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Wastewater Treatment Of Freshwater Crayfish (*Cherax quadricarinatus*) Culture With Lettuce (*Lactuca sativa*)

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ABSTRACT

The objective of this study was to treat freshwater crayfish Red Claw (*Cherax quadricarinatus*) culture wastewater containing organic material of residual feed and feces by bioremediation using aquaponic recirculating system with lettuce (*Lactuca sativa*). Aquaponic bioremediation of crayfish culture wastewater with lettuce attained ammonia and nitrate reduction up to 90.1% and 23.3%, respectively, during three weeks observation. At the end of experiment, the average length of crayfish was 6.27cm (control) and 7.12cm (treatment). Crayfish weight of control was 6.12g, and crayfish weight of treatment was 8.12g. Crayfish growth rate ranged 0.95-1.01 g/day. The length of lettuce during observation ranged 8.9 - 12.0 cm. Lettuce RGR was 0.04 cm/day.

Keywords: Aquaponic, Ammonia, Bioremediation, Freshwater crayfish, Lettuce

INTRODUCTION

Aquaculture generates organic waste from food remains and feces of biota. The presence of organic materials that do not decompose properly will produce ammonia and sulfide, and can reduce levels of dissolved oxygen in the water of media cultivation.

Furthermore, it can inhibit the growth of cultured aquatic biota (Anonymous 2007, Ling and Weimin 2010, Yeo *et al.* 2004).

Bioremediation is a process of biodegradation to remove or detoxify pollutants entering the environment as contaminants in soil, water, and sediment (Hazra *et al.* 2011, Hinchman *et al*. 1996, Thapa *et al*. 2012). Bioremediation is closely related to the biological activity of microorganisms and plants. The principle of bioremediation by aquatic plants is the process of filtering and absorption by the roots and stems of aquatic plants, as well as the process of exchange and absorption of dissolved ions used for plant growth (Anonymous 2007, Turcios and Papenbrock 2014, Yusuf 2008).

Red Claw is native to the waters of the river in the northeast of Australia and Papua New Guinea. Crayfish is endemic to the tropics of northeastern Australia. Freshwater crayfish is easily cultivated, not susceptible to disease, eating plants and animals (omnivores), fast-growing, and high fecundity (Anonymous 2011, FAO 2011, Sukmajaya dan Suharjo 2003).

Fertilizers and feeds are applied to ponds to promote shrimp and fish production, and no more than 25% to 30% of the nitrogen and phosphorus applied to ponds in fertilizers and feeds is recovered in fish or shrimp at harvest (Boyd and Tucker 1998). Ponds often have higher concentrations of nutrients, plankton, suspended solids, and oxygen demand than the water bodies into which they discharge (Schwartz and Boyd 1994b). Thus, pond effluents are potential sources of pollution in receiving waters (Boyd 2003).

Aquaponic system is a combination of aquaculture and hydroponics cultivation. In this system, fish and plants grow in one integrated system and creating a symbiotic (Allsopp *et al.* 2009). This technology is an applied technology saving land and water in aquaculture, so it can serve as a fishery model in tight areas or shortage of land as urban or residential complex (Diver 2006, Kumar and Sierp 2003). Aquaponic system uses recirculation concept with an aqueous medium for the production of plants without soil. Recirculation system is designed to increase the number of fish or shrimp produced in the relatively small volume of water, organic waste products are absorbed by plants (Allsopp *et al.* 2009, Diver 2006, Qin *et al*. 2005, Wik *et al*. 2008). Aim of experiment was to treat freshwater crayfish (*Cherax quadricarinatus*) culture wastewater with lettuce (*Lactuca sativa*) in recirculating system of aquaponic.

MATERIALS AND METHODS

Experimental Design

Several treatments consisted of (1) Crayfish without lettuce, (2) Crayfish and lettuce, (3) Crayfish, lettuce, and bacteria. Experiment was performed at recirculating aquaculture systems, consisting of aquarium (80 x 40 x 40 cm^3), ditch, container, and submersible pump.

