

Article

## Development of Aeration-Assisted Combined Nitrification and Solid Removal Unit for a Compact Recirculating Aquaculture System

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**Abstract.** This work focused on the development of water treatment unit, called aeration-assisted combined nitrification and solid removal (ACNS), for compact aquaculture system. Results of preliminary study indicated that aerated filtration could extend filtration time significantly and capture more solids as compared to non-aerated filtration. Operating parameters of ACNS, including screen pore size, influent flow rate, aeration rate and position of air diffuser, were determined at 130  $\mu\text{m}$ , 750 L/h, 120 L/h and 1.0 cm below the base of screen, respectively. ACNS was subsequently evaluated for its performance in nitrogen and solid removal during the 60-days closed-water tilapia cultivation. Performance of aquaculture system with ACNS, as the only treatment unit, was comparable to the aquaculture system having both nitrifying biofilter and ACNS, as separated units. Specifically, ACNS could maintain suspended solids ( $28 \pm 7.6$  mg SS/L), ammonia ( $< 0.5$  mg N/L) and nitrite ( $< 0.5$  mg N/L) concentration below the recommended limits for extended period. ACNS also produced relatively high and constant effluent flux close to initial value despite increasing the filtration time in aerated compartment up to 7 days. This led to continuous filtration, without stopping the system to remove solids on screen, for 50 days.

**Keywords:** Solid, nitrification, aquaculture, RAS, tilapia.

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## 1. Introduction

Aquaculture has moved from extensive cultivation in opened system toward intensive cultivation in recirculating system in response to increasing food consumption and stringent environmental regulation [1]. Significant amount of high protein feed is used during the cultivation to obtain animal high growth rate, leading to large amount of solid accumulation in cultured system. Excessive solids in aquaculture system promotes pathogen and causes gill irritation [2, 3]. The presence of solids also increases biochemical oxygen demand of water and requires intensive aeration to maintain sufficient oxygen concentration [4]. Meanwhile, solid accumulation at the bottom of cultured tank can establish the local anaerobic environment, required for toxic metabolite (e.g., hydrogen sulfide) formation [5]. Further, successive biodegradation of protein in unconsumed feed and urea in animal excretion yields varieties of inorganic nitrogen compounds, namely ammonia, nitrite and nitrate, in water. Ammonia and nitrite concentration greater than 1.0 mg N/L can cause negative health effects on cultured animals, including high stress, lower oxygen transport capability, lower growth, weaken immunity, and even death [1]. As a result, the simplest solution for farmers is discharging water from cultivating system into aquatic environment and replacing with water from natural resource. This practice increases the risk of disease infection and creates adverse environmental problems, such as oxygen depletion in receiving water, eutrophication and toxicity to aquatic animals and human [1, 6]. Clearly, solid removal and inorganic nitrogen treatment are important issues for achieving the sustainable aquacultures.

Filtration is one of the most popular methods for solid removal in aquaculture due to high removal efficiency [7]. Its main setback is the rapid accumulation of solids on filtered medium that leads to frequent stopping to clean the systems [8]. Filtration units with sophisticated cleaning mechanics such as floating bead clarifier and granular media filter, can be expensive to build and operate and need high skilled labors. These issues are the main bottleneck for employing such filtration units in developing countries [7-9]. For inorganic nitrogen treatment, nitrification is the conventional process and has gained more popularity among aquaculture farmers in developing countries. It is an aerobic biological process that converts ammonia and nitrite to, far less toxic, nitrate. Nitrifying systems are available in both attached-growth and suspended-growth configurations. Attached-growth systems, for examples, trickling filter, fluidized-sand filter, moving-bed filter and rotating biological contactor, immobilize nitrifying bacteria on the surface of inert materials [10-14]. In spite of high treatment efficiency, attached-growth systems are often sophisticated in their designs, costly to operate and need high skilled operators [14]. Suspended-growth systems are more simple, demand less investment and incur less operational cost, yet they require the suitable

range of suspended solid concentration to mediate nitrification. Previous works recommended the range of suspended solid concentration in suspended-growth aquaculture systems (e.g., biofloc technology system) from 200 to 500 mg SS/L in order to avoid high ammonia and nitrite concentration [4, 15-18].

Therefore, it is apparent that the simple and low-cost treatment unit, which combines solid removal and nitrification is needed. Aeration is the important factor that influences both nitrification and solid removal processes. Nitrification occurs under aerobic condition, while providing aeration was reported to increase the solid removal efficiency of crossflow filtration [19-21]. Therefore, the proposed treatment unit must integrate aeration as a mean to enhance nitrification and solid removal, and combined both processes into a single unit. Herein, this paper describes the development of such unit, referred to as the aeration-assisted combined nitrification and solid removal unit (ACNS), as well as the results of ACNS evaluation during the closed-water aquaculture.

## 2. Materials and Methods

### 2.1. Preliminary Study

#### 2.1.1. Property of solids in aquaculture tank

Cultivation of tilapia (*Oreochromis niloticus*) in 500-L tank provided suspended solids in the experiment. Tilapia, stocked in cultured tank to obtain the initial weight about 3.0 kg/m<sup>3</sup>, was fed twice daily with 18% protein feed at 3% of total fish weight per day. Cultivating tanks were located indoor under room temperature (25 to 28 °C). The pH of water in cultured tanks was maintained between 7.0 and 8.0 by periodic addition of NaHCO<sub>3</sub>, as needed. A centrifugal submersible pump, providing water flow rate at 8,500 L/h, was placed on the tank floor to circulate water in the first treatment (T1). No water pump was used in the second treatment (T2). Both treatments were repeated in triplicate. Aquarium diffuser supplied dissolved oxygen in cultured tank greater than 4.0 mg/L in both treatments. Water samples (1.0 L) were collected at the start of tilapia cultivation and later periodically, and were analyzed for the sludge volume index (SVI) and turbidity. The average size of suspended solids at the beginning and the end of tilapia cultivation were also determined.

#### 2.1.2. Description of ACNS

Figure 1 illustrates the schematic drawing of ACNS, which was assembled from two transparent coaxial acrylic cylinders with cone shape bottom. The small cylinder was inserted into the large cylinder to create two compartments (i.e., inner and outer) that separated influent water from filtered water. Two ACNS were built, small and large, with the working volume of 1.5 and 30 L, respectively. The inner cylinder of ACNS was cut to

make the rectangular opening (Fig. 1), which was covered by stainless steel screen with pore size ranged from 100 to 160  $\mu\text{m}$ . One and three openings were made for small and large ACNS, respectively. Aquarium diffuser was placed at the bottom of inner cylinder to provide aeration. Influent entered ACNS over the top of inner cylinder and passed through screen, which was also subject to aeration from diffuser. Filtered water in the outer compartment was discharged via effluent port. Settled solids were removed from the inner compartment through drained valve.

