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AMMONIA REMOVAL OF CATFISH (*CLARIAS* SP) CULTIVATION WASTEWATER USING VETIVER GRASS (*VETIVERIA ZIZANIOIDES*)

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ABSTRACT

Ammonia concentration of catfish culture wastewater underwent reduction up to 90.73% under vetiver treatment. Length and weight range of catfish under vetiver treatment was higher than that of without vetiver. Similarly, survival rate of catfish at vetiver treatment was higher compared with that at the control. Vetiver was able to remove ammonia and grow under recirculating system of catfish culture by utilizing nutrient resulted from organic substance decomposition of uneaten fish food, feces, and urine. Nitrate and total phosphate concentration remained high at the end of experiment (third week) denoting that decomposition process has still been taking place.

KEY WORDS: Ammonia, Catfish, Phytoremediation, Vetiver.

INTRODUCTION

Catfish (*Clarias* sp.) is one of fisheries commodity that is much in demand for aquaculture since it has a high economic value. Catfish farming is performed not only to meet the needs of the domestic market, but also for export. Catfish can be cultivated with a relatively easy cultivation technology and a relatively low venture capital. The farmers often face problems in the management of organic matter from the rest of the feed or feces that settles in the water bottom. Water that has been mixed with organic wastes in aquaculture will affect the survival of cultured organisms. The existence of organic materials that are not treated properly will produce ammonia.

Phytoremediation consists of a set of technologies that exploit the ability of some plants to absorb, accumulate, metabolize, volatilize or stabilize pollutants that are present in soil, air, water or sediments such as: heavy metals, radioactive, organic compounds, compounds derived from petroleum, etc (Alkorta *et al*., 2004, Alvarez *et al*., 2015; Gupta *et al*., 2012; López *et al*., 2011; Mudgal *et al*., 2010; Qixing *et al*., 2011).

Plant species used in recirculating system as phytoremediator of catfish culture wastewater was vetiver grass (*Vetiveria zizanioides*). Vetiver grass role has been successfully extended to environmental protection, particularly in the field of wastewater treatment, due to its prominent morphological and physiological characteristics and tolerance to adverse conditions (Darajeh *et al*., 2014a; Gupta *et al*., 2008; Kede *et al*., 2014; Troung, 2013). Vetiver is known for its effectiveness in a sediment erosion control due to its unique morphological and physiological characteristics. Vetiver is also a high biomass plant with remarkable photosynthetic efficiency which renders it tolerant against various harsh environmental conditions. Vetiver with deep-rooted and higher water-use can effectively stabilize soluble metals in sediments (Chen *et al.,* 2004; Madhavi *et al.,* 2014; Yehand Lin, 2014). The plant has a strong and massive root system, which is vertical in nature descending 2-3 meters in the first year, ultimately reaching some 5 meters under tropical conditions (Bushan *et al.,* 2013; Gupta *et al.,* 2012; Truong and Loch, 2004).

Vetiver is effective for soil and water conservation, and is endowed with excellent biological features to ameliorate wastewater and pollution mitigation (Chen *et al.,* 2012; Lavania *et al.,*

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2004). Vetiver tolerated and accumulated the greatest amount of heavy metals (Jayashree *et al.,* 2011; Mukhopadhyay and Maiti, 2010; Oh *et al.,* 2014, Sampanpanish *et al.,* 2008). Vetiver has favorable qualities for animal feed and handicraft, biofuel etc. From the roots, oil is extracted and used for cosmetics and aromatherapy (Hosamane, 2012; Jayashree *et al.,* 2011; Lavania *et al.,* 2004).

The purpose of this study was to process organic liquid waste of catfish culture using vetiver grass in the recirculation system. Ideal phytoremediator is a species that creates a large biomass, grows quickly with an extensive root system and can be easily cultivated and harvested (Kakoli *et al.,* 2014; Liu *et al.,* 2013). Suitable plant species used for phytoremediation should have high uptake of both organic and inorganic pollutants, grow well in polluted water and be easily controlled in quantitatively propagated dispersion (Darajeh *et al.,* 2014b; Oh *et al.,* 2014).