Freshwater crayfish/Red Claw (*C. quadricarinatus*) aging 2 month was acclimated for 24 hours. As much as 30 freshwater crayfish (length 4-5 cm) was grown at each treatment. Lettuce (*L. sativa*), 5 bunds was planted at the floating sterofoam to avoid lettuce leaf submerged under water. Commercial Bacteria combination of Microplus (*Aerobacter* sp, *Nitrobacter* sp, *Nitrosomonas* sp, *Lactobacillus* sp, *Saccharomyces* sp) was inoculated to the system.

Water from crayfish aquarium containing organic substances (residual feed and feces) was flowed into a ditch planted with lettuce. Water was further flowed into a container which was previously inoculated with bacterial mixture (0.5 l). Then water was pumped back into crayfish aquarium (Figure 1), without water replacement.

Water quality parameters analyzed included temperature, pH, Dissolved Oxygen, Ammonia, Nitrate, Orthophosphate, dan Total Sulfide (APHA 2008). Bacterial abundance (Cfu/ml) was determined. Lettuce length measurement was performed at leaf. Crayfish length was measured from rostrum until tail end (**Figure 1**).

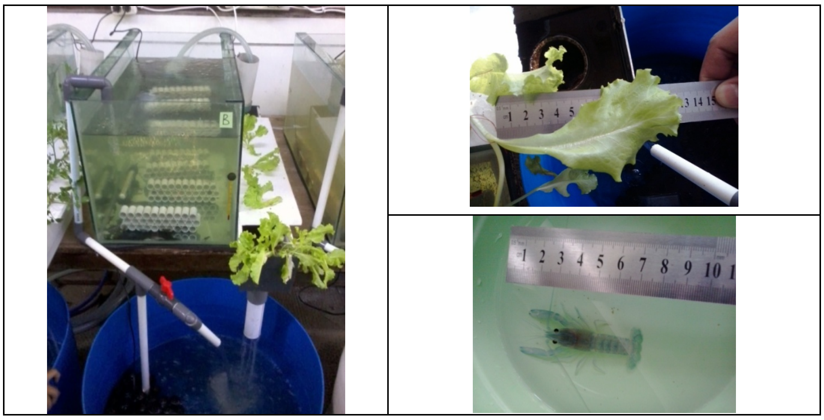

Figure 1. Recirculating aquarium system, lettuce leaf and crayfish length measurement.

Survival Rate

$$SR = \frac{Nt}{N0} \times 100\%$$

Where

SR : Survival rate

No : Number of crayfish at the beginning of experiment

Nt : Number of crayfish at the end of experiment

Growth Rate

$$\propto = \left[\sqrt[t]{\frac{Wt}{Wo}} - 1 \right] \times 100\%$$

Where

$\propto$: Growth rate (%)

W_t : Weight of crayfish at the end of experiment (g)

W_0 : Weight of crayfish at the beginning of experiment (g)

t : Experimental duration (day)

Relative Growth Rate

$$RGR = \frac{\text{Ln Xt} - \text{Ln Xo}}{\Delta t}$$

Where
RGR : Relative growth rate
Xt : Lettuce length at the end of experiment
Xo : Lettuce length at the beginning of experiment
Δt : Experimental duration

Water Quality Reduction

% Reduction = [(a-b)/a] x 100%

Where
a: Control concentration of water quality parameter at time t
b: Treatment concentration of water quality parameter at time t

RESULT AND DISCUSSION

Temperature and pH

Temperature influences growth of biota and chemical reaction at aquatic ecosystem (Tchobanoglous *et al.* 2004). Observed temperature ranged 27-29 ^{0}C. Freshwater crayfish culture grows optimum at temperature of 24-29 ^{0}C (Budiardi *et al.* 2008).