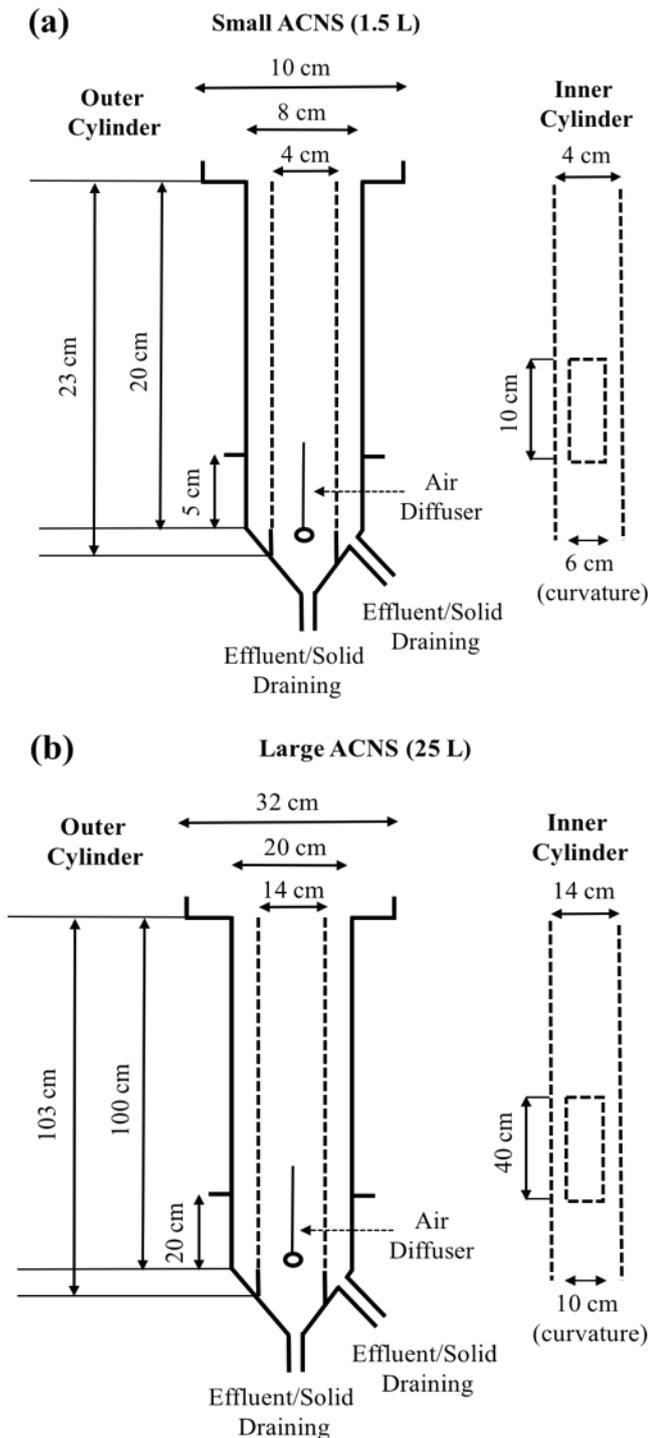


Fig. 1. Schematic drawing (not to scale) of ACNS: (a) small ACNS 1.5 L and (b) large ACNS 30 L.

### 2.1.3. Operating parameters of ACNS

The source of suspended solids was from tilapia cultivation in the same laboratory (Center of Excellence in Marine Biotechnology, Chulalongkorn University). Tilapia, stocked in 2,000-L tank to achieve the initial weight about 3.0 kg/m<sup>3</sup>, was fed twice daily with 18% protein feed at 3% of total fish weight per day. Solids from tilapia cultivating tank were collected weekly and stored in 1,000-L aerated tank. Suspended solids in aerated tank was adjusted to 200 mg SS/L before supplying to ACNS.

The first experiment aimed to verify aeration as a mean to improve filtration performance of ACNS. Small ACNS (1.5 L) was used. Influent was fed into ACNS at 250 L/h. Stainless steel screen with three pore sizes (100, 130 and 160  $\mu\text{m}$ ) was tested in both aerated and non-aerated filtration. Air flow through diffuser was 70 L/h. Filtration proceeded until the complete solid clogging, as indicated by zero effluent flux. Volumes of filtrate were obtained periodically to determine effluent flux and suspended solids concentration. The next experiment intended to determine influent flow rate. Large ACNS (30 L) was used to accommodate higher influent flow, which varied from 250 to 1,000 L/h. Screen pore size was based on the results of previous experiment while the aeration rate was maintained at 70 L/h for the entire experiment. The effects of aeration rate and position of air diffuser were also studied in large ACNS after the influent flow rate experiment. Screen pore size and influent flow rate were based on the findings of previous experiments, whereas aeration rate varied from 60 to 300 L/h given that the position of air diffuser was 1.0 or 15.0 cm below the base of screen. Filtration continued until approximately 5% reduction of effluent flux was reached. The experiment was repeated three times for each combination of aeration rate and diffuser position. Volumes of filtrate were obtained regularly to determine effluent flux and suspended solid concentration.

### 2.1.4. Continuous filtration

Large ACNS was used to assess the possibility of continuous filtration. Operating parameters, including screen pore size, influent flow rate, aeration rate and position of air diffuser, were based on the findings of previous section. As illustrated in Fig. 2(a), influent, containing suspended solids approximately 200 mg SS/L, was fed into the inner cylinder. Filtration continued until 5% reduction of effluent flux was reached, then the filtration chamber was switched from inner to outer compartments. Specifically, aeration and influent inlet were moved from inner to outer compartments. This resulted in reversed liquid flow direction, while effluent and solid discharges were from the inner and outer compartments, respectively (Fig. 2(b)). The switch of filtration chamber was conducted whenever the effluent flux attained 5% reduction. Volumes of filtrate were measured regularly during the experiment.

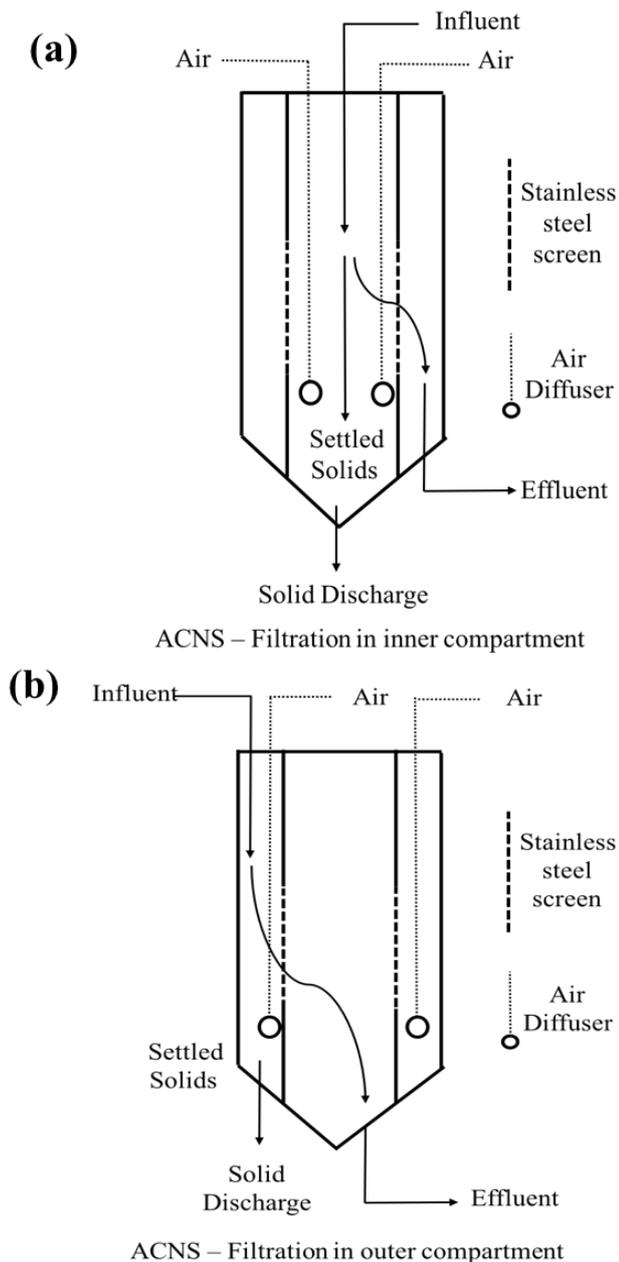


Fig. 2. Liquid flow direction and solid discharge during the continuous filtration: (a) filtration using the inner compartment and (b) filtration using the outer compartment.

