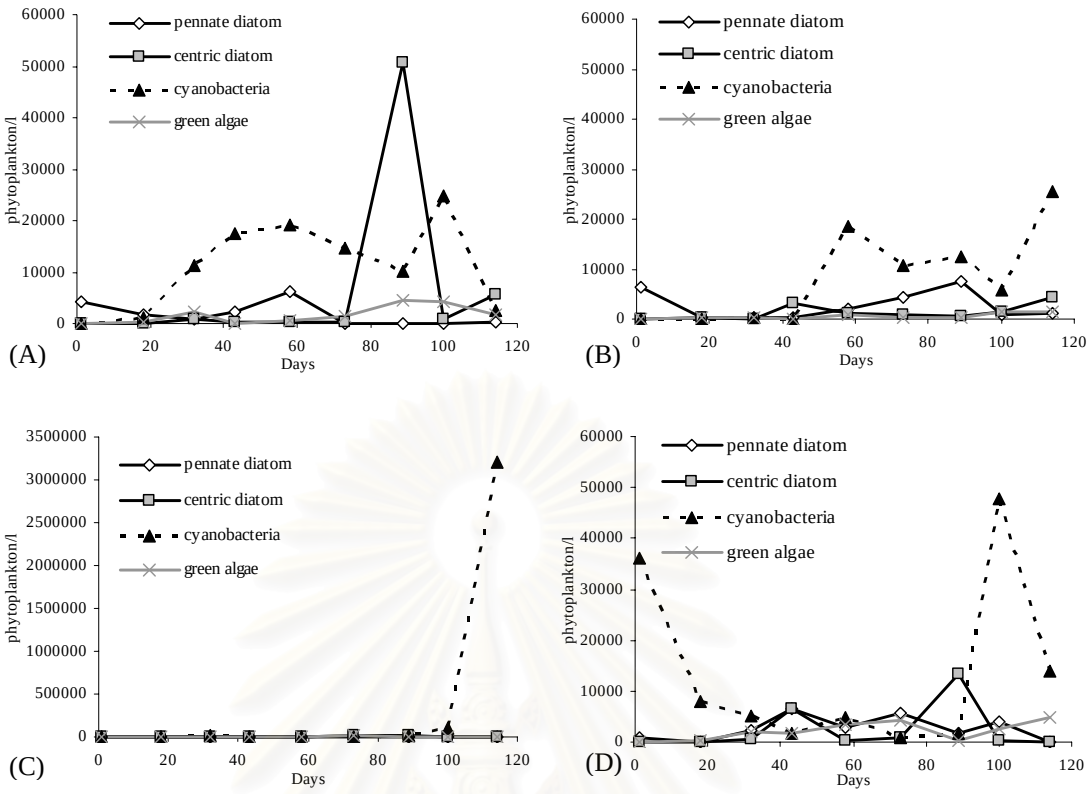


Figure 4-24: Dominant phytoplankton in pond 1 (A), pond 2 (B), pond 3 (C), and pond 4 (D) in trial II

4.2.3.2.2 Zooplankton

Dominant zooplankton in both control and treatment ponds of trial I was rotifer, copepod and naupleus in which rotifer was the dominant zooplankton in all ponds (Figure 4-25). With this trial, control pond 1 had the highest rotifer concentration. A peak of rotifer was found in day 44-58 with the concentration of 3,987-5,087 rotifer/L. The lowest rotifer concentration was found in the treatment pond 1 that rotifer concentration was lower than 1200 rotifer/L throughout the experiment.

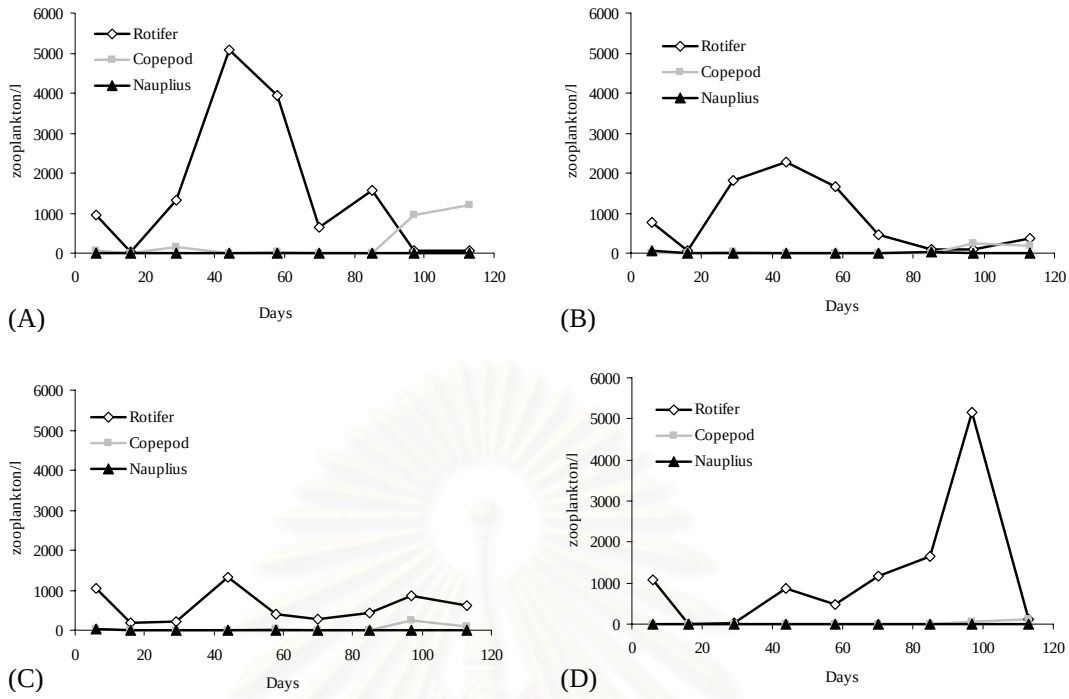


Figure 4-25: Dominant zooplankton in control ponds, (A) and (B), and treatment ponds, (C) and (D) in trial I

The zooplankton results of trial II, as illustrated in Figure 4-26, showed that dominant zooplankton in all ponds was rotifer, copepod and nauplius. Similarly to trial I, rotifer was the dominant group of zooplankton in all ponds of trial II but with higher concentration than that found during trial I. The highest number of rotifer was found in pond 4 (day 100) with the concentration of 25,000 rotifer/L.

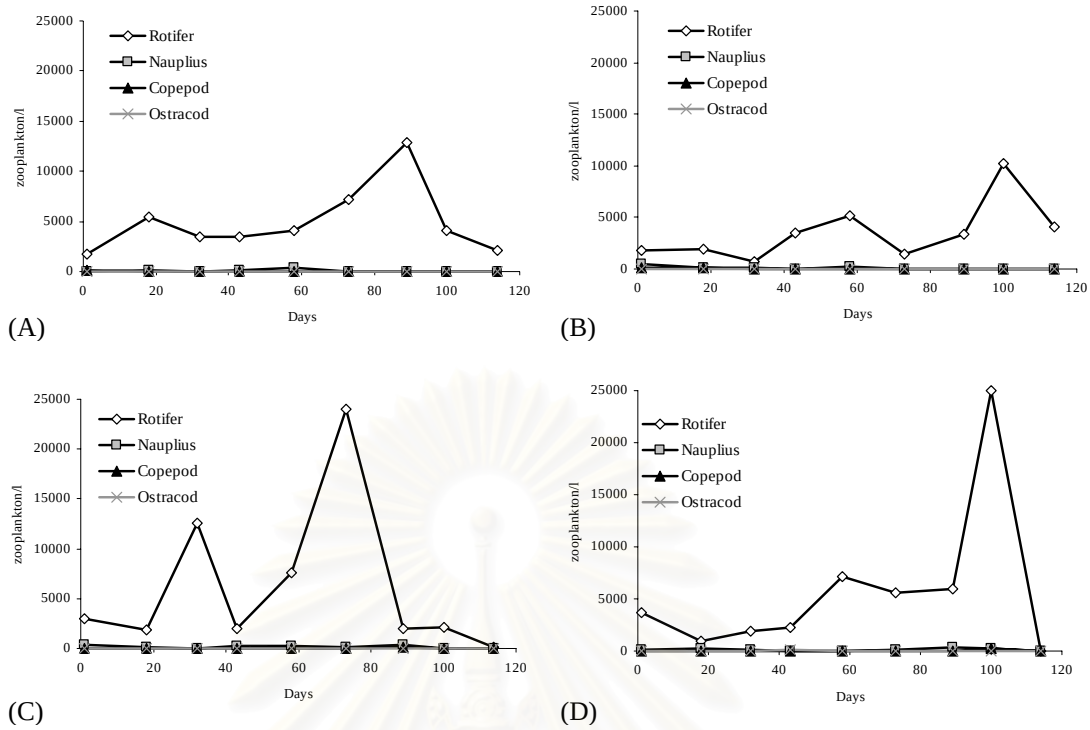


Figure 4-26: Dominant zooplankton in pond 1 (A), pond 2 (B), pond 3 (C), and pond 4 (D) in trial II

Relationship between phytoplankton, zooplankton and some environmental factors are showed in Figure 4-27. Regression analysis showed that there was no relationship between total phytoplankton and total zooplankton in shrimp ponds. However, after separating phytoplankton into groups, significant relation (Figure 4-28) between rotifer and total diatom (P=0.004) and between rotifer and centric diatom (P=0.005) were found. The other significant relationships were found between total phytoplankton and cyanobacteria (P=1.80E-115) and between copepod and nauplius (P=2.24E-21). Both relationships strongly suggested that cyanobacteria were the dominant phytoplankton group and most of the nauplius was the larval stage of copepods.

