การประเมินสมรรถนะของระบบเลี้ยงสัตว์น้ำแบบปิดในการควบคุมปริมาณตะกอนและสารประกอบอินทรีย์ไนโตรเจนระหว่างการเลี้ยงปลานิลระดับความหนาแน่นสูง

นายเอกพจน์ แขมมี

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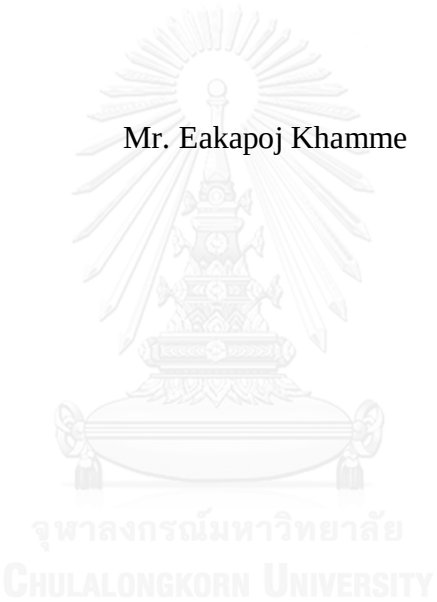
PERFORMANCE EVALUATION OF CLOSED AQUACULTURE SYSTEM IN CONTROLLING SOLIDS AND INORGANIC NITROGEN COMPOUNDS DURING INTENSIVE TILAPIA CULTIVATION

Mr. Eakapoj Khamme



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เอกพจน์ แขมมี : การประเมินสมรรถนะของระบบเลี้ยงสัตว์น้ำแบบปิดในการควบคุมปริมาณตะกอนและสารประกอบอนินทรีย์ไนโตรเจนระหว่างการเลี้ยงปลานิลระดับความหนาแน่นสูง (PERFORMANCE EVALUATION OF CLOSED AQUACULTURE SYSTEM IN CONTROLLING SOLIDS AND INORGANIC NITROGEN COMPOUNDS DURING INTENSIVE TILAPIA CULTIVATION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.กษิดิศ หนูทอง, 104 หน้า.

การทดลองนี้มุ่งในการประเมินสมรรถนะของระบบเลี้ยงสัตว์น้ำขนาดเล็กซึ่งดัดแปลงจากการออกแบบของ Sesuk et al. (2009) โดยเพิ่มหน่วยแยกตะกอนแขวนลอยเข้ามารวมในระบบ จากการทดลองส่วนแรกพบว่าการมีตัวกรองชีวภาพไนตริฟิเคชันที่พร้อมต่อการใช้งานและการแยกตะกอนเป็นปัจจัยที่สำคัญในการควบคุมระดับสารอนินทรีย์ไนโตรเจนและของแข็งแขวนลอยในถังเลี้ยง ระบบเลี้ยงสัตว์น้ำขนาดเล็กที่มีความยาวตัวกรองชีวภาพไบโอคอร์ด 20 m และการใช้งานหน่วยแยกตะกอนสามารถรองรับความหนาแน่นของสัตว์น้ำในถังเลี้ยงได้สูงสุดที่ 5 kg/m^3 หรือเทียบเท่าภาระโหลดไนโตรเจนที่ 8.4 mg N/L/day ในการทดลองส่วนที่สองทำการศึกษาถึงแนวทางการจัดการตะกอนที่เหมาะสมซึ่งประกอบด้วยวิธีการแยกตะกอนและความถี่ในการซักทำความสะอาดตัวกรองชีวภาพ โดยมีวัตถุประสงค์เพื่อเพิ่มประสิทธิภาพของระบบเลี้ยงสัตว์น้ำขนาดเล็กให้สูงกว่าการทดลองแรก ผลการทดลองเมื่อทำการเลี้ยงปลานิลที่ระดับ 7 kg/m^3 โดยไม่ถ่ายน้ำในระบบเลี้ยงสัตว์น้ำขนาดเล็ก พบว่าการกรองโดยใช้ใยญี่ปุ่นมีประสิทธิภาพสูงกว่าการใช้งานหน่วยแยกตะกอนจำนวน 1 หน่วย การทำความสะอาดตัวกรองชีวภาพไบโอคอร์ดและใยญี่ปุ่นทุก 4 วัน สามารถควบคุมคุณภาพน้ำในถังเลี้ยงให้อยู่ในระดับที่เหมาะสมแก่การเลี้ยงสัตว์น้ำได้ สำหรับการทดลองสุดท้ายได้ทดสอบสมรรถนะของระบบเลี้ยงสัตว์น้ำขนาดเล็กโดยเลี้ยงปลานิลที่ความหนาแน่นเริ่มต้นประมาณ 3 kg/m^3 โดยไม่ถ่ายน้ำเป็นเวลา 60 วัน และใช้แนวทางการจัดการตะกอนที่ได้รับจากการทดลองส่วนที่สอง ผลการทดลองในส่วนสุดท้ายพบว่าระบบสามารถควบคุมความเข้มข้นของแอมโมเนียและไนไตรท์ได้ต่ำกว่า 1.0 mg N/L ตลอดการทดลอง และมีระดับตะกอนแขวนลอยในถังเลี้ยงในช่วงระหว่าง 20 ถึง 35 mg SS/L อัตรารอดและอัตราการเจริญของปลานิลมีค่าเป็นที่น่าพอใจที่ 97% และ 3.45 g/day ตามลำดับ การแยกตะกอนของแข็งเป็นปัจจัยที่สำคัญต่อความสำเร็จในการใช้งานระบบเลี้ยงสัตว์น้ำขนาดเล็กที่นำเสนอในงานวิจัยนี้แม้ว่าระดับความหนาแน่นของสัตว์น้ำอยู่ในระดับกึ่งพัฒนา (Semi-intensive Cultivation) นอกจากนี้ผลการวิเคราะห์โดยใช้การสมดุลมวลไนโตรเจนในระบบเลี้ยงสัตว์น้ำพบว่าไนตริฟิเคชันและดีไนตริฟิเคชันเป็นกระบวนการทางชีวภาพหลักที่ใช้บำบัดสารประกอบอนินทรีย์ไนโตรเจน ขณะที่การแยกตะกอนของแข็งช่วยยืดอายุการใช้งานตัวกรองชีวภาพไนตริฟิเคชัน

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557

5471044021 : MAJOR CHEMICAL ENGINEERING

KEYWORDS: AQUACULTURE / DENITRIFICATION / NITRIFICATION / RAS / SOLIDS

EAKAPOJ KHAMME: PERFORMANCE EVALUATION OF CLOSED AQUACULTURE SYSTEM IN CONTROLLING SOLIDS AND INORGANIC NITROGEN COMPOUNDS DURING INTENSIVE TILAPIA CULTIVATION. ADVISOR: ASST. PROF. KASIDIT NOOTONG, Ph.D., 104 pp.

This experiment aimed to evaluate the performance of compact aquaculture system modified from the original design by Sesuk et al. (2009) by integrating suspended solids removal unit. In the first part, the presence of active nitrifying biofilters and solid removal were important aspect for controlling inorganic nitrogen and solids concentrations. The compact aquaculture system given the length of Biocord^{TM} biofilters at 5.6 m and operation of solid separating unit was able to accommodate the total aquaculture weight as high as 5 kg/m^3, which corresponded to the nitrogen loading rate of 8.4 mg N/L/day. In the second experiment, suitable management strategies namely method of solid separation and frequency of biofilter cleaning were determined in order to improve the aquaculture system capacity. By maintaining the aquaculture weight at 7 kg/m^3, it was found that filtration by Japanese mats filtered media was more effective in solid-liquid separation than using the gravitational sedimentation in solid separating unit. Moreover, cleaning the filtration unit and Biocord^{TM} by scratching and rinsing with clean water every 4 days was able to maintain good water quality within acceptable range for practical aquaculture cultivation. In the final experiment, the compact aquaculture system with filtration unit was operated using the strategies determined previously to grow tilapia without water exchange for 60 days. Under the described operating condition, ammonium and nitrite concentrations were significantly less than 1.0 mg N/L while suspended solid concentrations varied between 20 and 35 mg SS/L. Survival and growth rates of tilapia were measured at 97% and 3.45 g/day, respectively. Finally, solid removal appeared as critical factor for successful aquacultures in the compact aquaculture system even under extensive or semi-intensive aquaculture cultivation, and finally nitrogen mass balance performed after the conclusion of the tilapia cultivation indicated that nitrification-denitrification were main treatment pathways while solid removal only prolonged the activity of nitrifying biofilters.

Department: Chemical Engineering Student's Signature .....

Field of Study: Chemical Engineering Advisor's Signature .....

Academic Year: 2014

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Chapter 1

Introduction

1.1 Motivation

The annual increases in protein consumption as well as the increase production of animal feeds have driven the aquaculture industry in Thailand to change its production strategy from extensive low-density opened system toward intensive high-density closed system cultivation. Closed system aquaculture cultivation is advantageous over the opened system in term of reducing the risk of microbial and viral infection from polluted water and lowering the nutrient (i.e., nitrogen and phosphorus) discharge from farming facility to natural water resource.

Accumulation of inorganic nitrogenous compounds, especially ammonium and nitrite, is undesirable for good aquaculture practice. These compounds at excessive levels (i.e., > 1.0 mg N/L) can cause negative health effect toward cultured animals, namely inducing stress, weakening the animal immune system, retarding growth or even death. As a result, farmers are forced to exchange water from culture tanks or ponds frequently as high as 5 to 6 times daily. Discharge of aquaculture effluent containing nitrogenous compounds even at low concentrations also creates various environmental concerns, namely depletion of oxygen in receiving water, toxicity towards aquatic animal plants, eutrophication and contamination of drinking water.

From these reason, employment of recirculating aquaculture system (RAS) is perceived as a sustainable solution for aquaculture. Conventionally, nitrifying biofilters and, to the lesser extent, the combination of nitrifying and denitrifying biofilters in separated compartments have been successfully employed to treat aquaculture effluent in many configurations, for example fluidized sand filters, packed-bed bioreactors, moving-bed bioreactors, rotating biological contactors and trickling filters. The mentioned biofilter systems are usually coupled with expensive and complex solid-liquid separators to enhance the system performance. In spite of high treatment efficiency, nitrifying and denitrifying aquaculture systems remain quite costly to operate due to the energy requirement for pumping water through series of unit operations, redundant aeration provided in both cultured tanks and nitrifying biofilter unit and the requirement for high-skilled operators to operate and maintain the systems.

A compact aquaculture system, initially developed by Sesuk et al. (2009), integrates the fibrous nitrifying biofilter directly into the rearing tank. The concept of such design intends to use fibrous nitrifying biofilters for nitrogenous treatment and separation of suspended solids (SS) from water simultaneously as well as to reduce water circulation demand, with the final aims of creating a simple and affordable aquaculture system for small-scale budget-limited farmers or for urban aquacultures. Early assessment of the proposed system, which employed acclimated nitrifying biofilters (Biocord^{TM}) of 5.6 m in length, revealed that the aquaculture system was able to produce good quality effluent with ammonium and nitrite concentrations less than 1.0 mg N/L and suspended solid concentrations less than 70 mg SS/L. However,

the setback of that study includes relatively short experimental period of less than 40 days and low nitrogen loading rates ranged from 1.24 to 2.78 mg N/L/day, which were equivalent to fish weight from 0.68 to 2.6 kg/m^3. In addition, significant solid accumulation was observed on the surface of fibrous nitrifying biofilters at the end of the experiment that consequently led to lower ammonium degradation rates.

In the present study, the original design by Sesuk et al. (2009) is modified by combining with low-budget solid separating unit to increase the system treatment capacity at higher nitrogen loading rates and to prolong nitrifying activity of fibrous biofilters. Solid separating unit, based on the work by Nootong et al. (2013), is operated using gravitational sedimentation to separate suspended solids from water column with the maximum removal efficiencies reported around 70 to 72% at the optimal volumetric flow rates.

Therefore, the present study aims to evaluate the performance of the compact aquaculture system modified according to the original design of Sesuk et al. (2009) by integrating the solid removal unit. The assessment is conducted under higher nitrogen loading rates and longer cultivating period as compared to those of the original study. Management and economic aspects of the compact aquaculture system are also analyzed and presented.

1.2 Objectives

1. Design the compact aquaculture system able to treat nitrogenous compounds and separate suspended solids from bulk liquid during aquaculture cultivation.
2. Evaluate the performance of compact aquaculture system integrated solid-liquid separators in controlling inorganic nitrogen and suspended solid concentrations during zero-water exchange aquaculture cultivation.

1.3 Scopes of Work

This work can be broadly divided into four sections. The first part describes the acclimatization of biofilters to establish nitrification. The second part is the preliminary evaluation of the compact aquaculture system to identify the system capacity in term of nitrogen loading rate and aquaculture weight. The third part extended the results of the second part to improve the system capacity and to determine the appropriate system management strategy, and finally the final part evaluates the performance of the compact aquaculture system during the zero-water exchange aquaculture cultivation. The scopes of this work can be listed as follows.

1. Perform single sex tilapia (*Oreochromis niloticus*) cultivation without water exchange in the compact aquaculture system at the fish weights from 3 to 11 kg/m^3 using the commercial feed.

2. Evaluate the performance of the compact aquaculture system in controlling ammonium and nitrite concentrations lower than 1.0 mg N/L and suspended solid concentrations lower than 70 mg SS/L.
3. The following parameters are used to assess the system performance: concentrations of ammonium, nitrite, nitrate and suspended solids in tilapia rearing tank, tilapia mortality (i.e. survival) and growth rates, and physical parameter including pH, DO, and alkalinity.
4. Economic feasibility of the compact aquaculture systems is based on investment cost, operational cost, utility cost, and revenue from fish sale.

1.4 Benefits

1. Obtain the design of the compact aquaculture system for the semi-intensive or higher density aquaculture cultivation.
2. Obtain the optimal operating condition for the compact aquaculture system.
3. Reduce the nitrogenous waste discharge into natural receiving water.
4. It is possible to use the data from the present work to apply with the cultivation of other economical aquaculture species.

Chapter 2

Literature Review

2.1 Intensive aquacultures

Intensification of aquaculture cultivation is necessary in order to satisfy the increasing demand for consumption. For the minimal fed ponds, which are common practice for Thai aquaculture farmers, cultured animals are usually kept without aeration and fed with small quantity of feed made of grains and residues from home activities, thus resulting in low productivity of less than 2,000 kg/ha/year. Fed ponds are similar to minimal fed pond except that commercial feeds are used and thus results in higher productivity ranged from 2,000 to 4,000 kg/ha/year. Depletion of oxygen early in the morning could also be observed with fed pond cultivation. Night time aeration ponds are set up to overcome the limitation of fed pond that is the depletion of oxygen in the early morning. Cultured animals are fed with commercial feeds to supply sufficient amounts of proteins to promote growth. Aerators with the power ranged from 1 to 5 hp/ha are usually employed to provide oxygen at night or during emergency situation. Productivity of night time aeration ponds ranges from 4,000 to 10,000 kg/ha/year. However, night time aeration ponds are associated with excess sludge accumulation on the pond bottom, creating undesirable anaerobic condition. In the intensive mixed aerated ponds, aeration and mixing are provided continuously for the entire day as well as using commercial feed to provide sufficient proteins for animals. Productivity

of intensive mixed ponds is reported to be in the range from 20,000 to 100,000 kg/ha/year. Due to high animal density in the ponds, water quality, specifically the excessive concentrations of ammonium, nitrite and suspended solid and solid accumulation on pond bottom, are the main difficulty for the successful operation. Table 2.1 summarizes the pond intensification for different pond systems.

Table 2.1 Pond intensity levels, annual fish yields and limiting factors in operating the pond (Avnimelech, 2006)

Pond type	**Intervention**	**Yields (kg/ha/year)**	**Limiting factors**
Minimal Feed	Minimal feed with grains, farm and home residues	< 2000	Limits of primary production food chain efficiency
Fed ponds	Feeding by complete diet pellets	2,000 – 4,000	Early morning oxygen depletion
Night time aeration	Night time or emergency aerators, <1-5 hp/ha	4,000 – 10,000	Sludge accumulation, anaerobic pond bottom
Intensive mixed aerated ponds	24 h aeration, >20 hp/ha, well-mixed	20,000-100,000	Water quality control

Three different approaches were employed as means to maintain acceptable water quality: (1) replacing pond water with freshwater as frequent as 5 times per day (2) treating and recycling water through external nitrifying and denitrifying biofilters and (3) treating water within pond using microalgae or activated bacterial communities (i.e., biofloc technology system).

2.2 Water quality parameters

The knowledge about water chemistry and suitable range of operating parameters are important for the successful cultivation at semi-intensive or intensive levels. Table 2.2 lists important water quality parameters required for aquacultures.

Table 2.2 Water quality parameters in aquaculture (Adapted from Timmons et al., 2002)

Parameters	**Concentration (mg/L)**
Chlorine (Cl)	< 0.003
Hydrogen sulfide (H_2S)	< 0.002
Nitrite (NO_2)	< 1.0
Nitrate (NO_3)	0 – 400
Oxygen Dissolved (DO)	> 5
Alkalinity (as $CaCO_3$)	50 – 300
Ammonium (TAN) Cool-water fish	< 1.0

Parameters	Concentration (mg/L)
Ammonium (TAN) Warm-water fish	< 3.0
Carbon Dioxide (CO_2)	20 – 60 depending on species
Ozone (O_3)	< 0.005
pH	6.5 – 8.5
Phosphorous (P)	0.01 – 3.0
Salinity	depends on salt or fresh species
Sodium (Na)	< 75
Sulfate (SO_4)	< 50
Sulfur (S)	< 1.0
Total suspended solids (TSS)	< 80

2.3 Tilapia

Tilapia *(Orechromis niloticus*) is a native animal of Africa. Tilapia has fairly conventional, laterally compressed and deep body shape with the body that is quite large and hard-to-removed scales (Ross, 2000). The dorsal and anal fins have hard spines and soft anterior in an advanced configuration. Numbers of scales, gill rakers and fins are widely used to identify different sub-species. The body of the fish is generally characterized by vertical bars with relatively subdued colors and with little contrast over the body colors. This provides tilapia with a modest ability to change

their body colors in response to stress by controlling skin chromatophores (Ross, 2000).