## 2.2. Evaluation of ACNS

Large ACNS was integrated into different aquaculture systems to evaluate its performance in solid removal and inorganic nitrogen control. The initial stocking density of tilapia in 5,000-L cultivating tank was about 3.0 kg/m<sup>3</sup>. Tilapia was fed twice daily with 18% protein feed at 3% of the total fish weight per day. Cultivating tanks were located indoor under room temperature (25 to 28 °C). The pH of water in cultivating tanks was maintained between 7.0 and 8.0 by adding NaHCO<sub>3</sub>, as needed. The commercial plastic media BCN-012 (ENEXIO Germany, HDPE, specific gravity

0.95 and specific surface area 859 m<sup>2</sup>/m<sup>3</sup>) were packed into PVC column (inner diameter 30 cm) to attain bed height of 50 cm. Acclimation of these plastic media to establish nitrification followed the method described in earlier work [14]. Three treatments of aquaculture systems were set up. Water in the first treatment (T1) was circulated between cultivating tank and nitrifying biofilter only. In the second treatment (T2), water from cultivating tank was directed to ACNS, then nitrifying biofilter, before returning to cultivating tank. Water circulation in the third treatment (T3) was between the cultivating tank and ACNS only. Filtration of influent water from tilapia cultivating tank used the inner compartment from the start of fish cultivation, and continued until the effluent flux reached 5% reduction, when it was stopped to remove clogged solids from screen. Cleaning of screen was simple by light scraping and spraying water from low-pressured hose. After operation resumed, switching filtration chamber between inner and outer compartments, along with the solid discharge, were conducted on the daily (1 day) and weekly (7 days) interval for T2 and T3, respectively. However, effluent discharge from ACNS operated continuously in both treatments. It should be mentioned that water pumps, air pumps and valves, which regulated influent and air flow in ACNS, were automatically controlled by Arduino micro-controller. Operating parameters of ACNS, including screen pore size, influent flow rate, aeration rate and position of air diffuser, followed the results of previous sections. Water samples (100 mL) from cultivating tanks were collected daily and analyzed for the concentration of ammonia, nitrite, nitrate and suspended solids. Volumes of filtrate from ACNS were also obtained regularly to determine effluent flux. Tilapia in cultivating tanks were caught and weighed biweekly to obtain weight and determine survival rate.

## 2.3. Determination of Ammonia Degradation Rate

Samples of nitrifying biofilter (500 mL) from T1 and T2 were taken on the first and final day of tilapia cultivation to determine ammonia degradation rate (i.e., nitrification rate). Batch experiment was performed in 1-L glass bottle (n = 3 bottles for each treatment), which was completely wrapped with aluminum foils. Each bottle had a diffusive stone aerator to provide liquid mixing and dissolved oxygen greater than 4.0 mg/L. NH<sub>4</sub>Cl was added into glass bottle to achieve the initial ammonia concentration at 2.0 mg N/L. The range of pH (7 to 8) and alkalinity (100 to 150 mg CaCO<sub>3</sub>/L) was maintained by adding NaHCO<sub>3</sub>. Water samples (10 mL) from glass bottles were obtained periodically and analyzed for ammonium, nitrite and nitrate concentration. For the remaining treatment (T3), liquid samples (1.0 L), containing suspended solids, were collected from aerated compartment on day 1, day 22 and day 60 to determine the rate of ammonia degradation. Batch experiment was conducted in 1-L glass bottle (n = 3 bottles for each sampling date). Ammonium concentration in water

samples was adjusted by adding  $\text{NH}_4\text{Cl}$  to attain the initial concentration at 2.0 mg N/L. Experimental procedure followed the details as described in earlier batch experiments.

## 2.4. Analytical Techniques

Concentration of ammonia, nitrite, nitrate and suspended solids was determined according to APHA, method 4500-NH<sub>3</sub>-D, 4500-NO<sub>2</sub>-B, 4500-NO<sub>3</sub>-B and 2540-D, respectively [22]. Measurement of sludge volume index (SVI) was performed by allowing solids in water sample to settle in an Imhoff cone for 30 minutes, according to APHA method 2540-F [22]. Particle sizes were measured using laser light scattering technique (Malvern, Mastersizer 3000) [8]. Water turbidity was determined based on the light absorbance using microplate spectrophotometer (Biotek, PowerWave XS2) at 760 nm [8]. Results were statistically compared by one-way ANOVA with Tukey HSD post hoc test with significant level of 0.05 [23]. For the nitrogen mass balance calculation, content of nitrogen in feed and solids in cultivating tanks was determined by CHN analyzer (Perkin Elmer, PE2004) [15]. The nitrogen content in tilapia was assumed at 6.35% based on the wet weight basis according to the previous work [24]. Dissolved inorganic nitrogen was calculated using the final concentration of ammonia, nitrite and nitrate in cultivating tanks.

## 3. Results and Discussion

### 3.1. Preliminary Study

#### 3.1.1. Property of solids in aquaculture tank

The values of SVI from T1, which had submergible pump to circulate water, were 6.0 mL/L during the first hour, then decreased to relatively constant level at  $5.0 \pm 0.78$  mL/L (Fig. 3). Relatively unchanged SVI values, measured at  $6.0 \pm 0.07$  mL/L, were observed in T2, which operated without submergible pump. However, water from T1 was more turbid despite lower SVI values (Fig. 3). Shearing and compression by pump moving parts, producing finer solid particles, seemed to be the likely explanation for higher water turbidity. This explanation concurred with the results that showed the reduction in size of suspended solids from 250  $\mu\text{m}$  at the beginning of tilapia cultivation to about 170  $\mu\text{m}$  after 1,440 minutes in T1, as compared to larger suspended solids (235  $\mu\text{m}$ ) in T2. Clearly, sedimentation was less effective for solid-liquid separation when finer solid particles were present. This problem is likely to intensify when larger pumps, paddles and aerators are employed to achieve good water mixing and sufficient oxygenation during the intensive aquaculture. This setback led to the decision to proceed with filtration in the future development of ACNS.

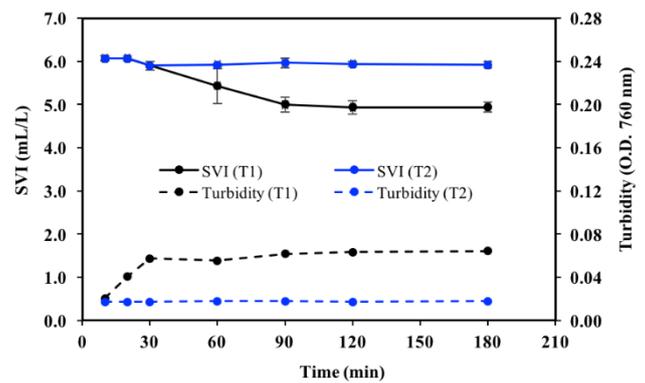


Fig. 3. Sludge volume index (SVI) after 30-minutes sedimentation and turbidity of water samples from cultivating tank: T1 with submergible pump and T2 without pump.