Figure 4-27: Relationship between phytoplankton, zooplankton and some environmental factors

	total rotifer	total phytoplankton	total cyanobacteria	total diatom	total centric diatom	total pennate diatom	total green algae	Ammonia	Nitrate	Phosphate	Chlorophyll-a	Total copepod	total nauplius
total rotifer													
total phytoplankton	0.554												
total cyanobacteria	0.511	**1.80E-115**											
total diatom	**0.004**	0.622	0.5										
total centric diatom	**0.005**	0.804	0.686	**1.64E-21**									
total pennate diatom	0.432	0.577	0.524	**0.0003**	0.412								
total green algae	0.421	0.492	0.428	0.138	0.289	0.317							
Ammonia	0.85	0.659	0.65	0.628	0.274	0.365	0.827						
Nitrate	0.252	0.826	0.838	0.6	0.946	0.198	0.969	0.352					
Phosphate	0.057	0.53	0.535	0.262	0.399	0.553	0.078	0.035	0.784				
Chlorophyll-a	0.995	**0.026**	**0.026**	0.633	0.356	0.28	0.3	0.076	0.005	0.336			
total copepod	0.428	0.854	0.863	0.918	0.673	0.245	0.124	0.561	0.102	0.123	0.732		
total nauplius	0.896	0.707	0.771	0.978	0.678	0.518	0.332	0.189	0.662	0.117	0.164	**2.24E-21**	

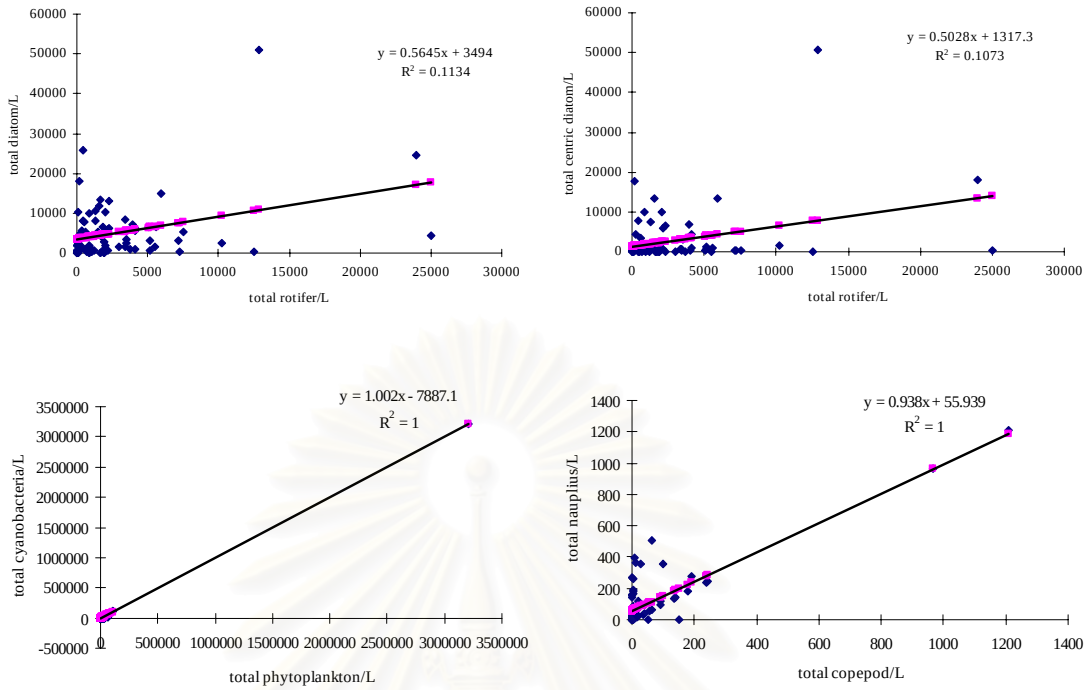


Figure 4-28: Significant relation between total rotifer and total diatom (A), total rotifer and total centric diatom (B), total phytoplankton and total cyanobacteria (C) and total copepod and total nauplius (D)

4.2.4 Shrimp growth and production yielded

4.2.4.1 Growth rate determination

4.2.4.1.1 Growth of shrimp in trial I (April 4, 2003 to August 14, 2003)

In the first trial which ponds number 2 and 3 were assigned as control ponds and ponds number 4 and 5 were assigned as treatment ponds, the result in Figure 4-29 showed no significant difference between average length of shrimps in both control and treatment pond.

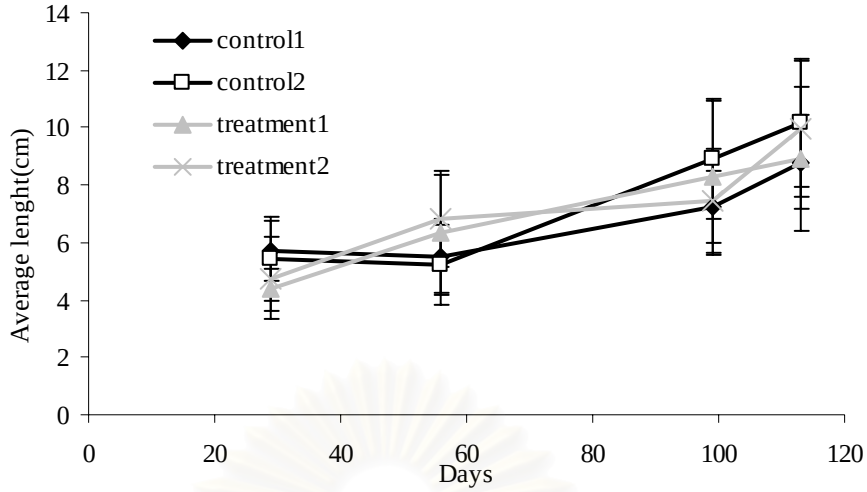


Figure 4-29: Shrimp average length in control and treatment pond in trial I

Shrimp weight as illustrated by size frequency plots in Figure 4-30 showed that during the first month (day 29), shrimps in control ponds had larger size than shrimps in treatment ponds. However, in the second month (day 56), trend of shrimp growth was changed that shrimps in treatment ponds had better growth than control ponds. At the third (day 85) and the forth month (day 113) size of shrimp in all pond had very high variation in length, ranged from 5-15 cm. Large shrimps with more than 12 cm length were found only in treatment ponds. Frequency of weight distribution of shrimps in day 113 (Figure 4-30E) clearly showed that shrimps with weight higher than 23 g was predominant in treatment ponds and large size shrimps, up to 37 g in weight, were found only in treatment pond 1.

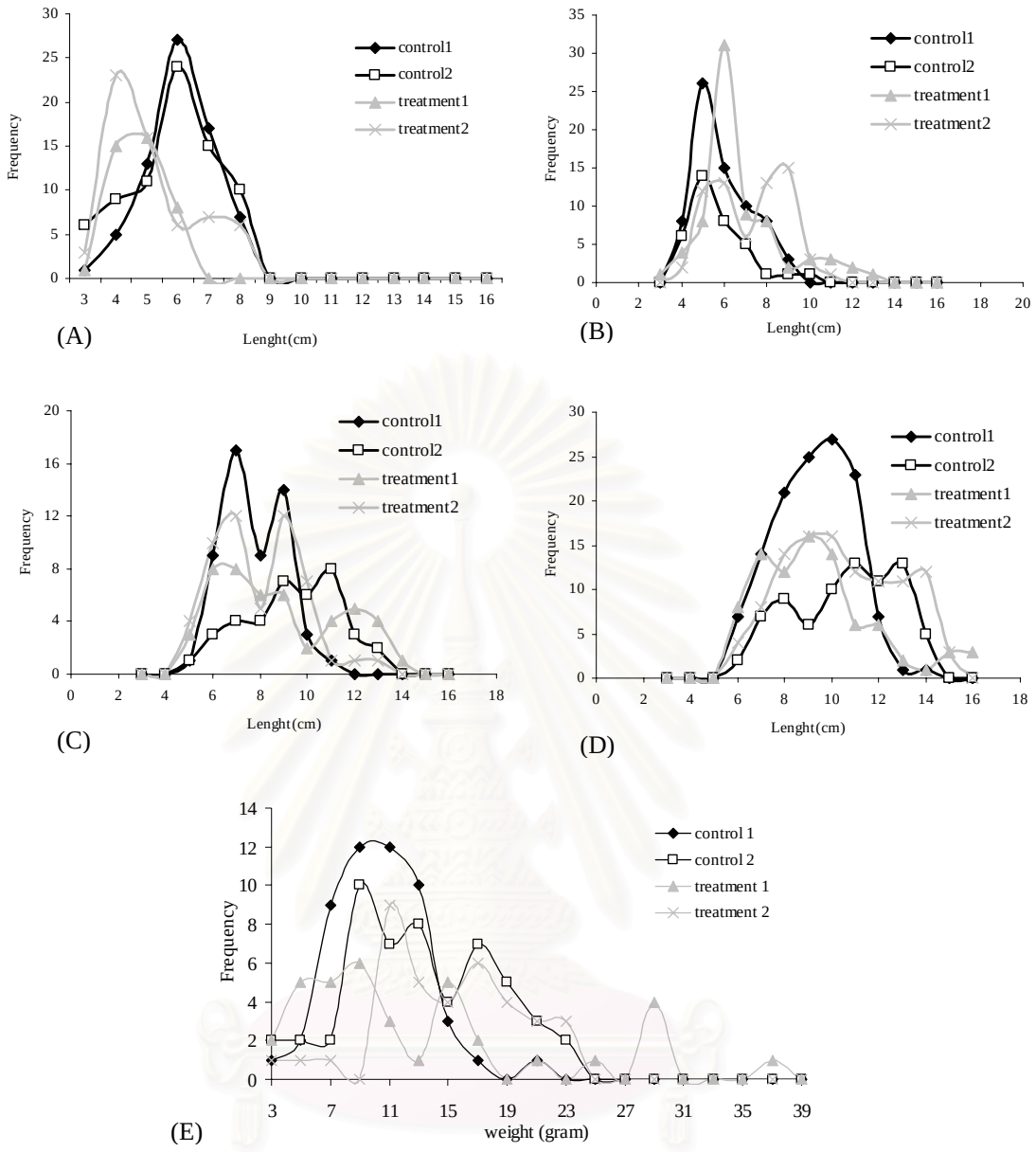
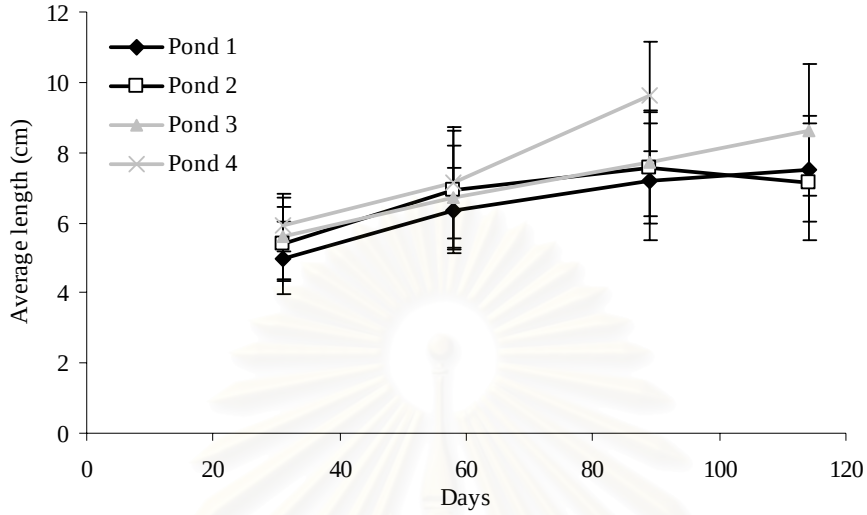


Figure 4-30: Frequency of shrimp length in trial I at day 29 (A), day 56 (B), day 85 (C), and day 113 (D) and frequency of shrimp weight at day 113 (E). Remark: the data was plotted with unequal number of shrimps from each pond.

