

*Article*

## Control of Inorganic Nitrogen and Suspended Solids Concentrations in a Land-Based Recirculating Aquaculture System

Kasidit Nootong<sup>1\*</sup>, Siwaruek Nurit<sup>1</sup>, and Sorawit Powtongsook<sup>2,3</sup>

<sup>1</sup> Chemical Engineering Research Unit for Value Adding of Bioresources, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, 10330, Thailand

<sup>2</sup> National Center for Biotechnology and Genetic Engineering, National Science and Technology Development Agency, Pathum Thani, 12210, Thailand

<sup>3</sup> Center of Excellence in Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

\* E-mail: kasidit.n@chula.ac.th (Corresponding author)

**Abstract.** This study intended to evaluate the performance of land-based aquaculture system integrating solids separating units, nitrifying biofilters, and tubular denitrifying reactor during the closed-water tilapia cultivation for 98 days. Operation of both solids separating units was able to maintain suspended solids (SS) concentrations in the rearing tank below 35 mg SS/L. Simultaneous operation of nitrifying biofilters, ORP-controlled denitrifying reactor, and two solids separating units was capable of maintaining total ammonia nitrogen (TAN) and nitrite concentrations substantially below 1.0 mg N/L and nitrate concentrations less than 12.0 mg N/L despite nitrogen loading rate and tilapia weight density were as high as 21.6 mg N/L/day and 17.2 kg/m<sup>3</sup>, respectively. Without solids separating units, TAN and nitrite concentrations drastically increased above the acceptable limits of 1.0 mg N/L. Finally, the result of nitrogen balance analysis indicated that nitrification and denitrification were the primary treatment pathways in this recirculating system capable of removing 49.8% of total nitrogen input, whereas solids separating units removed 9.5% of total nitrogen input yet their presence was essential for sustaining the activity of nitrifying biofilters for an extended period.

**Keywords:** Nitrification, denitrification, solids, tilapia, aquaculture.

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## 1. Introduction

Recirculating aquaculture systems are the effective means for environmentally friendly and high productivity of fish and shrimp production [1-2]. Two related problems occur frequently during an operation of recirculating aquaculture systems, namely excessive production of solids and accumulation of inorganic nitrogen compounds [3-5]. Solids in aquaculture system are originated from excretion of cultured animals, unconsumed feed, and new microbial biomass formed in cultured units. Controlling the amounts of solids is necessary to the successful aquaculture cultivation because biodegradation of solids by different organisms produce various forms inorganic nitrogen compounds such as ammonia and nitrite, which can be acutely and chronically harmful towards aquatic animals at concentrations above 1.0 mg N/L [6]. In addition, discharges of production water containing high solids and inorganic nitrogen concentrations can exert negative effects to aquatic environment, namely toxicity to fish, depletion of oxygen in water, and eutrophication [7-9].

In order to control the quantity of solids in production water, different unit operations namely centrifugation pumps, microbeads filters, rotating drum filters, swirl separators, and microscreening are available to reduce the amounts of solids in water with the removal efficiency depending on solids loadings and solids particle sizes [3]. Setbacks of these solids removal devices, however, are high investment and operational costs, which are primary concerns for farmers with limited budget such as those in developing countries. For the control of inorganic nitrogen compounds, biofiltration based on nitrification has been generally employed to convert ammonia to intermediate nitrite, and finally to nitrate under aerobic condition [6]. In spite of successful ammonia and nitrite treatment, prolonged operation of nitrifying biofilters leads to high nitrate accumulation in cultured unit that can cause acute health effects in aquatic animals as well as create environmental concerns if the discharged water is not properly treated [7, 9-10]. Heterotrophic denitrification is often cited as a biological process to complete inorganic nitrogen treatment because it reduces nitrate into nitrogen gas with organic carbon served to provide electrons for heterotrophic bacteria under anaerobic condition [10]. Employment of heterotrophic denitrification for nitrate removal in domestic and industrial wastewater is common, yet reports on the application for aquaculture effluent have been limited [2, 10]. Another operational difficulty associated with employing biofilters, especially during intensive cultivation with the animal weight density greater than 5.0 kg/m<sup>3</sup>, is solids accumulation on biofilters surface, leading to the decrease in nitrifying activities and subsequently failure to control water quality [10-11].

