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Nitrogen and Phosphorus Removal in the Recirculating Aquaculture System with Water Treatment Tank Containing Baked Clay Beads and Chinese Cabbage

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Abstract

This research aims to describe the nitrogen and phosphorus removal in Recirculating Aquaculture System (RAS) by crop plants biomass production. The 3 experiment systems consisted of 1 treatment (fish tank + baked clay beads + Chinese cabbage) and 2 controls as control-1 (fish tank only) and control-2 (fish tank + baked clay beads), were performed. With all experimental RAS, Nile tilapia (*Oreochromis niloticus*) was cultured at 2 kg/m^3 density. The baked clay beads (8-16 mm in diameter) were filled as a layer of 10 cm in the water treatment tank of control-2. While in the treatment tank, Chinese cabbage (*Brassica pekinensis*) was planted at 334 plants/m^2 in baked clay beads layer. During 35 days of experiment, the average fish wet-weight in control-1, control-2 and treatment systems increased from 16.31±1.49, 15.18±1.28 and 11.31±1.49 g to 29.43±7.06, 28.65±3.12 and 27.20±6.56 g, respectively. It was found that the growth rate of 0.45±0.15 g-wet weight/day in a treatment tank was higher than in those 2 controls, which were rather similar at 0.37±0.16 and 0.38±0.05 g-wet weight/day, respectively. The fish survival rate of all experimental units was 100%. The average Chinese cabbage wet-weight in treatment system increased from 0.15±0.02 g to 1.00±0.38 g. For water quality, all parameters were within the acceptable range for aquaculture. The assimilation inorganic nitrogen in a treatment tank showed a slower rate and lower nitrite accumulation relative to those in control tanks. The nitrogen and phosphorus balance analysis illustrated that most of the nitrogen and phosphorus input in all systems was from feed (82-87% and 21-87%) while at the final day of experiments, nitrogen and phosphorus in tilapia culture revealed at 15-19% and 4-13%. The accumulation of nitrogen and phosphorus in the water, up to 56% and 70%, was found in control-1 while water in the tank with baked clay beads had substantial lower nitrogen and phosphorus concentration. The most important part was unaccounted nitrogen and phosphorus as high as 60% and 17% in treatment and 53% and 10% in control-2 systems. Nitrogen and phosphorus incorporated in plant (treatment) was only 1.31% and 0.11%, respectively. It can be implied from the results that the assimilation in plant was a minor process for nutrient removal in this RAS. On the other hand, the nitrification and denitrification occurred in the sediment layer of baked clay beads tank were the major treatment processes to maintain water quality in the recirculating system. Without baked clay bead, nitrogen waste was accumulated as nitrate in the water while in treatment tank with backed clay beads, nitrogen was significantly removed by denitrification process.

Keywords: Recirculating Aquaculture System; RAS; nitrogen removal; phosphorus removal; nitrification; denitrification; Chinese cabbage

1. Introduction

Recently, aquaculture industry is expanding rapidly due to an increase of the world food demand. Environmental friendly aquaculture system is therefore essential for sustainable development. The closed-recirculating aquaculture technology has been developing for decades, but mostly is under research. Recirculating Aquaculture System (RAS) uses water treatment technologies to treat wastewater from aquaculture tank and reuse the water for a long period. Common water treatment processes in the RAS, apart from aeration, are sediment removal and nitrification processes. In general, toxic nitrogen compounds such as ammonia and nitrite derived from aquatic animal's excretion, feed residues, and microbial degradation (Crab *et al*., 2007) must be regulated below 0.5 mg-N/L. High ammonia and nitrite can cause adverse health effects in aquatic animals and create environmental concerns if effluent is not properly treated. Apart from nitrogen waste treatment, phosphorus accumulation in the RAS is also concerned but phosphorus removal requires sophisticated sequential anaerobic-aerobic process which is not yet commercial available (Burut-Archanai *et al*., 2013). In this research, we studied the possibilities of combining wastewater treatment with the production of crop plants biomass for phosphate removal which could not be treated by conventional treatment system. The advantage of using plant is that it is not only assimilating nitrogen waste but it also remove phosphate from the water with the absorption of the root system (Beven, 2010). In an aquaponic RAS with substrate support for planting, the nitrogen assimilation process by plant and the nitrogen degradation process by nitrifying/denitrifying bacteria were combined for nitrogen removal from fish wastewater. Unlike the aquaponic concept with floating plants (Rakocy and Hargreaves, 1993; Timmons *et al*., 2002; Wilson, 2005), the proposed system applied baked clay beads layer in a tank, that was not only for supporting plant root but also performed as the media for microbial nitrogen removal via nitrification and denitrification processes. Moreover, the optimization of nitrogen and phosphorus removal processes was necessary for water quality control in fish tank and yield of plant (Graber and Junge, 2009). In our systems, Nile tilapia and Chinese cabbage were chosen for this study as they are economical important species and fast growth rates. The experimental system was carried out in partial controlled condition in which light, temperature, DO, moisture, nutrients and pests were regulated to suit for both fish and plant living.