4.2.4.1.2 Growth of shrimp in Trial II (December 29, 2003 to April 20, 2004)

In trial II, the result in Figure 4-31 showed no significant different of shrimp length of all ponds. However, shrimps in pond 4 which had the highest average length in day 90 suffered by mass mortality during day 96-100 there was no shrimp left in day 114. Frequency of shrimp length distribution in trial II is showed in Figure 4-32. This frequency delineated that shrimp had similar in growth during the

first three months (day 0-89). But in the last month (day 90-114), shrimp in pond 3 had slightly better growth than shrimps in the other ponds.



.

Figure 4-31: Shrimp average length in the experimental ponds in trial I

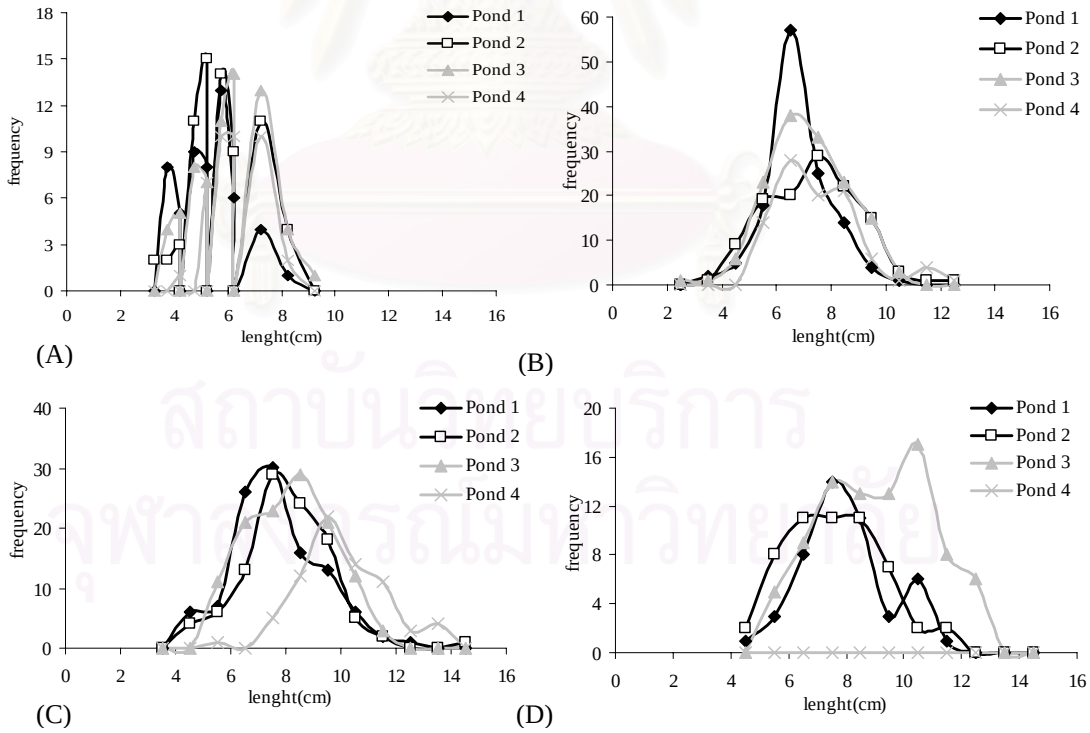


Figure 4-32: Frequency of shrimp length in trial II at day 31 (A), day 58 (B), day 89 (C), and day 114 (D). Remark: the data was plotted with unequal number of shrimps from each pond.

4.2.4.2 Shrimp production

Shrimp production can be delineated as biomass production, survival rate, total feed used, and feed conversion ratio (FCR). In trial I, total feed used in treatment ponds were much higher than control ponds (Figure 4-33). Biomass production was 67.1 kg and 48.1 kg in control pond 1 and control pond 2 while it was 153 kg and 88.7 kg in treatment pond 1 and treatment pond 2, respectively. The highest survival rate was 43.04 % found in treatment pond 1. The lowest feed conversion ratio (FCR) was 1.49 in treatment pond 1 (Table 4-2).

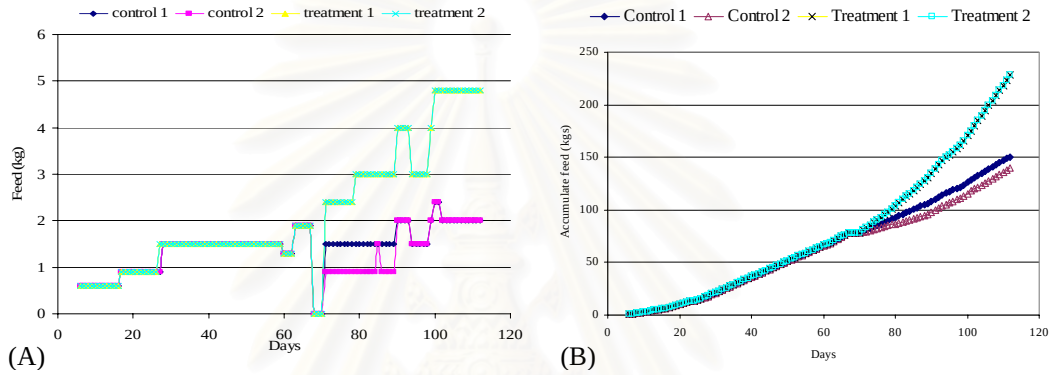


Figure 4-33: Feed used (A) and accumulate feed (B) during trial I

Table 4-2: Biomass production, survival rate, total feed used, and feed conversion ratio in trial I.

	Control pond 1	Control pond2	Treatment pond1	Treatment pond2
Biomass Production (kg)	67.1	48.1	153	88.7
Survival Rate (%)	20.14	11.12	43.04	26.4
Total feed used (kg)	150.7	139.9	228.1	228.1
Feed Conversion Ratio (FCR)	2.24	2.91	1.49	2.4

In trial II, biomass production was 36 kg, 45 kg, and 109 kg in pond 1, pond 2, and pond 3 respectively while it was no biomass production in pond 4 due to mass mortality. The highest survival rate of 32.7 % was found in pond 3. Total feed used was 320.2 kg, 320.2 kg, 259 kg, and 285.7 kg in pond 1, pond 2, pond 3, and pond 4 respectively (Figure 4-34) . It has to be noted that all pond was contaminated by

tilapia fish from nearby fish ponds after flooding in day 41. Hence, feed conversion ratio (FCR) in this trial was very high. The lowest FCR of 2.38 was found in pond 3. Feed conversion ratio in pond 4 was invalid because of no biomass production (Table 4-3).

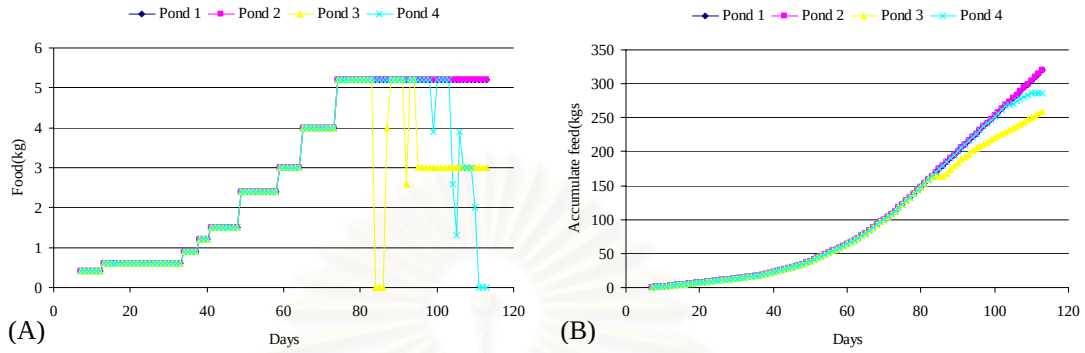


Figure 3-34: Feed used (A) and accumulate feed (B) during trial II

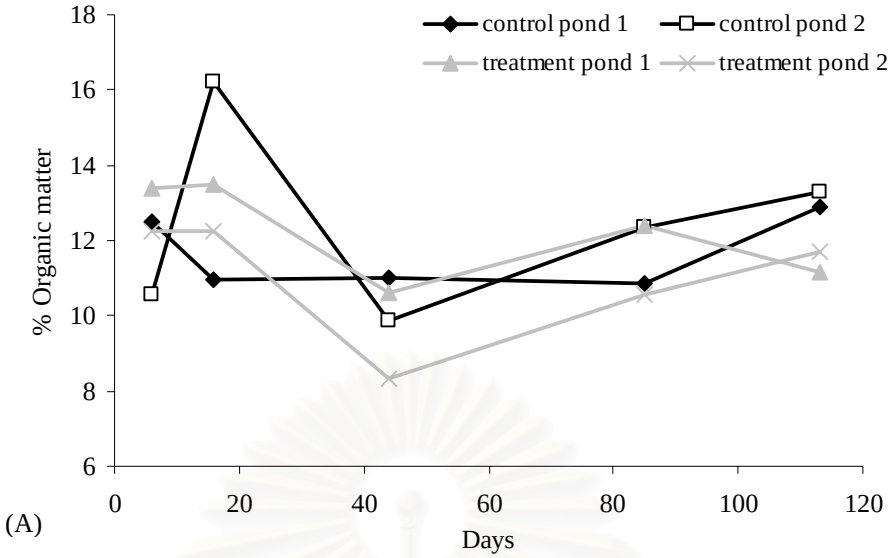
	Pond 1	Pond 2	Pond 3	Pond 4
Biomass Production (kg)	36	45	109	0
Survival Rate (%SR)	10.76	13.5	32.7	0
Total feed used (kg)	320.2	320.2	259	285.7
Feed Conversion Ratio(FCR)	8.89	7.12	2.38	invalid

Table 4-3: Biomass production, survival rate, total feed used, and feed conversion ratio during trial II.

