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The effectiveness of biofilter and density of different stocking in aquaponic recirculation systems in the integration of tilapia (*Oreochromis niloticus* L.) and pakchoy plants (*Brassica rapa* L.)

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Abstract. The availability of land and water for the cultivation process is limited, thus aquaponics cultivation technique is necessary to be considered. This study aimed to determine the effectiveness of bio filter at different stocking densities and the effects on the growth of tilapia and pakchoy plants in aquaponic systems. This research was conducted at Romangpolong Village, Bontomarannu District, Gowa Regency, South Sulawesi, from August to October 2018. The study was conducted in the form of factorial experiment with two factors arranged according to a randomized block design. The first factor was the treatment of biofilter with bioball filter media which consisted of three levels: without bioball, 25 bioballs, 50 bioballs. The second factor was the density of fish stock consisting of two levels namely density of fish stocking with 6 fish per 50 L water per container and with 12 fish per 50 L water per container. The data were analyzed by analysis of variance followed by the Least Significant Difference test at the level of 5%. The results indicate that the interaction of 25 bioballs filter and the fish density of 6 fish per 50 L container produced the best water quality, thus can support the optimum metabolic process and producing the best growth and survival of fish reaching 100 % and was able to supply the nutrient needs of pakchoy plants. The best results of growth and production of pakchoy shown were leaf area of 70.22 cm² and fresh weight of 65.99 g/plant.

16 Introduction

Aquaponics is a bio-integration of a 17 aculture with the principle of recirculation and vegetable produ 8 on that can be done on narrow land and limited water resources, including in urban areas [1-3]. The aquaponic system reduces ammonia through absorption of aqua 9 lture wastewater by using plant roots that function as biofilter. Ammonia that is absorbed through oxidation 5 process, with the help of oxygen and nitrifying bacteria, converted into nitrates [4]. The content of ammonia car 3 be reduced by plants up to 90% of the existing levels, therefore the water is still suitable to be reused as a



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medium for raising fish [5]. One of the plants that can be used in the aquaponic system is pakchoy (*Brassica rapa* L.).

In intensive fish farming, the use of fish stocking densities and high doses of feed results in a decrease in aquaculture water quality that is triggered by high feed residues and fish metabolic waste. In addition, intensive cultivation also produces secondary product in the form of ammonia which has a negative effect on the water quality. The quantity and quality of water supply is the main factor that determines the success of fish cultivation avoiding disease attacks.

Efforts that can be made to overcome these problems is by applying aquaculture recirculation systems with filtration techniques in fish cultivation that reuse water that has been used by recirculating it through a filter, hence the system is water-efficient [6]. Filters in this system serve to purify water and biologically functional to neutralize toxic ammonia compounds into less toxic nitrate compounds in a process called nitrification [7]. The success or failure of fish cultivation in the recirculation system is largely determined by the well-functioning of the system. Based on the description above, a research on the effectiveness of biofilter in the aquaponic recirculation system in the integration of tilapia (*O. niloticus*) and pakchoy (*B. rapa*) was conducted.

2. Research methods

The research was conducted from August to October 2018, located in Romangpolong Village, Bontomarannu District, Gowa Regency. Laboratory tests were carried out at the Water Quality Laboratory, Department of Fisheries, Faculty of Marine Sciences and Fisheries, Universitas Hammadin, Makassar.

This research was conducted in the form of a two-factor factorial experiment arranged according to the Randomized Blocked Design (RBD) pattern. The first factor was the treatment of biofilter with bioball filter media (p) consisted of three levels, i.e. without bioball, 25 bioballs and 50 bioballs filters. The second factor was fish stock treatment consisted of two levels i.e. fish stocking density of 6 fish per 50 L of water per container and fish stocking density of 12 fish per 50 L of water per container.

3. Results

3.1. Preliminary Research

Table 1 shows the results of water quality observations in the Preliminary Study which included the parameters of acidity degree (pH), temperature, dissolved oxygen (DO), carbon dioxide (CO₂), ammonia (NH₃), and nitrate (NO₃). Existing water quality must be at the optimum level as a requirement for a system to work well. The results of measurements of raw water samples indicate the quality of raw water that will be used in research is still suitable for aquaculture activities. The measurements results of sample q1 (fish stocking density of 6 fishes per 50 L per container) and sample q2 (fish stocking density of 12 fishes per 50 L per container) without recirculation showed an increase in concentration levels of several water quality parameters that exceeded or did not meet water quality standards, thus this shows that there is a need for implementing a recirculation system.