Tilapia are able to grow in wide range of temperature from 20 to 35 ºC (Balarin and Hatton, 1979). Tilapia was observed to stop eating when the temperature of water was below than 15 °C and die at the temperature less than 8 °C (Abdel-Fattah and El-Sayed, 2006). Alkalinity of water should be maintained from 20 to 300 mg $CaCO_3$/L while the optimal pH range was reported from 6.5 to 8.5 (Ross, 2000; Abdel-Fattah and El-Sayed, 2006). Ammonium is toxic towards tilapia when the concentrations are greater than 1.0 mg N/L while the threshold for nitrite concentrations is reported at 2.1 mg N/L but recommended to keep the concentration below 1.0 mg N/L (Abdel-Fattah and El-Sayed, 2006).

2.4 Inorganic Nitrogen Compounds and Toxicities

An annual increase in human population implies more demand on protein consumption from aquaculture. Discharge of effluent containing ammonium, nitrite and nitrate from aquaculture facilities into natural receiving water creates various environmental and health effects, which are briefly discussed as follows:

Toxicity. Nitrogenous compounds can be toxic towards human and aquatic life. Free ammonia at the concentration above 0.2 mg/L has been demonstrated to be fatal to several fish species (Sawyer and McCarty, 1978). Equilibrium concentrations of free ammonia (NH_3) and ammonium ion (NH_4^+) depend on pH in the way that ammonia

dominated when the pH is greater than 7. Ammonia toxicity was not a concern when the pH of receiving water and ammonia concentration are less than 8 and 1.0 mg N/L, respectively (Sawyer and McCarty, 1978). Nitrite (NO_2^-) is a potential mutagen and carcinogen when it reacts with proteins products such as amines to form nitrosamines. Infant under the age of six months may become seriously ill and die, if untreated, after drinking water contaminated with nitrite. For nitrate (NO_3^-), it is poisonous when the concentrations are greater than 90 mg N/L because it causes methanoglobinemia (i.e., blue baby syndrome), the disease that prevents effective binding of hemoglobin in red blood cells with oxygen in the infant under four months old (Metcalf and Eddy, 2003). This disease usually occurs in the regions where the usage of groundwater is common. USEPA (United States Environmental Protection Agency) and WHO (World Health Organization) regulate nitrite and nitrate concentrations in drinking water at 1.0 and 10 mg N/L, respectively (Tsao, 2003).

Dissolved Oxygen Depletion. Bacteria in water are able to oxidize ammonium to nitrite and nitrate successively in a biological process called nitrification when the dissolved oxygen (DO) concentrations are greater than 2 mg/L. Oxygen requirement to complete nitrification referred to as the nitrogenous oxygen demand (NOD) equals to 4.6 mg O_2 per mg NH_3 consumed (Metcalf and Eddy, 2003). NOD accounts for the additional oxygen requirement to the biochemical oxygen demand (BOD). The combining effect of BOD and NOD can lead severe oxygen depletion in the receiving water especially when the re-aeration rates are not adequate. Moreover, aquatic plants can also utilize ammonium, nitrite and nitrate as nutrients to support their growths that consequently lead to serious problems after their death because the remains of those

plants will magnify BOD requirement for that particular water body, thereby resulting in severe oxygen depletion (Tsao, 2003).

Eutrophication. Eutrophication refers to the natural aging of freshwater reservoir such as lakes to become organically rich, leading to the domination of weeds and eventually transforming into mash land (Metcalf and Eddy, 2003). Discharge of nitrogenous compounds into lakes and natural water reservoirs accelerates eutrophication process by promoting the growth of aquatic plants and microalgae. The onset for the rapid bloom of microalgae in well-mixed lake was predicted when the concentrations of inorganic nitrogen and phosphorus exceed 0.3 mg N/L and 0.01 mg P/L, respectively (Metcalf and Eddy, 2003). Eutrophication is detrimental for the aquatic life especially during the night-time when oxygen production by photosynthesis is halted, whereas the competition for oxygen from fish and other species (e.g., bacteria and aquatic plants) is fierce.

2.5 Biological Pathway for Nitrogen Treatment in Aquaculture Cultivation

2.5.1 Nitrification

Nitrification is the biological process in which ammonium is oxidized under aerobic condition to nitrite and nitrate successively in a two-step reaction scheme as show equation 2.1 and 2.1.

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

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

The first step of nitrification, which involves the conversion of ammonium to nitrite (i.e. equation 2.1), is carried out by the ammonium oxidizing bacteria (AOB) such as *Nitrosomonas*, *Nitrosolobus*, *Nitrosococcus*, *Nitrovibrio* and *Nitrospira* (Painter, 1977). Enzyme ammonium monooxygenase (AMO) is identified as a catalyst for the oxidation of ammonium to hydroxylamine, which is further oxidized to nitrite by enzyme hydroxylamine oxidoreductase (HAO)(Shrestha et al., 2002). *Nitrobacter* is commonly recognized as a species responsible for the second step of nitrification that is the conversion of nitrite to nitrate (i.e. equation 2.2). Other nitrite oxidizing bacteria (NOB) include *Nitrospina* and *Nitrococcus*, which are marine species. *Nitrospira*-like bacteria was found as common NOB in various wastewater treatment plants (Persson et al., 2002). It was postulated that *Nitrobacter* is unable to compete for nitrite at low concentrations, whereas *Nitrospira*-like bacteria can grow in such environment (Daims et al., 2000). The overall stoichiometry of nitrification including cell synthesis (i.e., $C_5H_7NO_2$) is written in equation 2.3.

$$62NH_3 + 5CO_2 + 117O_2 \rightarrow C_5H_7NO_2 + 59H_2O + 61NO_3^- + 61H^+ \quad (2.3)$$

Environmental Factor Affecting Nitrification

Dissolved Oxygen (DO). The optimal DO concentrations for nitrification are greater 2.0 mg/L. Pure cultures of *Nitrosomonas* and *Nitrobacter* as well as the activated sludge show the complete inhibition of nitrification when the DO concentration is less

than 0.5 mg/L (Painter, 1977). In a full-scale nitrification plant, nitrite accumulation was eliminated when the supply of dissolved oxygen was sufficient (Hart et al., 1986). The nitrite oxidizer *Nitrobacter* has been suggested to possess lower affinity for dissolved oxygen than the ammonium oxidizers *Nitrosomonas* (Laanbroek et al., 1994). As the result, *Nitrosomonas* would outgrow *Nitrobacter*, leading to significant buildup of nitrite when the availability of oxygen is limited. Hanaki et al. also found that, at the DO concentration of 0.5 mg O_2/L, ammonium oxidizing bacteria had double growth yield compared to nitrite oxidizing bacteria (Hanaki et al., 1990).

Temperature. The rate of nitrification is directly proportional to temperature with the optimal range between 28 and 36 °C. Temperature-dependent growth of nitrifying bacteria can be described by the van't Hoff Arrhenius equation up to 30 °C. By using the data from pure culture from both domestic and industrial wastewater, the specific growth rate (μ) of nitrifying bacteria can be written in equation 2.4 as:

$$\mu = 0.18e^{0.0729(T-15)} \qquad (2.4)$$

where T is absolute temperature in K (Painter and Loveless, 1983). The rate of ammonium oxidation can be improved by raising temperature to compensate with the substrate availability as suggested by Groeneweg et al. (1994) who reported the ammonium oxidation rates for *Nitrosomonas* of 260 and 165 mg N/g VSS/L at 30 °C and 15 °C, respectively.

pH. The pH range between 7.5 and 8.5 was suggested as the optimal pH for nitrification. Ammonium and nitrite oxidations respond differently when the pH of bulk liquid is changing. Ammonia oxidation rate decreases while the rate of nitrite oxidation is observed to increase when the pH is less than 7. Under this condition, hydrogen ions generated by nitrification along with the inward bicarbonate gradient and outward CO_2 gradient may cause the pH in the bulk liquid to decrease. Further decrease in pH is also noticed in the deeper region of biofilm layer. United States Environmental Protection Agency (USEPA) recommended the pH for nitrification from 6.5 to 8.6 (USEPA, 1993). The optimal pH of nitrifying bacteria from pure and activated sludge cultures is reported around 8 ± 0.05 (Painter and Loveless, 1983). Nitrifying activities decreases when the pH went into the acidic range. Low pH values from 5.0 to 5.5 were demonstrated to effectively inhibit nitrification yet the process could be reversible after the pH was returned to the optimal level (Ford, 1980). From these reasons, sufficient alkalinity in the wastewater is necessary to prevent the sharp decline of pH.

Inhibitory Compounds. Various compounds have been demonstrated to retard or completely inhibit nitrification. Among those compounds are organic compounds such as acetate, methanol or ethanol. It was hypothesized that organic compounds could stimulate the growth of heterotrophic bacteria, which were known to consume oxygen at the significant faster rates than nitrifying bacteria, thereby resulting in oxygen depletion and eventually decreasing of nitrification rate (Hargreaves, 2006). Nitrification can also be inhibited by its substrates (*e.g.* ammonia and nitrite) that followed the pattern of Haldane inhibition kinetics. Nitrite was also reported to

irreversibly inhibit ammonium oxidizing bacteria at the concentration higher than 80 mg N/L at pH 8 and 30 °C (Groeneweg et al., 1994). Heavy metals such as silver, mercury, copper, zinc, nickel and chromium are toxic toward nitrification (Tsao, 2003). Nickel was shown to assert more toxicity toward NOB more than AOB, (Randall and Buth, 1984). Sato et al. reported a magnified toxic effect of copper on the culture of *Nitrosomonas europaea* when NH_4^+-N concentration increases from 3 to 23 mg N/L (Sato et al., 1988), while Lu et al. found that *m*-cresol and *p*-cresol were toxic at concentration beyond 15 mg/L (Lu et al., 1984). Other inhibitory substances include cyanides, halogenated compounds, phenols, mercaptans, and thiourea. Table 2.3 illustrates inhibitory concentration of various compounds on nitrification.

Table 2.3 Examples of inhibitory compounds and inhibitory concentrations on nitrification (Sesuk, 2008).

Chemicals	**Inhibitory Concentration (mg/L)**
Cobalt	0.08 – 0.5
Chromium	0.25
Copper	0.05 – 0.56
Nickel	0.25
Zinc	0.08 – 0.5
Cadmium	14.3
Sulfide	5.0
Sodium Chloride	35,000

Chemicals	Inhibitory Concentration (mg/L)
Sodium Cyanide	1
Hydrogen Sulfide	50
Potassium Dichromate	6

2.5.2 Heterotrophic Denitrification

Heterotrophic denitrification is the biological process in which NO_2^- and/or NO_3^- are reduced to gaseous N_2, NO, or N_2O under the oxygen-limited or anoxic conditions. Heterotrophic denitrification requires external organic carbon source as the electron donors to provide energy for heterotrophic denitrifying bacteria. Common microorganisms responsible for heterotrophic denitrification include Achromobacter, Bacillus denitricans, Flavobacterium, Micrococcus denitrificans, Proteus and Pseudomonas and Pseudomonas stutzeri (Metcalf and Eddy, 2003). Heterotrophic denitrification also requires appropriate electron donors, which are normally organic carbon such as methanol, ethanol and acetate. The stoichiometry of denitrification depends on which the type of organic carbon substrate but can be generalized as shown in equation 2.5.

$$5(\text{organic-C}) + 2H_2O + 4NO_3^- \rightarrow 2N_2 + 4OH^- + 5CO_2 \qquad (2.5)$$

According to equation (2.5), denitrification generates one hydroxyl ion (OH^-) for every nitrate being reduced. The hydroxyl ions can neutralize the hydrogen ions (H^+) produced during nitrification to maintain appropriate pH for establishing the simultaneous nitrification and denitrification (SND). In general, DO concentrations for heterotrophic denitrification are maintained below 0.5 mg O_2/L (Van Rijn et al., 2006). For higher DO concentrations, heterotrophic denitrifying bacteria would switch from nitrite and nitrate to employ oxygen as the final electron acceptor, thus leading to the accumulation of nitrite and nitrate in the effluent. Methanol is the most common electron donor for heterotrophic denitrification due to its relatively low cost (Metcalf and Eddy, 2003). Other organic compounds such as acetic acid, citric acid, acetone, ethanol, methane, benzoate n-alkanes, molasses or even organic residues in wastewater can be employed as external electron donors (Nguyen, 1994). By using methanol as an example, denitrification can be described by the following equations:

$$NO_3^- + 0.33CH_3OH \rightarrow NO_2^- + 0.33CO_2 + 0.67H_2O \quad (2.6)$$

$$NO_2^- + 0.5CH_3OH \rightarrow 0.5N_2 + 0.5CO_2 + 0.5H_2O + OH^- \quad (2.7)$$

$$NO_3^- + 0.83CH_3OH \rightarrow 0.5N_2 + 0.83CO_2 + 1.17H_2O + OH^- \text{ (Overall reaction)} \quad (2.8)$$

The required dose of methanol to achieve the complete heterotrophic denitrification when waste stream contains both nitrite and nitrate can be calculated as follows:

$$Cm = 2.47Na + 1.53Ni + 0.87D \quad (2.9)$$

Where *Cm* is the required methanol dosage in mg/L; *Na* is the Initial NO_3^--N concentration in mg/L; *Ni* is the initial NO_2^--N concentration in mg/L, and D is the initial DO concentration in mg O_2/L in waste stream (Metcalf and Eddy, 2003).

Environmental Factors Affecting Heterotrophic Denitrification

Dissolved Oxygen. Low oxygen concentrations must be maintained in order to establish the successful heterotrophic denitrification. Many reports suggested different thresholds for the DO concentrations, for example ranging from 0.2 to 2.0 mg O_2/L according (Christensen and Harremoës, 1977) and completely absence of oxygen based on Payne (1981). Varying reports on the optimal DO concentrations for heterotrophic denitrification perhaps arise from different microorganisms residing in the cultures, the specific nature of the bioflocs that microorganisms reside and finally the degree of oxygen penetration into bioflocs (Payne, 1981).

pH. The optimal pH for heterotrophic denitrification depends on specific bacteria or source of sludge but it is generally accepted that the optimal pH varied from 7 to 8 (Winkler, 1984). The rate of denitrification was observed to decrease approximately 30% when the pH is outside that range. The optimum pH for heterotrophic denitrification was determined at 7.5 at 25 °C when methanol was the electron donor (Beccari et al., 1983). The level of pH during denitrification can also determine the species of the end products, for instance, the majority of end product is nitrous oxide when the pH is below 7.3 while nitrogen gas is dominant beyond that pH level

(Christensen and Harremoës, 1977). Knowles showed that N_2O is a major product of denitrification at pH 4 (Knowles, 1982).

Temperature. The temperature-dependency of heterotrophic denitrification can be described by Arrhenius equation similar to that of nitrification. Denitrification is active under wide temperature range between 0 and 50 °C with the optimal values reported between 35 and 40 °C (Winkler, 1984). The temperature-dependency of denitrification in the attached growth systems was shown to be less sensitive than the suspended growth system (Christensen and Harremoës, 1977).

Inhibitory Compounds. Heterotrophic denitrification is inhibited by similar compounds to nitrification. Acetylene and pesticides were shown to inhibit heterotrophic denitrification (Winkler, 1984). Metal chelating agents such as potassium cyanide, dithiol, and o-phenanthroline can inhibit nitrate reductase enzyme in *Pseudomonas aeruginosa*. The nitrite reductase and hydroxylamine reductase enzymes in *P. aeruginosa* were also inhibited by copper (Painter, 1977). Additional study by Abeling and Seyfried revealed that heterotrophic denitrification was inhibited by nitrous acid when the concentration exceeded 0.13 mg HNO_2/L in a 2-staged anaerobic treatment of high strength ammonium wastewater (Abeling and Seyfried, 1992).

2.5.3 Anaerobic Ammonium Oxidation (Anammox)

Anammox converts ammonium to nitrogen gas by using nitrite as terminal electron acceptors under strictly anaerobic condition. Anammox was originally discovered in

a methanogenic denitrification fluidized bed bioreactor that shown the disappearance of ammonium and nitrate from the effluent while concomitantly increasing the production of N_2 (Mulder et al., 1995). Anammox process is mediated by the group of *Plantomycete* bacteria, which is identified as *Candidatus* Brocadia *anammoxidan* and closely related *Candidatus* Kuenenia stuttgartientsis (Jetten et al., 2001; Schmidt et al., 2003).

