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Nitrogen removal of aquaculture wastewater in aquaponic recirculation system

[1]Sri Wahyuningsih, [2]Hefni Effendi, [1]Yusli Wardiatno

[1] Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia; [2] Center for Environmental Research, Bogor Agricultural University, Bogor, Indonesia. Corresponding author: H. Effendi, hefni_effendi@yahoo.com

Abstract. Nitrogen wastes in the culture system are still difficult to handle. Aquaponic system can be an alternative to reduce the impact of the inorganic nitrogen accumulation that can be a limiting factor to the fish growth. At aquaponic system plant can absorb nutrient from farming waste, whereas bacteria functions in reducing the ammonia through the nitrification process. The aim of study was to assess aquaculture nitrogen waste reduction in aquaponic system. The result showed that the nutrient concentration fluctuated during the observation periods, and the highest nutrients accumulation were 6.489, 3.601, and 0.933 mg L^{-1} for TAN (ammonia and ammonium), nitrate, and nitrite in the control, respectively. Integration of tilapia (*Oreochromis niloticus*) fish farming, romaine lettuce (*Lactuca sativa*), and bacteria can reduce inorganic nitrogen with the best removal efficiency. There was 91.50, 34.41, 22.86, and 49.74% for TAN, nitrate, and nitrite, respectively. All results showed that treatment with the bacteria addition was the best treatment to reduce nitrogen waste, optimizing the fish and romaine lettuce plants production.

Key Words: ammonia, aquaponic, nitrification, tilapia, romaine lettuce.

Introduction. The main problem in fisheries is water quality degradation caused by the accumulation culture waste. According to Rakocy et al (2006), fish excretes nitrogenous waste such as ammonia directly discharged into the aquatic environment. Subsequently, according Francis-Floyd et al (1996) ammonia becomes the second limiting factor after oxygen and this parameter gives an effect to the fish growth.

Feed is a major source of ammonia in the culture system (Hargreaves & Tucker 2004), because the fish can only absorb 20-30% of nutrients from the feed, while the remaining is excreted into the environment in ammonia and organic protein form (Avnimelech 2006; Hargreaves 1998). According to Ebeling et al (2006), from 80% of nitrogen excreted, 90% contained as ammonia and 10% as urea.

Total ammonia nitrogen (TAN) in the water consists of ammonia unionized (NH_3) and ammonia ionized (NH_4^+) (Francis-Floyd et al 1996; Körner et al 2001; Rahmani et al 2004; Eshchar et al 2006; Van Rijn et al 2006; Titiresmi & Sopiah 2006). Temperature and pH increment will shift the equilibrium of TAN into ammonia which is a more toxic element. Ammonia toxicity is manifested by hyperactivity, convulsions, loss of equilibrium, lethargy, and coma (Hargreaves 1998). Chen et al (2006) reported that a high level of ammonia that can be tolerated in the culture system was 0.025 mg N L^{-1}. In general, to maintain good water quality, 5-10% volume of water containing nitrogen should be replaced with fresh water (Masser et al 1999). According to Hu et al (2015) untreated water containing ammonia discharged into the ecosystem will lead to eutrophication and other environmental problems.

Aquaponic becomes alternative of nitrogen waste treatment in farming systems, especially in areas with limited water supply. Aquaponic system is known as a combination of aquaculture with hydroponic plant in recirculation systems (Diver 2006; Rakocy et al 2006; Endut et al 2010; Roosta & Hamidpour 2011; Zheljazkov & Horgan 2011; Liang & Chien 2013). Ammonia in aquaponic system is changed into ammonium and nitrate (NO_3^-) by nitrification bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.). Ammonium and nitrate are absorbed by plants as nutrients (Rakocy et al 2006; Tyson et al 2011; Liang & Chien 2013). Plants can provide a biofiltration role by absorbing ammonium, whereas nitrification bacterial provides the dual role of reducing ammonia concentration through oxidation and converting ammonia to nitrate (Tyson et al 2011). With this system, water and nutrients can be reused maximally and environmentally friendly and simultaneously producing two cash corps (Diver 2006; Tyson et al 2011). This research was intended to assess the nitrogen reduction of aquaculture waste in recirculating aquaponic system.