In our previous work, the recirculating aquaculture system integrated with submerged nitrifying biofilters in the rearing tanks, denitrifying biofilters, and solids filtration unit was built to support tilapia cultivation without water exchange for 81 days [12]. Result of that study indicated extremely high concentrations of suspended solids that led to unstable performance of nitrifying and denitrifying biofilters in maintaining acceptable concentrations of TAN, nitrite, and nitrate. As a result, the following modifications were made in this study to the original design of the recirculating aquaculture system including (1) placement of nitrifying biofilters outside the rearing tank; (2) changing the type of nitrifying biofilters media; and (3) replacing crossflow filtration unit with the newly developed solids separating unit, which was operated based on gravitational sedimentation. The modified recirculating aquaculture system was then tested for its performance by growing tilapia without water exchange for 98 days. Therefore, this article aims to describe the preliminary evaluation of the laboratory scale recirculating aquaculture system featuring submerged nitrifying biofilters, tubular denitrifying reactor, and solids separating units for its ability to control inorganic nitrogen and solids concentrations.

## 2. Materials and Methods

### 2.1. Description of Recirculating Aquaculture System

Figure 1 illustrates a schematic diagram of the recirculating system used in this experiment.

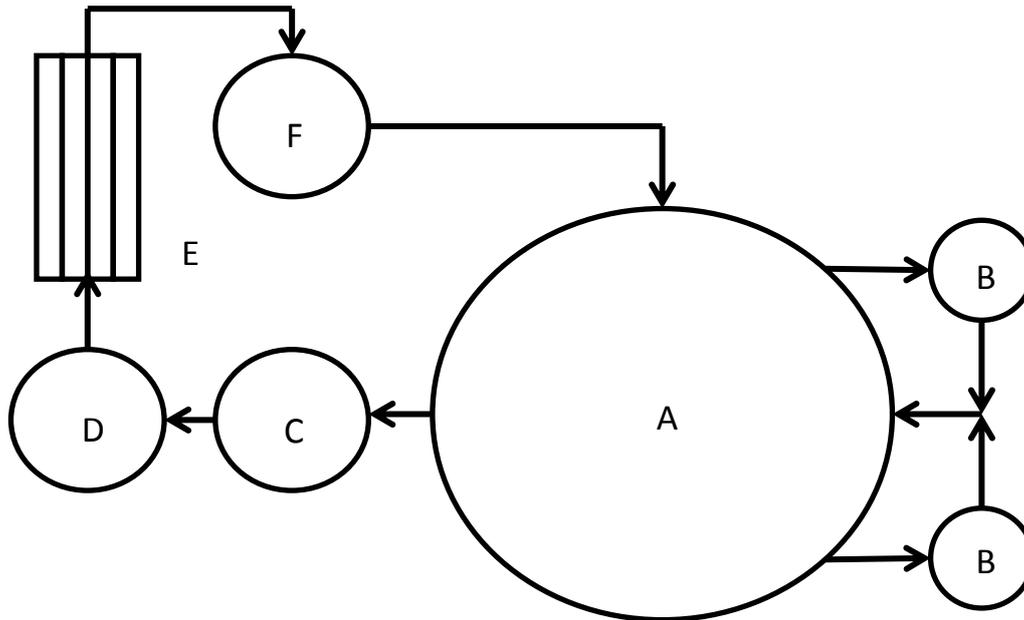


Fig. 1. Schematic diagram of the recirculating aquaculture system: (A) rearing tank (B) solids separating units (C) nitrifying biofilters unit (D) methanol addition and mixing unit (E) tubular denitrifying reactor and (F) aeration unit.

