Phytoremediation Technology Using Water Lettuce (*Pistia stratiotes*) and Blood Cockle Shell Biofilters (*Anadara granosa*) in the Treatment of Aquaculture Waste

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Abstract: *Aquaculture activities with high stocking densities produce liquid and solid waste rich in organic matter, turbidity, and suspended solids. This study aimed to evaluate the effectiveness of the aquatic plant water lettuce (Pistia stratiotes) as a phytoremediator and the blood cockle shell (Anadara granosa) as a biofilter in treating aquaculture wastewater to enhance water quality and reduce nutrient loads. The research employed a Completely Randomized Design (CRD) with two factors: water lettuce density and observation time. Three treatments and three replications were conducted for 30 days. Water quality parameters measured included temperature, pH, dissolved oxygen (DO), nitrate, ammonia, and phosphate. The results showed that the combination of water lettuce and blood cockle shell biofilters with ten clumps of water lettuce was more effective in reducing phosphate levels, while five clumps were more effective for nitrate reduction. Both treatments produced similar plant growth performance, indicating flexibility in system design.*

Keywords:*biofilter; phytoremediation; water quality; wastewater treatment*

1. INTRODUCTION

Aquaculture activities with high stocking densities have the potential to produce liquid and solid waste containing high levels of organic matter, turbidity, and suspended solids. The increase in organic elements such as nitrate and phosphate (N and P) generally originates from fish metabolic waste and uneaten feed (Dewi et al., 2023). Lukman & Bidawati (2023) also stated that wastewater from catfish farming contains nitrogen (N) and ammonia (NH_3), which are formed from the decomposition of proteins and amino acids derived from feed residues and fish feces. Feed residues contribute the largest portion of N and P waste in aquatic environments. Continuous increases in N and P can lead to nutrient enrichment, hyper-nutrification, changes in community structure, and alterations in aquatic productivity.

The waste generated from aquaculture activities must be carefully considered to avoid disturbing the surrounding environment. The direct and continuous discharge of liquid or solid waste into natural water bodies causes pollution (Harihastuti et al., 2015). Therefore, aquaculture waste treatment is necessary as a standard of production success and as a form of responsible aquaculture that supports environmental sustainability (Rahim, 2018). In efforts to maintain the sustainability of fish farming, water quality management becomes a crucial focus.

Shells have been proven effective in filtering solid particles and organic compounds in water, as well as having the ability to neutralize acids. Shells can also function as a water filtration medium, providing dual benefits by reducing waste and being cost-effective, while positively contributing to environmental sustainability. The main component found in blood clam shells is calcium carbonate ($CaCO_3$). Blood clam shells are waste products derived from leftover food-processing materials from blood clams. The use of blood clam shells as a filter has been proven to be superior in improving water quality by 99.5% (Renitasari et al., 2023). In addition to using blood clam shells as a biofilter, phytoremediation techniques can also be applied to prevent pollution or losses caused by the discharge of aquaculture wastewater in a cost-effective and environmentally friendly manner. The use of plants to treat aquaculture waste has begun to be widely implemented. The principle of this system is the bioremediation of inorganic waste in fish farming media using plants. Water from fish culture tanks, which is rich in N and P, is channeled into planting media so that it can be utilized by aquatic plants.

The use of the aquatic plant Pistia stratiotes (water lettuce) for treating liquid waste is being developed in Indonesia. This is because water lettuce has strong capabilities in reducing the levels of organic and inorganic compounds in wastewater. This plant is effective in absorbing and reducing various heavy metals such as Hg, Cd, Mn, Ag, Pb, and Zn in aquatic environments,

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making it a suitable phytoremediation agent for improving water quality in polluted ecosystems.

The use of water lettuce and blood clam shell biofilters to reduce waste levels in aquaculture through phytoremediation and filtration techniques has not been previously conducted. Therefore, this study is needed to measure the effectiveness of water lettuce as a phytoremediator and blood clam shell biofilters in processing organic aquaculture waste to improve pond water quality and reduce pollutant levels.