Material and Method. This experiment was carried out from February to April 2015 at Center for Environmental Research of Bogor Agricultural University (PPLH IPB). It consisted of three treatments, each had three randomly assigned replications. (1) tilapia (*Oreochromis niloticus*) without romaine lettuce (*Lactuca sativa*) as control, (2) tilapia and romaine lettuce (*Lactuca sativa* L. var. *longifolia*) (T1), (3) tilapia, romaine lettuce, and inoculation with nitrifying bacteria (T2), were assigned.

Nine aquariums (80 x 40 x 40 cm^3) with 100 L water volume were chosen as the fish culture. Reservoir (60 L) and the aquaponic chamber (100 x 15 x 15 cm^3) as planting romaine lettuce were set up. Water from fish cultivation aquarium was drained with the discharge of 187 L h^{-1} to the chamber (volume ±1.4 L), passing through the roots of romaine lettuce. The water then flew from the chamber to the reservoir for being homogenized. The water was then piped vertically back into the aquarium (Figure 1).

As much as 20 fishes, average weight of 20 g, size ranged from 9 to 10 cm, were initially cultivated in each aquarium. Fishes were cultured for 35 days, and fed with commercial food around 3% of body weight with 30% of protein content. Frequency of feeding was three times a day.

Commercial nitrifying bacteria were added to the system (except control and T1) as much as 32 mL per week, containing around 10^6 CFU m L^{-1} *Nitrobacter* sp. and *Nitrosomonas* sp.

Two week old romaine lettuce seedlings (height 11 cm) were planted in small pots (diameter 5 cm). In each chamber was planted 5 romaine lettuces with the distance of 20 cm between plants. Only part of the plant roots was touched by the flowing water as recommended in nutrient film technique (NFT).

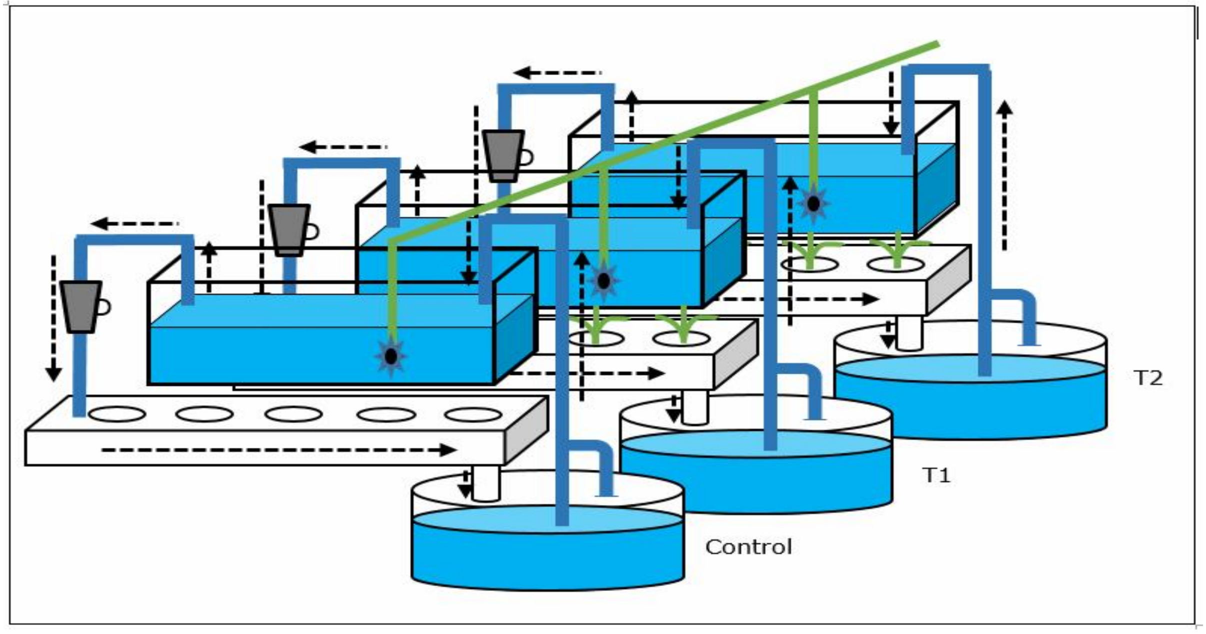


Figure 1. The series of experimental installations.