Table 1. The value of water quality in the Preliminary Research

Water quality parameters	Samples of raw water	Sampel q1	Sampel q2	Water quality standards	Library Resources
pH	8	7.8	7.6	6 - 9	Boyd [17]
Temperature (°C)	28	29	29	25 -29	Boyd [17]
DO (mg/L)	5.44	6.40	2.88	> 3	Boyd [17]
CO ₂ (mg/L)	16.0	23.9	35.5	< 30	Wedemeyer [7]
NH ₃ (mg/L)	0.002	0.069	0.068	< 0.02	Wedemeyer [7]
NO ₃ (mg/L)	1.291	0.166	0.043	< 1	Wedemeyer [7]

3.2. Main Research

3.2.1. *Temperature and acidity.* Table 2 shows the observations on mean temperature and degree of acidity (pH) in the treatment of bioball filters and different fish stocking densities in the aquaponic system during the study. The highest temperature during this research was in the range of 20.5 - 30.1 °C while the lowest temperature was in the range of 20.2 - 29.2 °C. Observation on the temperature and water pH in a combination of bioball treatment and stocking density showed that the average of water temperature in the maintenance container for each treatment combination ranged from 24.02 - 25.03 °C and the average of water acidity level (pH) at maintenance containers for each treatment combination ranged from 7.15 - 7.19.

Table 2. Results of observations of average temperature (°C) and degree of acidity (pH) in the treatment of bioball filters and stocking densities of fish differed in the aquaponic system.

Combination of treatments	Temperature (°C)	Acidity (pH)
p1q1	24.29	7.12
p1q2	24.43	7.16
p2q1	25.03	7.19
p2q2	24.31	7.19
p3q1	24.11	7.23
p3q2	24.02	7.15

3.2.2. *Dissolved oxygen and carbon dioxide.* Table 3 shows the levels of dissolved oxygen and carbon dioxide in the treatment of bioball filters and fish stocking density of in the aquaponic system during this research. The results of the measurement of dissolved oxygen concentration showed a sufficient value in the range of 5.80 - 7.03 mg/L at 3 weeks after seedling (WAS) and decreased at 6 WAS in the range of 3.37 - 4.87 mg/L. Contrarily, the results of carbon dioxide measurements obtained during this research showed fluctuating values between each treatment combination. The results of carbon dioxide measurements obtained during the research showed a decrease in the concentration of carbon dioxide in 6 WAS, namely in the combination of treatments p2q1, p3q2, p2q2 and p1q1. The opposite occurs in the combination of p1q2 and p3q1 treatments, which shows an increase in carbon dioxide levels.

Table 3. The results of observations of dissolved oxygen (mg / L) and carbon dioxide (mg/L) in the treatment of bioball filters and stocking density of fish differed in the aquaponic system.

Combination of treatments	Dissolved Oxygen (mg/L)		Carbon dioxide (mg/L)	
	3 WAS	6 WAS	3 WAS	6 WAS
p1q1	6.73	3.93	31.96	29.56
p1q2	5.87	3.37	21.97	29.56
p2q1	6.63	4.87	23.97	19.18
p2q2	6.03	4.47	31.96	25.57
p3q1	7.03	4.80	23.97	31.96
p3q2	5.80	3.63	23.97	19.98

WAS = Weeks after seedling

3.2.3. *Ammonia.* Table 4 shows ammonia levels at the inlet and outlet sampling points of 3 WAS and 6 WAS in the treatment of bioball filters and different stocking densities in the aquaponic system