Vetiver system is based on the use of vetiver grass, which was first recognized early in the 1990s for having "super absorbent" characteristics suitable for the treatment of wastewater and leachate generated from landfill (Troung and Stone, 1996). Even though it is not an aquatic plant, vetiver can be established and survive under hydroponic conditions (Chomchalow, 2003; Gupta *et al.,* 2012). Vetiver grass has been found to be highly tolerant to drought, flood, submergence, extreme temperature, a wide range of soil pH, and toxicities of various heavy metals (Chomchalow, 2003; Oh et al., 2014).

MATERIALS AND METHODS

As much as 400 catfish (*Clarias* sp.) average size of 2 cm length was introduced on each aquarium (80 x 40 x 40 cm). 4 pots planted 3 bunds Vetiver grass (*Vetiveria zizanioides*) was placed in each gutter. Vetiver leaves were cut off to be 20 cm length. Wastewater from the aquarium flows into gutters grown by vetiver. Furthermore, the water flows into the drum, and then pumped back into the aquarium. Treatment of catfish wastewater quality with vetiver and control without vetiver.

Ammonia, Nitrate and Total Phosphate

Measurement of water quality, catfish, and vetiver was held 3 times (once a week). Catfish length measurement started from the mouth end to the tail tip. Catfish length and weight measurement was done for 30 fishes on each sampling. Meanwhile Vetiver grass length measurement was carried out from the base of the leaf close to the ground to the leaf tip for 12 leaves on each sampling. Water quality parameters measured included temperature, pH, dissolved oxygen, ammonia, nitrate, and total phosphate (APHA, 2008).

Survival rate (SR) = N_{t+1}/N_t x 100 %, N_{t+1}: Catfish number at time$_{t+1}$, N_t: Catfish number at time t.

$$\propto = \left[\sqrt[t]{\frac{\bar{L}_t}{\bar{L}_0}} - 1\right] \times 100$$

Specific Growth Rate (SGR), the percentage growth rate of weight and length.

: Specific growth rate (%), L_t: Average weight or length at time t_1 or t_2 or t_3, L_o: Average weight or length at time t_o, t: Observation time interval between t_o and t_1 or t_2 or t_3.

The percentage decline of ammonia, % Reduction= [(a-b)/a] x 100, a: Ammonia concentration at time t_3, b: Ammonia concentration at time t_o.

Pearson correlation was applied in determining correlation among parameters. One-way Anova was performed to compare the means of treatment and control parameter at each sampling ($p < 0.05$).

RESULTS AND DISCUSSION

Temperature, pH and Dissolved Oxygen

The temperature range was 28 - 28.4°C during the study. Water temperatures tend to be stable and in accordance with the optimum temperature for catfish growth. Optimum temperature range for growth and survival of catfish is 26-31 °C (Madinawati *et al.,* 2011). Meanwhile Boyd (1990) suggested that the optimal temperature for fish growth is 25-32°C. Vetiver flourish rapidly at temperature above 25°C (Zhang, 1992).

Maffei (2002) describes vetiver as growing luxuriantly in areas with temperatures ranging 21-45!. Vetiver grass can tolerate extreme climatic variations such as prolonged drought, flooding and temperatures between -14°C to 55°C. In China, it has survived at -22°C and in the U.S. state of Georgia it was able to tolerate temperatures to -10°C (Mondyagu *et al.,* 2012; Troung, 1996).

The pH of the vetiver treatment (5.33-7.18) was higher than that of control (5.50-6.97). It indicates that Vetiver grows and maintains the pH towards neutral which might be due to the root secretions produced to avoid negative effect of wastewater (Deval *et al.,* 2012). Hence, Vetiver may function as pH stabilization (Pazoki *et al.,* 2014). Vetiver is able to grow at pH range of 3.0 – 10.5 (Troung and Baker, 1998).