Water quality parameters such as TAN, nitrite (NO_2^-), nitrate (NO_3^-), temperature, pH, dissolved oxygen (DO), turbidity, and alkalinity were recorded during the experimental period with an interval of 7 days using the standard methods outlined in APHA (2008). Bacterial abundance (CFU m L^{-1}) was determined using total plate count (TPC) method. Growth performance of tilapia and romaine lettuce was also observed. The concentration of NH_3 can be computed from the following equation (Strickland & Parsons 1972):

$$\% \text{ un-ionized ammonia} = 100/[1 + \text{antilog } (pKa - pH)]$$

Where: pKa - the multiplication factor;
pH - the measured pH of the solutions.

Table 1 is used to find the value of pKa.

Table 1

p*K*a value based on temperature between 5-30°C

Temperature (°C)	*5*	*10*	*15*	*20*	*25*	*30*
p*K*a	9.90	9.73	9.56	9.40	9.24	9.09

Percentage reduction using formula proposed Bay Effendi et al (2015):

$$\% \text{ Reduction} = [(a-b)/a] \times 100\%$$

Where: a - control concentration of water quality parameter at time t;
b - treatment concentration of water quality parameter at time t.

Data analyses were performed using statistical package for the social sciences (SPSS) version 15 with an alpha set at 0.05 (significant at $P<0.05$). If there were significant differences at significant level 0.05, then Duncan multiple comparison test was used to compare means in order to identify significant difference between treatments.

Results and Discussion

Water quality parameters. Table 2 shows the average of water quality during the study in aquaponic system.