#### 3.1.2. Operating parameters of ACNS

Operating parameters of ACNS, including screen pore size, influent flow rate, aeration rate and position of air diffuser, were considered. The first experiment aimed to verify the concept of aeration as a means to improve filtration performance of ACNS. As shown in Fig. 4(a), the complete solid clogging on screen, as indicated by zero effluent flux, occurred after 90 to 160 s for non-aerated filtration. Significantly longer filtration time up to 210 to 400 s was observed for aerated filtration. Providing aeration created higher liquid turbulence over the screen surface, thereby reducing the rate of solid deposition on screen (i.e., prolonging filtration time). Besides aeration, screen pore size also affected filtration performance, as demonstrated in Fig. 4(a) to 4(c). This experiment selected the screen, with pore size varied from 100 to 160  $\mu\text{m}$ , according to the average size of solids (170  $\mu\text{m}$ ) obtained in the previous section. The longest filtration times were associated with the largest screen pore size (160  $\mu\text{m}$ ). Filtration using smaller screen pore size (130  $\mu\text{m}$ ) also yielded relatively long filtration time about 320 s. As expected, the highest solid removal efficiency was obtained when using the smallest screen pore size (100  $\mu\text{m}$ ). However, the highest amount of solid retention ( $804 \pm 35.1$  mg SS) was achieved when using 130  $\mu\text{m}$  screen under aerated condition. The amount of retained solids was significantly higher ( $p < 0.05$ ) than the results from other screen pore sizes that captured solids in range  $240 \pm 12.3$  to  $703 \pm 26.8$  mg SS. Clearly, aeration had the positive impact on filtration performance of ACNS by increasing filtration time. By using filtration time and the total solid retention as main criteria, it was deemed reasonable to use aerated filtration and 130  $\mu\text{m}$  screen in future experiments.

Influent flow rate was another important parameters. Large ACNS was used to accommodate higher influent flow rate. Other operating parameters were maintained: screen pore size at 130  $\mu\text{m}$ , aeration rate at 70 L/h and air diffuser at 1.0 cm below the base of stainless steel screen. Under the described condition, approximately 50% and 75% of screen area were under water when

influent flow rates were 250 and 500 L/h, respectively. Complete utilization of screen area, defined as the total submerging of screen in water, was observed when maintaining the influent flow rate at 750 L/h. However, overflow of influent occurred when the flow rate was 1,000 L/h.

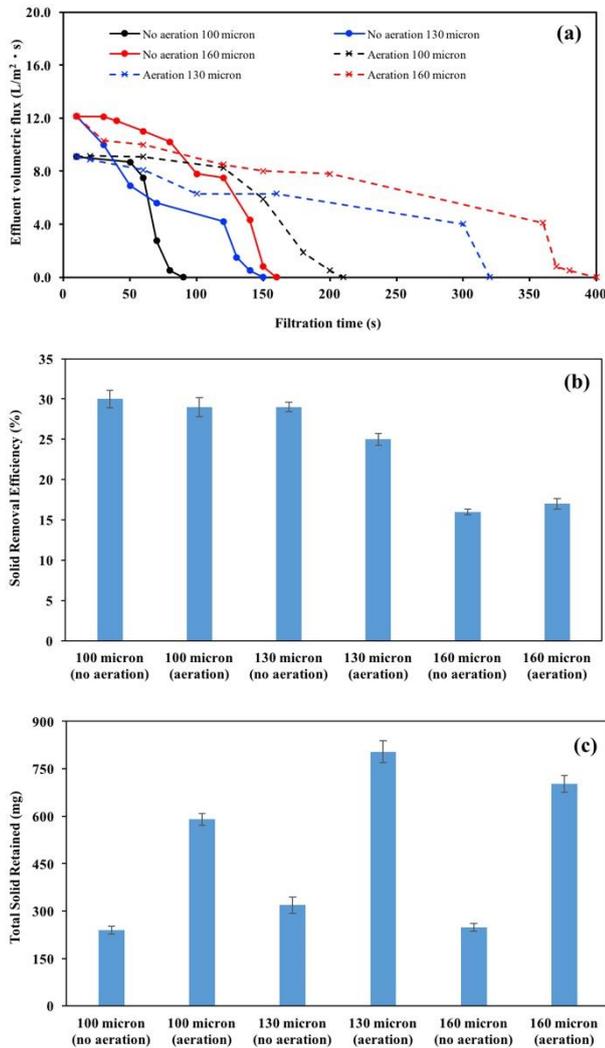


Fig. 4. Effects of aeration and screen pore size on performance of ACNS: (a) flux and filtration time (b) solid removal efficiency and (c) total solid retention.

Aeration rate also influenced ACNS performance (Fig. 5). With the following parameters, namely the screen pore size at 130  $\mu m$ , the influent flow rate at 750 L/h and the position of diffuser at 1.0 cm below the base of screen, the solid removal efficiency increased with higher aeration rate, reaching the maximum at  $45.3 \pm 3.06\%$ , when the aeration rate was 120 L/h. Lower solid removal efficiency was observed after the aeration rate exceeded 120 L/h. The removal efficiency as low as  $23.8 \pm 0.39\%$  was obtained when the aeration rate was 300 L/h. Past work reported the positive relationship between aeration rate (i.e., gas superficial velocity) and liquid shear rate in bubble column [25]. The rise in gas superficial velocity produced higher shear force, which in

turn created stronger liquid turbulence over the screen. However, excessive aeration rate yielded the negative impact on solid removal efficiency. High liquid shear force from increased gas flow, along with other forces such as vibration from bubble breaking, could reduce the size of suspended solids and shred biofilm (i.e., layer of solids) on surface into small pieces. These small solid fragments were subsequently forced through screen into the outer compartment, resulting in lower solid removal efficiency.

The position of air diffuser also affected the performance of ACNS. For example, when air diffuser was placed at the upper position (i.e., 1.0 cm below the base of screen), ACNS, subject to 120 L/h aeration rate, was capable of producing relatively constant effluent flux from 6,122 to 6,162  $L/m^2 \cdot h$  throughout the filtration period (160 minutes). Comparable flux was obtained for a short period when moving the air diffuser to the lower position (i.e., 15.0 cm below the base of screen). The effluent flux was measured at 5,688  $L/m^2 \cdot h$  (about 7.4% reduction) after 45 minutes. Less amount of air bubbles was observed near the screen when the diffuser was placed at the lower position, yet considerable amount of air bubbles was present near the solid settling zone. Breaking of bubbles and their upward movement disturbed the quiescent condition of settling zone at the bottom of ACNS and caused the settled solids to re-suspended into the bulk liquid. This effect became more noticeable as the aeration rate intensified.

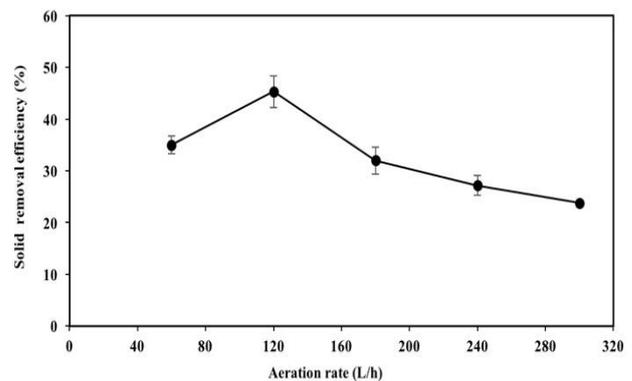


Fig. 5. Effects of aeration rate on the solid removal efficiency of ACNS subject to the following operating parameters: screen pore size at 130  $\mu m$ , influent flow rate 750 L/h, and air diffuser at 1.0 cm below the base of screen.

### 3.1.3. Continuous filtration

Up to now, ACNS had to be halted temporarily to remove accumulated solids on screen. Although the solid removal was simple, it could become problematic when applying ACNS in large scale operation. As a result, continuous operation was introduced by changing filtration chamber from inner to outer compartments, and *vice versa*. Operating parameters of ACNS, including screen pore size (130  $\mu m$ ), influent flow rate (750 L/h), aeration rate (120 L/h) and position of air diffuser (1.0

cm below the base of screen), were based on the findings of previous sections. The average initial flux of both compartments was comparable, determined at  $6,093 \pm 185$  L/m<sup>2</sup>·h. ACNS produced relatively high effluent flux (> 95% of initial flux) for 217 h (i.e., about 9 days) when the compartment switching was required. However, large solid accumulation and formation of small bubbles in settling zone were noticed when the filtration time exceeded 8 days. Therefore, it was recommended to reduce the filtration time to 7 days or less in order to maintain high effluent flux and avoid excessive solid accumulation.

