Chapter IV

Results and Disccussion

4.1 Parameters Affecting Nitrifying Biofilter Acclimation

Non-acclimated biofilters (i.e., Biocord™ and BCN-009) were subjected to different operating conditions, which were believed to exert significant effects on nitrifying biofilter startup. Type of ammonium sources was the first parameter that was considered. From the experimental result, which was shown in Figure 4-1, 4-2, 4-3, 4-4 and 4-5, it was apparent that the chosen ammonium sources did not demonstrate any significant differences in achieving the complete nitrification. For a given type of ammonium source, ammonium and nitrite accumulations were observed in consequence. As an example, in the experiment utilizing tilapia excretion as ammonium source (Figure 4-1), the peaked ammonium concentration was found at 1.75 mg N L^{-1} on day 7 and the nitrite accumulation as high as 0.9 mg N L^{-1} was observed later on from day 13 – 20. Sequential buildups of ammonium and nitrite were common characteristics for non-acclimated biofilters subjecting to a continuous ammonium addition. Ammonium peak observed on day 7 was the result of protein degradation (i.e., ammonification) that released ammonium as product. Ammonium oxidizing bacteria (AOB) were responsible for a rapid ammonium decline and lengthy nitrite buildup. A sudden decrease of nitrite concentration on day 22, followed by increasing nitrate concentration, was the result of nitrite oxidizing bacteria (NOB) carrying out nitrite conversion to nitrate. According to inorganic nitrogen profiles shown in Figure 4-1, a complete nitrification was established after day 22. In constrast to shrimp diets, NH_4Cl was able to dissociate into ammonium instantly once in solution so that ammonium peaks were always observed right after each NH_4Cl addition (Figure 4-2 and Figure 4-3). For the experiment involved supplying 2 mg N L^{-1} of shrimp diets (Figure 4-4), only nitrite buildup as high as 0.9 mg N L^{-1} was found on day 8, whereas no distinct ammonium peak was noticeable throughout the experiment. Low ammonium concentration observed in Figure 4-4 was explainable since shrimp diets slowly released ammonium into water so that it can be utilized by AOB instantly. The effects of elevating ammonium concentrations from 2 – 10 mg N L^{-1} as means to accelerate nitrifying biofilter acclimation were not pronounced regardless of ammonium sources applied. All tests revealed that the complete nitrification was established after approximate 1 month (Figure 4-2, 4-3, 4-4, and 4-5). The only difference between using

the low and high ammonium dosages (i.e., 2 and 10 mg N L^{-1}) was the final nitrate concentrations measured on the last day of the experiment. For a given ammonium source (i.e., shrimp diets and NH_4Cl), providing higher ammonium concentrations resulted in higher nitrate concentration at the end of the experiment. For instance, the final nitrate concentrations on day 56 were measured at 26 and 62 mg N L^{-1} when using 2 and 10 mg N L^{-1} of NH_4Cl as ammonium source, respectively.

Based on the inorganic nitrogen profiles (i.e., Figure 4-1 to 4-5), no conclusion can be made to indicate which parameters were the most likely to affect nitrifying biofilter startup. For this reason, samples of acclimated biofilters (i.e., 10 cm of Biocord™; 150 pieces of BCN-009) from all operating conditions were obtained to perform batch experiments to determine the rate of ammonium degradation. The result is displayed in Table 4-1. The maximum ammonium degradation rate was observed at between 133.33 and 138.88 mg N m^{-2} day^{-1} when the Biocord™ biofilters were subjected to a periodic addition of 10 mg N L^{-1} NH_4Cl. Based on the data in Table 4-1, several comments can be made. It appeared that longer acclimating period was able to enhance ammonium degradation rates regardless of ammonium sources, ammonium concentrations, and type of biofilters. Moreover, Biocord™ biofilters were more effective than BCN-009 as can be confirmed by greater degradation rate for each ammonium concentration tested. This was due Biocord™ biofilter possessing higher surface area for nitrifying bacteria to attach than BCN-009. The specific surface area of Biocord™ biofilters and BCN-009 was estimated at 4,200 and 864 m^2 m^{-3}, respectively. The result from batch experiments indicated that all acclimating conditions were able to enrich biofilters to achieve the complete nitrification. However, the batch result was inconclusive to specify which acclimating conditions were the most suitable to nitrifying bacteria. On separated note, the scanning electron micrograph (SEM) also revealed that microorganisms tended to form dense colonies over the surface Biocord™. In contrast, significant amount of dense bacterial colonies was noticed mostly on the inner surface of BCN-009 in comparison to outer surface. The reason for this was linked to excessive shear force created by liquid circulation and air bubbles.

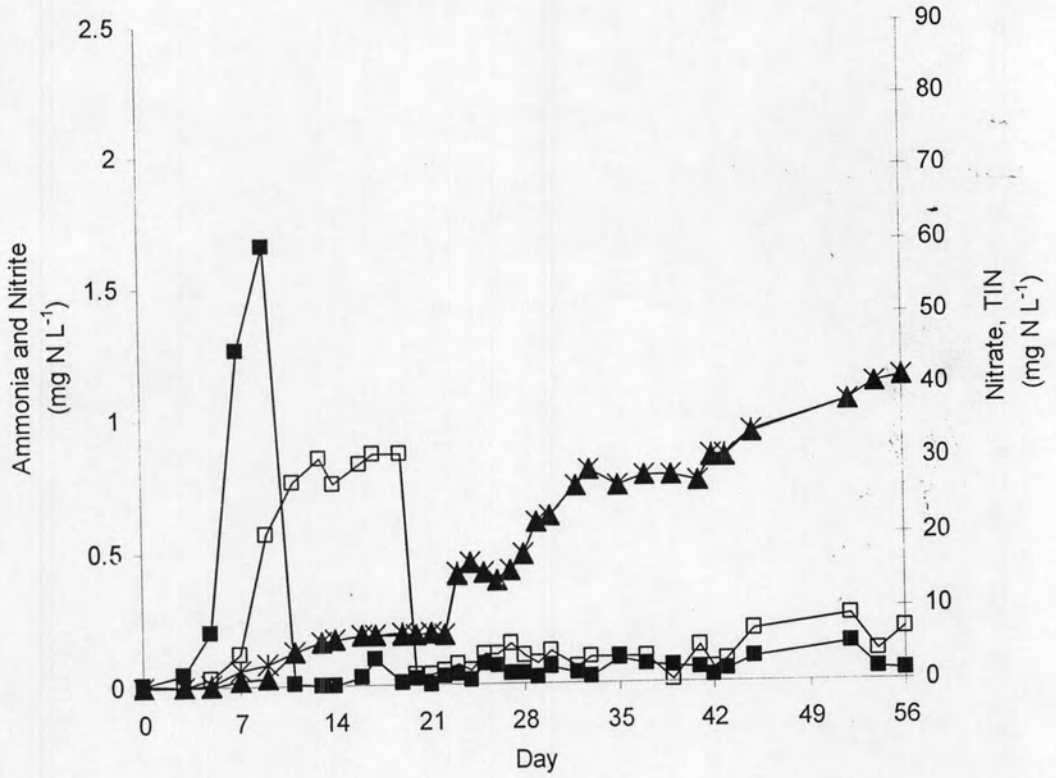

Figure 4-1 Inorganic nitrogen profiles during the Biocord^{TM} biofilter acclimation with tilapia excretion as ammonium source.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents the total inorganic nitrogen (TIN) concentration, mg N L^{-1}

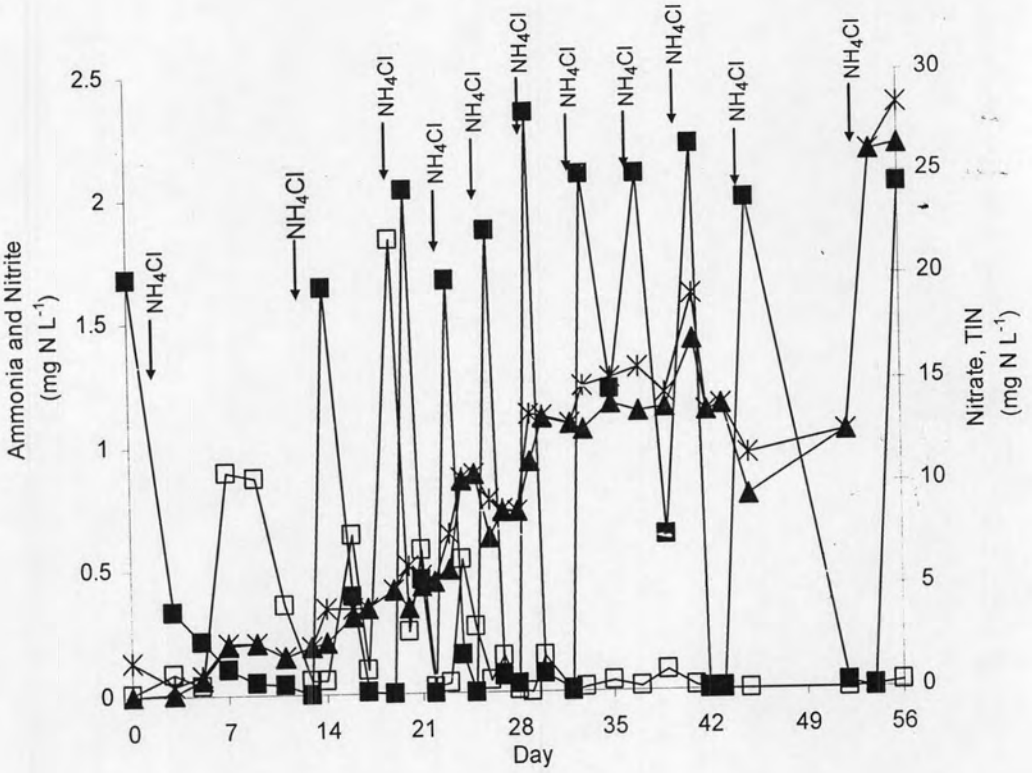

Figure 4-2 Inorganic nitrogen profiles during the Biocord^{TM} biofilter acclimation 2 mg N L^{-1} of NH_4Cl as ammonium source.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents the total inorganic nitrogen (TIN) concentration, mg N L^{-1}

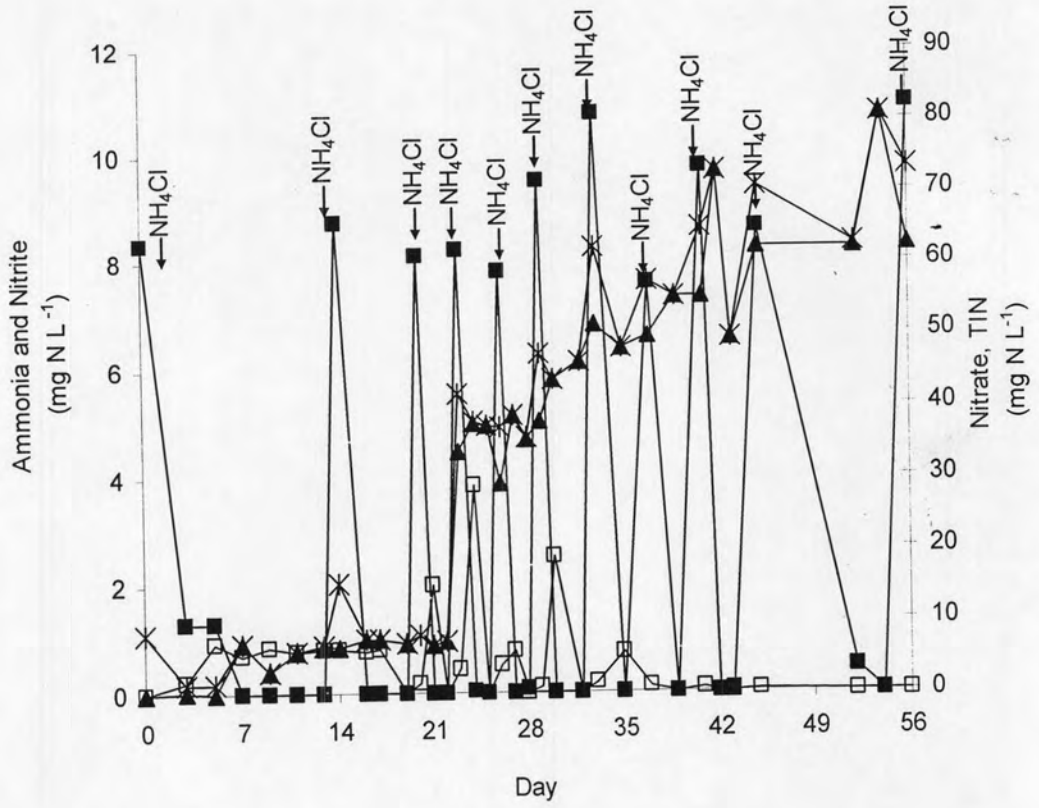

Figure 4-3 Inorganic nitrogen profiles during the Biocord™ biofilter acclimation with 10 mg N L^{-1} of NH_4Cl as ammonium source.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents the total inorganic nitrogen (TIN) concentration, mg N L^{-1}