Table 2

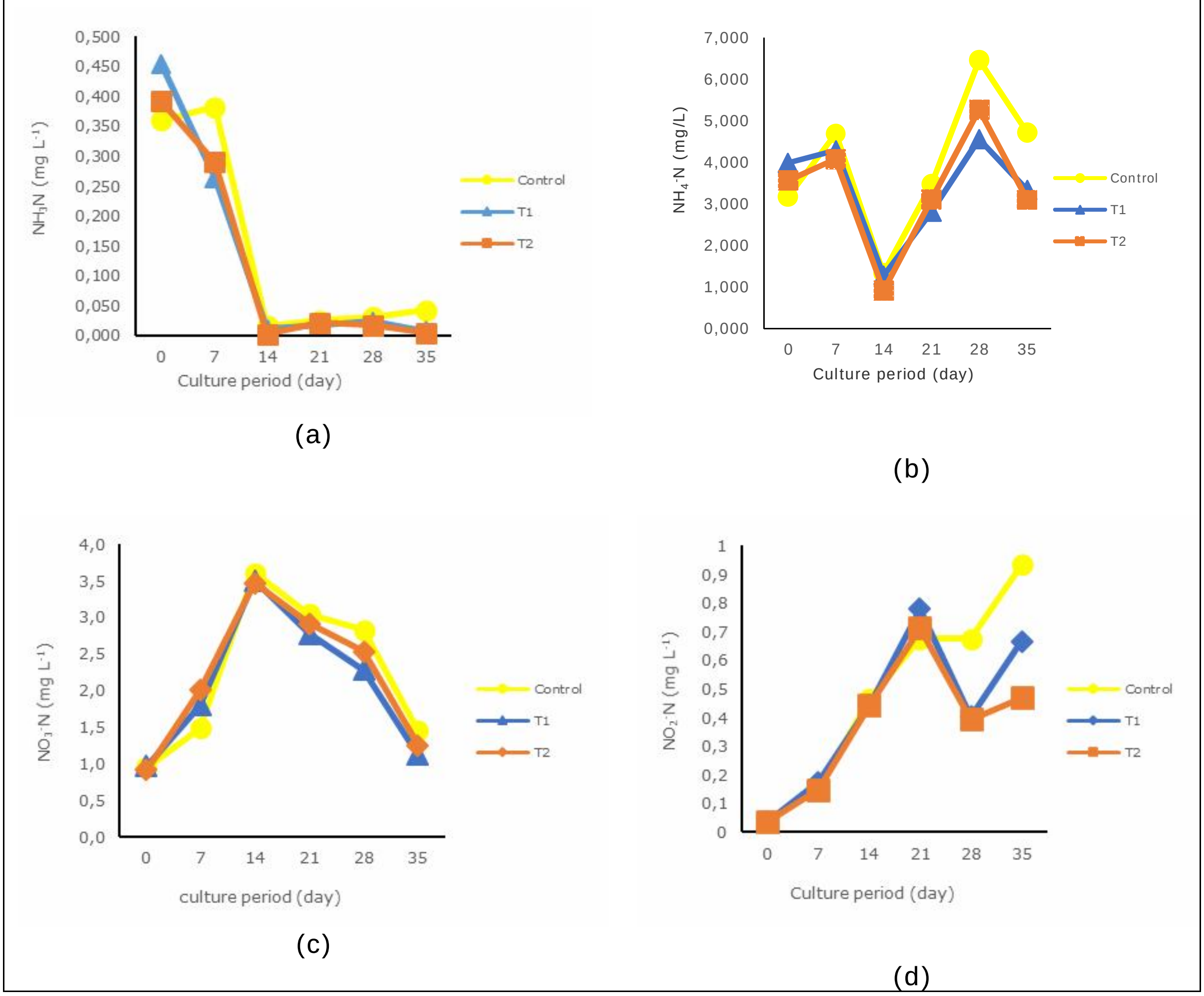
Water quality parameter observed during experiment

Parameters	*Control*	*T1*	*T2*
Temperature (°C)	29.18 ± 0.23^a	29.63 ± 0.28^b	29.14 ± 0.36^a
pH	7.28 ± 0.21^a	7.11 ± 0.24^b	7.05 ± 0.17^b
DO (mg L^{-1})	5.33 ± 0.53^a	5.20 ± 0.50^a	5.23 ± 0.39^a
Alkalinity (mg L^{-1})	20.07 ± 3.25^a	18.80 ± 3.94^a	18.54 ± 1.21^a
Turbidity (NTU)	17.51 ± 6.78^a	23.02 ± 8.46^a	21.57 ± 10.72^a
TAN (mg L^{-1})	4.12 ± 1.33^a	3.50 ± 0.71^a	3.46 ± 0.92^a
Ammonia (mg L^{-1})	0.14 ± 0.06^a	0.13 ± 0.04^a	0.12 ± 0.05^a
Ammonium (mg L^{-1})	3.97 ± 1.28^a	3.37 ± 0.67^a	3.34 ± 0.89^a
Nitrate (mg L^{-1})	2.24 ± 0.44^a	2.10 ± 0.27^a	2.18 ± 0.51^a
Nitrite (mg $L^{-1)}$	0.49 ± 0.13^a	0.42 ± 0.12^a	0.37 ± 0.13^a

T1 - tilapia and romaine lettuce; T2 - tilapia, romaine lettuce and inoculation with nitrifying bacteria - different letters in the same column are significantly different at $P<0.05$ level.