### 3.2. Evaluation of ACNS

ACNS was integrated into different aquaculture systems and evaluated for its performance in solid removal and inorganic nitrogen control during the 60-days closed-water tilapia cultivation. Screen pore size (130 µm), influent flow rate (750 L/h), aeration rate (120 L/h) and position of air diffuser (1.0 cm below the base of screen) were based on the findings of preliminary study.

#### 3.2.1. General water parameter

The average values of general water parameters, including dissolved oxygen (DO) concentration, pH and alkalinity in T1, which was operated with only nitrifying biofilter, were  $7.3 \pm 0.26$  mg/L,  $7.5 \pm 0.15$  and  $134 \pm 12.4$  mg CaCO<sub>3</sub>/L, respectively. Average values of these parameters in T2, which had nitrifying biofilter and ACNS as separated unit, were  $7.3 \pm 0.23$  mg/L,  $7.2 \pm 0.08$  and  $120 \pm 15.2$  mg CaCO<sub>3</sub>/L, respectively. Comparable results (DO =  $7.0 \pm 0.30$  mg/L, pH =  $7.2 \pm 0.08$  and alkalinity =  $125 \pm 12.8$  mg CaCO<sub>3</sub>/L) were found in T3, which had only ACNS. Alkalinity in each treatment was also founded decreasing gradually during the cultivation so that periodic addition of NaHCO<sub>3</sub> was carried out. The decline of alkalinity was one of the indicators that confirmed the occurrence of nitrification. Nonetheless, the magnitudes of water parameters from all treatments remained within the recommended ranges for nitrifying bacteria and tilapia cultivation [26].

#### 3.2.2. Effluent flux

The ability of ACNS to produce high and relatively constant effluent flux for extended period was important for effective filtration. Filtration was carried out using the inner compartment of ACNS since the start of tilapia cultivation in T2 and T3, producing the average initial flux at  $6,110 \pm 35$  L/m<sup>2</sup>·h. Filtration proceeded for the next 11 days, when it was stopped after the 5% reduction of effluent flux. After solid removal, operating procedure of ACNS was modified; specifically, alternating filtration chamber between inner and outer compartments daily for T2 and weekly for T3. Under the new operating

condition, continuous filtration was possible until the end of tilapia cultivation, without stopping the ACNS to remove solids on the screen. The resulting effluent flux of remaining period was relatively constant in both treatments, fluctuating from 5,925 to 6,105 L/m<sup>2</sup>·h for T2 and from 5,905 to 5,990 L/m<sup>2</sup>·h for T3. Clearly, extending the filtration time did not adversely affect the performance of ACNS in term of producing high and relatively constant flux, and allowed filtration to continue for extended period without interruption to remove clogged solids. These features offer benefits for small-budget farmers in developing countries, namely decreasing the frequency of maintenance and lowering operational expense. Another advantage of ACNS was the ability to process more influent and produce up to 3 times higher effluent flux, in comparison to other compact solid separators reported earlier, namely gravitational settling column, drum screen filter, and rapid-and-slow sand filter [8, 27, 28].

#### 3.2.3. Suspended solid concentration

Besides producing high and relatively constant flux, ACNS must be able to control suspended solid concentration within the acceptable range. As shown in Fig. 6(a), suspended solids concentration in T1 increased as high as  $754 \pm 20$  mg SS/L, with the average value for the entire cultivation measured at  $377 \pm 205$  mg SS/L. Large amount of solids was also observed between biofilter voided spaces and on tank floor. In contrast, suspended solid concentration from the remaining treatments was low and relatively constant. Average values of suspended solid concentration over the entire cultivation were  $23 \pm 8.9$  and  $28 \pm 7.6$  mg SS/L for T2 and T3, respectively, which were significantly less than the recommended limits for tilapia cultivation (60 mg SS/L) and local discharge regulation for inland aquaculture (70 mg SS/L) [26, 29]. The daily and weekly solid wastage from T2 and T3 was estimated at  $47.5 \pm 16.21$  and  $305 \pm 35$  g, respectively.

#### 3.2.4. Inorganic nitrogen concentration

Accumulation of ammonia, followed by nitrite, was observed during the first two weeks in T1 and T2, as illustrated in Fig. 6(b) and 6(c). Such profiles were common during the startup period of aquaculture system [14-16, 30]. In spite of similar trends, ammonia and nitrite concentration in T2 decreased to the negligible level (< 0.5 mg N/L) quicker than T1. The maximum concentration of ammonia ( $0.750 \pm 0.120$  mg N/L) and nitrite ( $1.14 \pm 0.018$  mg N/L) in T2 was also significantly lower ( $p < 0.05$ ) than the maximum concentration in T1. This observation seemed to be linked with ACNS operation. With ACNS, substantial solid accumulation in nitrifying biofilter column could be avoided. This decreased organic carbon availability for heterotrophic bacteria, which possessed higher oxygen affinity than nitrifying bacteria. [31]. Effective control of ammonia

and nitrite occurred in T1 after day 8, as indicated by insignificant concentration of these compounds ( $< 0.5$  mg N/L) and rising nitrate concentration (Fig. 6(d)). However, effective nitrification proceeded until day 50, when the decline of nitrate from  $69 \pm 2.2$  to  $48 \pm 4.2$  mg N/L was observed. The decreasing trend of nitrate suggested the occurrence of heterotrophic denitrification. It was possible that the biodegradation of solids provided dissolved organic carbon for denitrifying bacteria while the accumulation of solids established anaerobic environment, as required by denitrification. Although this study did not measure oxygen concentration within the biofilm layer, the presence of thick biofilm patches on the tank surface was the clear indicator that the establishment of anaerobic environment was possible within the biofilm layer [32]. Other works also reported zero DO concentration at the biofilm depth about 0.5 mm from the liquid-biofilm interface [33, 34]. Similar findings (i.e., decline of nitrate) were also reported in previous works, which operated nitrifying biofilter without solid removal [5, 32, 35, 36]. It should be pointed out that simultaneous nitrification and denitrification was undesirable in this study because it was difficult to predict the onset of denitrification and, moreover, the decreasing nitrate under anaerobic environment risked the formation of hydrogen sulfide, which is highly toxic to fish [5]. In contrast, the effective control of ammonia and nitrite by nitrification occurred in T2 after day 6. This was demonstrated by increasing nitrate concentration, as high as  $84 \pm 3.4$  mg N/L, along with negligible ammonia and nitrite ( $< 0.5$  mg N/L) until the end of cultivation (Fig. 6(b) to (d)). The positive impact of ACNS on inorganic nitrogen control was further supported by the results of ammonia degradation rate (Fig. 7). Ammonia degradation rate (i.e., nitrification rate) of biofilter from T1, which had no solid removal, decreased substantially from  $14.1 \pm 0.76$  mg N/m<sup>2</sup>·h at the beginning of tilapia cultivation to  $7.0 \pm 0.58$  mg N/m<sup>2</sup>·h at the end of cultivation. This was opposite to the rates from T2, which employed ACNS to lower the solid loading on biofilter, that showed the slight reduction to  $12.8 \pm 0.88$  mg N/m<sup>2</sup>·h. Thus, relatively the same nitrifying activity of biofilter could be maintained with ACNS operation, and this offered the possibility of effective ammonia and nitrite control for extended period. Without ACNS, nitrifying biofilter was clearly susceptible to high solid accumulation after short time, and became less effective in mediating nitrification due to the occurrence of anaerobic reactions.