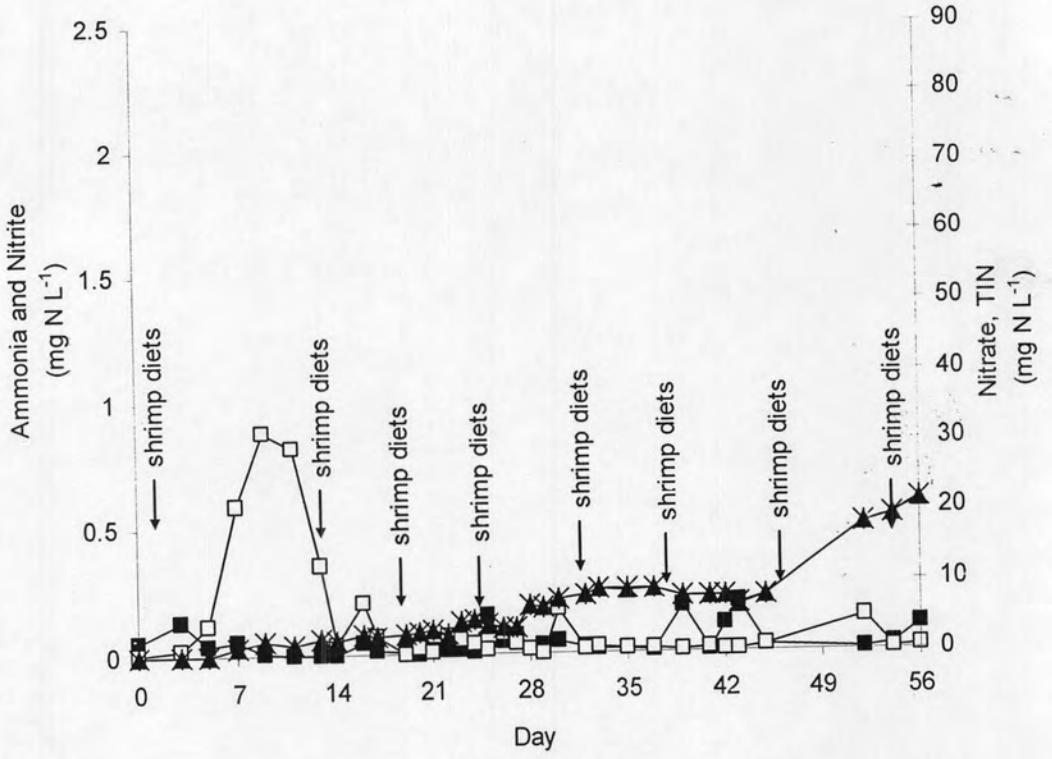

Figure 4-4 Inorganic nitrogen profiles during the Biocord™ biofilter acclimation with 2 mg N L^{-1} shrimp diets as ammonium source.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents the total inorganic nitrogen (TIN) concentration, mg N L^{-1}

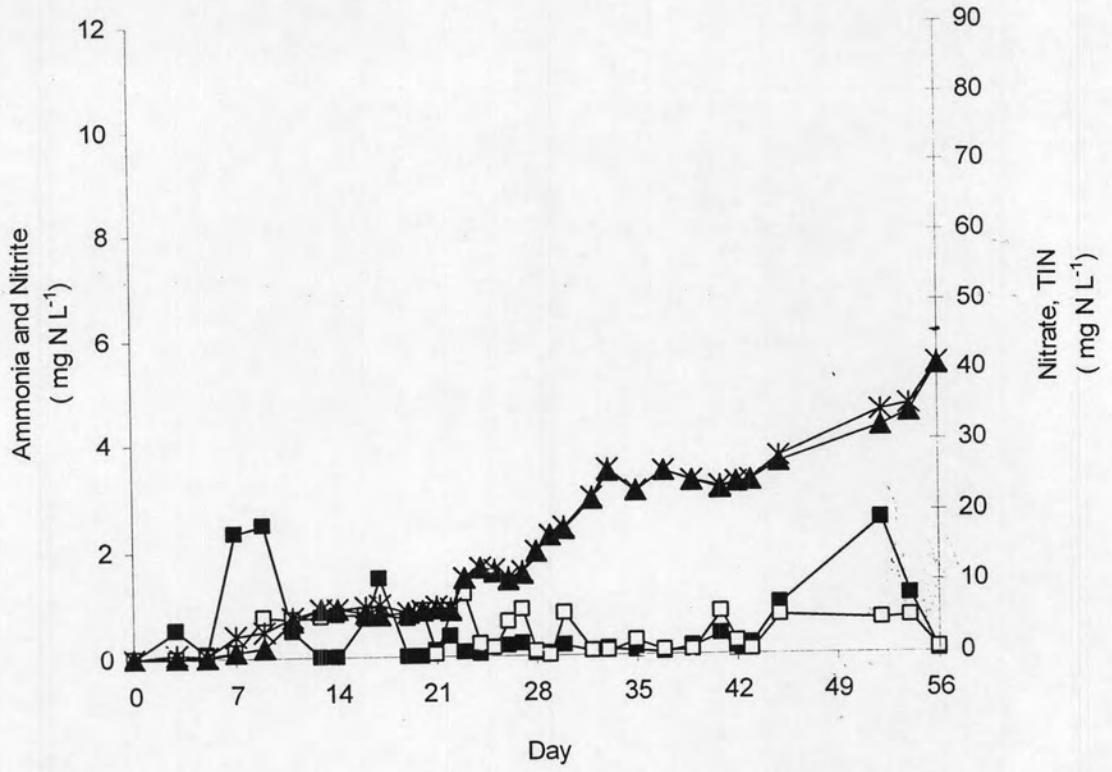

Figure 4-5 Inorganic nitrogen profiles during the Biocord^{TM} biofilter acclimation with 10 mg N L^{-1} shrimp diets as ammonium source.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents the total inorganic nitrogen (TIN) concentration, mg N L^{-1}

Table 4-1 Ammonium degradation rate of acclimated Biocord™ and BCN-009 subjected to different acclimating conditions.

Biofilters type	Substrate type	Concentration (mg N L^{-1}.)	Ammonia removal rate (mg N $m^{-2}day^{-1}$)			
			24 day		40 day	
			V_{max}	K_M	V_{max}	K_M
Biocord™	Shrimp diets	2	4.77	0.45	15.40	0.22
		10	46.08	2.86	64.10	4.61
	NH_4Cl	2	9.01	0.25	15.10	0.22
		10	133.33	5.40	138.88	2.97
	Aquaculture system		4.86	0.36	70.92	1.4
BCN-009	Shrimp diets	2	49.26	0.4	31.94	0.12
		10	29.85	0.37	65.78	1.16
	NH_4Cl	2	27.62	0.1	26.88	0.62
		10	34.01	0.39	23.36	0.14
	Aquaculture system		26.59	0.73	32.94	0.47

4.2 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 Biocord™ biofilters. Sediment was assumed to contain active mixed cultures of nitrifying bacteria because it had been continuously exposed to ammonium from shrimp diets and animal excretions 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 mg N L^{-1}. Shrimp diet was chosen to accelerate nitrifying reactions in this work because it is easy to purchase or 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 sequentially 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 Figure 4-6. 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 mg N L^{-1} on day 13 for ammonium and 0.79 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 mg (41%) of added nitrogen were unaccountable. The phytoplankton uptake of inorganic nitrogen was insignificant because the acclimating tank was completely covered to prevent 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., DO > 4.0 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 mg N L^{-1} as more shrimp diet (25 g for each addition) was replenished once every 5 – 10 days (Figure 4-6). Ammonium and nitrite concentrations were also lower than 1.0 mg N L^{-1} for the remainder of 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 Figure 4-6, mixed nitrifying cultures only required about 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 acclimated biofilters lower than the intended value of 1.85 mg N L^{-1}. For this reason, shrimp diet was substituted by NH_4Cl to provide instant ammonium concentrations in the water at 3.0 and 4.5 mg N L^{-1} on day 64 and day 71, respectively. The results displayed in Figure 4-6 confirmed the instant dissociation of NH_4Cl on day 64 and day 71 and further indicated the effective removal of ammonium and nitrite that led to a rapid climb of nitrate concentration from 9.3 – 19.1 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 presence of attached microorganisms (Figure 4-7). On the other hand, microorganisms 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 (Figure 4-7). Detailed examination of acclimated biofilter surface found filamentous microorganisms entangled with each other, creating mesh-like networks placed on top of smaller microorganisms. These mesh-like networks was likely to enhance the cell retention capability because they protected small microorganisms from being washout, and simultaneously acted as supporting backbones for small microorganisms to bind. 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. Stable nitrification

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observed during the biofilter enrichment could have been the consequence of successful immobilization that allowed slow growing nitrifying bacteria to establish onto 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 acclimating tank, denitrification rate was unlikely to match that of nitrification as can be shown by the increasing nitrate concentration observed in the acclimating tank.

At the end of acclimating period, small pieces (10 – 15 cm) of shrimp diet acclimated biofilters were taken to perform batch experiments to determine nitrification rates (Figure 4-8). Results from batch experiments revealed that the biodegradation of ammonium by acclimated biofilters finished within 1 – 2 days for each initial ammonium concentration tested (i.e., 2, 4 and 6 mg N L^{-1}). Ammonium oxidation appeared to follow the zero order reaction and displayed an average degradation rate of 24.1 mg N m^{-2} 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. 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. Another reason is related to pre-existing NOB in the sample biofilters that were unable to keep up with ammonium oxidation by AOB. The balance between AOB and NOB was reestablished after about 24 hour 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.

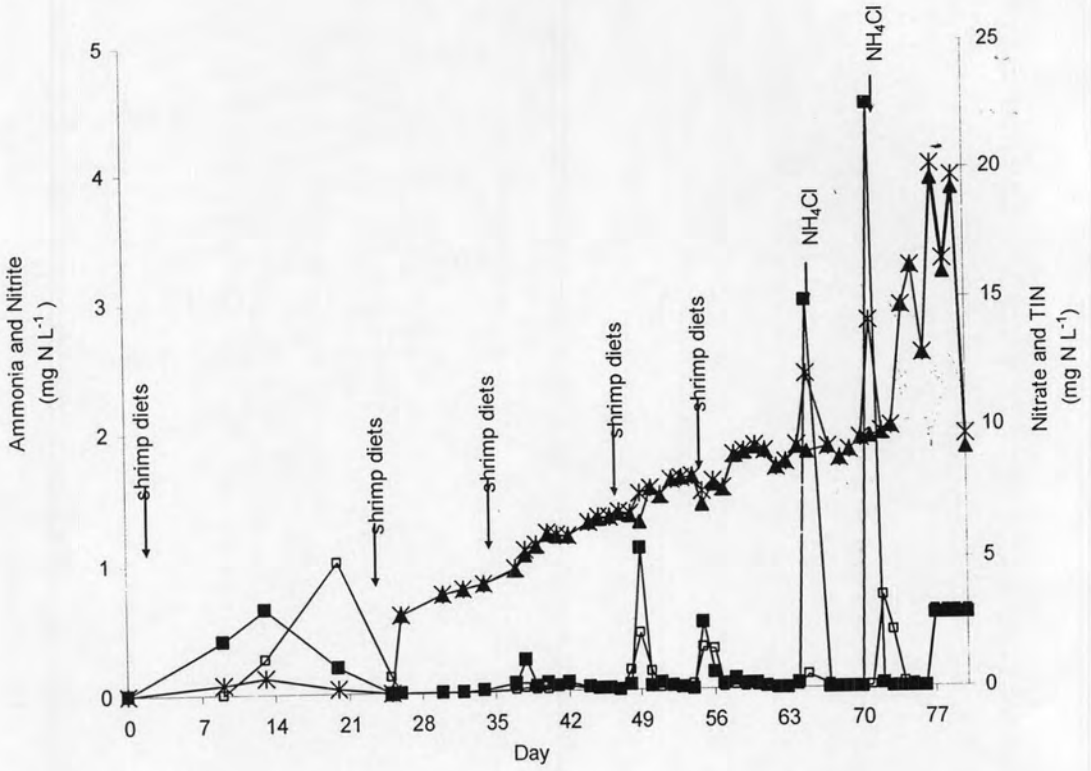

Figure 4–6 Inorganic nitrogen profiles in acclimating tank filled with Biocord™ biofilters. Biofilter acclimation was carried out in the acclimating tank without any water exchange. Arrows indicate shrimp diets and NH_4Cl addition.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents the total inorganic nitrogen (TIN) concentration, mg N L^{-1}

