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Original Article

Performance evaluation of the compact aquaculture system integrating submerged fibrous nitrifying biofilters

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Abstract

This experiment assessed the performance of submerged fibrous nitrifying biofilters (SFNBs) to carry out the zero-water exchange tilapia cultivation. Without biofilter cleaning, the SFNBs (21 m of biofilter length) were susceptible to hydrogen sulfide production when operating beyond the aquaculture density of 13.62 kg/m^3. The SFNBs were able to maintain total ammonia nitrogen (TAN) and nitrite below 1.0 mg N/L throughout the experiment and could handle inorganic nitrogen loading as high as 38.6 mg N/L/day when the solid removal from biofilters was performed biweekly. Ammonium degradation rate measured at the end of this study was 380±66 mg N/m^2/day for biofilters subjected to cleaning. A significantly lower rate of 41.4±2.86 mg N/m^2/day was associated with biofilters without any solid removal. Finally, the SFNBs should be attractive for budget-limited farmers since they are simple to build and operate and can provide alternatives to cage-cultured systems.

Keywords: biofilters, nitrification, nitrogen, tilapia, wastewater

1. Introduction

Closed-water recirculating systems are perceived as the solution for sustainable aquacultures (Crab *et al.*, 2007). Conventional closed-water recirculating systems utilizing nitrifying biofilters have been used successfully in many configurations including trickling filters, rotating biological contactors and fluidized sand filters (Kamstra *et al.*, 1998; Brazil, 2006; Summerfelt, 2006; Timmons *et al.*, 2006). The majority of these recirculating systems uses expensive solid-liquid separators to enhance the system performance. In spite of the success in many aquacultural applications, nitrifying biofilters and suspended solid separators remain costly to operate due to (1) the requirement to circulate water through aerated biofilters located outside production ponds (2) the intensive energy used and (3) the need for high-skilled labor for operation and maintenance. In a previous study, a proposed aquaculture system (Figure 1), referred to herein as the submerged fibrous nitrifying biofilters (SFNBs), was conceptualized and tested for its performance (Sesuk *et al.*, 2009). The design of SFNBs was intended to be simple and easily adoptable among budget-limited Thai farmers. The preliminary evaluation showed that the SFNB was able to control TAN and nitrite effectively (i.e., TAN and NO_2-N < 1.0 mg N/L) and was also capable of separating suspended solids (SS) from water, thereby producing a clear effluent

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containing less than 20 mg SS/L. Setbacks of the preliminary study were a short cultivating period of 44 days and relatively low aquaculture densities between 0.68 and 2.60 kg/m^3. These fish densities corresponded to inorganic nitrogen loadings between 1.24 and 2.78 mg N/L/day. Therefore, the objectives of the present study were to assess the performance of the SFNBs over a longer cultivating period and under higher inorganic nitrogen loadings. Specifically, the system ability to sustain acceptable levels of ammonia, nitrite, nitrate and suspended solid was assessed. Additional information about ammonium degradation rate, suspended solid retention by biofilters and tilapia growth is also presented.

2. Materials and Methods

2.1 Experimental system

Fibrous Biocord^{TM} biofilters (polypropylene; 2.8 m^2/m or 82.35 m^2/kg biofilter) were chosen to immobilize mixed nitrifying bacteria. Acclimation of biofilters was performed by a weekly addition of 25 g of 35% shrimp diets into the acclimating tank (working volume 800 L) according to the procedure described in Sesuk *et al.* (2009). Operating conditions of the acclimating tank were maintained within the optimal range for nitrification (i.e., pH = 7–8, DO > 2.0 mg/L, alkalinity = 100–150 mg/L $CaCO_3$) by a constant aeration and periodically addition of $NaHCO_3$. In this experiment, circular plastic tanks (500 L working volume) were used to accommodate acclimated nitrifying biofilters and tilapia (Figure 1). Acclimated biofilters (10 pieces; 70 cm per piece) were completely submerged below the water surface within a hollow cylindrical plastic net (inner diameter 30 cm; outer diameter 30.6 cm; height 90 cm), which was entirely wrapped by a thin plastic sheet except for the top and bottom. Biofilters inside the plastic net were connected to a metal frame lying on the tank floor to ensure that the biofilters were able to align vertically. A total of three plastic nets was used for each tank to provide the total biofilter length of 21 m. Two diffusive stone aerators were installed inside each biofilter net to provide constant aeration for nitrifying bacteria and to maintain water circulation in the tank.