The profile of ammonia and nitrite in T3 during the first three weeks were apparently higher than other treatments (Fig 7(b) and 7(c)). The maximum concentration of ammonia ( $1.25 \pm 0.12$  mg N/L) and nitrite ( $3.52 \pm 0.16$  mg N/L) in this treatment was substantially higher ( $p < 0.05$ ) than those in T1 and T2. Ammonia and nitrite in this treatment also required longer time to reach the negligible levels ( $< 0.5$  mg N/L)

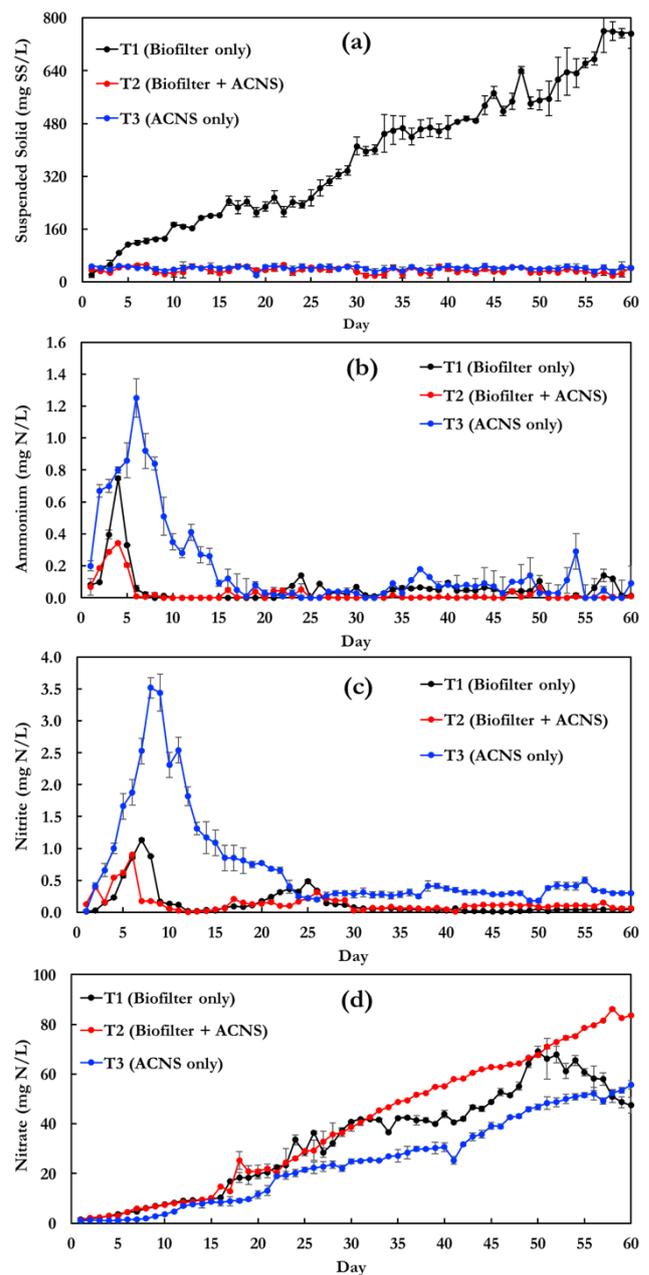


Fig. 6. Water parameters during the closed-water tilapia cultivation in different aquaculture systems: (a) suspended solids (b) ammonia (c) nitrite and (d) nitrate

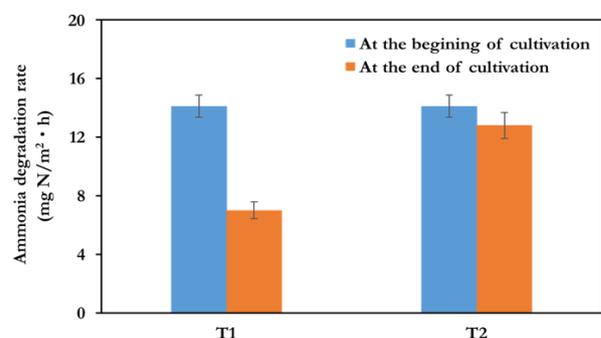


Fig. 7. Ammonia degradation rate of nitrifying biofilter at the beginning (day 1) and the end (day 60) of tilapia cultivation

as compared to earlier treatments. Longer startup period as well as higher ammonia and nitrite concentration were probably linked to the absence of active nitrifying sludge [14]. This was in contrast to the nitrifying biofilter in T1 and T2 that were acclimated to establish nitrification prior to the tilapia cultivation. Nonetheless, the effective nitrogen control via nitrification was possible in this treatment after day 21 and was responsible by solids in aerated compartment of ACNS as well as those in cultivating tank. Solids in aerated compartment, fluctuated between  $100 \pm 21.1$  and  $431 \pm 44.7$  mg SS/L during the weekly filtration period, were largely within the recommended range (200 to 500 mg SS/L) for effective control of ammonia and nitrite for aquaculture system with fish weight in tank less than  $11.0 \text{ kg/m}^3$  [4, 16-18]. Moreover, the rate of ammonia degradation of solids from aerated compartment increased from  $8.7 \pm 2.17$  mg N/h·g on the first day to  $58.1 \pm 8.83$  mg N/h·g on day 23 and remained relatively unchanged at  $63.8 \pm 8.63$  mg N/h·g at the end of cultivation (Fig. 8). The significant increase of ammonia degradation rate (i.e., about 7 times) indicated that solids had changed to more active nitrifying sludge after long exposure to ammonia from fish feed. This observation explained the longer startup period as well as the higher ammonia and nitrite concentration during the first three weeks. Based on the results presented, ACNS had the potential to perform simultaneous nitrification and solid removal effectively in the single unit for extended period when applying proper operating condition.

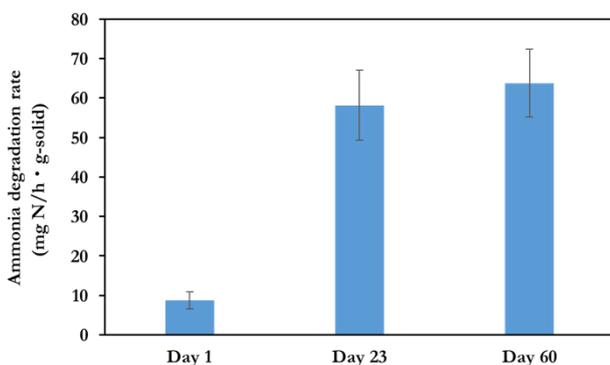


Fig. 8. Ammonia degradation rate of solids in aerated compartment of ACNS in T3.

Results of nitrogen mass balance calculation are demonstrated in Table 1. Nitrogen contents of biological solids from cultivating tanks and fish feed, determined by CHN analyzer, were 3.20% and 3.27% based on the dried weight basis, respectively. The majority of nitrogen input (> 99%) was from feed. Nitrate accounted for the large fraction (24.72%) of nitrogen output in T1, suggesting that nitrification was the major pathway for inorganic nitrogen treatment. Nitrification had more influence on the nitrogen treatment in T2 and T3, as indicated by the higher nitrogen fraction in the form of nitrate at 40.76% and 28.19%, respectively. The absence of ACNS in T1 yielded approximately 12.57% of

nitrogen as suspended solids. This was in contrast to the remaining treatments, where nitrogen in suspended solids accounted for less than 1% of nitrogen output. Continuous filtration by ACNS in T2 and T3 removed 25.91% and 22.31% of total nitrogen input, respectively. Based on the calculation, solid removal was not the primary pathway of inorganic nitrogen control since the nitrogen mass fraction associated with filtration was smaller than the values associated with nitrification. Yet, ACNS remained integral to the success of aquaculture system because it extended the activity of nitrifying biofilter and maintained the acceptable level of suspended solids in cultivating tanks. Unaccounted nitrogen was estimated at 38.17% in T1. The magnitude of nitrogen loss in this treatment was comparable to the previous works, which reported unaccounted nitrogen in range 28% to 55% [15, 37, 38]. Heterotrophic denitrification was the most probable pathway for nitrogen loss due to high level of suspended solids (i.e., organic carbon source), availability of nitrate and formation of anaerobic zone from high solid accumulation. Smaller nitrogen loss at 20.36% was determined in T3. Although the weekly solid wastage was carried out in this treatment, it was observed that considerable amount of suspended solids settled into the bottom cone region, away from the discharging port, and remained undisturbed by air bubbles. The accumulated solids became denser due to gravity compression, thereby likely to form anaerobic zone as required by denitrification. This observation suggested that the design and operation of ACNS required modification to reduce solid buildup in such region. Finally, the fraction of unaccounted nitrogen in T2, which operated both nitrifying biofilter and ACNS concurrently, was only 5.53%. Relatively balanced nitrogen in this treatment suggested that the effect of solid accumulation induced denitrification was unlikely.

