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Treatment of aquaculture wastewater using *Vetiveria zizanioides* (Liliopsida, Poaceae)

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Abstract. To determine the effectiveness of vetiver grass (*Vetiveria zizanioides*) to absorb organic waste and nutrient from fish aquaculture, an experiment using aquaponic with recirculating aquaculture system (RAS) was carried out. Nile tilapia fish (*Oreochromis niloticus*) was used and three treatments were conducted, i.e. P0 (tilapia without vetiver grass), P1 (tilapia with vetiver grass of 160 grams wet density), and P2 (tilapia with vetiver grass of 320 grams wet density). The experiment was carried out for 42 days. Observation and data retrieval were weekly conducted. The Relative Growth Rate (RGR) and Feed Conversion Ratio (FCR) in P2 were better than other treatments, i.e. 0.025±0.000 g day^{-1} and 1.6±0.1 g day, respectively. The growth of vetiver grass in P1 and P2 was not significantly different. The concentration of ammonia (NH_3-N) among the three treatments showed significant difference ($p < 0.05$) on day 14, i.e. 48.36% Ammonia (NH_3-N) removal in P2 and 31.33% in P1. Ammonium (NH_4-N) and nitrate (NO_3) showed no significant difference among the three treatments. Orthophosphate showed significant difference among treatments, particularly on day 28 with 19.94% orthophosphate removal in P2 and 15.27% in P1.
Key Words: aquaponic, Nile tilapia, vetiver grass, water quality.

Introduction. In mid-1980, the World Bank developed the concept of Vetiver Grass Technology for soil and water conservation in India (Chomchalow 2000). Vetiver grass (*Vetiveria zizanioides*) is a protruding plant and its physiologycal characteristics are capable of absorbing soluble nutrients such as N and P, capable of absorbing heavy metal, and tolerant towards various extreme environmental conditions (Truong et al 2011). Several studies showed that vetiver grass is capable of absorbing organic wastewater (containing N and P) from mills and urban areas (Indrayatie et al 2013; Chua et al 2012). In addition, the grass also has high economic value. The root of which can be used as raw material for perfume and cosmetic (Bhatia et al 2008), medicine (Chou et al 2012; Saikia et al 2012), insect repellant (Jain et al 1982; Aarthi & Murugan 2012), active carbon (Gaspard et al 2007), and biogas (Li et al 2014). The grass can also be used as material for unique handicraft (Tripathy et al 2014). Therefore, vetiver grass is not only an effective waste absorber media but also can be harvested as high economic value plant.

One of the problems still difficult to handle in fishery is aquaculture waste management (for fish and shrimp). Intensive aquaculture activities often increase organic matter and nutrients (N and P) in water. The increase is from residual feeds that are not consumed by fish and fish metabolic waste. Karakassis et al (2005) reported that aquaculture activities in the Mediterranean account for about 5% of N and P from total annual anthropogenic waste. The same problem occurred in China where water quality degrades from year to year as the result of aquaculture activities (Cao et al 2007). Another areas also experienced increasing organic materials due to aquaculture activities, such as Bolinao Coast in Philippines (Diego-McGlone et al 2008), Malawi Lake in Africa (Gondwe et al 2011), and Yemlo and Allage reservoirs in Ethiopia (Degefu et al 2011). Therefore, to reduce the negative impact of aquaculture, aquaculture waste treatment method must be developed.

Nowadays, various aquaculture waste treatment methods using biofiltration system have been developed. Water media for fish aquaculture rich of N and P is flown to planting medium and then utilised by plants by absorbing the water nutrients and eventually reducing the nutrients. The water then can be reused for fish aquaculture, indicating that this method can effectively reduce water use. Several successful studies using this method are Graber & Junge (2009) using tilapia, eggplant, tomato, and cucumber; Mariscal-Lagarda et al (2012) using white shrimp and tomato; and Liang & Chien (2013) using tilapia and water spinach. The results showed that the method is effective and profitable, in addition to effectively transform nutrients into biomass. This method is known as aquaponics (Bakiu & Shehu 2014; Goddek et al 2015). Although aquaponics usually uses vegetables, study using vetiver grass has yet to be conducted. Therefore, this study aimed to measure the effectiveness of vetiver grass as biofilter in fish aquaculture organic waste treatment which eventually produce good water quality, as well as to provide additional benefit through fish and vetiver grass crops.