Turbidity and total alkalinity did not show any significant difference among all treatments. However, values in control were lower for turbidity and higher for alkalinity. Dissolved oxygen decreased during the study, but the value was above 5 mg L^{-1}, within acceptable limits for tilapia (Villarroel et al 2011). Alkalinity and dissolved oxygen were important to eliminate ammonia through nitrification process. Alkalinity was needed as a carbon source in this process. It was estimated that for every g conversion of ammonia into microbial biomass, it was needed 4.71 g dissolved oxygen and 3.57 g alkalinity (Ebeling et al 2006). Temperature and pH fluctuated and showed significant differences ($P<0.05$). This condition causes a shift in the equilibrium between ammonia and ammonium (Hargreaves & Kucuk 2001). When the temperature was 22°C, ammonia fractionation of TAN was 0.46% and 4.4% at pH of 7.0 and 8.0, respectively (Francis-Floyd et al 1996).

The changes of inorganic nitrogen in control, T1, and T2 during the experiment are shown in Figure 2.



(a)

(b)

(c)

(d)

Figure 2. The changes from NH_3N (a), NH_4^+N (b), NO_3^-N (c) and NO_2^-N (d) in all treatments.

Nutrient concentrations increased along with the time of cultivation, where the highest accumulation levels in control were 6.489, 3.601, and 0.933 mg L^{-1} for TAN, nitrate, and nitrite, respectively. Effluent concentration generally increased with time (Endut et al 2011). However, towards the end of experiment period, the concentration of nutrients were gradually reduced (except nitrite), with the same pattern of decline. Different concentrations of nutrients during the experiment showed no significant difference ($P<0.05$). However the overall best value of decrease of nitrogen compound was indicated at T2. This tendency was also shown by results of Shete et al (2013), where the value of nitrogen reduction on treatment with aquaponic system showed better results than the control without aquaponic system. The nutrient concentrations decline in experiments related to the utilization by plants and bacteria. On aquaponic systems, plants and bacteria play a role in sewage purification. Nutrient-rich waste functions as fertilizer for plant growth, whereas nitrification bacteria associated with the roots of plants and play an important role in the nutrient cycle (Diver 2006). Hu et al (2015) adds that most of the nitrification bacteria attach to the plant roots.

According to the scientific literature (El-Shafai et al 2004; Hargreaves & Kucuk 2001; Chen et al 2006) ammonia in this experiment exceeded the safe limits for tilapia. El-Shafai et al (2004) reported that the concentration of ammonia should be maintained under 0.1 mg L^{-1}. According to Hargreaves & Kucuk (2001), chronic toxicity level of ammonia for tilapia ranging from 0.035 to 0.092 mg L^{-1}. Ammonia toxicity hinders the ability diffusion among cell membranes (Colt 2006). Ammonia and nitrite are toxic to fish, but relatively harmless for nitrate (Rakocy et al 2006). Nitrite will be dangerous when part of Cl^- uptake was replaced with nitrite (*i.e.* fish absorbs nitrites at the expense of chlorides). This is due to nitrite demonstrates a degree of affinity to the Cl^- or HCO_3^- ion exchange (Svobodová 2005). The toxicity of nitrate to freshwater fish is very low (96 h $LC50_s$ >1000 mg L^{-1} as N) and may be related to potential osmoregulation problems (Colt 2006).

Based on the results of the abundance calculation of bacteria, there were significant differences ($P>0.05$), where the T2 value was two times higher (10^6) than the control and T1 at the end of the experiment (Figure 3). This value was much lower compared with the results of Hu et al (2015), which reached 10^8-10^{12} for aquaponic system. One of the cause which causing the low number of bacteria in this study was likely the deliberate disposal of fish excreta out of the aquarium during the study through siphoning everyday. Liu & Han (2004) reported that the dynamics of bacterial populations is limited by the availability of nutrients in the system. Decline in organic matter will further hinder the rate of nitrification, resulting in low conversion and high accumulation of TAN (Chen et al 2005). It is different delivered by Tyson et al (2008) that the optimal pH operating system will more affect loss of TAN than nitrification bacteria population.

