Effectiveness of nitrogen absorption and growth performance of *Colossoma macropomum* with different density of nitrifying bacteria

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Abstract. Intensive *Colossoma macropomum* cultivation tends to have a high stocking density, thus causing the metabolic wastes of fish organisms to increase. The right solution to overcome this is to cultivate fish by adding bacterial density to the bioball to improve and maintain water quality in the culture media. The aims of this study were: to analyze the density of bacteria contained in the bioball, as well as to analyze the optimal bacterial density in increasing the effectiveness of nitrogen absorption and the growth performance. Test animals used in this research were as many as 300 specimens of *C. macropomum* with a length of 5.74-6.27 cm. Fish meal ingredients used were commercial feed with a composition of 46% protein content, 10% fat, 13% ash content, 2% crude fiber, and 10% water content. Nitrifying bacteria probiotics were used as a starter filter. The method used is the experimental method of Completely Randomized Design (CRD) with 1 factor, 5 treatment levels and 3 replications. The treatment levels in this study were: A) without the addition of nitrifying bacteria, B) by adding bacteria 2 mL 80 L^{-1} of water, C) 3 mL 80 L^{-1} of water, D) 4 mL 80 L^{-1} of water and E) 5 mL 80 L^{-1} of water respectively. The best result was found for the treatment with 4 mL 80 L^{-1} of nitrifying bacteria, generating a bacterial density of 1.857±0.022X108 CFU mL^{-1}, with a nitrogen absorption efficiency of 91.71±5.48%, for a temperature ranging from 26–29.7^{0}C, a pH of 6–7.5, a DO of 5.4–5.5 mg L^{-1}, determining levels of ammonia (NH_3) between 0.00011 and 0.05430 mg L^{-1}, levels of nitrite (NO_2) between 0.0549 and 0.2956 mg L^{-1} and levels of nitrate (NO_3) between 0.2000 and 9.0833 mg L^{-1}, and consequently an absolute weight growth of 23.29±1.41 g, an absolute length growth of 5.14±0.05 cm, a specific growth rate of 3.30±0.06%, a feed efficiency of 93.83±5.72%, a feed conversion rate of 1.07±0.06 and a survival rate of 100%.

Key Words: biological filter, water qualiy, survival rate, ammonia, stocking density.

Introduction. *Colossoma macropomum* is a relatively new aquaculture species in Indonesia. Nevertheless, the demand for this fish continues to increase both as a consumption fish and as an ornamental fish. The fish was originally used as an ornamental fish, but as an adult it tends to be used as fish for consumption. As a consumption fish, *C. macropomum* has a delicious and savory meat taste so it is liked by consumers (Santoso & Agumansyah 2011). This species, that have high adaptability, is easy to cultivate and have important economic value. One of the cities that produces high enough *C. macropomum* fry quantities in Indonesia is Bogor Regency. In 2016 there were 77,873 tons of the fish seed production in this district, while in 2017 it was recorded that the fish seed production reached 97,952 tons (Bogor Regency Marine and Fisheries 2017).

The production target for *C. macropomum* can be achieved through intensification of aquaculture. Intensive aquaculture activity tends to have a high stocking density, resulting in narrow space for fish movement and increased competition for oxygen and feed, so that the potential for fish to become stressed is greater. As a result, fish growth will be stunted. High density also accelerates the decline in water quality due to the accumulation of metabolic waste and feed residues, having a major effect on growth. In aquaculture activities, supporting factors affecting the success of culture activity and fish growth are optimal feeding and water quality. One of the technologies used to maintain water quality in fisheries is the aquaculture recirculation systems, in order to make the aquatic media suitable for aquatic organisms and to support the optimization of water use. The recirculation system is able to reduce the level of ammonia concentration, with up to 31-43% (Djokosetiyanto et al 2006; Putra & Pamukas 2011). According to Lekang (2008), Fadhil et al (2010) and Jacinda (2021), the use of a recirculation system has advantages including: a more efficient use of water, a higher flexibility on the culture locations, more hygienic culture systems, with relatively small space or land requirements, ease of controlling and maintaining cultivated organisms, ease of maintaining temperature and water quality, a friendly environment, protected against the pollution. Recirculating aquaculture system can also be used to control the dissolved solids for adjusting them to the aquaculture systems and filter substrates (Fadhil et al 2010). The filter substrate serves to filter dissolved solids. The use of the right type and material will determine the success of fish rearing in the recirculating system because it will determine the growth of non-pathogenic bacteria. Currently, biological filter substrates are widely used because they are more environmentally friendly.