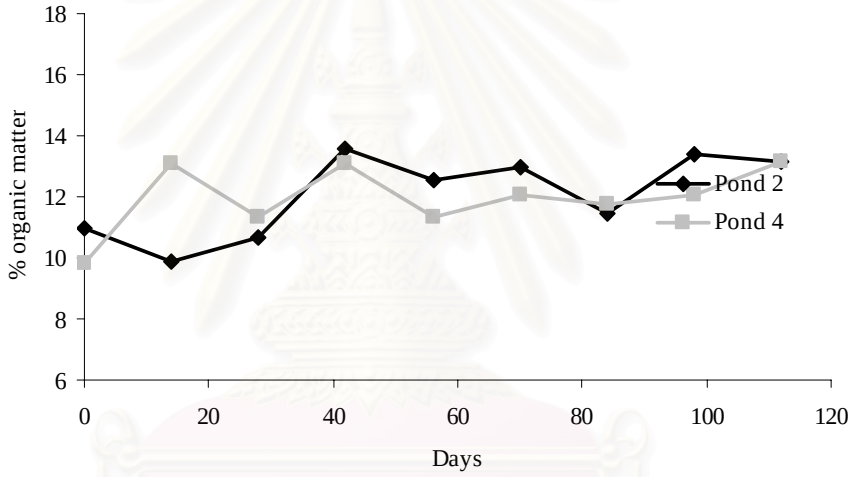
4.2.5 Soil analysis

4.2.5.1 Organic matter in soil

The results in Figure 4-35 show that organic matter in both trial I and trial II were fluctuate within 8-16 % organic matter. Slightly increase in organic matter content was found in trial II and organic matter in pond 4 which have shrimp mass mortality was not different from pond 2.



(A)

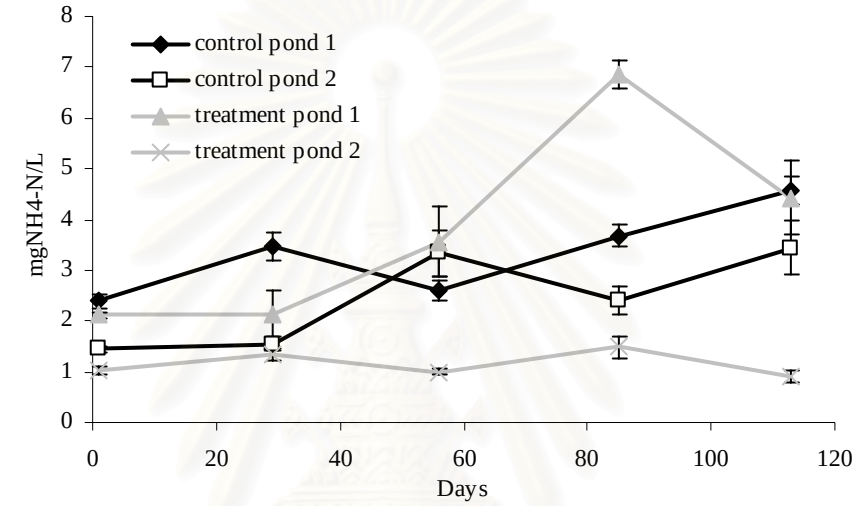


(B)

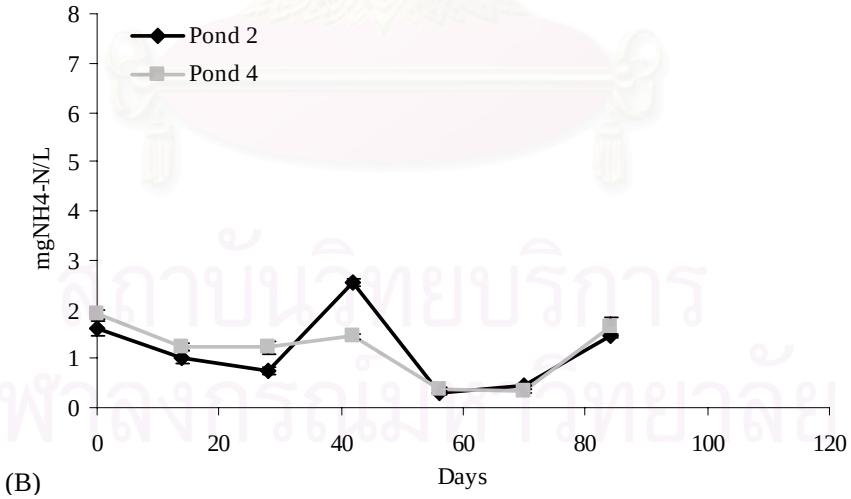
Figure 4-35: Organic matter in soil during trial I (A) and trial II (B)

4.2.5.2 Nutrients analysis in soil

Ammonia concentration in soil from trial I was between 1-7 mg NH_4-N/L with constant or slightly increase with time. The highest soil ammonia was found in treatment pond 1 on day 85 while the lowest soil ammonia was in treatment pond 2 at most of the experimental period. In trial II which only soil in pond 2 and pond 4 were analyzed, the highest ammonia concentration was found in pond 2 at 2.5 mg NH_4-N/L (Figure 4-36).



(A)



(B)

Figure 4-36: Ammonia concentration in soil during (A) trial I (B) trial II

Accumulation of nitrite in soil from all ponds was found in trial I (Figure 4-37A). The concentration increased from approximately 0.001 mg NO_2-N/L to 0.010 mg NO_2-N/L at the end of the culture period. However the highest nitrite concentration was found in treatment pond 1 at 0.017 mgNO_2-N/L was still low when compared with nitrite concentration in water. In trial II, soil nitrite concentration in pond 2 and pond 4 was close to that found in trial I. The highest soil nitrite concentration in pond 4 was 0.018 mgNO_2-N/L (Figure 4-37).

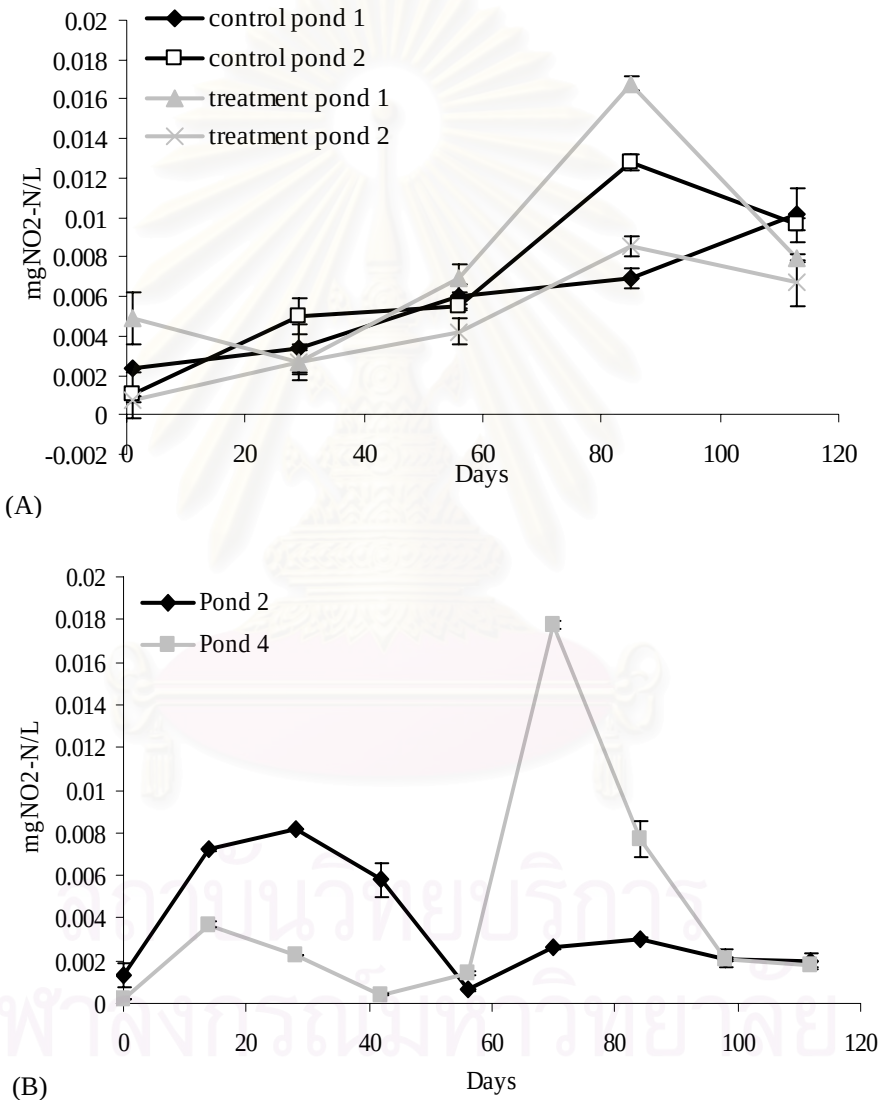


Figure 4-37: Nitrite concentration in soil during (A) trial I (B) trial II

Figure 4-38 illustrated that nitrate concentration in soil from all ponds in both trials increased rapidly after 60 day of shrimp culture and the soil nitrate found in trial II was much higher than in trial I. Trend of soil nitrate was similar in all ponds of the same trial. The highest nitrate concentration was found in pond 2 of trial II that was 2.23 mg NO_3-N/L.