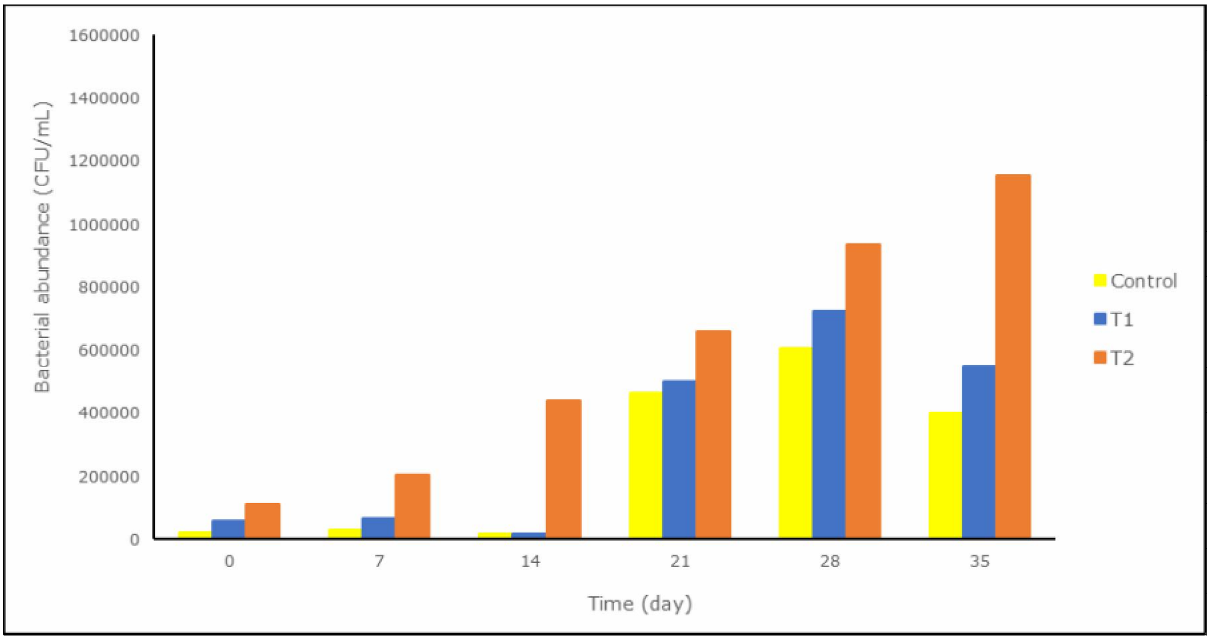


Figure 3. The abundance of bacteria in the control, T1, and T2.

Percentage of nitrogen reduction. The reduction percentage of nitrogen compared to the nitrogen in the controls is presented in Table 3. The best reduction percentage at the end of the experiment was T2 of 91.50%, 34.41%, and 49.74% for TAN, and nitrite, respectively. This result compares favorably with previous studies conducted by Effendi et al (2015) on the use of the lettuce in aquaponic system, *i.e.* 90.1% and 23.3% for ammonia and nitrate, respectively. The results of Snow & Ghaly (2008) study using aquatic plants showed that aquatic plants can significantly decrease the nitrogen waste in aquaculture amounted 55.9 to 76.0%, 34.5 to 54.4%, and 49.6 to 90.6% for ammonium, nitrate and nitrite, respectively.

Table 3

Percentage of nitrogen reduction

Time (day)	Percentage reduction (%)							
	NH_3N		NH_4^+N		NO_3^-N		NO_2^-N	
	T1	T2	T1	T2	T1	T2	T1	T2
7	30.96	24.16	8.62	13.06	-20.71	-34.80	-15.33	2.14
14	37.76	86.23	4.14	31.90	2.90	3.87	4.89	4.66
21	27.60	14.13	19.19	10.51	8.77	4.26	-15.74	-5.78
28	26.45	47.25	29.48	18.47	19.35	10.56	40.17	41.61
35	83.59	91.50	29.36	34.41	22.86	13.58	28.64	49.74