Plantomycetales are a separate division within the bacteria domain that are more diverse than the nitrifying counterparts. They display distinctive phenotypic properties namely the absence of peptidoglycan on the cell wall, budding reproduction and internal cell compartmentalisation (Fuerst, 1995). Anammox catabolism occurs partly within a permeable lipid membrane-bound intracytoplasmic compartment called anammoxsome, which contains no RNA or DNA. For each Anammox cell, only one anammoxsome can be detected (Lindsay et al., 2001). The nucleoid of anammox bacteria is found outside the anammoxsome and appears very dense similar to other *Plantomycetes*. The main product of Anammox is nitrogen gas but approximately 10% can be identified as nitrate. Strous et al. proposed the stoichiometry for anammox as displayed in equation (2.10) (Strous et al., 1998).

$$NH_4^+ + 1.31NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O0.5N_{0.15} + 2.03H_2O \qquad (2.10)$$

Oxygen completely inhibited Anammox process after it was freely delivered into the enrichment cultures (Van de Graaf et al., 1997). However, oxygen inhibition on

anammox activity was shown to be reversible in the subsequent work by the same research group. Jetten et al. (1998) indicated that oxygen concentration as low as 2 mM and nitrite concentrations from 5 to 10 mM were able to hinder the activity of anammox. Anammox is inactive after exposure by gamma radiation, heating of pilot plant sludge and incubation with acetylene and phosphate at the concentrations above 10 mg/L (Van de Graaf et al., 1996). The optimal pH and temperature ranges of Anammox are similar to nitrifying bacteria, ranging from 6.4 and 8.3 and from 20 and 43 °C, respectively (Jetten et al., 1998). Anammox bacteria grow very slowly, showing the doubling time from 11 to 14 days. Both purified and unpurified cultures of anammox were active only when the cell count was higher than 10^{10}-10^{11} cells/mL. The activity of Brocadia *anammoxidans* was completely inhibited when exposing to nitrite and phosphate concentrations greater than 70 mg N/L and 60 mg P/L, respectively for more than several days (Jetten et al., 1998). The rate of ammonium oxidation for anammox bacteria was reported to be higher than aerobic nitrifying bacteria for about 20 folds under anaerobic environment with nitrite as terminal electron acceptor yet it was approximately 7 times slower than the conventional aerobic ammonium oxidation (Jetten et al., 2001). Table 2.2 illustrates various parameters associated with aerobic and anaerobic ammonium oxidation. Table 2.4 illustrate values of different parameters for aerobic and anaerobic ammonium oxidation

Table 2.4 Various parameters of aerobic and anaerobic ammonium oxidation

Parameter	Nitrification $NH_4^+ + O_2 \rightarrow NO_2^-$	Anammox $NH_4^+ + NO_2^- \rightarrow N_2$	Unit
Free Energy	-275	-357	kJ/mol
Biomass yield	0.08	0.07	mol/mol C
Aerobic rate	200 - 600	0	nmol/min/mg protein
Anaerobic rate	2	60	nmol/min/mg protein
Growth rate	0.04	0.003	hr^{-1}
Doubling time	0.73	10.6	Days
K_s NH_4^+	5 - 2,600	5	µM
K_s NO_2^-	N/A	< 5	µM
K_s O_2	10 - 50	N/A	µM

N/A, not applicable; K_s, affinity constant

2.5.4 Nitrogen Assimilation

Nitrogen can be removed from water based on the direct immobilization (assimilation) into cells to synthesize new biomass. Assimilation of nitrogen by microalgae during the photosynthesis is the important control measures for extensive earthen ponds. Hargreaves estimated that microalgae with the composition C:N:P of 106:16:1 could uptake nitrogen into cells from 150 to 1,500 mg N/m^2/day depending

on temperature (Hargreaves, 1998). Microalgae were able to utilize all forms of nitrogen in water but prefer ammonium over nitrate (De Boer et al., 1981). Hargreaves also pointed out that microalgae would first assimilate ammonium until the concentrations were less than 0.3 mg N/L before switching to acquire nitrate (Hargreaves, 1998). Heterotrophic bacteria can also assimilate ammonium and nitrite into cell as materials to be used in the protein synthesis during cell growth. Addition of organic carbon compounds can rapidly promote the assimilation provided that oxygen is available in sufficient quantity. Nitrogen assimilation by heterotrophic bacteria and microalgae can be described by equation 2.11 and equation 2.12, respectively with the symbols $C_5H_7O_2N$ and $C_{106}H_{263}O_{110}N_{16}P$ representing the proximate compositions of bacteria and microalgae, respectively (Ebeling and Timmons, 2007).

$$NH_4^+ + 1.18C_6H_{12}O_6 + HCO_3^- + 2.06O_2 \rightarrow C_5H_7O_2N + 6.06H_2O + 3.07CO_2 \quad \text{(Bacteria)} \qquad (2.11)$$

$$16NH_4^+ + 92CO_2 + HPO_4^{2-} + 92H_2O + 14HCO_3^- \rightarrow C_{106}H_{263}O_{110}N_{16}P + 106O_2 \text{ (Microalgae)} \qquad (2.12)$$

2.6 Nitrogen Removal Process in Aquaculture Cultivation

Design of nitrogen removal process in aquaculture cultivation utilizes the fundamentals of nitrogen removal pathways described in section 2.5. Specifically,

design of nitrogen removal process tries to provide suitable environment to support relevant microorganisms responsible for nitrogen removal (e.g., nitrifying and denitrifying bacteria or microalgae). Based on our literature survey, the nitrogen removal process can be categorized based on the cell distribution in the system, specifically the system with cells attached on media surface (i.e., attached-growth System) and the system with cells freely suspended in water (i.e., suspended-growth system). Moreover, nitrogen removal process can be categorized according to the biological pathway responsible for the nitrogen treatment such as nitrifying or denitrifying systems.

2.6.1 Attached-Growth System

In general, attached-growth systems are associated with nitrification and denitrification. Media, referred to as biofilters, are used for cell attachment. Media can be natural materials such as oyster shell or loofa sponge or synthetic materials such as polyethylene and polypropylene, which generally come in the forms of commercial packing media, for example Biocord™, Bioball™, HyperDrain™ and BCN series. Important characteristics of biofilters should be durable and possessed high surface area for bacteria to attach to encounter slow growth rate. Biofilters systems employing anammox are available in literature but their commercial applications remain limited due to complex operation and difficulty in maintain suitable environment to anammox bacteria.

Nitrifying Attached-Growth System

In general, effluent from cultured tanks or ponds containing ammonium is circulated through nitrifying biofilter units or column (i.e. bioreactor), which are continuously aerated (DO > 4.0 mg/L) to convert ammonium to nitrate. It should be pointed out that biofilters must be acclimated to establish nitrification before employing otherwise accumulations of ammonium and nitrite will result. Disadvantages of nitrifying biofilters are high operational cost for pumping and oxygenation as well as solid clogging between biofilters pore space. Nitrifying biofilters are successful in treating aquaculture effluent for indoor aquaculture but their application for outdoor system (e.g., lining ponds or cement tank located outdoor without shade) remained limited. Nitrifying biofilters are available in many configurations as described below:

Rotating Biological Contactors (RBC): RBCs are generally built from PVC disks submerging approximately 50% under water. Rotation of disks at low speed approximately 2 - 5 rpm gave nitrifying bacteria the opportunity to contact with ammonium substrate in aquaculture effluent as oxygen in air. RBCs are less susceptible to solid clogging. Laboratory scale utilizing RBCs revealed ammonium degradation rates from 190 to 790 mg $N/m^2/day$ (Miller and Libey, 1985; Brazil, 2006; Crab et al., 2007).

Trickling Filter: Trickling filters utilize fixed media such as stone in the past and more recently light plastic packing. Surface area for bacterial attachment for tricking filters was estimated from 100 to 1,000 m^2/m^3 depending on type of media (Crab et

al., 2007). Aquaculture effluent from cultured tanks or ponds containing ammonium is introduced to trickling filters from the top and flows through void space between media surface where nitrifying bacteria are attached in the form of biofilm. Disadvantages of tricking filters are clogging between pored space by solids from aquaculture effluent and detached biofilms. Odors could become the problem if oxygenation is insufficient. Ammonium degradation rates in trickling filters ranges from 240 to 640 mg $N/m^2/day$ (Eding et al., 2006; Crab et al., 2007).

Fluidized Bed Bioreactor: In fluidized bed bioreactor, nitrifying bacteria are attached to small media particles namely sand or polystyrene beads (i.e., 1 - 3 mm in diameter), resulting in high surface area for bacterial attachment ranged from 4,000 to 20,000 m^2/m^3 (Shieh and Keenan, 1987). Liquid is pumped upward into media bed at high flow rate, resulting in fluidization and well mixed condition of media bed. Strength of fluidized bed bioreactor is the high treatment capacity for large amount of wastewater and less susceptible to solid clogging as compared to fixed-bed systems. Efficient oxygenation unit may be required to support nitrification as well as the recycle for effluent stream. Disadvantage of fluidized bed bioreactor is associated with high energy requirement to fluidize media bed. Lab-scale fluidized bed bioreactor revealed the ammonium degradation rate from 240 to 550 mg $N/m^2/day$ in treating aquaculture effluent (Sandu et al., 2002; Summerfelt and Sharrer, 2004).

Microbead Filters: Microbead filters are similar to the trickling filters but the sizes of media are significantly smaller with the diameter ranged from 1 to 3 mm. This leads significantly higher surface area estimated from 1,260 to 3,780 m^2/m^3 (Greiner and

Timmons, 1998). The benefit of microbead filters is high treatment capacity for large amount of wastewater as well as efficient solid-liquid separation leading to less clogging problem. Laboratory-scale experiment using microbead filter systems indicated the ammonium removal rate from 300 to 600 mg N/m^2/day (Greiner and Timmons, 1998; Sastry et al., 1999; Crab et al., 2007).

Nitrifying and Denitrifying Attached-Growth System

In spite of the effectiveness of nitrifying biofilter systems in converting ammonium and nitrite to a far less toxic nitrate, the prolong operation of nitrifying biofilter systems can cause extremely high nitrate accumulation in the cultured system that eventually exerts negative effects to animals. Moreover, the discharge of nitrate into natural water resource also creates various concerns as described in previous sections. It is generally known that denitrification can occur naturally by bacteria residing in the sediment but due to extremely high nitrate concentrations that are associated with intensive cultivation the treatment capacity of natural treatment process becomes limited. From these reasons, the development of nitrifying coupled with denitrifying biofilter systems becomes necessary. Environmental conditions in denitrifying bioreactor must be free of oxygen or at least oxygen limited as indicated by the DO concentrations less than 0.5 mg/L. Organic carbon source addition is also required in denitrifying bioreactor because heterotrophic bacteria responsible for denitrification need organic carbon as energy source for their metabolisms.

The development of recirculating aquaculture systems based on nitrification and denitrification in Thailand can be found in the Center of Excellence in Marine Biotechnology at the Chulalongkorn University. Researchers have developed the nitrifying treatment system using Biocord^{TM} biofilters to obtain satisfactory results. Works also focus on the nitrate removal in the system called tubular denitrification bioreactor. Aquaculture effluent from cultured tanks containing high nitrate and DO levels is circulated at low flow rate through long tubular bioreactor containing Bioball^{TM} biofilters, which is used as media to immobilize heterotrophic denitrifying bacteria. The source of denitrifying bacteria can be obtained from various sources for example sediments from shrimp pond or activated sludge. Methanol as organic carbon source is added into the bioreactor at the entry zone, resulting in the quick decrease of DO concentrations from approximately 4.0 mg/L to less than 0.7 mg/L, which is considered suitable for denitrification (Painter, 1977). Evaluation of this system by growing shrimp at 350 shrimp/m^2 revealed that the recirculating system was capable of maintaining good water quality for 7 months with average ammonium and nitrite concentrations less than 0.06 mg N/L and the maximum nitrate concentrations measured at 39 mg N/L (Menasveta et al., 2001). Subsequent research by Suwannarat (2010), which integrated the compact nitrifying system proposed by Sesuk et al. (2009) and replaced Bioball^{TM} with BCN-009 biofilters to increase surface area for microbial attachment, indicated that the recirculating system was still capable of maintaining inorganic nitrogen concentrations within acceptable range for practical aquaculture despite the total weight of animal (i.e., tilapia) reached approximately 10 kg/m^3. Fig. 2.1 illustrates the diagram of the aquaculture system of Suwannarat (2010).

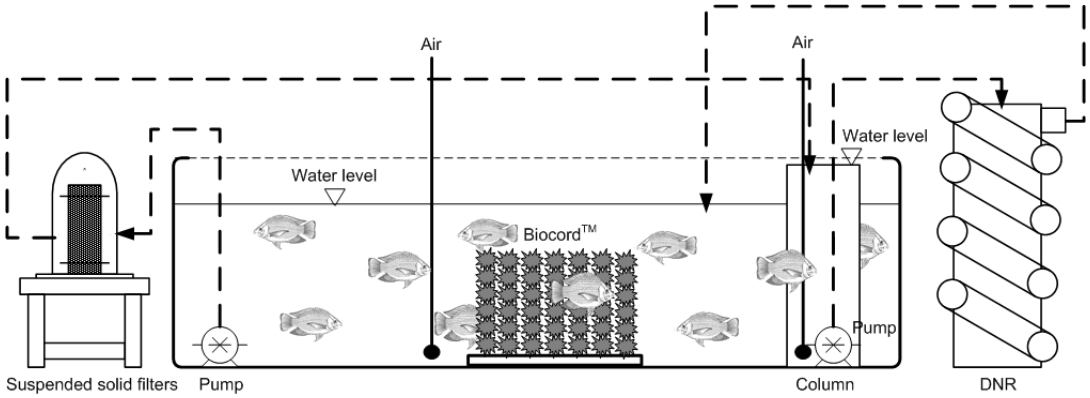

Fig. 2.1 Recirculating aquaculture system based on nitrification and denitrification in the work of Suwannarat (Suwannarat, 2010).

The recent work by Nootong et al. (2013) modified the system of Suwannarat (2010) as follows: (1) placing nitrifying biofilters outside cultured tank to reduce the solid accumulation on biofilter surface, (2) building the solid separating unit to control suspended solid concentrations in cultured tank as well as to lower solid loading rate to nitrifying biofilters, (3) adding oxygenation unit to increase DO concentrations of effluent from tubular denitrifying bioreactor, and (4) integrating oxidation-reduction potential (ORP) probes to control the rate of organic carbon addition to prevent formation of hydrogen sulfide in tubular bioreactor. Performance of the recirculating aquaculture system after the latest modification was effective in maintaining good water in cultured tank (i.e., ammonium and nitrite concentrations < 1.0 mg N/L, nitrate < 12 mg N/L and suspended solids concentration < 35 mg SS/L) for 60 days operation without water exchange. Moreover, the maximum fish weight obtained was

approximately 18 kg/m^3. Fig. 2.2 illustrates the diagram of the aquaculture system of Nootong et al. (2013).

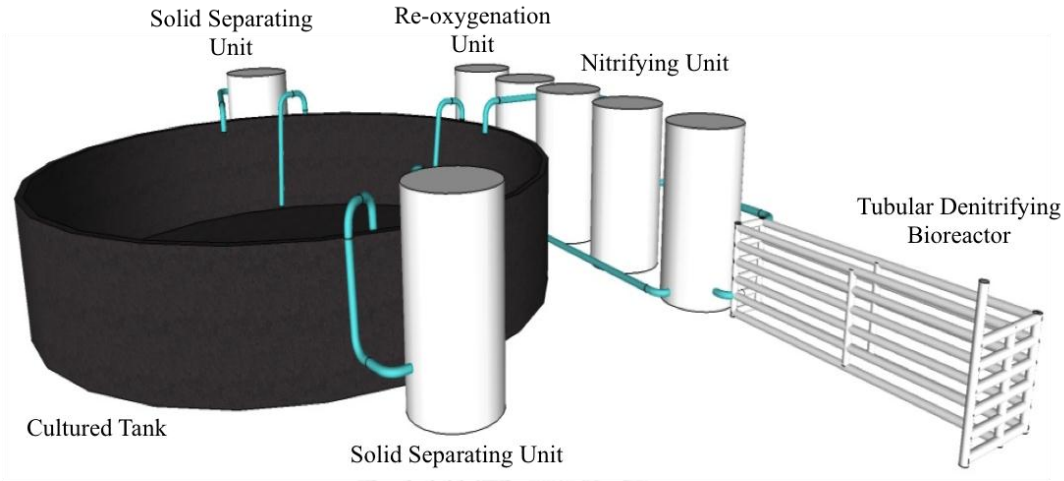

Fig. 2.2 Recirculating aquaculture system based on nitrification and denitrification in the work of Nootong et al. (2013).

Chapter 3

Materials and Methods

This study can be broadly divided into four main sections. The first section describes the preparation of biofilters to establish the complete nitrification. The second section describes the preliminary evaluation of the compact aquaculture system to identify the maximum treatment capacity in term of nitrogen loading rate and aquaculture weight. The third section extends the experimental results of the second section with aims to determine the appropriate system management strategy in order to improve the effectiveness of the compact aquaculture system in maintaining acceptable water quality. Finally, the final section describes the performance of the compact aquaculture system during the zero-water exchange aquaculture cultivation. The experiment was conducted at the aquaculture farm of Mr. Eakapoj Khamme, Samut saakhon Province, Thailand. Water analysis was performed at the Center of Excellence in Marine Biotechnology, Faculty of Science, Chulalongkorn University.

3.1 Biofilter Preparation

Fibrous Biocord^{TM} biofilters (polypropylene, surface area 2.8 m^2/kg biofilter) as shown in Fig. 3.1 were prepared to establish nitrification according to the method available in Sesuk et al. (2009). Biocord^{TM} biofilters (33 pieces at 0.6 m per piece) were submerged under the water surface in plastic circular acclimating tank (200 L

working volume). Ammonium chloride (NH_4Cl) (7.1 g) and 35% protein by weight shrimp diets (28.4 g) were added into acclimating tank to provide nitrogen source and essential minerals to mixed nitrifying bacteria sludge (4 g). The source of mixed nitrifying sludge was from the sediment of nitrifying biofilters unit treating aquaculture effluent at the Center of Excellence in Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University. A black plastic cover was placed over the top of the acclimating tank to prevent rainwater and sunlight from promoting the growth of phytoplankton. Acclimation of Biocord^{TM} biofilters was carried out in the acclimating tank without water exchange for 68 day. Repeated additions of NH_4Cl and shrimp diets were carried out after ammonium and nitrite concentrations in the tank were lower than 1.0 mg N/L. Aeration and liquid mixing were provided by several diffusive stones. Operating condition of acclimating tank were maintained within the optimal range of nitrification (i.e., DO > 4 mg/L, pH = 7 - 8 and alkalinity = 100 - 150 mg $CaCO_3$/L). Daily water samples (10 mL) were obtained from acclimating tank, refrigerated and later analyzed for the concentrations of ammonium, nitrite and nitrate according to APHA (1998).

