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Original article

Ammonia and orthophosphate removal of tilapia cultivation wastewater with *Vetiveria zizanioides*

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ABSTRACT

Vetiver is an environmentally friendly plant since it has non-invasive characteristic, has a high level of heavy metal tolerance, and could reduce N and P content originated from organic water pollutants. Tilapia (*Oreochromis niloticus*) cultivation wastewater, containing high concentration of N and P, was treated with vetiver (*Vetiveria zizanioides*) in aquaponics with NFT technique. Treatment consisted of triplicate of P1 (tilapia without vetiver); P2 (tilapia and 400 g of wet vetiver) and P3 (tilapia and 800 g of wet vetiver). Treatment of fish cultivation wastewater with vetiver was capable of lowering concentration of NH_3 (65.16%), NO_2 (27.51%), NO_3 (25.05%) in day 7, and NH_4 (30.17%), PO_4 (42.75%) in day 14. More vetiver density removed more N and P of tilapia culture wastewater.

1. Introduction

Aquaculture has been rapidly expanding industry that requires bulk quantities of water. As much as 200–600 m^3 water is needed for the production of 1 kg fish. Many aquaculture production facilities operate as flow through or open systems, hence releasing sizeable quantities of nutrient rich water into a receiving water body. Recent innovations such as denitrification reactors, sludge thickening technologies and ozone treatments led to a further decrease in water use, waste discharge and energy use in Recirculating Aquaculture System (RAS) (Kofinas and Kioussis, 2003; Martins et al., 2010).

Waste of aquaculture mostly originates from uneaten food and feces which is normally biodegradable waste. Thus BOD is determined instead of COD. The main constituents of concern from aquaculture include pH, biochemical oxygen demand (BOD), total suspended solids (TSS), turbidity, nitrogen, and phosphorus species (Steicke et al., 2002). In an aquaculture system without water exchange (zero water exchange) such as stagnant water pond, concentration of aquaculture waste such as ammonia (NH_3) and nitrite (NO_2) will increase rapidly and toxic to the cultured organisms. Aquaculture waste resulting from metabolic activity contains ammonia (Purwandari et al., 2017; Effendi, 2003). Out of several forms of water-soluble nitrogen, ammonia (NH_3) is the most harmful to fish, and most tropical fish species are generally more sensitive to ammonia (Effendi et al., 2015a; Wang and Leung, 2015). Meanwhile PO_4 is not harmful to fish, but causing eutrophication of water environment when available in an excessive concentration.

Feed as the main source of ammonia in the cultivation system because fish is only able to absorb 20–30% of nutrients derived from feed while the rest is excreted into the environment in the form of ammonia and organic protein (Avnimelech, 2006). Residual feed and feces discharged into waters has the potential to be organic contaminants in the form of N and P that can affect fertility levels and quality of water. Aquaponic system can be used as an alternative solution as fish farming waste treatment which effectively reduce total ammonia (Effendi et al., 2015b). Feed impact on the environment may also be reduced by selecting ingredients from a low trophic level (e.g. proteins and lipids from phytoplankton rather than from fish), provided feed digestibility does not decrease (Martins et al., 2010).

Phytoremediation is the utilization of plant to remove and accumulate contaminants from environment, including the use of plants to mitigate, transfer, stabilize or degrade pollutants in soil, sediments and water (Ojoawo et al., 2015).

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Vetiver (*Chrysopogon zizanioides* (L) Roberty), a medicinally important perennial plant, known to control soil erosion, tolerates a wide range of pH and elevated levels of toxic metals (Gautam and Agrawal, 2017). Vetiver is hydrophilic terrestrial plant which has physiological characteristics like the ability to absorb dissolved nutrients such as N and P, reduce BOD, COD, TSS, oil spill, accumulate heavy metals, batik production wastewater, tofu production wastewater, and high tolerance to herbicides and pesticides (Effendi et al., 2015d, 2017a; Seroja et al., 2018; Truong et al., 2011; Tambunan et al., 2018).