Material and Method. The study was conducted on 3 May to 14 June 2015 in the Laboratory of Centre for Environment Research (PPLH-IPB). Recirculating Aquaculture System (RAS) with vetiver grass as filter to reduce organic matter and nutrient was used. A total of 9 experimental units were employed, each with aquarium of 80 x 40 x 32 cm in size, gutter of 15 x 15 x 50 cm as the place to plant vetiver grass, and tank as water meeting point before entering the aquarium. Total water used was 160 L and the water was not changed during the experiment. The water was only added when water loss took place due to evaporation and transpiration. The design of recirculation system can be seen in Figure 1.

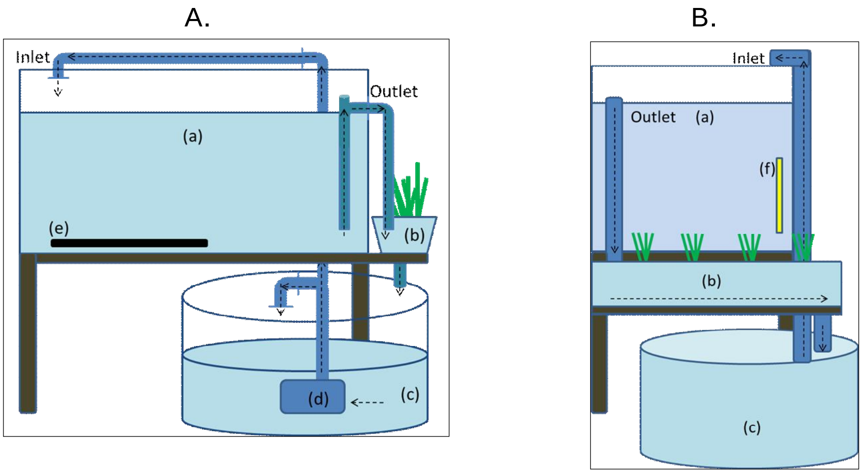


Figure 1. A. Instalation's side view, B. Instalation's front view. Note: (a) Aquarium for fish culture, (b) Gutter for vetiver grass planting, (c) Tank, (d) Water pump, (e) Water heater, (f) Thermometer, and -----► = water current flow.

Nile tilapia (*Oreochromis niloticus*), 8-10 cm in length, average weight of 25 grams were cultivated in aquariums, in each of which consisted of 20 Nile tilapia. Before the experiment started, Nile tilapia acclimatisation for five days was carried out, aiming to familiarise the fish to new environment and to accumulate organic materials so that the nutrients for the growth of vetiver grass were sufficient. After acclimatisation, one month vetiver grasses of 10 cm in height were planted on gutters with different planting densities according to their respective treatment. Before planting, vetiver grasses were cleaned from soil and sludge using water. The treatments given were as follows:
P0 = Nile tilapia without vetiver grass;
P1 = Nile tilapia with 4 clumps of vetiver grass (160 grams wet density);
P2 = Nile tilapia with 8 clumps of vetiver grass (320 grams wet density).
Each treatment was repeated three times.

After vetiver grass planted, Nile tilapia were grown for six weeks. The Nile tilapias were fed with pellets (commercial feeds) three times a day (morning, noon, and afternoon); the amount of which was 4% of fish body weight with 40% protein content. During the experiment, additional nutrients were not given to vetiver grass assuming that the grasses were capable of using available nutrients in the water cultivation media. The growth of Nile tilapia and vetiver grass was observed weekly for survival rate (SR), relative growth rate (RGR), and feed conversion ratio (FCR).

Survival rate (SR) was caculated using the formula:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Where:
SR = survival rate
Nt = number of fish or plant at the end of experiment
No = number of fish or plant at the onset of experiment

Relative growth rate (RGR) was calculated using the formula:

$$RGR = \frac{\ln W_t - \ln W_0}{\Delta t}$$

Where:
RGR = relative growth rate
Wt = weight of fish or plant at the end of experiment
Wo = weight of fish or plant at the onset of experiment
Δt = observation period.