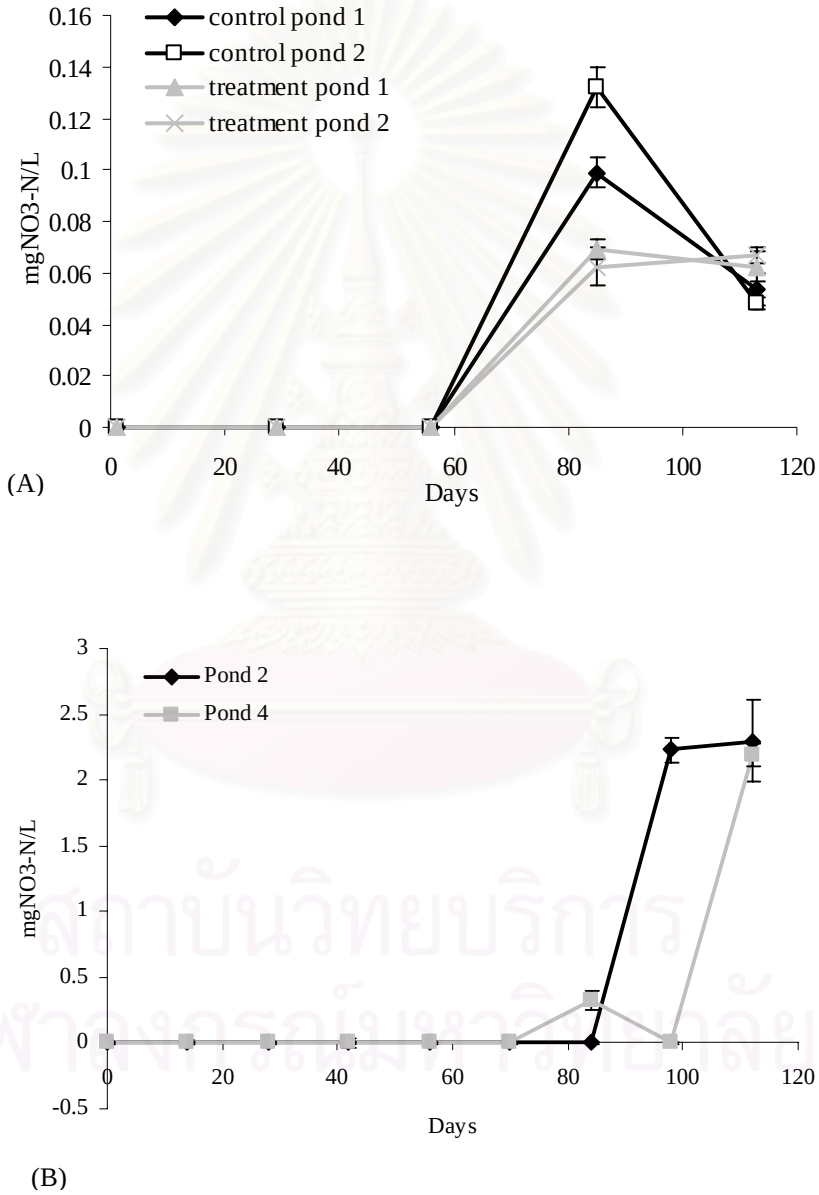


Figure 4-38: Nitrate concentration in soil during (A) trial I (B) trial II

CHAPTER V

DISCUSSION

5.1 Efficiency of Biofilter in ammonia removal

5.1.1 Ammonia removal under laboratory condition

The results from both laboratory trials strongly indicated that bacterial biofilm had the efficiency in ammonia removal via nitrification process. Under normal condition, the start-up period of the nitrification biofilter in aquaculture ponds usually takes 28-60 days (Carmignani and Bennett, 1977 cited in Grommen, 2002). The biofilters used in this experiment were incubated by immerging in shrimp pond for more than 30 days. This was to ensure that nitrifying bacteria in the biofilm had enough time for growing and being active.

The average nitrification rate as determined under laboratory condition of 1month-old biofilter from an outdoor shrimp pond was 447.5 (±205.58) mg-N/m (biofilter length)/day. This rate was equal to 447.5 mg-N/m^2 surface area/day since the biofilter had the specific surface area of approximately 1 m^2 per meter length. Range of ammonia removal rates found with this study was between 230 to 693 mg-N/m/day. Moreover, nitrification rate of the same type biofilter, as reported in Wutikampol (2003), was average 769.6 mg-N/m/day which was higher than that found in this study. Hence, variation of the rates was possibly due to the age and composition of microorganisms in the biofilm from each pond.

5.1.2 Ammonia removal under field condition

The efficiency of ammonia removal by nitrification biofilter was investigated in a PVC chamber placed in the shrimp pond. Increase in nitrite and nitrate concentrations was the parameter used for indicating nitrification process. Thompson *et al.* (2002) suggested that the decrease in ammonia in parallel with the increase in nitrite and nitrate concentrations indicated that nitrifying bacteria was present in the

biofilm. Decreased in ammonia concentration with significant increased in nitrate concentration in treatment chambers containing biofilter, as seen in Figure 4-8 and 4-9, indicated the occurrence of nitrification process in the chambers. However, the different in nitrification rate between 2 cm and 4 cm biofilters was still unclear.

It has to be noted that nutrients cycle in aquaculture pond is very complex. The ammonia removal process is a combination of several processes such as phytoplankton uptake, bacterial assimilation and nitrification.

5.2 Use of biofilter in the outdoor closed-recirculating shrimp pond

5.2.1 Physical and chemical water quality

The results from this study showed that temperature, pH, DO, alkalinity, transparency and BOD in all ponds were in the acceptable value for aquaculture, according to Tookwinas (2000). The detail is shown in Table 5-1.

Table 5-1: Comparison of water quality found in this study and the water quality in shrimp pond as recommended by Tookwinas (2000).

Parameters	Level recommended	Trial I (113 days)	Trial II (114 days)
Dissolved oxygen	>3.5 mgO_2/L	5-14 mgO_2/L	5-14 mgO_2/L (except the last date in pond 4)
pH	7.8-8.5	6-9	6-9
Transparency	30-80 cm	20-90 cm	20-70 cm
Alkalinity	>80 $mgCaCO_3/L$	40-120 $mgCaCO_3/L$	80-200 $mgCaCO_3/L$
Total ammonia	1.0 $mgNH_3$-N/L	0-1.0 $mgNH_3$-N/L (except the first date)	0-1.5 $mgNH_3$-N/L (except the last date in pond 4)
Nitrite	0.2 $mgNO_2$-N/L	0-0.1 $mgNO_2$-N/L	0-0.16 $mgNO_2$-N/L
BOD	<30 mgO_2/L	4-20 mgO_2/L	17-24 mgO_2/L

Some water quality parameters shown in Table 5-1 had high variation especially during day and night. Therefore, parameters such as pH and D.O. which were measured at 10.00 hr could not represent the fluctuation of pH and D.O. in the ponds. To maintain D.O., this experiment used 5 aerator discs running at least 18-20 hours per day in all ponds. However, aerator was usually still operated during

daytime when the D.O. concentration was above 5 mgO_2/L. Boyd (1998) suggested that concentrations of DO in aquaculture ponds usually above saturation during the day therefore aerator might not necessary to be operated during daytime. However, the aim of the aerator in this study was not only to provide oxygen, but it also to maintain mixing through the water column with some oxygen supplement to the biofilter. Aerator is thus one of the important components that maintain the efficiency of ammonia treatment in this system.

5.2.2 Nutrient concentration

The ammonia results showed that continuous aeration in shrimp ponds could maintain the low ammonia and nitrite concentrations (less than 0.5 mg-N/L) throughout the experiment. Significant different between ammonia concentration in control ponds and treatment ponds of trial I was not found. Nitrate which is the less toxic form of inorganic nitrogen was usually low (less than 1.0 mgNO_3-N/L) except very high nitrate concentrations found in the last period of trial I. This nitrate level was very high (up to more than 10 mg-N/L) compare with most studies in the shrimp ponds. After confirmation by repeating nitrate analysis, it could be stated that this was an unusual phenomenon which could not be found in the outdoor aquaculture ponds. This has been reported by Hargreaves (1998) that nitrification may increase temporarily following phytoplankton die-offs in response to elevated ammonia concentration. For this reason, it might be summarized that high nitrate in this trial might be the result from nitrification process.

5.2.3 Chlorophyll and plankton dynamics

The aim of this study was to evaluate the efficiency of biofilter under the extreme condition of shrimp culture. The ponds were set up with unpleasant condition for an ordinary shrimp culture such as no pond bottom drying or cleaning before the start of new crop, no water exchanged during culture and no addition of any chemical or biological substances except adding $CaCO_3$ to maintain alkalinity. The ponds therefore had high nutrients substantially for plankton growth and the anaerobic condition in the sediment. The bloom of phytoplankton was thus found since the early day of shrimp culture.

Regression analysis between chlorophyll_*a* and total phytoplankton and between chlorophyll_*a* and transparency (Secchi disc depth) had significant correlation (P=0.026 and P=0.000, respectively). This indicated that an increase of turbidity in the pond was due to phytoplankton population not by other suspended solids. It is well known that an excessive supply of nutrients will promote the bloom of phytoplankton and growth of some macrophytes. Additionally, excess nutrients will alter phytoplankton composition with a resulting change of dominant species; such changes imply the substitution of larger species for smaller ones (Rodriguez *et al*., 2003). Algae blooms can induce hypoxia and anoxia conditions. This could be prevented by sufficient aeration during night and improve water mixing during day time.

In both trials, all ponds had the same dominant groups of phytoplankton including of pennate diatom, centric diatom, green algae and cyanobacteria. Those groups of phytoplankton are usually found in most shrimp ponds in Thailand (Thai Farm Zone, 2002). Cyanobacteria, especially *Oscillatoria sp*, was most found over the other three groups through the trial. In general, cyanobacteria is found relatively to high concentration of nitrogenous waste and anaerobic condition in pond bottom. Bloom of theses algae usually made dark green and high viscosity water. One of the factors affecting continuously bloom of the cyanobacteria in this experiment was low salinity which Funge-Smith and Briggs (1998) reported that low salinity shrimp farm probably dominated by cyanobacteria.

During last month of trial II, bloom of *Microcystis sp*. was found in pond 3. This bloom made water in pond 3 became very dark milky-green color unlike the color of *Oscillatoria* bloom which mostly found in other ponds. Lightner (1978) cited in Kankaanpaa et al. (2005) suspected that cyanobacteria could cause mortality of prawns. Several species of cyanobacteria, *e.g*., *Microcystis*, *Nodularia, Lyngbya* and *Oscillatoria* can release the harmful cyanobacterial toxins. The concern with harmful effects of cyanobacterial toxins arose since the late 1970s. However, cyanobacteria bloom found in this experiment seemed to have no effect on shrimp production. In trial I, treatment pond 1 which had the bloom of *Oscillatoria sp*. produced the highest shrimp yield. In trial II, pond 3 which had *Microcystis* bloom also yielded the highest

shrimp production. However, the occurrence of cyanobacteria may result bad muddy smell or poor taste which affect to shrimp meat quality.

Dominant zooplankton in all ponds were rotifer, copepod, and nauplius in which rotifer was the dominant group. The result illustrated the relationship theoretically expected from the food-chain (see section 4.2.3.2). In fact, other groups of phytoplankton found in the ponds such as the cyanobacterium *Oscillatoria* or even some species of pinnate diatom had too large cell size which could not be consumed by rotifer.

5.2.4 Soil analysis

The results from soil analysis showed that concentration of soil ammonia in control and treatment ponds of trial I were not significant difference. The highest ammonia concentration in soil in trial I (7 mgNH_4-N/L) was much higher than in trial II (2.5 mgNH_4-N/L). On the other hand, nitrate concentration in soil in trial II (2.23 mgNO_3-N/L) was much higher than trial I (0.13 mgNO_3-N/L) while nitrite concentration in soil from both trials was similar. Comparing nutrients concentration in soil and nutrients in the water (Table 5-2), it was found that ammonia in the bottom soil was higher than that found in the water. These data can be defined that the concentration of nutrient in bottom soil were magnitude higher than in the water and organic substances accumulated in shrimp pond sediment were in highly reduced condition. Even when oxygen was depleted after aerobic decomposition (so called ammonification process), many anaerobic processes that taking place in the pond bottom could also lead to the production of ammonia (Avnimelech and Ritvo, 2003).

Increase in nitrate concentration in the bottom soil during the last month (after day 90, see Figure 4-36) of the culture period was very interesting result and has never been reported elsewhere. The accumulation of nitrate could possibly come from several processes such as the conversion from nitrite via nitrification, nitrate exchange between water and sediment and nitrate assimilation by microorganisms in the soil.

Table 5-2: Comparison between nutrients in water and in soil during both trials.