Biological filters depend on the bacteria activity of degrading organic and inorganic materials in the culture media. The filter functions as a reformer of toxic nitrogen compounds (ammonia and nitrite) into non-toxic compounds (nitrates) with the help of microorganisms. This filter also functions as a water purification mechanism and biological function to neutralize toxic ammonia compounds into less toxic nitrate compounds in a process called nitrification. Wiradana et al (2018) stated that biofiltration using a bacterial system uses a lot of bioball media as bacterial living media (a place to attach to for bacteria). Selection of the right biofilter media will provide a surface on which the microorganisms grow.

The bacteria that grow on the bioball are nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.). *Nitrosomonas* plays a role in oxidizing ammonia to nitrite, while *Nitrobacter* plays a role in oxidizing nitrite to nitrate, this nitrate being a source of nutrients for the development of plankton, which is a natural fish feed. The larger the microorganism population, the higher the nitrification process. In the research carried out by Mulyadi et al (2020) it was found that the optimal number of bioballs given to one fish rearing container was 55 bioballs/filter containers. However, the optimal value of nitrifying bacteria density for supporting the growth of nitrifying bacteria, in order to maintain good water quality in aquaculture fish farming containers, has not been found. Naturally, nitrogen from fish farming waste produces ammonia (NH_3) from feed residues and fish metabolism. This can result in the accumulation of organic matter, which causes a decrease in water quality (Putra & Pamukas 2011; Prayogo al 2012). According to Ardiansyah (2004), the effectiveness of the density of nitrifying bacteria can be analyzed by observing the quality of the water produced in the culture media container. Based on the description above, a series of studies are needed on the effectiveness of nitrogen absorption, the number and types of bacteria and the growth performance of *C. macropomum* in recirculating system cultivation with different doses of additional nitrifying bacteria density. Therefore, further research needs to be carried out on the effectiveness of nitrogen absorption and growth performance of *C. macropomum* in aquaculture recirculating system, with different nitrifying bacteria densities in bioballs.

Material and Method

Research location and time. This research was carried out for approximately 7 months, from March to September 2021, in several laboratories within the Faculty of Fisheries and Marin Science, University of Riau, namely the Aquaculture Technology Laboratory, Aquaculture Department, regarding the fish rearing activities, the Chemical Oceanography Laboratory, Marine Sciences Department, regarding the water quality analysis, and the Laboratory of Fish Diseases and Parasites, Department of Aquaculture, Faculty of Fisheries and Marine Science, Riau University, regarding the bacteria count.

Materials and tools. The main ingredients used in the study were as follows: 300 *C. macropomum* fish specimens originating from AMAMA hatchery in Pekanbaru, with a size of 5.74-6.27 cm and a weight of 4.01-5.11 g. The feed used was commercial feed, namely PF-800 pellets with a composition of 46% protein, 10% fat, 13% ash, 2% crude fibre and 10% water. The test container is a round bucket with a diameter of 60 cm, a height of 45 cm, a volume of 150 L. A bioball with a diameter of 4 cm, a specific area of ±230 $m^2\ m^{-3}$ with a cavity priority of 0.92, made of PVC was used as a filter substrate, being contained in a gutter of PVC measuring 50 cm x 14 cm x 14 cm.

Experimental design. The research method used in this study was an experimental method of Completely Randomized Design, 1 factor, 5 treatment levels and 3 replications (Steel & Torrie 1993). The factor in the study is the difference in the number of nitrifying bacteria. the determination of the level of treatment in this study was done based on the recommended dose by DDL Company (Dwi Devi Lancar), a fish cultivation business unit engaged in the production of fish and shrimp medicines. The standard dose of 2.5 mL 200 L^{-1} $week^{-1}$ is also supported by the research carried out by Hartini et al (2013). The levels of treatment in the current study were (1) without additional KP Super N (0 mL 80 L^{-1}), or with KP Super N: (2) 5 mL 200 L^{-1} water (2 mL 80 L^{-1}), (3) 7.5 mL 200 L^{-1} water (3 mL 80 L^{-1}), (4) 10 mL 200 L^{-1} of water (4 mL 80 L^{-1}), and (5) 12.5 mL 200 L^{-1} of water (5 mL 80 L^{-1}).