Dissolved oxygen (DO) at vetiver treatment ranged 1.39-2.34 mg/L, while at control ranged 3.59-5.78 mg/L. Low oxygen content at treatment might be due to being consumed by bacteria in decomposition of organic substances resulting from metabolism process of catfish which was regularly fed. Yi *et al.,* (2003) reported DO concentrations decreased steadily from initial levels of 1.63–3.93 to 0.17–0.30 mg/L over the 87-day culture period of catfish. Basically as much as 3 mg/L oxygen in the media is needed to maintain good bacterial growth for decomposition process in aerobic condition (Darajeh *et al.,* 2014). A process of treating lake water with vetiver as an alternative of natural absorbent, to achieve a drinking water quality, reduced dissolved oxygen from 6.30-7.97 mg/L to 4.2-4.8 mg/L (Razia *et al.,* 2014). Therefore, vetiver usage in wastewater treatment might decrease DO in the water.

Ammonia, Nitrate and Total Phosphate

Levels of ammonia, nitrate, and total phosphate are presented in Fig. 1. Organic substance in the aquaculture system is caused by leftover food, feces, and urine of fish. The organic compounds reduction takes place with biological decomposition processes by microorganisms (Effendi, 2003). Plant rhizosphere stimulates microbial activity and community density by providing root surface area for their growth (Tanner 2001; Vymazal and Kropfelova, 2009). Phytodegradation of organic substance by plants and associated microbes degrade organic pollutants (Burken and Schnoor, 1997). Subsequently it can increase concentrations of ammonia, nitrite, nitrate, orthophosphate, etc in water (Sindilariu *et al.,* 2008). Phytodegradation is also known as phytotransformation, and is a contaminant (such as herbicide, BTEX) destruction process (Mukhopadhyay and Maiti, 2010).

Ammonia concentration at control ranged 1.4077 - 2.7126 mg/L and at treatment ranged 0.2657 - 2.8648 mg/L. The concentration of ammonia in the water for cultivation should be 0.2 – 3.0 mg/L (Boyd, 1990). Ammonia in water exists as two compounds: ionized (NH_4^+) and un-ionized (NH_3) ammonia. Unionized ammonia is extremely toxic to fish. The amount of unionized ammonia present depends on pH and temperature of the water (Boyd, 1982). Statistical analysis showed that on the 3rd week, vetiver treatment significantly ($p<0.05$) affected the decrease of ammonia levels up to 90.73%, greater than the decrease of ammonia levels at the control of 18.74% (Fig. 1). High ammonia reduction at vetiver treatment was in accordance with low dissolved oxygen range at vetiver treatment. Much dissolved oxygen was required to convert ammonia to nitrate. Domestic wastewater treated with vetiver system is able to reduce ammonia from 1.7-9.1 mg/L to 0.07 – 0.57 mg/L and total phosphorus from 4.6-8.8 mg/L to 1.2-2.1 mg/L (Ash and Troung, 2003). 48.36% Ammonia (NH_3-N) removal in P2 (tilapia with vetiver grass of 320 grams wet density) (Delis *et al.,* 2015). Mean while, integration of tilapia (*Oreochromis niloticus*) fish farming, romaine lettuce (*Lactuca sativa*), and bacteria can reduce inorganic nitrogen concentration. There was a removal of 91.50%, 34.41%, 22.86%, and 49.74% for TAN, nitrate, and nitrite, respectively (Wahyuningsih *et al.,* 2015).

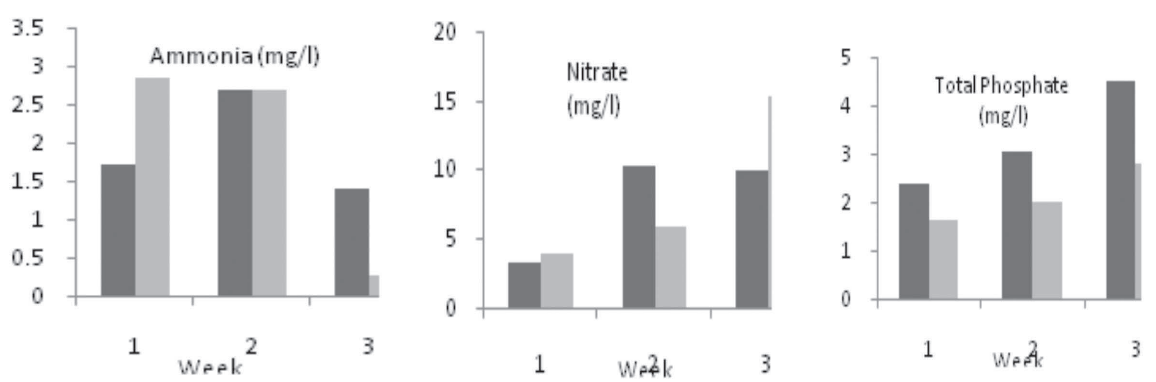

Fig. 1. Ammonia, Nitrate, Total phosphate at control (black) and vetiver treatment (grey).