### 3.2.5. Fish growth

High fish survival rate (>96%) were obtained from all treatments (Table 2). The reported survival rates were similar to the previous studies, which employed nitrifying and denitrifying biofilter to control water quality [5, 8, 9, 14, 39]. All tilapia death occurred during the first three weeks, perhaps, due to initial condition of tilapia, inability to adapt to new environment and high ammonia and nitrite concentration for several days during that period. The average daily weight gains of tilapia in T2 and T3 were similar, and were roughly 10% to 13% higher than that of T1. However, the average growth rate obtained in this work was about 2 to 3 times lower than the numbers reported elsewhere [5, 14, 39]. Several reasons might contribute to the lower growth rate, namely the condition of fish, protein in feed, and feeding rate. Lower fish growth rate in T1 was probably related to the lengthy exposure to high suspended solid levels. Lower growth rate and survival rate of tilapia were reported when suspended solid concentration in cultured tanks

exceeded 850 mg SS/L [17]. Excessive suspended solid concentration in cultivating tanks might reduce the visibility of fish to find feed [17].

### 3.2.6. Other consideration

The total cost to assemble the large ACNS was 18,000 baht, approximately 553 US dollars. The cost was categorized into pumping equipment (e.g., water and air pumps and diffuser) and construction material (e.g., transparent acrylic and screen) for 10,000 baht and micro-controller for 8,000 baht. It should be mentioned that the ACNS was built in special shop, which incurred extra expense than normal. Moreover, use of micro-controller is optional since switching filtration chamber could be performed manually. Micro-controller is useful when increasing the screen cleaning frequency due to higher solid loading or applying ACNS to larger

aquaculture facilities. The cost of construction material can be reduced as high as 25% by changing the transparent acrylic to PVC. The objective of using transparent acrylic was for observation during the experiment only. From the operational aspect, ACNS, as the single treatment unit, simplifies the recirculating aquaculture systems, typically containing unit operations such as biofilter, solid separator, foam fractionator and oxygenator, to be more compact that can be operated in limited space (e.g., urban area). ACNS also reduces water recirculation expense due to less complexed piping and fewer unit operations. Based on the author experience, the stainless steel screen can be rinsed with water using water-hose and scraped gently using sponge to remove trapped solids. The cleaning process was quick and did not require sophisticated system or intensive energy as in the backwash of existing process such as sand-filter or microbead filter.

Table 1. Nitrogen mass balance calculation displaying nitrogen distributions in different treatments.

Parameter	Treatment		
	T1	T2	T3
<i>Total nitrogen input</i>			
Feed (g N)	381.0 (99.2%)	392.4 (99.22%)	392.4 (99.40%)
Inorganic nitrogen (g N)	3.1 (0.80)	3.1 (0.78%)	2.4 (0.60%)
<i>Total nitrogen output (day 60)</i>			
Ammonia (g N)	0.026 (0.007%)	0.016 (0.004%)	0.620 (0.157%)
Nitrite (g N)	0.102 (0.027%)	0.122 (0.031%)	0.600 (0.152%)
Nitrate (g N)	94.9 (24.72%)	161.2 (40.76%)	111.3 (28.19%)
Solid discharged (g N)	12.2 (3.16%)	102.5 (25.91%)	88.1 (22.31%)
Suspended solid (g N)	48.3 (12.57%)	2.8 (0.714%)	2.8 (0.716%)
Nitrogen gained in fish (g N)	72 (18.75%)	97 (24.53%)	99 (25.08%)
Unaccounted nitrogen (g N)	156.6 (38.17%)	31.9 (5.53%)	92.4 (20.36%)

Table 2. Fish growth data from different treatments.

Parameter	Treatment		
	T1	T2	T3
Initial fish weight (g/fish)	62.45(3.83)	63.44(5.40)	63.12(5.11)
Final fish weight (g/fish)	152.03(25.38)	161.57(22.17)	164.34(20.19)
Initial total fish weight in tank (kg/m <sup>3</sup> )	3.00	2.96	3.00
Final total fish weight in tank (g/fish)	5.51	6.05	6.12
Average weight gained (g/day)	1.49	1.64	1.69
Survival rate (%)	100	96	98

## 4. Conclusions

Significantly longer filtration time and higher solid retention were achieved when performing aerated filtration. Therefore, ACNS, with the following parameters, namely pore size of screen (130  $\mu\text{m}$ ), influent flow rate (750 L/h), aeration rate (120 L/h) and position of air diffuser (1.0 cm below the base of screen), was evaluated during the 60-days closed-water tilapia cultivation. Performance of ACNS, in terms of controlling suspended solids and inorganic nitrogen concentration, was comparable to the recirculating system, which operated nitrifying biofilter and ACNS as separated units. Without ACNS, large solid accumulation was observed on the surface of nitrifying biofilter. In contrast, operation of single ACNS could maintain significantly lower suspended solid concentration ( $28 \pm 7.6$  mg SS/L) than the recommended limits for aquaculture cultivation and legal discharge, and produced negligible ammonia and nitrite concentration ( $< 0.5$  mg N/L). Moreover, ACNS yielded relatively high and constant effluent flux despite extending the filtration time up to 7 days. This feature allowed ACNS to operate for extended period without stopping to remove clogged solids on screen, hence reducing frequency of maintenance and operation cost. Finally, the longer startup period could be reduced by seeding the fully acclimated nitrifying sludge in aerated compartment prior to starting the cultivation.