2. Materials and Methods

The experiment was conducted at the Center of Excellence for Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University. The treatment recirculating aquaculture system consisted of fish tank growing Tilapia and plant tank packed with baked clay beads and Chinese cabbage. The fish tank without plant tank and fish tank + baked clay beads tank (no plant) were assigned as control-1 and control-2, respectively (Table 1). All experimental systems were performed with 3 replicates and placed in the greenhouse.

2.1. Recirculating aquaculture system

The experimental aquaculture system consisted of 38 x 58 x 31 cm^3 fish tank (working volume 45 L) connected to the overlay water treatment tank. The water treatment tank (plant tank) was 38 x 58 x 24 cm^3 plastic tank packed with 10 cm layer of spherical shape baked clay beads (8-16 mm in diameter) and Chinese cabbage with approximately 4.83±0.35 cm height planted at 334 plants/m^2. This bead packing performed as suspended solids retainer and biological filtration media for inorganic nitrogen treatments (Fig. 1). The effluent from fish tank was pumped by submersible pump (Resun SP-6600) through PVC pipes lying over the treatment tank. Water was spray into treatment tank at 3 L/min for 10 minutes thereafter pump was pause for 60 minutes before the next pumping round. Water from treatment tank was flow back to the fish tank by gravity. Continuous aeration in fish tank was provided through diffusive stone aerators in order to maintain proper environmental conditions for fish growth and nitrifying process (i.e., well-mixed, DO > 4.0 mg O_2/L, pH = 7-8 and alkalinity = 120-160 mg $CaCO_3$/L by adding sodium bicarbonate).

Nile tilapia with an average initial wet-weight of 14.27±1.42 g and length of 9.44±0.27 cm was stocked in all fish tanks to obtain the initial density of approximately 2 kg/m^3. Fish was fed twice daily at 8.00 am and 3.00 pm with 25% protein commercial feed pellets at 5% of total fish weight per day (feeding was adjusted every week following fish biomass). Fish growth was monitored by length and weight measurement every week and the experimental period was 35 days. Growth of Chinese cabbage was measured by weighing at the initial and the end of 35 days experiment. Leaf width, length and canopy size was measured every week.

2.2. Water quality parameters and analytical methods

During the experiment, water samples were taken out daily for ammonia, nitrite, nitrate, alkalinity, phosphate, total nitrogen and total phosphorus analysis following standard method for water and wastewater

Table 1. Experimental systems performed in this study

	Control-1	Control-2	Treatment
Experimental conditions	Fish tank only	Fish tank + Baked Clay Beads	Fish tank + Baked Clay Beads + Chinese cabbage