Tilapia (*Oreochromis niloticus*) can be cultured in aquaponic systems (Delis et al., 2015; Liang and Chien, 2013; Love et al., 2015; Wang et al., 2016). Tilapia has a good level of tolerance to various environmental conditions, is able to be cultivated in aquaponic system with vegetables (Effendi et al., 2015a), and has a high economic value (Diver, 2006). This study was aimed to analyze the effectiveness of vetiver in removing nitrogen and phosphorous of tilapia cultivation waste water in recirculation systems of aquaponics by comparing treatment of different vetiver planting density.

2. Materials and method

The study applied recirculation aquaculture system of aquaponics with nutrient film technique (NFT). NFT is a hydroponic method with a thin water flow (15.34 ml/s) as high as ±1 cm, so the roots grow in a shallow nutrient layer, while the non-submerged roots can absorb oxygen through diffusion (Rakocy et al., 2006).

Aquarium (80 x 40 x 60 cm^3, and 200 L), gutter (80 x 15 x 15 cm^3, and 1.2 L), and water tank (80 x 40 x 60 cm^3, and 200 L) were used. The system utilized peristaltic pump to control water flow. Water exchange was not performed during the study. The water in the aquarium before usage was aerated for 1 week to enhance dissolve oxygen in the water (Effendi et al., 2017e).

Prior to use, the vetiver was stored in a 40 x 35 x 25 cm^3 tank with a floating raft system in 25 L water added 5 ml commercial hydroponic nutrient solution (AB mix) per 1 L media and acclimated to wetland conditions for 1 month. Vetiver height of 10 cm was planted in several pots filled with rockwool and placed in a gutter. Vetiver utilized the available nutrients resulting from decomposition of uneaten fish food and feces.

A total of 20 tilapias (*Oreochromis niloticus*), average weight of 14 g, average length of 8–9 cm, was used. Density of 20 fish/200 L refers to Sace and Fitzsimmons (2013). There was no addition of artificial nutrients for vetiver during six weeks experiment. Treatment consisted of triplicate of P1 (tilapia without vetiver); P2 (tilapia and 400 g of wet vetiver) and P3 (tilapia and 800 g of wet vetiver) (Fig. 1). 400 and 800 g vetiver as wet weight means life vetiver grass weight as treatment.

Seven days fish acclimatization would accumulate organic matter, which later provided nutrient for the growth of vetiver. The fish age was 3 months (with average length of 9 cm and average weight of 14 g). Fishes were fed by pellets three times a day as much as 3% of body weight. Average feed per day: 3.27 g (1st week), 3.88 g (2nd week), 4.43 g (3rd week), 4.79 g (4th week), 5.67 g (5th week), 6.72 g (6th week). Floating pellet, size 2 mm, contained 33% protein with 5.97% N and 1.10% P.

Water quality was measured weekly for six weeks in tank (c). Parameters analyzed were N (TAN, nitrate, nitrite), P (orthophosphate), Dissolved Oxygen (DO), pH, temperature, and turbidity, referring to APHA (2012). All data were analyzed by ANOVA using SPSS (Saltman, 2015). Moreover, varimax factor was determined by comparing all variables to scrutinize correlation among variables.

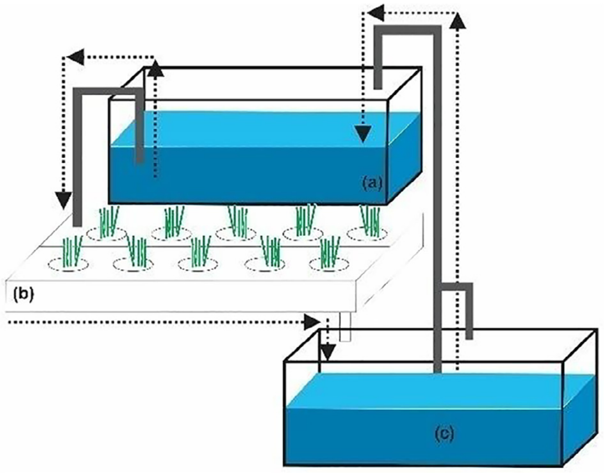

Fig. 1. Aquaponic installation (a) Fish tank, (b) Vetiver gutter, (c) Water tank after vetiver treatment.