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## References

- [1] R. Crab, Y. Avnimelech, T. Defoirdt, P. Bossier, and W. Verstraete, "Nitrogen removal techniques in aquaculture for a sustainable production" *Aquacult.*, vol. 270, pp. 1-14, 2007.
- [2] J. Davidson and S. T. Summerfelt, "Solids removal from a cold water recirculating system – comparison of a swirl separator and radial-flow settler," *Aquacult. Eng.*, vol. 33, no. 1, pp. 47-61, 2005.
- [3] G. S. Bilotta, and R. E. Brazier, "Understanding the influence of suspended solids on water quality and aquatic biota," *Water Res.*, vol. 42, pp. 2849-2861, 2008.
- [4] Y. Avnimelech, "Bio-filters: the need for a new comprehensive approach," *Aquacult. Eng.*, vol. 34, pp. 172-178, 2006.
- [5] K. Nootong and S. Powtongsook, "Performance evaluation of compact aquaculture system integrating fibrous nitrifying biofilters" *Songkla. J. Sci. Technol.*, vol. 34, no. 1, pp. 53-60, 2011.
- [6] G. Tchobanoglous and F. L. Burton, *Wastewater Engineering: Treatment, Disposal and Reuse*, 3rd ed. New York: McGraw-Hill, 1991, pp. 85-86.
- [7] S. J. Cripps and A. Bergheim, "Solids management and removal for land-based aquaculture production system," *Aquacult. Eng.*, vol. 22, pp. 33-56, 2000.
- [8] K. Nootong, S. Nurit, and S. Powtongsook, "Control of inorganic nitrogen and suspended solids concentrations in a land-based recirculating aquaculture system," *Eng. J.*, vol. 17, no. 1, pp. 46-59, 2013.
- [9] A. Khammi, M. Kutako M, C. Sangwichien, and K. Nootong, "Development and evaluation of compact aquaculture system for the application of zero-water exchange inland aquaculture," *Eng. J.*, vol. 19, no. 2, pp. 15-27, 2015.
- [10] A. Kamstra, J. W. van der Heul, and M. Nijhof, "Performance and optimization of trickling filters on eel farms," *Aquacult. Eng.*, vol. 17, pp. 175-192, 1998.
- [11] B. L. Brazil, "Performance and operation of rotating biological contactor in a tilapia recirculating aquaculture system," *Aquacult. Eng.*, vol. 34, pp. 261-274, 2006.
- [12] S. T. Summerfelt, "Design and management of fluidized-sand biofilters," *Aquacult. Eng.*, vol. 34, pp. 275-302, 2006.
- [13] M. B. Timmons, J. L. Holder and J. M. Ebeling, "Application of microbead biological filters," *Aquacult. Eng.*, vol. 34, pp. 332-343, 2006.
- [14] T. Sesuk, S. Powtongsook, and K. Nootong, "Inorganic nitrogen control in a novel zero-water exchanged aquaculture system integrated with airlift-submerged fibrous nitrifying biofilters," *Bioresour. Technol.*, vol. 100, pp. 2088-2094, 2009.
- [15] K. Nootong, S. Powtongsook, and P. Pavasant, "Effect of organic carbon addition in controlling inorganic nitrogen concentrations in biofloc systems," *J. World Aquacult. Soc.*, vol. 42, no. 3, pp. 339-346, 2011.
- [16] P. Sittplangkoon, "Efficiency of inorganic nitrogen treatment of biological sludge from biofloc aquaculture system," M.S. Thesis, Dept. Envir. Eng., Chulalongkorn Univ., Bangkok, Thailand, 2013.
- [17] M. E. Azim and D. C. Little, "The biofloc technology (BFI) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*)," *Aquacult.*, vol. 283, pp. 29-35, 2008.
- [18] D. C. Little, F. J. Murray, M. E. Azim, W. Leschen, K. Boyd, A. Watterson, and J. A. Young, "Option for producing a warm water fish in the UK: limit to "green growth"?" *Trends Food Sci. Technol.*, vol. 19, pp. 255-264, 2008.

- [19] I. Chang, and S. J. Judd, "Air sparging of submerged MBR for municipal wastewater treatment," *Process Biochem.*, vol. 37, pp. 915-920, 2002.
- [20] P. Le-Clech, B. Jefferson and S. J. Judd, "Impact of aeration, solids concentration and membrane characteristics on the hydraulic performance of membrane bioreactor," *J. Membrane Sci.*, vol. 218, pp. 117-129, 2003.
- [21] J. P. Nywening and H. Zhou, "Influence of filtration conditions on membrane fouling and scouring aeration effectiveness in submerged membrane bioreactors to treat municipal wastewater," *Water Res.*, vol. 43, pp. 3548-3558, 2009.
- [22] APHA, AWWA and WEF, *Standard Methods for the Examination of Water and Wastewater*, 20<sup>th</sup> ed. Washington D.C.: American Public Health Association, 1998.
- [23] D. C. Montgomery and G. C. Runger, *Applied Statistics and Probability for Engineers*, 3rd ed. New York: John Wiley & Sons, 2002, pp. 278-306.
- [24] P. Phinitthanaphak, "Development of combined nitrification – denitrification reactor for nitrogen treatment from aquaculture systems," M.S. Thesis, Dept. Envir. Eng., Chulalongkorn Univ., Bangkok, Thailand, 2013.
- [25] Y. Chisti and M. Moo-Young, "On the calculation of shear rate and apparent viscosity in airlift and bubble column bioreactors," *Biotechnol. Bioeng.*, vol. 34, pp. 1391-1392, 1989.
- [26] M. B. Timmons, J. M. Ebeling, F. W. Wheaton, S. T. Summerfelt, and B. J. Vinci, *Recirculating Aquaculture Systems*, 2nd ed. Ithaca, NY: Cayuga Aqua Ventures, 2002, pp. 24-31.
- [27] S. A. Ali, "Design and evaluate a drum screen filter driven by undershot waterwheel for aquaculture recirculating systems," *Aquacult. Eng.*, vol. 54, pp. 38-44, 2013.
- [28] T. Ermukdakul, "Application of rapid and slow sand filtration for water treatment in aquaculture system," M.S. Thesis, Dept. Envir. Eng., Chulalongkorn Univ., Bangkok, Thailand, 2012.
- [29] Pollution Control Department Thailand. "Notification of Ministry of Natural Resources and Environment: Standards for Effluent Discharge from Freshwater Inland Aquacultures." [infofile.pcd.go.th](http://infofile.pcd.go.th). [http://infofile.pcd.go.th/law/3\\_56\\_water.pdf?CFID=8937914&CFTOKEN=47135004](http://infofile.pcd.go.th/law/3_56_water.pdf?CFID=8937914&CFTOKEN=47135004) (accessed 13 April 2020).
- [30] B. Hari, B. M. Kurup, J. T. Varghese, J. W. Schrama, and M. C. J. Verdegem, "The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems," *Aquacult.*, vol. 252, pp. 248-263, 2006.
- [31] T. Khin, and A.P. Annachatre. "Novel microbial nitrogen removal processes," *Biotechnol. Adv.*, vol. 22, pp. 519-532, 2004.
- [32] M. Kutako and K. Nootong, "Evaluation of upflow bioreactor system for treating low-strength nitrogenous wastewater under low-shear environment," *Songkla. J. Sci. Technol.*, vol. 37, no. 1, pp. 73-80, 2015.
- [33] B. Tang, H. Song, L. Bin, S. Huang, W. Zhang, F. Fu, Y. Zhao, and Q. Chen, "Determination of the profile of DO and its mass transferring coefficient in a biofilm reactor packed with semi-suspended bio-carriers," *Bioresour. Technol.*, vol. 241, pp. 54-62, 2017.
- [34] Z. Xiao-Hong, Z. Ming-Kai, Y. Tong, L. Yan-Chen, and S. Han-Chang, "Oxygen profiles in biofilm undergoing endogenous respiration," *Chem. Eng. J.* vol. 220, pp. 452-458, 2013.
- [35] M. S. Sales, and W. K. Shieh, "Performance of an aerobic/anaerobic hybrid bioreactor under nitrogen deficient and low F/M condition," *Water Res.*, vol. 40, pp. 1442-1448, 2006.
- [36] E. V. Munch, P. Lant, and J. Keller, "Simultaneous nitrification and denitrification in bench-scale sequencing batch reactor," *Water Res.*, vol. 30, pp. 277-284, 1996.
- [37] D. P. Thakur, and C. K. Lin, "Water quality and nutrient budget in closed shrimp (*Penaeus monodon*) culture systems," *Aquacult. Eng.*, vol. 27, pp. 159-176, 2003.
- [38] H. V. Daniels and C. E. Boyd, "Chemical budgets for polyethylene-lined brackish water ponds," *J. World Aquacult. Soc.*, vol. 20, pp. 53-60, 1989.
- [39] Y. S. Al-Hafedh, A. Alam, and M. Alam, "Performance of plastic biofilter media with different configuration in a water recirculation system for the culture of Nile tilapia (*Oreochromis niloticus*)," *Aquacult. Eng.*, vol. 29, pp. 139-154, 2003.



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