The experiment to determine the ammonium degradation rates was conducted after the complete nitrification was achieved. Small pieces (15 cm in length) of 60 days old acclimated nitrifying biofilters from previous paragraph were taken from acclimating tank to perform the batch experiment to determine ammonium degradation rates. Analytical grade NH_4Cl was used as nitrogen source to prepare the initial ammonium concentration of 1.0 mg N/L. The batch experiment was performed

in 2 L plastic bottle (3 replications) equipped with a diffusive stones to provide thoroughly mixed condition and DO concentrations greater than 4 mg/L. Alkalinity and pH were maintained between 100 and 150 mg $CaCO_3$/L and from 7 to 8, respectively. Approximately 10 mL of water from plastic bottle were collected at predetermined interval and later analyzed for the concentrations of ammonium, nitrite and nitrate according to APHA (1998).

Fig. 3.1 Fibrous Biocord^{TM} biofilters

3.2 Preliminary Evaluation

Schematic diagram of the compact aquaculture system with one solid separating unit is illustrated in Fig. 3.2. Circular plastic tank (1,000 L working volume) was used to accommodate aquacultures, nitrifying biofilters and aeration equipment. Fibrous nitrifying Biocord^{TM} biofilters (total length of 20 m) as described in section 3.1 were connected to a metal frame lying on the tank floor to ensure that Biocord^{TM} biofilters were able to align vertically under water. Water circulation inside the plastic tank

was accomplished by using several diffusive stones and submergible pump. The solid separating unit was built from hollowed plastic PVC cylinder (inner diameter 30 cm; height 100 cm) with inside being inserted by 8-leveled horizontal plastic PVC discs (diameter 28 cm; thickness 0.5 cm), which were mounted to plastic PVC pipe (inner diameter 2.5 cm; outer diameter 3.0 cm; height 1.5 m) with spacing between discs at 8.5 cm. Openings for water inlet and outlet were located at 5 and 90 cm from the bottom of plastic cylinder, respectively. Water circulation between cultured tank and solid separating unit was maintained using pump at the optimal volumetric flow rates of 80 L/h (i.e. upflow superficial velocity of 3.15×10^{-2} cm/s) that resulted in solid removal efficiency approximately 70% (Nootong et al., 2013).

The following experimental design was used to obtain the preliminary data. During the preliminary evaluation, the compact aquaculture system (Biocord^{TM} biofilters 20 m) with one solid separating unit was subjected to different nitrogen loading rates. The nitrogen loading rate can be calculated by using the amounts of feed given to the animals daily and the total weights of animals in cultured tanks. It was also assumed that 1 g of protein in aquaculture feed contained 0.16 g of nitrogen (Nootong et al., 2013). Nile tilapia (*Oreochromis niloticus*) with average weight of 118 ± 4.6 g/fish were stocked in cultured tanks to attain initial fish weight of approximately 3.0 kg/m^3, which were equivalent to the nitrogen loading rates of 5.1 mg N/L/day. Feeding was performed twice daily using 35% protein commercial feed pellets at 3% of the total fish weight in the tank per day. Relatively constant fish weights (i.e., nitrogen loading rates) in the cultured tank were maintained by removing tilapia from cultured tank periodically. During the assessment that lasted for 21 days, the solid separating unit

was operated continuously for the first 14 days and then disconnected from cultured tank for the remainder of the experiment (i.e., Day 15 - 21). Conditions in cultured tank during the experiment were maintained as follows: pH = 7 – 8, DO > 4.0 mg/L and alkalinity = 100 – 150 mg $CaCO_3$/L. Daily water samples (25 mL) were obtained from cultured tank and analyzed for the concentrations of ammonium, nitrite, nitrate and suspended solids according to the Standard Methods (APHA, 1998). The same procedure was repeated for higher tilapia weights of 5, 7, 9 and 11 kg/m^3.

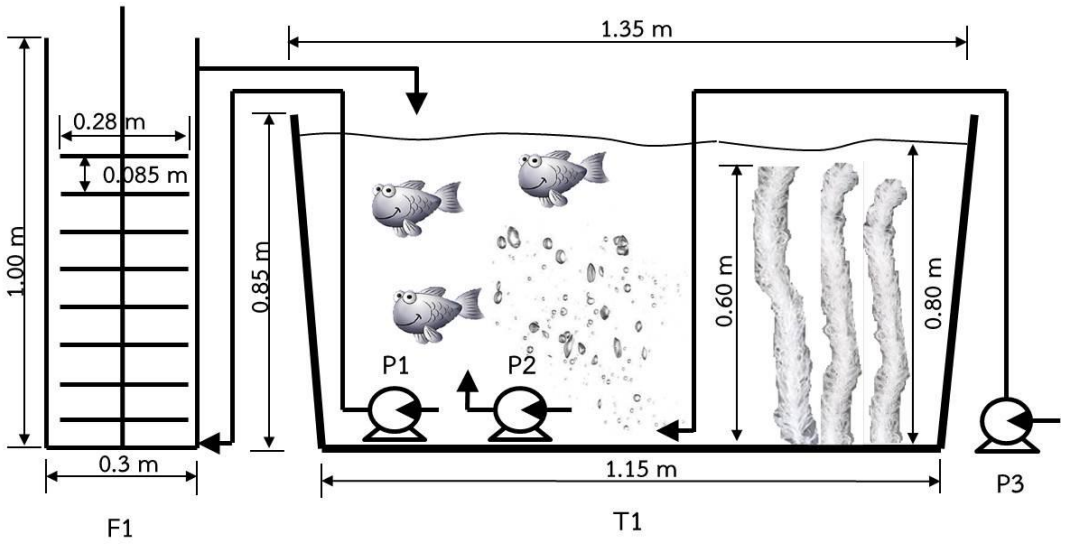

Fig. 3.2 Schematic diagram (not to scale) of the compact aquaculture system: (T1) cultured tank, (P1) water pump between cultured tank and solid separating unit, (P2) water circulating pump in cultured tank, (P3) air pump, and (F1) solid separating unit.

3.3 System Management Strategy

In this section, the compact aquaculture system with one solid separating unit was employed to cultivate tilapia without water exchange with the fish weights in cultured tank maintained relatively constant at 7 kg/m^3. From Day 1 - 14, the compact aquaculture systems (BiocordTM biofilters 20 m) with one solid separating unit were operated as normal using the optimal water flow rates of 80 L/h. In the next period, which extended from Day 15 - 29, the solid separating unit was still operated as normal at the water flow rates of 80 L/h but the fibrous nitrifying biofilters (Biocord^{TM}) were taken out of the cultured tank on a daily basis and cleaned manually by light scratching and rinsing with clean water using low pressure water hose. Cleaning process required approximately 20 to 30 minutes. From Day 31 - 45, the solid separating unit was replaced by filtration unit with the material called Japanese mats (polyester, thickness 3 cm, diameter 28 cm) employed as filtered media. Fig. 3.3 illustrates the Japanese mats media. Japanese mats were placed in plastic basket (diameter 0.3 m). The water flow rates through the basket remained at 80 L/h. Cleaning of Japanese mats and Biocord^{TM} nitrifying biofilters during this period was performed periodically in every 4 days by light scratching by hands and rinsing with clean water. For the remainder of the experiment (i.e., Day 46 - 60), the frequency of cleaning was reduced by half to every 2 days. Table 3.1 summarizes the operation taken during the experiment. Tilapia feeding was still performed twice daily using 35% protein commercial pellets at 3% of the total fish weight in the tank per day. Condition in cultured tanks was maintained under suitable range for nitrification and tilapia growth (i.e., pH = 7 – 8, DO > 4.0 mg/L and alkalinity = 100 – 150 mg

$CaCO_3$/L) for the entire cultivation. Daily water samples from cultured tanks were obtained and analyzed for the concentrations of ammonium, nitrite, nitrate and suspended solids according to APHA (1998).

Table 3.1 Operation condition for different period for the experiment to determine suitable management strategy.

Period	Day	Operating condition
1	1 - 14	Operating nitrifying biofilter; Operating solid separating unit (flow rate = 80 L/h); No biofilter cleaning.
2	15 - 29	Operating nitrifying biofilter; Operating solid separating unit (flow rate = 80 L/h); Cleaning biofilters daily by scratching and rinsing with water.
3	30 - 45	Operating nitrifying biofilters; Replacing solid separating unit with filtration unit (Japanese mats); Flow rate maintained at 80 L/h; Cleaning biofilters and filtration unit every 4 days.
4	46 - 60	Operating nitrifying biofilter; Operating filtration unit; Flow rate maintained at 80 L/h; Cleaning biofilters and filtration unit every 2 days.

Fig. 3.3 Japanese mats media

3.4 Tilapia Cultivation in the Compact Aquaculture System

Design of the compact aquaculture system used in this section was identical to that described in section 3.2 except that the solid separating unit was replaced by the filtration unit with Japanese mats as filtered media. Fig. 3.4 illustrates the modified aquaculture system with filtration unit.

Zero-water exchange tilapia cultivation was carried out for 60 days in the modified compact aquaculture system, which removed suspended solids by filtration unit. Nile tilapia with average weight of 81.4 ± 5.2 g were stocked in cultured tanks to attain initial fish weight of 3.0 kg/m^3. Tilapia were fed twice daily with 35% protein commercial feed pellets at 3% of the total fish weight per day. Approximately 30% of tilapia population was sampled and measured for its weight and length in every 3

weeks. Constant aeration by diffusive stones and $NaHCO_3$ addition were carried out to maintain proper conditions for nitrifying bacteria and tilapia (i.e., DO > 4.0 mg/L, pH = 7 – 8 and alkalinity = 100 – 150 mg/L $CaCO_3$). Cultivating tanks were located outdoors under shade and covered with black plastic sheet (0.5 cm thick) to prevent rainwater and sunlight. The compact aquaculture system was operated without solid removal for the first two weeks (i.e., Day 1 to Day 14) while it was operated normally (i.e., nitrifying biofilters and solids removal by filtration) for the remainder of the experiment (i.e., Day 16 to Day 60). Water samples (25 mL) from cultured tank were obtained daily and analyzed for the concentrations of ammonium, nitrite, nitrate and suspended solid according to APHA (1998). After the completion of the cultivation, the nitrogen mass balance analysis and economic feasibility was conducted and the results were presented in the next chapter.

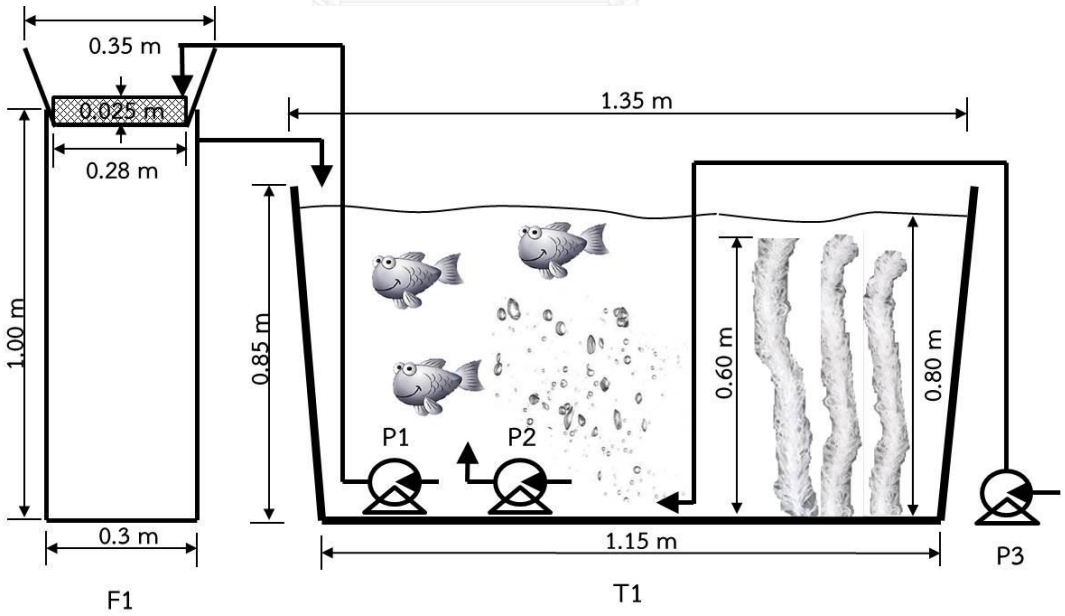

Fig. 3.4 Compact aquaculture System with the filtration unit.

3.5 Analytical Methods

Prior to water sampling, water in cultured tank was agitated manually using a long wooden stick to suspend solids settled on the tank floor. Water samples (25 mL) were obtained from cultured tank, refrigerated and taken to the Center of Excellence in Marine Biotechnology at Chulalongkorn University for analysis. Water samples were filtered using Whatmann filter (pore size = 0.5 μm). Filtered water samples were subsequently analyzed for total ammonia nitrogen (TAN) using APHA method 4500-NH_3-D, for nitrite (NO_2-N) using APHA method 4500-NO_2-B and for nitrate (NO_3-N) using APHA method 4500-NO_3-B. For the Whatmann filter, it was taken to determine the suspended solids according to APHA method 2540-Solids-D. In addition, approximate 1.0 L of water sample from cultured tank were obtained at the end of the cultivation and settled in an Imhoff cone. Settled sludges were then taken to determine the proximate CHN contents at the Scientific and Technologic Research Equipment Center of the Chulalongkorn University. During the third experiment, the statistical analysis (t-test: paired two-sample for means) for TAN and suspended solid concentrations between the data from Day 31 – 45 and those from Day 46 – 60 was performed using Microsoft Excel 2007.

Chapter 4

Result and Discussion

This chapter is divided into four main sections according to the way the experiment was conducted as described in the methodology. The first section shows the results of biofilter acclimatization to establish nitrification and the rate of ammonium degradation by the prepared nitrifying biofilters. The second section demonstrates the results of the preliminary evaluation that determined the maximum capacity of the compact aquaculture system to handle nitrogenous waste generated from feeding and animal excretion. The third section describes the appropriate system management strategy as means to increase the system capacity. Finally, the last section demonstrates the results of closed-water tilapia cultivation in the compact aquaculture system, which was modified and operated according to the results, obtained the third section. The results of nitrogen mass balance analysis and the economic feasibility are also presented.

4.1 Biofilter Acclimatization

Fig. 4.1 displays the trend of physical parameters including temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in the acclimating tank. The trends of these parameters were relatively constant with the average values of temperature (29.5 ± 1.1 $^{\circ}$C), pH (7.5 ± 0.2), DO concentration (5.1 ± 0.6 mg/L) and

alkalinity (96 ± 15 mg $CaCO_3$/L) within the practical range for nitrification (Timmons et al., 2002).

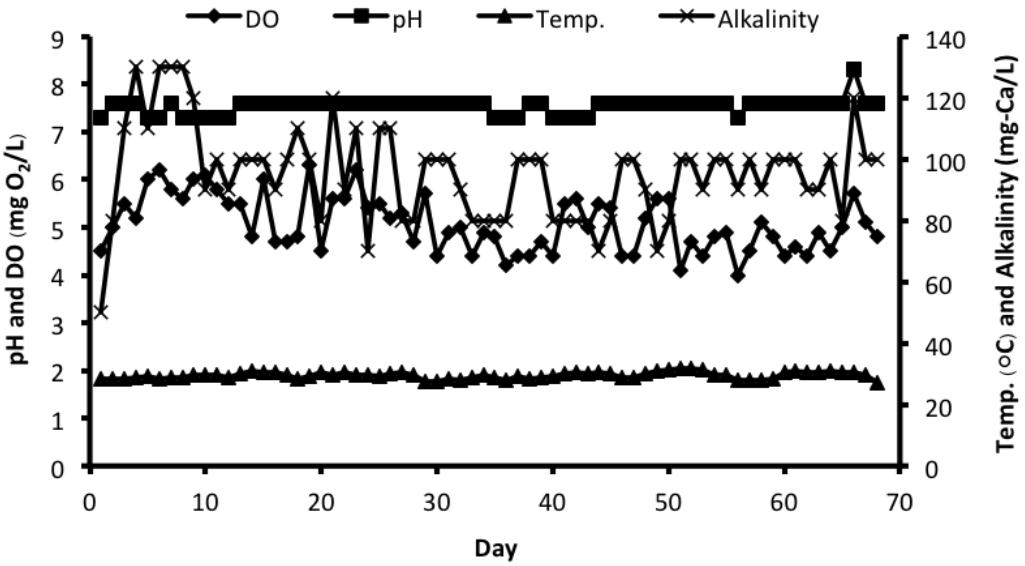

Fig. 4.1 Trend of temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in acclimating tank.

The seeding sludge was expected to contain active mixed nitrifying bacteria because it has been exposed to the aquaculture effluent containing ammonium from degraded fish diets and from fish excretion for more than a year. Shrimp diets were also chosen in the preparation process because they contained proteins as nitrogen source for bacteria as well as trace metals and vitamins essential for microbial growth (Sesuk et al., 2009). Fig. 4.2 demonstrates the profiles of inorganic nitrogen concentrations in acclimating tank. Ammonium peak at 10 mg N/L was observed on Day 6 was the result of ammonification that converted proteins in animal feed consequently into urea and ammonium. Ammonium concentrations began to decrease after Day 6 and

remained less than 1.0 mg N/L since Day 20 until the preparation process was concluded. For nitrite, the maximum concentrations of 4.5 mg N/L were observed on Day 13 before decreasing to the level below 1.0 mg N/L for the remainder of the experiment. Nitrate increased gradually from negligible level (< 1.0 mg N/L) since Day 10 but the rate of increase was apparent after Day 20. The final nitrate concentrations were measured at 58 mg N/L on Day 63. The increase of ammonium followed by the successive accumulation of nitrite followed by nitrate was the characteristic of nitrifying system startup that can be explained by ammonification and the difference in growth rate of ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (Sharma and Ahlert, 1977). In this study, the complete nitrification of Biocord^{TM} biofilters was established after one month as can be seen by insignificant concentrations of ammonium and nitrite and continuous increase of nitrate (Fig. 4.2). Moreover, the repeated addition of sodium bicarbonate ($NaHCO_3$) as means to prevent the decrease of water alkalinity in acclimating tank further confirmed the occurrence of nitrification. The production of hydrogen ions and decrease in alkalinity are main characteristics for identifying nitrification (Metcalf and Eddy, 2003). It should also be pointed out that Biocord^{TM} biofilters were efficient in intercepting and retaining suspended solids as can be seen in Fig. 4.3, which compared the difference in colors of Biocord^{TM} biofilter before and after acclimating process. Similar observations were noted in the previous work by Sesuk et al. (2009).

