ISSN 2347-6893

Combination of water spinach (*Ipomea aquatica*) and bacteria for freshwater cryfish red claw (*Cherax quadricarinatus*) culture wastewater treatment in aquaponic system

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ABSTRACT

The purpose of the study was to treat wastewater of freshwater crayfish (*Cherax quadricarinatus*) culture in aquaponic system using bacteria combination and water spinach (*Ipomea aquatica*). 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. Negative correlation occurred between ammonia and cryfish growth. Positive correlation existed between dissolved oxygen and the length of cryfish. Negative correlation occurred between growth of water spinach with ammonia and orthophosphate concentration. Negative correlation occurred between the abundance of bacteria and ammonia concentration. Positive correlation occurred between the abundance of bacteria and the length as well as weight of the cryfish.

Indexing terms/Keywords

Aquaponic, water spinach, crayfish, water quality

Academic Discipline And Sub-Disciplines

Wastewater treatment

SUBJECT CLASSIFICATION

Water quality

TYPE (METHOD/APPROACH)

Laboratory experiment

INTRODUCTION

Freshwater crayfish red claw (*Cherax quadricarinatus*) is a aquaculture commodity which is currently quite attractive to be cultured by the community. This cryfish superiority when compared with others is not susceptible to disease, eating plants and animals (omnivores), relatively rapid growth, and high fecundity (Sukmajaya and Suharjo, 2003). This cryfish is easily cultivated, so it has a chance to be developed in the aquaculture activities (Budiardi et al., 2008).

Although aquaculture has the potential to feed millions of people, some types of aquaculture production may severely degrade aquatic ecosystems (Klinger and Naylor, 2012; Mithra et al., 2012). Farmed shrimp is the most profitable commodity and also the most polluting (Naylor et al., 2000). Aquaculture activities generate waste from feces and food remains. Fed fish farms produce large amounts of wastes, including dissolved inorganic nitrogen and phosphorus (Chung et al. 2002; Zhou et al. 2006). Approximately 25% of the feed given to fish is excreted as solid waste (Rakocy, 1997).

If water is not replaced, it will decrease the water quality, hence it will cause an effect on survival of cultured organisms. Incomplete decomposition of organic materials can produce ammonia and sulfides and can reduce levels of dissolved oxygen in the water. If waste products remain in the aquaculture system, they can accumulate to levels that are toxic to fish and other organisms (Klinger and Naylor, 2012), subsequently inhibit the growth of cultured organisms (Cao et al., 2007; Tumembouw, 2011). Concerns are evoked about the possible effects of ever increasing aquaculture waste both on productivity inside the aquaculture system and on the ambient aquatic ecosystem. Therefore, it is apparent that appropriate waste treatment processes are needed for sustaining aquaculture development (Cao et al., 2007). Phytoremediation technology has proved to be a viable option to purify water contaminated with trace elements since it is cost-effective and has a positive impact on the environment (Chaudhary and Sharma, 2014).

Fish excreta contains rich amount of inorganic nutrients such as nitrogen, phosphorus, potassium, and organic matter which are useful for plants (Waqar et al., 2013). Increasing production cost and stricter environmental regulations have let to the production requirements of an efficient and closely managed system. Aquaponic systems are recirculating aquaculture systems that incorporate the production of plants without soil. Recirculating systems are designed to raise large quantities of fish in relatively small volumes of water by treating the water to remove toxic waste products and then reusing it (Rakocy et al., 2006).

Aquaponics is suitable for environments with limited land and water because it produces about three to six times the vegetables (Resh, 2004) and uses about 1% of the freshwater used by traditional aquaculture (Rakocy, 1989). Aquaponics on a small scale can serve as a family's solution to the need for an inexpensive, nutritious and reliable food source that has the capacity to provide a full meal (vegetables and protein) without many inputs (Connolly and Trebic, 2010).

This study aims to process residual waste feed and feces of freshwater crayfish red claw (*C. quadricarinatus*) cultivation using water spinach (*I. aquatica*) and bacteria combination on aquaponic system.

MATERIALS AND METHODS

The study consisted of a control (only cryfish) and treatment (cryfish, water spinach, and bacteria combination) in the aquarium (80 x 40 x 40 cm) with a recirculation system. Freshwater crayfish Red Claw (*C. quadricarinatus*) 2 months old was acclimatized for 24 hours. A total of 30 cryfish (4-5 cm length) is given in each aquarium. Water spinach (*I. aquatica*) was grown on aquaponic.

A combination of microorganism MicroPlus (*Aerobacter*, *Nitrobacter*, *Nitrosomonas*, and *Lactobacillus*) was added to the cryfish cultivation medium at week 3. Bacterial inoculation from external source is essential (Amit et al., 2003). Bioaugmentation has been employed to improve effluent quality by treating the water during the production process. This technology consists basically of the addition of microorganisms able to degrade or remove polluting compounds, especially organic matter and nutrients (Lopes et al., 2011).

