



## Short Communication

# Inorganic nitrogen control in a novel zero-water exchanged aquaculture system integrated with airlift-submerged fibrous nitrifying biofilters

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## ABSTRACT

This work examined the feasibility of applying shrimp diets to establish nitrification on submerged fibrous biofilters. It also investigated the performance of a proposed zero-water exchanged aquaculture system, which integrated growing of aquatic stocks and operation of acclimated biofilters in the same environment. Addition of shrimp diets fully established nitrification within 3 weeks as indicated by continuous increase of nitrate and trivial levels of ammonium and nitrite. A series of batch experiment revealed an average ammonium degradation rate of  $24.1 \text{ mg N m}^{-2} \text{ day}^{-1}$ . Zero-water discharged tilapia cultivation could be carried out in the proposed aquaculture system for at least 44 days when daily inorganic loadings increased from  $1.24$  to  $10.78 \text{ mg N l}^{-1} \text{ day}^{-1}$ . The corresponding daily growth rates of tilapia from the proposed aquaculture systems integrated with acclimated biofilters varied from  $3.01$  to  $3.35 \text{ g day}^{-1}$ , which was approximately 7–16% better than numbers from the systems using non-acclimated biofilters.

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## 1. Introduction

An excessive accumulation of inorganic nitrogenous compounds especially in the forms of ammonium and nitrite is a common problem often encountered during intensive aquacultures in plastic lining ponds. These nitrogenous compounds are produced primarily from the rigorous use of high protein feeds and the lack of complete biological pathways that are able to convert toxic nitrogenous compounds into inert forms (Avnimelech and Ritvo, 2003). The physiology of aquatic stocks is also partly responsible for ammonium and nitrite accumulation because the animals are able to metabolize, on average, only 25–30% of proteins available in feeds while the rest is released in the form of ammonia (Avnimelech and Ritvo, 2003). A buildup in these inorganic nitrogenous compounds to above  $1.0 \text{ mg N l}^{-1}$  can assert negative effects on aquatic animals including greater stress, a lowering oxygen transport in the blood, a weakening of the immune system and even death (Crab et al., 2007). For this reason, farmers are forced to exchange water from external sources at high rates more frequently in order to dilute toxic nitrogenous concentrations and this practice tremendously magnifies the risk of disease infections and outbreaks.

Nitrification is a well-studied biological process that aerobically transforms ammonium and nitrite into nitrate, which is far less toxic to aquatic animals (Timmons et al., 2002). In earthen ponds,

complete nitrification of ammonium to nitrate occurs naturally in the sediments and to lesser extent in the water columns. This process, however, is not entirely possible in the case of plastic lining ponds, which are often reported to encounter excessive nitrite accumulation in water. As a result, the plastic lining pond aquaculture systems that successfully mediate nitrification should be able to maintain good water characteristics for extended periods without any water exchange. Different design configurations of attached-growth nitrifying systems such as trickling filters, fluidized-sand filters, biological rotating contactors and downflow microbead filters have been proposed and successfully employed to carry out nitrification in varieties of aquaculture applications (Kamstra et al., 1998; Brazil, 2006; Summerfelt, 2006; Timmons et al., 2006). In spite of their successful nitrogen treatment, existing attached-growth nitrifying systems are sophisticated in their design and are costly to operate due to: (1) the requirement to recirculate water through aerated carriers (e.g., sand) located outside production ponds, (2) deposition from suspended solids between carrier pored spaces, (3) intensive energy requirements for pumping, fluidizing plastic carriers or backwashing and (4) the need for high skills from operators. An additional operating difficulty of attached-growth nitrifying systems is the lengthy startup period that is related to the limited growth rate of nitrifying bacteria and improper microbial seeding strategies. In addition to the conventional biofilter systems, the biofloc technology is recently proposed as the alternative for water treatment and feed reutilization (Avnimelech, 2006; De Schryver et al., 2008), yet it

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is not completely suitable for small farms due to their intensive aeration, regular solid removal and requirement for carbon source to stimulate heterotrophic bacterial growth.

Therefore, this article generally describes experimental results obtained during the initial phase of developing an efficient closed-water aquaculture system for plastic lining ponds that is inexpensive and easy to adopt in Thailand. The specific objectives of this paper are: (1) describing the feasibility of applying shrimp diet as a new strategy to establish nitrifying biofilters and (2) presenting the preliminary results of the zero-water exchanged tilapia cultivation in a novel yet simplified aquaculture system, which combines nitrifying biofilters and aquacultures in the same environment.