Feed convertion ratio (FCR) was calculated using the formula:

$$FCR = \frac{W_f}{W_b}$$

Where:
FCR = feed convertion ratio
Wf = weight of food given (g)
Wb = weight of fish (g)

Water quality characteristics i.e. dissolved oxygen, pH, turbidity, temperature, N (ammonia, ammonium, and nitrate), and P (orthophosphate) were observed every week. Dissolved oxygen and temperature were measured using DO meter, pH using pH meter, and turbidity using turbiditymeter. Analysis of ammonia, nitrate and orthophosphate was conducted in the laboratory using spectrophotometric method which refers to APHA (2005). The nutrient loss due to treatment in cultivation media was measured using the following formula:

$$NR = \frac{C_a - C_b}{C_a} \times 100\%$$

Where:
NR = nutrient removal (%)
C_a = nutrient concentration in the treatment of fish without vetiver grass (mg L^{-1})
C_b = nutrient concentration in the treatment of fish with vetiver grass (mg L^{-1})

All data collected were statistically analysed using ANOVA with time series followed with Duncan's test using SAS 9.1.3 portable software.

Results and Discussion

The growth of Nile tilapia and vetiver grass. The growth of Nile tilapia and vetiver grass can be seen in Table 1. The results showed that the Nile tilapia survival rate for all treatments was above 90% with the highest survival rate belonged to P1 (98.33%). Although the survival rate value of P1 was the highest, statistically the survival rate of each treatment were not significantly different ($p > 0.05$). Relative growth rate (RGR) of the Nile tilapia by weight (grams), however, showed significant difference for each treatment ($p < 0.05$). The RGR of P2 was higher than P1 and P0. RGR for P2, P1, and P0 were respectively 0.025±0.000 g day^{-1}; 0.022±0.002 g day^{-1}; and 0.021±0.001 g day^{-1}, indicating that the daily weight gain of Nile tilapia in P2 was faster than in P1 and P0, and the growth rate of Nile tilapia in P1 was faster than in P0. The growth of cultivated fish using recirculating system with additional plants was faster than recirculating system without plants (Dediu et al 2011).

FCR of Nile tilapia showed significant difference for the three treatments ($p < 0.05$) where the lowest value belonged to P2. It indicates that more effective feeding occurred in P2 where to produce 1 kg of cultivated Nile tilapia requires 1.6 kg feed. FCR in P1 also lower than in P0. The smaller FCR value indicates the more effective fish culture because to produce one kilogram of fish needs only smaller amount of feed, and eventually the production cost can be reduced.

Table 1

Performance of Nile tilapia and vetiver grass culture at the end of experiment

Growth indicator	*P0*	*P1*	*P2*
Nile tilapia			
Survival rate (%)	93.33 ± 7.6^{a}	98.33 ± 2.9^{a}	91.66 ± 4.7^{a}
Relative grow rate (g day^{-1})	0.021 ± 0.001^{a}	0.022 ± 0.002^{ab}	0.025 ± 0.000^{b}
Feeding convertion ratio	2.0 ± 0.1^{a}	1.9 ± 0.3^{ab}	1.6 ± 0.1^{b}
Vetiver grass			
Survival rate (%)	-	100^{a}	100^{a}
Relative grow rate (g day^{-1})	-	0.01 ± 0.006^{a}	0.01 ± 0.007^{a}