Circular tank (inner diameter 2.3 m; height 0.8 m; working volume 4 m<sup>3</sup>) was connected to an enclosed plastic PVC column (inner diameter 0.3 m; height 1.0 m), which was used as a nitrifying unit to convert ammonium to nitrate. Inside the nitrifying unit was filled with BCN-009 media (polypropylene; specific surface area 864 m<sup>2</sup>/m<sup>3</sup>) to attain the bed height of 0.6 m. BCN-009 media were already acclimatized to attain the complete nitrification before starting the experiment according to the procedure available in previous report [11]. Aeration of nitrifying biofilters was accomplished by using air diffusers located at the bottom of the media bed. In this experiment, nitrate removal system consisted of three main components namely methanol addition column, tubular denitrifying reactor, and aeration column. Design of tubular denitrifying reactor was based on the previous work except that peristaltic pumps were replaced by geared pump to reduce cost [12-13]. BCN-009 media were also chosen to immobilize heterotrophic bacteria responsible for denitrification and were packed into horizontal PVC tubes (inner diameter 0.038 m; length 4.0 m; 6 tubes), which were connected together to attain the total length of 24.0 m. Preparation of denitrifying biofilters followed the procedure available in literature [12]. Methanol (2% by volume) at 0.03 L/hr was mixed with the effluent from nitrifying biofilters unit and the liquid started to accumulate in plastic PVC column (inner diameter 0.3 m; height 1.0 m). Once water in the column reached the leveled set point, the controller signaled pumping to stop and water started to flow by gravity through tubular denitrifying reactor, and accumulated in another column (inner diameter 0.3 m; height 1.0 m), where aeration by air diffusers is provided to increase DO concentrations before water returned to the rearing tank. Solids removal was carried out by using two solids separating units, which were hollowed plastic PVC cylinders (inner diameter 0.3 m; height 1.10 m) with the inside being inserted by the 8-leveled plastic PVC discs (diameter 0.3 m; thickness 0.001 m; disk orientation 15° to the ground). Discs were mounted to plastic PVC pipe (inner diameter 0.025 m; outer diameter 0.03 m; height 1.80 m) with spaces between discs at 0.1 m. Water inlet and outlet were located at 0.1 and 1.0 m from the bottom of plastic cylinder, respectively. The highest range of solids removal efficiency for the described solids separating unit was determined from  $71.3 \pm 2.4$  to  $73.7 \pm 1.1\%$  when the upflow volumetric flow rate was maintained at 110 L/hr [14]. Water was circulated between the rearing tank and solids separating units by maintaining the flow rate at 110 L/hr. Figure 2 displays the schematic diagram of the solids separating unit.

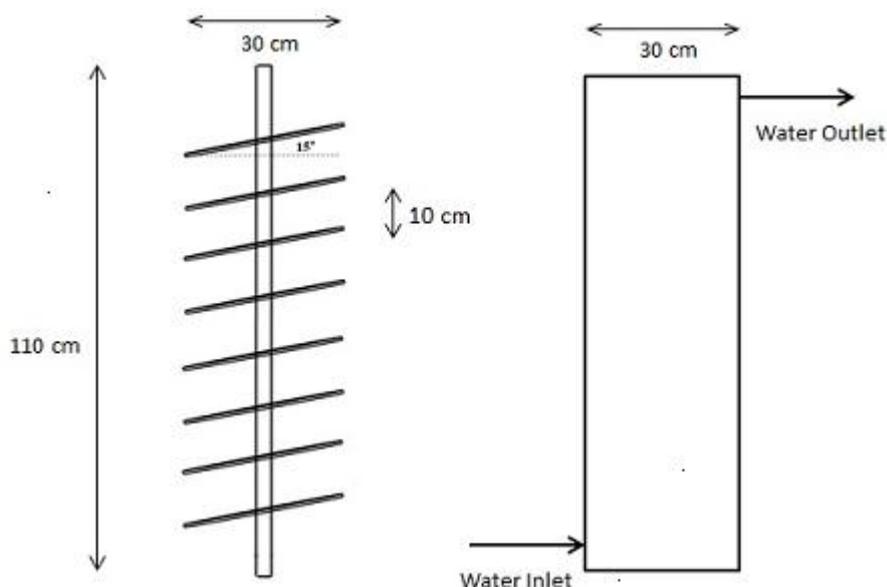


Fig. 2. Schematic diagram of the solids separating unit: (left) the interior design with 8-levelled discs (right) exterior sketch showing water inlet and outlet.

It should be mentioned that for the last three weeks of the experiment probe for oxidation-reduction potential (ORP) and controller (Hanna model BL932700) were installed at the outlet of tubular denitrifying reactor to control the rate of methanol addition.