## 2. Experimental approach

### 2.1. Biofilter preparation

Commercial fibrous Biocord™ biofilters (polypropylene; specific surface area:  $2.8 \text{ m}^2 \text{ m}^{-1}$  or  $82.35 \text{ m}^2 \text{ kg-biofilter}^{-1}$ ) were cut into 30 pieces (60 cm each), and fixed with weighting stones to ensure the total submergence under water in a 1000 l plastic acclimating tank. Approximately 25.0 g of  $37 \pm 2\%$  protein shrimp diets were grounded and added into an acclimating tank filled with 800 l water to provide the initial dose of ammonium concentration at  $1.85 \text{ mg N l}^{-1}$ . About 2.0 g of the sediments from a Pacific white shrimp cultivating tank in the same laboratory were also added into the acclimating tank to supply nitrifying bacterial seeding. 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™ biofilters was carried out in the acclimating tank without any water exchange for 78 days. Water samples, taken at least four times a week from acclimating tank, were analyzed for  $\text{NH}_4^+ \text{-N}$ ,  $\text{NO}_2^- \text{-N}$ , and  $\text{NO}_3^- \text{-N}$  concentrations according to the Standard Methods (1998). Identical amounts of shrimp diet (25.0 g) were replenished in the acclimating tank once an ammonium concentration in the water was undetectable. In order to examine the ability of acclimated biofilters to sustain nitrification at higher ammonium loadings, the shrimp diets were replaced by 9.17 and 13.76 g of an analytical grade  $\text{NH}_4\text{Cl}$  on day 64th and 71st, respectively. In this experiment, a completely mixed hydraulic regime in the acclimating tank was maintained by constant aeration to provide dissolved oxygen (DO)  $> 4 \text{ mg l}^{-1}$ . Alkalinity and pH were controlled at between 100 and  $150 \text{ mg l}^{-1}$  and from 7.0 to 8.2, respectively by adding  $\text{NaHCO}_3$ . In order to investigate changes in the biofilter surface, small pieces ( $\approx 5 \text{ cm}$ ) of new and a month old acclimated Biocord™ biofilters were obtained to undergo SEM examination at the Scientific and Technological Research Equipment Centre of the Chulalongkorn University.

### 2.2. Determination of nitrification rate

Small pieces ( $\approx 15 \text{ cm}$ ) of 60 days old acclimated biofilters from Section 2.1 were taken to perform batch experiments to determine ammonium degradation rates in comparison to new biofilter samples. Batch experiments were performed at the initial ammonium concentrations of 2, 4 and  $6 \text{ mg N l}^{-1}$ . For each initial ammonium concentration tested, batch experiments were setup in two replicates in 6 l plastic bottles equipped with a stone aerator to provide thoroughly mixed conditions and  $\text{DO} > 4 \text{ mg l}^{-1}$ . Alkalinity and pH were maintained at between 100 and  $150 \text{ mg l}^{-1}$  and from 7.0 to 8.2, respectively. Approximately 9 ml of water from 6 l plastic bottles were collected at predetermined intervals and later analyzed for  $\text{NH}_4^+ \text{-N}$ ,  $\text{NO}_2^- \text{-N}$  and  $\text{NO}_3^- \text{-N}$  concentrations according to the Standard Methods (1998).

### 2.3. Fish cultivating system

A circular plastic tank (500 l) was employed to accommodate the acclimated biofilters described in Section 2.1 and fish. The total of nine pieces (60 cm each) of acclimated biofilters were completely submerged under the water surface within a hollow cylindrical plastic net (inner diameter = 30 cm, outer diameter = 30.6 cm and height = 90 cm), which was entirely wrapped in thin plastic sheet except for the top and bottom ends. Acclimated biofilters located inside the net were connected to a metal frame lying on the tank floor to ensure that the acclimated biofilters were able to align vertically. Only a single net was set up for each plastic tank. Within this net, the acclimated biofilters were free from fish interferences and were fully oxygenated by the diffusive stone aerator to provide an upflow water movement by means of airlift actions. Water circulation between inside and outside of the plastic net was made possible by making a small opening (width = 1 cm and length = 8 cm) as water outlet on thin plastic sheet about 0.5–1.0 cm above water surface. The aeration of biofilters also served to maintain aerobic conditions for the aquatic stocks. Additional aeration outside the biofilter net could be installed to ensure good animal welfare. Clearly, the proposed aquaculture system was different from the conventional designs, which normally located the treatment unit (i.e., biofilters) outside production ponds. In this study, acclimated biofilters were installed in the same tank as aquacultures so that the rearing of aquatic stocks, water treatment, and separation of suspended solids were able to be performed simultaneously.

### 2.4. Tilapia cultivation in the zero-exchanged aquaculture system

The closed aquaculture system described in Section 2.3 was fabricated and tested by growing tilapia without any water exchange for 44 days. Tilapia with average initial weights of  $116 \pm 3.96 \text{ g}$  were stocked in four replicated sets in 500 l plastic tanks (450 l working volume) to produce an average initial biomass density at  $772 \pm 26.41 \text{ g m}^{-3}$ . The fish were fed twice daily with 30% protein commercial feed at 3% fish weight per day. Growth data was determined by measuring the weights and lengths of the fish every 3 weeks. Tanks 1 and 2 (T1 and T2) were two replicated experimental systems, which integrated the acclimated biofilters from Section 2.1 based on the design of the proposed aquaculture system. Tank 3 (T3) featured no biofilters and was considered to be control 1. Tank 4 (T4), arranged with non-acclimated Biocord™ biofilters and constructed following the scheme of the proposed aquaculture system, was considered to be control 2. After the cultivation was complete on day 44, all surviving tilapia from T2, T3 and T4 were transferred into T1 to continue testing the proposed aquaculture system at higher nitrogen loading (i.e., higher fish loading). All the cultivating tanks were located outdoors adjacent the laboratory building and were hardly penetrated by sunlight. Inorganic nitrogen concentrations (i.e.,  $\text{NH}_4^+ \text{-N}$ ,  $\text{NO}_2^- \text{-N}$  and  $\text{NO}_3^- \text{-N}$ ) in the water columns for all tanks were constantly monitored according to the Standard Methods (1998). The hydraulic regime was completely mixed for all tanks. Operating conditions were maintained as the following:  $\text{DO} > 4 \text{ mg l}^{-1}$ ,  $\text{pH} = 7\text{--}8$ , salinity = 5 ppt, temperature =  $28\text{--}31 \text{ }^\circ\text{C}$  and alkalinity =  $100\text{--}150 \text{ mg l}^{-1}$ .

