Design and function of a nitrogen and sediment removal system in a recirculating aquaculture system optimized for aquaponics

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ABSTRACT

Aquaponic systems (APSs) are based upon the sustainable utilization of nitrogen and phosphorus from a recirculating aquaculture system (RAS) as the nutrient source for plant production. Since the proper management of nitrogen and suspended solids are important for nutrient utilization efficiency, their optimization was evaluated. The RAS integrated with filtration unit and nitrifying biofilter provided complete nitrification without solid accumulation. Under the optimal treatment condition for 16 d, the treated water was low in ammonia (0 mg-N/L) and high in nitrate (> 6 mg-N/L) concentrations, which was appropriate for use in the cultivation of lettuce (Lactuca sativa). The RAS was then incorporated with hydroponics at a 5:1 fish: plant weight ratio. Moreover, the simplified APS was compared with a typical APS system that incorporated filtration unit and nitrifying biofilter. The natural growth of nitrifying microorganisms in the simplified APS could perform complete nitrification after 20 d of operation giving low ammonia and nitrite concentrations. The nutrient removal efficiency of the simplified APS resembled the typical system. During the aquaponics, the hydroponic unit in the aquaculture system was sufficient to control the nutrient concentrations within the appropriate levels for fish cultivation, i.e. nitrate (< 20 mg-N/L) and phosphate (< 3 mg-P/L).

Keywords: Aquaponics, Green Oak Lettuce, Nitrification, Nitrifying biofilter, Sediment filtration

1. Introduction

Nutrient enrichment is a major problem in fish cultivation and is directly associated with waste excretion where 50–80% of the feed nitrogen and 35–85% of the feed phosphorus are retained in an aquaculture system [1]. Inorganic ammonia is the main nitrogen excretory product of fish and it affects their growth even at a low concentration [2]. Advanced water treatments, such as membrane filtration, anammox process [3] or heterogeneous photocatalysis [4], are limited due to their complexity and cost of operation. In the typical operation of nitrogen treatment in a recirculating aquaculture system (RAS), ammonia is sequentially oxidized into nitrite and nitrate via nitrification by the cooperation of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), while the addition of a nitrate removal system by heterotrophic bacteria through anoxic denitrification is required in intensive fish cultivation for long-term operation [5, 6]. With phosphorus, on the other hand, the concentration of dissolved phosphate in the RAS is not high enough to adversely influence fish health since a large portion of the phosphate is removed from solution in both deposited and suspended sediments. Therefore, the removal of phosphate can be achieved either by a sediment separation process or by incorporating a complex anoxic treatment with either phosphate accumulating organisms (PAOs) or heterotrophic denitrifiers [7].

Instead of using anoxic treatments for nitrate and phosphate removal, an aquaponic system (APS) is an alternative sustainable system to eliminate excess nutrients in the RAS by hydroponic
cultivation of plants [8]. With the combination of plant cultivation in the aquaculture system, the main pathway of nutrient recycling is the utilization of nitrogen and phosphorus as a source of internal nutrients for plant growth. In general, the efficiency of nutrient absorption in hydroponics depends on many factors, such as the nutrient concentration, plant species, growth rate, development stage, and environmental factors, e.g. light intensity [9]. Previous studies reported that an increased nutrient concentration resulted in an improved nutrient utilization efficiency of the APS [9, 10].

For nitrogen utilization, although plants can uptake nitrogen in the form of both ammonium and nitrate ions, most studies have evaluated nitrate as the major soluble nitrogen source for plant cultivation [8-10]. This is because of the decreased level of ammonium ions produced in the RAS after 20–30 d cultivation [8-10]. Apart from the decreased level of ammonium ions, previous studies have verified the efficiencies of various types of media applied for ammonia treatment in the RASs [13-15].