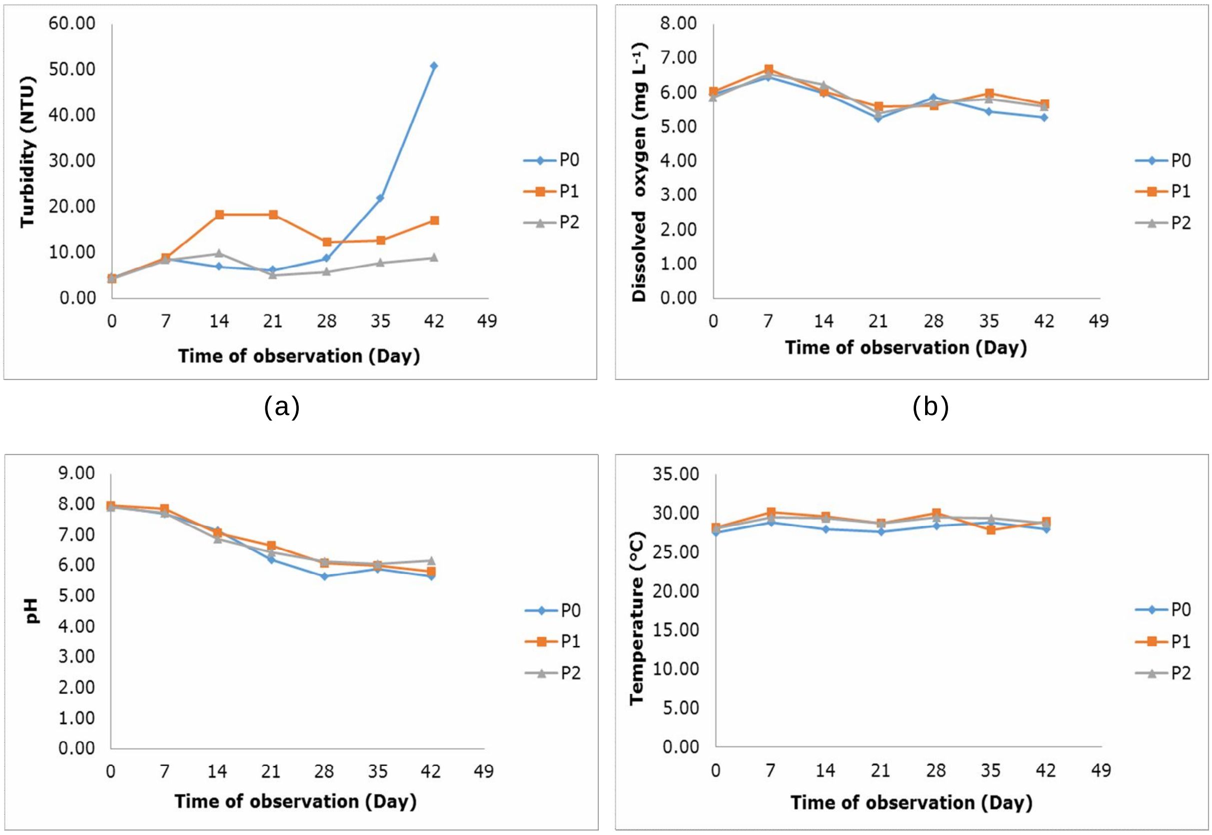
Values were expressed as mean ± SD; Values with the same superscript letters are not significantly different ($p > 0.05$); Analysis for Nile tilapia used one-way ANOVA analysis, whereas vetiver grass used independent-sampled T-test.

During the cultivation period, there was no dead vetiver grass (Table 1), resulting in the survival rate of P1 and P2 reached 100% and indicating that vetiver grass is potential to be planted in cultivation media along with fish aquaculture. The vetiver grass growth observed was the rate of growth of vetiver plants by weight (grams). The results showed that the weight of vetiver grass in P1 and P2 increased 0.01% per day. The RGR value of vetiver grass in P1 and P2 showed no significant difference ($p > 0.05$) where RGR for P1 was 0.01 ± 0.006 g day^{-1} and P2 was 0.01±0.007 g day^{-1}. This revealed that the density of vetiver grass brings about no significant impact on the survival rate and growth of the grass. However, RGR of 0.01 day^{-1} is still lower than the RGR of vetiver grown in artificial nutrient media, i.e. 0.02 day^{-1} (Jampeetong et al 2012).

Water quality. During the cultivation period, turbidity was fluctuating (Figure 2a). The turbidity of P2 tended to be more stable, while in P1 the value increased on day 14 and 21, and then decreased on the following days. In P0 stable turbidity was seen on day 0 to 28 and increased very sharply on day 42. The difference of turbidity in each treatment, however, was not significantly different ($p > 0.05$). Turbidity was allegedly affected by the high abundance of phytoplankton (Figure 3).