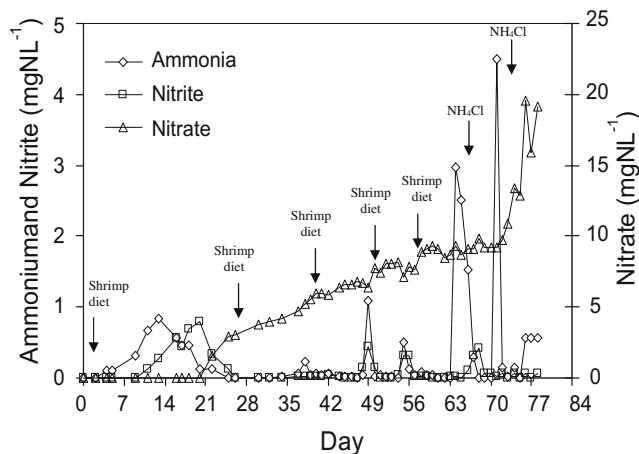
## 3. Results and discussions

### 3.1. Biofilter acclimation

Approximately 2.0 g of sediment taken from the Pacific white shrimp cultivation tank were employed as the initial seeding to establish the nitrifying activity for the Biocord™ biofilters. Sediment

was assumed to contain active mixed cultures of nitrifying bacteria because it had been continuously exposed to ammonium from shrimp diet and animal excretion for an extended period of more than one year. In order to investigate biofilter startup, 25 g of 37% protein shrimp diets, which is equivalent to 1.5 g of nitrogen, were introduced into the acclimating tank to provide the initial inorganic nitrogen concentration at  $1.85 \text{ mg N l}^{-1}$ . Shrimp diet was chosen to accelerate the nitrifying reactions in this work because it is easy to purchase and readily available in many aquaculture farms, but most importantly, shrimp diet contains traced elements and vitamins necessary for microbial growth and also significant amounts of proteins that later degrades into ammonium. Biofilter preparation based on the addition of shrimp diet was carried out in the acclimating tank without any water exchange, and the results are illustrated in Fig. 1. The first dosage of shrimp diet (25 g) was slowly degraded into ammonium and nitrite as shown by the gradual increase in their concentrations that successively reached the peaked values at  $0.85 \text{ mg N l}^{-1}$  on day 13 for ammonium and  $0.79 \text{ mg N l}^{-1}$  on day 20 for nitrite. The ammonium peak came from the microbial decomposition (ammonification) of shrimp diets, while the nitrite accumulation could have been the result of ammonia oxidizing bacteria (AOB) possessing greater growth rate in comparison to nitrite oxidizing bacteria (NOB) (Sharma and Ahler, 1977; Smith et al., 1997; Vadivelu et al., 2007). For this reason, more AOB populations would be present in the acclimating tank to produce nitrite, which remained accumulated in the water until sufficient NOB populations had been established.

Inorganic nitrogen mass balance up to the third week of biofilter acclimation revealed that  $756 \text{ mg}$  ( $\approx 41\%$ ) of added nitrogen were unaccountable. The phytoplankton uptake of inorganic nitrogen was insignificant because the acclimating tank was completely covered to prevent the penetration of sunlight. Heterotrophic denitrification was also unlikely to be the main mechanism in this case because the bulk liquid was constantly kept at high DO concentration (i.e.,  $\text{DO} > 4.0 \text{ mg l}^{-1}$ ) and there was insufficient organic carbon source for denitrifying bacteria to use. As a result, it was logical to assume that unaccountable amounts of added nitrogen had been incorporated into bacterial cells to synthesize new proteins during their growth. After an initial period of 3 weeks, nitrate concentration became more apparent, and continued to increase reaching a level as high as  $20 \text{ mg N l}^{-1}$  as more shrimp diet (25 g for each addition) was replenished once every 5–10 days (Fig. 1).



**Fig. 1.** The concentration profiles of inorganic nitrogenous compounds in the acclimating tank filled with the fibrous Biocord<sup>®</sup> biofilters during the startup. Biofilter acclimation was carried out in the acclimating tank without any water exchange. Arrows indicate the shrimp diet and  $\text{NH}_4\text{Cl}$  addition.