Apart from nitrogen treatment, the excess suspended solids (SS) should also be removed to maintain the efficiencies of nitrification and nutrient assimilation. The installation of a solid removal system, such as a sludge clarifier and filtration unit, can prevent the clogging of solid particles on the biofilter and plant roots, as well as minimize the sedimentation in the APS [12, 16, 17]. Thus, with the incorporation of nutrient and solid managements, it was previously reported that the flow sequence of a typical APS normally begins with the aquaculture tank, followed by solid separation, nitrification bioreactor, and hydroponic system, respectively [8].

Nevertheless, since the APS involves multiple disciplines, e.g. aquaculture, agriculture, microbiology and engineering, the complex system design and operation are still needed to be simplified [8]. Under the normal operation of a RAS, the microorganisms involved in nitrification occur naturally along with fish cultivation [18]. Thereafter, the population level and diversity of nitrifying bacteria can be enhanced by the explicit conditions such as high ammonium, oxygen and inorganic carbon (e.g. bicarbonate) supplementation. Microbial cell growth is not only found on the artificial biofilter media, but they also in suspension or attached on the tank wall, pipeline, and, especially, on plant roots in the APS [12]. Therefore, with proper management, the natural occurrence of ammonia conversion to nitrate by nitrification is possible after a certain period (e.g. > 30 d) of RAS operation.

Due to these natural treatment processes, the simplification of the APS design was performed and evaluated in this study by replacing the sophisticated nitrification biofilter device with a natural nitrification treatment in both the aquaculture tank and the hydroponic unit. Accordingly, removal of nutrients in the simplified APS was evaluated with the experimental scale units against a typical APS with a sediment removal and nitrification device. Apart from laboratory scale, the experimental scale was maintained close to a practical system to more realistically evaluate the precise efficiency and to estimate the exact cost of each system. With the minimal component of this simplified APS design, the outcomes (advantages) will be useful for the design and operation of an efficient system that is less complicated and minimizes the space required for reactor installation.

2. Material and Methods

2.1. Optimization of Nitrogen and Solid Removal in the RAS

To optimize the nitrogen and solid removal, four types of the recirculating aquaculture system (RAS) were compared, i.e. (i) control, (ii) treatment-1 (T1) with a filtration unit, (iii) treatment-2 (T2) with a moving bed nitrifying biofilter media, and (iv) treatment-3 (T3) with both a filtration unit and a nitrifying biofilter. Schematic diagrams of the experimental units are shown in Fig. 1, while the costs of the experimental unit components are summarized in Table S1.

Each RAS unit consisted of a 120-L aquaculture tank for Nile tilapia (Oreochromis niloticus) cultivation at an initial density of 1 kg/m³ with the average initial weight and length of 6.67 ± 0.91 g and 7.06 ± 0.28 cm, respectively. The filtration unit was installed next to the aquaculture tank in T1 and T3. This unit comprised of three sets of cylinder-shaped 150-μm stainless-steel screen filters (22 cm in length, 10 cm in diameter), in order to trap the suspended solids (SS) from the overflowed effluent. Within the 120-L under-neath treatment tank, a total volume of 1-L of 3-cm porous cylinder shaped plastic media (BCN-012 KLL; 2H GmbH, Germany), with a specific surface area of 859 m²/m³, specific weight of 150 kg/m³, and density of 0.95 g/cm³, was used as the moving bed nitrifying biofilter in T2 and T3. The nitrifying biofilter was pre-acclimated in synthetic wastewater containing 2 mg-N/L NH₄Cl (99.5% pure, 30 d operation when
AR grade, Loba Chemie, Mumbai, India), and maintaining the alkalinity between 150–200 mg-CaCO₃/L by the addition of NaHCO₃ (99.0% pure, food grade, Haohua Honghe Chemical, Sichuan, China). The process of biofilter acclimation was performed in advanced for 60 d to achieve complete nitrification prior to use in the experiment [19].