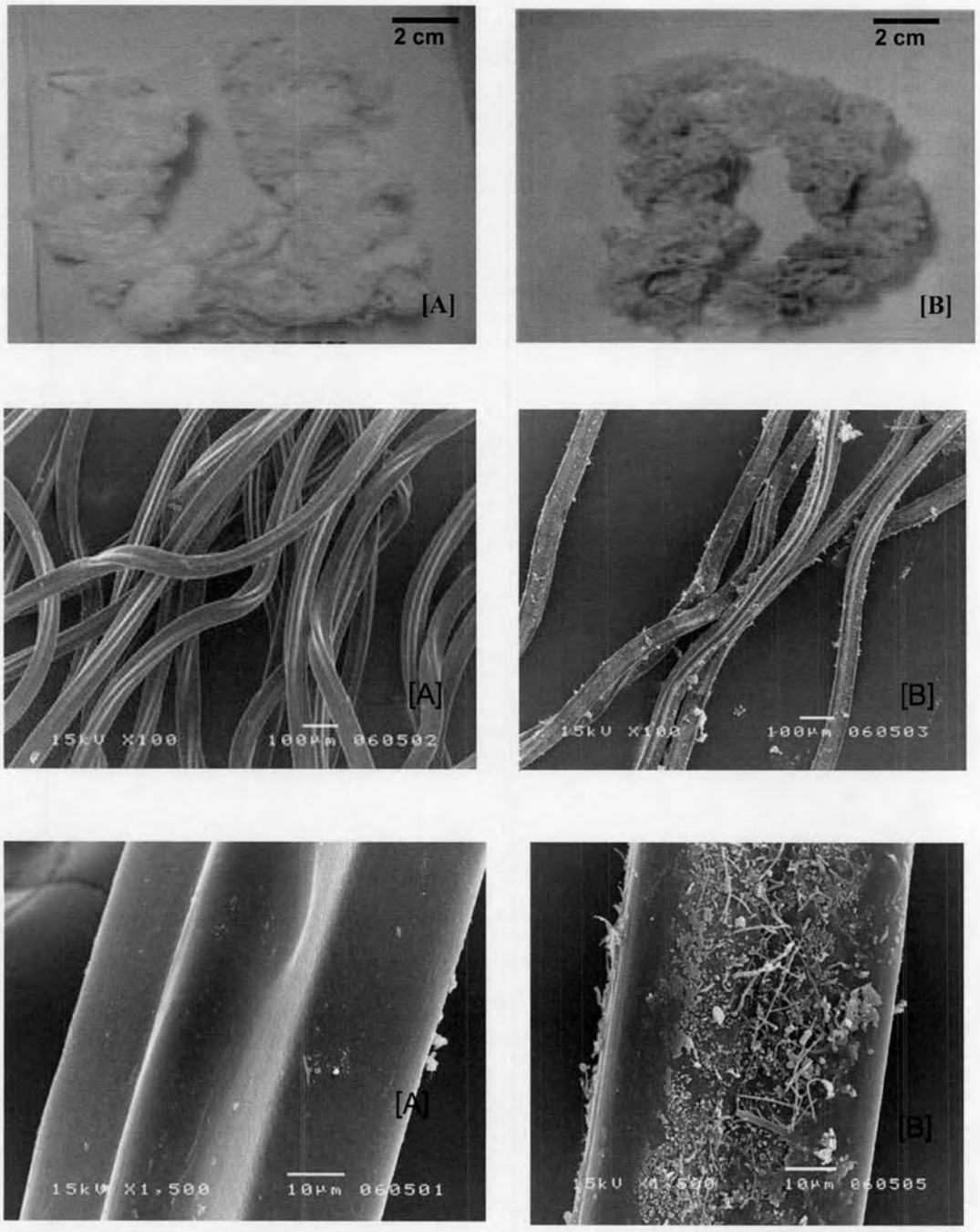

Figure 4–7 Comparison between non-acclimated (new) and 30-day acclimated Biocord™ biofilters; [A] non-acclimated biofilters, [B] acclimated biofilters.

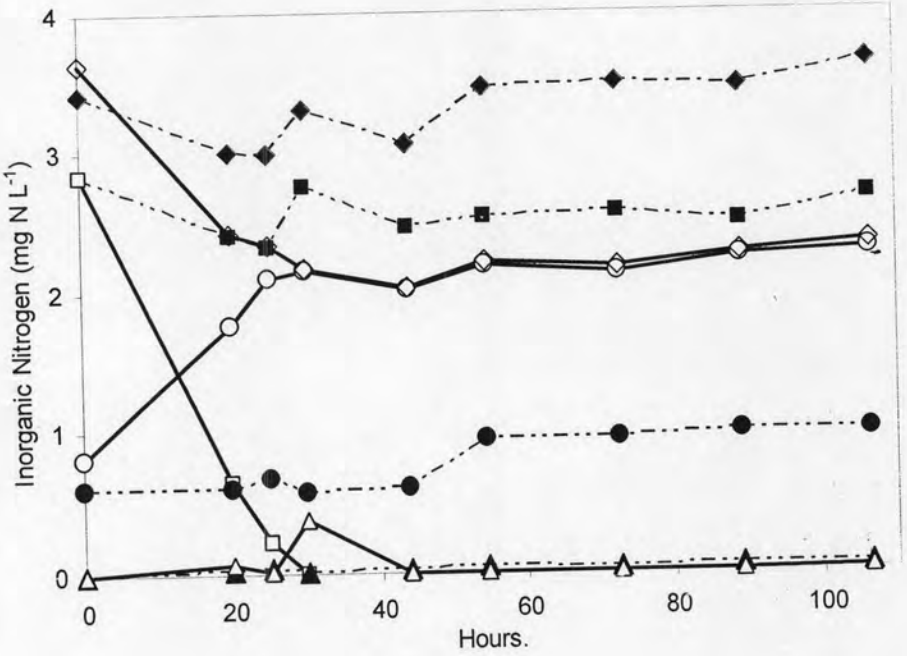

Figure 4–8 Inorganic nitrogen profiles during the batch experiments to determine nitrification rate of acclimated biofilters described in section 4.2. This illustration shows the result when using 4 mg N L^{-1} NH_4Cl.

(■) represents NH_4^+-N concentration by non-acclimated biofilters, mg N L^{-1}.

(▲) represents NO_2^--N concentration by non-acclimated biofilters, mg N L^{-1}.

(●) represents NO_3^--N concentration by non-acclimated biofilters, mg N L^{-1}.

(◆) represents the total inorganic nitrogen (TIN) concentration by non-acclimated biofilters, mg N L^{-1}.

(□) represents NH_4^+-N concentration by acclimated biofilters, mg N L^{-1}.

(△) represents NO_2^--N concentration by acclimated biofilters, mg N L^{-1}.

(○) represents NO_3^--N concentration by acclimated biofilters, mg N L^{-1}.

(◇) represents the total inorganic nitrogen (TIN) concentration by acclimated biofilters, mg N L^{-1}.

4.3 Evaluation of the Proposed Aquaculture System at Different Tilapia Stocking Densities

4.3.1 Initial Stocking Density at 0.7 kg m^{-3}

The zero-water exchanged tilapia cultivation was carried out at the initial stocking density of 0.7 kg m^{-3} as can be shown in Figure 4-9. Figure 4-10, 4-11, and 4-12 illustrate the results of water analysis from each tilapia cultivating tank. Clearly, the proposed 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 – 2.78 mg N L^{-1} day^{-1}. This ability to accomplish the complete nitrification led to the low concentrations of ammonium and nitrite under 1.0 mg N L^{-1}, while the nitrate concentration continued to increase reaching the levels as high as 39.5 mg N L^{-1} on day 44. 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 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 and no biofilters) indicated that the daily addition of tilapia feeds did not generate an ammonium accumulation in water above 1.0 mg N L^{-1} during the initial period of 3 weeks. Based on feeding record that produced the daily inorganic nitrogen loadings from 1.16 – 1.67 mg N L^{-1} day^{-1}, 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_4^+-N, NO_2^-N, NO_3^--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 trivial (NO_2^--N < 0.25 mg N L^{-1}; NO_3^--N < 1.0 mg N L^{-1}). The photoautotrophic assimilation of inorganic nitrogen was also unlikely because phytoplankton was not presence 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 SS L^{-1} 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_2^-- N = 2.0 – 16.2 mg N L^{-1}) 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_3^--N = 2.3–27.2 mg N L^{-1}) 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^{-1}) 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 – 1.38 mg N L^{-1} day^{-1}. 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^{-1} 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 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.

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 see through to observe the tilapia swimming in the tank. At the end of the cultivation on day 44, the total suspended solids in T3 were determined at 160 mg SS L^{-1}, which was almost 40-folds higher than the numbers obtained from T1, T2 and T4 (i.e., TSS < 5.5 mg SS L^{-1}). 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 suspended solid concentrations from the proposed aquaculture systems (i.e., T1 and T2) were well below the discharged limitation set at 80 mg SS L^{-1} (The Pollution Control Department, Thailand).

Table 4-2 demonstrates tilapia growth data during the zero-water exchanged cultivation. Tilapia biomass density in T1 and T2 increased from 680 – 2,589 g m^{-3} during the 44 day period, and this corresponded to the average daily growth rates of 3.01 and 3.35 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 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 (NO_2^--N_{max} = 16.2 mg N L^{-1}) as well as that in T4 (NO_2^--N_{max} = 30.6 mg N L^{-1}) were many magnitudes higher than the acceptable limitation of 1.0 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 mg N L^{-1}), exhibited higher growth rates and all survived at the end of the experiments. The occurrence of other harmful organic residues (e.g., H_2S) 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).

Biofilter Solid Retention

During the zero-water exchanged tilapia cultivation, significant amount of suspended solids was intercepted and retained by Biocord™ biofilters. Therefore, an independent experiment was performed to determine the ability of Biocord™ biofilters in retaining suspended solids. The result from an independent experiment confirmed the effectiveness of Biocord™ biofilters in solid-liquid separation (Figure 4-13). It appeared that gravitational sedimentation did not attribute as much to the solid removal because as high as 75% (≈ 600 mg SS L^{-1}) of available suspended solids still remained in water when Biocord™ biofilters were not featured. Enhanced solid removal efficiency up to 99% was accomplished after Biocord™ biofilters were integrated into the experiment. Another interesting observation that arose from an independent experiment was the rate at which suspended solids were removed. Acclimated Biocord™ biofilters required about 10 hours to separate 85% of the total suspended solids from water, whereas unused Biocord™ biofilters needed as long as 3 days to achieve the same efficiency.

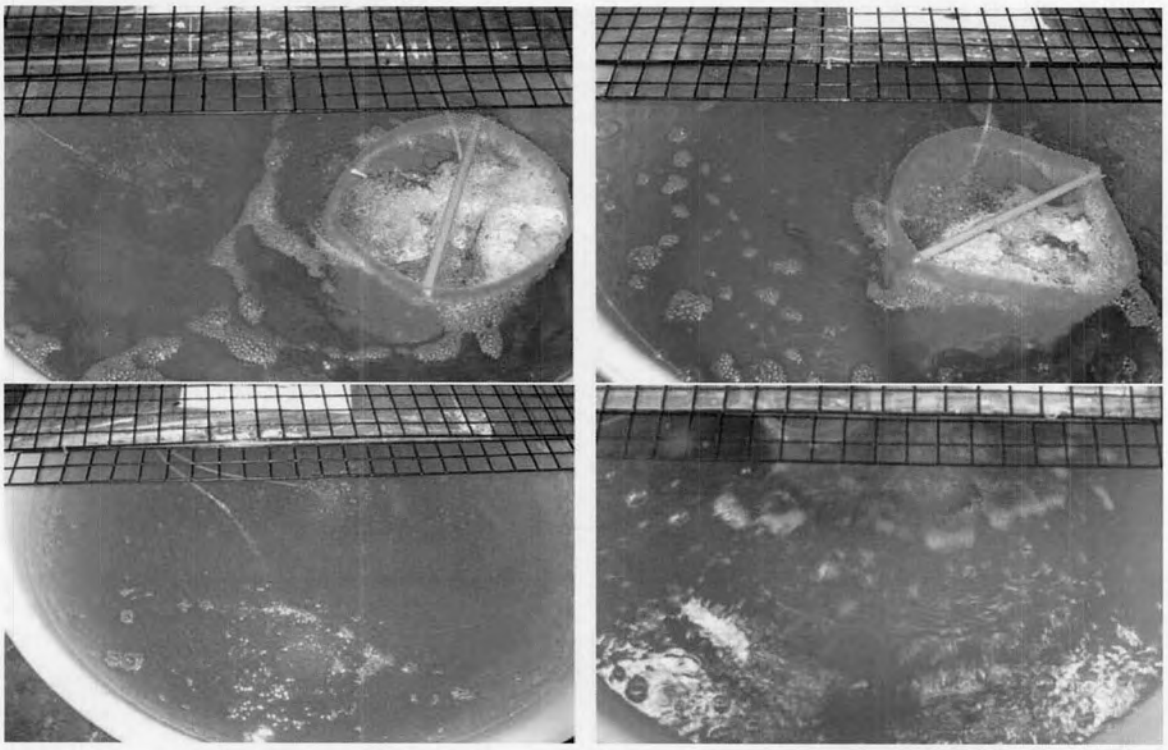

Figure 4-9 The zero-water exchanged tilapia cultivation at the initial stocking density of 0.7 kg m^{-3}; T1 and T2 represent the proposed aquaculture system integrated with shrimp diet acclimated Biocord^{TM} biofilters, T3 is regular fish without biofilter, and T4 is the proposed aquaculture system integrated with non-acclimated (new) Biocord^{TM} biofilters.