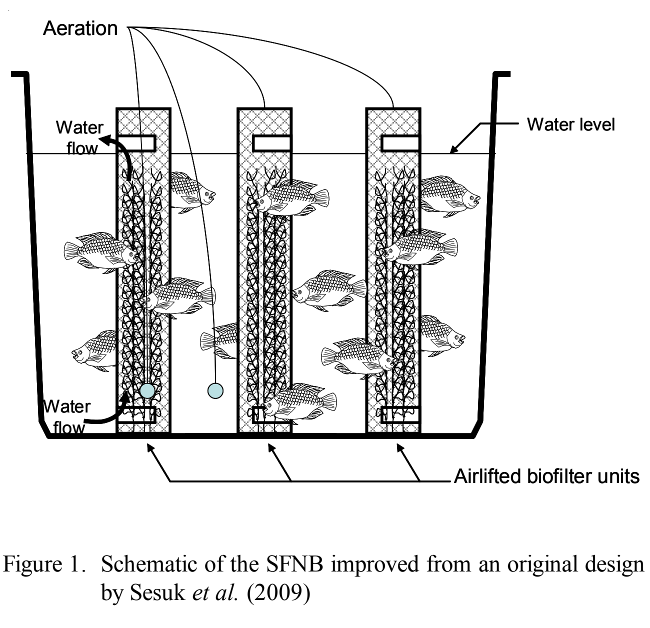

Figure 1. Schematic of the SFNB improved from an original design by Sesuk *et al.* (2009)

2.2 Zero-water exchanged tilapia growout

The zero-water exchanged tilapia cultivation was conducted for 90 days in the controls (3 replications) and the treatments (3 replications), all of which were fabricated following the design of SFNB as shown in Figure 1. Nile tilapia (*Oreochromis niloticus*) with an average weight of 57.3±5.83 g were stocked in the controls to attain an initial density of about 3.0 kg/m^3. No removal of suspended solids from biofilters was performed for the controls. Similarly, Nile tilapia with an average weight of 50.3±4.23 g were stocked in the treatments to attain higher density of about 5.0 kg/m^3. Unlike the controls, biofilters in the treatments were cleaned by scratching and rinsing with tap water to remove trapped suspended solids once every 14 days. Tilapia in the controls and treatments were fed twice daily with 30% protein commercial feed at 3% of the total fish weight per day. Approximately 30% of the tilapia population was sampled and measured for its weight and length about every 3 weeks. Constant aeration by diffusive stone aerators and $NaHCO_3$ addition were carried out to maintain proper conditions for nitrifying bacteria and tilapia (i.e., well-mixed, DO > 4.0 mg/L, pH = 7–8 and alkalinity = 100–150 mg/L $CaCO_3$). Cultivating tanks were located outdoors under heavy shade and each covered with a plastic lid to prevent the entry of rainwater and sunlight. Water samples from the controls and treatments were obtained at least 3 to 4 times a week and immediately analyzed for total ammonia nitrogen (TAN), NO_2-N, NO_3-N and suspended solid (SS) according to APHA (1998).