	Nutrient concentration in water		Nutrient concentration in soil	
	Trial I	Trial II	Trial I	Trial II
Ammonia ($mgNH_4$-N/L)	0.01-2.43	0.01-4.20	1-7	0.25-25
Nitrite ($mgNO_2$-N/L)	0.01-0.10	0.01-0.16	0.001-0.018	0.001-0.018
Nitrate ($mgNO_3$-N/L)	0.02-18.36	0.01-0.80	0.001-0.013	0.01-2.23
Phosphate ($mgPO_4$-P/L)	0.01-0.07	0.01-0.09	0.01-0.08	No data

Organic content in the sediment from both trials were not high and the concentrations were constant over shrimp culture period. Martinez-Cordava *et al* (1997) suggested that organic matter were significantly higher in ponds with low aeration rate. Aeration perhaps moved the organic matter from the sediment to the water column where the oxidation occurs. This might be an explanation of the results found in this study that the water in all ponds was well mixed by the continuous aeration.

5.2.5 Shrimp growth determination

Although the water quality data in trial I did not showed significant different between control and treatment ponds, but shrimp production in treatment ponds were higher than control ponds. The highest yield of 153 kg was found in treatment pond 1 following by 88.7 kg in treatment pond 2. Beside that, shrimp in treatment ponds showed the higher survival rate and better FCR.

It has to be mentioned that shrimp ponds in this study were set at the extreme condition because the main objective was to determine the efficiency of the biofilter. Shrimp length histogram in Figures 4-28 and 4-30 illustrated that shrimp growth rate in all ponds had high variation in size and growth rate of shrimp in both trials were quite low. Large variation in shrimp size found in the same pond or slow growth syndrome is one of the most concern problems for black tiger shrimp culture in Thailand (Yuwabenjapol, 2003). The reason of slow growth is still unclear but it has been proposed that viral diseases, high nitrogenous waste in the water, bloom of toxic algae, low quality feed, improper pond preparation and even feeding practice are among the possible causes of the syndrome (Chanratchakul, 2002). With this study, two different sizes of shrimps, large size over 14 cm length and small size below 5 cm length, collected from the same pond were checked for Monodon Baculovirus (MBV)

and Hepatoprancreatic Parvovirus (HPV) which might be related with slow growth rate (Grasslund and Bentsson, 2001). The results showed that MBV and HPV virus in all shrimp samples were negatively detected. Therefore variation in growth might be the result from environmental or improper feeding management. Unfortunately, an improve of feeding management in trial II by increase the feeding area to cover most of the pond area was disrupted because the contamination of tilapia fish from nearby fish ponds into the experimental shrimp ponds during the second month of culture period. Shrimp growth was therefore suffered from insufficient feeding since large amount of shrimp feed was consumed by fish.

Unexpected mass mortality of shrimp was found in pond 4 of trial II. The collapses of the environmental condition suddenly occurred in the last week of shrimp culture in which the pond environment was turned into sulfide reduction within two to three days. Unfortunately, the change in environmental parameters could not be detected because the occurrence was in the middle of the routine sampling interval. This incident could be hypothesized that waste loading was higher than the carrying capacity of the shrimp pond. In general, more than 80% of the nitrogen input was come from high protein feeding and only 25% of this fraction was incorporated in fishes while the rest was retained and accumulated in the water and pond bottom (Hargreaves, 1998; Avnimelech and Ritvo, 2003). Seo and Boyd (2001) concluded that aquaculture pond bottom soils are the recipient of large amount of nutrients and organic matters. These substances tend to accumulate in bottom soil especially in the old ponds. Similarly, Munsiri *et al* (1996) cited in Avnimelech and Ritvo (2003) reported that high concentration of organic matter in bottom soil of the old pond might result an anaerobic condition in the top layer of soil and at the soil-water interface. Hence, the study in shrimp pond carrying capacity is needed.

Because of the limitation of this research, all ponds were continuity used for the second experimental crop without pond drying and bottom cleaning. In fact, drying pond between crops has beneficial in lowering organic matter concentrations and reducing the likelihood of anaerobic condition at the soil-water interface during the next crop. This factor might induce the bottom sediment of all ponds in trial II which was rich in organic content became strong anaerobic condition. When it was

incorporated with other incidents especially phytoplankton collapse, sudden oxygen depletion and following by sulfate reduction at the pond bottom could be happen.

Pond bottom conditions are more critical for shrimp than for other aquaculture species because shrimp spend most of their time at the bottom or even immersing in the soil and ingest the sediment (Boyd, 1989; Chien, 1989 cited in Avnimelech and Ritvo, 2003). In this experiment, feeding management was mainly depending on sampling pens which were installed in the edge region of the pond. Chanratchakul (2002) reported that only feeding pen checking did not enough for the proper feeding practice. Most shrimp farmers fed shrimp depending on their own traditional experience and did not realize on shrimp survival rate in the early month. Feeding pen checking without the proper monitoring on shrimp survival rate could not provide the suitable feeding and therefore led to over or under feeding.

5.2.6 Using horizontal net as an additional surface for shrimp attachment

From diving observation, shrimp were found attach on most materials in the pond including horizontal net, vertical bamboo poles, aerator discs and biofilter structure. Boyd (1998) suggested that fishes become conditioned to high DO concentrations around an aerator and they usually swam around this area when low DO concentrations occur in the other part of the pond. Although observation of shrimp behavior was not as easy as fish because of high turbidity, but attachment behavior were found in both small and large size of shrimps throughout the experiment These results can be concluded that black tiger shrimp had the attachment behavior and the concept of providing an additional surface for shrimp attachment in order to rise the pond carrying capacity is possible. However, the problem of this concept was that shrimp which attached on the material might lack of the occasion on feeding which mostly found at the pond bottom.

5.2.7 Nitrification process in an outdoor earthen pond

Several processes or techniques have been reported to be used for reducing ammonia concentration in the water. This includes nitrification/denitrification processes and ammonia assimilation by both phototrophic and heterotrophic

microorganisms. Using the nitrification rate calculated from laboratory experiment (see section 4.1.1), nitrification biofilter installed in each pond with the total length of 82.5 m could have a capability to treat 36926.12 mg NH_3-N/day which was lower than estimated amount of NH_3-N that produced from daily feeding.

Many studies concluded that nitrification play a minor role in ammonia removal in most aquaculture ponds. For example, Brune *et al*, 2003 reported that only 15% of ammonia in the pond was converted to nitrate by nitrification process while the greater fraction of the ammonia (43%) being converted to heterotrophic bacterial biomass. Burford and Lorenzen (2004) also concluded that the phytoplankton uptake and subsequence sedimentation were among the key processes in nitrogen dynamics in shrimp pond operated at low water exchange rate.

In this experiment, ammonia removal in an outdoor earthen pond was also mainly depend on ecological processes especially phytoplankton uptake and sedimentation, but the concept of using biofilter via nitrification process could facilitate excess amount of ammonia that over the natural treatment capability of the pond itself particularly at night. This has been suggested by Brune *et al.* (2003) that nitrification in the pond was stimulated after the depletion of photosynthesis. The concept of nitrification biofilter in an outdoor shrimp pond, as illustrated in this study, must be the supplementary treatment system. More research and development on biofilter design and construction, pond design, feeding practice and basic study of ecological processes relating with nitrogen cycle are needed.

CHAPTER VI

CONCLUSION AND RECOMMENDATION

1. Under laboratory condition, activated biofilter from shrimp pond had a significant capability in ammonia treatment via nitrification process. The average ammonia removal rate was 447 mg NH_4-N/m/day.

2. Activated biofilter also showed a potential of ammonia treatment in the experimental chambers installed within the shrimp pond. Significantly higher ammonia reduction, together with higher increase in nitrate concentration, was found in the chambers containing biofilter.

3. Water quality especially inorganic nitrogen concentration in shrimp ponds with biofilter was not apparently different from control ponds. The results indicated that nitrification could occur naturally within all ponds. However, since nutrients cycle in aquaculture pond is very complex process, the aim of using biofilter in shrimp pond must be the additional system to reduce the risk of ammonia peak during shrimp culture.

4. The nitrification capability of the biofilter as estimated from laboratory test suggested that the number of biofilter installed in treatment ponds (the shrimp pond with biofilter sets) was not enough to treat ammonia produced as estimated from daily feeding basis. Therefore, increase in the number of biofilter sets, improvement of water flow pass through the biofilter and intensively vertical mixing of water in the pond are among the possibly factors that enhance the biofilter capability. Further study of nitrification biofilter design under field condition is therefore needed.

5. Water sampling interval of every two weeks in this study was not enough to detect the rapid change in water quality especially a peak of ammonia after phytoplankton drop in the pond. Hence, more sampling frequency at least

twice a week is recommended. This could also integrate with an automatic water quality monitoring system.

6. Because the experiment was designed to test the biofilter capability under extreme culture condition. Shrimp culture was performed without regular pond drying and cleaning process between each crop. This made the pond bottom turn into anaerobic condition with high risk of hydrogen sulfide that could directly affect growth of shrimps and inhibit the activity of nitrifying bacteria.

REFERENCES

Al-hafedgh, Y. S., Alam, A. and Alam, M. A. 2003. Performance of plastic biofilter media with different configuration in a water recirculation system for the culture of Nile tilapia (*Oreochromis niloticus*). *Aquacultural Engineering* 29: 139-154.

Alonzo-Rodriguez. R and Paes-Osuna, F. 2003. Nutrients, phytoplankton and harmful Algal blooms in shrimp ponds: a review with special reference to the situation in the Gulf of California. *Aquaculture* 219: 317-336.

Avnimelech, Y. and Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. *Aquaculture*: 549-567.

Azim, M. E., Verdegem, M. C. S., Khatoon, H., Wahab, M. A., Van Dam, A. A. and Beveridge, M. C. M. 2002. A comparison of fertilization, feeding and three periphyton substrates for increasing fish production in freshwater pond aquaculture in Bangladesh. *Aquaculture* 212: 227-243.

Azim, M. E., Milstein, A., Wahab, M. A. and Verdegem, M. C. J. 2003. Periphyton -water quality relationships in fertilized fishponds with artificial substrates. *Aquaculture* 228: 169-187.

Boyd, C. E. 1998. Pond water aeration systems. *Aquacultural engineering* 18: 9-40.

Brune, D. E., Schwartz, G., Eversole, A. G., Collier, J. A. and Schwedler, T. E. 2003. Intensification of pond aquaculture and high rate photosynthetic system. *Aquacultural Engineering* 28:65-86.

Burford, M. A., Thompson, P. J., McIntosh, R. P., Bauman, R. H. and Pearson, D. C. 2003. Nutrient and microbial dynamic in high-intensity, zero exchange shrimp pond in Belize. *Aquaculture* 219: 393-411.

Burford, M. A. and Lorenzen, K. 2004. Modeling nitrogen dynamic in intensive shrimp ponds: the role of sediment remineralization. *Aquaculture* 229: 129-145.