For experimental operation, the aquaculture was performed in a recirculating system. Fish were fed twice daily at 1–3% (w/w) of the total fish weight per day with an artificial feed containing 20% (w/w) protein. The water in the control and T2 fish tanks directly flowed down to the underneath treatment tank by gravity. On the other hand, the gravity flowed water in T1 and T3 was passed through the filtration unit while the solids deposited on the stainless-steel screen were removed weekly to prevent filter clogging. For nitrogen removal, the nitrifying biofilter media in the T2 and T3 treatment tanks was operated under a moving bed reactor scheme, whereas there was no plastic media in both the control and T1 tanks. The treated water from the underneath treatment tank in each system was finally pumped up to the respective aquaculture tank using a submersible pump (Resun SP-600, China) with an adjusted flow rate of approximately 100 L/h. This experiment was continuously operated for 16 d without water exchange with the following conditions: dissolved oxygen (DO) of > 5 mg/L [20, 21], pH of 6.4–9 [22], and alkalinity of > 100 mg-CaCO₃/L [18].

2.2. Efficiency Assessment of the APS

The efficiency of the aquaponic system (APS) was compared between the control, which was a typical APS with a filtration unit and a nitrifying biofilter (Fig. 2(a)), and a simplified APS system with natural nitrification derived from the control system in the previous experiment (Fig. 2(b)). Each APS consisted of two components, i.e. a hydroponic unit for Green Oak lettuce (Lactuca sativa) and an aquaculture unit for the Nile tilapia cultivation. Within the hydroponic unit, the lettuce seeds were sown in a wet sponge for 5 d, and then the sprouts were cultivated in a 20-L open top plastic box containing 1% (v/v) liquid fertilizer (Forfarm, Pathum Thani, Thailand) for 20 d prior to use in the study. The lettuce growing conditions were as follows: light-dark cycle of 12:12 h [23], light-emitting diode intensity of 10,000 lux [24], pH of 5.8–6 [22, 25], and electrical conductivity (EC) of 1,000 μS/cm [25]. The fish were reared in an aquaculture unit at an initial density of 1 kg/m³ with the average initial weight and length of 22.16 ± 5.09 g and 10.59 ± 0.66 cm, respectively. With respect to the typical APS, a 150-μm stainless-steel screen filter in the filtration unit coupled with the 1-L pre-acclimated BCN-012 biofilter media in the nitrification tank were applied in order to remove excess solid and nitrogen. Meanwhile, there was no treatment unit in the simplified APS system.

The experimental period was divided into two phases. During phase I (day 0–30), the RAS was operated solely at a fish feeding rate of 1–3% (w/w) of the total fish weight per day. Water in the typical APS (control) flowed through the filtration unit to the underneath nitrification tank. In contrast, the gravity flowed water in the simplified APS (treatment) was directly pumped back to the aquaculture tank at a recirculation rate of 100 L/h. Thereafter, a hydroponic unit containing lettuce at a total fresh (wet) weight (FW) of 17.92 ± 1.88 g was installed into both experimental systems in phase II (day 31–52), which was equivalent to a 5.1 (w/w) fish: plant weight ratio. So that the water from the underneath tank in the typical and simplified APSs was shifted to the hydroponic box before being gravity fed down to the fish tank. This APS was continuously operated in triplicate for 52 d without water exchange under the same conditions as in section 2.1.

2.3. Sampling and Analytical Methods

The experimental units, with 240 L total water volume, were operated at a high recirculating rate of 100 L/h or 1.200% per day. Complete mixing of water in both the RAS and APS was assumed to occur, and so the water sampling was performed only on water from the aquaculture tank. Water samples were filtered with 25-mm Whatman® GF/C glass fiber filters and then stored at -20°C prior to analysis. The samples were examined for total ammonia nitrogen (TAN) [26], nitrite [27], nitrate [28], and phosphate [29] concentrations using a microplate spectrophotometer (BioTek PowerWave XS2, Winooski, USA). The alkalinity was analyzed by the titration method [30], while the SS was determined by the filtration method [28]. The DO, pH, temperature, EC, and light intensity were measured daily using portable probes (DO meter; HANNA HI 9147, pH/temperature meter; HANNA HI 9125 and EC/TDS meter; HANNA HI 99301P and light meter; Digicon LX72). Finally, the total nitrogen and phosphorus contents in the artificial feed, aquatic animals (Nile tilapia), plants (Green Oak lettuce), and sediment were analyzed by the authorized outsourcing service (Betagro Science Center, Thailand). Calculation of the nitrogen and phosphorus mass balances was performed by multiplying the percentage by weight of nitrogen and phosphorus with the initial and final biomass in grams.