Aquaponic bioremediation of crayfish culture wastewater with lettuce attained ammonia and nitrate reduction up to 90.1% and 23.3%, respectively (Effendi *et al.,* 2015c).

Vetiver can grow normally in water and is powerful to remove nitrogen and phosphorus from water and, therefore, is a good plant for purifying eutrophic water (Xia and Shu, 2001; Zheng, 1998) and garbage leachates (Xia *et al.,* 1999). Total nitrogen content in the water reduced 83-99% after sewage treatment using vetiver grass (Truong, 2011). Vetiver can reduce 67% ammonia and 80% total nitrogen (Xiao et al.,*2009*).

Levels of nitrate in the treatment and control showed an increase from the beginning of cultivation until the end measurement from 3.83 to 15.33 mg/L and from 3.25 to 10.00 mg/L, respectively. The increase of nitrate concentration of vetiver treatment at 3rd sampling was significantly higher than that of control ($p<0.05$). This was in line with the significant decline of ammonia concentration at the 3rd sampling, due to the conversion of ammonia to nitrate occurring much more intensive at vetiver treatment. Hence, at third week of experiment with vetiver, ammonia conversion into nitrate was in the highest level. Ammonia negatively correlated (-0.99) with nitrate, hence the decrease of ammonia concentration was followed by the increase of nitrate concentration. Treatment of hospital wastewater with vetiver results in removal rate of total nitrogen (88.46%) and BOD (88.54%) (Sani and Dareini, 2014).

Furthermore, experiment of palm oil mill effluent wastewater treatment with vetiver reduced total nitrogen up to 94% (Darajeh *et al.,* 2014).

Total phosphate also increased either at vetiver treatment or control. However total phosphate concentration range at vetiver treatment (1.6403 – 2.7941 mg/L) was lower than control (2.3927 – 4.4986 mg/L). This depicted that vetiver treatment had an ability to absorb phosphorus released by the catfish culture. However, vetiver requirement for P was not as high as for N (Gupta *et al.,* 2012). Boonsong and Chansiri (2008) reported 63.85% removal of total nitrogen and 36.34% removal of total phosphorus of domestic wastewater after seven days of hydroponic treatment in the green house. Truong and Hart (2001) used vetiver for domestic effluent treatment for 4 days and the removal in total nitrogen was 94%, total phosphorus was 90%.

Nitrogen and phosphorus absorption is expedited because roots of vetiver have direct exposure to effluents (Gupta *et al.,* 2012). Previously the total phosphorous level for the plant effluent varied between 4.6 to 8.8 mg/L and the results dropped to 1.2 mg/L after vetiver treatment (Ash and Truong, 2003). Wagner *et al.* (2003) used vetiver in hydroponic system using sewage effluent and observed that both N and P removal over 90% from the effluent. Aquaponic system applying water spinach can reduce freshwater crayfish culture wastewater particularly ammonia ($NH3$) until 84.6%, and nitrate ($NO3$) until 34.8%. Orthophosphate underwent reduction of 44.4% under spinach treatment (Effendi *et al.,* 2015b). Aquaculture wastewater treatment using water spinach and bacteria combination in aquaponic system can improve water quality of cultivation media of freshwater crayfish, indicated by the decrease of 81% ammonia, 33% nitrate, and 89% orthophosphate (Effendi *et al.,* 2015d)

Plants have shown the capacity to withstand relatively high concentrations of organic chemicals without toxic effects, and they can uptake and convert chemicals quickly to less toxic metabolites in some cases. In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes and the build-up of organic carbon in the soil (Mukhopadhyay and Maiti, 2010). It involves uptake, metabolization and degradation of contaminants within the plant, or the degradation of contaminants in the soil sediments, sludges, groundwater or surface water by enzymes produced and released by the plant. Razia *et al.* (2014) used vetiver in purifying water quality for drinking purpose, and showed a promising outcome. In order to remove COD, BOD, TDS, TSS, TOC the best result was obtained in the region's soil planted with Vetiver plant (Pazoki *et al.,* 2014).