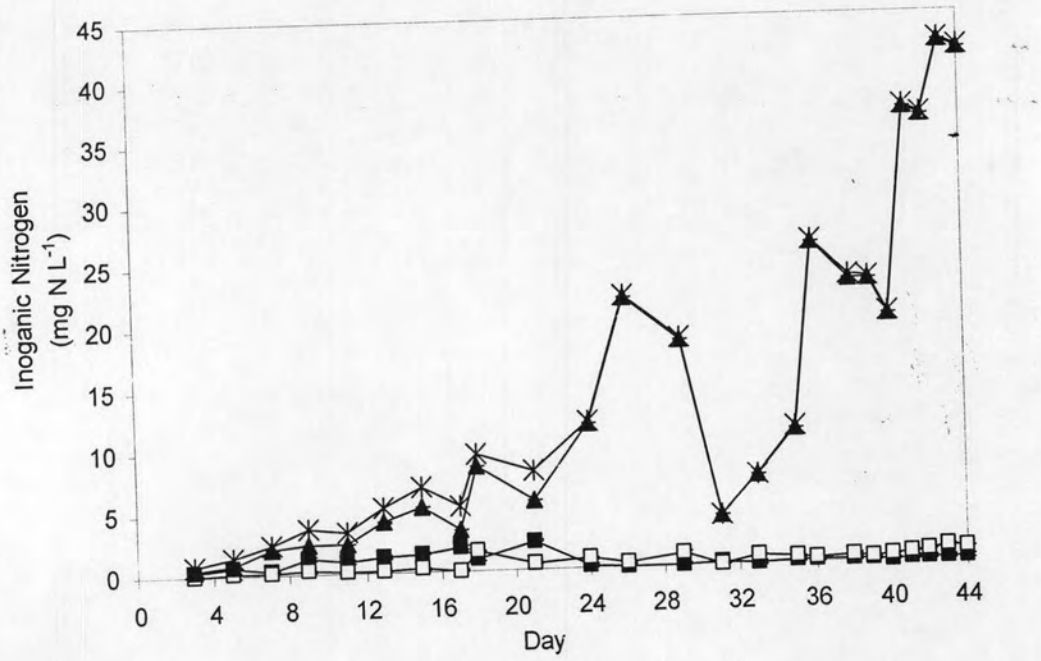

Figure 4-10 Inorganic nitrogen analysis of water from T1 and T2, which were the proposed aquaculture systems integrated with shrimp diet acclimated Biocord™ biofilters. The initial tilapia stocking density was approximately set at 0.7 kg m^{-3}.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents total inorganic nitrogen (TIN) concentration, mg N L^{-1}.

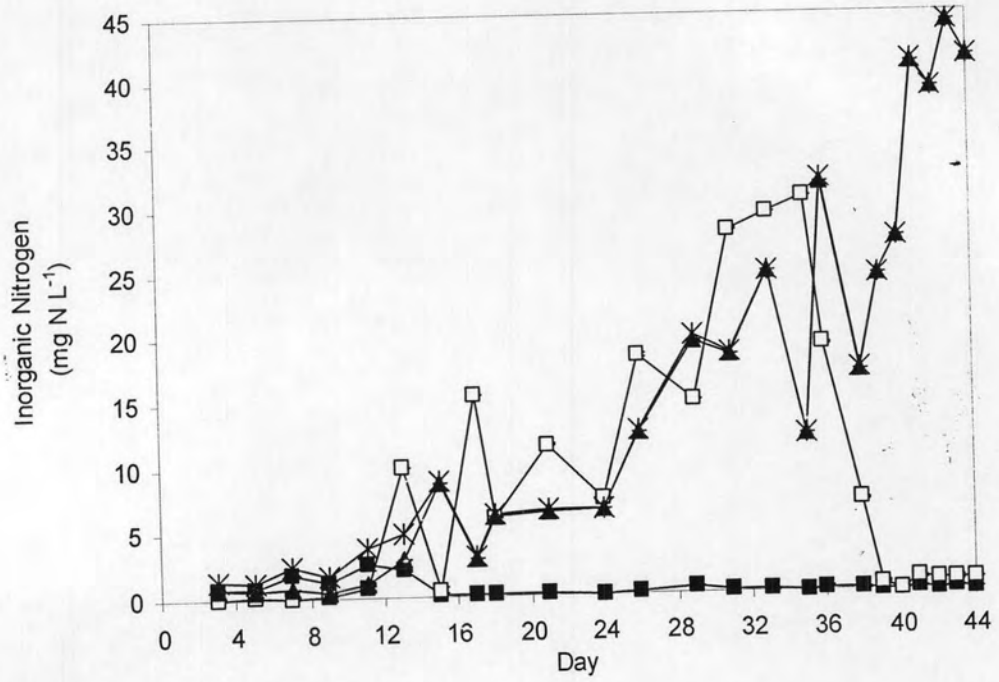

Figure 4-11 Inorganic nitrogen analysis of water from T3, which was the regular suspended-growth tank (i.e., no biofilters). The initial tilapia stocking density was approximately set at 0.7 kg m^{-3}.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents total inorganic nitrogen (TIN) concentration, mg N L^{-1}.

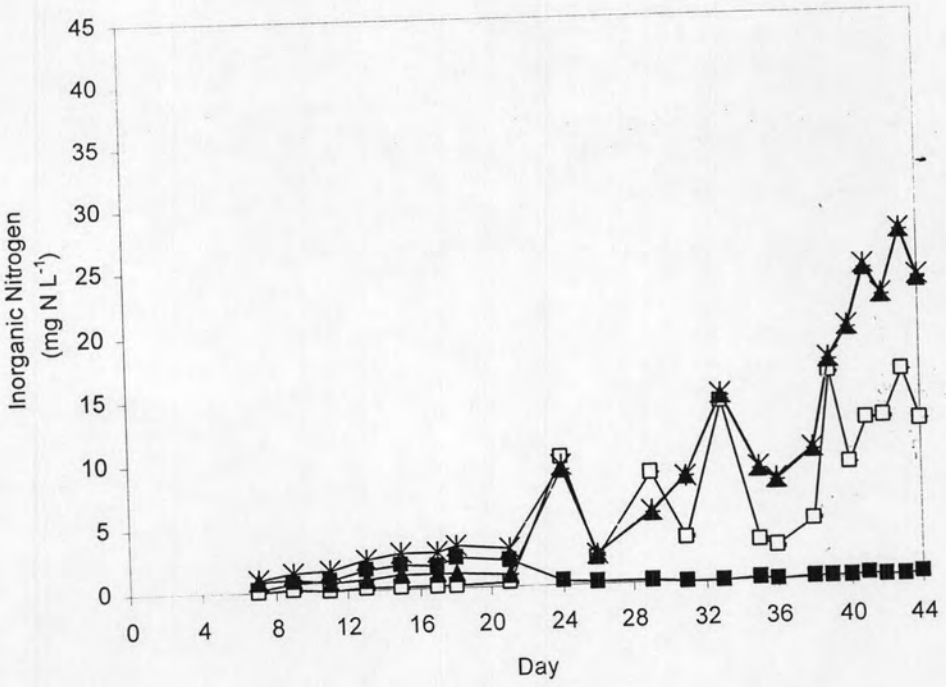

Figure 4-12 Inorganic nitrogen analysis of water from T4, which was the proposed aquaculture systems integrated with non-acclimated (new) Biocord™ biofilters. The initial tilapia stocking density was approximately set at 0.7 kg m^{-3}.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents total inorganic nitrogen (TIN) concentration, mg N L^{-1}.

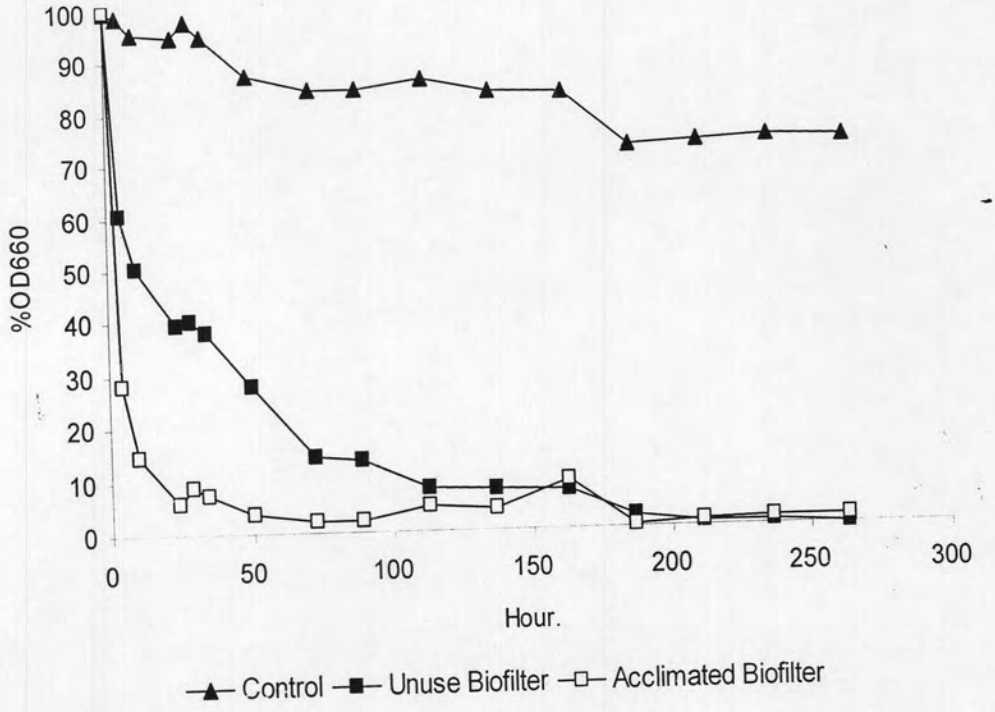

Figure 4-13 Ability of acclimated and unused Biocord^{TM} biofilters in retaining suspended solids measured in term of light absorption at 660 nm. Original wastewater sample contained approximately 800 mg SS L^{-1} (i.e., t = 0 hour). Control; experiment performed without Biocord^{TM} biofilters (i.e., sedimentation only); Acclimated Biofilters – experiment performed using Biocord^{TM} biofilters taken from cultivating tanks; Unused Biofilters – experiment performed using new Biocord^{TM} biofilters

Table 4-2 Tilapia growth data from the zero-water exchanged tilapia cultivation for the initial stocking density of 0.7 kg m^{-3}.

Parameters	Average ± SD (min - max)			
	T1	**T2**	**T3**	**T4**
Average initial weight (g)	113.3 ± 11.5 (101.8 -124.8)	118.33 ± 7.6 (110.73 -125.93)	120 ± 17.3 (102.7 - 137.3)	111.7 ± 10.4 (101.30 - 122.1)
Average initial length (cm)	17.5 ± 0.50 (17.0 - 18.0)	18.17 ± 0.58 (17.59 - 18.75)	17.7 ± 0.77 (16.93 - 18.47)	18 ± 0.87 (17.13 - 18.87)
Initial density (g m^{-3})	680	710	720	670
End of Experiment				
Average final weight (g)	246 ± 15.3 (230.7 -261.3)	266 ± 25.2 (240.8 -291.2)	190 ± 25.49*(164.5 -215.49)	235 ± 5.77 (229.2 -240.7)
Average final length (cm)	20.9 ± 0.55 (20.35 -21.45)	21.33 ± 1.17 (20.16 -22.55)	20.8 ± 4.27*(16.53 -25.07)	19.9 ± 0.67 (19.2 -20.5)
Final density (g m^{-3})	2411	2589	1689*	2148
Survival rate (%)	100	100	0	100
ADG (g day^{-1})	3.01	3.35	2.06*	2.81
FCR	1.27	1.28	2.15*	1.37
Suspended olids (mg SS L^{-1})	2.86	5.28	160	2.59
Average ammonium (mg N L^{-1})	0.32 ± 0.02 (0.03 - 1.28)	0.55 ± 0.05 (0.05 - 2.34)	0.56 ± 0.69 (0.04 - 2.67)	0.52 ± 0.82 (0.06 - 2.31)
Average nitrite (mg N L^{-1})	0.30 ± 0.04 (0.00 -0.71)	0.49 ± 0.05 (0.02 - 1.54)	4.77 ± 5.82 (0.1 - 30.59)	8.52 ± 10.46 (0.05 - 16.2)
Average nitrate (mg N L^{-1})	13.81 ± 11.62 (17.0 - 18.0)	15.01 ± 13.77 (0.51 - 42.64)	7.78 ± 8.89 (0.50 - 44.18)	16.27 ± 14.67 (0.68 - 27.21)

(*) Measured on day 33 before tilapia mortality

4.3.2 Initial Stocking Density at 3.0 kg m^{-3}

The setback of the first evaluation included short cultivation period for 44 days and relatively low inorganic nitrogen loadings that range from 1.24 – 2.78 mg N L^{-1} day^{-1}. As a result, an additional evaluation of the proposed aquaculture systems was carried out at higher initial stocking density of 3.0 kg m^{-3}. The result indicated that the proposed aquaculture systems were able to maintain physical parameters including DO, temperature, pH, and alkalinity within the optimal range for nitrifying bacteria (Table 4-3). These parameters were also favorable for tilapia growth, which required desirable pH from 7 – 8 and temperature from 20 – 35 °C (Fattah and El-Sayed 2006). In this work, high suspended solid contents were observed for all cultivating tanks (Figure 2). Average suspended solid concentrations in T1 and T2 increased from 5 – 114 mg SS L^{-1} during the experiment. Lower suspended solid concentrations varying from 5 – 22 mg SS L^{-1} were observed in T3 due to lower tilapia stocking. The result from T4, which was fabricated to resemble suspended growth systems, was highly different from those tanks described previously. In this case, water became tremendously turbid by the 2nd week, and the suspended solid concentrations increased rapidly from about 10 mg SS L^{-1} to reach an extremely high level measured at 1,340 mg SS L^{-1} on the final day. The source of inorganic nitrogen was primarily from an ammonification of unconsumed feed proteins as well as from animal excretion. The proposed aquaculture systems (i.e., T1, T2, and T3) were able to maintain ammonium and nitrite concentrations under 1.0 mg N L^{-1} for the entire experiment (Figure 4-14, 4-15, 4-16). Lower nitrate production in T3 compared to T1 and T2 was due to lower animal stocking. Inorganic nitrogen profiles for T4 were fairly different from those tanks mentioned earlier. In this case, ammonium concentrations can be kept under 1.0 mg N L^{-1} throughout the cultivation, but nitrite concentrations were excessive at approximately 5.0 mg N L^{-1} from day 14 – 36 before their concentrations diminished after day 36. Acceptable inorganic nitrogen concentrations in the proposed aquaculture systems were partly linked to a complete nitrification that had been established on Biocord™ biofilters although inorganic nitrogen loadings increased more than 3-folds from 5 – 18.3 mg N L^{-1} day^{-1}. Proper biofilter preparation to achieve a complete nitrification before an initial deployment was another factor that contributed to the system efficient performance. With fully acclimated Biocord™ biofilters installed, the proposed aquaculture systems were able to establish nitrification almost immediately and thus overcome the possibility of excessive nitrite

accumulation especially during the system startup from day 1 – 30. An initial evaluation of the systems by Sesuk et al. (2009) demonstrated that using non-acclimated Biocord™ biofilters can produce an excessive nitrite buildup (i.e., NO_2^--N > 10 mg N L^{-1}) for more than 3 weeks. The proposed aquaculture systems also featured microbial immobilization (i.e., adsorption) to retain significant amount of bacterial cells within cultivating tanks. Microbial attachment to Biocord™ biofilters can be accomplished after 3 weeks of acclimation by adding shrimp diets (Sesuk et al., 2009). Immobilization was known as a particularly useful technique to encounter nitrifying bacteria slow growth (Nicolella et al., 2000). In fact, many domestic and industrial wastewater treatment facilities have been used immobilization successfully for years to improve nitrogen-rich wastewater treatment (Tchobanoglous et al., 2003). Lack of microbial immobilization in suspended growth systems such as T4 produced several undesirable consequences including the extreme water turbidity and more importantly the occurrence of partial nitrification, which was described as a cause of excessive nitrite accumulation for extended period.