Dissolved oxygen (DO) were also not significantly different for each treatment ($p > 0.05$). DO range values of P0, P1, and P2 were 5.74±0.40 mg L^{-1}, 5.94±0.35 mg L^{-1}, and 5.87±0.35 mg L^{-1} respectively (Figure 2b). The DO is still tolerable for Nile tilapia (DeLong et al 2009). pH of each treatment were also not significantly different (p >

0.05). The decreasing pH during the cultivation period can be seen in Figure 2c. At the beginning of the experiment, the initial pH of each treatment was 7.92±0.02. At the end of the observation, the pH of P0, P1, and P2 decreased to 5.64±0.70, 5.80±0.12, and 6.15±0.30, respectively. From the data, it appears that the best pH belonged to P2. The reducing pH in water occurs due to high organic materials in the water. Decomposition process requires dissolved oxygen and produces dissolved carbon dioxide. pH is strongly affected by the presence of carbon dioxide, the higher the dissolve carbon dioxide, more acidic the pH will be. According to DeLong et al (2009), the optimum pH for the growth of Nile tilapia is 6-9, while the optimum pH for the growth of aquatic plants is less than 7 (Owens et al 2005). In this system, it is very important to maintain the pH in the range of 6-7. Temperatures in each treatment also showed no significant difference (p > 0.05). Temperatures during the cultivation period of P0, P1, and P2 were 28.10±0.53°C, 28.99±0.82°C, and 28.95±0.51°C, respectively. . Temperatures in the range of 27-29°C is the optimum temperature for growth of Nile tilapia (DeLong et al 2009) (Figure 2d).



(c) (d)

Figure 2. Alteration of water quality parameters (a) Turbidity, (b) Disolved oxygen, (c) pH, (d) Temperature.

Chlorophyll-a abundance fluctuated over time (Figure 3), although the abundance of chlorophyll-a of each treatment were not significantly different (p > 0.05). The result shows that the abundance of chlorophyll-a in P0 tended to increase and peaked on day 42 while in P1 peaked on day 21 and then decreased until day 42. The abundance of chlorophyll-a in P2 also peaked on day 21 and then decreased until day 42 but not as sharp as P1. Chlorophyll-a indicates the presence of phytoplankton, organisms that appear in waters rich of organic matter. Phytoplankton is often used as the indicator of water fertility (Veronica et al 2014). The presence of chlorophyll-a can also affect the turbidity of the water. In conditions of phytoplankton bloom, the water may become very turbid that could result in a bad effect on appetite and fish health.

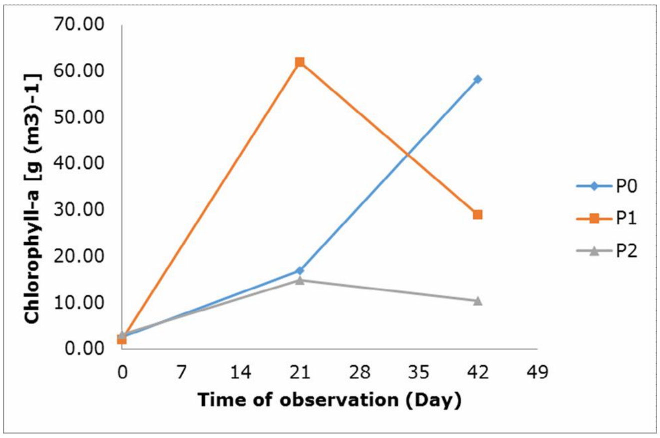


Figure 3. Alteration of chlorophyll-a.

Dissolved nutrients analyzed during the experiment were N (ammonia, ammonium and nitrate) and P (orthophosphate). Ammonia in water consists of two forms, i.e. unionized ammonia (NH_3-N) and ionized ammonia (NH_4-N). Unionized ammonia, or commonly called ammonia, is dangerous because it is toxic to aquatic organisms, while the ionized one, or ammonium, is harmless to aquatic organisms and is a nutrient that can be used directly for plants. Plants can function as biofilters by absorbing NH_4^+, and thus reducing toxic NH_3 through TAN equilibrium (Tyson et al 2011).

The content of ammonia (NH_3-N) increased on day 7 and then decreased on day 14 and the following days (Figure 4). The content of ammonia in P0, P1, and P2 at the end of experiment were respectively 0.014±0.02 mg L^{-1}, 0.011±0.00 mg L^{-1}, and 0.029±0.02 mg L^{-1}. The ammonia concentrations were below fish tolerance threshold (1.0 mg L^{-1}) (DeLong et al 2009). Based on the results of the study, a significant difference for ammonia (NH_3-N) was seen on day 14 ($p < 0.05$), but not significant in the other days. The concentration of ammonia (NH_3-N) in P2 was lower than in P1 and P0.