2.4. Growth of Fish and Plants

The fish growth parameters of the body weight and length were measured to determine the density, daily weight gain (DWG), and feed conversion ratio (FCR). The DWG and FCR were calculated from Eqs. (1) and (2), respectively:

\[
\text{DWG (g/d)} = (W_f - W_i)/\text{Experimental period (d)} \quad (1)
\]

where \(W_f\) is the total final weight (g) and \(W_i\) is total initial weight (g).
FCR = Feed intake (g)/ Weight gain (g) (2)

For plant growth in the APS, the FW, height, and number of leaves, were recorded on the first and last days of the experiment. The dry weight (DW) was determined after the lettuce was dried in an electric oven at 70°C for 48 h. Finally, the survival rate of the fish and plants was estimated at the end of the experiment to evaluate the performance of the RAS and APS.

3. Results and Discussion

3.1. Control of the Inorganic Nitrogen and Suspended Solids in the RAS

Among the four conditions of RAS operation, the nitrogen profile indicated the important role of a pre-acclimated nitrifying biofilter. Without the artificial media for nitrifying bacteria in the underneath treatment tank, high TAN concentrations were observed in the control (2.19 ± 0.59 mg-N/L; day 12) and T1 (1.93 ± 0.21 mg-N/L; day 11) tanks, as shown in Fig. 3. These TAN concentrations were higher than the acceptable level (0.42 mg-N/L) and so might adversely affect the Nile tilapia under long-term exposure [3, 4]. An increase in the ammonia concentration during the first period of RAS operation was directly related to the nitrogen mineralization process, in which organic nitrogen in the uneaten feed and feces is converted to inorganic ammonia by the cooperation between autotrophs and heterotrophs [31]. Meanwhile, the growth and metabolism of microorganisms involved in ammonia oxidation, i.e. AOB, are normally stimulated thereafter by the natural source of ammonia supplement [15]. As a result, the TAN level tended to decrease, while the nitrite and nitrate levels began to increase from day 14 of the experiment onwards. This indicated the sufficient growth of nitrifying bacteria that multiplied naturally and required approximately 2–4 weeks to establish [12, 32].

For the T2 and T3 tanks (Fig. 3), the pre-acclimated nitrifying biofilter, especially with AOB, effectively controlled the TAN concentrations at 0.25 ± 0.03 and 0.11 ± 0.08 mg-N/L, respectively, throughout the 16-d RAS operation. Incomplete nitrification with nitrite accumulation was found during day 4–14. The concentrations of nitrite observed in our RASs, however, were still safe for fish as they were mostly maintained below 0.5 mg-N/L [33]. This accumulation of nitrite might be related to the acclimation procedure in which only ammonia was supplied, and nitrite was then derived from ammonia oxidation. Hence, NOB could begin to develop only after complete ammonia oxidation under nitrite available condition. Moreover, nitrite accumulation in this study was also caused by the lower growth rate of NOB in comparison with the AOB. It was previously reported that the balance between AOB and NOB was established after approximately 4 weeks of experimental operation as indicated by the occurrence of the complete oxidation of ammonia to nitrate [15]. With complete nitrification, the nitrate concentrations in the T2 and T3 tanks finally increased continuously to 9.87 ± 0.77 and 7.43 ± 0.58 mg-N/L, respectively, at the end of the experiment.

With respect to the removal of the SS, installation of the filtration unit in both the T1 and T3 tanks could remove the excess SS at average DWs of 3.02 ± 0.60 and 3.47 ± 1.08 g/week, respectively. This contributed to keep the SS concentration within the standard.