It should reemphasize that the proposed aquaculture systems were originally designed for nitrification and were operated without any means of suspended solid removal in this work. Besides nitrification, other biological processes, which were capable of inorganic nitrogen control, were also established in the proposed systems. Substantial production of suspended solids in all cultivating tanks was partly the result of microbial growth, which required the direct assimilation of dissolved nitrogenous and carbonaceous matters for the synthesis of new biomass. Inorganic nitrogen treatment based on direct assimilation was similar to the principle of biofloc technology, which required intensive aeration and organic carbon addition to sustain the system (Avnimelech, 2006). At the moment, the extent of inorganic nitrogen removal based on the direct assimilation remained unknown because it was difficult to distinguish the new biomass from unconsumed feeds and tilapia feces. Heterotrophic denitrification was another biological process that may be responsible for nitrate utilization observed in T1 and T2 after day 57 (Figure 4-14). During this period from day 57 – 90, nitrate concentrations in T1 and T2 decreased from 109 – 13 mg N L^{-1} while ammonium and nitrite concentrations still remained under 1.0 mg N L^{-1}. Moderate septic odors were also detectable from Biocord™ biofilters after day 85. The ability of Biocord™ biofilters in intercepting and retaining suspended solids was a crucial factor that facilitated the onset of heterotrophic denitrification. Trapped suspended solids acted as organic electron donor and their accumulation on biofilter surface can create anaerobic pockets required

for denitrifying population. Heterotrophic denitrification was likely to continue as long as there was sufficient nitrate. Other anaerobic metabolisms that produced toxic metabolites may develop after nitrate was exhausted. Moderated septic odors from Biocord™ biofilters from day 85 – 90 suggested the development of sulfate reduction process, which yielded hydrogen sulfide as end product. Hydrogen sulfide was toxic towards aquacultures and its presence even as low as 0.002 mg L^{-1} was able to affect aquaculture welfare (Timmons et al., 2002). In fact, tilapia reared in the proposed aquaculture systems (i.e., T1 and T2) was unable to survive by the end of experiment, with the majority of death observed when septic odors were strongly detectable after day 87. Production of toxic residues was less likely in T3 due to lesser solid production. Nonetheless, the occurrence of heterotrophic denitrification or other anaerobic processes in this tank was expected if the zero-water exchanged tilapia cultivation was to continue. The likelihood of anaerobic process occurring in T4 was also small since well-mixed condition and constant aeration were maintained to prevent substantial solid sedimentation on tank floor.

From an operational point of view, occurrence of heterotrophic denitrification in the proposed aquaculture systems were undesirable although this anaerobic process was able to transform inorganic nitrogen completely to nitrogen gas. Achieving simultaneous nitrification and denitrification was currently impractical for the proposed systems because suitable denitrifying conditions were quite difficult to maintain without employing sophisticated controlling equipments. System operators should perform a regular suspended solid removal from the surface of Biocord™ biofilters. Elimination of trapped suspended solids prevented the formation of anaerobic pockets and reduced the amount of organic electron donors available. Data from an independent experiment confirmed the necessity of removing trapped suspended solid regularly. Both the clean and unclean Biocord™ biofilters were able to convert approximately 5.0 mg N L^{-1} of ammonium completely to nitrate in less than 60 hours without showing a substantial nitrite accumulation (i.e., NO_2^--N < 0.5 mg N L^{-1}). However, clean Biocord™ biofilters exhibited an average nitrification rate of 41.4 mg N m^{-2} day^{-1}, which was approximately 33% greater that the rate from unclean Biocord™ biofilters (Figure 4-17). Enhanced nitrification rate from clean Biocord™ biofilters was related to providing optimal conditions (i.e., DO > 3 mg L^{-1}; pH = 7 – 8; and lesser organic compounds) to favor nitrifying bacteria. With lesser amount of suspended solids on biofilter surface, heterotrophic bacteria might not have sufficient substrate to sustain their growth, and that

presented the opportunity for nitrifying bacteria residing in the same biofilms to be more competitive in acquiring oxygen for their metabolisms. Based on inorganic nitrogen profiles (Figure 4-14), nitrification occurred effectively up to day 57. The cumulative nitrogen input up to this point was determined at 0.24 kg N, while the corresponding inorganic nitrogen loading on this day (i.e. day 57) was calculate at 14.56 mg N L^{-1} day^{-1}, which translated into fish density at roughly 10 kg m^{-3}. Based on this information, it was not recommendable to carry out the zero-water exchanged aquacultures in the proposed systems over 10 kg m^{-3} (biofilter length = 21.6 m) without performing a regular biofilter cleaning. Continued operation beyond the recommended aquaculture density would make the proposed systems more susceptible to heterotrophic denitrification and other unwanted anaerobic processes. Nitrification rates achieved in this work (i.e., 27.6 – 41.2 mg N m^{-2} day^{-1}) was clearly in the low range compared to other recirculating systems, which reported areal nitrification rates above 200 mg N m^{-2} day^{-1} (Delos Reyes and Lawson, 1996; Brazil, 2006; Timmons et al., 2006; Tal et al., 2009). The maximum inorganic nitrogen that the proposed aquaculture systems were able to handle before heterotrophic denitrification started to dominate should increase upon the regular biofilter cleaning.

Table 4-4 depicts tilapia growth data from this work. For the proposed aquaculture systems (i.e., T1, T2, and T3), the average daily growth (ADG) of tilapia was in the range from 1.81 – 2.31 g day^{-1}, which was approximately 30 – 45% better than the result from suspended growth systems (i.e., T4). The ADG from the proposed aquaculture systems was also comparable to other works employing nitrifying biofilters that demonstrated ADG in the range from 1.2 – 2.3 g day^{-1} (Ridha and Cruz, 2001; Al-Hafedh, et al., 2003). In term the feed conversion ratio (FCR), the result from the proposed aquaculture systems was similar to previous works, which reported FCR from 1.86 – 2.57 (Suresh and Lin, 1992; Ridha and Cruz, 2001; Al-Hafedh et al., 2003). Despite reporting the complete mortality in T1 and T2, the animals in T1 and T2 in were able to survive for 85 days. After this point, tilapia in these tanks was unable to eat normally as can be shown by unconsumed feed pellets, which floated on the water surface the morning after feeding had been performed. The majority of tilapia death in T1 and T2 was observed between day 86 and 89 when nitrate concentrations in these tanks were almost entirely disappeared (i.e., NO_3^--N < 20 mg N L^{-1}) and septic odors from Biocord™ biofilters were strongly detectable. The mortality rate of tilapia was determined at 23% for T4. The entire death of animals in this tank concurred with the period from day 14 – 26

when excessive nitrite concentrations at roughly 5.0 mg N L^{-1} were detectable. Tilapia growth data were additional information that pointed to the disadvantage of employing suspended growth systems for the application of closed-water aquacultures. The proposed aquaculture systems appeared more capable of producing suitable water characteristics (i.e., inorganic nitrogen concentrations and suspended solids) in comparison to suspended growth tanks. With better water quality, it was unsurprised to discover that tilapia reared in the proposed aquaculture systems was able to grow better than animals in suspended growth tanks.

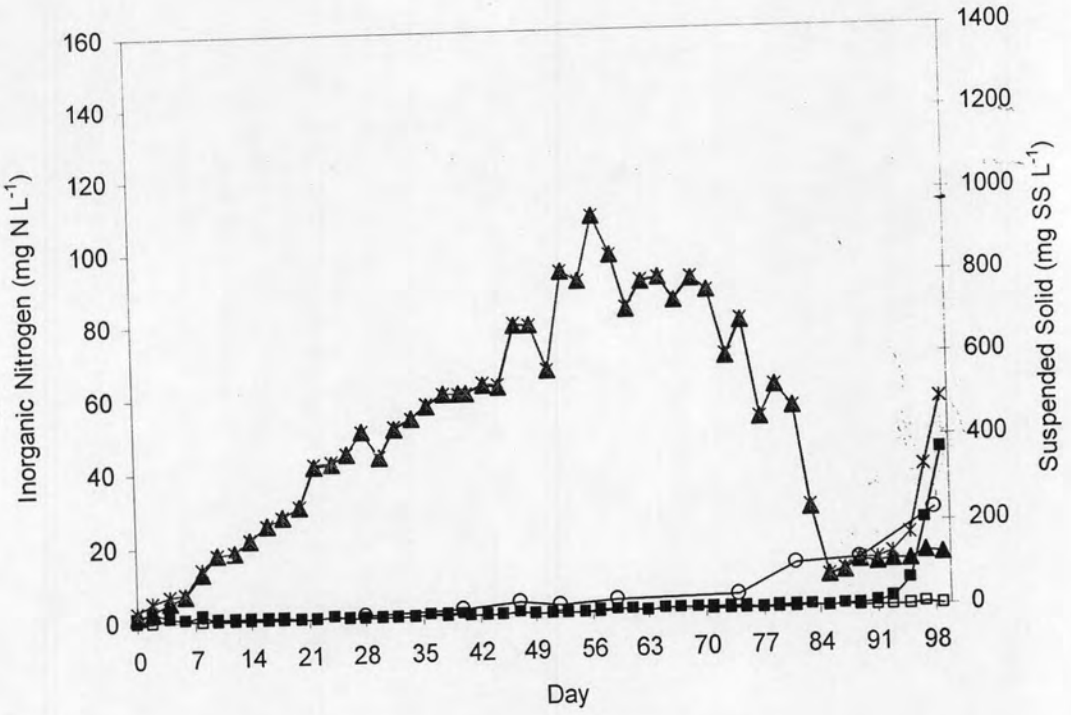

Figure 4-14 Inorganic nitrogen analysis of water from T1 and T2, which were the proposed aquaculture systems integrated with Biocord™ biofilters. T1 and T2 were initially stocked with tilapia at approximately 3.0 kg m^{-3}.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents total inorganic nitrogen (TIN) concentration, mg N L^{-1}.

(○) represents total suspended solid concentration, mg SS L^{-1}.

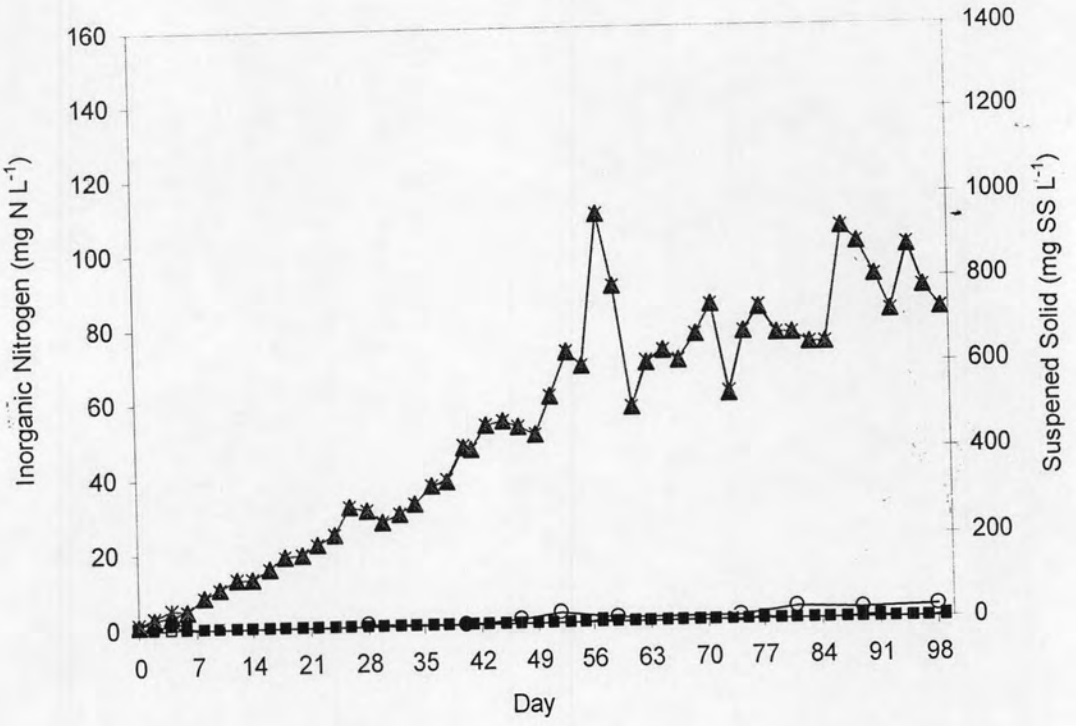

Figure 4-15 Inorganic nitrogen analysis of water from T3, which is the proposed aquaculture systems integrated with Biocord™ biofilters. T3 was initially stocked with tilapia at half the density of T1 and T2.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents total inorganic nitrogen (TIN) concentration, mg N L^{-1}.