Research procedure. *C. macropomum* measuring 5.74-6.27 cm and 4.01-5.11 g weight was obtained from the AMAMA hatchery in Pekanbaru. Fish rearing containers use a closed recirculation system. Each rearing tank was filled with 80 L of fresh water. The rearing tank was connected to a 50 cm x 14 cm x 14 cm PVC gutter as a filter container, which was placed at the top of the fish rearing tanks. In each filter container, 55 bioballs were introduced (Mulyadi 2021). In the bioball of the filter container, bacteria with the appropriate amount were added to the treatment. Furthermore, the water from the gutter filter was flowing back through a PVC pipe with a diameter of 2.5 cm to the rearing pond for *C. macropomum*. The water from the fish rearing container was channeled to the filter media (talang) using an 18 watt water pump. After passing through the filter media (gutters) the water was returned to the fish rearing tank through the channel pipe contained in the filter container. In the rearing media, the starter bacteria trademarked KP-SUPER N (*Nitrosomonas* sp. and *Nitrobacter* sp.) were added at a dose of 2.5 mL 200 L^{-1} $week^{-1}$, to accelerate the growth of bacteria in the bioballs.

(a) (b)

Figure 1. (a) Bioball used as filter substrate; (b) Design of culturing recirculation system (original photos).

The test fish that had been adapted to the rearing media were then randomly put into 15 rearing containers. The fish were fed with a feed containing 46% protein, 10% fat, 13% ash content, 2% crude fiber, and 10% water content, ad satiation at 07.00, 12.00 and 17.00 WIB. Every 14 days the fish were weighed to see their growth. Fish rearing was carried out for 56 days. All dead fish during the rearing period was counted and weighed to calculate the mortality rate.

Measured response. The responses measured in the study were: the density of the filter container was observed at the beginning, middle and end of the study (SNI.01-2332.3-

2006 and SNI.01-2332.3-2006). The nitrogen uptake efficiency was measured according to the procedure recommended by Zonneveld et al (1991). The temperature and pH were observed daily, while the DO, total ammonia nitrogen (TAN), nitrite (NO_2) and nitrate (NO_3) were observed weekly. The procedure for measuring water quality refers to the SNI of the Department of Public Works (1990). The growth performance of *C. macropomum* includes: survival rates, absolute growth weight and growth length, referring to Effendi (2003). The specific growth rate and feed efficiency according to Zonneveld et al (1991) and feed conversion according to the National Research Council (NRC 2011).

Data analysis. Data on bacterial density, feed efficiency and growth performance were analyzed according to the RAL model (Steel & Torrie 1993). To determine the effect of the KP SUPER N nitrifying bacteria on each variable measured, a diversity analysis was carried out using the SPSS 17.0 software. If $p<0.05$, the Newman-Keuls test was carried out to see the difference between treatments. Water quality data were analyzed descriptively using Microsoft Excel and displayed in the form of tables and graphs.

Results and Discussion

Bacterial density in the filter container. Biological filters or bio media function as habitat for nitrifying bacteria colonies. These filters usually have pores like bioballs, which are an important component in the filtration system. Biological filters play a role in filtering the harmful ammonia (NH_4) originating from the fish waste and food remnants, and converting it into nitrate (NO_3), which is safe for fish. This bioball can work optimally if you add starter bacteria to accelerate the growth of good bacteria, namely micro-organisms or bacterial seeds that help fighting against bad bacteria for fish. The results of observations of bacterial density in bioballs at the beginning of the study (day 1), 28 and 56 are presented in Table 1.

Table 1

Density of bacteria in the filter container in all treatments

No	*Number of bacteria*	*Bacterial density on day of (10^8 CFU mL^{-1})*		
		1	*28*	*56*
1	0 mL 80 L^{-1}	0.042	1.332 ± 0.004^a	1.027 ± 0.004^a
2	2 mL 80 L^{-1}	0.052	1.606 ± 0.026^b	1.285 ± 0.037^b
3	3 mL 80 L^{-1}	0.054	1.965 ± 0.038^c	1.609 ± 0.020^c
4	4 mL 80 L^{-1}	0.050	2.039 ± 0.004^d	1.857 ± 0.022^d
5	5 mL 80 L^{-1}	0.042	3.045 ± 0.002^e	1.594 ± 0.026^c

Different superscript letters (a, b, c, d and e) on the same line show marked differences between treatments ($p<0.05$).