during the research. The measurement results for the range of ammonia values showed a fluctuating concentration values between each treatment combination at the inlet and outlet channel sampling points at the time of observation of 3 WAS and 6 WAS. The measurement results obtained at 3 WAS showed that the highest ammonia concentration in the inlet channel found in the combination of treatment of p1q2 (0.0213 mg/L) then followed by the treatment combination of p2q2 (0.0097 mg/L), p2q1 (0.0093 mg/L), p3q2 (0.0087 mg/L), p1q1 (0.0080 mg/L), respectively. Lowest ammonia concentration at 3 WAS found in the p3q1 treatment combination (0.0068 mg/L). On the contrary, ammonia concentration in the outlet channel was highest in the p2q2 treatment combination (0.0303 mg/L), followed by the p2q1 treatment combination (0.0177 mg/L), p3q2 (0.0177 mg/L), p1q1 (0.0163 mg/L), and the lowest in p2q1 treatment combination (0.0127 mg/L). Furthermore, observation at 6 WAS showed the highest ammonia levels obtained in inlet channel in the treatment combination p2q2 (0.0127 mg/L), then followed by the treatment combination p1q1 (0.0113 mg/L), p1q2 (0.0103 mg/L), p3q2 (0.0103 mg/L), p3q1 (0.0097 mg/L), and the lowest in the p2q1 (0.0083 mg/L) treatment combination. In contrast, the highest ammonia concentration in the outlet channel was found in the combination of treatment p1q2 (0.0127 mg/L), followed by the combination of treatment p1q1 (0.0110 mg/L), p3q2 (0.0100 mg/L), p2q2 (0.0097 mg/L), p2q1 (0.0093 mg/L), and the lowest in the p3q1 treatment combination (0.0067 mg/L).

Table 4. Average of ammonia levels (mg / L) at the inlet and outlet sampling points 3 Weeks After Seedling (WAS) and 6 WAS in the treatment of different bioball filters and fish stocking densities in the aquaponic system.

Combination of treatments	3 WAS		6 WAS	
	Inlet	Outlet	Inlet	Outlet
p1q1	0.0080	0.0163	0.0113	0.0110
p1q2	0.0213	0.0303	0.0103	0.0127
p2q1	0.0093	0.0093	0.0083	0.0080
p2q2	0.0097	0.0177	0.0127	0.0097
p3q1	0.0068	0.0143	0.0097	0.0067
p3q2	0.0087	0.0177	0.0103	0.0100

WAS = Weeks after seedling

3.2.4. *Nitrate.* Table 5 shows nitrate levels at the inlet and outlet sampling points at 3 WAS and 6 WAS in the treatment of bioball filters and different stocking densities in the aquaponic system during the research. The results of nitrate concentration measurements showed fluctuating values between each treatment combination at the inlet and outlet channel sampling points at 3 WAS and 6 WAS. The measurement results obtained at 3 WAS showed the highest nitrate concentrations in the inlet channel was found in the treatment combination of p2q1 (0.906 mg/L), then followed by treatment combination of p3q1 (0.857 mg/L), p3q2 (0.763 mg/L), p2q2 (0.752 mg/L), p1q1 (0.711 mg/L), and the lowest in the p1q2 treatment combination (0.627 mg/L). On the contrary, the highest nitrate concentration in the outlet channel was found in the combination of treatment p3q1 (1.079 mg/L), then followed by a combination of treatments p3q2 (1.073 mg/L), p2q2 (1.709 mg/L), p2q1 (0.959 mg/L), p1q1 (0.882 mg/L), and the lowest in the p1q2 treatment combination (0.796 mg/L). The measurement results obtained at the time of observation of 6 WAS also gave the highest nitrate concentration in the inlet channel in the p3q2 treatment combination (1.395 mg/L), followed by a combination of p1q1 (1.318 mg/L) treatment, p3q2 (1.300 mg/L), p2q2 (1.270 mg/L), p1q2 (1.181 mg/L), and the lowest in the p2q1 treatment combination (1.124 mg/L). The highest nitrate concentration in the outlet channel was found in the combination of p1q1 (1.525 mg/L) treatment, followed by the combination of p1q2 (1.369 mg/L), p3q2 (1.352 mg/L), p2q2 (1.250 mg/L), p3q1 (1.205 mg/L), p3q2 (1.352 mg/L), p2q2 (1.250 mg/L), p3q1 (1.205 mg/L), and the lowest in the p2q1 treatment combination (1.148 mg/L).

Table 5. The results of observations of nitrate (mg / L) at the inlet and outlet sampling points 3 weeks after seedling and 6 Weeks After Seedlings (WAS) in the bioball filter and stocking density treatment of fish differed in the aquaponic system.