(○) represents total suspended solid concentration, mg SS L^{-1}.

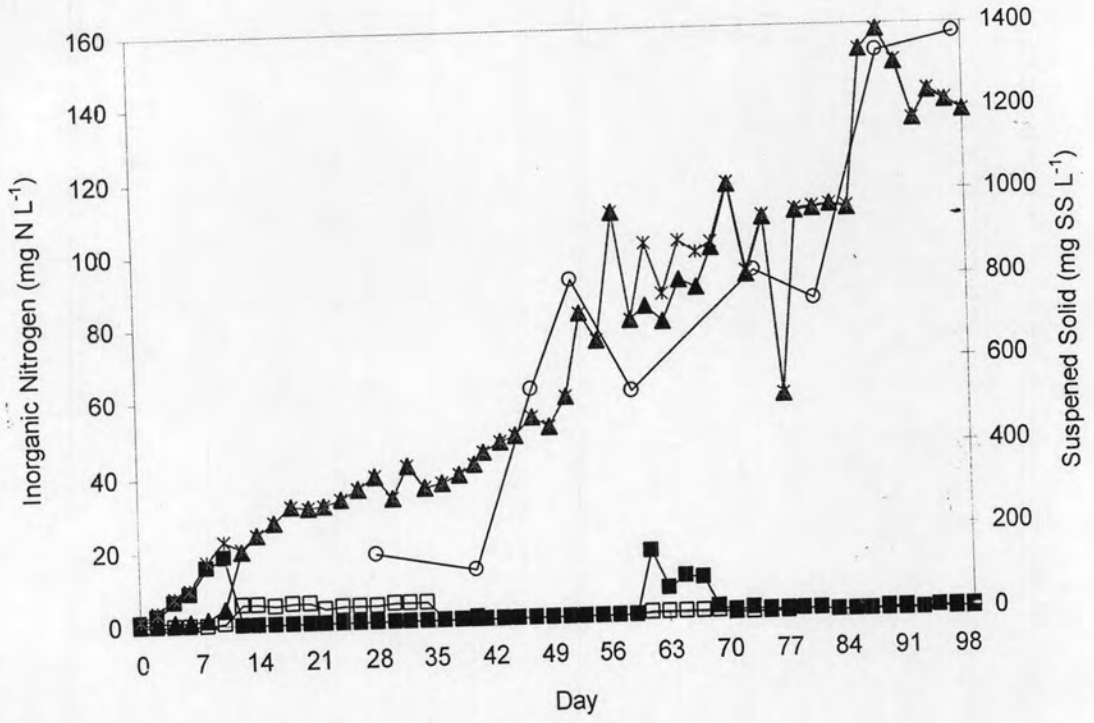

Figure 4-16 Inorganic nitrogen analysis of water from T4, which was the conventional suspended-growth system (i.e., no biofilters). T4 was initially stocked with tilapia at approximately 3.0 kg m^{-3}.

(■) represents NH_4^+-N concentration, mg N L^{-1}

(□) represents NO_2^--N concentration, mg N L^{-1}

(▲) represents NO_3^--N concentration, mg N L^{-1}

(*) represents total inorganic nitrogen (TIN) concentration, mg N L^{-1}.

(○) represents total suspended solid concentration, mg SS L^{-1}.

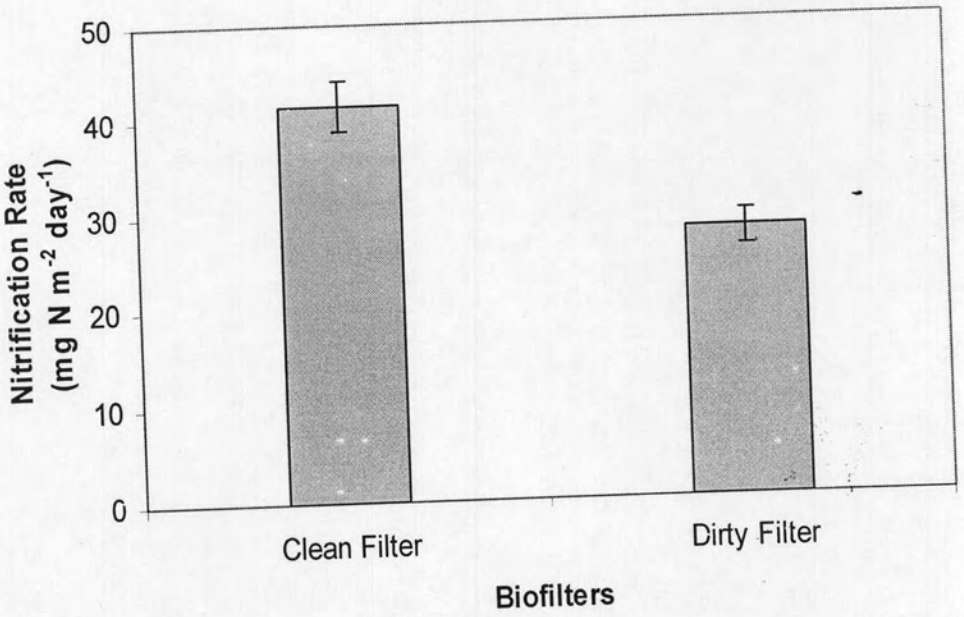

Figure 4-17 Comparison of nitrification rates between the clean and unclean (dirty) Biocord™ biofilters.

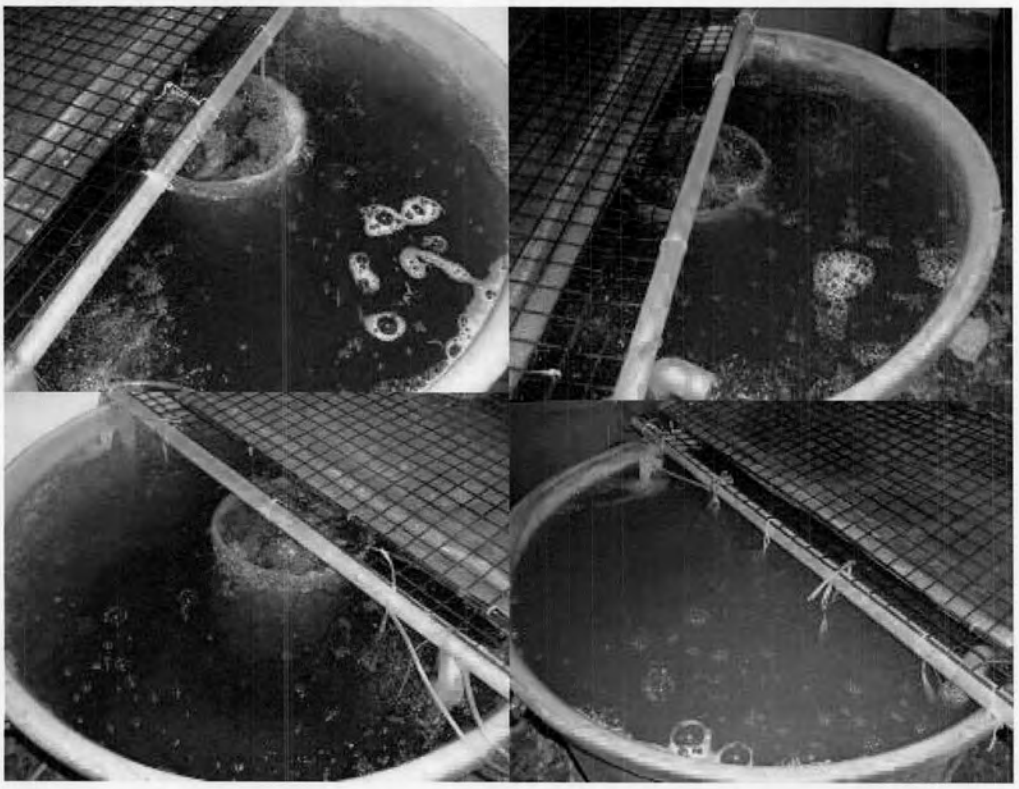

Figure 4-18 Illustration of the systems used during the zero-water exchanged tilapia cultivation at the initial stocking density of 3.0 kg m^{-3}; T1 and T2 (upper) were the proposed aquaculture systems initially stocked with tilapia at 3.0 kg m^{-3}, T3 (lower left) was the proposed aquaculture system initially stocked with tilapia at half density of T1 and T2, and T4 (lower right) was suspended growth system initially stocked with tilapia at 3.0 kg m^{-3}.

Table 4-3 Physical parameters measured from each tilapia cultivating tank. T1 and T2 were replicated experimental systems, which integrated with acclimated Biocord™ biofilters. T3 was identical to T1 and T2 with only half of initial tilapia biomass density. T4 featured no Biocord™ biofilters.

Parameters	**T1 and T2**	**T3**	**T4**
Temperature (°C)	27.7 ± 0.73	27.8 ± 0.55	27.7 ± 0.70
DO (mg L^{-1})	6.40 ± 0.26	6.29 ± 0.11	6.35 ± 0.12
Alkalinity (mg L^{-1} $CaCO_3$)	111 ± 44	95 ± 27	98 ± 36
pH	7.5 ± 0.21 (day 1 – 57) 8.2 ± 0.17 (day 58 – 90)	7.8 ± 0.27	7.3 ± 0.36

Table 4-4 Tilapia growth data from the zero-water exchanged tilapia cultivation for the initial stocking density of 3.0 kg m^{-3}.

Parameter	Average ± SD (Max - Min)			
	T1	T2	T3	T4
Average initial weight (g/fish)	58.93 ± 5.31 (40 - 70)	55.74 ± 7.17 (40 - 70)	55 ± 7.60 (40 - 70)	52.78 ± 10.03 (40 - 70)
Average initial length (cm/fish)	14.85 ± 0.63 (13.9 - 16.2)	14.72 ± 0.65 (13.3 - 15.8)	14.56 ± 0.78 (13.1 - 16.2)	14.49 ± 0.82 (13 - 16.2)
Initial density (g m^{-3})	3,535.56	3,344.44	1,711.11	3,166.67
Average final weight (g/fish)	242.00 ± 73.76* (140 - 400)	211.85 ± 66.39 (100 - 350)	253.18 ± 79.19 (100 - 340)	197.24 ± 37.42 (120 - 270)
Average final length (cm/fish)	22.85 ± 2.44* (20 - 27)	21.15 ± 2.45 (16.9 - 25.2)	22.85 ± 3.02 (17.6 - 25.3)	21.22 ± 1.54 (18 - 23.7)
Final density (g m^{-3})	14,520*	12,711.11	6,188.89	9,204.44
Average daily growth (g day^{-1})	2.63	1.58	2.00	1.46
Feed conversion ratio (FCR)	2.26	2.04	1.69	2.23
Initial TSS (mg SS L^{-1})	2.86	5.28	2.59	160.00
Final TSS (mg SS L−1)	377.42	72.29	28.00	1,375.00
Survival rate (%)				
Day 0 - 12	100	100	100	88.88
Day 13 - 43	100	100	100	74.27
Day 44 - 75	96	100	100	74.27
Day 77 - 99	0	0	78.51	74.27
Overall (Day 1 - 99)	0	0	78.51	74.27

(*) Measured on day 85 before mass tilapia mortality

4.3.3 Initial Stocking Density at 5.0 kg m^{-3}

One of the conclusions from section 4.3.2 pointed to the importance of suspended solid removal from Biocord™ biofilters as means to enhance nitrification rates and to prevent the formation of anaerobic metabolites. For these reasons, an occasional biofilter cleaning, which was repeated once every 2 weeks, was carried out simultaneously to the zero-water exchanged tilapia cultivation in the proposed aquaculture systems, and the result of this experiment was demonstrated in Figure 4-19 to 4-23 and in Table 4-5. The result of inorganic nitrogen analysis from L1, L2, D1, and D2 were comparable in the way that ammonium and nitrite concentrations can be kept at low levels throughout the experiment while continuous production of nitrate was also observable during the same period. Increasing nitrate concentration indicated the occurrence of complete nitrification in all cultivating tanks. The detailed inspection of ammonium and nitrite concentrations in each cultivating tanks revealed that they were greater than an acceptable levels of 1.0 mg N L^{-1} in many occasions. The reason for this may be linked to high initial stocking density applied in this experiment in comparison to earlier trials (i.e., initial stocking at 0.7 and 5.0 kg m^{-3}).