![Fig. 3. Variations in the nitrogen concentration during 16-d of fish cultivation in the RAS of the (a) control, (b) T1 with a filtration unit, (c) T2 with a nitrifying biofilter, and (d) T3 with a filtration unit and a nitrifying biofilter.](image)
level of 80 mg/L [19]. The elimination of solid debris could prevent biofilter clogging and maintain their nitrification ability for the long-term operation of the RAS. Nevertheless, SS was removed from the RAS at the same time as the elimination of nitrogen from the system, and so led to a lower nitrate concentration in the system. Comparison between the presence (T3) and absence (T2) of the filtration unit in the RASs with the nitrification tank, the final concentration of nitrate in T3 (7.43 ± 0.58 mg-N/L) was lower than that in T2 (9.87 ± 0.77 mg-N/L). This indicated that the integration of the filtration unit in RAS might be less favorable for APS due to the low concentration of residual nitrate, which is the main nitrogen source for plant cultivation [11, 12]. In addition, the low nutrient level might have a negative effect on plant adsorption efficiency as many previous studies on nutrient uptake kinetics have demonstrated that a higher concentration of the nitrogen source can increase the uptake rate in the APS [9, 10].

3.2. Efficiency of the Simplified APS

In the typical APS (Fig. 4(a)), the acclimated nitrifying biofilter functioned to keep the TAN and nitrate concentrations at safe levels, with the average concentrations of 0.05 ± 0.02 and 0.05 ± 0.01 mg-N/L, respectively, throughout the 52-d period of aquaponics operation. A similar result was previously observed where the concentrations of inorganic nitrogen (ammonia and nitrite) were controlled by the integration of nitrification treatment in the aquaculture system for two weeks of fish cultivation even though the stocking density in the fish tank reached 17.0 ± 8.0 kg/m³ [34]. For the simplified APS (Fig. 4(b)), without a nitrifying biofilter, the TAN and nitrate levels during the first two weeks of the experiment were slightly increased to 0.67 ± 0.10 and 1.88 ± 0.01 mg-N/L, respectively, due to the ammonia excreted by fish. However, the complete removal of ammonia and nitrite occurred from day 20 onwards, which was related to the growth and function of natural nitrifying microorganisms that were suspended and/or attached on system components, especially the tank walls. This was supported by a previous study that reported the loss of ammonia in the APS not only resulted from both plant adsorption and nitrification process, but was also due to the amount of microbial biomass that developed on the plant roots [12].

For the nitrate and phosphate (nutrients) concentrations, the concentration profiles clearly demonstrated that the nutrient removal efficiency of the simplified APS (treatment) resembled that of the control system with a filtration unit and nitrifying biofilter. During phase I (day 0–30) of the RAS operation, the increase in the nitrate concentration was directly related to the nitrification process. Nonetheless, although a hydroponic unit was connected to the experimental systems during phase II (day 31–52), the trend in the nitrate concentration was still to increase continuously and reached nearly 20 mg-N/L. This might be because the 5:1 (w/w) fish: plant ratio used in this study was insufficient to absorb the nitrogen from the aquaculture system. Indeed, previous studies have recommended a higher plant ratio of 1:2 (w/w) fish: plant as being more suitable for the effective operation of an APS [35, 36]. Thus, the elevated concentration of nitrate produced in this study might have exceeded the plant requirement [37].

In terms of phosphate, likewise, the concentration increased gradually during both the RAS and the following APS operations. At the end of the experiment, the final concentrations of phosphate were 2.25 ± 0.62 and 2.62 ± 0.23 mg-P/L in the control and treatment systems, respectively. The increased phosphate level was related to the lower adsorption capacity of the plants than the phosphate generation rate from organic phosphorus in the artificial fish feed. This problem was also found in a previous study, where a fish: plant ratio of 1:1 (w/w) was insufficient to control the level of total phosphate in the APS, as indicated by the higher concentration of remaining phosphate compared to that at a 1:2 and 1:3 (w/w) fish: plant ratio, respectively [36]. Therefore, the density of lettuce in the hydroponic unit should be increased to improve the nutrient (nitrate and phosphate) removal efficiency.