## 2.2. Evaluation of Recirculating Aquaculture System

Recirculating aquaculture system described in section 2.1 was tested without replication by growing male tilapia (*Oreochromis niloticus*) without water exchange for 98 days. Volumetric flow rate of recirculating aquaculture system and solids separating units were maintained at 200 and 110 L/hr, respectively for the entire experiment. Tilapia with average weight of 4.8 g/fish was stocked in the rearing tank to obtain an initial weight density of 3.0 kg/m<sup>3</sup> (2,500 tilapia/tank). Tilapia fed twice daily in the morning (9:00 hours) and afternoon (17:00 hours) with 30% protein commercial feed pellets at 3% of total fish weight per day. The remaining feeds were collected, dried overnight in oven, and weighed. Approximately half of tilapia population from the rearing tank was caught monthly to measure weight, which was used to calculate fish growth data. Constant aeration by air diffusers and NaHCO<sub>3</sub> addition were carried out in the rearing tank to maintain proper condition for tilapia and nitrifying bacteria (i.e., well-mixed; DO > 4.0 mg O<sub>2</sub>/L; pH = 7 – 8; alkalinity = 100 – 150 mg CaCO<sub>3</sub>/L). The rearing tank was covered with plastic lid to prevent rainwater and sunlight. Water samples from the rearing tank were obtained daily and immediately analysed for total ammonia nitrogen (TAN), nitrite, nitrate, and suspended solids according to APHA (1998) [15]. In addition, at the end of experiment, solids samples from the rearing tank, inside the solids separating units, and effluent of solids separating units were collected and examined for particle sizes using the laser particle size distribution analyser (Malvern Model Mastersizer-S). Nitrogen contents of solids were determined using CHN analyzer based on the Pregl-Dumas method [16].

## 3. Results and Discussion

### 3.1. General Water Quality

Table 1 demonstrates that ranges of pH, temperature and alkalinity were within the acceptable ranges for practical aquacultures [6].

Table 1. Water quality in the rearing tank throughout the experiment.

Parameters	Range	Average	Recommended Range [6]
DO (mg O <sub>2</sub> /L)	4.83 – 7.20	5.88 ± 0.89	> 4.0
Temperature (°C)	25.30 – 28.90	26.49 ± 1.17	20 – 35
pH	7.30 – 7.88	7.61 ± 0.18	6.5 – 8.5
Alkalinity (mg CaCO <sub>3</sub> /L)	130 – 150	140 ± 8.8	50 – 300

DO concentrations in the rearing tank decreased from 7.2 to 4.83 mg O<sub>2</sub>/L during the 98-days cultivating period but still be considered a non-limiting growth factor in this experiment. Decreasing DO concentration was likely the results of increasing oxygen consumption by tilapia as the weight of animals increased as well as microbial requirement for respiration, aerobic biodegradation of organic compounds, and nitrification [12, 17-18]. Temperature remained quite constant at 26.5 ± 1.27 °C but exhibited a slight increase to about 29 °C during the final two weeks of the experiment. Average pH in the rearing tank was measured at 7.61 ± 0.18. The values of pH were observed to decrease gradually from 7.70 to 7.30 since Day 60. Increasing CO<sub>2</sub> production due to animal respiration and nitrification by in suspended solids was the likely explanation for the decreasing pH [17-18]. Alkalinity was initially set at 150 mg CaCO<sub>3</sub>/L and later fluctuated between 120 and 150 mg CaCO<sub>3</sub>/L throughout the experiment.

### 3.2. Control of Suspended Solids Concentrations

The source of solids in this experiment came from unconsumed feed pellets, animal feces and microbial biomass formed in cultured tank. Performance of solids separating unit in controlling suspended solids concentrations in the rearing tank is presented in Fig. 3(a).