3. Results and discussion

Initial water quality characteristic and average water quality in each treatment are presented in Tables 1 and 2**.**

3.1. Temperature, Turbidity, pH, and Dissolved oxygen

Treatment without plants (P1), vetiver of 400 g (P2), and vetiver of 800 g (P3) had relatively similar temperature, pH and Dissolved Oxygen (DO). Moreover, turbidity and inorganic nutrient content including Total Ammonia Nitrogen (TAN), Ammonia (NH_3), Ammonium (NH_4), Nitrite (NO_2) in P3 tended to be lower than those in P1 and P2.

Turbidity in P3 was lower than in P2 and P1 (Table 2). Furthermore, turbidity in each treatment was not significantly different ($p > 0.05$). Time of observation had significant impact on turbidity ($p < 0.05$).

Value of pH was significantly different among treatments ($p < 0.05$) and decreased at the end of observation. During observation period, pH ranged 6.00–6.04. Process of organic matter breakdown in waters produces CO_2 which causes acidic water. According to DeLong et al. (2009), optimum pH for tilapia growth is 6–9, while optimum pH for the growth of aquatic plants is <7 (Owens et al., 2005). For aquatic plants, pH influences the metabolic process as well as the absorption of nutrients and carbon (Mitchell, 1974). Moreover, fish living in environments with low pH (<5.0) may die from a decrease in plasma ion and osmoregulation process failure (Evans and Claiborne, 2006). Thus, maintaining the pH in the range of 6–7 in this system is necessary. In addition to optimize the growth of fish and plants, pH in the range of 6–7 can maintain

Table 1
Initial water quality characteristic.

Parameter	Control (P1)	Treatment (P2)	Treatment (P3)
Temperature (°C)	30.17	29.50	29.47
Turbidity (NTU)	1.12	1.08	1.11
pH	6.93	6.83	7.00
DO (mg L^{-1})	6.10	6.17	6.37
NH_3 (mg L^{-})	0.0034	0.0026	0.0030
NH_4(mg L^{-1})	0.4859	0.5067	0.3813
NO_2 (mg L^{-1})	0.05	0.05	0.05
NO_3 (mg L^{-1})	0.13	0.13	0.15
PO_4 (mg L^{-1})	0.04	0.04	0.03

Table 2
Average and standard deviation of water quality parameter during experiment.

Parameter	Control (P1)	Treatment (P2)	Treatment (P3)	Limit
Temperature (°C)	30.34 ± 0.39	29.50 ± 0.32	29.86 ± 0.27	11–42 °C (FAO, 2012)
Turbidity (NTU)	7.13 ± 4.17^{a}	5.57 ± 3.43^{a}	5.26 ± 3.38^{a}	–
pH	6.00 ± 0.28^{a}	6.04 ± 0.22^{b}	6.04 ± 0.23^{b}	6–9 (Popma and Masser, 1999)
DO (mg L^{-1})	5.24 ± 0.63^{a}	5.42 ± 0.69 ^{a}	5.45 ± 0.77^{a}	≥5 (Lloyd, 1992) 3–5 (Anita and Pooja, 2013)
NH$_3$ (mg L^{-})	0.026 ± 0.08^{a}	0.020 ± 0.03^{b}	0.015 ± 0.05^{b}	0.05 (Lawson, 1995) 0.1 max.tolerable level (Pillay and Kutty, 2005)
NH$_4$(mg L^{-1})	1.486 ± 0.15^{a}	1.48 ± 0.33 ^{a}	1.35 ± 0.05^{b}	0.2–2 (Boyd,1998)
NO$_2$ (mg L^{-1})	0.41 ± 0.06^{a}	0.33 ± 0.03 ^{a}	0.32 ± 0.03^{a}	0.5 (Swann, 1997) ≤1 (Pillay and Kutty, 2005)
NO$_3$ (mg L^{-1})	0.79 ± 0.05^{a}	0.77 ± 0.07 ^{a}	0.72 ± 0.03^{a}	≤10 (Pillay and Kutty, 2005)
PO$_4$ (mg L^{-1})	1.29 ± 0.06^{a}	1.23 ± 0.04^{b}	1.19 ± 0.04^{b}	0.03–2 (Anita and Pooja, 2013)

Different letters (a and b) in the same row are significantly different at $P < 0.05$ level.

ammonia in the form of NH$_4^+$, thus lowered toxicity level of NH$_3$ (Goldman and Horne, 1983).