The most effective ammonia removal i.e. on day 14. Ammonia (NH_3-N) removal on day 14 was 48.36% in P2 and 31.33% in P1. Nevertheless, the percentage of ammonia removal using vetiver grass is lower than using tomatoes (69%) (Graber & Junge 2009), spinach (84.4%) (Effendi et al 2015a), lettuce (91.5%) (Effendi et al 2015b), and romaine lettuce (91.50%) (Wahyuningsih et al 2015). In contrast to the ammonia (NH_3-N), the concentration of ammonium (NH_4-N) continued to increase over time. During the observation, significant difference of ammonium (NH_4-N) concentration was unseen in each treatment ($p > 0.05$). However, the trend in P2 showed that the concentration of ammonium was lower than in P1 and P0, particularly on day 35. Ammonium removal on day 35 of P2 and P1 were 4.06% and 3.06% respectively. This system denotes that vetiver grass takes advantage of ammonium in water, but in relatively small quantities.

Nitrate (NO_3) is harmless compound to fish and is one of nutrient source for plants beside ammonium (NH_4-N). Nitrate toxicity can occur if the level of which in water reuse systems exceeds 300 to 400 mg L^{-1} nitrate-nitrogen range (DeLong et al 2009). During the experiment, the concentration of nitrate was still supportive for the life of Nile tilapia. The concentrations of nitrate in each treatment were not significantly different ($p > 0.05$). Nitrate concentration tended to fluctuate each day (Figure 4c) and the lowest concentrations occurred on day 0 (P0: 2.970±0.08 mg L^{-1}; P1: 2.817±0.16 mg L^{-1}; and P2: 2.978±0.06 mg L^{-1}) and the highest on day 21 (P0: 5.267±0.63 mg L^{-1}; P1: 5.684±0.32 mg L^{-1}; and P2: 5.922±0.43 mg L^{-1}). Vetiver grass brought no change on the concentration of nitrate in each treatment, indicating that vetiver grass is unlikely effective to absorb nitrates. Instead, the grass is more effective to absorb ammonium (NH_4-N) than nitrate (NO_3) (Jampeetong et al 2012). NO_3 must first be reduced to NH_4-N before it can be assimilated by the plants because the energy necessary to assimilate nitrogen is lowest for NH_4-N and increase for NO_3 (Wetzel 2001).