2.3 Solid retention by biofilters

A significant suspended solid retention on Biocord^{TM} biofilters was observed during the tilapia cultivation. Therefore, an independent experiment was conducted in parallel to the cultivation to demonstrate the ability of biofilters to retain suspended solids. The experiment was performed in 6 L glass bottles, which were filled with turbid water containing approximately 800 mg SS/L to achieve a working volume of 5 L. Three pieces ($\approx$ 10 cm each) of new Biocord^{TM} biofilters and three pieces ($\approx$ 10 cm each) of acclimated Biocord^{TM} biofilters randomly obtained from the controls and treatments were submerged under the water surface in the glass bottles. Aeration by a diffusive stone aerator was provided to create water circulation in each glass bottle. Water turbidity was regularly monitored at predetermined intervals by measuring optical density (OD) at 660 nm.

2.4 Determination of ammonium degradation rate

Six pieces of Biocord^{TM} biofilters (10 cm each) were randomly sampling from the controls and treatments after the conclusion of tilapia cultivation. These biofilters were subjected to batch experiments to determine the rate of ammonium degradation. The batch experiment was set up in 6 L glass bottle (3 replications for controls and 3 replications for treatments). Each glass bottle was equipped with a diffusive stone aerator to provide a thoroughly mixed condition and DO greater than 4.0 mg/L. Addition of NH_4Cl (i.e., 0.086 g) was carried out to achieve an initial ammonium concentration at 5.0 mg N/L. Alkalinity and pH of liquid in glass bottles were maintained between 100 and 150 mg/L $CaCO_3$ and between 7 and 8, respectively. Approximately 9 mL of water from each bottle was collected at predetermined intervals and immediately analyzed for the TAN, NO_2-N and NO_3-N according to APHA (1998).

3. Results and Discussion

3.1 Suspended solids

The source of suspended solids during the zero-water exchange tilapia cultivation was from the unconsumed feeds, the tilapia feces, and the formation of suspended bioflocs as a result of microbial growth. Suspended solids in the controls and treatments increased steadily from 5 to 116 mg N/L and from 4 to 110 mg N/L, respectively (Figure 2). Clearly, suspended solid profiles from both systems were comparable to suggest that the biofilter cleaning had insignificant effect on the ability of these particular biofilters to separate suspended solids from water. However, it was interesting to note that substantial quantity of suspended solids was retained on the surface of nitrifying biofilters especially in the controls. Rigorous shaking, scratching and rinsing of biofilters from the controls released significant amount of trapped suspended solids into water. This observation led to an independent experiment, as described in section 2.3, to assess the ability of Biocord^{TM} biofilters in retaining suspended solids. The result from an independent experiment confirmed the effectiveness of the chosen biofilters in solid-liquid separation (Figure 3). It appeared that gravitational sedimentation did not contribute as much to the solid removal because much as 75% (≈ 600 mg SS/L) of initial suspended solids remained in water after 10 days. Enhanced solid removal efficiency up to 99% was accomplished after the 10 cm Biocord^{TM} biofilters were integrated into the experiment. Another interesting observation from an independent experiment was the rate at which suspended solids were removed from water by acclimated and non-acclimated biofilters. For instance, acclimated biofilters required about 10 hours to separate 85% of suspended solids from water, whereas the unused biofilters without attached bacteria needed as long as 3 days to achieve the same efficiency. Enhanced solid removal by acclimated biofilters may be linked to biomass adsorption onto the charged biofilm formed on the surface (Shieh and Keenan, 1987).

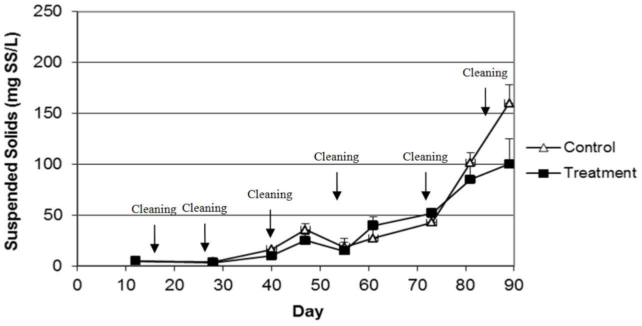

Figure 2. Suspended solid concentrations in the controls and treatments during the zero-water exchange tilapia growout. Arrows indicate biofilter cleaning.