2. MATERIALS AND METHODS

2.1. Experimental Design

This study was conducted in the Laboratory of Aquatic Productivity and Environment, University of Lampung. A Completely Randomized Design (CRD) was used with two factors: plant density and observation time. The treatments were: P0 (no plants), P1 (5 clumps of water lettuce), and P2 (10 clumps of water lettuce), each with three replications. The experiment lasted for 30 days using 90 liters of aquaculture wastewater. Samples were taken from the outlet for water quality testing.

2.2. Water and Plant Measurements

Water samples were collected every 10 days. Parameters measured included nitrate, ammonia, phosphate, dissolved oxygen (DO), pH, and temperature. DO and temperature were measured using a DO meter, pH using a pH meter, and chemical parameters were analyzed using spectrophotometry (APHA, 2005). Plant growth was assessed by measuring leaf width and fresh weight.

Table 1. Parameters for measuring water and plant quality

Parameter	Unit	Method	Location
a. Physic			
1. Suhu	^{0}C	Thermistor pada DO meter	*In-situ*
b. Kimia			
1. pH	mg/L	pH meter	*In-situ*
2. DO	mg/L	DO meter	*In-situ*
3. Amonia	mg/L	Spektrofotometri	*Ex-situ*

2.3. Data Analysis

Data were analyzed using ANOVA (*Analysis of Variance*). Significant differences among treatments were further analyzed using Duncan's multiple range test at a 5% significance level.

3. Results and Discussions

3.1. Results

3.1.1. Dissolved Oxygen (DO)

The results of observations of dissolved oxygen values during 30 days of observation can be seen in Figure 1.

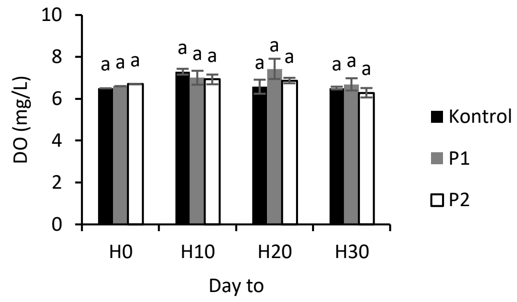

Figure 1. Changes in DO values.

The results of the study showed that dissolved oxygen concentrations did not differ significantly between treatments ($p > 0.05$). Dissolved oxygen concentrations tended to decrease at the end of the observation period. DO values ranged from 6.5-6.7 mg/L at H0; 6.71-7.41 mg/L at H10; 6.19-7.80 mg/L at H20; and 6.03-7.0 mg/L at H30.

3.1.2. pH

The results of observations of water pH values during 30 days of observation can be seen in Figure 2.

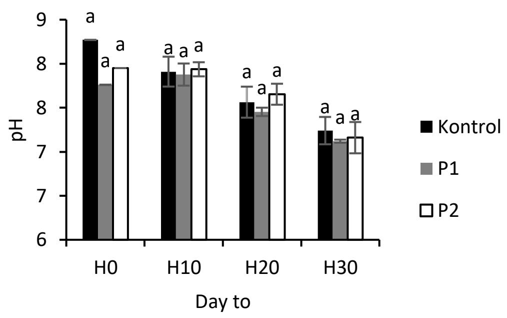

Figure 2. Changes in pH value.

The research results showed that the water pH concentration did not differ significantly between treatments ($p > 0.05$). The pH values ranged from 7.76-8.27 at H0; 7.76-8.03 at H10; 7.36-7.79 at H20; and 7.02-7.40 at H30.

3.1.3. Temperature

The results of water temperature observations over 30 days can be seen in Figure 3.

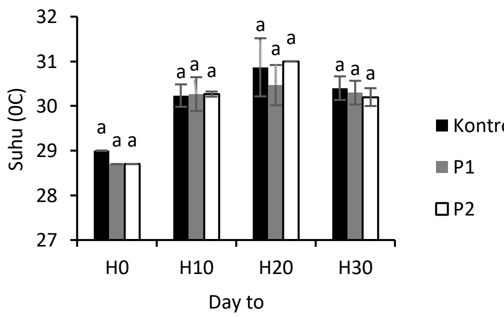

Figure 3. Changes in temperature values.