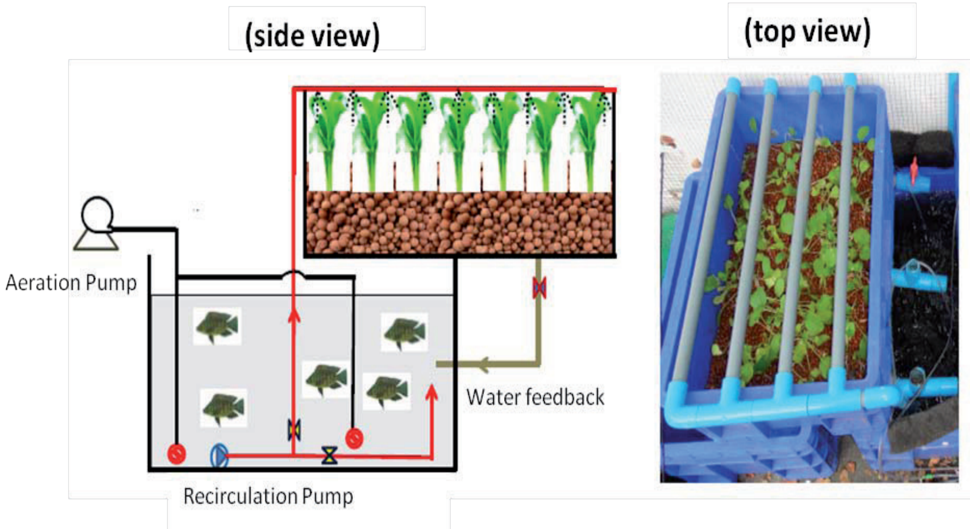

Figure 1. Schematic diagram and photo of the treatment recirculating aquaculture system consisted of fish tank and overlay treatment tank with baked clay beads and Chinese cabbage.

Table 2. Growth characteristics of Tilapia during the experiment (a, b shows was differed significantly)

Parameter	Control-1	Control-2	Treatment
Initial tilapia wet-weight (g)	16.31±1.49	15.18±1.28	11.31±1.49
Final tilapia wet-weight (g)	29.43±7.06	28.65±3.12	27.20±6.56
Initial tilapia length (cm)	9.96±0.23	9.46±0.27	8.89±0.32
Final tilapia length (cm)	11.79±0.73	11.92±0.62	11.37±1.02
Initial biomass density (kg/m^3)	2.04	2.01	2.14
Final biomass density (kg/m^3)	3.68	3.81	5.11
Total feed (g)	135.33	176.12	200.50
Average daily growth (g/day)	0.37±0.16^a	0.38±0.05^a	0.45±0.15^b
Feed conversion ratio; FCR	2.06	1.96	1.69
Survival rate (%)	100	100	100

analysis (APHA, 2005). Suspended solids in the water was analyzed every three days. Physical parameters including DO, pH, temperature and ORP were measured using portable meters. Nitrogen and phosphorus in feed, fish, suspended solids in fish tank, solid retained in baked clay beads tank, Chinese cabbage, and baked clay beads were determined at the initial and the end of the experiment for nitrogen and phosphorus budget analysis. Nitrogen content in dried samples were analyzed by CHN analysis using dynamic flash combustion, CHNS-O analyzer. Phosphorus was analyzed using inductively coupled plasma optical emission spectrometry, at the Scientific Equipment Center, Prince of Songkla University, Thailand. Statistical analysis (ANOVA) between the controls and treatments was calculated using Microsoft Excel 2007.

3. Results and Discussion

3.1. Growth of fish and Chinese cabbage

During 35 days of experiment, the average fish wet-weight in control-1, control-2 and treatment systems increased from 16.31, 15.18 and 11.31 g to 29.43, 28.65 and 27.20 g, respectively (Table 2). It was found that treatment tank with baked clay beads and Chinese cabbage had the highest growth of 0.45 g/day while fish growth rate in control-1 and control-2 were rather similar at 0.37 and 0.38 g/day, respectively. These growth rates were within acceptable range due to the proper fish density between 2-5 kg/m^3 while growing fish at higher density e.g. 12 kg/m^3 could reduce average daily growth to 0.16±0.09 g/day as reported by Azim and Little (2008). Statistical analysis indicated that the fish in treatment tank had significantly higher growth rate than control-1 and control-2. Feed conversion ratio (FCR) in treatment system was 1.69 while FCR in control-1 and control-2 were 2.06 and 1.96, respectively. This indicated better feed utilization of the fish in treatment tanks. Survival rate of all control and treatment units was 100%. The average Chinese cabbage wet-weight in treatment system increased from 0.15±0.02 g to 1.00±0.38 g and length increased from 4.83±0.35 cm to 8.04±1.13 cm (0.11 cm/day). Leaf width increased from 0.77±0.10 cm to 3.26±0.54 cm (0.09 cm/day) and canopy size expanded from 1.64±0.18 cm to 11.43± 3.22 cm (0.35 cm/day). The average growth rate of Chinese cabbage was equivalent to 0.02±0.01 g/day.