Ammonium and nitrite concentrations were also lower than  $1.0 \text{ mg N l}^{-1}$  for the remainder of the acclimating period which lasted until day 78. The only exception was for ammonium that revealed small concentration peaks shortly after every shrimp diet addition. According to the experimental outcome presented in Fig. 1, mixed nitrifying cultures used in this work only required approximately 3 weeks of startup period to grow and adjust to a new environment before displaying effective nitrification.

Based on this initial finding, adding shrimp diet seemed to be a practical strategy that could be easily employed to establish nitrifying biofilters. It should point out that the shrimp diet slowly released organic nitrogen (proteins) into the water, thereby making the actual ammonium concentration exposed by the acclimated biofilters lower than the intended value of  $1.85 \text{ mg N l}^{-1}$ . For this reason, shrimp diet was substituted by  $\text{NH}_4\text{Cl}$  to provide instant ammonium concentrations in the water at  $3.0$  and  $4.5 \text{ mg N l}^{-1}$  on day 64 and day 71, respectively. The results displayed in Fig. 1 confirmed the instant dissociation of  $\text{NH}_4\text{Cl}$  on day 64 and day 71, and further indicate the effective removal of ammonium and nitrite that led to a rapid climb in nitrate concentration from  $9.3$  to  $19.1 \text{ mg N l}^{-1}$ . Based on this preliminary results, shrimp diet acclimated biofilters were capable of sustaining nitrification even when different sources of ammonium were applied at higher nitrogen loadings.

The microscopic examination revealed that the surfaces of non-acclimated (new) biofilters were relatively clean and smooth without the attached microorganisms. On the other hand, the microbial presences in various sizes and shapes (e.g., rod, sphere and filament) were clearly noticeable on the surface of a month old acclimated biofilters suggesting the occurrence of microbial immobilization. Detailed examination of the acclimated biofilter surface found filamentous microorganisms entangled with each other creating mesh-like networks placed on top of smaller microorganisms. These mesh-like networks could possibly enhance the cell retention capability because they protected small microorganisms from being washout, and simultaneously acted as supporting backbones for small microorganisms to bind to. Cell attachment also tended to populate around the deep-inner regions of each individual filament rather than the near edges. It is possible that the fluid shear forces created by aeration were less severe around the deep-inner regions of biofilter filament to cause substantial cell detachment in comparison to those near edges. The stable nitrification observed during the biofilter enrichment could have been the consequence of successful immobilization that allowed slow-growing nitrifying bacteria to establish on to the biofilter surface at a high density. Despite the advantages, excessive microbial immobilization forming thick biofilm layers can create oxygen mass transfer limitation to cells located far from bulk liquid, thereby lowering the overall nitrification rate that can be achievable and allowing the likelihood of denitrification to occur. Due to insufficient organic carbon in the acclimating tank, the rate of denitrification was unlikely to match that of nitrification as can be shown by the increasing nitrate concentration observed in the acclimating tank.

### 3.2. Nitrification rate of acclimated biofilters

Results from the batch experiments revealed that the biodegradation of ammonium by 60 days old acclimated biofilters finished within 1–2 days for each initial ammonium concentration tested (i.e.,  $2$ ,  $4$  and  $6 \text{ mg N l}^{-1}$ ). Ammonium oxidation appeared to follow the zero order reaction, and displayed an average degradation rate of  $24.1 \text{ mg N m}^{-2} \text{ day}^{-1}$ . For each initial ammonium concentration examined, the nitrifying intermediate product (i.e., nitrite) rapidly emerged to reach the maximum concentrations, and later declined once nitrate production was in progress. Clearly, the accumulation

of nitrite suggested that ammonium and nitrite oxidations did not proceed at the same rates during the batch experiments. Since oxygen availability and pH were kept at the optimum, higher ammonium loading enhancing AOB growth was perhaps the possible explanation for the nitrite accumulation in the water. Another reason is related to pre-existing NOB in the sample biofilters that were unable to keep up with ammonium oxidation by AOB in order to maintain negligible nitrite concentration in the water. The balance between AOB and NOB was reestablished after a certain period ( $\approx 1$  day) as indicated by the occurrence of complete nitrification. In contrast, the batch experiments of non-acclimated biofilters did not reveal appreciable nitrifying activity since the concentrations of ammonium, nitrite and nitrate remained relatively unchanged from their initial values.

### 3.3. Inorganic nitrogen control in the zero-water exchanged tilapia cultivation

The important outcomes from earlier sections were: (1) the ability of shrimp diet to establish nitrifying biofilters within a reasonable period and (2) the necessity of preparing the biofilters to achieve the complete nitrification before their deployment. It was also clear that the experimental conditions applied during the biofilter acclimation were different from the actual aquaculture conditions. In order to investigate the performance of shrimp diet acclimated biofilters in controlling inorganic nitrogenous compound toxicity in a real situation, the zero-water exchanged tilapia cultivation was carried out in the proposed aquaculture system integrated with acclimated biofilters.