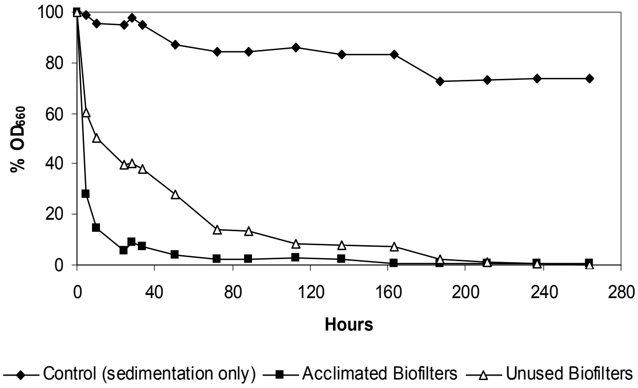

Figure 3. The ability of Biocord^{TM} biofilters in retaining suspended solids measured in terms of light absorption at 660 nm. The initial suspended solid concentration (i.e., time = 0 hour) was at 800 mg SS/L.

3.2 Inorganic nitrogen compounds

The sources of inorganic nitrogen compounds in this experiment were entirely from feeds and tilapia excretion. The general water parameters during the experiment were within the optimal range (i.e., temperature from 25.29 to 28.33°C; pH from 7.32 to 8.36; alkalinity from 74 to 148 mg/L $CaCO_3$; DO > 4 mg/L) for nitrifying bacteria and tilapia (Hagopian and Riley, 1998; Timmons *et al.*, 2002). Figure 4 illustrates inorganic nitrogen profiles during the tilapia growout. For the controls, the SFNBs integrated with 21 m of biofilters were effective in maintaining TAN and nitrite with their average concentrations measured at 0.24±0.09 and 0.26±0.07 mg N/L, respectively. Acceptable levels of harmful inorganic nitrogen compounds in the controls were partly linked to a complete nitrification that had been established on biofilters even though inorganic nitrogen loading of the controls increased more than 3-fold from 4.95 to 19.61 mg N/L/day. Proper biofilter preparation to achieve the complete nitrification before an initial deployment was another factor that contributed to the system performance. With fully acclimated biofilters, the SFNBs were able to convert TAN and nitrite into nitrate almost immediately, thereby avoiding excessive inorganic nitrogen buildups during the startup period (i.e., day 1 to 30). An initial evaluation of the SFNBs by Sesuk *et al.* (2009) indicated that using non-acclimated biofilters can produce elevated nitrite accumulation at dangerous levels (i.e., NO_2-N > 10 mg N/L) for 3 weeks.

Other biological processes, which were capable of inorganic nitrogen treatment, were also probable in the controls. Substantial production of suspended solids was partly linked to suspended microbial activity, which assimilated the dissolved nitrogenous and carbonaceous matters directly into cells for new biomass synthesis. Inorganic nitrogen control based on the direct assimilation was similar to the concept of biofloc technology (Avnimelech, 2006). At the moment, the extent of direct assimilation remained unknown because it was difficult to distinguish new biomass from unconsumed feeds and tilapia feces. Heterotrophic denitrification was responsible for the nitrate utilization observed after day 57, and during this period nitrate decreased from 109 to 18 mg N/L while TAN and nitrite were still less than 1.0 mg N/L (Figure 4). The effectiveness of Biocord^{TM} biofilters in retaining suspended solids was a crucial factor that facilitated the onset of denitrification. Trapped suspended solids provided electrons for denitrifying bacteria and the solid accumulation on biofilter surface created anaerobic pockets required for denitrification. The occurrence of simultaneous nitrification and denitrification is currently undesirable in the SFNBs despite the fact that harmful inorganic nitrogen compounds can be completely transformed into nitrogen gas and the equipment sizing and operating cost were reduced (Munch *et al.*, 1996). The reasons for the difficulty were the inability to consistently predict the occurrence of denitrification and to maintain a stable denitrifying rate without employing sophisticated controlling equipment. Denitrification was likely to continue in the controls as long as nitrate was available in sufficient quantity (i.e., NO_3-N > 20 mg N/L). Sulfate reduction might develop to produce hydrogen sulfide after nitrate is exhausted. Strong septic odors from biofilters as well as blackish water color were observed between day 87 and 90, supporting the hypothesis of hydrogen sulfide production in the cultivating tank (Tchobanoglous *et al.*, 1991).