Table 1 shows that the bacterial density increased from the first day and reached its peak on day 28, then decreased again on day 56 (Figure 2). The highest increase in bacterial density on day 28 was found in the administration of KP-SUPER N nitrifying bacteria at 5 mL 80 L^{-1}, the resulted density being of 3.045 x 108 CFU mL^{-1}. On the 56th day, the highest bacterial density was found for KP-SUPER N of 4 mL 80 L^{-1}. The magnitude of the decrease in bacterial density following the administration of 5 mL L^{-1} nitrifying bacteria on the 56th day was due to organic matter originating from the unconsumed feed and fish feces. The source of nutrients from the bioball is not sufficient for bacteria development, because it has been maximally utilized at the time of peak population, on day 28.

The results of the analysis of variance showed that the administration of KP-SUPER N nitrifying bacteria on the bioball filter affected the bacterial density on days 28 and 56 ($P<0.05$). Based on the further test of Student Newman Keuls (SNK) it was found that on day 28 there was a significant difference in bacterial density between treatments. On day 56 the bacterial density for the administration of KP-SUPER N starter bacteria was 3 mL 80 L^{-1}, not different from 5 mL 80 L^{-1}, while the other treatments were significantly different.

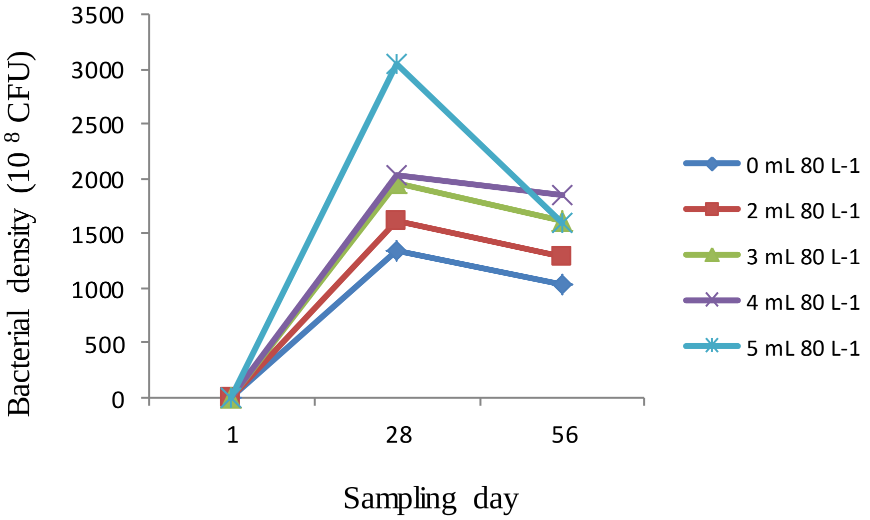


Figure 2. Fluctuations in bioballs bacterial density during the study.

According to Sunarto (2003), dissolved organic matter is needed by living bacteria. Sidharta (2000) explains that organic matter contains carbon, nitrate, phosphate, sulfur, ammonia, and several minerals which are nutrients for bacterial growth. The provision of KP SUPER N which is given routinely in a culture container causes bacteria to continue to grow due to the availability of organic materials that can be utilized optimally.

Nitrogen absorption efficiency. Applying different densities of nitrifying bacteria to the filter containers with bioball substrates will result in different nitrogen absorption levels for each treatment. This aquaculture system involves various trophic levels, namely the role of fish and bacteria as ammonia breaker. The results of the analysis of the nitrogen absorption are presented in Table 2.