It was found that growth of Chinese cabbage in this experiment was much slower than conventional vegetable planting in soil but comparable to aquaponic system by Graber and Junge (2009) which had the average 2.07 g of wet-weight and 18 cm in length after 55 days. This was probably due to the limitation of nutrients and improper environmental condition in the experiment. Low light intensity between 980-25,410 Lux due to building shade on the experiment green house in the afternoon was also another factor affecting growth. Decrease of Oxidation-Reduction Potential (ORP) from +290 to +110 mV in baked clay bead layer indicated that accumulation of sediment in baked clay beads caused an increase of oxygen consumption in the bead layer (Fig. 2).

3.2. Water quality

According the Fig. 3 illustrates inorganic nitrogen and phosphate concentrations in control and treatment systems. High peak of total ammonia nitrogen (TAN) up to 1.2±0.3 mg-N/L was found in control-1 during the first 5 days, while small TAN peaks at 0.31±0.1 mg-N/L were found in control-2 and treatment system which was within the safety range [below 0.5 mg-N/L (Liao and Mayo, 1972)]. During day 3-8, the accumulation of nitrite was found in all tanks after TAN peaks disappearance. This indicated the occurrence of nitrification process via ammonia oxidizing bacteria. The highest peak of nitrite, up to 3.84±0.8 mg-N/L, was also found in control-1, while smaller peaks were found in control-2 and treatment systems (0.86±0.1 and 0.38±0.25 mg-N/L, respectively), and these concentrations were under the safety range [below 1 mg-N/L (Hart and O,Sullivan, 1993)]. The nitrification was complete within 10 day when the accumulation of nitrate, the end product of nitrification, occurred without nitrite accumulation. At the end of the experiment, concentration of nitrate was as high as 100.37±5.6 mg-N/L in control-1. Accumulation of nitrate is generally found in closed aquaculture system in which the major water treatment process is nitrification (Nootong *et al*., 2011; Nootong and Powtongsook, 2012). This nitrate concentration was higher than the safety concentration of 50 mg-N/L so water exchange is

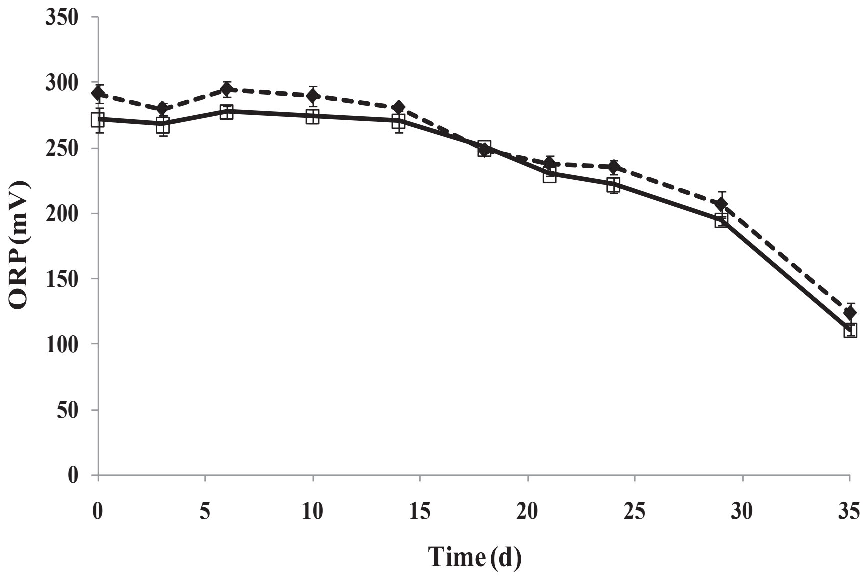

Figure 2. The variation of Oxidation-Reduction Potential in baked clay bead layer of control-2 (☐) and treatment (◆) system

therefore needed (Hart and O,Sullivan,1993).On the other hand, nitrate in control-2 and treatment systems were 47.24±4.1 mg-N/L and 26.66±3.7 mg-N/L respectively. The lower nitrate accumulation in control-2 and treatment systems indicated that baked clay beads played a significant role in nitrate removal.