For the treatments, the SFNBs were subjected to a biweekly biofilter cleaning. The average TAN and nitrite concentrations were measured at 0.57±0.22 and 0.70±0.24 mg N/L, respectively, a statistically significant difference ($P < 0.05$) from those of the controls (Figure 4). Detailed examination of inorganic nitrogen profiles found that TAN and nitrite concentrations in the treatment appeared to increase gradually as tilapia cultivation progressed but sharply declined to be within safety threshold of 1.0 mg N/L after biofilter cleaning had been performed to remove trapped suspended solids. Unlike the controls, nitrate in the treatments increased from insignificance to reach high level measured at 234 mg N/L on day 90 (Figure 4). Slight septic odors of hydrogen sulfide were also detectable during biofilter cleaning performed on day 70 and 84. Clearly, the described result points to the importance of periodic biofilter cleaning as it reduces the solid accumulation and allows the complete nitrification to continue despite increasing the applied inorganic nitrogen loading from 7.23 to 38.6 mg N/L/day. Detection of slight septic odors during biofilter cleaning further implies that unwanted anaerobic reaction (i.e., sulfate reduction) was already established around the inner region of biofilm where oxygen and nitrate transports were limited. Without any

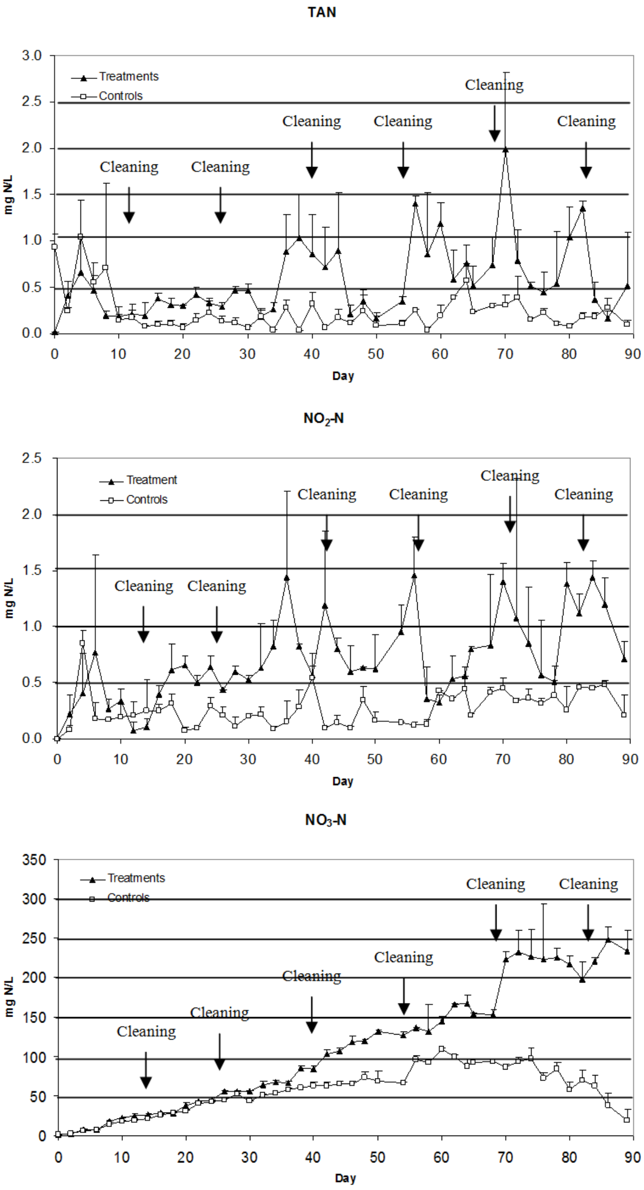

Figure 4. Inorganic nitrogen profiles in the controls and treatments during the closed-water tilapia growout. Arrows indicate biofilter cleaning.

biofilter cleaning, more suspended solids were likely to accumulate on biofilter surface causing more hydrogen sulfide odors and an unhealthy environment for tilapia cultivation.