Lowest control aquarium pH was 6.46. Range of pH suitable for growth of aquatic biota and plant was 6.8-8.5 (Gjesteland 2013, Pescod 1973), 6-9 (Boyd 2003). Range of pH supporting crayfish media growth ranges 7-8 (Lukito and Prayugo 2007). pH <5 does not accommodate crayfish life, meanwhile pH >9 decreases crayfish feeding appetite (Budiardi *et al.* 2008).

Dissolved Oxygen (DO)

Dissolved oxygen are required by all living things for respiration, metabolism, and exchange of substances, which then produce energy for growth and reproduction, and also needed to oxidize organic materials and inorganic in aerobic process (Boyd 1990). Dissolved oxygen in water comes from the photosynthesis of phytoplankton and aquatic plants as well as diffusion of air (Eaton *et al.* 2005).

Oxygen in the rooting medium is necessary for the metabolic processes involved in root formation and growth (Soffer dan Burger 1988). Low dissolved oxygen concentrations can decrease the absorption of nutrients by the roots and causes growth inhibition of lettuce (Yoshida *et al.* 1997).

Dissolved oxygen levels were never <5 mg/l during the observation, which denoted that the dissolved oxygen levels are in good condition. Dissolved oxygen for supporting the growth of freshwater crayfish is >5 mg/l (Lukito and Prayugo 2007, Boyd 2003). Range of temperature, pH and dissolved oxygen in the experiment was conducive to the growth of crayfish. Temperature range of 25-29^{o}C facilitates the pH and oxygen levels in the water occurring in favorable conditions.

Ammonia

Ammonia in waters derived from the breakdown of organic nitrogen and inorganic nitrogen contained in the water originating from the decomposition of organic matter by microbes and fungi. Ammonia is the major toxic nitrogenous metabolic product excreted from fish in dissolved form (Yeo *et al.* 2004).

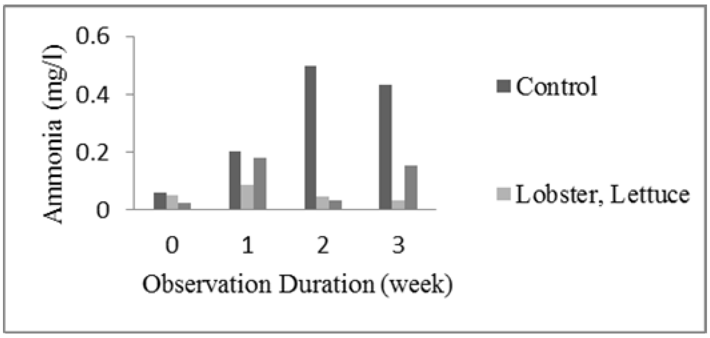

Figure 2. Ammonia concentration of control and treatment aquarium.

Ammonia concentration of both treatments (0.0249-0.1830 mg/l) was lower compared with that of control aquarium (0.0626-0.4996 mg /l) (**Figure 2**). Since there was no lettuce and bacteria combination at control aquarium that can help the process of nitrification. Decrease in the concentration of ammonia can occur because of ammonia conversion into nitrite and nitrate through the nitrification process. The concentration of ammonia in water for cultivation is 0.2-3 mg/l (Boyd 1990). Percentage reduction of ammonia concentration reached 91.5% for the treatment aquarium. This is consistent with the statement of Ika and Rifa'i (2012), the concentration of ammonia in the pond will be filtered out to 80% by the plant. The root zones are superb micro-sites for bacterial communities. These bacteria carry out nitrification and thus make N available for absorption (Baruah et al. 2006).

Depending on the pH and temperature of the water, a certain percentage of the total ammonia will be in the ionized non toxic form (NH_4^+) and some will be in the highly toxic unionized form (NH_3), the higher the pH or water temperature, the greater the percentage of ammonia remaining unionized (Yeo *et al.* 2004).