DO is one of the important parameters and a limiting factor for fish life. Low DO will disrupt the lives of fish cultured since DO is not only needed by the fish, but also required by microbes in oxidizing organic materials. Nitrifying bacteria are aerobic and require oxygen to produce NO$_3$ in nitrification process (Henriksen et al., 1981). Average DO for all treatments was >5 mg L^{-1} with a range of 5.24 to 5.45 mg L^{-1}. DO in P1, P2, and P3 did not show any significant differences, whereas observation duration significantly affected the DO ($p < 0.05$).

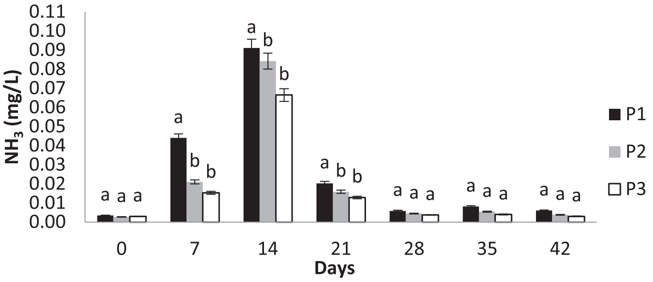

Fig. 2. Ammonia (NH$_3$) fluctuation during experiment. Different letters (a and b) are significantly different at $P < 0.05$ level.

3.2. Ammonia, ammonium, nitrite, and nitrate

NH$_3$ in water is usually measured as total ammonia nitrogen/ TAN (NH$_3$+ NH$_4$). The toxicity of NH$_3$ is primarily attributable to the un-ionized form (NH$_3$), as opposed to the ionized form (NH$_4$). In general, more NH$_3$ and greater toxicity exist at higher pH. Toxicity increases as pH increases and as temperature increases. Plants are more tolerant of NH$_3$ than animals, and invertebrates are more tolerant than fish (Anonymous, 2014).

NH$_3$ in all treatments increased from day 0 to day 14 due to accumulation of uneaten feed and fish feces. NH$_3$ ranged from 0.03 to 0.91 mg L^{-1}. NH$_3$ in P3 was lower than that in P2 and P1 (Fig. 2). Meanwhile a research by Effendi et al. (2017b) on aquaponics of guoramy and romaine lettuce found that NH$_3$ ranged 0.02–0.04 mg L^{-1}. Tilapia cultivation resulted in more NH$_3$. In addition, Effendi et al. (2017c) reported that NH$_3$ removal of catfish cultivation wastewater using vetiver ranged 0.2657–2.8648 mg L^{-1}. Therefore, catfish cultivation produced more NH$_3$ than tilapia and guoramy cultivation. TAN fluctuations in each treatment had similar pattern. Concentration of NH$_3$ sharply declined on day 21 of