At the end of tilapia cultivation, another independent experiment was carried out to determine ammonium degradation rates of biofilters. Biofilters from the controls exhibited ammonium degradation rate at 41.4±2.68 mg N/m^2/day, a low value compared to other recirculating systems, which have reported areal nitrification rates in the range from 250 to 640 mg N/m^2/day (Delos Reyes and Lawson, 1996; Brazil, 2006; Timmons *et al.*, 2006; Tal *et al.*, 2009). In contrast, cleaned biofilters demonstrated a remarkable increase of ammonium degradation rate, which was measured at 380±66 mg N/m^2/day. This result suggested the higher nitrifying efficiency in cleaned biofilters and reconfirmed the importance of periodic biofilter cleaning as means to maintain suitable nitrifying conditions. With less solids on the biofilter surface, heterotrophic bacteria might have insufficient carbon source to sustain their growth and that presented the opportunity for nitrifying bacteria residing in the same biofilm to be more competitive in acquiring oxygen for their metabolisms.

3.3 Tilapia growth

Table 1 displays tilapia growth data from the controls and treatments. The final tilapia biomass obtained in this experiment varied from 12.35 to 14.89 kg/m^3 and from 24.97 to 30.35 kg/m^3 for the controls and treatments, respectively. These biomass yields were apparently greater than harvesting densities of land-based aquacultures in Thailand that ranged from 0.5 to 2.5 kg/m^3 (Triyarat, 2003). The average daily growth rates (ADG) of tilapia were determined in the range from 1.75 to 2.17 g/day and from 2.04 to 2.66 g/day for the controls and treatments, respectively. Higher growth performance observed in the treatments was likely the result of better water quality. The daily growth rates reported in this study were slightly higher than the results of Ridha and Cruz (2001) and Al-Hafedh et al. (2003) that indicated the ADG in the range from 1.2 to 2.3 g/day. In contrast, the ADG of this study remained significantly lower than in other recirculating systems, which obtained ADG in the range from 3.07 to 3.6 g/day (Little *et al.*, 2009). It is also important to point out that periodic biofilter cleaning performed in the treatments was able to sustain high tilapia survival rate as high as 97%, whereas an entire death of tilapia occurred in the controls between day 87 and 90 when strong septic odors of hydrogen sulfide were clearly detectable from biofilters. The death of tilapia in the control was likely linked to the release of trapped hydrogen sulfide gas from anaerobic region of biofilters into water column. Only minute concentration (i.e., 0.002 mg/L) of this particular gas is extremely dangerous to aquaculture (Timmons *et al.*, 2002). Based on the result described, it seemed that the SFNBs at the given biofilter length of 21 m finally reached their maximum waste handling capacity on day 85 as signaled by the presence of hydrogen sulfide and the massive tilapia mortality. The corresponding tilapia biomass and inorganic nitrogen waste loading on this particular day (i.e., day 85) was determined at 13.62 kg/m^3 and 19.61 mg N/L/day, respectively. Periodic biofilter cleaning in the treatments removed accumulated solids from the surface of nitrifying biofilters and expanded the system capability in treating inorganic nitrogen compounds via nitrification for a longer period, thereby leading to higher tilapia biomass and survival rate.

Table 1. Tilapia growth data and water characteristics during the zero-water exchange cultivation.