Combination of treatments	3 WAS		6 WAS	
	Inlet	Outlet	Inlet	Outlet
p1q1	0.711	0.882	1.318	1.525
p1q2	0.627	0.796	1.181	1.369
p2q1	0.926	0.959	1.124	1.148
p2q2	0.752	1.060	1.270	1.250
p3q1	0.857	1.079	1.395	1.205
p3q2	0.763	1.703	1.300	1.352

WAS = Weeks after seedling

4. Discussion

In the Preliminary Study, ammonia concentration increased in the water sample q1 by 0.069 mg/L and in the sample q2 by 0.068 mg/L. According to Wedemeyer [8], the maximum limit of the concentration of ammonia in fish maintenance containers is <0.02 mg / L.

The observation results in the sample q1 show that the dissolved oxygen content was 6.40 mg/L and the carbon dioxide (CO₂) content was 23.9 mg/L, whereas for the q2 sample the dissolved oxygen content was 2.88 mg/L and the carbon dioxide (CO₂) content was 35.5 mg/L. According to Hephher & Pruginin [9], an increase in stocking density will be followed by an increase in the amount of feed, the body's metabolic waste, oxygen consumption, and can reduce water quality. According to Syamsuddin [10], an increase in CO₂ concentration is always accompanied by a decrease in the concentration of dissolved oxygen needed for the breathing of aquatic animals. Although CO₂ has not yet reached lethal concentrations for cultivating animals, increased concentrations can kill aquaculture animals due to reduced oxygen concentration.

The results of the preliminary research observations indicate a decrease in the value of water quality as a negative effect of intensive fish cultivation on water quality. In order to maintain water quality for it to remain proper for fish, a recirculation system with biofilter is used in the process of maintaining cultivation fish. Recirculation is a system that uses water continuously by turning it to be cleaned inside the filter and then flowed back into the cultivation container [11].

In the main research, water quality parameters observed during the research included temperature, degree of acidity (pH), dissolved oxygen, carbon dioxide, ammonia, and nitrates. The process of nitrification in accelerating or inhibiting bacterial growth and propagation will affect cell metabolism thereby reducing the ability of bacteria. The ability of oxidation by bacteria is influenced by several factors, such as the presence of toxic compounds (bactericidal) water, temperature, pH, dissolved oxygen, salinity, and surface area for bacteria to attach [12]. The results of observations of average temperatures during the study ranged from 20.2 - 30.1 °C and the average observations of pH ranged from 6.60 - 7.93. The optimal temperature for tilapia seeds is between 25 - 32 °C. Tilapia growth will usually be disrupted if the habitat temperature is lower than 14 °C or at high temperature of 38 °C. Tilapia will die at a temperature of 6 °C or 42 °C [13,14].

According to Sucipto and Prihartono [15], tilapia seeds can grow well in a pH range of 7-8. Optimal acidity [20] can cause fish to be stressed, prone to disease, and low productivity and growth. In addition, pH plays an important role in the field of fisheries because it is related to the ability to grow and reproduce. Fish can live at a minimum of pH 4 and will die at pH above 11 [16]. Water quality also affects the growth of pakchoy plants, especially on its temperature and pH. According to Muliawati [17] most nutrients are more soluble and are available to plants in the pH range of 6.0 - 7.0, and temperatures that can support plant growth range from 5 - 35 °C. According to Susanto [18] the

temperature of nutrient solution must be kept optimal to support root growth and maintain the effectiveness of nutrient absorption by roots.

The observations during the research showed that the treatment of biofilter media used worked well. The system works marked by fluctuations in dissolved oxygen, carbon dioxide, nitrates and ammonia. The results of measurement of dissolved oxygen content during the research ranged from 5.80 - 7.03 mg/L at 3 WAS and 3.37 - 4.87 mg/L at 6 WAS. Dissolved oxygen levels at 3 WAS per treatment combination unit tend to be higher than oxygen levels in the treatment combination unit at 6 WAS. Decrease in dissolved oxygen levels is thought to be caused by the oxidation process of organic and inorganic materials by bacteria that require oxygen during the oxidation process.

Measurement results during the research showed that carbon dioxide levels in 3 WAS and 6 WAS fluctuated in each combination unit of treatment and during the study tended to increase. According to Wedemeyer [8] to ensure good health and physiological conditions, warm water fishes should not be maintained at carbon dioxide levels of more than 20-30 mg/L for a long time. Boyd [19] states that dissolved carbon dioxide levels correlate with dissolved oxygen requirements. Fish are still able to tolerate carbon dioxide at a concentration of 60 mg/L provided that dissolved oxygen is enough in waters.