Ammonia toxicity to aquatic organisms increases if there is a decrease in dissolved oxygen levels, an increase in pH, and an increase in water temperature. Ammonia is a compound resulting from the process organic matter decomposition in water. This compound can be used by water plant after being converted into nitrite and nitrate by nitrifying bacteria (Kordi dan Tancung 2007). Unionized ammonia is the more toxic form attributable to the fact that it is uncharged and lipid soluble and consequently traverses biological membranes more readily than the charged and hydrated NH_4^+ ions (Crab *et al.* 2007, Körner *et al.* 2001). When NH_4^+ and nitrate (NO_3^+) are present simultaneously, plant removes NH_4^+ faster than NO_3^+. NH_4^+ is the preferred form for plant growth because the incorporation of NO_3^+ requires additional metabolic energy and enzymatic activity (Baruah *et al.* 2006).

Nitrate

Nitrate is a compound that is formed from the oxidation of nitrite (NO_2) to nitrate (NO_3) by the *Nitrobacter* bacteria. Goldman and Horne (1983) stated that nitrite in the water will be converted into nitrate form under sufficient oxygen concentration, and nitrate in the water is more stable than nitrite.

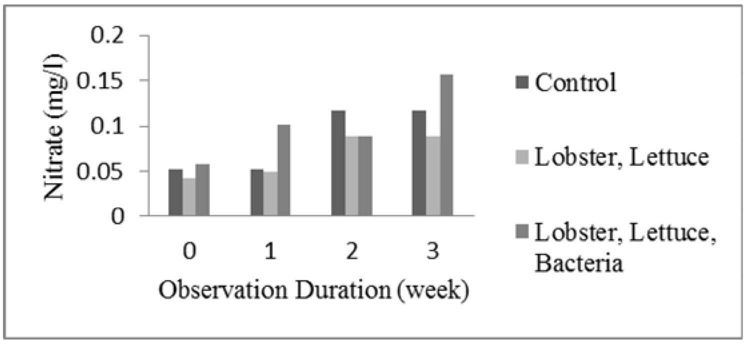

Figure 3. Nitrate concentration of control and treatment aquarium.

The concentration of nitrate in the control aquarium tended higher than that in treatment aquarium (**Figure 3**). This is because of the usage of most nitrate by lettuce as nutrient for plant growth. Nitrate levels safe for aquaculture range of 0-3 mg/l (Bansal *et al*. 2007).

Percentage change of nitrate concentration is lower than the percentage change of ammonia concentration (**Table 1**). Largest percentage change of 91.5% ammonia occurred at observation 3 and 23.3% nitrate at the observation 2 and 3 for treatment with crayfish and lettuce. Meanwhile, for treatment with crayfish, lettuce, and bacteria combination, nitrate concentration increased. This might relate with the existence of inoculated bacterial combination to system undergoing much intensive organic matter degradation. Ammonia concentration of treatment with crayfish, lettuce and bacteria combination was also slightly higher.

Table 1. Percentage of ammonia and nitrate reduction.

Observation	Reduction Percentage (%)			
	Crayfish and Lettuce		Crayfish, Lettuce, and Bacterial combination	
	Ammonia	Nitrate	Ammonia	Nitrate
1	57.1	18.5	9.2	Increased 92.7
2	90.1	23.3	92.7	23.3
3	91.5	23.3	64.5	Increased 33.8