#### 3.3.1. Inorganic nitrogen control

Fig. 2 illustrates the results of water analysis from each tilapia cultivating tank. Clearly, the aquaculture systems integrated with acclimated biofilters (i.e., T1 and T2) were effective in sustaining the complete nitrification during the period of 44 days, when the daily inorganic nitrogen loadings from feed pellets were increased from 1.24 to 2.78 mg N l<sup>-1</sup> day<sup>-1</sup>. This ability to accomplish the complete nitrification led to the low concentrations of ammonium and nitrite under 1.0 mg N l<sup>-1</sup>, while the nitrate concentration continued to increase reaching the levels as high as 39.5 mg N l<sup>-1</sup> on day 44. It is important to note that all surviving fish from T2, T3 and T4 were transferred to T1, and the zero-water exchange tilapia cultivation continued for 3 more weeks. Feeding continued at 3% of fish weight, while the water samplings were performed occasionally. The results of water analysis during this period indicated that the ammonium and nitrite concentrations remained below 1.0 mg N l<sup>-1</sup> even though the daily inorganic nitrogen loadings further increased from 2.78 to 10.78 mg N l<sup>-1</sup> day<sup>-1</sup>. The complete nitrification observed in the proposed aquaculture systems could have been the results of proper biofilter acclimation that successful attained the complete nitrification before the actual operation had taken place. It appears that the acclimated biofilters can initiate the nitrifying reactions almost immediately once they have been deployed as long as the substrates are available.

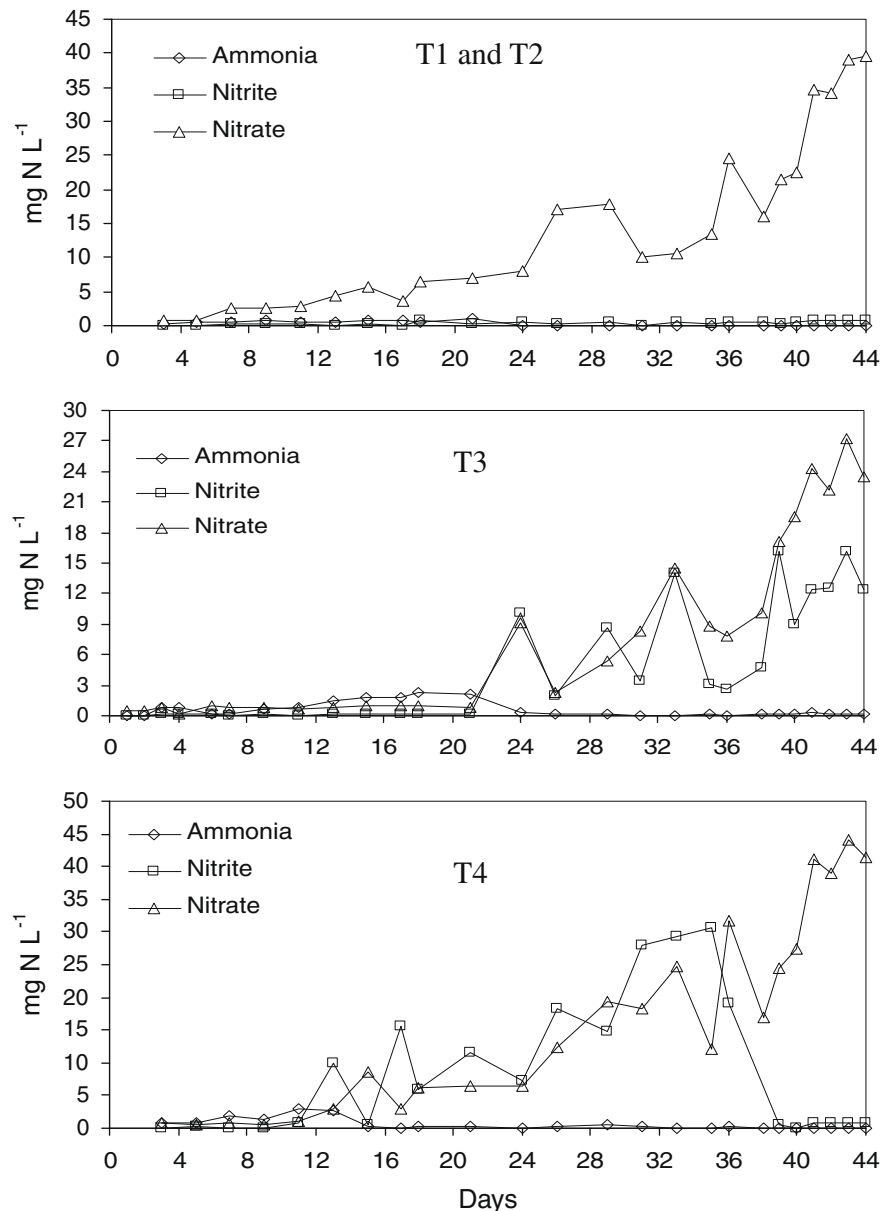
The analysis of water samples from T3 (i.e., suspended-growth system, no biofilters) indicated that the daily addition of tilapia feeds did not generate an ammonium accumulation in water above 1.0 mg N l<sup>-1</sup> during the initial period of 3 weeks. Based on the feeding record that produced the daily inorganic nitrogen loadings from 1.16 to 1.67 mg N l<sup>-1</sup> day<sup>-1</sup>, the cumulative inorganic nitrogen mass in water up to the third week should be about 12.0 g N, yet the total dissolved inorganic nitrogen in water (i.e., NH<sub>4</sub><sup>+</sup>-N + NO<sub>2</sub><sup>-</sup>-N + NO<sub>3</sub><sup>-</sup>-N) was only at 1.44 g N. Clearly, nitrification did not contribute significantly to the fate of added inorganic nitrogen compounds during the initial period because both nitrite and nitrate concentrations in the water remained triv-