From this study, the simplified APS using the activity of natural microorganisms clearly demonstrated the ability to control inorganic nitrogen compounds (ammonia and nitrite) in the aquaculture system through nitrification. However, although the trends in the nitrate and phosphate levels in the control and treatment systems were rather similar, the excess SS in the simplified APS was still in need of proper management. The elevated SS concentration, which reached as high as 830.0 ± 90.0 mg/L, was found in the treatment system and this could adversely affect the fish gill in long-term exposure [19]. In contrast, with the integration of a filtration unit in the typical APS, the SS debris at approximately 1.45 ± 0.52 g DW was trapped and removed weekly, which contributed to keep the SS level at only 60.0 ± 10.0 mg/L. This demon-

Fig. 4. Variations in the nitrogen and phosphorus concentrations during 52-d of fish and plant cultivation in the APS of (a) the control, a typical APS system with a filtration unit and a nitrifying biofilter, and (b) the simplified APS treatment system. Phase I is the operation of the aquaculture and treatment units. Phase II is the additional installation of the hydroponic unit in the experimental system. Data are shown as mean ± 1SD, derived from three replicates.
strated the important role of a filtration unit in the APS that could minimize excess solid with a removal efficiency of approximately 68.2%. However, as discussed in section 3.1, the integration of only the filtration unit might increase the level of the nutrient loss from solid removal. Therefore, the use of the filtration unit coupled with the anaerobic sludge digestion unit is recommended to enhance the ability of nutrient recovery from these excess solid particles [38].

### 3.3. Production Performances of Fish and Plant

The fish (Nile tilapia) and plant (Green Oak lettuce) productions obtained from the APS are shown in Table 1. During the 52-d period of the aquaponics operation, the total fish biomass in control system increased by 182.3 ± 59.4 g, while in the simplified APS increased by 127.2 ± 9.4 g. Nevertheless, there were no statistically significant difference in the fish weight gain between the control and treatment systems. Likewise, the DWG and FCR in the simplified APS resembled the typical system, indicating the similar potential of fish production in both systems. However, the observed DWG was rather low when compared with the previously reported values, where values of between 0.5 and 0.7 g/d were reported at a feed rate of 5% (w/w) [13]. Thus, the low DWG in this study was probably due to the lower utilized feed rate of only 1–3% (w/w) in the APS. Finally, the survival rates showed that both the typical and simplified APSs were efficient at maintaining a suitable condition for fish cultivation with 100% survival.

During the hydroponic scheme, the lettuce was transferred to culture in the APS during phase II at an initial FW of 1.73 ± 1.16 g in the control and 1.32 ± 1.38 g in the treatment. After 22 d of plant cultivation, the increased biomass of lettuce in both the control and treatment systems were not statistically significant, being 8.19 ± 4.84 g of plant production in the control system and 5.16 ± 2.62 g in the simplified APS. Nonetheless, with respect to the outward appearance of the lettuce, the harvested plants were under-sized and not perfectly shaped. A similar problem was also previously found in tomato cultivation, where the low quality of the fruit was related to potassium limitation in the aquaculture water [39]. Likewise, the low productivity of the harvested lettuce in this study was possibly due to either the insufficient nutrient concentration, especially potassium, or lack of micro-nutrients, e.g. ferrous, in the experimental system. Thus, it has previously been suggested that the ferrous concentration in the APS should be maintained above 2 mg/L [40].