Plants can lower 98% and 94% of the total nitrogen and inorganic nitrogen respectively. In addition, the plant is able to decrease 97% total phosphorus and 97% dissolved phosphorus (Brown *et al.* 1999). Treatment of water spinach (*Ipomoea aquatic*) for 120 days can reduce 30.6% total nitrogen and 18.2% total phosphorus (Li and Li 2009). Treatment with aquatic plants can reduce NH_4-N (55.9-76.0%), NO_2-N (49.6-90.6%), NO_3-N (34.5-54.4%), and PO_4-P (64.5-76.8%) (Snow and Ghaly 2008), decrease of NO_3-N (82.9 - 98.1%), NO_2-N (95.9 - 99.5%), and phosphate (54.5 - 93.6%) (Ghaly *et al.* 2005). Anonymous (2007) found that reduction of total phosphate was also lower than that of ammonia reduction of aquaculture wastewater treated with plant. The macrophyte cultivating area and open area formed a purification system. Their purification mechanisms are described as follows (Baruah *et al.* 2006), (1) Nitrogen removal process via nitrification (aerobic condition) converting $NH_4^+ \rightarrow NO_2 \rightarrow NO_3$, denitrification (anaerobic condition) converting $NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$, and (2) Phosphorus removal process.

Orthophosphate

Orthophosphate is utilized by bacteria, phytoplankton, and aquatic plants. Phosphorus absorption by aquatic plants is slower than absorption by phytoplankton, but aquatic plants can absorb and store phosphorus in greater numbers (Boyd 1990).

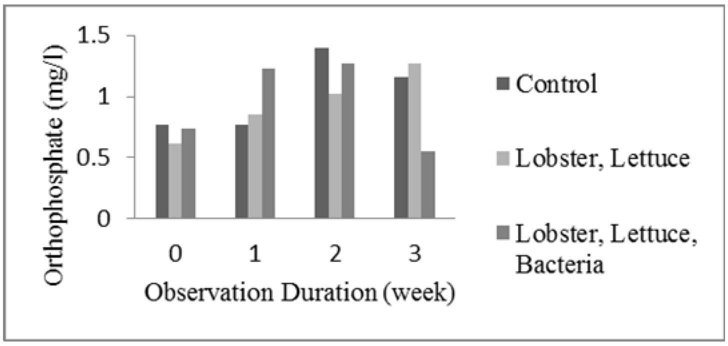

Figure 4. Orthophosphate concentration of control and treatment aquarium.

Orthophosphate levels in both treatments (0.5512-1.2691 mg/l) tended to be lower than controls (0.771-1.4019 mg/l) (**Figure 4**). This is due to the orthophosphate used by lettuce for growth. According to Boyd (1990), plants can absorb and store phosphorus in greater numbers. Safe orthophosphate concentration in the water is from 0.01 to 3 mg/l (Bansal 2007), <0.5 mg/l total phosphorous (Boyd 2003).

Studies in India showed that about one million litres per day of domestic sewage could be treated over an area of one hectare by using water hyacinth which reduces the BOD and COD by 89 and 71% along with removal of 89 % nitrogen and 50 % of phosphorus (Kumar and Sierp, 2003).

A recent review of the waste from large cage farms operating in Sweden noted on average 6.4 kg phosphorus and 55.0 kg nitrogen per tonne of fish produced, whereas large land-based farms released 11.3 kg phosphorus and 86.2 kg nitrogen per tonne of fish produced. The calculated average discharge was 8.2 kg phosphorus and 64.7 kg nitrogen per tonne of fish produced (Alanara *et al.* 2006 in Anonymous 2007). Hence, phosphorus and nitrogen are a prominent aquatic waste problem in aquaculture need to be removed.

Total Sulfide

Sulfide derived from the decomposition of organic compounds, can also be of industrial waste, but mainly from the reduction of sulfate compounds (Sugiarti *et al.* 2011). Total sulfide concentration in both treatments and control was 0.0019 mg/l. H_2S concentration was positively correlated with total sulfide concentration meaning that the total sulfide concentration increase will be followed by the increase in the concentration of H_2S. Levels of total sulfide (H_2S, HS^-, and S_2^-) <0.002 mg/l is considered not harmful to aquatic organisms survival (Effendi 2003, Sugiarti *et al.* 2011). The concentration of contaminants should not be in excess to ensure that they do not affect the growth rate of the plant species as excess may cause toxicity (Hazra *et al.* 2011).