It could be summarized that nitrification was the major process for water quality control in fish tanks without baked clay beads and plant. This nitrification activity occurred with the natural biofloc (suspended solids) that accumulated during fish culture (Nootong *et al*., 2011). In contrast, when baked clay beads tank was applied to the fish culture system, nitrate was significantly removed by denitrification process in the anaerobic layer of the baked clay bead tank. Moreover, phosphate concentration was also low in control-2 and treatment system. At the end of the experiment, accumulation of phosphate (8.84±0.4 mg-P/L) was found in control-1. Lower phosphate concentrations were found in control-2 and treatment systems at 5.72±0.1 mg-P/L and 3.38±0.5 mg-P/L, respectively. It is generally known that plant can take up inorganic nitrogen and phosphorus compounds as nutrients for growth, however, phosphate concentration in treatment tank containing Chinese cabbage was slightly higher than control-2 which had only baked clay beads. Hence, the role of phosphorus uptake by plant in this experiment was still unclear and further detailed study is therefore recommended.

It was found that the baked clay beads tank was not only remove inorganic nitrogen but it also retain suspended solids (Fig. 4). Water in the fish tank without beads filtration (control-1) had the suspended solids concentration as high as 352.22±56.01 mg/L below the safety concentration of 80 mg/L (Timmons *et al*., 2002) throughout the experimental period. In general, suspended solids higher than 500 mg/L must be avoided due to it obstruct visibility while it was only 46.11±8.55 mg/L and 55.00±22.55 mg/L in control-2 and treatment system, respectively. Hence, baked clay beads tanks in this experiment were successfully maintain suspended solids in fish tank. Other water quality parameters were within the acceptable range for aquaculture (i.e., pH = 8.23-8.55; alkalinity = 100-163.33 mg $CaCO_3$/L; DO