### 3.4. Mass Balance of Nitrogen and Phosphorus

The schematic flow diagrams for the mass balance of nitrogen and phosphorus in the APSs are illustrated in Figs. S1 and S2, respectively, while the overall nutrients budget is summarized in Table 2. The total nitrogen inputs were mainly obtained from artificial feed (approximately 70%) and fish (approximately 30%). With the aquaculture operation, the amount of nitrogen incorporated into the fish body accounted for 32.4% and 20.4% in the control and the treatment systems, respectively, which were within the optimal range of 12–36% [41, 42]. Meanwhile, only 0.25 and 0.17% were converted to accumulate in plant tissue in the control and treatment systems, respectively. Compared with another APS (Table 3), a higher proportion of nitrogen in lettuce was reported (3.7%), while the average values for plants are normally between 5–22% [42]. The inferior percentage of nitrogen content in this study was probably due to the low potassium and ferrous concentrations that limited the efficiency of nutrient adsorption [39, 40]. To improve the nitrogen content (%) in the harvested plant, as generally recommended by commercial fertilizer, the N: P: K: Fe ratio for a hydroponic system should be maintained at 1:2.5:0.3:0.5:1–3:0.01–0.05. Apart from nitrogen accumulation in fish and plant tissues, approximately 30% of the total nitrogen was dissolved in water, especially in form of nitrate, while 35.0% was deposited as SS and settled solids in the simplified APS. With the integration of a filtration unit in the control system, the results demonstrated that the filtration unit contributed to the control of SS, unfortunately, the nitrogen content was also simultaneously lost by solid removal. The 8.40% of nitrogen was removed from the typical APS, while only 3.92% remained as solid debris. Likewise, the undetectable nitrogen in a typical APS (20.0%), which combined a filtration unit and a nitrification tank, was 2.1 times higher than that in the simplified system (9.4%). A previous study reported that the nitrogen loss was possibly accumulated in microbial cells on the biofilter and/or released as nitrogen gas from ammonia volatilization and denitrification processes [43]. In general, more than 30% of the nitrogen input can be lost during the operation of an APS, with approximately 5.2–36.0% being eliminated in the gas phase [44–46].

In terms of phosphorus, similar to nitrogen, the same proportions of phosphorus inputs were calculated from both the artificial feed and fish. During the experiment, the level of phosphorus that accumulated in the fish body was 34.1% in the control and 23.9% in the treatment. Consistent with previous studies (Table 3), the typical range of phosphorus contained in fish was between 20–42 % and could reach 50%, depending on fish species and feed quality [41, 42, 47]. On the other hand, with the limitation of nutrient assimilation, the level of adsorbed phosphorus in the plant tissue
in this study was only 0.1%, which was lower than the previously reported 0.7% [42]. As it is generally known that phosphate is mainly deposited in the sediment, a large portion (60.1%) of phosphorus was observed as settled solids in the simplified APS. Meanwhile, the filtration unit could discharge approximately 14.3%, resulting in only 6.7% being retained in the control system. Finally, the undetectable phosphorus in the control system was high at 26.1%, while only 1.9% of phosphorus was lost in the simplified APS. This demonstrated that the undetectable phosphorus in the typical APS (control) was 13.6 times higher than that in the simplified system. From these results, focusing on nutrient utilization, the recovery of nutrient from lost elements by applying the anaerobic digestion system in the APS is a recommended [38].

4. Conclusions

Although the nitrifying biofilter was the major component in the RAS, integration of hydroponics with the aquaculture system was adequate to perform complete nitrification by the natural growth of microorganisms. With the proper design and operation of a sim-

Table 2. Overall Nitrogen and Phosphorus Budgets in the APS during a 52-d Operation Period.