Parameters	Controls	Treatments
Initial individual weight (g)	57.3 ± 5.83	50.3 ± 4.23
Initial fish density (kg/m^3)	3.44 ± 0.14	5.02 ± 0.02
Final individual weight (g)	217 ± 51.28[a]	257 ± 27.86
Final fish density (kg/m^3)	13.62 ± 1.27[a]	26.8 ± 2.98
Average daily growth (g/day)	1.96 ± 0.21[a]	2.35 ± 0.31
Survival rate (%)		
Day 1 – 85	100	98.52
Day 86 – 90	0	96.96
Temperature (ºC)	27.6 ± 0.73	26.8 ± 1.51
pH	7.89 ± 0.38	7.84±0.52
DO (mg/L)	6.20 ± 0.48	6.35 ± 0.63
Alkalinity (mg/L $CaCO_3$)	112 ± 38	120 ± 28

[a] Measured on day 85 when the entire tilapia in the controls was still alive

3.4 System management aspects

In the present study, the SFNBs were intended for nitrification. The special feature of the proposed systems was clearly the integration of aquaculture production, inorganic nitrogen treatment and solid-liquid separation in a single tank, thereby allowing process simplification by eliminating the external aerated biofilters and solid separating devices. Without solid removal, nitrification seemed to occur effectively up to day 57 and the corresponding inorganic nitrogen loading for this particular day (i.e., day 57) was calculated at 14.56 mg N/L/day, which was equivalent to fish biomass at 10 kg/m^3. Based on this information, it is not recommended to use the SFNBs to perform closed-water aquacultures beyond 10 kg/m^3 given the biofilter length of 21 m and without any solid removal. Continued use of the systems beyond the suggested density would reduce nitrifying capability of the biofilters and risk the occurrence of denitrification and sulfate reduction. The capacity of SFNBs was expandable with a periodic solid removal. Based on the author's experience, the manual removal of solids from Biocord^{TM} biofilters can be easily accomplished by light scratching and rinsing with water. In practice, a moderate-pressure water spray could be used to remove the trapped solids from biofilters for a larger scale operation. Further experiments to determine an appropriate procedure for biofilter cleaning as well as cleaning frequency should be investigated in the future.

At the reported aquaculture density (i.e., 24.97 to 30.35 kg/m^3), the SFNBs with biweekly biofilter cleaning should be able to compete with the cage-culture of tilapia in Thailand that is known to be environmentally unfriendly and susceptible to water quality variation. With better management strategies such as optimized biofilter cleaning interval, suitable cleaning method, improved diets and feedings, the productivity of SFNBs should be further improved to match other recirculating systems with efficient solid separators that reported the final aquaculture density in the range from 20 to 100 kg/m^3 (Timmons *et al.*, 2006; Little *et al.*, 2008). Simplicity is another feature contributing to the system's attractiveness. The proposed system consisted of two main units: biofilters for inorganic nitrogen treatment and solid-liquid separation and aeration devices to provide sufficient oxygenation for aquaculture and nitrifying bacteria. In contrast, other designs usually segregated aquaculture production from water treatment units, which may consist of various unit operations, 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; Tal *et al.*, 2009). This complexity leads to costly investment and the need for highly skilled operators, all of which are often lacking for local Thai farmers. Finally, an elevated nitrate concentration in the effluent of SFNBs was an important issue that must be addressed. 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 the SFNBs.

4. Conclusions

The SFNBs integrated with acclimated Biocord™ biofilters (21 m) were effective in controlling TAN and nitrite concentrations with an average of less than 1.0 mg N/L for the entire experiment. Without solid removal, the SFNBs were not recommended to grow aquaculture at high biomass density due to the possible release of hydrogen sulfide from anaerobic regions of biofilters. Biweekly biofilter cleaning was able to increase the inorganic nitrogen handling capacity of the proposed aquaculture systems from 19.61 to 38.6 mg N/L/day and attain an aquaculture density as high as 30 kg/m^3. An independent experiment confirmed the importance of periodic biofilter cleaning. Finally, the SFNBs are simple to build and should be attractive for budget-limited farmers.

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