The result in L1 (i.e., located outdoor and with biofilter cleaning) indicated that the proposed aquaculture system was able to maintain the concentrations of ammonium and nitrite below 1.0 mg N L^{-1} for the majority of the cultivation. Increasing nitrate concentrations from negligible level to as high as 250 mg N L^{-1} were clearly the consequence of complete nitrification. In L1, suspended solid concentrations were found to increase after day 70 from about 20 – 180 mg SS L^{-1}. Detailed inspection of nitrate profile revealed that it started to decrease from the maximum level as the concentration of suspended solid rapidly surge from 60 – 180 mg SS L^{-1} in the final week of the cultivation. This observation was similar to the result described in section 4.3.2, which pointed to the onset of denitrification. For L2 (i.e., located outdoor and without biofilter cleaning), the proposed aquaculture system was able to maintain low ammonium and nitrite concentrations despite some occasional failures especially after day 65. The concentrations of ammonium and nitrite were found to increase sharply to 5.0 mg N L^{-1}. On this day, it also appeared that Biocord™ biofilters lose the ability to retain suspended solids as can be shown by a rapid increase of suspended solid concentrations from 130 mg SS L^{-1} on day 65 to as high as 650 mg SS L^{-1} on the final day of the experiment. After day 65, nitrate concentrations continued to increase from about 125 – 200 mg N L^{-1}.

The complete nitrification observed after day 65 was unlikely to be accountable solely by Biocord™ biofilters, but also by the ability of formed suspended solid (i.e., bioflocs) to carry out nitrification. It was quite interesting to point out that L1 and L2 received full sunlight only from 8:00 – 10:00 am, and that was confirmed by a presence of phytoplankton at minimum level (i.e., $< 10^4$ cell mL^{-1}).

The result of water analysis for D1 (i.e., located in the dark with biofilter cleaning) indicated that the proposed aquaculture system was able to control ammonium and nitrite concentrations within safety levels. A steady increasing of nitrate contrations (i.e., 1 – 225 mg N L^{-1}) signaled an occurrence of nitrification. In this case, cleaning biofilters at once every 2 weeks was able to sustain nitrification throughout the experimental period of 99 days. This was opposed to the result in the previous section (i.e., section 4.3.2), which indicated the occurrence of heterotrophic denitrification. In this section, apparent decline of nitrate concentrations were not observed, but this result did not imply that heterotrophic denitrification was absence. The author strongly believed that an excellent ability of Biocord™ biofilters in retaining solid was a crucial factor facilitating the formation of anaerobic pockets required for denitrifying bacteria. Therefore, the future work must also focus on the determination of released gas from the proposed aquaculture system. For D2 (i.e., located indoor without biofilter cleaning), the profiles of inorganic nitrogen were similar to those found in L2 (located out without biofilter cleaning). Ammonium and nitrite concentrations appeared to fluctuate from 0.5 – 3 mg N L^{-1}. As expected, nitrate concentration increased from significance to reach the maximum measured on day 70 at 225 mg N L^{-1} before starting to diminish. Suspended solid concentrations were found to rapidly surge from 5 – 420 mg SS L^{-1} during the declination of nitrate. From the experimental result obtained, it was clear that biofilter cleaning was able to prolonge an activeness of Biocord™ biofilters to nitrify and separate solids from liquid. These features were crucial for the sucesss of the proposed aquaculture system.

Table 4-5 illustrates tilapia growth data. The survival rate of tilapia in each cultivating tank was high, ranging from 90 – 98%. The highest mortality rate (≈ 10%) was noticed in L2, which was located outdoor and without biofilter cleaning. Tilapia mortality in this tank was observed from day 62 – 70 when ammonium and nitrite concentrations were measurable at high concentrations about 5.0 mg N L^{-1}. Other causes of tilapia mortality may be related tilapia biting with each other. From Table 4-5, the maximum average daily growth (ADG) was calculated at 2.10 g day^{-1}, which was

comparable to the values reported in earlier section. The feed conversion ratio (FCR) was determined to range from 1.35 – 1.60, which was considered noncompetitive in comparison to earthen ponds or biofloc technology ponds in which natural food source was available. The readers should keep in mind that this experiment was performed to test the ability of the proposed aquaculture systems in sustaining the closed-water cultivation only. No optimization such as feeding, feed nutrition, vaccination, and improved tilapia strain, was applied during the experiment. Despite inferior growth performance, the most impressive feature that emerged from this section was high tilapia production, which was able to reach the level as high as 30 kg m^{-3}. Clearly, the rate of tilapia production in this experiment was almost 30 folds higher than that from land-based aquacultures in Thailand, and moreover, it was comparable to caged cultivation or even to the biofloc productions, which reported harvesting density around 30 – 50 kg m^{-3} (Azim et al., 2008).

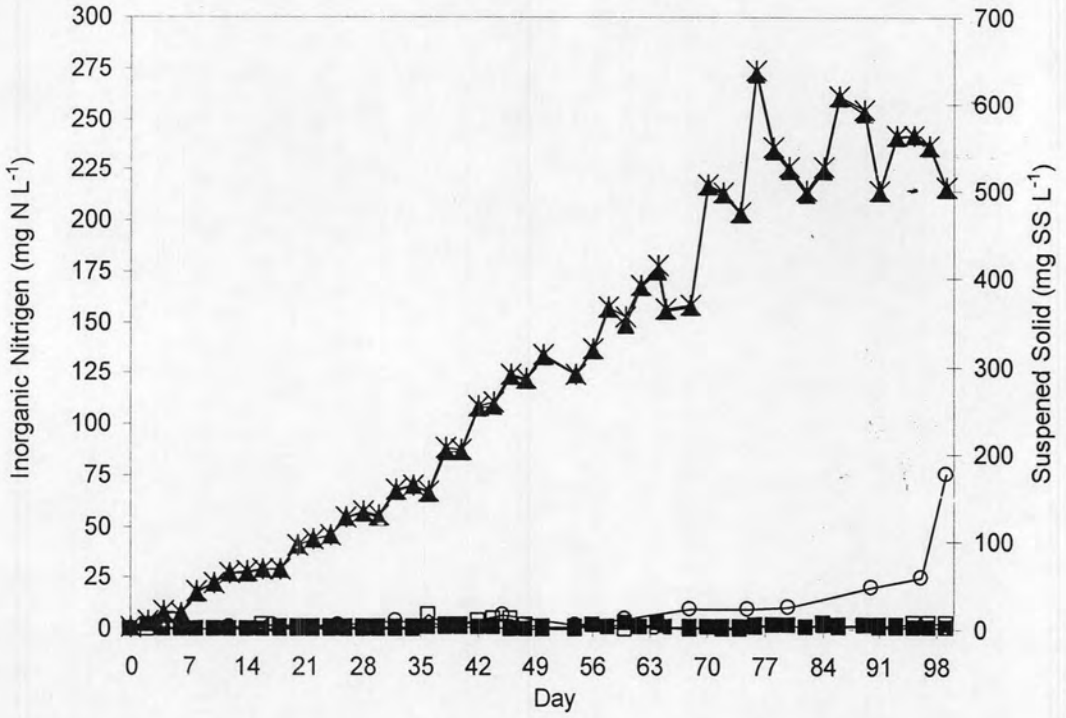

Figure 4-19 Inorganic nitrogen profiles in L1, which was the proposed aquaculture system located outdoor and subjected to biofilter cleaning every 2 weeks.

(■) represents NH_4^+-N concentration, mg N L^{-1}.

(□) represents NO_2^--N concentration, mg N L^{-1}.

(▲) represents NO_3^--N concentration, mg N L^{-1}.

(*) represents inorganic nitrogen (TIN) concentration, mg N L^{-1}.

(○) represents total suspended solid concentration, mg SS L^{-1}.

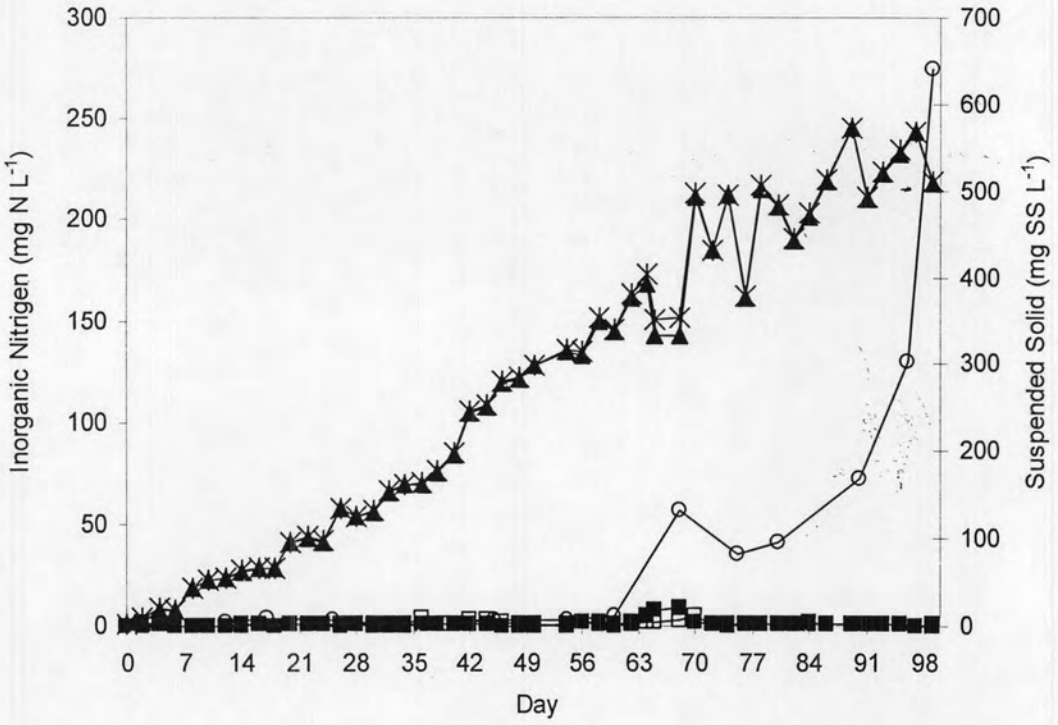

Figure 4-20 Inorganic nitrogen profiles in L2, which was the proposed aquaculture system located indoor and subjected to no biofilter cleaning.

(■) represents NH_4^+-N concentration, mg N L^{-1}.

(□) represents NO_2^--N concentration, mg N L^{-1}.

(▲) represents NO_3^--N concentration, mg N L^{-1}.

(*) represents inorganic nitrogen (TIN) concentration, mg N L^{-1}.

(○) represents total suspended solid concentration, mg SS L^{-1}.

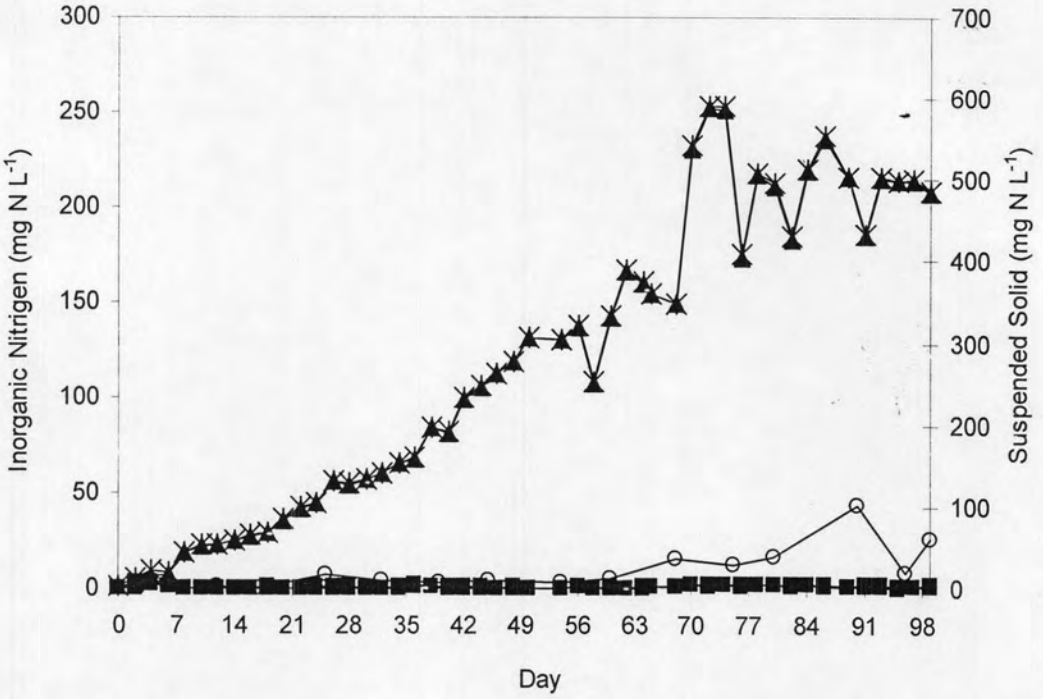

Figure 4-21 Inorganic nitrogen profiles in D1, which was the proposed aquaculture system located indoor and subjected to biofilter cleaning every 2 weeks.
(■) represents NH_4^+-N concentration, mg N L^{-1}.

(□) represents NO_2^--N concentration, mg N L^{-1}.

(▲) represents NO_3^--N concentration, mg N L^{-1}.

(*) represents inorganic nitrogen (TIN) concentration, mg N L^{-1}.

(○) represents total suspended solid concentration, mg SS L^{-1}.