Nitrate and total phosphate concentration remained high at the end of experiment compared that of at the initial experiment. This might denote that decomposition has still been taking place.

The observed differences in the nitrogen and phosphorus removal efficiencies at literatures might be related to differences of vetiver application method of experiment such as soil as a growing medium or recirculating (hydroponic) with or without supporting medium. In addition other factors that could differentiate the result namely variation of wastewater concentration or source of wastewater, setting up the hydroponic system in an open space or green house, quantity of vetiver applied, volume, concentration, and type of wastewater (Darajeh *et al.,* 2014).

Intensive farming adjacent to water bodies, the quantities of N and P in the water are bound to increase. Removal of these elements can be achieved by (i) planting vetiver on the edges of the streams or in the shallow parts of the lakes where usually high concentrations of soluble N and P occurred, and (ii) growing vetiver hydroponically on floating platforms which could be moved to the worse affected parts of the lake or pond (Chomchalow, 2003).

Length, Weight and Survival Rate of Catfish

The average length and weight of catfish increased at each sampling, both in the control and treatment (Fig. 2). Length of catfish throughout experiment ranged 5.50-7.54 cm (vetiver treatment) and4.72-7.04 cm (control). Meanwhile catfish weight of vetiver treatment and control ranged 1.20-3.32 gr and 0.79-3.03 gr, respectively. Linear increase relation between length and weight of catfish with correlation of 0.99 described growth normality and proportionally, although dissolved oxygen at vetiver treatment was quite low (1.39- 2.34 mg/L), however catfish could thrive under this low oxygen content and quite high ammonia concentration at both vetiver treatment (0.2657 - 2.8648 mg/L) and control (1.4077 – 2.7126). This pointed out that catfish is a quite tolerant fish that remain flourishing at the harsh environment. Survival rate of catfish at vetiver treatment (90.17 - 97.25 %) was higher than that of control (67.25 - 69.00 %). Low survival rate was more likely becauseof cannibalism whichwas the main problem in fry rearing as well as competition for feed rather than poor water quality. Cannibalism was the major factors affecting the pond nursing of *C. gariepinus* (De Graaf *et al.,* 1995; Francisca and Mgbenka, 2013).

Specific Growth Rate of Catfish and Vetiver

Catfish weight SGR (7.55 – 8.95) higher than catfish length SGR (2.14-2.42) pointed out that weight addition percentage per day was higher than length addition percentage per day. *Vetiver zizanioides* can grow well under recirculating system with highest SGR of 9.22% at the first week (Table 1). Vetiver length increased at each sampling, vetiver initial length was 20 cm and the length at the end of the study (week 3) was 51.8 cm. Vetiver leaf is 45 - 100 cm long and 6 - 12 cm wide (Darajeh *et al.,* 2014b). After 14 months of growth, Xia and Bing (2003) reported Vetiver plant height ranged from 160 cm to 170 cm, meaning average additional length was 2.95 cm per week (0.42 cm/day). At this experiment vetiver length addition ranged 1.15-1.84cm/day was higher than that reported by Xia and Bing (2003). This Vetiver growthwas likely supported by continuous nutrient supply from organic substances decomposition originating from catfish feces, urine, and uneaten food. Nile tilapia planted concurrently with vetiver grass in higher density (P2) has the best Relative Growth Rate (RGR) and Feed Conversion Ratio (FCR) (Effendi *et al.,* 2015a). The research of Effendi *et al.* (2016) showed that tilapia weight reached 48.49 ± 3.92 g of T3 (tilapia, romaine lettuce, and inoculated bacteria), followed by T2 (tilapia and romaine lettuce) and T1 (tilapia) of 47.80 ± 1.97 and 45.89 ± 1.10 g after 35 days of experiment. Tilapia best performance in terms of growth and production occurred at T3 of 3.96 ± 0.44 g/day, 12.10 ± 0.63 %/day, 96.11 ± 1.44 % and 1.60 ± 0.07 for GR, SGR, SR and FCR, respectively.