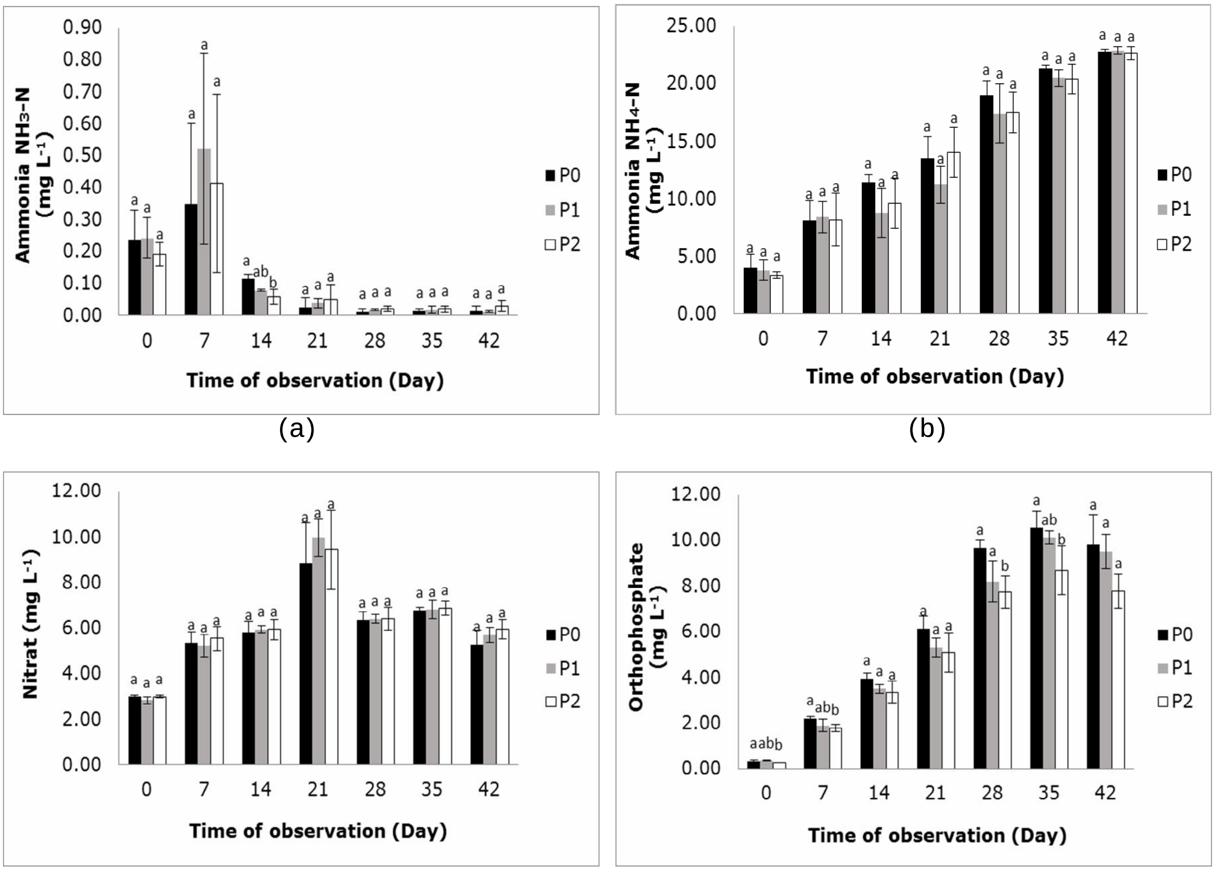


Figure 4. Alteration of water quality parameters (a) Ammonia NH_3-N, (b) Ammonia NH_4-N, (c) Nitrat, (d) Orthophosphate.

Orthophosphate (PO_4) brought no negative impact on fish growth, however, in high amount can lead to fertile the water and to cause algal bloom which then eventually can affect fish health. During the cultivation period, orthophosphate increased from day 0 to day 35 (Figure 4d), and decreased on day 42. The concentrations of orthophosphate were significantly different for each treatment ($p < 0.05$), i.e. on day 0, 7, 28, and 35. The content of orthophosphate in P2 was lower than in P1 and P0. Nutrient removal was the highest on day 28, i.e. reached 19.94% for P2 and 15.27% for P1. Phosphor is a main nutrient required by plants, and orthophosphate is phosphorus in dissolved form that can be directly absorbed by plant due to its simpler form. In this experiment, it reveales that the vetiver grass is more effective in lowering phosphorus compared to nitrogen.

In this experiment, the nutrient removal (NR) was relatively low. This is more likely due to the amount of nutrients in the cultivation medium (excess feed and metabolic waste) is higher than the absorption of the plant, and therefore the higher density of vetiver grass is needed. The ratio of fish feed per gram of vegetable biomass that is optimum for aquaponics system is 1:5 (Simeonidou et al 2012). This might be also related to the fact that vetiver is a plant which can be grown in dry areas and infertile (Mondyagu et al 2012), so that the nutritional needs of vetiver is not too high. In general, based on the results of the present study, vetiver grass is a potential biofilter used to manage organic waste which further decomposes to produce high nutrient, especially phosphorus in aquaculture activities. Considering that the vetiver grass provides various benefits, a more advance study is necessary to be carried out in the future.

Conclusions. Fish cultivated using aquaponic system (by planting vetiver grass) shows better growth than without vetiver grass, indicated by the higher RGR and more efficient feed consumption. The growth of vetiver grass is not affected by its density, as shown by the coefficient of vetiver grass planted with density of 160 grams which was not different with density of 320 grams. The turbidity in P2 was lower than in P1 and P0, and the dissolved oxygen and pH of which were also better than P1 and P0 although not statistically different. Vetiver grass is capable of reducing ammonia up to 48.36% and orthophosphate up to 19.94%, with the exception of nitrate. In this study, vetiver grass was unlikely to be effective to absorb nitrogen (N) particularly nitrate, but effective in absorbing orthophosphate. This might relate to the amount of nitrogen in the water that was still too high, compared to absorption capability of vetiver grass. Overall, vetiver grass is potential to be used as biofilter to absorb organic materials and nutrient, and suitable to be planted along with Nile tilapia in aquaponics system. Further studies are necessary to be carried out, especially with vetiver grass in higher density.

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