The decline rate of ammonia was much larger than the ammoniums, nitrites, and nitrates (Table 3). This decrease related to pH fluctuations during the experiment. According to Van Rijn et al (2006) pH and temperature influence the TAN fractionation, because at pH 7, TAN will form ammonium. At pH>8.75, 30% of TAN will turn out to be relatively toxic ammonia. The best reduction percentage of nitrogen occurred in T2, where in this treatment were added nitrifying bacteria. It is known that nitrification bacteria play an important role in converting ammonia into nitrate. Ammonia is firstly oxidized to nitrite by ammonia oxidizing bacteria (AOB), and then converted to nitrate by nitrite oxidizing bacteria (NOB) (Hu et al 2015). Decrease in ammonia level greater than ammonium, nitrite and nitrate was also associated with the growth rate of AOB, predicted much faster than the NOB. AOB population will increase faster than NOB when the temperature is above 25°C (Yamamoto et al 2008). In addition to bacteria, plants also play a crucial role in the decline of nutrients. Nutrients can be reduced at the lowest level when the number of plants more (Tyson et al 2008). Overall, the concentration and percentage reduction in nitrogen were observed lower in aquaponic systems, especially T2. This indicates that aquaponic system could potentially be a nitrogen waste treatment technologies.

Aquaponic performance. Increase in water quality at aquaponic system might bring about the performance of cultivated tilapias at treatment better than that of control. Tilapia highest survival rate was 96.1% at T2, followed by T2 and control were 94.4% and 89.17%, respectively. The growth of tilapia increased during the observation period. Final weight reached double from the initial weight. They were 45.89, 47.80, and 48.49 g for the control, T1, and T2 respectively. Fish survival and growth were influenced by the level of ammonia concentration, wherein ammonia causes a decrease in the sublethal of fish growth (Hargreaves 1998). Feed conversion rate were 2.02, 1.70, and 1.60 for the control, T1 and T2, respectively. Higher feed conversion rate phenomenon at control was similar with higher ammonia concentration at control than that of T1 and T2. Ammonia will give an effect on energy utilization suppress growth (Hargreaves & Kucuk 2001).

At the end of the experiment, romaine lettuce fresh weight reached 61.9-57.7 g for T1 and T2, with average growth rate of 0.39 and 0.37 cm d^{-1} for both T1 and T2. This result was better than in the experiments conducted by Effendi et al (2015) *i.e.* 0.04 cm d^{-1}, and lower than in experiments conducted by Endut et al (2011) *i.e.* 1.91 and 1:32 cm d^{-1} for water spinach and mustard green, respectively. The growth that has been shown by the romaine lettuce showed the high absorption of nutrients. A high level of plant uptake showed a high degree of absorption, thus decreasing the concentration of nutrients in the system (Hu et al 2015).

Conclusions. Plants and bacteria in aquaponic system play an important role in the processing of nitrogenous wastes. This study showed that the application of the aquaponic system has significant influence at nitrogen transformation. Nutrient concentration during the experiment fluctuated, where treatment with the addition of romaine lettuce and bacteria showed better results than the control, and treatment of romaine lettuce without the addition of bacteria. Aquaponic system was able to decrease the inorganic nitrogen amounted 91.50%, 34.41%, 22.86%, and 49.74% for ammonia and ammonium, nitrate, and nitrite, respectively. Performance of tilapia and romaine lettuce production also showed promising results, and can adapt to fluctuating conditions. All results showed that aquaponic system can be used as alternative for fish cultivation waste treatment, and subsequently for plant and fish biomass production.

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Authors:
Sri Wahyuningsih, Bogor Agricultural University (IPB), Faculty of Fisheries and Marine Science, Department of Aquatic Resources Management, Indonesia, Bogor 16680, Kampus IPB Dramaga, e-mail: s_yuni18@yahoo.com
Hefni Effendi, Bogor Agricultural University (IPB), Centre for Environmental Research, Indonesia, Bogor 16680, Kampus IPB Dramaga, e-mail: hefni_effendi@yahoo.com
Yusli Wardiatno, Bogor Agricultural University (IPB), Faculty of Fisheries and Marine Science, Department of Aquatic Resources Management, Indonesia, Bogor 16680, Kampus IPB Dramaga, e-mail: yusli@ipb.ac.id

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