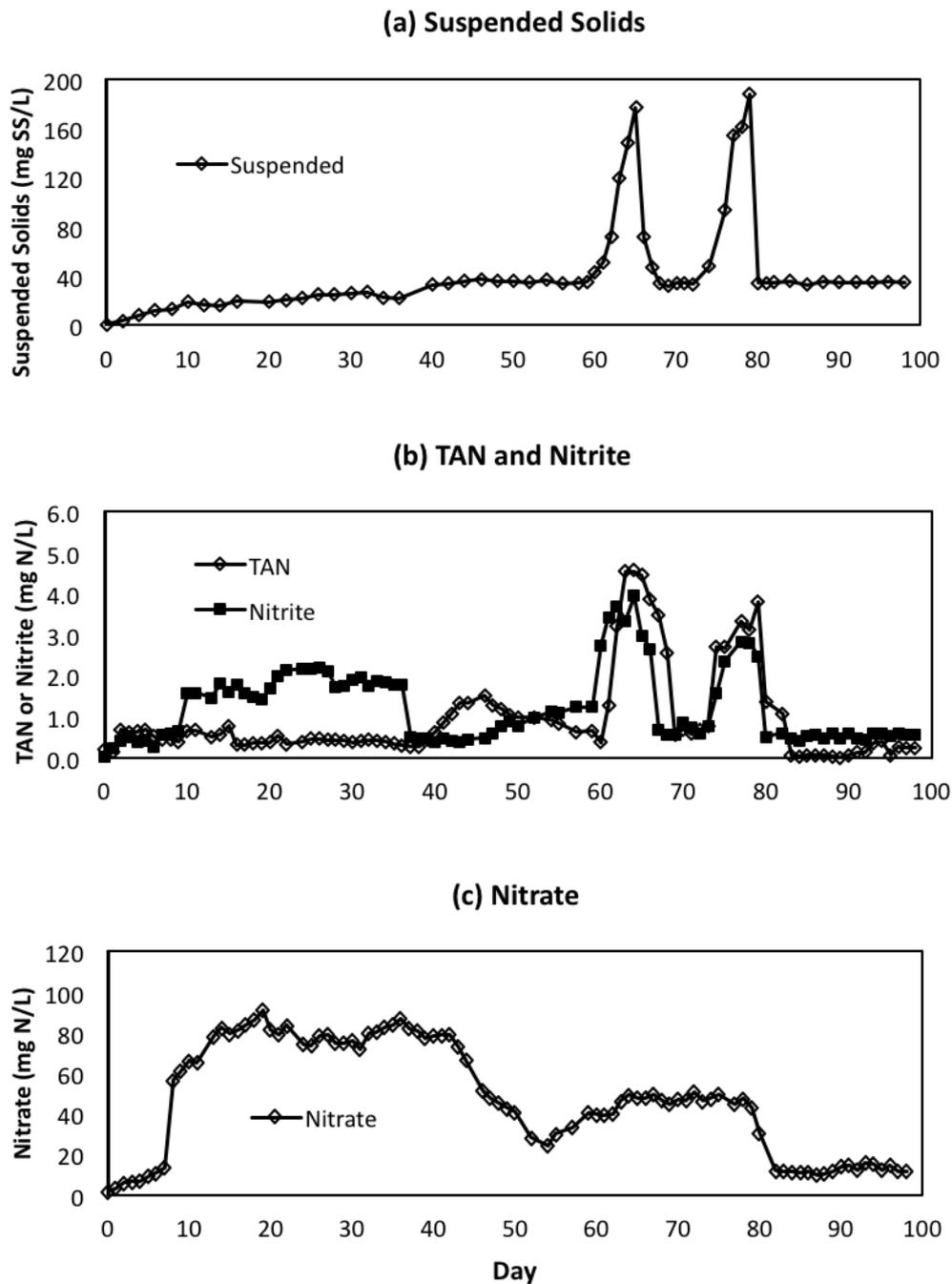


Fig. 3. (a) Suspended solids concentrations in the rearing tank; (b) TAN and nitrite concentrations in the rearing tank; and (c) nitrate concentrations in the rearing tank.