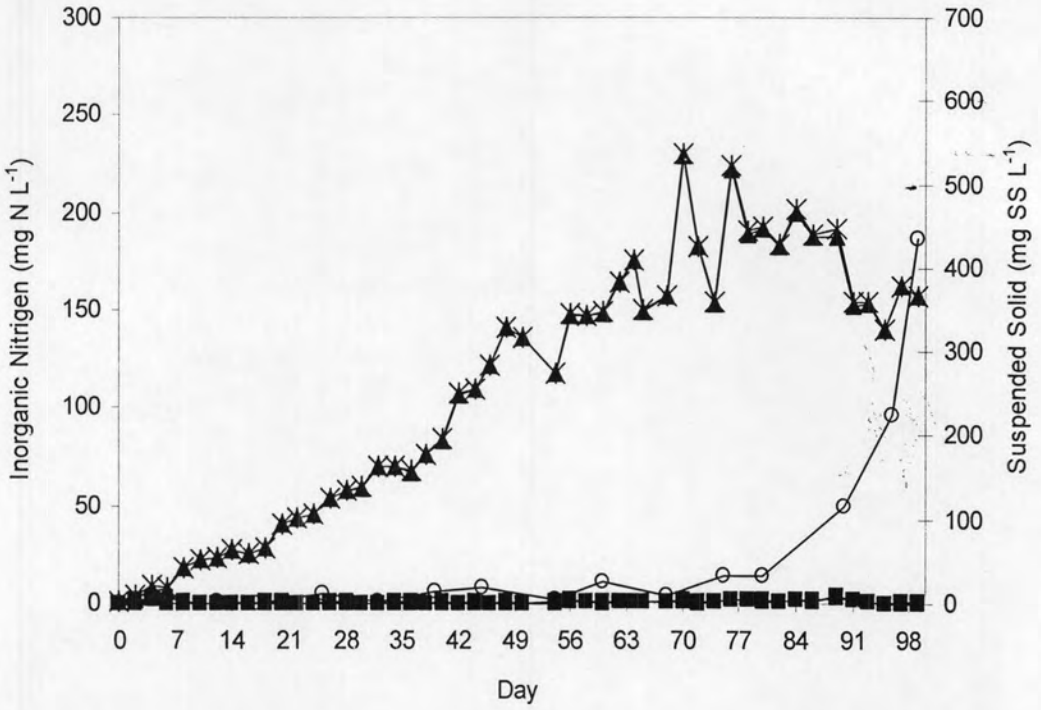

Figure 4-22 Inorganic nitrogen profiles in D2, which was the proposed aquaculture system located indoor and subjected to no biofilter cleaning.

(■) represents NH_4^+-N concentration, mg N L^{-1}.

(□) represents NO_2^--N concentration, mg N L^{-1}.

(▲) represents NO_3^--N concentration, mg N L^{-1}.

(*) represents inorganic nitrogen (TIN) concentration, mg N L^{-1}.

(○) represents total suspended solid concentration, mg SS L^{-1}.

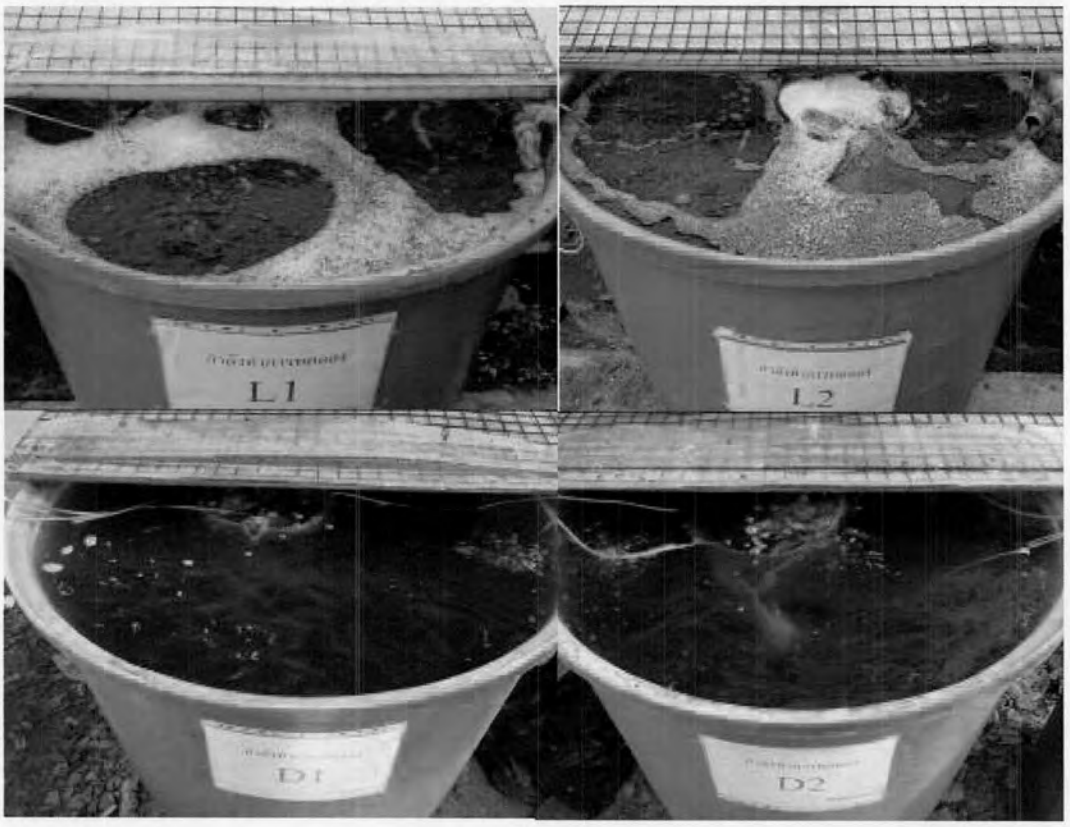

Figure 4-23 Proposed aquaculture systems used during the zero-water exchange tilapia cultivation for initial stocking of 5.0 kg m^{-3}. L1 (upper left), the proposed aquaculture system located outdoor and subjected to biofilter cleaning; L2 (upper right), the proposed aquaculture system located outdoor with no biofilter cleaning; D1 (lower left), the proposed aquaculture system located in the dark and subjected to biofilter cleaning; D2 (lower right), the proposed aquaculture system located in the dark with no biofilter cleaning.

Table 4-5 Tilapia growth data from the zero-water exchanged tilapia cultivation for the initial stocking density of 5.0 kg m^{-3}.

Parameter	Average ± SD (Max - Min)			
	L1	**L2**	**D1**	**D2**
Average initial weight (g/fish)	34.37 ± 5.27 (25.0 - 50.8)	31.77 ± 5.72 (16.20 - 50.7)	33.12 ± 5.18 (21.9 - 51.2)	33.28 ± 4.95 (24.4 - 45.8)
Average initial length (cm/fish)	12.40 ± 0.64(11.0 - 13.9)	12.53 ± 0.61(11.2 - 14.5)	12.27 ± 0.64 (10.5 - 14.0)	12.40 ± 0.61(10.9 - 14.2)
Initial density (g m^{-3})	5,041.33	5,012.66	5,005.11	5,028.22
Average final weight (g/fish)	212.68 ± 68.68 (91.20 - 385.6)	177.16 ± 58.55(86.9 - 325.0)	167.73 ± 66.66 (86.9 - 355.2)	167.15 ± 48.27 (93.4 - 278.4)
Average final length (cm/fish)	21.41 ± 2.23 (16.7 - 26.0)	20.18 ± 2.29 (15.7 - 24.5)	19.75 ± 2.33 (16.2 - 25.7)	19.81 ± 1.84 (16.7 23.6)
Final density (g m^{-3})	30,248.00	25,196.66	24,972.44	24,515.33
Survival rate (%)	96.96	90.14	98.52	97.05
Average daily growth (g day^{-1})				
Day 0 - 34	1.59	1.25	0.99	0.68
Day 35 - 71	2.52	1.74	2.56	2.39
Day 72 - 99	1.81	1.27	1.11	1.08
Overall (Day 1 - 99)	2.10	1.75	1.66	1.65
Feed conversion ratio (FCR)				
Day 0 - 34	1.08	1.14	1.25	1.75
Day 35 - 71	1.04	1.15	1.12	1.09
Day 72 - 99	1.37	1.33	1.57	1.52
Overall (Day 1 - 99)	1.57	1.48	1.37	1.38

4.4 Proposed Aquaculture Systems

The special feature of the proposed aquaculture system was the integration of growing aquatic stocks, inorganic nitrogen treatment, and solid-liquid separation in a single tank. The main advantage of this particular design was a simplification of process scheme by eliminating external aerated biofilters and solid separating devices, all of which are typically located outside production tanks. The proposed aquaculture systems should be attractive for local Thai farmers with limited-budget because the system performance greatly exceeded the current harvesting density (i.e., $\approx 1.0 - 2.5$ kg m^{-3}) of land-based aquacultures in Thailand (Kittisak Harsup, personal communication). With better management strategies such as periodic solid removal, optimized feeding, and disease control, the proposed systems should be able to replace the caged production, which is known to release nitrogen waste directly into natural water resources and often suffered from variation in water quality. Simplicity of the proposed aquaculture systems was another feature attributed for the system attractiveness. The proposed system consisted of two main units, Biocord^{TM} biofilters and aeration device. Biocord^{TM} biofilters were responsible for inorganic nitrogen treatment as well as solid-liquid separation, while aeration devices provided sufficient oxygenation for both aquacultures and nitrifying bacteria. In contrast, the designs from other works usually segregated aquaculture production from water treatment units, which may consist of many sophisticated equipments namely nitrifying biofilters, aeration devices, solid separators, disinfection units, protein skimmers, CO_2 strippers and denitrifying biofilters (Delos Reyes and Lawson 1996; Jegatheesan et al., 2007; Park et al., 2008; Kim et al., 2008; Tal et al., 2009). This complexity led to costly investment and the need for high skill operators, all of which was often lacked for local Thai farmers. In addition, the fibrous Biocord^{TM} biofilters were available in the form of rope so that they are relatively easy to be applied in different situations. Based on the author experience, another advantage of the selected biofilters was the ease of removing suspended solids from 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 exisiting systems such as microbead filters and pack-bed filters, which required intensive energy for backwash (Steicke et al., 2007). Finally, an elevated nitrate concentration in the effluent of the proposed aquaculture systems was an important issue that must be considered. As mentioned in Sesuk et al. (2009), nitrate accumulation can be overcome by retaining

nitrate-rich effluent in segregated earthen ponds or denitrifying bioreactors before recirculating treated water back to production.

4.5 Nitrogen Balance

The nitrogen balance was performed for the initial stocking density of 5.0 kg m^{-3}, and the result was displayed in Table 4-6. For each cultivating tank, the majority of nitrogen input (≈ 95 – 96%) was from tilapia feed while the rest of nitrogen can be founded in water column and initial tilapia stocking. At the end of the cultivation, the nitrogen input was redistributed and can be found in tilapia, water column, and unaccountable portion. Nitrogen in water column, entirely inorganic nitrogen, was the product of ammonification and nitrification. Their distribution was determined from 13 – 18% of total nitrogen input for all cultivating tanks. It was generally known that aquacultures were able to utilize approximately 20 – 30% of available proteins in feed (Avnimelech and Ritvo, 2003). Thus, a portion of feed nitrogen must be found in tilapia biomass. From the calculation, nitrogen in tilapia biomass ranged from 20 – 25% of total nitrogen input. It was necessary to point out that significant portion (≈ 60%) of nitrogen input was unaccountable. Unidentified nitrogen was likely to be in the forms of suspended solid trapped on biofilter surface, suspended solid deposited on the tank floors, suspended solid removed by biofilter cleaning, and the product of denitrification (i.e., N_2). Nitrogen uptake by phytoplankton should be insignificant in this experiment since extremely small amount of microalgae (< 10^4 cell mL^{-1}) was observed as cultivating tanks (L1 and L2) only received full sunglight early in the morning. Finally, it was interesting to note that the differences between unaccountable nitrogen from tank subjected to biofilter cleaning (i.e., L1 and D1) and those without biofilter cleaning (L2 and D2) were trivial. From this observation, biofilter cleaning did not serve as means to remove excess suspended solids from aquaculture tanks but only required to maintain optimal condition for nitrification.

Table 4-6 Nitrogen balance during the zero-water exchange tilapia cultivation for the initial stocking density of 5.0 kg m^{-3}.

	L1			
	Nitrogen (g $tank^{-1}$)		Total nitrogen (g $tank^{-1}$)	Total nitrogen (%)
Input	Water	0.42	780.89	100
	Feed	750.91		
	Tilapia	29.56		
Output	Water	117.63	295.02	15.06
	Tilapia	177.39		22.72
	Others		485.87	62.22

	L2			
	Nitrogen (g $tank^{-1}$)		Total nitrogen (g $tank^{-1}$)	Total nitrogen (%)
Input	Water	0.37	613.55	100
	Feed	583.78		
	Tilapia	29.4		
Output	Water	111.42	259.18	18.15
	Tilapia	147.76		24.08
	Others		354.37	57.77

	D1			
	Nitrogen (g $tank^{-1}$)		Total nitrogen (g $tank^{-1}$)	Total nitrogen (%)
Input	Water	0.043	708.493	100
	Feed	679.1		
	Tilapia	29.35		
Output	Water	107.35	253.8	15.15
	Tilapia	146.45		20.66
	Others		454.693	64.19

	D2			
	Nitrogen (g $tank^{-1}$)		Total nitrogen (g $tank^{-1}$)	Total nitrogen (%)
Input	Water	0.043	656.133	100
	Feed	626.6		
	Tilapia	29.49		
Output	Water	85.11	226.19	13.05
	Tilapia	141.08		21.68
	Others		429.943	65.27