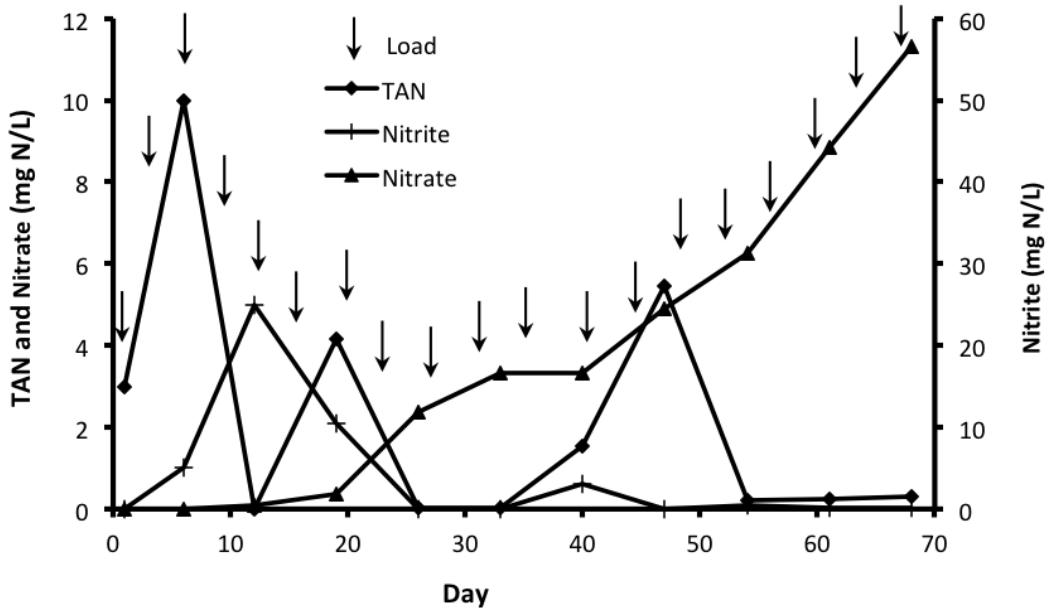

Fig. 4.2 Profiles of inorganic nitrogen concentrations in the acclimating tank (Arrows indicates the addition of NH_4Cl and shrimp diets).

Fig. 4.3 Comparison of color of Biocord^{TM} biofilters before and after the acclimating process to attain the complete nitrification

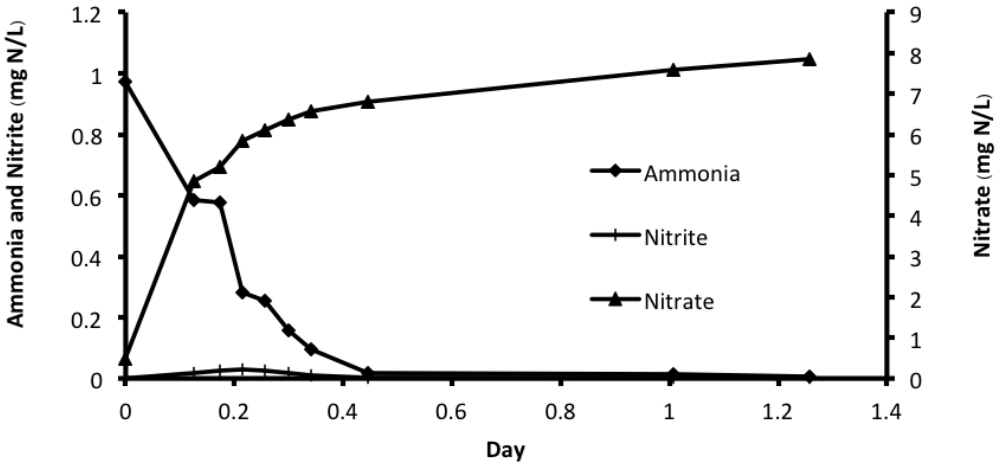

Fig. 4.4 Profiles of inorganic nitrogen concentrations during the batch experiment to determine the ammonium degradation rate.

Profiles of inorganic nitrogen concentrations during the batch experiment to determine the ammonium degradation rates of acclimatized Biocord^{TM} biofilters are illustrated in Fig. 4.4. Nitrifying reaction was complete by Day 5 as can be seen by the relatively unchanged concentrations of inorganic nitrogen compounds with respect to reaction time. Ammonium degradation appeared to follow the zero order reaction with the degradation rates determined at 72.7 mg N/m^2/day. The ammonium removal rates by Biocord^{TM} biofilters were comparable the previous works employing Biocord^{TM} biofilters that reported the ammonium degradation rates in the range from 24 to 106 mg N/m^2/day (Malaphol, 2009; Sesuk et al., 2009). Moreover, it appeared that ammonium and nitrite oxidation did not proceed at the same rates, thereby resulting in the accumulation of nitrite. Since the environmental conditions (i.e., DO and pH) in the plastic bottle were optimal for nitrification, higher ammonium loading enhancing AOB growth was perhaps the possible explanation for nitrite accumulation observed. Another reason was related to the pre-existing NOB population in the

sample biofilters that were unable to keep up with ammonium oxidation by AOB. The balance between AOB and NOB population was reestablished after certain period, approximately on Day 5 in this study, as indicated the occurrence of complete nitrification (Sesuk et al., 2009).

4.2 Preliminary Evaluation

Ranges of physical parameters namely temperature (26 – 29 $^{\circ}$C), pH (6.8 – 8.1), DO concentration (6.8 – 7.1 mg/L) and alkalinity (90 – 160 mg $CaCO_3$/L) were within the practical range for tilapia cultivation and nitrification (Timmons et al., 2002). The nitrogen loading rate, selected as the main experimental variable, can be calculated by using the amounts of feed given to tilapia on the daily basis. This resulted in nitrogen loading rates varying from 5.1 to 18.5 mg N/L/day. The specified nitrogen loading rates corresponded to increasing fish weights from 3 to 11 kg/m^3. Clearly, the range of nitrogen loading rates applied in this experiment was greater than the maximum reported values of 2.78 mg N/L/day during initial evaluation by Sesuk et al. (2009). At a given nitrogen loading rate, tilapia cultivation was carried out in the compact aquaculture system in parallel to the continuous solid removal by solid separating unit during the first 14 days followed by disconnecting the solid separating unit for the remainder of the experiment (i.e., Day 15 - 21). According to Fig. 4.5, increasing the nitrogen loading rates from 5.1 to 18.5 mg N/L/day led to the rapid increase of suspended solid concentrations regardless of whether solid removal was conducted. The increase in suspended solids in cultured tank was expected because solid formation in aquaculture tank was the direct consequence of feeding that in turn led to

animal excretions, unconsumed feed residues and formation of microbial biomass or bioflocs (Crab et al., 2007).

Operation of solid separating unit also influenced the levels of suspended solids in cultured tank. With solid separating unit running continuously, suspended solid concentrations in cultured tank was relatively constant and low (i.e., 20 - 40 mg SS/L) given that nitrogen loading rate from feeding did not exceed 8.4 mg N/L/day. Beyond the mentioned nitrogen loading rates (i.e., 8.4 mg N/L/day), suspended solid concentrations increased gradually at first and began to rise more quickly to 184 mg SS/L. Without operating the solid separating unit, suspended solid concentration was found to increase very rapidly from 65 to 708 mg SS/L with respect to increasing nitrogen loading rates. Excessive accumulation of solids on biofilter surface was also observed during non-operational period of solid separating unit. The presence of solids on biofilter was undesirable because their biodegradation yielded dissolved organic carbon that could promote the growth of heterotrophic bacteria, thereby leading to lower nitrifying activity of biofilters (Hargreaves, 2006; Malone et al., 2006).

Nitrogen loading rate and mode of operation of solid separating unit were key parameters influencing the ability to control ammonium concentrations in the compact aquaculture system (Fig. 4.5). With a single solid separating unit in operation, ammonium concentrations in cultured tank could be effectively maintained below the practical limit of 1.0 mg N/L given that nitrogen loading rates from tilapia feeding were less than 8.4 mg N/L/day. Further increase in nitrogen loading rate to the range

between 15.1 and 18.5 mg N/L/day led to a significant increase of ammonium concentrations in cultured tank to approximately 20 mg N/L. Such extremely high ammonium concentrations were dangerous toward aquatic animals and required an immediate water exchange to lower the concentrations (Timmons et al., 2002). Without solid removal, the compact aquaculture system was clearly ineffective in maintaining acceptable TAN concentrations, which were measured in the range from 6.4 to 22.3 mg N/L for all nitrogen loading rates applied except at 5.1 mg N/L/day (i.e., 3 kg/m^3 of fish weight). Nitrite concentrations in cultured tank were negligible and fluctuated in the narrow range between 0.1 and 0.5 mg N/L regardless of whether the solid removal was conducted (Fig. 4.5). Comparable profiles of nitrate between both operating conditions were also noticeable (Fig. 4.5). Nitrate concentrations increased to the maximum (i.e., 25 – 28 mg N/L) when the nitrogen loading rate was maintained at 11.8 mg N/L/day, followed by the decreasing trend after the nitrogen loading rate from tilapia feeding continued to rise. Such concentration profiles of nitrite and nitrate (i.e., relatively constant nitrite and decreasing nitrate concentrations) might imply that the occurrence of heterotrophic denitrification in the compact aquaculture system was possible. This scenario can be related to the excessive solid accumulation on biofilter surface that created anaerobic zone required by heterotrophic denitrifying bacteria while the local organic carbon source was available from the biological degradation of accumulated solids. Occurrence of simultaneous nitrification and denitrification was reported elsewhere in biofilter systems treating aquaculture effluent as well as domestic and industrial nitrogenous wastewater (Münch et al., 1996; Silapakul et al., 2005; Rahimi et al., 2011; Wang et al., 2012).

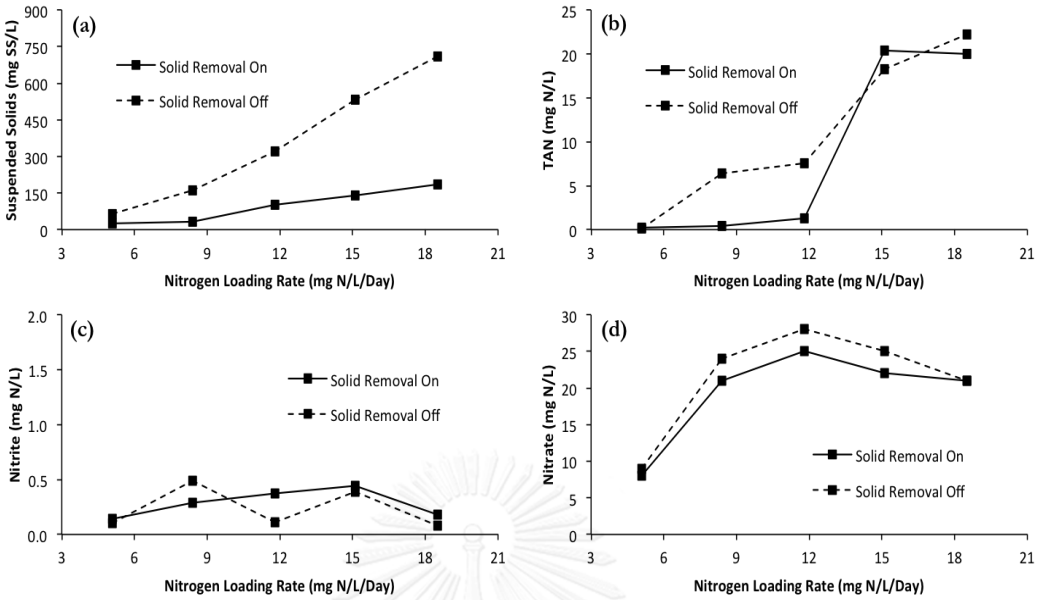

Fig. 4.5 Concentrations of suspended solids, ammonium, nitrite and nitrate in cultured tank at different nitrogen loading rates during the preliminary evaluation.

4.3 System Management Strategy

Based on the results of the preliminary evaluation, the performance of compact aquaculture system depended primarily on the operation of solid separating unit as well as the presence of nitrifying biofilters. This led to the conclusion that the compact aquaculture system should not be employed to accommodate aquaculture weights above 5 kg/m^3. This is equivalent to the nitrogen loading rates of 8.4 mg N/L/day. In order to improve on that result, various management strategies, specifically the method of solid-liquid separation and the frequency of biofilter cleaning, were studied during the closed-water tilapia cultivation in which tilapia weights in cultured tank were maintained at approximately 7 kg/m^3. It should be

pointed that the total weight of tilapia in this experiment was higher than the recommended value in section 4.2. Different operations were applied to the compact aquaculture system as depicted in Table 3.1. Results of water analysis including the concentrations of inorganic nitrogen compounds and suspended solids under different management strategies are illustrated in Fig. 4.6.

The compact aquaculture system given the total biofilter length of 20 m and one solid separating unit were operated normally for the first 20 days. During this period, suspended solid concentrations in cultured tank increased rapidly, exceeding the legal discharged limit of 70 mg SS/L on Day 4 and finally reaching 352 mg SS/L on Day 20. Ammonium and nitrite concentrations were relatively constant and remained low (i.e. < 0.6 mg N/L) during the first two weeks but started to exhibit the increasing trend after Day 16. Ammonium and nitrite concentrations were measured at 1.88 and 0.94 mg N/L on Day 20, respectively. The obtained results during the first period agreed with earlier outcomes during the preliminary evaluation that did not recommend using the compact aquaculture system coupled with one solid separating unit to grow aquacultures when animal weights in cultured tank were greater than 5 kg/m^3.

In order to improve on the system effectiveness, fibrous nitrifying biofilters were taken out of cultured tank to remove accumulated solids on biofilter surface by rinsing with clean water and scratching by hands. This operation was performed daily from Day 21 to Day 30 while the solid separating unit was still operated as normal. The removal of solids from biofilters was conducted to reduce the negative effect of

organic carbon source on nitrifying activity of biofilters. However, the combination of daily biofilter cleaning and operating solid separating unit was unable to improve the system performance as can be seen by the increasing suspended solid concentrations to as high as 457 mg SS/L measured on Day 30 and the detection of ammonium concentrations at high levels (i.e., 1.78 ± 0.10 mg N/L). It should be pointed out that heterotrophic denitrification might be established in the compact aquaculture system during this period since the sharp decline of nitrate from 33 to 9 mg N/L was observed.

Apparently, the method for solid removal should be rectified and therefore, in the next period (i.e., Day 31 - 45), solid-liquid separation by the solid separating unit was replaced by filtration unit with Japanese mats employed as filtered media. Japanese mats were commonly used in solid filtration during ornamented fish rearing. During this period, cleaning of filtration unit and nitrifying Biocord^{TM} biofilters were carried out in every 4 days by light scratching by hands and rinsing with clean water. Under the new management strategy, the turbidity of water in cultured tank improved considerably as indicated by the clear observation of tilapia swimming in cultured tank. Results of water analysis as shown in Fig. 4.6 confirmed that by displaying the significant decrease of suspended solid concentrations in cultured tank in comparison to those reported during the last period. Suspended solid concentrations were measured at 44 ± 5 mg SS/L on average while inorganic nitrogen concentrations varied within the narrow ranges from 0.41 to 0.89 mg N/L for ammonium and from 0.12 to 0.41 mg N/L for nitrite.

Comparable profiles of suspended solids and inorganic nitrogen compounds were observed after the frequency of biofilter cleaning were reduced by half to once in every 2 days. Under the latest management strategy, ammonium and nitrite concentrations were insignificantly different ($p < 0.05$) to those of earlier period (i.e., Day 31 - 45) while suspended solids decreased slightly to about 27 ± 6 mg SS/L (Fig. 4.6). For nitrate, its concentrations increased from 18 to 40 mg N/L during the filtered period, which extended from Day 31 to Day 60. Increasing nitrates as well as negligible ammonium and nitrite concentrations were clear indications that nitrification was fully established and acted to control inorganic nitrogen concentrations in cultured tank within acceptable range.