Water sampling, water spinach measurement, cryfish weighing were done every week for 12 weeks. Water quality parameters measured included: temperature, pH, dissolved oxygen, ammonia, nitrate, orthophosphate, bacterial abundance (APHA, 2008). Length measurement of spinach was carried out on stems and leaves. Cryfish length measurement starts from the rostrum to the tip of tail.

Percentage reduction of water quality parameters were analyzed by the formula.

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

Where a: Initial concentration of water quality parameter, b: End concentration of water quality parameter.

The survival rate of freshwater crayfish was calculated by the formula.

$$SR = N_t/N_0 \times 100\%$$

Where SR: Survival rate (%), N_0: Cryfish number at the onset of experiment, N_t: Cryfish number at the end of experiment.

Pearson correlation coefficient test was used when normality test results showed the Sig >0.05 (normal data distribution), with the help of SPSS 20.00.

RESULTS AND DISCUSSIONS

Temperature, pH, and Dissolved Oxygen

The average temperature in control and treatment 27.8°C and 27.4°C, respectively with a range of 25-28°C. The average pH in control 6.8 and treatment 6.9, with a range of 6-7. The average concentration of dissolved oxygen (DO) in treatment (6.80 mg/l) was higher than control (6.32 mg/l). DO range was 5.78-7.67 mg/l (treatment) and 5.18-7.77 mg/l (control). Freshwater crayfish grew best in the temperature range of 24-29°C (Rouse, 1977 dan Rouse et al., 1988). The good temperature range for the fish in the tropics is 25-32°C (Boyd, 1990).

The pH for the growth of freshwater crayfish range 6.5-9. The pH <5 is very bad for the life of shrimp, because it can lead to death, and pH>9 may decrease appetite (Iskandar 2003; Sukmajaya dan Suharjo, 2003). Good concentration of DO to support the growth of freshwater crayfish is >5 mg/l (Budiardi et al., 2008; Rakocy, 1997). An adequate levels of DO is also essential to maintain healthy populations of nitrifying bacteria, which convert toxic levels of ammonia and nitrite to relatively non-toxic nitrate ions (Rakocy, 1997). Aquaponic systems should always operate under aerobic conditions (Lennard, 2012).

Ammonia, Nitrate, and Orthophosphate

The average concentration of ammonia in control 0.1828 mg/l and treatment 0.0962 mg/l. The range of ammonia concentration in control 0.05-0.50 mg/l and in treatment 0.03-0.39 mg/l (Figure 1). The principal sources of ammonia are fish excretion and sediment flux derived from the mineralization of organic matter and molecular diffusion from reduced sediment (Antony and Philip, 2006). The content of ammonia is still feasible for the life of freshwater crayfish. The maximum concentration of ammonia in the cryfish medium cultivation should not be >1.2 mg/l (Tumembouw, 2011). Nitrate levels fluctuated in control and treatment. The average nitrate concentration in control and treatment 0.0866 mg/l and 0.0814 mg/l, respectively. Range of nitrate in control was 0.0471-0.1713 mg/l and treatment was 0.0332-0.1871 mg/l (Figure 2).

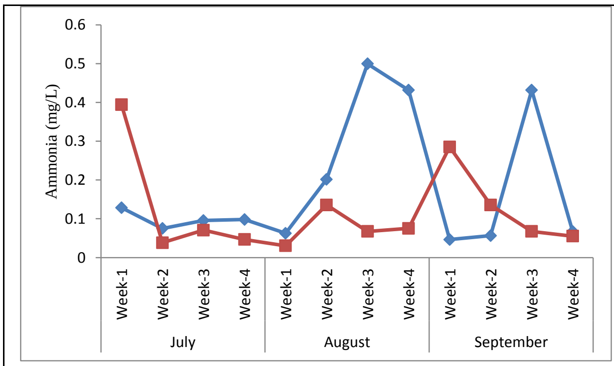
Decomposition of organic matter may result in ammonia. In addition, the urine and feces from the aquatic animals can cause high content of ammonia nitrogen and an increase of BOD. Ammonia is the main nitrogenous waste produced by fish via metabolism and is excreted across the gills (Cao et al., 2007).

Orthophosphate is a form of inorganic phosphorus that can be utilized immediately and easily absorbed by autotrophic organisms such as plants and algae for growth (Effendi, 2003). Orthophosphate range in control and treatment was 0.0421-1.4019 mg/l and 0.0571-0.9540 mg/l, respectively. The average orthophosphate concentration in control and treatment was 0.7359 mg/l and 0.4418 mg/l, respectively (Figure 3). Ammonia, nitrate, and orthophosphate concentration fluctuated during the study. Phosphorus is recognized as one of the major nutrients required by living organisms, involved in vital physiological process. At the same time it can also be considered as a pollutant at the high concentration under specific environmental conditions. It contributes to increase eutrophication process of lakes and natural waters (Usharani et al. 2009). Phosphorus is usually the limiting nutrient in freshwater, nitrogen is the limiting nutrient in seawaters (Jegatheesan et al., 2006).