The results of the average measurement of ammonia concentration at 3 WAS at the inlet point showed that all treatment combinations were still in optimum condition ranging from 0.0068 - 0.0080 mg/L, except for the p1q2 treatment combination reaching 0.0213 mg/L. Likewise, at the outlet point, all treatment combinations were still in optimum condition, ranging from 0.0127 - 0.0177 mg/L, except for the p1q2 treatment combination reaching 0.0303 mg/L. The high concentration of ammonia in the combination of p1q2 treatment is suspected to be influenced by an increase in stocking density which will be followed by an increase in the amount of feed, metabolic waste of body, oxygen consumption, and this can reduce water quality. According to Sidik [6], intensive cultivation using stocking densities and high doses of feed will have an impact on decreasing aquaculture water quality due to the increasing level of waste from feed and feces. In addition, the combination of the p1q2 treatment is a treatment without bioball where bioball is a place for the growth of bacteria that play a role in the nitrification process allegedly caused by the absence of the nitrification process because there is no bioball filter in the p1q2 treatment.

The measurement results of the average ammonia concentration at 6 WAS showed fluctuating concentration values between each treatment combination, both from the inlet sample point and the outlet sample point and were still at the optimum condition that is <0.02 mg/L. Ammonia concentration increased at 6 WAS due to the longer maintenance time the higher the concentration of ammonia produced. In 6 WAS, the combination of p1q1 and p1q2 treatments showed a more stable concentration value at the inlet and outlet points which ranged from 0.0103 - 0.0127 mg/L and the pakchoy plant had good root growth therefore it could absorb nutrients from the flow of nutrients along the gutter maintenance

The results of nitrate concentration (NO₃) measurements in each treatment combination showed different results on the inlet and outlet channels, both at 3 WAS and 6 WAS. Nitrate concentrations in the inlet channel at 3 WAS ranged from 0.627 to 0.906 mg/L, whereas at 6 WAS it ranged from 1.181 to 1.395 mg/L. The range of nitrate concentrations in the outlet channel at 3 WAS is 0.796 - 1.079 mg/L, whereas at 6 WAS is 1.148 - 1.525 mg/L. Nitrate content in each treatment combination unit at 3 WAS and 6 WAS showed fluctuating concentration values but there was no significant difference between nitrate levels before and after the filtration process. Overall the results of measurements of nitrate values are still at optimum conditions for the growth of pakchoy plants and the growth of tilapia.

The tendency of decreasing water quality in the preliminary research shows that in the main research, the highest effectiveness of biofilter was found in the combination of 25 bioball treatments and 6 stocking densities per 50 L per container. The results showed that the number of 25 bioball was more effective in the size of the filter used during the research where the number of bioballs was not too dense, which gave oxygen the opportunity to enter the bioball cavity thus the bacteria attached to

the bioball get enough oxygen. If enough oxygen is obtained by bacteria, then many bacteria will grow in the bioball cavity. Nitrifying bacteria can grow if the supply of oxygen for life is fulfilled, as stated by Nut [20], which states that nitrifying bacteria work more optimally if they get a rich supply of oxygen. In accordance with its function, bioball is a place for bacteria to grow. Bacteria that grow on bioball are nitrifying bacteria (*Nitrosomonas* sp and *Nitrobacter* sp). *Nitrosomonas* has the role of oxidizing ammonia to nitrite, while *Nitrobacter* has the role of oxidizing nitrite to nitrate.

The impact of the accumulation of organic and inorganic materials in tilapia maintenance containers. In contrast, the results of the water quality analysis test in the main research show that all biofilter treatments in the tilapia recirculation system and the pakchoy plant can effectively help produce water quality in the optimum range.

4. Conclusions

Based on the research results obtained, it can be concluded that the interaction of the use of 25 bioball filters and stocking density of 6 fish per 50 L per container effectively produces the best water quality therefore it supports the optimum metabolic process and produces the best growth and survival of tilapia (100%) and able to supply the nutrient needs of pakchoy plants thus it provides the best results, namely leaf area of 70.22 cm² and fresh weight of 65.99 g/plant.

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