Chanratchakul, P. 2002. Shrimp growth problems. Available from: http://www.thaifarmzone.com/shrimp/modules.php

Chow, V. T., Eliassen, R. and Linsley. 1972. *Biological unit process*. Wastewater Engineering. Mcgrow-Hill, Inc., USA 660-668.

Chen, J. C. and Lin, C. Y. 1992. Effect of ammonia on growth and molting of *Penaeus monodon* juveniles. *Comp. Biochem. Physiol*. 101C (3): 449-452.

Fdz-Polanco, F., Mendez, E. and Villaverde, S. 1995. Study of nitrifying biofilms in Submerged biofilters by experimental design methods. *Water Science and*

Technology 32 (8):227-233.

Funge-Smith, S. J. and Briggs, M. R. P. 1998. Nutrient budget in intensive shrimp ponds: implications for sustainability. *Aquaculture* 164: 117-133.

Grasslund, S. and Bengtsson, B. E. 2001. Chemical and biological products used in south-east Asian shrimp farming, and their potential impact on the environment -a review. *The Science of the Total Environment* 280: 93-131.

Greiner, A. D. and Timmons, M. B. 1998. Evaluation of the nitrification rates of microbead and trickling filters in intensive recirculating tilapia production facility. *Aquacultural engineering* 18:189-200.

Grommen, R., Hauteghem, I. V., Wembeke, M. V. and Verstraete, W. 2002. An improved nitrifying enrichment to remove ammonium and nitrite in fresh water aquaria system. *Aquaculture* 211: 115-124.

Gross, A., Nemirovsky, A., Zilberg, D., Khaimov, A., Brenner, A., Snir, E., Ronen, Z. and Nejidat, A. 2003. Soil nitrifying enrichment as biofilter starters in intensive recirculating saline water aquaculture. *Aquaculture* 223: 51-62.

Hapogian, D. S. and Riley, J. G. 1998. A closer look at the bacteriology of nitrification. *Aquacultural Engineering* 18: 223-244.

Hargreaves, J. A. 1998. Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture* 166: 181-212.

Hargrove, L. L., Westerman, P. W. and Losordo, T.M. 1996. Nitrification in Three-stage and Single-stage Floating Bead Biofilters in Laboratory-scale Recirculating Aquaculture System. *Aquacultural engineering* 15 (1): 65-80.

Huchette, S. M. H. and Beveridge, M. C. M. 2003. Technical and economical evaluation of periphyton-based cage culture of tilapia (Oreochromis niloticus) in tropical freshwater cages. *Aquaculture* 218: 219-234.

Hwang S. C., Lin, C. S., Chen, I. M., Chen, J. M., Liu, L. Y. and Dodds, W. K. 2004. Removal of multiple nitrogenous wastes by *Aspergillus niger* in a continuous fixed-slab reactor. *Bioresource Technology* 93: 131-138.

Jiang, L. X., Pan, L. Q. and Bo, F. 2004. Effect of dissolved on immune parameters of the white shrimp *Litopenaeus vannamei*. *Fish & Shell fish Immunology* 18: 185-188

Kamstra, A., Heul, J. W. and Nijhof, M. 1995. Performance and optimization of trickling filter on eel farms. *Aquacultural engineering* 17: 175-192.

Kankaanpaa, H. I., Holliday, J., Schroder, H., Goddard, T. J., Fister, R. and

Carmichael, W. W. 2005. *Toxicology and Applied Pharmacology* 203: 243-256.

Keshavanath, P., Gangadhar, B., Ramesh, T. J., Van Dam, A. A., Beveridge, M. C. M. and Vertegem, M. C. J. 2004. Effects of bamboo substrate and supplement feeding on growth and production of hybrid red tilapia fingerlings (*Oreochromis mossambicus* X *Oreochromis niloticus*). *Aquaculture* 235: 303-314.

Lekang, O. I. and Kleppe, H. 2002. Efficiency of nitrification in trickling filters using difference filter media. *Aquacultural engineering* 21: 181-199.

Ling, J. and Chen, S. 2005. Impact of organic carbon on nitrification performance of nitrification biofilters. *Aquacultural engineering* XX: XXX.

Martinez-Cordova, L. R., Villarreal-Colmenates, H., Porchas-Cornejo, M. A., Naranjo-Paramo, J. and Aragon-Noriega, A. 1997. Effect of aeration rate on growth, survival and yield of white shrimp *Penaeus vannamei* in low water exchange ponds. *Aquacultural engineering* 16:85-90.

Menasveta, P. 2002. Improved Shrimp Growout Systems for Disease Prevention and Environmental Sustainability in Asia. *Reviews in Fisheries Science* 10 (3&4): 391-402.

Montoya, R.A., Lawrence, A.L., Grant, V.E. and Velaso, M. 1999. Simulation of nitrogen dynamic and shrimp growth in an intensive shrimp culture system: effect of feed and feeding parameters. *Ecological modeling* 122: 81-95.

Na-anan, P., Hendeen, M. 2000. *Culture of tiger shrimp, Penaeus monodon by closed-recycled system*. Marine shrimp research and development. Technical paper No. 14/2000. 13 p.

Franco-Nava, M. A., Blancheton, J. P., Deviller, G., Charrier, A. and Le-Gall, J. Y. 2004. Effect of fish size and hydraulic regime on particulate organic matter dynamics in a recirculating aquaculture system: elemental carbon and nitrogen approach. *Aquaculture* 239: 179-198.

Ostrensky, A. and Wasielsky Jr., W. 1995. Acute toxicity of ammonia to various life stages the Sao Paulo shrimp, Penaeus paulensis Perez-Farfante, 1967. *Aquaculture* 132: 339-347.

Otoshi, C. A., Arce, S. M. and Moss, S. M. 2003. Growth reproductive performance of broodstock shrimp reared in the biosecure recirculating aquaculture system versus a flow-through pond. *Aquacultural Engineering* 29: 93-107

Paphavasit, N. 2003. *Rapid Assessment Methodology as a Tool for Integrated Coastal Zone Management in Thailand*: Mangrove Ecosystem. United Nations

Educational, Scientific and Cultural Organization.

Pruder, G. D. 2004. Biosecurity: application in aquaculture. *Aquacultural Engineering.* 32: 3-10.

Ridha, M. T. and Cruz, E. M. 2001. Effect of biofilter media on water quality and biological performance of the Nile tilapia Orechromis niloticus L. reared in a simple recirculating system. *Aquacultural Engineering* 24: 157-166.

Sandu, S. I., Boardman, G. D., Watten, B. J. and Brazil, B. L. 2002. Factors influencing the nitrification efficiency of fluidized bed filter with a plastic bead medium. *Aquacultural Engineering* 26: 41-59.

Sastry, B. N., DeLosReyes Jr., A. A., Rusch, K. A. and Malone, A. F. 1999. Nitrification performance of a bubble-washed bead filter for combined solid removal and biological filtration in a recirculating aquaculture system. *Aquacultural Engineering* 19: 105-117.

Shan, H. and Obbard, J. P. 2001. Ammonia removal from prawn aquaculture water Using immobilized nitrifying bacteria. *Appl. Microbiol. Biotechnol*. 57: 791-798.

Seo, J. and Boyd, C. E. 2001. Effects of bottom soil management practices on water quality improvement in channel catfish *Ictalurus punctatus* ponds. Aquaculture Engineering 25: 85-97.

Shrimp center. 2003. Management in shrimp growth problems. Available from: http://www.shrimpcenter.com/shrimp/t-shrimp077.html

Silapakul, S. 2002. *Nitrogen compounds removal in closed recirculating seawater system for shrimp pond by external loop airlift bioreactor*. Thesis in Master of Engineering in Chemical Engineering, Department of Chemical Engineering. Faculty of Engineering, Chulalongkorn Univerity.

Smith, P. T. 1996. Toxic effect of blooms of marine species of Oscillatoriales on farmed prawns (*Penaeus monodon* and *Penaeus japonicus*) and brine shrimp (*Artemia salina*). *Toxicon* 34 (8): 857-869.

Songsangjinda, P., Intramontree, C., La-ongsiriwong, L. and Tookwinas, S. 1999. *Treatment of the water from closed –recycled marine shrimp pond using oxidation pond and sand filtration*. Available from: http://www.thaifarmzond.com/shrimp/modules.php

Songsangjinda, P. 2002. *Nitrogen dynamic in shrimp pond*. Available from: http://www.thaifarmzone.com/shrimp/modules.php

Strickland, J. D. H. and Parson, T. R. 1972. *A Practical handbook of seawater analsis*. Fisheries research board of Canada. Bulletin 167, 2nd edition: 71-94.

Summerfelt, S.T. and Sharrer, M. J. 2004. Design implication of carbon dioxide production within biofilters contain in recirculating salmonid culture system. *Aquacultural Engineering* 32: 171-182.

Sundstorm, D. W. and Klei, H. E. 1979. *Wastewater treatment processes*. Wastewater treatment. Prentice-Hall Inc., USA 28-32.

Suzuki, Y., Maruyama, T., Numata, H., Sato, H. and Osakawa, M. 2003. Performance of a closed system with foam separation, nitrification and nitrification units for intensive culture of eel: towards zero emission. *Aquacultural Engineering* 29: 165-182.

Thakur, D. P. and Lin, C. K. 2003. Water quality and nutrient budget in closed shrimp (Penaeus monodon) culture systems. *Aquacultural engineering* 27: 159-176.

Thai farm zone. 2002. Problems from phytoplankton to black tiger shrimp culture. Available from: http://www.thaifarmzone.com/shrimp/modules.php

Thompson, F. L., Abreu, F. C. and Wasielesky, W. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture* (203): 263-278.

Tookwinas, S. and Sangrungruang, C. 1998. Study on Bio-filtering treatment for improving the water quality in an intensive marine shrimp pond. *Thai Fisheries Gazette* 51(6): 535-540.

Tookwinas, S. 2000. *Closed-recirculating shrimp farming system*. State-of-the-art series. Southeast Asian Fisheries Development Center. Association of Southeast Asian Nations. : 27 pp.

Tookwinas, S. 2001. *Shrimp culture in Thailand- Present Status and Future Directions for Research.* Avialable form: http://www.aciar.gov.au/web.nsf/doc/

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

Tseng, K. F. and Wu, K. L. 2004. The ammonia removal cycle for a submerged biofilter used in a recirculating eel culture system. *Aquacultural engineering* 31: 17-30.

Twarowska, J. G., Westerman, P. W. and Losordo, P. M. 1997. Water treatment and waste characterization evaluation of an intensive fish production system. *Aquacultural Engineering* 16: 133-147.

Wutikumpoln, M. 2003. *Effects of bio-filter on water quality in closed recirculating system for tilapia culture*. Thesis in Master of Science in Environmental Science (Inter-department). Graduate School. Chulalongkorn University.