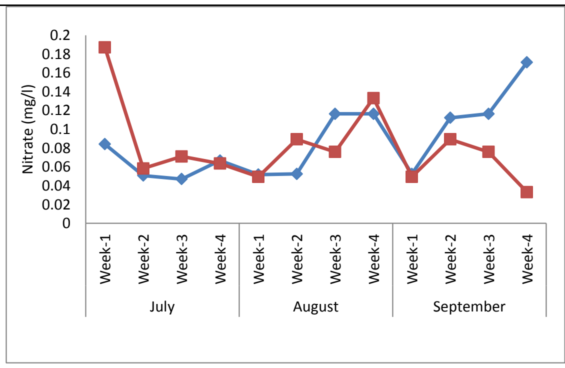
Decrement percentage of water quality parameter before the addition of bacteria was 81% ammonia, 29% nitrate, 11% orthophosphate. Decrease percentage after bacteria addition was, 81% ammonia, 33% nitrate, 89% orthophosphate (Figure 4). One of successful bioremediation involves optimizing nitrification rates to keep low ammonia concentration (Antony and Philip, 2006). Phosphorus and biologically active nitrogen are valuable nutrient resources (Sode et al., 2013). Phytoremediation in marine aquaculture using *Gracillaria longissima* removed 96.8% ammonia, 86.6% nitrate, and 77.1% orthophosphate (He et al., 2014). Bioremediation studies using aquatic plants *Eichhornia crassipes* in domestic wastewater treatment showed 74% ammonium-nitrogen and 41% orthophosphate (Mangunwardoyo et al., 2013). The concentration of the nutrients decreased significantly, indicating that macroalgae have large nutrient removal capacity. The concentration of orthophosphate reduced 94.5%, 49% nitrate removal, and 42% ammonia removal (Mithra et al., 2012).

Five plants were examined for their ability to remove nutrients from aquaculture wastewater and suitability as fish feed: alfalfa, white clover, oat, fall rye, and barley. The plants were fed wastewater from Tilapia production facility. The total reductions in total solids, COD, NO_3–N, NO_2–N, phosphate and potassium ranged from 54.7% to 91.0%, 56.0% to 91.5%, 82.9% to 98.1%, 95.9% to 99.5%, 54.5% to 93.6% and 99.6% to 99.8%, respectively (Ghaly et al., 2005). **C**omplete fish survival of Tilapia (*Oreochromis mossambicus*) and Carp (*Cyprinus carpio*) was recorded in bioremediated sewage effluent after phytoremediation with Coontail (*Ceratophyllum demersum*) plant that has potential of removing ammonia, nitrates and chlorides from sewage waste water (Waqar et al., 2013).

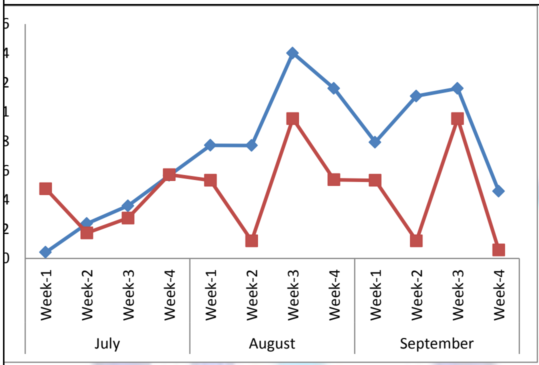
ISSN 2347-6893



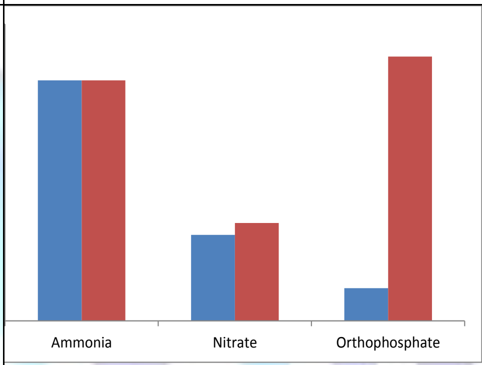
gure 1. Ammonia (control, treatment)



re 2. Nitrate (control, treatment)



Orthophosphate (control, treatment)



Percentage of water quality reduction (before bacteria addition, after bacteria addition)

Length, Weight, and Survival Rate of Crayfish

The average length of crayfish increased in control and treatment, 5.5 cm and 5.1 cm, respectively. Crayfish length range was 3.1-7.9 cm in control and of 3.1-7.4 cm in treatment. Average weight of crayfish in control and treatment was 4.85 gr and 3.66 gr, respectively. Crayfish weight ranged 0.76-10.52 gr (control) and 0.75-8.70 gr (treatment). Crayfish survival rate in control (75-78%) and treatment (78-95%).