The presence of solids separating units was essential to the control of suspended solids concentrations in the rearing tank. Operation of two solids separating units led to effective control of suspended solids concentrations below 35 mg SS/L even though cumulative feed and tilapia weight density reached 112.5 kg and 17.2 kg/m<sup>3</sup>, respectively. Suspended solids concentrations were also substantially less than the effluent discharged limitation set at 80 mg SS/L [19]. In contrast, the rapid increase of suspended solids concentrations was observed shortly after the operation of solids separating units were halted. Peak concentrations at 177 and 188 mg SS/L were measured on Day 65 and Day 79, respectively. Effective control of suspended solids concentrations observed in this study was different to our previous work, which employed crossflow filtration unit for potable water to remove solids in the rearing tank [12]. Results of that work indicated heavy solids accumulation on filters surface and rapid increase of suspended solids

concentrations in the rearing tank above 500 mg SS/L just after four weeks into the tilapia cultivation at similar weight densities [12]. High suspended solids levels above 500 mg SS/L was also known to exert negative effects on cultured animals, namely clogging of gills, hindering oxygen transport capability and reducing the visibility of animals to find feeds [20]. Although the inclusion of solids separating units improves the capability of the recirculating aquaculture system in maintaining acceptable suspended solids concentrations, the solids separating units were still unable to remove tiny solids particles from water. Result of particle size analysis revealed that the solids separating units allowed passage of particles with the size smaller than 60  $\mu\text{m}$  while the range of particle size in the rearing tank varied from 20 to 120  $\mu\text{m}$ . Tiny solids particles could attach to gill filament of fish and affected respiration [20-21]. Aggregation of solids particles might also take place inside the solids separating units, resulting in larger particle sizes ranged from 220 to 360  $\mu\text{m}$ .

### 3.3. Control of Inorganic Nitrogen Concentrations

Nitrifying and denitrifying biofilters were employed as primary means for treatment of TAN, nitrite, and nitrate concentrations. In the original design of the recirculating aquaculture system, nitrifying biofilters were submerged in the rearing tank with aims to reduce space requirement, and yet the performance of nitrifying biofilters in the original design deteriorated significantly after the second month into operation due to heavy deposition of solids on biofilters surface [12]. Causes of solids depositions were directed to clogging of filtration unit and characteristics of nitrifying biofilters media (Biocord™), which were effective in intercepting and retaining solids [11-12, 22]. Based on these reasons, the following modifications were made to the recirculating aquaculture system in this study including (1) development of solids separating units; (2) changing biofilters media from Biocord™ to BCN-009, which was less susceptible to solids retention; and (3) locating nitrifying biofilters outside the rearing tank to lower solids accumulation on biofilters surface.

The first period of cultivation, which extended from Day 1 to 36, involved simultaneous operation of solids separating units and nitrifying biofilters. Effluent from nitrifying biofilters was allowed to flow through tubular denitrifying reactor to prevent drying of denitrifying biofilters before returning to the rearing tank. According to Figs. 3(b) and 3(c), which illustrate the results of inorganic nitrogen analysis, it appeared that nitrifying biofilters could mediate nitrification process almost immediately, resulting in TAN and nitrite concentrations below 1.0 mg N/L. Addition of sodium nitrate at 50 mg N/L was conducted into the denitrifying reactor after the first week to prevent the production of hydrogen sulfide and this led to a rapid increase of nitrate concentrations. The gradual decrease of nitrate concentrations from 91 to 74 mg N/L accompanied by a fluctuation of nitrite in range from 1.0 to 2.2 mg N/L were observed from Day 11 to 36 and could have been the result of incomplete denitrification with decayed biomass served as organic carbon source for heterotrophic bacteria [13].

In the next period from Day 37 to 46, methanol at 0.95 g/hr was supplied into the tubular denitrifying reactor while solids separating units and nitrifying biofilters remained operated as normal. Although the experiment did not conduct the DO measurement in the tubular bioreactor, establishment of heterotrophic denitrification could be confirmed by the concentration profiles of inorganic nitrogen compounds that indicated the reduction of nitrate concentrations from 86 to 51 mg N/L while nitrite concentrations were negligible (nitrite < 0.5 mg N/L) (Fig. 3). Another confirmation of heterotrophic denitrification was the range of oxidation-reduction potential (ORP = -215 to -333 mV), which concurred within the range reported for heterotrophic denitrification between -200 and -400 mV [10, 23]. The rate of methanol addition might be excessive at the moment and likely to exert negative effects on tilapia because significant amounts of feed pellets were left uneaten. Excess methanol could also promote the growth of heterotrophic bacteria in the rearing tank to start degrading proteins in solids into ammonia, thereby explaining the slight increase of TAN concentrations from 0.4 to 1.51 mg N/L in this period. Methanol addition was stopped from Day 47 to 60 to reduce the effects towards tilapia. It was noticed that tilapia started consuming more feed pellets after stop methanol feeding and were able to consume the entire feeds provided after Day 50.