Xie, W., Wang Q., Song, G. Condo, M., Teraoka, M., Ohsumi, Y. and Okawa H. I. 2004. Upflow biological filtration with floating filter media. *Process Biochemistry* 39: 765-770.

Yuwabenchapol, A. 2003. Marketing, Production and Situation of World shrimp and Thai shrimp. Available from: http://www.sittogroup.com/t-shrimp031.html

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

Zhu, S. and Chen, S. 2002. The impact of temperature on nitrification rate in fixed film biofilters. *Aquacultural Engineering* 26: 221-237.

APPENDICES

Appendix A: Shrimp length in trail I and trial II

Date	Day	control1		control2		treatment1		treatment2	
		average(cm)	SD	average(cm)	SD	average(cm)	SD	average(cm)	SD
5/22/2546	29	5.69	1.06	5.42	1.44	4.38	0.73	4.74	1.42
6/18/2546	56	5.49	1.31	5.22	1.39	6.31	2.04	6.80	1.68
7/31/2546	99	7.26	1.24	8.9	2.06	8.29	2.70	7.45	1.81
8/14/2546	113	8.80	1.64	10.15	2.19	8.93	2.52	9.99	2.41

Date	Date	Pond2		Pond3		Pond4		Pond5	
		average(cm)	SD	average(cm)	SD	average(cm)	SD	average(cm)	SD
1/28/2547	31	4.98	1.02	5.38	1.05	5.60	1.22	5.94	0.78
2/24/2547	58	6.35	1.21	6.94	1.66	6.71	1.49	7.13	1.56
2/26/2547	89	7.17	1.66	7.58	1.59	7.69	1.52	9.61	1.57
4/20/2547	114	7.53	1.49	7.16	1.64	8.64	1.90	0	0

Appendix B: Comparison of ammonia removal ($mgNH_3$-N/L) in the first trial of laboratory nitrification test

Hours	tank without biofilter		tank with new biofilter		tank with one month biofilter 1		tank with one month biofilter 2	
	average	SD	average	SD	average	SD	average	SD
0	1.78	*	1.81	*	2.51	*	1.90	*
12	1.77	*	1.82	*	0.82	*	1.70	*
14 adding NH4Cl	3.46	0.27	2.96	0.23	3.82	0.14	3.64	0.17
16	4.09	0.18	4.05	0.27	2.40	0.23	3.52	0.25
18	3.08	0.10	3.44	0.17	1.41	0.09	2.27	0.06
20	2.87	0.17	2.84	0.06	0.68	0.06	1.97	0.09
22	2.81	0.14	2.54	0.07	0.69	0.03	2.05	0.14
24	2.65	0.07	2.63	0.06	0.67	0.11	1.86	0.06
26	2.25	0.11	2.33	0.14	0.31	0.03	1.77	0.08
28	1.96	0.10	1.96	0.09	0.30	0.02	1.44	0.21
40	1.53	0.06	1.54	0.06	0.02	0.01	1.58	0.05
44	1.48	0.11	1.38	0.07	0.02	0.00	1.41	0.09
48	1.86	0.01	1.77	0.07	0.03	0.00	1.17	0.14
64	1.54	0.03	1.82	0.04	0.01	0.00	0.91	0.14

***** Use test kit

Appendix B (cont): Comparison of ammonia removal ($mgNH_3$-N/L) in the second laboratory nitrification test

Hours	tank without biofilter		tank with new biofilter		tank with one month biofilter 1		tank with one month biofilter 2	
	average	SD	average	SD	average	SD	Average	SD
0	2.60	0.04	2.71	0.06	2.74	0.05	2.75	0.06
18	2.17	0.07	2.17	0.03	0.77	0.17	1.93	0.04
19 adding NH4Cl	3.04	0.11	3.24	0.04	2.93	0.02	3.14	0.11
23	3.07	0.01	2.93	0.08	1.30	0.12	2.80	0.08
27	2.55	0.01	2.50	0.06	0.76	0.04	2.64	0.08
43	2.08	0.03	1.86	0.04	0.09	0.02	0.42	0.02
47	2.16	0.11	1.73	0.07	0.04	0.00	0.58	0.02
51	1.99	0.06	1.87	0.07	0.04	0.03	0.45	0.03
71	1.89	0.06	1.86	0.01	0.00	0.00	0.04	0.00

Appendix C: Comparison of ammonia removal ($mgNH_3$-N/L) in field nitrification test

First test

Hours	without biofilter chamber		2cm biofilter chamber		4cm biofilter chamber	
	average	SD	average	SD	average	SD
0	3.10	0.55	2.67	0.17	3.63	0.93
4	2.62	0.10	2.58	0.23	2.46	0.15
8	2.52	0.01	2.41	0.06	2.48	0.22
15	2.73	0.09	2.71	0.19	3.05	0.01
19	2.58	0.12	2.56	0.11	2.57	0.24
22	2.51	0.08	2.22	0.02	2.84	0.37
43	2.50	0.16	2.44	0.22	2.49	0.43
358	1.13	0.31	0.55	0.01	0.41	0.39

Second test

Day	without biofilter chamber		2cm biofilter chamber		4cm biofilter chamber	
	average	SD	average	SD	average	SD
initial	2.68	0.82	3.23	0.11	2.18	0.04
2	1.45	0.21	1.55	0.03	0.89	0.72
5	2.29	0.19	1.66	0.04	1.57	0.20
16	0.49	0.29	0.10	0.05	0.08	0.04

Appendix D: Statistical analysis output from biofilter efficiency test in an outdoor shrimp pond

Trial I

Ammonia (day 15)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	4.527874	1.131968	0.067223
2 cm	4	2.191756	0.547939	0.001686
4 cm	4	1.634284	0.408571	0.102243

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.178424	2	0.589212	10.32784	0.004673	4.256492
Within Groups	0.513457	9	0.057051			
Total	1.691881	11				

Nitrite (day 15)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	0.304084	0.076021	0.000116
2 cm	4	1.581587	0.395397	0.001643
4 cm	4	1.071108	0.267777	5.86E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.206744	2	0.103372	170.6371	6.99E-08	4.256492
Within Groups	0.005452	9	0.000606			
Total	0.212196	11				

Nitrate (day 15)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	0.188547	0.047137	0.000114
2 cm	4	0.109743	0.027436	7.78E-05
4 cm	4	0.260447	0.065112	0.005324

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.002841	2	0.00142	0.772673	0.490137	4.256492
Within Groups	0.016546	9	0.001838			
Total	0.019386	11				

Trial II

Ammonia (day 5)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	9.174844	2.293711	0.02556
2 cm	4	6.637121	1.65928	0.001345
4 cm	4	6.267806	1.566952	0.028708

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.252275	2	0.626138	33.77656	6.55E-05	4.256492
Within Groups	0.166839	9	0.018538			
Total	1.419114	11				

Ammonia (day 16)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	1.950266	0.487567	0.062728
2 cm	4	0.399202	0.0998	0.001902
4 cm	4	0.324351	0.081088	0.001481

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.42125	2	0.210625	9.557772	0.005941	4.256492
Within Groups	0.198333	9	0.022037			
Total	0.619584	11				

Nitrite (day 5)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	0.237688	0.059422	0.000496
2 cm	4	1.902645	0.475661	0.000289
4 cm	4	0.994357	0.248589	0.029653

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.347468	2	0.173734	17.12356	0.000856	4.256492
Within Groups	0.091313	9	0.010146			
Total	0.438781	11				

Nitrite (day 16)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	0.518612	0.129653	0.017484
2 cm	4	0.708159	0.17704	1.96E-05
4 cm	4	0.085768	0.021442	9.53E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.050888	2	0.025444	4.337179	0.047978	4.256492
Within Groups	0.052798	9	0.005866			
Total	0.103686	11				

Nitrate (day 5)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	0.128294	0.032074	5.6E-06
2 cm	4	1.485403	0.371351	0.001379
4 cm	4	0.715975	0.178994	0.017224

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.231594	2	0.115797	18.66826	0.000627	4.256492
Within Groups	0.055826	9	0.006203			
Total	0.28742	11				

Nitrate (day 16)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
no biofilter	4	0.124368	0.031092	0.001291
2 cm	4	0.604195	0.151049	0.008199
4 cm	4	0.099761	0.02494	0.000829

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.040441	2	0.020221	5.878099	0.023277	4.256492
Within Groups	0.03096	9	0.00344			
Total	0.071401	11				

Appendix E: Calculation for rate of ammonia removal by biofilter (%) in the treatment pond

Each treatment pond had 5 sets of biofilter which each set contained 11 of 1.5 m of cylinder shape of plastic media

So, total length of plastic media in each treatment 11x1.5x5= 82.5 m

Average efficiency of biofilter (lab experiment) is 447.5894 mgNH_4-N/m/day

Biofilter efficiency in each treatment pond is 447.5894x82.5= 36926.1255 mgNH4-N/day

For example, if daily feeding in treatment pond was 2.4 kg, ammonia released should be (2.4x40.6/100)/6.25= 0.155904 kg-N or 155904 mg-N

*This experiment used 40.6% protein feed and 6.25 was factor constant value for converted protein to nitrogen

As the result, percentage for ammonia removal by biofilter was

(36926.1255/155904)*100= 23.68517 %

BIOGRAPHY

Miss Suttikarn Sutti was born on June 25, 1981 in Udornthanee. She graduated with the primary education at Anuban Songkhla School, secondary education at Mahawachiravut School at Songkhla and bachelor degree in Biological Science from Department of Biological Science, Faculty of Science, Prince of Songkhla University in 2001. She continued her further study for Master's degree in Marine Science at the Faculty of Science, Chulalongkorn University in 2002.

During M.Sc. study, she was granted by the Thailand Graduated Institute of Science and Technology scholarship. The results from this thesis were presented at the conferences as following:

Sutti, S., Powtongsook, S. and Menasveta, P. (2005) Relationship between phytoplankton, zooplankton and environmental parameters in low salinity shrimp culture pond. **The Second National Conference on Algae and Plankton**, 23-25 March 2005, Chiang Mai, Thailand.

Sutti, S., Powtongsook, S. and Menasveta, P. (2004) Efficiency of nitrification biofilter for ammonia treatment in shrimp culture pond. **Proceedings of 30th Congress on Science and Technology of Thailand**, 19-21 October 2004, Impact Exhibition and Convention Center, Muang Thong Thani, p. 67.

[Suthi, S., Powtongsook, S., Chairatana, C., Ngampongsai, C., Nupasant, P., Piyateratitivorakul, S., Menasveta, P. (2004) Integration of biofilter and horizontal net in the closed system shrimp pond. **The 5th National Symposium on Marine Shrimp**, 29-30 March 2004, National Center for Genetic Engineering and Biotechnology, pp.333-334.