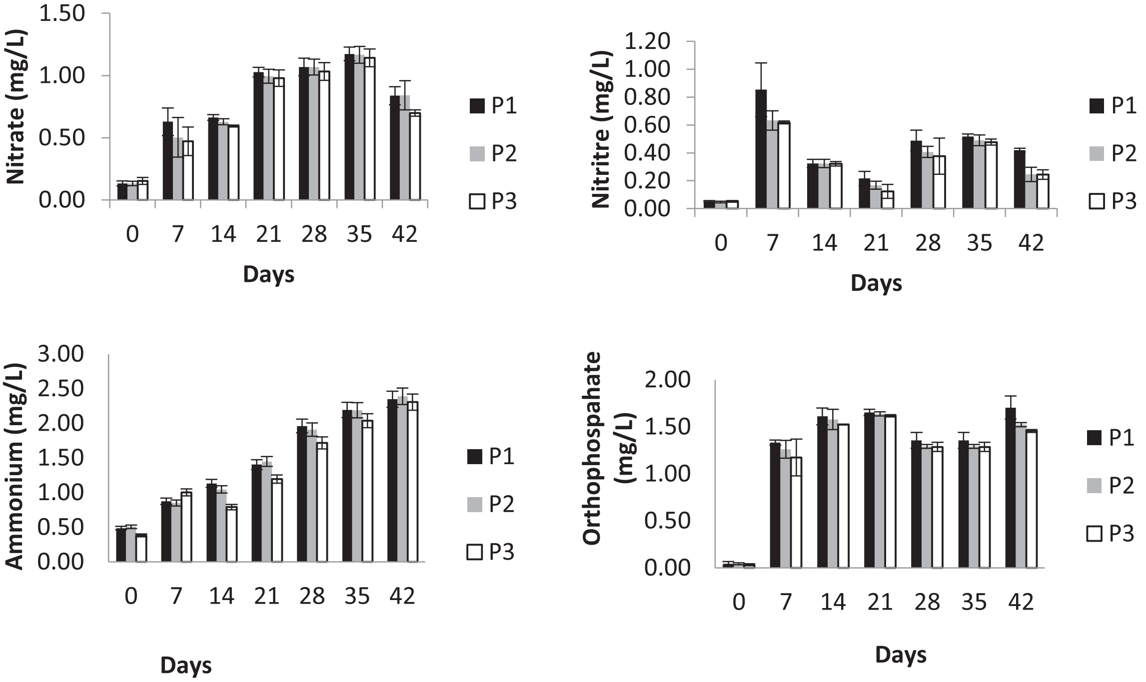

Fig. 3. Nitrate, nitrite, ammonium, and orthophospahte fluctuation during experiment. No significant difference among treatment.

observation and continued to decline until day 42. This sharp decline of NH_3 was followed by the increase of NH_4 and NO_3, suggesting that the convertion of NH_3 to NO_3 through nitrification process occurred much more intensive (Fig. 3). NH_3 contained in P1 tended to be higher than in P2 and P3. Furthermore, NH_3 in P1, P2, and P3 were significantly different ($p < 0.05$) (Fig. 2). Plants can act as phytoremediation to absorb NH_4^+; thus, toxic NH_3 can be reduced through the balance of TAN (Tyson et al., 2011).

At the beginning, NH_3 is oxidized to nitrite by ammonia oxidizing bacteria (AOB), later converted to nitrate by nitrite oxidizing bacteria (NOB) (Hu et al., 2015). Removal of NH_3 concentration greater than NH_4, NO_2 and NO_3 was likely associated with much faster growth of AOB than NOB. This is supported by Yamamoto et al. (2008), growth of AOB population will be faster than NOB when the temperature is above 25 °C.

Changes in temperature and pH during observation period affected the equilibrium of NH_3 and NH_4. At the beginning of experiment (day 0), pH reached 7.0 and affected the equilibrium of TAN. Therefore, concentration of NH_3 on day 0 tended to be normal. Later, pH decreased to 6.0 on day 14, caused NH_3 in all treatments to decline sharply. P3 had lower average concentration of NH_3 than that of P2 and P1 (Table 2). NH_3 in P2 and P3 were significantly different ($p < 0.05$) and observation time significantly affected NH_3 ($p < 0.05$).

NH_4 ranged from 0.38 to 2.38 mgL^{-1}. At the end of the experiment, NH_4 in P1 (1.48 ± 0.15 mg L^{-1}) tended to higher than in P2 (1.48 ± 0.33 mgL^{-1}) and P3 (1.35 ± 0.22 mg L^{-1}). Furthermore, NH_4 in all treatments increased until the end of observation. P3 had lower NH_4 than P2 and P1 (Table 2). NH_4 in P2 and P3 was significantly different, observation time also had significant impact on ammonium ($p < 0.05$). Plants play as biofiltration by absorbing NH_4. Meanwhile nitrification bacteria reduce NH_3 concentration through oxidation and converting NH_3 to NO_3 (Tyson et al., 2011).

Average NO_2 in P3 was lower than that in P2 and P1 (Table 2). Concentration of NO_2 during observation period ranged from 0.05 to 0.85 mg L^{-1}. Concentration of NO_2 tended to rise and only decreased on day 42 of observation. NO_2 in P2 and P3 was not significantly different ($p > 0.05$), while observation time significantly affected NO_2 ($p < 0.05$). NO_2 is the intermediate product of nitrification process. Hence NO_2 concentration is generally lower than NH_3 and NO_3. Plants do not use nitrite as nutrient source and high concentration of nitrite leads to poisoning in fish. Thus, nitrite concentration should not exceed 5 mg L^{-1} (DeLong et al., 2009).