Performance of nitrifying biofilters was greatly affected when operation of solids separating units were stopped from Day 61 to 65. Without solids removal, suspended solids concentrations in this period increased significantly while the ability of nitrifying biofilters to maintain TAN and nitrite concentrations within acceptable limits became adversely affected as can be seen in Fig. 3. The average values of TAN and nitrite in this period were  $3.61 \pm 1.42$  and  $3.47 \pm 0.37$  mg N/L, respectively and were substantially higher

than the average TAN and nitrite concentrations from Day 20 to 60, which were measured at  $0.59 \pm 0.32$  mg N/L for TAN and  $1.19 \pm 0.69$  mg N/L for nitrite. Similar results were also observed after the solids separating units were halted again from Day 74 to 79. In contrast, the use of solids separating units not only decreased suspended solids concentrations in the rearing tank to the level observed previously (30 – 35 mg SS/L) but also reduced TAN and nitrite concentrations to under 1.0 mg N/L (Fig. 3). For nitrate, the concentrations varied between 40 and 51 mg N/L during the period from Day 61 to 79 with the average concentration determined at  $46 \pm 3.1$  mg N/L. Fluctuation of nitrate during the past two weeks could be linked to the offset between nitrifying and denitrifying reactions with remaining solids and decayed biomass served to provide carbon source for heterotrophic bacteria [13].

The final period, which extended from Day 80 until the end of experiment, involved simultaneous operation of solids separating unit, nitrifying biofilters, and tubular denitrifying reactor. Unlike earlier periods, ORP probe and controller were installed at the outlet of denitrifying reactor as means to maintain the rate of methanol addition. Installation of control equipment was made in response to the results observed earlier when tilapia feed consumption and the ability to maintain inorganic nitrogen were affected by the rate of methanol addition. ORP controller signalled methanol pump to supply methanol into denitrifying reactor when ORP was within the range between -200 and -400 mV and to stop methanol feeding when ORP was lower than -400 mV. Results of water analysis, as demonstrated in Fig. 3, indicated that integration of solids separating units, nitrifying biofilters, and ORP-controlled denitrifying reactor was effective in sustaining inorganic nitrogen concentrations within acceptable limits although the daily nitrogen loading rates were as high as 21.6 mg N/L/day (i.e., approximately 9 folds higher than the initial rate applied on Day 1). TAN and nitrite concentrations in this period were substantially less than 1.0 mg N/L with the average values determined at  $0.25 \pm 0.23$  and  $0.52 \pm 0.06$  mg N/L, respectively. Continuous nitrate removal was also possible as can be confirmed by low nitrate concentrations averaging at  $12 \pm 1.8$  mg N/L.

Finally, the nitrogen mass balance around the recirculating aquaculture system was performed at the end of the experiment by estimating nitrogen input and output from feeds, tilapia, solids, and dissolved inorganic nitrogen (TAN, NO<sub>2</sub>-N, and NO<sub>3</sub>-N) (Table 2).

Table 2. Nitrogen balance calculation displaying the distribution of nitrogen in the recirculating aquaculture system.

Parameters	Values
<i>Nitrogen input</i>	
Feed pellets (g N)	3,667 (92.6%)
Seeding water (g N)	4.6 (0.1%)
Tilapia	288 (7.3%)
Total input (g N)	3,959.6 (100%)
<i>Final Day (Day 98)</i>	
Tilapia (g N)	1,561 (39.4%)
Suspended solids (g N)	2.8 (0.1%)
Removed solids (g N)	375 (9.5%)
Dissolved inorganic nitrogen (g N)	49 (1.2%)
Unaccounted nitrogen (g N)	1971.8 (49.8%)