ial (NO<sub>2</sub><sup>-</sup>-N < 0.25 mg N l<sup>-1</sup> and NO<sub>3</sub><sup>-</sup>-N < 1.0 mg N l<sup>-1</sup>). The photoautotrophic assimilation of inorganic nitrogen was also unlikely because phytoplankton was not present in significant amounts. Based on this observation, the disappearance of added inorganic nitrogen compounds during the initial period was perhaps related to the onset of a lag period that allowed both autotrophic and heterotrophic microorganisms either suspended in water or attached to the tank surface to take up nitrogen and produce a new biomass. This was confirmed by the formation of thick biofilm layer on the tank surface and significant amounts of suspended solids as high as 100 mg l<sup>-1</sup> that turned the production water from transparent to turbidity. The lag period of nitrifying bacteria residing in T3 was presumably over after the third week as is demonstrable by the ascending concentration profiles of nitrite and nitrate. Unlike earlier results, the partial nitrification was established in this tank instead of the complete nitrification, thereby resulting in the considerable amounts of nitrite accumulation (NO<sub>2</sub><sup>-</sup>-N = 2.0–16.2 mg N l<sup>-1</sup>) in water. The faster growth rate of AOB relative to NOB was an important factor, which caused the unbalanced populations between AOB and NOB that ultimately produced the nitrite accumulation. The lack of immobilizing materials might also partially contribute to the nitrite buildup. Nitrifying bacteria were unable to colonize at a high density in the suspension system as they did not have any carriers to attach and support their growth. Past literatures also suggested that the attached-growth systems were able to improve the nitrifying capacity based on increasing biomass retention time and biomass density (Chen et al., 1998; Nicolella et al., 2000). Moreover, substantial amounts of nitrate (NO<sub>3</sub><sup>-</sup>-N = 2.3–27.2 mg N l<sup>-1</sup>) were detected in water to suggest significant nitrifying activities. The production of nitrate was the consequence of keeping the aerobic condition (i.e., DO > 4.0 mg l<sup>-1</sup>) in the tank that should be able to enhance the NOB ability to oxidize the excess nitrite into nitrate without difficulty.

The results of water analysis from T4, which integrated the new Biocord™ biofilters, indicated that nitrification did not take place during the initial period of 2 weeks despite increasing the daily inorganic nitrogen loadings from 0.53 to 1.38 mg N l<sup>-1</sup> day<sup>-1</sup>. Since the nitrogen uptake by phytoplankton was unlikely, the added inorganic nitrogen might be assimilated directly into new microbial biomass, which can be identified in the form of biofilm attached on biofilters or in the form of suspended solids. After the initial period, it appeared that the nitrifying bacteria in T4 became more active, causing the rapid accumulation of nitrite and nitrate over 25 mg N l<sup>-1</sup> by the fourth week. The limited growth rate of NOB relative to AOB can be recited as the possible reason to explain the excessive nitrite buildup in this tank. A sudden decline of nitrite concentration from the maximum value to the negligible level from day 36 to day 40 can signal the onset of the complete nitrification in this tank. Although the non-acclimated biofilters arranged in T4 finally achieved the complete nitrification after day 40, it is important to indicate that extremely dangerous levels of nitrite lingered in the tank for about 2 weeks that may have asserted unhealthy effects on aquacultures. As a result, it can be concluded that the non-acclimated biofilters were highly susceptible towards incomplete nitrification, and their deployment in a closed-water recirculating system should be avoided or done in a cautious manner.

#### 3.3.2. Total suspended solids

Since the commercial feeds with 30% protein content were used in this experiment, plus the fact that no water was exchanged during the 44 day period, the production of carbonaceous matters in the form of biofilm and suspended solids were likely. Significant amounts of suspended solids were noticeable in T3 after the third week producing extremely turbid water, which was impossible to



**Fig. 2.** The results of the water analysis from each tilapia cultivating tank showing the concentration profiles of inorganic nitrogenous compounds. The results from T1 and T2 (integrated with acclimated biofilters) were combined together, T3 has no biofilter, and T4 was arranged with non-acclimated biofilters.

see through to observe the tilapia swimming in the tank. At the end of the cultivation on day 44, the total suspended solids (TSS) in T3 were determined at  $160 \text{ mg TSS l}^{-1}$ , which was almost 40-folds higher than the numbers obtained from T1, T2 and T4 (i.e.,  $\text{TSS} < 5.5 \text{ mg TSS l}^{-1}$ ). The low suspended solid concentrations can be further observed in T1 after the surviving fish from other tanks were combined. The low suspended solid contents in these tanks can be explained by the fact that the fibrous Biocord™ biofilters were capable of intercepting and retaining the suspended matters. A rigorous shaking of biofilters from these tanks resulted in a release of the trapped suspended matters back into water. The formation of suspended solids was likely to be linked with the direct assimilation of dissolved carbonaceous and nitrogenous matters from feeds and animal excretions by heterotrophic and autotrophic bacteria. Finally, it should point out that the effluent TSS concentrations from the proposed aquaculture systems (i.e., T1 and T2) were well below the discharged limitation set at  $80 \text{ mg TSS l}^{-1}$  (The Pollution Control Department, Thailand).