Increased NO_3 occurred over time. NO_3 in all treatments increased until day 35 of observation then decreased until day 42. NO_3 concentration ranged from 0.13 to 1.17 mg L^{-1}. P3 had lower average of NO_3 than P2 and P1 (Table 2). Concentrations of NO_3 in P2 and P3 were not significantly different. Time of observation significantly affected NO_3 concentration ($p < 0.05$). NO_3 is relatively non toxic to most of fish, and does not cause any health hazard except at exceedingly high levels (>90 mg L^{-1}) (Stone and Thomforde, 2004). NO_3 toxicity for tilapia may occur if the concentration exceeds 300–400 mg L^{-1} (DeLong et al., 2009).

3.3. Orthophosphate

Phosphorus in the form of orthophosphate (PO_4) is an essential plant nutrient, resulting from decomposition of tilapia cultivation wastewater. Concentration of PO_4 in P3 was lower significantly than in P2 and P1. Low PO_4 in P3 might be attributable to usage by vetiver for their growth. *Eichornia crassipes* could reduce 63.3% TP in water (Wang et al., 2011). Maximum P reduction in this research was 42.75%. According to Li et al. (2013), phosphorus (P) accumulates in plant root tissues. Therefore, PO_4 concentration of tilapia cultivation wastewater underwent much more reduction in P3 (800 g vetiver) than in P2 (400 g vetiver) and P1 (control, without vetiver) (Table 2).

3.4. Correlation of water quality parameter

In aquaponics system, turbidity had strong positive correlation (0.907) with other parameters in component 1 (45.186% of total variance) and negatively correlated with NH_3, DO and pH (−0.079, −0.533, −0.579) (Table 3), suggesting that high turbidity might hinder DO penetration. pH in P3 was lower than in P2 and P1.

Turbidity (0.907) strongly correlated with TAN (0.921), NH_4 (0.915), PO_4 (0.808) in P3 component 1, suggesting that high turbidity might be associated with high concentration of those three parameters, but correlated negatively with NH_3. The same pattern occurred in P2 component 1. In control without vetiver (P1, component 1) positive correlation of turbidity was not only with three parameters but also with NO_3, likely indicating that the available nitrate as a result of decomposition of organic matter was not utilized, as did in P2 and P3.

In P3, DO was positively correlated (0.666) in component 2 (27.516% of total variance) with NH_3 (0.948), pH (0.742), suggesting that temperature and pH increment will shift the equilibrium of TAN into NH_3, which is a more toxic element. Shifting TAN to NH_3 was proved by negative correlation of DO with TAN, NO_3, NO_2, NH_4, PO_4 and turbidity (−0.261, −0.643, −0.424, −0.289, and −0.122, respectively) (Table 3).

3.5. N and P removal

Percentage of nutrient removal was calculated by comparing nutrient in treatment and control. Percentage of nutrient removal in P2 and P3 for ammonia (NH_3), ammonium (NH_4), nitrate

Table 3
Varimax rotated factor-loading matrix for P1, P2, and P3.

System	P1			P2			P3		
Component	1	2	3	1	2	3	1	2	3
NO_3	.832*	−.294	.051	.690	−.478	−.121	.504	−.643	.289
NO_2	.808*	−.441	−.130	.792*	.125	.002	.690	−.424	−.126
TAN	.922*	−.142	−.179	.853*	−.343	.320	.921*	−.261	.176
NH_3	−.200	.965*	.095	−.014	.963*	.100	−.079	.948*	.117
NH_4	.917*	−.185	−.181	.844*	−.376	.313	.915*	−.289	.170
PO_4	.787*	−.200	−.025	.655	−.479	−.244	.808*	−.315	−.103
DO	−.720	.319	.168	−.611	.633	−.047	−.533	.666	−.230
pH	−.453	.831*	.221	−.393	.843*	−.092	−.579	.742	.060
Temperature	.014	.210	.942*	.033	.053	.963*	.014	.008	.951*
Turbidity	.635	−.005	−.675	.750*	−.264	−.068	.907*	−.122	.004
% Explained variance	48.216	21.439	15.131	40.627	28.434	12.274	45.186	27.516	11.438
Cumulative%	48.216	69.655	84.786	40.627	69.060	81.335	45.186	72.702	84.140

Strong loading* ≥0.75, moderate loading (0.5–0.75) and weak loading 0.5–0.3) (Liu et al., 2003).