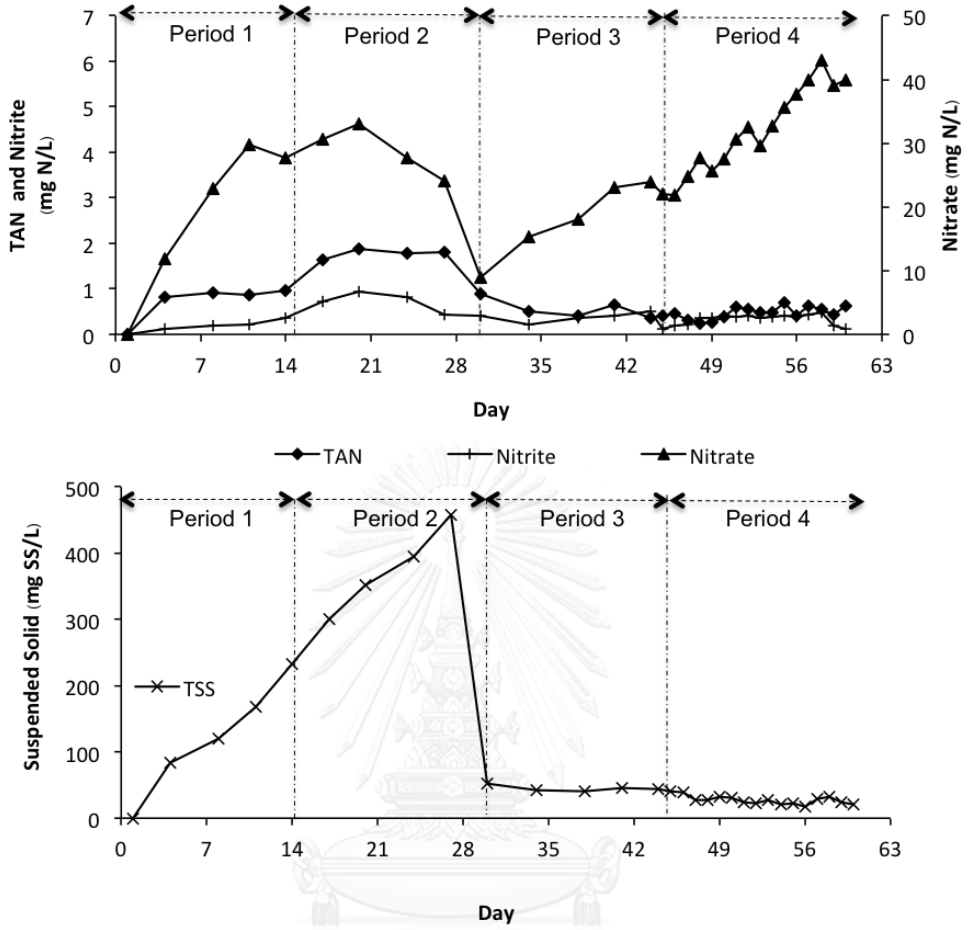

Fig. 4.6 Concentrations of suspended solids, TAN, nitrite and nitrate in cultured tank at different nitrogen loading rates.

4.4 Zero-Water Exchange Tilapia Cultivation

Based on the results of section 4.3, the compact aquaculture system was effective in maintaining inorganic nitrogen and solid concentrations when filtration and periodical biofilter cleaning were conducted in every 4 days. Therefore, in this section the compact aquaculture system operated with the described strategy was put to test by growing tilapia without water-exchange for 60 days. The initial fish weights in

cultured tank were approximately 3 kg/m^3, which corresponded to the nitrogen loading rates of 5.1 mg N/L/day.

As shown in Table 4.1, the ranges of water physical parameters in cultured tank including temperature, pH, DO and alkalinity were within the acceptable ranges for tilapia cultivation and nitrification (Timmons et al., 2002). Profiles for those parameters including temperature, pH and DO were also relatively constant throughout the cultivation except for alkalinity, which required lime addition on a weekly basis to prevent a decreasing trend. Addition of lime to maintain alkalinity is one of the characteristics of nitrifying system (Metcalf and Eddy, 2003).

Table 4.1 Physical parameters of water in cultured tank during the closed-water tilapia cultivation in the compact aquaculture system.

Parameters	**Range**	**Average**	**Recommended Range**
DO (mg/L)	5.5 – 6.6	5.9 ± 0.41	> 4.0
Temperature (°C)	29.7 – 30.9	30.2 ± 0.5	20 – 35
pH	7.1 – 7.6	7.3 ± 0.11	6.5 – 8.5
Alkalinity (mg $CaCO_3$/L)	90 – 130	106 ± 12	50 – 300

Table 4.2 Growth performance of tilapia during the zero-water exchange cultivation in the compact aquaculture system for 60 days.

Parameters	**Average**
Initial tilapia weight (g/fish)	81.4 ± 5.2
Final tilapia weight (g/fish)	287.6 ± 40.7
Initial total tilapia weight (kg/m^3)	3.0
Final total tilapia weight (kg/m^3)	10.4
Survival rate (%)	97
Growth rate (g/day)	
Entire cultivation (60 days)	3.45
Day 1 – 14	1.31
Day 14 – 60	4.08
Feed Conversion Ratio (FCR)	1.00

Table 4.2 shows tilapia growth data during the cultivation. High tilapia survival rate was reported at 97%. Fish death occurred on Day 10 when the compact aquaculture system had been operated without solid removal that resulted in excessive ammonium concentration (i.e., ammonium = 2.14 - 2.66 mg N/L) and increasing nitrite levels in water. Daily growth rates of tilapia reported at 3.45 g/day in this study were higher than those of the previous studies, which employed nitrifying biofilters or other means (e.g., biofloc system) to maintain good water quality that reported the growth rates ranged from 1.03 to 3.6 g/day (Ridha and Cruz, 2001; Al-Hafedh et al., 2003; Little et

al., 2008; Suwannarat, 2010; Pfeiffer and Wills, 2011). Higher growth rates obtained in the present study could be related to good water quality in most part of the cultivation as well as the utilization of high protein diets.

Profiles of suspended solid and inorganic nitrogen concentrations during the 60-day cultivation are demonstrated in Fig. 4.7. During the initial period from Day 1 - 14, the compact aquaculture system was operated without solid removal while the nitrogen loading rates from the daily tilapia feeding increased from 5.1 to 6.66 mg N/L/day. As expected, suspended solid and inorganic nitrogen concentrations increased from 5 to 69 mg SS/L and from 0.1 to 2.6 mg N/L, respectively. The rapid increase of those parameters to harmful levels confirmed the importance of providing sufficient solid-liquid separation as well as the presence of active nitrifying biofilters to maintain good water quality in closed-aquaculture system even at relatively low fish weights in cultured tank (i.e., < 5 kg/m^3). For the rest of the experiment (i.e., Day 15 - 60), the compact aquaculture system was operated based on the management strategy described in the previous section, specifically performing continuous filtration as well as cleaning nitrifying biofilters and filtration unit in every 4 days. Nitrogen loading rates applied to the compact aquaculture system during this period (i.e., Day 15 - 60) increased from 6.66 to 17.4 mg N/L/day. Suspended solid concentrations varied in the range between 20 and 35 mg SS/L for the remainder of the cultivation while ammonium and nitrite concentrations could be kept well below the practical limit (i.e., < 1.0 mg N/L) with the average values determined at 0.41 ± 0.19 and 0.57 ± 0.17 mg N/L for ammonium and nitrite, respectively. Clearly, the compact aquaculture system, which integrated nitrifying biofilters (20 m in length) and filtration unit, was

effective in maintaining good water quality for aquaculture cultivation although the nitrogen loading rates as high as 17.4 mg N/L/day were reached. It should also be pointed out that the maximum nitrogen loading rates applied in this cultivation were approximately 2 times higher than the recommended values (i.e., 8.4 mg N/L/day) obtained during the preliminary evaluation when only one solid separating unit was used.

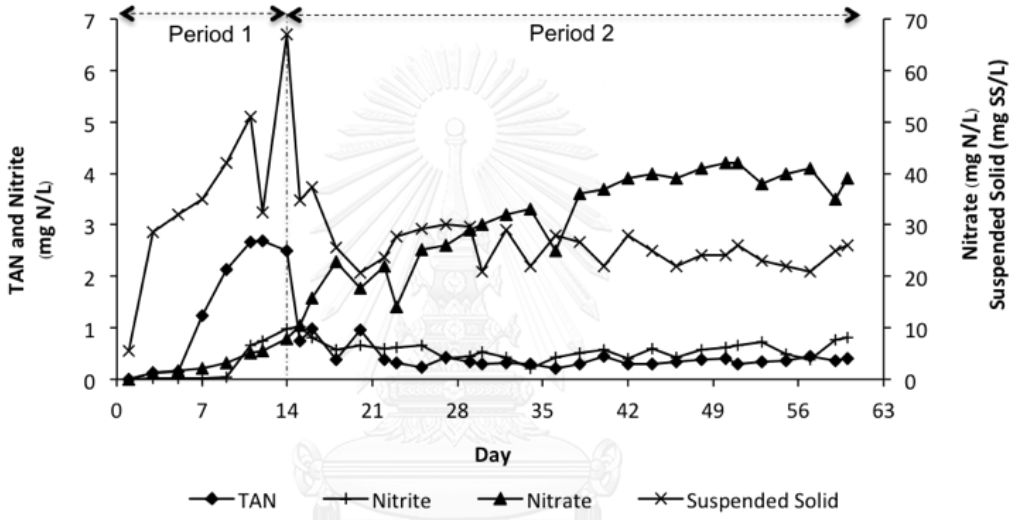

Fig. 4.7 Concentrations of suspended solids, ammonium, nitrite and nitrate in cultured tank at different nitrogen loading rates.

Results of the nitrogen mass balance calculation are displayed in Table 4.3. The majority of nitrogen input (99.6%) to the aquaculture system was primarily from daily fish feeding. Nitrogen distributions at the end of the cultivation were in various forms of dissolved inorganic nitrogen compounds (i.e., ammonium, nitrite and nitrate), biological solids and weight gains in tilapia. Nitrogen in tilapia was assumed at 2.6% of tilapia wet weight (Wutikumpoln, 2003). The percentages of nitrogen in biological

solids were from CHN analysis, which revealed the nitrogen contents of 3.5% on dried weight basis. The amounts of biological solids were the sum of suspended solids in water and those removed from cultured tank using filtration unit and in rinsing water. The percentages of nitrogen retention in fish (39.3%) reported in this study were comparable to those of other works, which showed the nitrogen retention ranged from 25% to 40% (Skjolstrup et al., 1998; Suzuki et al., 2003; Sesuk et al., 2009; Pfeiffer and Wills, 2011). Solid removal by filtration unit was apparently not the primary means for inorganic nitrogen control in the compact aquaculture system because it accounted for only 9.61% of the total nitrogen input. However, the presence of filtration unit remained critical for the effectiveness of nitrifying biofilters since it kept suspended solids at low levels in water, thereby preventing favorable condition required by heterotrophic bacteria.

Clearly, the obtained results from the 60-days cultivation indicated the necessity in providing sufficient solid-liquid separation in both extensive and semi-intensive aquaculture cultivations, whereas the past researches focused mainly on solid-liquid separation in intensive or super-intensive cultivation where the fish weights in the rearing system were greater than 40 kg/m^3 (Pfeiffer and Wills, 2011). In addition, large amounts (42.67%) of nitrogen input were unaccounted for and could be assumed in the form of nitrogen gas, which is the end of product of denitrification. Nitrogen loss via ammonia volatilization was not expected to be substantial in this case because ammonium concentrations were low and the pH values were suitable for ammonia to be in soluble form (i.e., NH_4^+). The magnitude of nitrogen loss in this cultivation was similar to Nootong et al. (2013), who reported the nitrogen loss as high as 49.8%

during the zero-water exchange tilapia cultivation in a nitrifying-denitrifying biofilter system. Nitrogen loss as high as 55% was also reported in brackish ponds with very limited water discharge (Daniels and Boyd, 1989). Occurrence of heterotrophic denitrification was possible due to the effectiveness of fibrous Biocord^{TM} biofilters in retaining solids on the surface (Sesuk et al., 2009), thereby creating anaerobic environment that facilitated the onset of denitrification while the trapped solids provided organic carbon source for denitrifying bacteria. Filtration unit was another likely source of heterotrophic denitrification due to high solid accumulation on filtered media. Nonetheless, the occurrence of simultaneous nitrification and heterotrophic denitrification in the compact aquaculture system was undesirable at the moment although it could lead to the complete nitrogen transformation to harmless compound (i.e., N_2) and possible reduction in operational cost and equipment sizing (Münch et al., 1996; Rahimi et al., 2011). The reasons for that were related to the difficulty in predicting the onset of denitrification by using the farmer existing equipment as well as the high cost of investment for sophisticated controlled equipment.

Table 4.3 Nitrogen mass balance calculation displaying the distribution of nitrogen in the compact aquaculture system.

Parameters	**Values**
Nitrogen input	
Feed pellets (g N)	485 (99.6%)
Seeding water (g N)	2.15 (0.4%)
Total input (g N)	487.15 (100%)
Nitrogen on Day 60	
Tilapia weight gain (g N)	191.6 (39.3%)
Suspended solids in water (g N)	0.875 (0.18%)
Removed solids (g N)	46.8 (9.61%)
Ammonium (g N)	0.37 (0.08%)
Nitrite (g N)	0.76 (0.16%)
Nitrate (g N)	39 (8%)
Unaccounted nitrogen (g N)	207.8 (42.67%)

The final aquaculture weights in this cultivation (i.e., 10.4 kg/m^3) were significantly higher than the harvesting weights of inland aquaculture in Thailand that reported the values ranged from 1 to 2 kg/m^3 (Vanitchanai, 2009). Final fish weights in this study remained considerably lower than those of the cage cultures (i.e., 30 - 40 kg/m^3), which were known to be highly susceptible to the variation in water quality upstream

and were environmental unfriendly due to the direct disposal of nitrogenous wastes into receiving water. With better system management namely appropriate solid removal method, optimal frequency for biofilter cleaning, improved fish strain, and improvement of feed quality as well as feeding methods, the final fish yields were expected to be significantly higher than the current ones obtained in this study and should be able to match with those intensive recirculating aquaculture systems equipped with efficient solid-liquid separators and other complicated equipments (e.g., skimmer, infection device, denitrifying biofilter and etc) that reported the final fish weights in the range from 20 to 100 kg/m^3 (Timmons et al., 2006; Little et al., 2008). In term of operation, the compact aquaculture system was relatively easy to build and operate. Fibrous nitrifying biofilters (Biocord^{TM}) came in the form of rope, which was easy to adapt under different situations. Based on the author experience, removal of attached solids from biofilter surface and from filtration unit was easy and could be accomplished by rinsing or spraying with clean water using low-pressure water hose and by light scratching by hands. The entire cleaning process required approximately 20 to 30 minutes and was not energy intensive as opposed to more complicated systems such as microbeads biofilters and fluidized-bed sand filters, which required intensive energy during backwashing (Steicke et al., 2007).

Despite the compact aquaculture system possessing many advantages, the revenue generated from selling tilapia (468 Baht) in this experiment was substantially less than the sum of total construction and operational costs as demonstrated in Table 4.4. Construction cost estimated at 10,190 Baht accounted for 96% of the total cost. Operational cost was only 434 Baht for the 60-days cultivation. Plastic rearing tank

and Biocord^{TM} biofilters were among the most expensive items, which incurred the expense of 6,800 Baht. However, the mentioned equipment were durable and could be used for many years. With appropriate adjustment to the compact aquaculture system, for example using local materials as biofilters or building fish tank from cement ponds or existing facilities, construction and equipment expense should be greatly reduced. In order to improve the economic feasibility as well as to increase the attractiveness to farmers, the compact aquaculture system might be employed to cultivate high-valued aquatic species such as sea bass, grouper or shrimp bloodstock.

Table 4.4 Construction cost of the compact aquaculture system and the operational cost incurred during the 60-day tilapia cultivation without water exchange in the compact aquaculture system.

Details	Cost (Baht)[1]	%
Construction Cost		
Plastic tank (1,000 L)	4,500	42
Biocord^{TM} biofilters (20 m)	2,300	22
Plastic PVC for solid separating unit	990	9
Water pumps (2)	1,150	11
Air pump (1)	1,100	10
Japanese mat filters (0.75 m^2)	150	1
Total cost of construction	10,190	95
Operational Cost [2]		
Tilapia	100	1
Feeds	259	3
Electricity	75	1
Total cost of operation	434	5
Total Cost	10,624	100
Revenue		
Tilapia (45 Baht/kg)[3]	468	

[1]1 US dollars = 32.5 Baht (6 June 2014)
[2]Excluding labor cost under the assumption of self-employment
[3]wholesale prices

Chapter 5

Conclusion and Recommendation

The present study aimed to assess the performance of the land-based compact aquaculture system, which integrated fibrous Biocord^{TM} nitrifying biofilters and suspended solids removal unit. The main findings from the study could be summarized as follows.

1. Management of suspended solids appeared as the critical aspect for successful operation of the compact aquaculture system. Without conducting solid removal, the rapid increase of suspended solids was observed along with significant increase of TAN and nitrite concentrations above the acceptable limit of 1.0 mg N/L even at low fish weights (i.e., < 5 kg/m^3).
2. Based on the original design of the compact aquaculture system that is based on employing the Biocord^{TM} nitrifying biofilters of 20 m in length and one solid separating unit, it was found that the compact aquaculture system was able to maintain the total aquaculture weight as high as 5 kg/m^3, which was equivalent to the nitrogen loading rate of 8.4 mg N/L/day.
3. Solid-liquid separation by filtration unit with the Japanese mats filter as media was more effective than using the single solid separation unit. Operation of the compact aquaculture system given the total biofilter length of 20 m along with employing the filtration unit as well as cleaning the Biocord^{TM} biofilters and

filtration unit in every 4 days could maintain TAN, nitrite and suspended solids concentrations within the acceptable ranges for aquaculture cultivation extended period, thereby leading to good water quality, high fish growth rate (i.e., 3.45 g/day) and relatively high harvesting weight of tilapia (i.e., 10.4 kg/m^3).

4. Based on the nitrogen mass balance calculation, significant portion of unaccounted nitrogen (42.67%) might imply that simultaneous nitrification and denitrification were the primary means for inorganic nitrogen treatment while the presence of filtration unit was essential for prolonging the activity of nitrifying biofilters.
5. Based on the data from this study, the compact aquaculture system was still economically infeasible because the revenue from selling tilapia was still significantly lower than the total cost. However, certain equipment were durable and could be used for many year, and with appropriate system adjustment such as using existing ponds or using local materials as biofilter or using the system to cultivating expensive species, the compact aquaculture system would become more attractive for low-budget farmers and help promoting the concept of sustainable aquacultures.