Pearson correlation test indicated positive relationship between DO and the length of cryfish, both in control (0,679) and treatment (0.673) ($p<0.01$). Negative correlation between cryfish growth ammonia and, both in control (-0603) and treatment (-0613) ($p<0.05$). It explains that elevated levels of ammonia in the cultivation media will inhibit the growth of crayfish. In the intensive cultivation, ammonia is often a limiting factor, because it is a toxic substance and its value depends on the density of fish and water management. Aquatic organisms can not tolerate too high ammonia, because it can interfere with the binding of oxygen by the blood and can ultimately lead to suffocation (Budiardi et al., 2008).

No significant correlation between the level of cryfish survival and water quality, both in control and treatment. Hence the condition of water quality in this experiment did not significantly affect the survival rate of cryfish. Water quality conditions are not a limiting factor for the survival of cryfish. Deaths that occurred during the cultivation are due to the nature of lobster cannibalism (Budiardi et al., 2008).

Bacteria play a role as decomposers, which reduces the organic matter contained in waste water. In this process the bacteria need oxygen, so that when the availability of oxygen in the water is not sufficient, there will be imperfect decomposition produces toxic gases such as H_2S , NH_3 , and CH_4, harmful to aquatic biota. The combination of bacteria and water spinach gave effect to the improvement of water quality. The concentration of ammonia can be reduced up to 81% (Figure 4).

Correlation test showed negative correlation (-0641) between the abundance of bacteria and ammonia. Therefore the decrease in ammonia concentration was influenced by the presence of bacteria ($p<0.05$). Laboratory experiments of aquaculture waste treatment with bacterial mats showed that the mats rapidly removed ammonia (from 4.1 to 0.2 mg/l) (Bender and Phillips, 2004). Michel and Garcia (2003) reported that average efficiencies of ammonia nitrogen removal from shrimp (*Litopenaeus vannamei*) effluent, treated with microbial mats, was 97% and 95% for nitrate nitrogen. The correlation coefficient between bacterial abundance and length as well as the average weight of the crayfish was 0.725. This suggests that the length and weight of cryfish was influenced by the presence of bacteria ($p<0.01$).

Negative correlation (-0595) between water spinach and ammonia explained that the decrease in the concentration of ammonia was influenced by the growth of water spinach ($p<0.05$). One of the plants that can lower ammonia concentrations up to 80% is water spinach (Ika and Rifa'i, 2012). Root of water spinach is a habitat for microorganisms that make the process of decomposition of organic matter. Furthermore, water spinach absorb nutrients via root, resulting from such decomposition (Lamber and Timothy, 2005).

Utilization of nutrients can be interpreted by an increase in the length of stem and leaf of water spinach. Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds (Iram et al., 2012).

Negative correlation (-0.780) between the growth of water spinach and orthophosphate denoted that the decrease in the concentration of orthophosphate was affected by the growth of water spinach ($p<0.01$). Decreased concentrations of orthophosphate before addition of bacteria 11% and 89 % after the addition of bacteria (Figure 4). Orthophosphate as form of inorganic phosphorus can be directly absorbed by plants and algae for growth (Effendi, 2003). Decrease in nitrate concentrations before the addition of bacteria was 29% and after the addition of the bacteria was 33%. There was a negative correlation between growth of water spinach and nitrate concentrations, but not significant.

Jegatheesan et al. (2006) reported that a variety of vegetables have been trialled with the aquaponics system and the results showed that cherry tomatoes, various species of basil, chillies and lettuces, strawberry and water spinach grew well with the aquaponics system. When the system is in balance, high production of fish and plant crops at high stocking densities can be obtained without the use of chemical fertilizers, herbicides or pesticides (Nelson, 2008).

CONCLUSION

Aquaculture wastewater treatment using water spinach and bacteria combination in aquaponic system can improve water quality of cultivation media of freshwater crayfish. This was indicated by the decrease of 81% ammonia, 33% nitrate, and 89% orthophosphate. Negative correlation occurred between ammonia and cryfish growth. Positive correlation existed between DO and the length of cryfish. Negative correlation occurred between growth of water spinach with ammonia and orthophosphate concentration. Negative correlation occurred between the abundance of bacteria and ammonia concentration. Positive correlation occurred between the abundance of bacteria and the length as well as weight of the cryfish.

ACKNOWLEDGMENTS

Our thanks to the Directorate General of Higher Education of Indonesia for providing research funding.

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ISSN 2347-6893

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