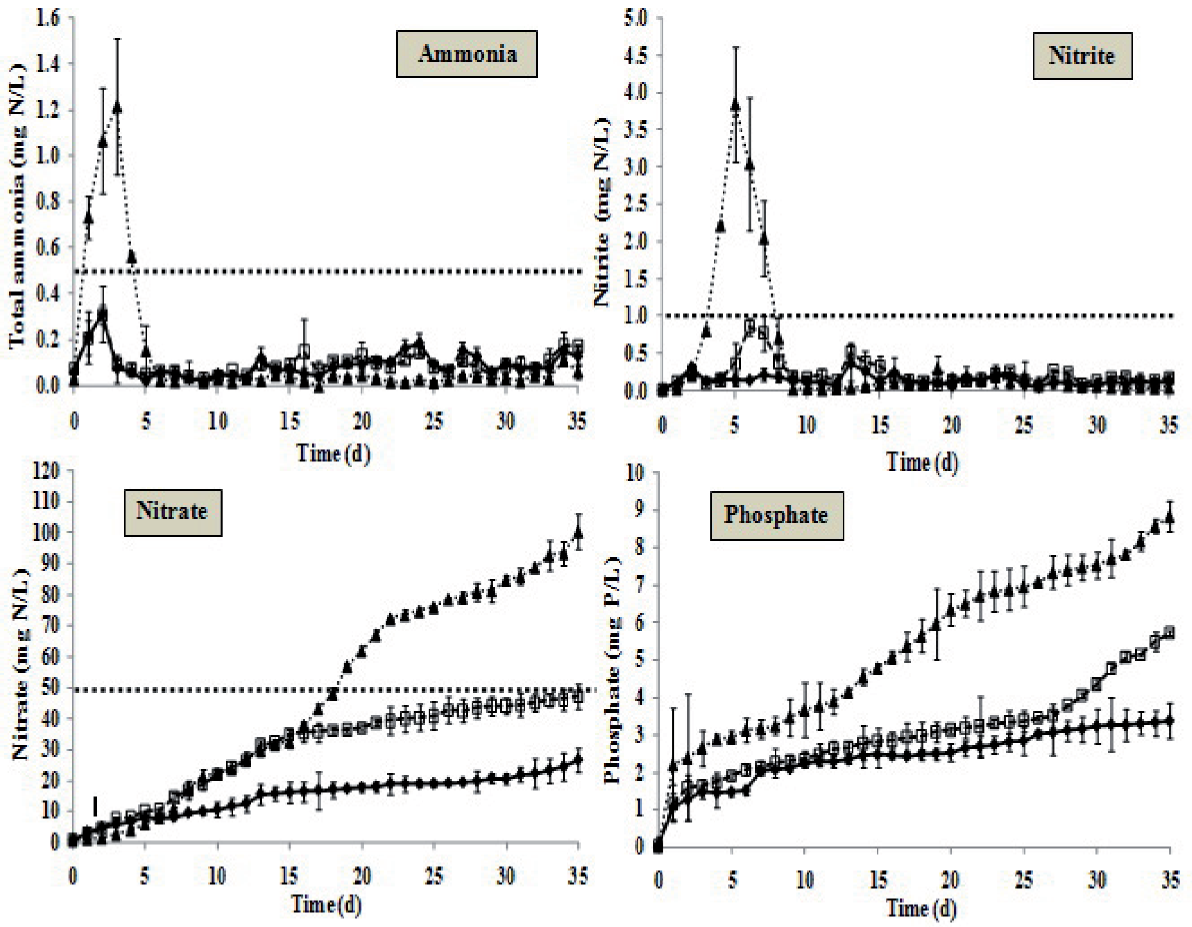

Figure 3. The water quality analysis in fish tanks from control-1 (▲), control-2 (□) and treatment (◆) systems. (The horizontal dot lines indicate safety concentration for aquaculture)

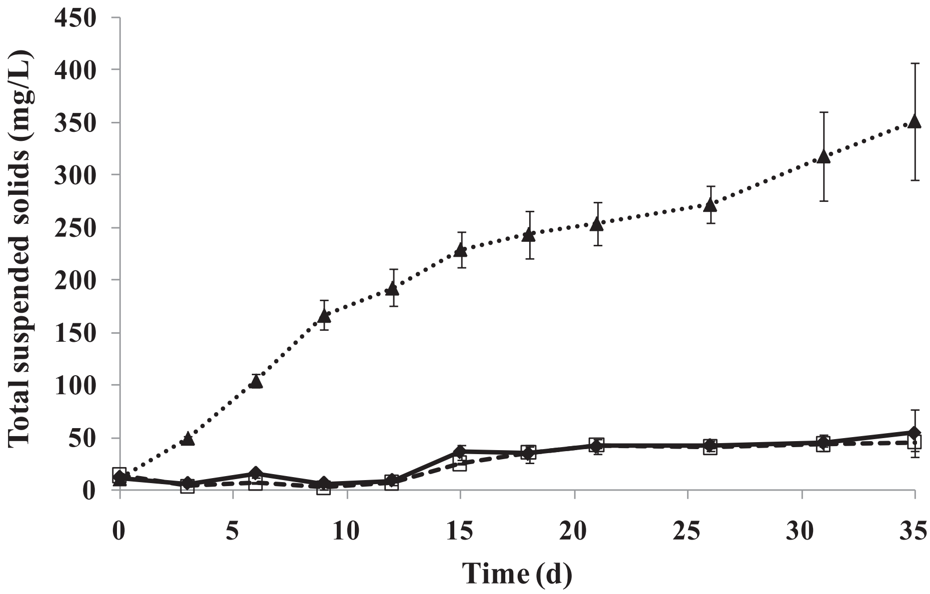

Figure 4. The suspended solids concentration in control-1 (▲), control-2 (□) and treatment (◆) systems

Table 3. The nitrogen balance in the recirculating aquaculture systems

Parameter	Control-l		Control-2		Treatment	
	Nitrogen per tank (g)*		Nitrogen per tank (g)*		Nitrogen per tank (g)*	
	In put	at final day	In put	at final day	In put	at final day
Feed	7.15 (82.00%)	-	9.31 (85.73%)	-	10.59 (86.45%)	-
Fish	1.53 (17.54%)	1.56 (17.89%)	1.51 (13.90%)	1.59 (14.64%)	1.60 (13.06%)	2.28 (18.61%)
TN in water	0.04 (0.46%)	4.83 (55.39%)	0.04 (0.37%)	2.18 (20.07%)	0.04 (0.33%)	1.21 (9.88%)
Suspended solid in fish tank	-	0.56 (6.42%)	-	0.07 (0.65%)	-	0.08 (0.65%)
Solid retained in baked clay beads tank	-	-	-	1.28 (11.79%)	-	1.19 (9.71%)
Chinese cabbage	-	-	-	-	0.02 (0.16%)	0.16 (1.31%)
Baked clay beads	-	-	-	-	-	-
Unaccounted	-	1.77 (20.30%)	-	5.74 (52.85%)	-	7.33 (59.84%)
Total	**8.72 (100%)**	**8.72 (100%)**	**10.86 (100%)**	**10.86 (100%)**	**12.25 (100%)**	**12.25 (100%)**

* CHNS-O Analyzer, CE Instruments Flash EA 1112 Series, Thermo Quest, Italy

= 7.07-9.07 mg/L; temperature = 26.50-30.07ºC).