Recommendation for Future Work

1. Testing of local materials such as plastic bottle caps or crushed marine shell should be conducted. Information from the test will be used to determine whether the chosen material is capable of replacing the more expensive commercial nitrifying biofilters.

2. Intensification testing of the compact aquaculture with the filtration unit operated under periodic system cleaning of every 4 days should be conducted to determine the maximum carrying capacity.
3. Application of the compact aquaculture system should be extended to bloodstocks or more expensive aquaculture species to justify the economic feasibility.

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APPENDICES

Appendix A

Standard Curve for Ammonia, Nitrite, and Nitrate

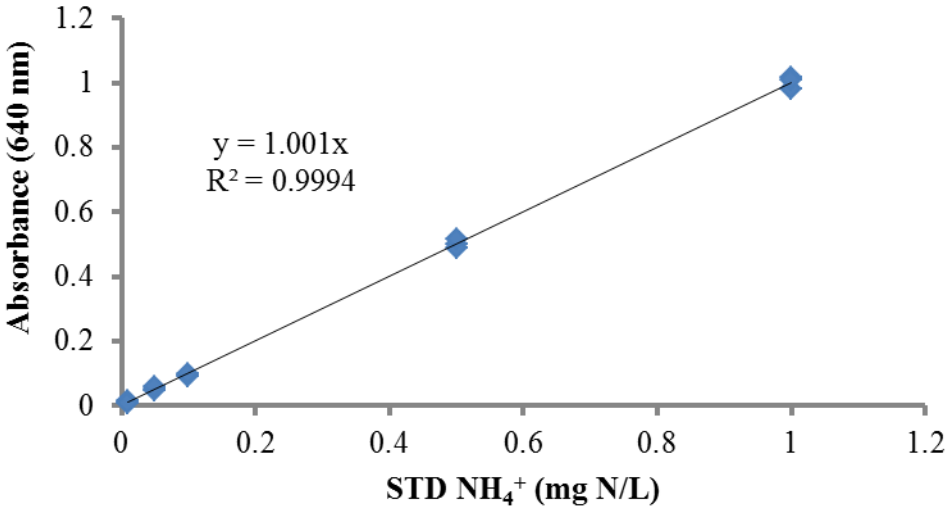

Fig. A-1 Standard curve for ammonia (NH_4^+-N)

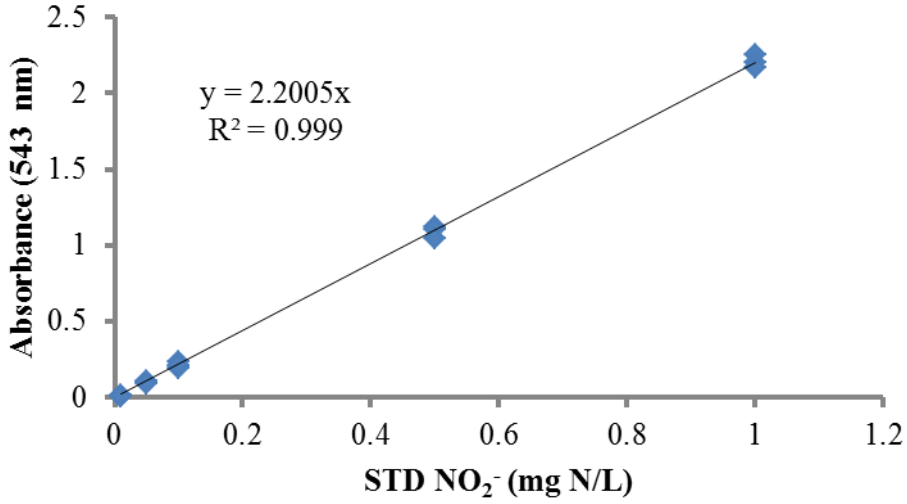

Fig. A-2 Standard curve for nitrite (NO_2^--N)

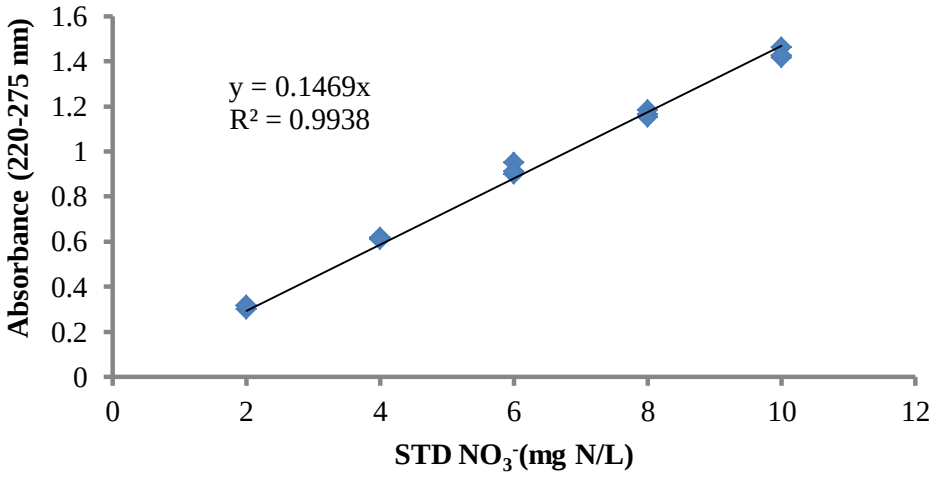

Fig.A-3 Standard curve of nitrate (NO_3^--N)

Appendix B

Information of the Proposed Aquaculture Systems

Table B-1 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in acclimating tank.

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
1	23/7/12	28.6	4.5	7.3	50	Add $CaCO_3$ 20 g
2	24/7/12	28.5	5.0	7.6	80	Add $CaCO_3$ 20 g
3	25/7/12	28.6	5.5	7.6	110	
4	26/7/12	28.8	5.2	7.6	130	Add Nutrients 6 g
5	27/7/12	29.1	6.0	7.3	110	
6	28/7/12	28.5	6.2	7.3	130	Add NH_4Cl 1.8 g
7	29/7/12	28.8	5.8	7.6	130	Add Nutrients 6 g
8	30/7/12	28.7	5.6	7.3	130	
9	31/7/12	29.6	6.0	7.3	120	
10	1/8/12	29.5	6.1	7.3	90	Add Nutrients 6 g, $CaCO_3$ 6 g
11	2/8/12	29.5	5.8	7.3	100	Add NH_4Cl 1.8 g
12	3/8/12	29.0	5.5	7.3	90	Add $CaCO_3$ 6 g
13	4/8/12	30.0	5.5	7.6	100	Add Nutrients 6 g
14	5/8/12	30.8	4,8	7.6	100	
15	6/8/12	30.5	6.0	7.6	100	Add NH_4Cl 1.8 g
16	7/8/12	30.7	4.7	7.6	90	Add Nutrients 6 g, $CaCO_3$ 6 g
17	8/8/12	29.5	4.7	7.6	100	
18	9/8/12	28.5	4.8	7.6	110	
19	10/8/12	29.2	6.3	7.6	100	Add NH_4C 4 g, Nutrients 15 g
20	11/8/12	30.5	4.5	7.6	80	Add $CaCO_3$ 10 g

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
21	12/8/12	29.7	5.6	7.6	120	
22	13/8/12	30.5	5.6	7.6	90	Add $CaCO_3$ 20 g
23	14/8/12	29.5	6.2	7.6	110	Add NH_4Cl 4 g
24	15/8/12	29.5	5.4	7.6	70	Add $CaCO_3$ 18 g
25	16/8/12	29.4	5.5	7.6	110	
26	17/8/12	30.1	5.2	7.6	110	
27	18/8/12	30.7	5.3	7.6	80	Add NH_4Cl 4 g, Nutrients 15 g, $CaCO_3$ 20 g
28	19/8/12	29.7	4.7	7.6	80	Add $CaCO_3$ 20 g
29	20/8/12	27.6	5.7	7.6	100	
30	21/8/12	27.7	4.4	7.6	100	Add $CaCO_3$ 20 g
31	22/8/12	28.2	4.9	7.6	100	Add NH_4Cl 4 g, $CaCO_3$ 20 g
32	23/8/12	28.1	5.0	7.6	90	
33	24/8/12	28.9	4.4	7.6	80	Add $CaCO_3$ 40 g
34	25/8/12	29.5	4.9	7.6	80	Add $CaCO_3$ 40 g
35	26/8/12	28.8	4.8	7.3	80	Add NH_4Cl 4 g, Nutrients 15 g
36	27/8/12	28.1	4.2	7.3	80	Add $CaCO_3$ 40 g
37	28/8/12	29.1	4.4	7.3	100	
38	29/8/12	28.2	4.4	7.6	100	
39	30/8/12	28.9	4.7	7.6	100	Add NH_4Cl 4 g
40	31/8/12	29.1	4.4	7.3	80	Add $CaCO_3$ 20 g
41	1/9/12	30.1	5.5	7.3	80	Add $CaCO_3$ 20 g
42	2/9/12	30.5	5.6	7.3	80	Add $CaCO_3$ 20 g
43	3/9/12	30.0	5.0	7.3	80	Add NH_4Cl 4 g, Nutrients 15 g, $CaCO_3$ 20 g
44	4/9/12	30.5	5.5	7.6	70	Add $CaCO_3$ 40 g
45	5/9/12	30.0	5.4	7.6	80	Add $CaCO_3$ 40 g
46	6/9/12	29.0	4.4	7.6	100	

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
47	7/9/12	29.0	4.4	7.6	100	Add NH_4Cl 4 g
48	8/9/12	30.1	5.2	7.6	90	Add $CaCO_3$ 20 g
49	9/9/12	31.1	5.6	7.6	70	Add $CaCO_3$ 40 g
50	10/9/12	31.5	5.6	7.6	80	Add $CaCO_3$ 40 g
51	11/9/12	31.8	4.1	7.6	100	Add NH_4Cl 4 g, Nutrients 15 g, $CaCO_3$ 20 g
52	12/9/12	31.6	4.7	7.6	100	
53	13/9/12	31.2	4.4	7.6	90	Add $CaCO_3$ 20 g
54	14/9/12	29.8	4.8	7.6	100	
55	15/9/12	29.5	4.9	7.6	100	Add NH_4Cl 4 g
56	16/9/12	28.1	4.0	7.3	90	
57	17/9/12	28.0	4.5	7.6	100	
58	18/9/12	27.9	5.1	7.6	90	Add $CaCO_3$ 20 g
59	19/9/12	28.3	4.8	7.6	100	Add NH_4Cl 4 g, Nutrients 15 g
60	20/9/12	30.5	4.4	7.6	100	Add $CaCO_3$ 20 g
61	21/9/12	31.0	4.6	7.6	100	
62	22/9/12	30.5	4.4	7.6	90	Add $CaCO_3$ 20 g
63	23/9/12	30.5	4.9	7.6	90	Add NH_4Cl 4 g, $CaCO_3$ 20 g
64	24/9/12	31.0	4.5	7.6	100	
65	25/9/12	30.5	5.0	7.6	80	Add $CaCO_3$ 40 g
66	26/9/12	30.4	5.7	8.3	120	
67	27/9/12	29.7	5.1	7.6	100	Add NH_4Cl 4 g, Nutrients 15 g
68	28/9/12	27.3	4.8	7.6	100	

Table B-2 Concentrations of ammonia, nitrite, and nitrate of water in the acclimating tank.

Day	D/M/Y	Ammonia (mg N/L)	Nitrite (mg N/L)	Nitrate (mg-N/L)
1	23/7/12	3.00	0.00	0.00
6	28/7/12	10.00	1.00	0.00
12	3/7/12	0.00	5.00	0.50
19	10/8/12	4.15	2.10	1.76
26	17/8/12	0.04	0.00	11.80
33	24/8/12	0.04	0.00	16.56
40	31/8/12	1.54	0.61	16.66
47	7/9/12	5.46	0.01	24.47
54	14/9/12	0.20	0.09	31.29
61	21/9/12	0.23	0.04	44.31
68	28/9/12	0.30	0.04	56.51

Table B-3 Inorganic nitrogen concentrations during the batch experiment to determine the ammonium degradation rate

Day	Ammonia (mg N/L)	Nitrite (mg N/L)	Nitrate (mg-N/L)
0.00	1.00	0.00	0.43
0.13	0.75	0.14	5.08
0.17	0.63	0.19	5.55
0.22	0.36	0.21	6.15
0.26	0.26	0.19	6.48
0.30	0.17	0.15	6.70
0.34	0.11	0.09	6.90
0.44	0.04	0.02	7.15
1.01	0.01	0.01	7.94
1.26	0.00	0.01	8.11

Table B-4 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in cultured tank at tilapia weights of 3 kg/m^3

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
1	25/1/13	28.7	5.8	7.6	100	
4	29/1/13	28.5	4.8	7.6	110	
7	1/2/13	29.1	4.4	7.6	130	
11	5/2/13	29.7	4.5	7.6	120	
14	8/2/13	29.5	4.5	7.6	100	Open Solid removed Unit
17	10/2/13	29.0	4.5	7.6	80	Add $CaCO_3$ 20 g
21	14/2/13	30.1	4.5	7.6	80	Add $CaCO_3$ 20 g
Max.		30.1	5.8	7.6	130.0	
Min.		28.5	4.4	7.6	80.0	
Aver.		29.2	4.7	7.6	102.9	
SD		0.6	0.5	0.0	18.9	

Table B-5 Concentrations of suspended solids, ammonium, nitrite and nitrate of water in cultured tank at tilapia weights of 3 kg/m^3

Day	D/M/Y	Ammonium (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	TSS (mg SS/L)	Remark
1	25/1/13	0.00	0.00	0.00	0.12	
4	29/1/13	0.19	0.04	5.36	25.00	
7	1/2/13	0.20	0.04	10.94	41.00	
11	5/2/13	0.21	0.07	14.17	40.00	
14	8/2/13	0.90	0.09	14.40	63.00	Open Solid removed Unit
17	10/2/13	0.30	0.10	14.75	100.00	
21	14/2/13	0.32	0.19	15.27	151.00	

Table B-6 Information of fishes weight in cultured tank at tilapia weights of 3 kg/m^3

No.	**Started Weight** (g)	**Ended Weight** (g)	**Remark**
Total	3083.6	3103.2	Total tilapia started = 26 fishes
Max.	127.5	182.6	Total tilapia stopped = 26 fishes
Min.	112.4	112.4	Total tilapia death = 0 fishes
Aver.	118.6	131.6	
SD	4.6	19.8	

Table B-7 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in cultured tank at tilapia weights of 5 kg/m^3

Day	**D/M/Y**	**Temp.** (◦C)	**DO** (mg/L)	**pH**	**Alkalinity** (mg $CaCO_3$/L)	**Remark**
1	16/2/13	29.5	7.1	7.6	100	
3	18/2/13	28.5	7.8	7.6	110	
7	22/2/13	29.2	6.3	7.6	100	
11	26/2/13	30.5	4.5	7.6	80	Add $CaCO_3$ 40 g
14	1/3/13	29.7	5.6	7.6	120	Open Solid removed Unit
17	4/3/13	30.5	5.6	7.6	90	Add $CaCO_3$ 20 g
21	8/3/13	29.5	6.2	7.6	110	
Max.		30.5	7.8	7.6	120.0	
Min.		28.5	4.5	7.6	80.0	
Aver.		29.6	6.2	7.6	101.4	
SD		0.7	1.1	0.0	13.5	

Table B-8 Concentrations of suspended solids, ammonium, nitrite and nitrate of water in cultured tank at tilapia weights of 5 kg/m^3

Day	D/M/Y	Ammonium (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	TSS (mg SS/L)	Remark
1	16/2/13	0.00	0.00	0.00	0.12	
3	18/2/13	0.45	0.07	6.34	25.00	
7	22/2/13	0.47	0.29	21.49	41.00	
11	26/2/13	0.54	0.37	33.73	40.00	
14	1/3/13	0.58	0.31	44.96	50.00	Open Solid removed Unit
17	4/3/13	2.00	0.50	50.00	120.00	
21	8/3/13	6.91	0.87	51.11	200.00	

Table B-9 Information of fishes weight in cultured tank at tilapia weights of 5 kg/m^3

No.	Started Weight (g)	Ended Weight (g)	Remark
Total	5013.8	5150.1	Total tilapia started = 35 fishes
Max.	148.0	271.6	Total tilapia stopped = 29 fishes
Min.	136.1	124.8	Total tilapia death = 0 fishes
Aver.	143.3	158.8	
SD	2.9	26.1	

Table B-10 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in cultured tank at tilapia weights of 7 kg/m^3

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
1	9/3/13	30.8	5.5	7.3	90	Add $CaCO_3$ 10 g
4	12/3/13	31.0	5.5	7.6	100	
8	16/3/13	30.8	4.8	7.6	100	
11	19/3/13	30.5	5.1	7.6	100	
14	22/3/13	30.7	4.7	7.6	90	Open Solid removed Unit
17	25/3/13	31.2	4.2	7.3	80	Add $CaCO_3$ 40 g
21	28/3/13	30.9	4.4	7.3	80	Add $CaCO_3$ 40 g
Max.		31.2	5.5	7.6	100.0	
Min.		30.5	4.2	7.3	80.0	
Aver.		30.8	4.9	7.5	91.4	
SD		0.2	0.5	0.2	9.0	

Table B-11 Concentrations of suspended solids, ammonium, nitrite and nitrate of water in cultured tank at tilapia weights of 7 kg/m^3

Day	D/M/Y	Ammonium (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	TSS (mg SS/L)	Remark
1	9/3/13	0.00	0.00	0.00	0.12	
4	12/3/13	0.84	0.96	13.00	40.00	
8	16/3/13	0.80	1.79	23.52	120.00	
11	19/3/13	1.91	2.59	25.10	190.00	
14	22/3/13	0.58	2.00	35.76	232.00	Open Solid removed Unit
17	25/3/13	2.00	0.50	50.00	290.00	
21	28/3/13	6.32	0.06	53.78	354.00	