Table 4
Percentage of nutrient removal.

Day	Percentage of reduction									
	Ammonia (NH_3)		Ammonium (NH_4)		Nitrite (NO_2)		Nitrate (NO_3)		Orthophosphate (PO_4)	
	P2	P3	P2	P3	P2	P3	P2	P3	P2	P3
7	52.10	65.16	2.77	−14.70	25.81	27.51	20.01	25.05	14.50	21.77
14	7.57	27.04	7.60	30.17	−0.02	0.68	5.39	10.48	28.50	42.75
21	21.58	36.53	−3.17	14.68	22.50	42.73	3.17	4.76	26.25	31.25
28	23.90	36.71	2.66	12.46	16.87	22.99	0.00	3.36	4.87	5.17
35	33.61	50.84	0.11	7.10	5.26	7.84	0.47	2.58	15.66	20.31
42	37.28	50.49	−1.79	1.76	41.34	41.85	−0.42	16.59	10.66	14.60

(NO_3), nitrite (NO_2), and orthophosphate (PO_4) fluctuated during the observation period (Table 4).

The highest removal percentage was found in the early experiments on P3, namely 65.16%, 27.51% and 25.05% for NH_3, NO_2 and NO_3, respectively. Meanwhile NH_3 removal of tilapia culture wastewater by butterhead lettuce was 45.49% (Effendi et al., 2017d). The highest NH_4 removal percentage was 30.17% which was obtained in P3 at day 14 (Table 4). P3 had higher rate of inorganic nutrients removal and was more effective than P2 for NH_3, NH_4, NO_2 and NO_3.

Growth performance of tilapia and vetiver was also better in P3 compared to that in P2 and elaborated elsewhere. Therefore, increasing number of vetiver population or plant density can be applied to increase the absorption of pollutants. NH_3 and NO_3 of crayfish culture wastewater was reduced 84.6% and 34.8% by spinach (Effendi et al. 2015c), 91.5% and 23.3% by lettuce (Effendi et al., 2015b). Meanwhile Wahyuningsih et al. (2015) found reduction of tilapia cultivation wastewater of 91.50%, 34.41%, 22.86%, and 49.74% for TAN, NO_2 and NO_3, respectively by lettuce and added bacteria.

NH_4, NO_2 and NO_3 are the main form of the element absorbed by most plants (Liu et al., 2014). Kennedy and Murphy (2004) stated that increase in plant density affected the decrease in nitrogen concentration. According to Garnett et al. (2003), various different types of plants in the form N source preferred to be absorbed depends on resources available. Fang et al. (2007) also stated that nitrogen intake is done by plant roots and leaves, if both N sources are available, plant prefers to take NH_4. Plants have the ability to take up several chemical forms of nitrogen. The most common are ammonium (NH_4^+), which has a positive charge; nitrate (NO_3^-), which has a negative charge; and urea, ($(NH_2)_2CO$), which has no charge (Mattson et al., 2009). Tea (*Camellia sinensis* (L.) O. Kuntze) prefers ammonium (NH_4^+) over nitrate (NO_3^-) as an inorganic nitrogen (N) source (Yang et al., 2013).

Fish farming wastewater treatment by using economically valuable crops such as vetiver is expected to provide value added. Fish farming waste treatment methods using aquaponics system is an efficient method because waste treatment can be done in one cycle of recirculation by applying this method. Moreover, apart from being used in organic waste treatment, there are several other advantages of using recirculation method including efficiency of water use, efficiency of space usage and production as well as double advantages of harvesting fish and plants (Datta, 2015).

4. Conclusion

Treatment of fish cultivation wastewater with vetiver was capable of reducing the concentration of NH_3 (65.16%), NO_2 (27.51%), NO_3 (25.05%) in day 7, and NH_4 (30.17%), PO_4 (42.75%) in day 14. More vetiver density removed more N and P of tilapia cultivation wastewater. Optimization of fish and plant density as well as searching the best fish and plant type combination in phytoremediation are our subsequence work.

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