| Composition          | Nitrogen (g) | Phosphorus (g) |
|----------------------|--------------|----------------|
|                      | Control      | Treatment      | Control      | Treatment      |
| **Input:**           |              |                |              |                |
| Feed                 | 12.02 (69.72%)| 12.02 (70.17%)| 4.19 (67.81%)| 4.19 (68.13%)  |
| Fish                 | 4.88 (28.31%)| 4.79 (27.96%)  | 1.98 (32.04%)| 1.95 (31.71%)  |
| Plant                | 0.07 (0.41%) | 0.07 (0.41%)   | 0.01 (0.15%) | 0.00 (0.16%)   |
| Dissolved in water   | 0.27 (1.57%) | 0.25 (1.46%)   | -            | -              |
| Sediment             | -            | -              | -            | -              |
| Sediment removal     | -            | -              | -            | -              |
| Total                | 17.24 (100%) | 17.13 (100%)   | 6.18 (100%)  | 6.15 (100%)    |
| **Gain:**            |              |                |              |                |
| Feed                 | -            | -              | -            | -              |
| Fish                 | 3.89 (32.36%)| 2.46 (20.43%)  | 1.43 (34.13%)| 1.00 (23.87%)  |
| Plant                | 0.03 (0.25%) | 0.02 (0.17%)   | 0.01 (0.13%) | 0.00 (0.11%)   |
| Dissolved in water   | 4.22 (35.11%)| 4.21 (35.02%)  | 0.78 (18.62%)| 0.59 (14.08%)  |
| Sediment             | 0.47 (3.92%) | 4.21 (34.99%)  | 0.28 (6.73%) | 2.52 (60.05%)  |
| Sediment removal     | 1.01 (8.40%) | -              | 0.06 (14.32%)| -              |
| Undetectable         | 2.40 (19.96%)| 1.13 (9.39%)   | 1.09 (26.08%)| 0.08 (1.90%)   |
| Total                | 12.02 (100%) | 12.02 (100%)   | 4.19 (100%)  | 4.19 (100%)    |

Table 3. Comparison of the Nitrogen and Phosphorus Accumulation in the Fish and Plants between Previous Studies and This Study

| Fish species         | Proportion in fish (%) | Plant species          | Proportion in plant (%) | References |
|----------------------|------------------------|------------------------|-------------------------|------------|
| **Nitrogen:**        |                        |                        |                         |            |
| Tilapia (Oreochromis niloticus) | 19.40–24.30            | Tomato (Lycopersicon esculentum) | 15.00–17.00          | [12]       |
|                      |                        | Pakchoi (Brassica chinensis) |                         |            |
| Tilapia (Oreochromis niloticus) | 12.00–36.00            | Water spinach (Ipomoea aquatica Forssk.) | 3.70          | [42]       |
|                      |                        | Lettuce (Lactuca sativa L) |                         |            |
|                       |                        | Canna (Canna glauca L)    |                         |            |
| Tilapia (Oreochromis niloticus) | 20.43–32.36            | Lettuce (Lactuca sativa) | 0.17–0.25              | This study |
| **Phosphorus:**      |                        |                        |                         |            |
| Tilapia (Oreochromis niloticus) | 20.00–42.00            | Water spinach (Ipomoea aquatica Forssk.) | 0.70          | [42]       |
|                      |                        | Lettuce (Lactuca sativa L) |                         |            |
|                       |                        | Canna (Canna glauca L)    |                         |            |
| Tilapia (Oreochromis niloticus) | 23.87–34.13            | Lettuce (Lactuca sativa) | 0.11–0.43              | This study |
plified APS, inorganic nitrogen (ammonium, nitrite, and nitrate) and phosphate could be controlled within the appropriate levels for fish cultivation. In terms of plant production, however, the lack of other nutrients, such as potassium and ferrous ions, in the aquaculture effluent might be at an insufficient level for the plant requirement. Hence, additional nutrients are needed to be supplied in the APS in order to increase the lettuce biomass. In addition, the utilization and management of excess solid were still required for long-term operation. The use of a filtration unit in cooperation with sludge digestion was recommended to improve the efficiency of nutrient recovery in the APS.

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Author Contributions

S.S. (Master student) performed experiments, analyzed data, and drafted the manuscript. P.S. (Postdoctoral researcher, BIOTEC) participated in experimental operation and wrote the manuscript. W.P. (Advisor) provided funding and stylistic revisions to manuscript. S.P. (Co-advisor) designed experiments and provided revisions to scientific content of manuscript. All authors read and approved the final manuscript.

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