Nitrogen in solids was calculated from the result of CHN analysis, which indicated the nitrogen content of 15% dried weight. Nitrogen in tilapia was assumed at 15% of tilapia wet weight [24]. Clearly, the majority of nitrogen input came from feed. At the end of the experiment, the majorities of nitrogen input were accounted in forms of fish biomass, solids, and unaccountable portion. Denitrification and ammonia stripping were normally assumed as pathways for unaccounted nitrogen. In this study, ammonia stripping was not expected to be significant because TAN concentrations were relatively low and pH was between 7 and 8 so that the major fraction of TAN was in the soluble ionized form ( $\text{NH}_4^+\text{-N}$ ), and thus denitrification was more likely the cause of nitrogen loss. Percentages of nitrogen retention in fish were also

comparable to other works, which reported nitrogen assimilation in fish varied from 25 to 40% regardless of fish species [2, 16, 25]. In addition, solids separating units were not the primary means of inorganic nitrogen treatment in this study because the portions of nitrogen removed by solids separating units were only 9.5% compared to 49.8% for denitrification. However, the presence of solids separating units was critical to the effectiveness of recirculating aquaculture system because it helped maintaining the activities of nitrifying biofilters for extended period and prevented excessive suspended solids concentrations in rearing water that can be harmful to tilapia.

### 3.4. Tilapia Growth

Growth performance of tilapia is displayed in Table 3.

Table 3. Growth performance of tilapia during the zero-water exchange cultivation.

Parameters	Average
Initial tilapia weight (g/fish)	4.8 ± 1.77
Final tilapia weight (g/fish)	86.2 ± 20.77
Initial tilapia length (cm/fish)	6.62 ± 0.86
Final tilapia length (cm/fish)	16.14 ± 2.91
Initial tilapia weight density (kg/m <sup>3</sup> )	3.0
Final tilapia weight density (kg/m <sup>3</sup> )	17.2
Survival rate (%)	84
Feed conversion ratio (FCR)	1.63
Growth rate (g/day)	
Entire cultivation (98 days)	0.83
Day 1 – 35	0.54
Day 36 – 50	0.55
Day 51 – 69	0.82
Day 70 – 84	1.00
Day 85 – 98	1.70

Tilapia survival rate was measured at 84% in this study and was comparable to previous studies, which nitrifying biofilters or combined nitrifying and denitrifying biofilters [11, 12, 22, 26]. Majority of tilapia death occurred during the first two weeks of the cultivation and could be linked to the conditions of tilapia, which appeared unhealthy and perhaps could not adjust to the new environment after releasing into the rearing tank. In spite of acceptable water parameters (i.e., DO, temperature, pH, and alkalinity), the average growth rates of tilapia for the entire cultivation (0.83 g/day) were lower than those from previous studies, which reported the growth rate in the range from 0.85 to 2.30 g/day for tilapia reared in cultivating systems with either nitrifying and denitrifying biofilters [11, 12, 22, 26-28]. Low growth rates of tilapia could be related to lengthy exposure to TAN and nitrite concentrations above the acceptable limits of 1.0 mg N/L as well as long exposure to high nitrate concentrations [6, 10]. However, tilapia growth rates were observed to increase as the cultivation progressed, increasing from 0.54 g/L during the first month to 1.70 g/day for the final period from Day 81 to 93. Significant improvement of tilapia growth rates concurred with the period when the solids separating units, nitrifying biofilters, and ORP-controlled denitrifying reactor were operated simultaneously. However, this observation contradicted to the results of previous experiments that indicated the retardation of tilapia growth with increasing body size [29-30].

## 4. Conclusions

Based on the findings of this study, the following conclusions could be drawn: (1) operation of two solids separating units was capable of maintaining suspended solids concentration below 35 mg SS/L despite the

tilapia weight density was as high as 17.2 kg/m<sup>3</sup>; (2) without the solids separating unit, the drastic increase of suspended solids concentrations was accompanied by significant increase of TAN and nitrite concentrations above the acceptable limits of 1.0 mg N/L; (3) controlling ORP proved to be the effective means to control the rate of methanol addition; (4) simultaneous use of solids separating units, nitrifying biofilters, and ORP-controlled denitrifying reactor could maintain the concentrations of TAN, nitrite, and nitrate within practical limits for aquacultures given that the nitrogen loading rate was as high as 21.6 mg N/L/day; and (5) nitrification and denitrification were the primary means for inorganic nitrogen treatment while the presence of solids separating units was essential for prolonging the activities of nitrifying biofilters.

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