The results of the study showed that the water temperature concentration did not differ significantly between treatments ($p > 0.05$). Temperature values ranged from 28.7-29 0C at H0; 30-30.7 0C at H10; 30-31.5 0C at H20; and 30-30.6 0C at H30.

3.1.4. Ammonia (NH_3)

The results of the ammonia (NH_3) observations over 30 days can be seen in Figure 4.

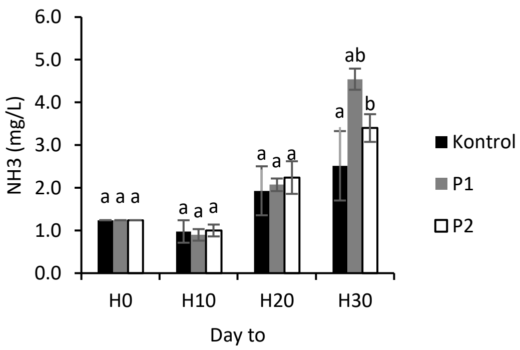

Figure 4. Changes in ammonia concentration (NH_3)

The research results showed that ammonia (NH_3) parameters differed significantly on day 30 of treatment ($p < 0.05$). Ammonia values were 1.24 mg/L at H0; 0.81-1.28 mg/L at H10; 1.27-2.54 mg/L at H20; and 1.94-4.72 mg/L at H30.

3.2. Discussions

Dissolved oxygen (DO) values did not show significant differences between treatments ($p > 0.05$). This may be due to the aeration of the culture medium during the study to maintain dissolved oxygen concentration. Dissolved oxygen also functions in the process of breaking down organic matter into dissolved inorganic nutrients that can be utilized by plants. Dissolved oxygen values in the study decreased at the end of the observation but remained within the tolerance range for the life of farmed fish. The optimum dissolved oxygen concentration for fish growth is around 5-7.5 mg/L (DeLong et al. 2009). The decrease in dissolved oxygen concentration is thought to be due to the high input of organic matter, which causes the process of organic matter decomposition by bacteria using oxygen.

Temperature did not show significant differences between treatments ($p > 0.05$). The temperature conditions in this study did not fluctuate significantly. Temperatures during the study ranged from 26.5 to 30.11 °C, a range still considered optimal for the growth of farmed fish (DeLong et al., 2009). The acidity (pH) values between treatments did not show significant differences ($p > 0.05$). The pH decreased at the end of the observation (Appendix 21c). The decrease in pH in the water is related to the high amount of organic matter entering the water. The process of breaking down organic matter in the water produces CO2 (Nugroho et al., 2012), which causes the water to become acidic. According to DeLong et al. (2009), the optimum pH for aquatic plant growth is below 7. A pH in the range of 6-7 will maintain the form of ammonia as NH_4^+, thus lowering ammonia toxicity.

Ammonia (NH_3) is toxic to aquatic organisms, while ammonium (NH_4^+) is harmless and is a form that can be utilized by plants as a nutrient source. The change in form between NH_3 and NH_4^+ in water is influenced by pH and temperature (Goldman and Horne, 1983). The percentage of NH3 in water will decrease as the pH and temperature of the water decrease. During the study, the concentration of NH3 increased from the 20th to the 30th day. A nutrient source for plants other than NH_4^+ is NO_3. For higher plants, NO_3 must first be reduced to NH_4^+ before being assimilated because the energy required to assimilate NH4+ is lower than NO_3 (Wetzel, 2001).

The process of managing fish farming waste using economically valuable plants such as water lettuce is expected to provide added value. Aquaponics is an effective and efficient method because it can be managed in a single recirculation cycle.

4. CONCLUSIONS

The conclusion of this study is that the water quality parameters in the form of temperature, dissolved oxygen, and pH are still within the standard quality range and are optimal for the growth of aquatic plants, and the ammonia parameter increased on the last day of observation because it came from the decay of dead apu-apu leaves.

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