3.3. Nitrogen and phosphorus mass balance

The nitrogen balance analysis in Table 3 shows that nitrogen input in all systems was mostly from feed (82-87%) and fish (13-18%) while at the end of experiments; nitrogen in fish was between 15-19%. These results were comparable to the report of Avnimelech and Rityo (2003), which explained that the input nitrogen and phosphorus was accumulate in fish 22% and 16% respectively. Moreover, in many research reports notified the proportion of ammonia nitrogen in RAS that 39.29% was from feed, 26-28% was from fish excretion while the final portion of 24% was accumulated in sludge suspended solids (Lin and Nash, 1996; Funge-Smith and Briggs, 1998). Accumulation of nitrogen in the water, up to 56%, was found in control-1 while water in the tank with baked clay beads had substantial lower nitrogen concentration. The most important part was unaccounted nitrogen as high as 53% in control-2 and 60% in treatment system. This was assumed as the nitrogen gas loss through denitrification process (Rafiee and Saad, 2005; Funge-Smith and Briggs, 1998). Nitrogen incorporated in Chinese cabbage (treatment system) was only 1.31%. Hence, results from this study illustrated that nitrogen removal in our RAS was mainly by nitrification-denitrification processes while nitrogen uptake by plant

Table 4. The phosphorus balance in recirculating aquaculture systems

Parameter	Control-l		Control-2		Treatment	
	Phosphorus per tank (g)*		Phosphorus per tank (g)*		Phosphorus per tank (g)*	
	In put	at final day	In put	at final day	In put	at final day
Feed	0.55 (87.02%)	-	0.50 (21.35%)	-	0.56 (20.47%)	-
Fish	0.08 (12.66%)	0.08 (12.66%)	0.08 (3.42%)	0.10 (4.27%)	0.09 (3.29%)	0.10 (3.65%)
TP in water	0.002 (0.32%)	0.439 (69.46%)	0.002 (0.08%)	0.298 (12.72%)	0.004 (0.15%)	0.186 (6.80%)
Suspended solid in fish tank	-	0.11 (17.41%)	-	0.02 (0.85%)	-	0.03 (1.10%)
Solid retained in baked clay beads tank	-	-	-	0.28 (11.96%)	-	0.21 (7.68%)
Chinese cabbage	-	-	-	-	0.001 (0.04%)	0.003 (0.11%)
Baked clay beads	-	-	1.76 (75.15%)	1.41 (60.21%)	2.08 (76.05%)	1.76 (64.35%)
Unaccounted	-	0.003 (0.47%)	-	0.234 (9.99%)	-	0.446 (16.31%)
Total	**0.632 (100%)**	**0.632 (100%)**	**2.342 (100%)**	**2.342 (100%)**	**2.735 (100%)**	**2.735 (100%)**

* Optical Emission Spectrometer, Optima 4300 DV, Perkin Elmer Instruments, USA

incorporated with the minor role.

The phosphorus balance in all systems is show in Table 4. It was found that phosphorus input was mostly from feed (21-87%) and fish (3-13%). At the end of experiments, phosphorus in fish was between 4-13% which was slightly lower than previous report (15.98%) by Rafiee and Saad (2005), phosphorus accumulation in the water was up to 70% in control-1, while tanks with baked clay beads had substantial lower phosphorus concentration. Unaccounted phosphorus was as high as 17% in treatment, but control-1 and control-2 system were lower at 1.0% and 10%, respectively. Phosphorus in suspended solids ranged between 1-18% while phosphorus incorporated in plant (treatment) was only 0.11%. Moreover, it was assumed that most of the nutrients were accumulated in suspended solids and solid deposited in baked clay beads tank.

4. Conclusion

With the proposed RAS, toxic nitrogenous compounds such as ammonia and nitrite were maintained within the safety level for fish. Significant amount of nitrogen compounds were removed mostly by degradation especially nitrification and denitrification processes while nutrients (nitrogen and phosphorus) assimilation in plant was the minor process. This RAS concept has high potential for further development.

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