The roots of Vetiver can extend downward and reach 2 to 4 meters in depth (USEPA, 2000). Vetiver

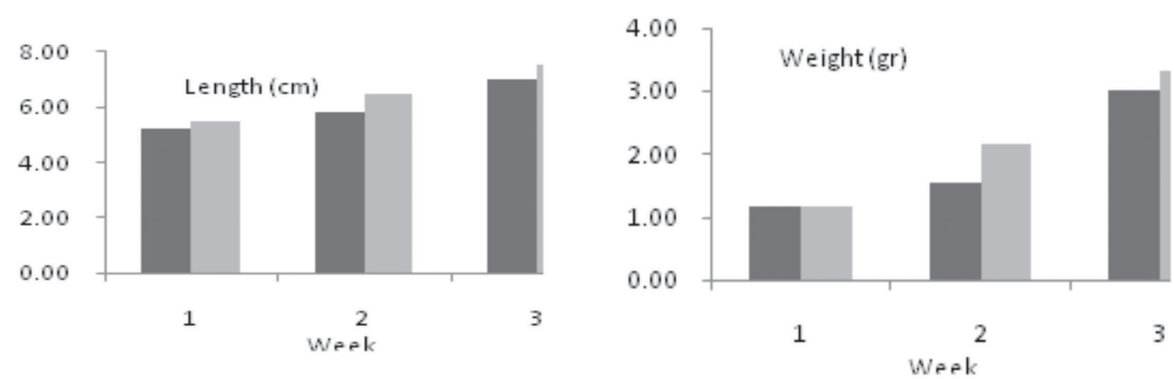

Fig. 2. Average length of catfish (black bar: control, grey bar: vetiver treatment).

Table 1. Specific growth rate of catfish and vetiver.

Observation week	Catfish		Vetiver
	SGR Weight	SGR Length	GR Length
1	8.95	2.42	9.22
2	7.55	2.28	7.03
3	7.66	2.57	5.77

does not belong to aquatic plants, but it can live in wet habitats, swampy, and will grow, although most of the shoots submerged, in a relatively long period of time in the water (Boonsong, 2008). Vetiver grass is touted for use in many soil and water conservation practice to reduce runoff, soil erosion, and to stabilize steep slopes (Barton *et al.*, 2011; Mondyagu *et al.,* 2012; Tawde and Bhalerao, 2012). Vetiver does not have stolon or rhizomes, roots and massive structured, and can grow very rapidly to a length of 3-4 m in the first year (Truong, 2011). Vetiver may be the best species used for vegetation rehabilitation in oil shale disposal piles (Xia, 2004).

Phytoremediation methods using aquatic macrophytes are the need for developing countries because they are environmentally friendly, effective and cheaper to establish and operate (Lema *et al.,* 2014). When compared with other plants, vetiver grass is more efficient in absorbing certain heavy metals and chemicals due to the capacity of its root system to reach greater depths and widths (Truong *et al.,* 1998).

Treating effluent with vetiver in industrial wastewater is actually a recycling process and not a treatment process, as in the process of treatment, the vetiver plant absorbs essential plant nutrient such as N, P, and cations, and store them for other uses. In Australia, this recycling plant is anticipated to provide high nutrient material for animal feed, mulch for gardens, manure for organic farming and organic source for composting (Lavania *et al.,* 2004).

CONCLUSION

Ammonia concentration of catfish culture wastewater underwent reduction up to 90.73% under vetiver treatment. Length of catfish throughout experiment ranged 4.72-7.04 cm (control) and 5.50-7.54 cm (vetiver treatment). Meanwhile catfish weight of control and vetiver treatment ranged 0.79-3.03 gr and 1.20-3.32 gr, respectively. Survival rate of catfish at vetiver treatment was higher than that of control. Vetiver was able to remove ammonia resulted from catfish cultivation.

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