Survival Rate

Survival rate (SR) is a measure denoting how many lobsters survive for observations in percent. SR in treatment aquarium (96.6%) was higher than that of control aquarium (76.6%). Crayfish deaths may be related to higher ammonia levels in control, and is also caused by cannibalism. This is consistent with the statement Budiardi *et al.* (2008), that the death occurred during the cultivation, one of them caused by cannibalism. An attempt to use extreme culture media of geothermal-heated water for farming of freshwater prawns (*Macrobrachium rosenbergii)* has been demonstrated to be feasible in a non-tropical climate (Johnson 1978). Hence prawn is quite tolerant.

Length, Weight, and Growth Rate of Crayfish

The average length of crayfish on the observations was 6.27cm (control) and 7.12cm (treatment). Freshwater crayfish grows and develops optimally in a good environment and adequate nutrition. Budiardi *et al.* (2008) suggested that intensive cultivation can be successful if four factors are controlled namely water temperature, feed, dissolved oxygen, and waste metabolites.

At observation 3, crayfish weight of control was 6.12g and crayfish weight of treatment was 8.12g. Crayfish growth rate ranged 0.95-1.01 g/day. Rouse and Khan (1998) stated that daily growth rate of crayfish weights ranges 0.3-0.5 g/day. Webster *et al.* (2004) stated crayfish daily growth rate ranged 0.6-1.0 g/day.

Lettuce Length

The length of lettuce during observation ranged 8.9 - 12.0 cm. RGR was 0.04 cm/day. This indicates that the lettuce grows in crayfish cultivation medium. Lettuce is a vegetable that should be free of water stress condition, because it is very sensitive to drought due to the shallow root system (Tsabedze dan Wahome 2010). Water stress is one factor that limits plant growth (Kizil *et al.* 2012).

The presence of organic matter also affects the growth of lettuce. Nutrient needs were fulfilled from decomposition of organic matter contained in the water used for crayfish cultivation. Nutrients resulting from decomposition of organic matter will be used by autotrophs organisms, such as aquatic plants and phytoplankton (Tchobanoglous *et al.* 2004). Phytoremediation takes advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant storage/degradation abilities of the entire plant body (Hinchman *et al*. 1996).

Bacterial Abundance

Total number of bacteria increased at the observation 2 from 5.1 millions CFU/ml to 32 millions CFU/ml and decreased at observation 3 to be 12 millions CFU/ml. The increase of total bacteria number at observations 1 and 2 showed that the bacteria are in the log phase of growth (rapid growth). This is due to the presence of organic matter content in the crayfish cultivation medium used by bacteria to make new cells and multiplication. Bacteria in the water can grow optimally at pH 6.5-8.5 (Badjoeri and Widiyanto 2008, Tchobanoglous *et al.* 2004). The biological community acts upon the dissolved wastes and helps to stabilize and recycle waste (Yeo *et al.* 2004)

In aquaponic recirculation system, the process of decomposition of organic matter took place in aerobic conditions, since the oxygen content is always >5 mg/l. If the availability of oxygen in the water is not sufficient, then there is the anaerobic decomposition, producing toxic gases (H_2S, NH_3, and CH_4) that are harmful to aquatic biota. The higher the organic matter, the more oxygen required by the bacteria.

CONCLUSION

Aquaponic bioremediation of freshwater crayfish (*Cherax quadricarinatus*) culture wastewater with lettuce (*Lactuca sativa*) attained ammonia and nitrate reduction up to 90.1% and 23.3%, respectively during three weeks observation. At the end of experiment, the average length of crayfish was 6.27cm (control) and 7.12cm (treatment). Crayfish weight of control was 6.12g and crayfish weight of treatment was 8.12g. Crayfish growth rate ranged 0.95-1.01 g/day. The length of lettuce during observation ranged 8.9 - 12.0 cm. Lettuce RGR was 0.04 cm/day.

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