### 3.3.3. Tilapia growth

Table 1 demonstrates tilapia growth data during the zero-water exchanged cultivation. Tilapia biomass density in T1 and T2 increased from  $680$  to  $2589 \text{ g m}^{-3}$  during the 44 day period, and this corresponded to the average daily growth rates of  $3.01$  and  $3.35 \text{ g day}^{-1}$  for tilapia in T1 and T2, respectively. Clearly, the fish growth rates from the proposed aquaculture systems utilizing the acclimated biofilters (i.e., T1 and T2) were approximately 7–16% better than the numbers obtained from T4, which was fabricated with non-acclimated biofilters. The effects of using acclimated biofilters mediated nitrifying reactions were even more impressive when considering tilapia reared in T3 (i.e., no biofilters) were unable to survive. It should be pointed out that after day 30 the tilapia reared in T3 was unable to eat as can be shown by the unconsumed feed pellets, which remained floating on the water surface the morning after the feeding had been performed, and this led to the first mortality of tilapia on day 35. Since ammonium was largely absent, the lower fish growth rate in T4 and the mortality in T3 can be related

**Table 1**

Tilapia growth data and average water quality during the cultivating period of 44 days. T1 and T2 using acclimated biofilter, T3 without any biofilter, and T4 using non-acclimated biofilters.

Parameters	T1	T2	T3	T4
Average initial weight (g/fish)	113.3 ± 11.5	118.33 ± 7.6	120 ± 17.3	111.7 ± 10.4
Average initial length (cm/fish)	17.5 ± 0.50	18.17 ± 0.58	17.7 ± 0.77	18 ± 0.87
Initial density (g m <sup>-3</sup> )	680	710	720	670
Average final weight (g/fish)	246 ± 15.3	266 ± 25.2	190 ± 25.49 <sup>a</sup>	235 ± 5.77
Average final length (cm/fish)	20.9 ± 0.55	21.33 ± 1.17	20.8 ± 4.27 <sup>a</sup>	19.9 ± 0.67
Final density (g m <sup>-3</sup> )	2411	2589	1689 <sup>a</sup>	2148
Survival rate (%)	100	100	0	100
Average daily growth (g day <sup>-1</sup> )	3.01	3.35	2.06 <sup>a</sup>	2.81
Feed conversion ratio (FCR)	1.27	1.28	2.15 <sup>a</sup>	1.37
TSS (mg TSS l <sup>-1</sup> )	2.86	5.28	160	2.59
Average NH <sub>4</sub> <sup>+</sup> -N (mg N l <sup>-1</sup> )	0.32 ± 0.021 <sup>b</sup>	0.55 ± 0.051 <sup>b</sup>	0.56 ± 0.692 <sup>b</sup>	0.52 ± 0.815 <sup>b</sup>
Average NO <sub>2</sub> <sup>-</sup> -N (mg N l <sup>-1</sup> )	0.30 ± 0.035 <sup>b</sup>	0.49 ± 0.047 <sup>b</sup>	4.77 ± 5.824 <sup>b</sup>	8.52 ± 10.457 <sup>b</sup>
Average NO <sub>3</sub> <sup>-</sup> -N (mg N l <sup>-1</sup> )	13.81 ± 11.621 <sup>b</sup>	15.01 ± 13.771 <sup>b</sup>	7.78 ± 8.893 <sup>b</sup>	16.27 ± 14.675 <sup>b</sup>

<sup>a</sup> Measured at the end of day 33rd when all fish remained in the tank.

<sup>b</sup> Indicate statistically significant differences ( $P < 0.05$ ).