Table B-12 Information of fishes weight in cultured tank at tilapia weights of 7 kg/m^3

No.	**Started Weight** (g)	**Ended Weight** (g)	**Remark**
Total	7012.7	7052.6	Total tilapia started = 41 fishes
Max.	183.0	309.9	Total tilapia stopped = 32 fishes
Min.	160.2	166.6	Total tilapia death = 4 fishes
Aver.	171.0	220.4	
SD	5.1	35.6	

Table B-13 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in cultured tank at tilapia weights of 9 kg/m^3

Day	**D/M/Y**	**Temp.** (◦C)	**DO** (mg/L)	**pH**	**Alkalinity** (mg $CaCO_3$/L)	**Remark**
1	30/3/13	28.7	5.8	7.6	100	
4	1/4/13	28.5	4.8	7.6	110	
8	5/4/13	29.1	4.4	7.6	130	
11	8/4/13	29.7	4.5	7.6	120	
14	12/4/13	29.5	4.5	7.6	100	Open Solid removed Unit
17	15/4/13	29.0	4.5	7.6	80	Add $CaCO_3$ 40 g
21	19/4/13	30.1	4.5	7.6	80	Add $CaCO_3$ 40 g
Max.		30.1	5.8	7.6	130.0	
Min.		28.5	4.4	7.6	80.0	
Aver.		29.2	4.7	7.6	102.9	
SD		0.6	0.5	0.0	18.9	

Table B-14 Concentrations of suspended solids, ammonium, nitrite and nitrate of water in cultured tank at tilapia weights of 9 kg/m^3

Day	**D/M/Y**	**Ammonium** (mg N/L)	**Nitrite** (mg N/L)	**Nitrate** (mg N/L)	**TSS** (mg SS/L)	**Remark**
1	30/3/13	0.00	0.00	0.00	0.12	
4	1/4/13	0.19	0.04	5.36	25.00	
7	5/4/13	0.20	0.04	10.94	41.00	
11	8/4/13	0.21	0.07	14.17	40.00	
14	12/4/13	0.90	0.09	14.40	63.00	Open Solid removed Unit
17	15/4/13	0.30	0.10	14.75	100.00	
21	19/4/13	0.32	0.19	15.27	151.00	

Table B-15 Information of fishes weight in cultured tank at tilapia weights of 9 kg/m^3

No.	**Started Weight** (g)	**Ended Weight** (g)	**Remark**
Total	9023.5	9069.4	Total tilapia started = 41 fishes
Max.	271.0	399.3	Total tilapia stopped = 34 fishes
Min.	166.6	176.6	Total tilapia death = 4 fishes
Aver.	220.1	266.7	
SD	22.8	72.1	

Table B-16 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in cultured tank at tilapia weights of 11 kg/m^3

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
1	20/4/13	28.7	5.8	7.6	100	
4	22/4/13	28.5	4.8	7.6	110	
7	26/4/13	29.1	4.4	7.6	130	
11	29/4/13	29.7	4.5	7.6	120	
14	3/5/13	29.5	4.5	7.6	100	Open Solid removed Unit
17	7/5/13	29.0	4.5	7.6	80	Add $CaCO_3$ 40 g
21	10/5/13	30.1	4.5	7.6	80	Add $CaCO_3$ 40 g
Max.		30.1	5.8	7.6	130.0	
Min.		28.5	4.4	7.6	80.0	
Aver.		29.2	4.7	7.6	102.9	
SD		0.6	0.5	0.0	18.9	

Table B-17 Concentrations of suspended solids, ammonium, nitrite and nitrate of water in cultured tank at tilapia weights of 11 kg/m^3

Day	D/M/Y	Ammonium (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	TSS (mg SS/L)	Remark
1	20/4/13	0.00	0.00	0.00	0.12	
4	22/4/13	0.19	0.04	5.36	25.00	
7	26/4/13	0.20	0.04	10.94	41.00	
11	29/4/13	0.21	0.07	14.17	40.00	
14	3/5/13	0.90	0.09	14.40	63.00	Open Solid removed Unit
17	7/5/13	0.30	0.10	14.75	100.00	
21	10/5/13	0.32	0.19	15.27	151.00	

Table B-18 Information of fishes weight in cultured tank at tilapia weights of 11 kg/m^3

No.	**Started Weight** (g)	**Ended Weight** (g)	**Remark**
Total	11080.0	10992.2	Total tilapia started = 42 fishes
Max.	279.0	479.3	Total tilapia stopped = 32 fishes
Min.	222.6	267.0	Total tilapia death = 8 fishes
Aver.	263.8	343.5	
SD	10.1	56.4	

Table B-19 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in cultured tank at tilapia weights of 7 kg/m^3 during the System Management Strategy 64 days

Day	**D/M/Y**	**Temp.** (◦C)	**DO** (mg/L)	**pH**	**Alkalinity** (mg $CaCO_3$/L)	**Remark**
1	1/7/13	29.9	5.5	7.3	100	
4	4/7/13	30.0	5.5	7.3	100	
8	8/7/13	30.8	4.8	7.3	100	
11	11/7/13	30.9	5.0	7.3	100	
14	14/7/13	30.1	4.6	7.3	120	
17	17/7/13	29.8	4.6	7.3	120	
20	20/7/13	29.7	4.5	7.3	130	
24	24/7/13	29.9	5.0	7.3	100	
27	27/7/13	29.7	5.0	7.3	100	
30	30/7/13	30.8	4.8	7.3	100	
34	3/8/13	29.9	5.5	7.3	110	
38	7/8/13	29.9	5.5	7.3	130	
41	10/8/13	30.9	5.0	7.3	130	
44	13/8/13	30.9	5.0	7.3	130	
45	14/8/13	30.1	4.6	7.3	110	

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
46	15/8/13	29.7	4.5	7.3	100	
47	16/8/13	29.9	5.5	7.3	100	
48	17/8/13	30.0	5.5	7.3	100	
49	18/8/13	30.8	4.8	7.3	100	
50	19/8/13	30.9	5.0	7.3	100	
51	20/8/13	30.0	5.5	7.3	100	
52	21/8/13	30.8	4.8	7.3	100	
53	22/8/13	30.9	5.0	7.3	100	
54	23/8/13	30.1	4.6	7.3	90	Add $CaCO_3$ 20 g
55	24/8/13	29.8	4.6	7.3	110	
56	25/8/13	29.7	4.5	7.3	110	
57	26/8/13	29.7	4.5	7.3	100	
58	27/8/13	29.6	4.5	7.3	100	
59	28/8/13	29.7	4.5	7.3	90	Add $CaCO_3$ 20 g
60	29/8/13	29.8	4.5	7.3	110	
61	30/8/13	29.8	4.5	7.3	110	
62	31/8/13	29.7	4.5	7.3	110	
63	1/9/13	29.7	4.5	7.3	110	
64	2/9/13	29.7	4.5	7.3	110	
Max.		30.9	5.5	7.3	130.0	
Min.		29.6	4.5	7.3	90.0	
Aver.		30.1	4.9	7.3	106.8	
SD		0.5	0.4	0.0	10.9	

Table B-20 Concentrations of suspended solids, ammonium, nitrite and nitrate of water in cultured tank at tilapia weights of 7 kg/m^3 during the System Management Strategy 64 days

Day	**D/M/Y**	**Ammonium** (mg N/L)	**Nitrite** (mg N/L)	**Nitrate** (mg N/L)	**TSS** (mg SS/L)
1	1/7/13	0.00	0.00	0.00	0.12
4	4/7/13	0.83	0.12	11.88	84.00
8	8/7/13	0.91	0.18	22.82	120.00
11	11/7/13	0.87	0.21	29.79	168.00
14	14/7/13	0.97	0.37	27.63	232.00
17	17/7/13	0.90	0.39	30.61	300.00
20	20/7/13	0.99	0.42	37.58	352.00
24	24/7/13	0.87	0.21	23.79	168.00
27	27/7/13	0.77	0.17	16.45	104.00
30	30/7/13	0.69	0.11	10.02	139.00
34	3/8/13	0.99	0.22	15.35	201.00
38	7/8/13	1.57	0.35	21.20	273.00
41	10/8/13	1.77	0.42	27.20	323.00
44	13/8/13	2.10	0.51	29.79	439.00
45	14/8/13	1.35	0.12	17.88	35.00
46	15/8/13	0.81	0.18	21.82	48.00
47	16/8/13	0.87	0.21	24.79	63.00
48	17/8/13	0.97	0.37	27.63	79.00
49	18/8/13	0.90	0.35	25.61	37.00
50	19/8/13	0.79	0.42	27.58	49.00
51	20/8/13	0.95	0.39	30.55	61.00
52	21/8/13	1.09	0.42	32.58	84.00
53	22/8/13	0.97	0.37	29.63	30.00
54	23/8/13	0.70	0.39	32.61	57.00
55	24/8/13	0.84	0.42	35.58	68.00

Day	D/M/Y	Ammonium (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	TSS (mg SS/L)
56	25/8/13	1.10	0.39	37.61	97.00
57	26/8/13	1.23	0.43	40.00	120.00
58	27/8/13	1.61	0.48	43.11	40.00
59	28/8/13	0.77	0.20	41.10	64.00
60	29/8/13	0.62	0.11	36.98	16.00
61	30/8/13	0.68	0.13	37.60	36.00
62	31/8/13	0.55	0.06	38.83	16.00
63	1/9/13	0.71	0.10	39.53	24.00
64	2/9/13	0.63	0.06	40.98	20.00

Table B-21 Information of fishes weight in cultured tank at tilapia weights of 7 kg/m^3 during the System Management Strategy 64 days

No.	Started (g)	14 Days (g)	28 Days (g)	42 Days (g)	64 Days (g)
Total (fishes)	30	26	22	20	17
Death (fishes)	-	-	-	-	-
Total weight (g)	7053.6	7077.1	7029.1	6993.6	6965.6
Max.	280.4	372.5	365.7	395.0	520.5
Min.	210.2	234.8	267.0	325.7	345.5
Aver.	235.1	272.2	319.5	349.7	409.7
SD	19.4	37.1	31.6	15.1	65.1

Table B-22 Temperature, pH, dissolved oxygen (DO) concentration and alkalinity of water in cultured tank at tilapia weights of 3 kg/m^3 during the Zero-Water Exchange Tilapia Cultivation 60 days

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
1	4/9/13	29.7	6.4	7.3	150	
3	6/9/13	30.0	5.5	7.3	120	
5	8/9/13	30.1	4.8	7.3	100	
7	10/9/13	29.8	4.8	7.3	100	
9	12/9/13	29.7	5.5	7.3	110	
11	14/9/13	29.7	5.5	7.3	90	Add $CaCO_3$ 20 g
12	16/9/13	29.6	5.0	7.3	100	
14	17/9/13	30.1	5.0	7.3	100	
15	18/9/13	29.8	4.6	7.3	80	Add $CaCO_3$ 40 g
16	20/9/13	30.1	4.5	7.3	100	
18	22/9/13	29.8	4.8	7.3	110	
20	24/9/13	29.7	5.0	7.3	90	Add $CaCO_3$ 20 g
22	25/9/13	29.7	4.6	7.3	100	
23	26/9/13	29.6	4.6	7.3	100	
25	28/9/13	29.7	4.5	7.3	110	
27	30/9/13	30.1	4.5	7.3	100	
29	2/10/13	29.8	4.5	7.3	100	
30	3/10/13	29.7	4.5	7.3	90	Add $CaCO_3$ 20 g
32	5/10/13	29.7	4.8	7.3	100	
34	7/10/13	29.6	4.8	7.3	100	
36	9/10/13	29.7	5.0	7.3	100	
38	11/10/13	30.1	4.6	7.3	100	
40	13/10/13	29.8	4.8	7.3	100	
42	15/10/13	29.7	4.8	7.3	90	Add $CaCO_3$ 20 g
44	17/10/13	29.7	5.0	7.3	110	

Day	D/M/Y	Temp. (◦C)	DO (mg/L)	pH	Alkalinity (mg $CaCO_3$/L)	Remark
46	19/10/13	29.6	4.6	7.3	110	
48	21/10/13	29.7	4.6	7.3	100	
50	23/10/13	29.8	4.5	7.3	100	
51	24/10/13	29.8	4.5	7.3	90	Add $CaCO_3$ 20 g
53	26/10/13	29.8	4.5	7.3	100	
55	28/10/13	28.2	4.2	7.3	100	
57	30/10/13	28.7	4.1	7.3	100	
59	1/11/13	28.3	4.2	7.3	90	Add $CaCO_3$ 20 g
60	2/11/13	28.0	4.0	7.3	100	
Max.		30.1	6.4	7.3	150.0	
Min.		28.0	4.0	7.3	80.0	
Aver.		29.6	4.8	7.3	101.2	
SD		0.5	0.5	0.0	11.5	

Table B-23 Concentrations of suspended solids, ammonium, nitrite and nitrate of water in cultured tank at tilapia weights of 7 kg/m^3 during the Zero-Water Exchange Tilapia Cultivation 60 days

Day	D/M/Y	Ammonium (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	TSS (mg SS/L)
1	4/9/13	0.00	0.00	0.00	0.12
3	6/9/13	0.13	0.03	1.32	28.67
5	8/9/13	0.15	0.02	1.77	44.67
7	10/9/13	1.24	0.03	2.13	44.33
9	12/9/13	2.14	0.05	3.17	42.33
11	14/9/13	2.66	0.67	5.19	39.33
12	16/9/13	2.69	0.74	5.51	32.33
14	17/9/13	2.08	0.98	7.96	34.67

Day	D/M/Y	Ammonium (mg N/L)	Nitrite (mg N/L)	Nitrate (mg N/L)	TSS (mg SS/L)
15	18/9/13	0.74	1.02	10.34	34.77
16	20/9/13	0.97	0.80	15.77	37.33
18	22/9/13	0.39	0.57	22.80	25.67
20	24/9/13	0.95	0.67	17.64	20.67
22	25/9/13	0.38	0.60	21.92	23.70
23	26/9/13	0.32	0.62	14.04	27.76
25	28/9/13	0.24	0.66	25.25	29.33
27	30/9/13	0.43	0.40	25.85	30.00
29	2/10/13	0.33	0.44	26.24	29.67
30	3/10/13	0.29	0.53	25.84	31.33
32	5/10/13	0.32	0.42	27.00	32.89
34	7/10/13	0.30	0.21	28.26	32.67
36	9/10/13	0.22	0.43	28.44	28.00
38	11/10/13	0.29	0.51	26.50	26.60
40	13/10/13	0.45	0.57	27.80	32.00
42	15/10/13	0.30	0.41	28.64	39.67
44	17/10/13	0.29	0.59	27.50	39.67
46	19/10/13	0.34	0.43	28.27	38.00
48	21/10/13	0.38	0.57	29.15	37.33
50	23/10/13	0.41	0.62	29.54	36.33
51	24/10/13	0.29	0.66	29.63	28.00
53	26/10/13	0.34	0.73	29.47	23.67
55	28/10/13	0.37	0.48	28.91	48.00
57	30/10/13	0.44	0.38	29.02	44.00
59	1/11/13	0.37	0.76	29.00	40.00
60	2/11/13	0.40	0.81	28.91	41.00

Table B-23 Different of Tilapia weights and Nitrogen loading rates during the Zero-Water Exchange Tilapia Cultivation 60 days

Day	**Tilapia weight** (kg)	**Nitrogen Loading rate** (mg-N/L/day)
1	3.01	3.60
14	3.69	4.42
28	5.06	6.05
42	6.69	8.02
60	10.35	13.97

Table B-23 Total suspended solid strainer isolated from Japanese Mats during the Zero-Water Exchange Tilapia Cultivation 60 days

No.	**Japanese Mats** (g-Dry)	**Japanese Mats + Suspensions Solid** (g-Dry)	**Suspensions Solid** (g-Dry)
1	24.6	149.1	124.5
2	31.1	207.6	176.5
3	26.4	175.1	148.7
4	23.6	114.7	91.1
5	26.7	150.8	124.1
6	33.4	217.6	184.2
7	19.4	107.5	88.1
8	20.2	62.5	42.3
9	18.4	46.4	28.0
10	25.8	137.9	112.1
11	19.5	92.6	73.1
Total	269.1	1461.8	1192.7

Table B-24 Total suspended solid strainer isolated from cleaning the Biocord™ biofilters during the Zero-Water Exchange Tilapia Cultivation 60 days

No	suspended solid from cleaning Biocord™ (g-Dry)
1	35.16
2	65.88
3	190.44
4	96.24
5	113.28
6	119.8
7	129.88
8	60.4
Total	811.08

Table B-25 Information of fishes weight in cultured tank at tilapia weights of 7 kg/m^3 during the Zero-Water Exchange Tilapia Cultivation 60 days

No.	Started (g)	14 Days (g)	28 Days (g)	42 Days (g)	64 Days (g)
Total (fishes)	37	36	36	36	36
Death (fishes)	-	1	-	-	-
Total weight (g)	3011.1	3691.9	5058.8	6693.2	10353.8
Max.	92.0	107.5	223.8	287.8	446.6
Min.	74.0	82.7	90.8	97.0	128.8
Aver.	81.4	99.8	140.5	173.5	287.6
SD	5.2	6.2	31.6	35.2	40.7

VITA

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Eakapoj, K. and Kasidit, N. Efficiency Of Closed Aquaculture System In Controlling Solids And Nutrients During Intensive Tilapia Cultivation. Processing of The Sixth SNRU International Conference on Cooperation for Development on the East – West Economic Corridor : Strategic Development for ASEAN Community, 30 August 2013, Sakon Nakhon Rajabhat University , Sakon Nakhon, Thailand, 2013 : 681 - 686.

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