to the lengthy exposure (>15 days) to harmful levels of nitrite. Excessive nitrite accumulations are generally known to lower oxygen transport capability and weaken aquatic animal immune responses, yet the maximum nitrite concentration reported in T3 ( $\text{NO}_2^- - \text{N}_{\text{max}} = 16.2 \text{ mg N l}^{-1}$ ) as well as that in T4 ( $\text{NO}_2^- - \text{N}_{\text{max}} = 30.6 \text{ mg N l}^{-1}$ ) were many magnitudes higher than the acceptable limitation of  $1.0 \text{ mg N l}^{-1}$  (Timmons et al., 2002). Tilapia raised in the proposed aquaculture systems (i.e., T1 and T2), where ammonium and nitrite were kept at low concentrations (i.e.,  $< 1.0 \text{ mg N l}^{-1}$ ), exhibited higher growth rates and all survived at the end of the experiments. The additional results of water analysis from T1 that was obtained after the original experiment was concluded on day 44 further confirms that low ammonium and nitrite concentrations (i.e.,  $\text{NH}_4^+ - \text{N}$  and  $\text{NO}_2^- - \text{N} < 1.0 \text{ mg N l}^{-1}$ ) are essential for fish survival. During this period, the average tilapia biomass density in T1 increased from 2411 to  $7000 \text{ g m}^{-3}$ . The occurrence of other harmful organic residues (e.g.,  $\text{H}_2\text{S}$ ) that might be attributable to the fish mortality in T3 was unlikely. This is due to the maintenance of fully aerobic and well-mixed conditions that prevented the development of anaerobic degradation and the sedimentation of suspended solids on the tank floor. Finally, the average feed conversion ratio (FCR) for T1 and T2 was calculated at 1.28, which was slightly higher than the value of 1.1 reported for the tilapia recirculating system (Little et al., 2008). The result was also approximately half of the value from the biofloc technology system rearing tilapia (Azim and Little, 2008).

### 3.4. Proposed Aquaculture System

The special feature of the proposed aquaculture system was the integration of growing aquatic stocks and operating nitrifying biofilters in the same tank, rather than circulating the production water through aerated biofilters located outside the production pond as is often done in conventional aquaculture systems. Oxygenation of nitrifying biofilters created the airlift movement in the hollow plastic cylinder that automatically provided the water circulation and maintained aerobic and well-mixed conditions in the tank. In the present study, the concept of bacterial immobilization on high surface area fibrous biofilters was employed to overcome the limited nitrifying bacterial growth. According to the experimental outcomes, significant amounts of suspended solids were generated during the zero-water cultivation as a result of fish excretions, uneaten feeds, and heterotrophic and autotrophic bacterial growths. The presence of suspended solids at excessive levels was undesirable because they can damage fish gills, increasing biochemical oxygen demand, and lowering nitrifying efficiency (Zhu and Chen, 2001). Based on the effluent data (i.e., effluent  $\text{TSS} < 5.5 \text{ mg TSS l}^{-1}$ ), it is apparent that the design of the proposed

aquaculture system was capable of separating suspended solids from production water, and might permit the process scheme to be simplified by integrating the solid separating unit into the production tank. The proposed aquaculture systems were also capable of accomplishing the complete nitrification even though the daily inorganic nitrogen loadings from feed pellets increased from  $1.24$  to  $10.78 \text{ mg N l}^{-1} \text{ day}^{-1}$ . Successful nitrification could have been the result of having already active biofilters and keeping them under the fully aerobic condition ( $\text{DO} > 3.0 \text{ mg l}^{-1}$ ). The maintenance of the completely mixed condition was also essential to the system performance because it can prevent the solid particles from settling and undergoing an anaerobic degradation on the tank floor to produce toxic metabolites, which are harmful to fish and nitrifying bacteria on the biofilters.

From the investment and operational aspects, the proposed aquaculture system may be beneficial because it requires lesser area for system construction and can reduce the water recirculation expense. Moreover, the fibrous biofilters are available in the form of rope so that they are relatively easy to be applied in the different situations. Based on the author experience, another advantage of the selected biofilters is the ease of removing suspended solids deposited on the biofilter surface manually. The fibrous biofilters can be rinsed with water and scratched gently to remove particulate matters without intensive energy requirements as opposed to existing systems such as microbead filters and pack-bed filters, which required intensive energy for backwash (Steicke et al., 2007).

Finally, nitrification has been chosen as a biological pathway to reduce inorganic nitrogen compound toxicity. Despite being relatively harmless to aquatic species, the presence of nitrate at extremely high concentrations may induce stress on aquacultures as well as creating environmental concern if proper treatment is not met. Currently, heterotrophic denitrification is perceived as the most likely method of nitrate removal in aquaculture systems. Typical wastewater retention times have been reported at around 3–10 days for stabilization ponds or even lesser in denitrifying bioreactors (Tchobanoglous and Burton, 2003); that is significantly shorter than the cultivating period of 44 days described in this work. As a result, nitrate-rich wastewater can be kept in denitrifying systems and should have sufficient time to undergo the complete denitrifying reaction before recirculating back into the proposed aquaculture systems.

### 4. Conclusions

Based on the preliminary findings from this work, the following conclusions can be drawn:

1. The shrimp diet is a practical substrate that can be employed to establish nitrifying biofilters. Successful microbial immobilization can be found on the biofilter surface and contributes to the efficient nitrification observed.
2. Acclimated biofilters are effective in maintaining acceptable ammonium and nitrite concentrations in water during the zero-water exchanged tilapia cultivation. Higher tilapia growth in a system arranged with acclimated biofilters is clearly related to the ability to maintain low ammonium and nitrite concentrations. This confirms the necessity for employing already active nitrifying biofilters to control inorganic nitrogen compound toxicity.
3. The design of the proposed aquaculture system is simple to operate and does not adversely affect the ability of acclimated biofilters to perform nitrification. As a result, the proposed aquaculture system can offer an alternative option for closed-water recirculating systems to address inorganic nitrogen compound toxicity and environmental conservation.

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