Table 2

Effectiveness of nitrogen uptake by *Colossoma macropomum* and filter media

Number of bacteria	*Effectiveness of nitrogen absorption (%)*		
	Fish	*Media*	*Total*
0 mL 80 L^{-1}	69.17±6.03^{a}	0.27±0.16^{a}	69.43±6.10^{a}
2 mL 80 L^{-1}	71.31±3.18^{ab}	0.45±0.10^{ab}	71.76±3.17^{a}
3 mL 80 L^{-1}	79.07±10.95^{ab}	1.22±0.21^{bc}	80.29±10.93^{ab}
4 mL 80 L^{-1}	90.10±6.18^{b}	1.61±0.76^{c}	91.71±5.48^{b}
5 mL 80 L^{-1}	83.51±9.46^{ab}	1.27±0.28^{bc}	84.78±9.73^{ab}

Different superscript letters on the same line show marked differences between treatments ($p<0.05$).

Table 2 shows the effectiveness of the greatest nitrogen absorption in fish, filter media and the total found. In the 4 Ml 80 L^{-1} treatment, the greatest density of bacteria was found, namely 90.10, 1.61 and 91.71%, determining the decomposition of organic matter and the nitrification process. Based on the analysis of variance, the addition of nitrifying bacteria had an effect ($P<0.05$) on the effectiveness of nitrogen absorption by fish and filter media. Newman Keuls further test showed that the addition of nitrifying bacteria 4 mL 80 L^{-1} was significantly different from other treatments.

In the filtration system, the bacteria remove the toxic compounds from the fish rearing media. In the intensive cultivation, ammonia is a limiting factor. By filtration, ammonia can be converted into non-toxic compounds. The amount of nitrogen absorption was greater in fish than in the filter media. Fish absorb nitrogen into the body and the rest is released into the water. Furthermore, the nitrogen in the waters is captured by the filtration system so that the amount of nitrogen in the filter media is low.

In the recirculation system, nitrogen waste will flow into the filtration tank and nitrogen removal occurs from the amount of nitrogen entering the filter tank, originating from the amount of feed nitrogen (Putra 2009). The nitrogen removal in the filter tank is absorbed by the bacteria found in the bioball. According to Rakocy et al (2006) the main concentration in the filter system is the transfer of ammonia, a product of metabolic processes through fish gills. Ammonia will accumulate and reach toxic levels if not removed by a nitrification process (biofiltration), where the process is that ammonia is first oxidized to toxic nitrite, then to nitrate which is relatively non-toxic. The above process involves the bacteria *Nitrosomonas* and *Nitrobacter*. Nitrite bacteria grow as a film (biofilm) on the surface of the material (filter medium) or adhere to organic particles. The biofilter consists of a medium with a large surface area for the growth of nitrite bacteria. The application of nitrifying bacteria 4 mL 80 L^{-1} in the bioball was able to eliminate the incoming nitrogen waste because the bioball, with its regular cavity structure, had the property of being able to absorb ammonia waste, also being a medium for attaching microorganisms (biofilm) and capturing various elements suspended in water, which feed these organisms (Nurhidayat et al 2012). Naturally, nitrogen comes from fish farming waste which produces ammonia (NH3) from uneaten food and metabolic processes, supplying the nitrification process of ammonia into nitrite and nitrate. Ammonia and nitrites are toxic to fish, while nitrates are harmless and will be used by plants. The nitrification process is chemically assisted by certain bacteria, so that fish rearing with recirculation can use biological filters (Andrews 2002).

Bioball is a biological filter as a growth medium for bacteria, by adding nitrifying bacteria such as *Nitrosomonas* it will oxidize ammonia (NH_3) to nitrite (NO_2^-) which will then be oxidized to nitrate (NO_3^-) by *Nitrobacter* bacteria. The addition of nitrifying bacteria as starter bacteria will be able to increase the number of bacteria in the bioball, so that the bioball can help improve water quality, especially by reducing the concentration of TAN and increasing the nitrate content (Figure 5) in *C. macropomum* rearing media. According to Putra & Pamukas (2011) filter media provides a surface for growing and developing media for microorganisms, in a biofilter system. The size and shape of the material used as a filter is very important because it affects the population of microorganisms during the nitrification process. Failures in biofilters can produce very high levels of ammonia or nitrite, both of which are toxic to aquatic animals and can cause health problems, suppress growth, and cause death (Kuhn et al 2010).

Water quality. Management of water quality for aquaculture is very important, because water is a living medium for aquaculture organisms, thus determining success in aquaculture. Poor water quality can cause fish growth to slow down even though the feed provided is of sufficient quality and quantity. The results of observations on temperature, pH and dissolved oxygen (DO) in all treatments are presented in Table 3.

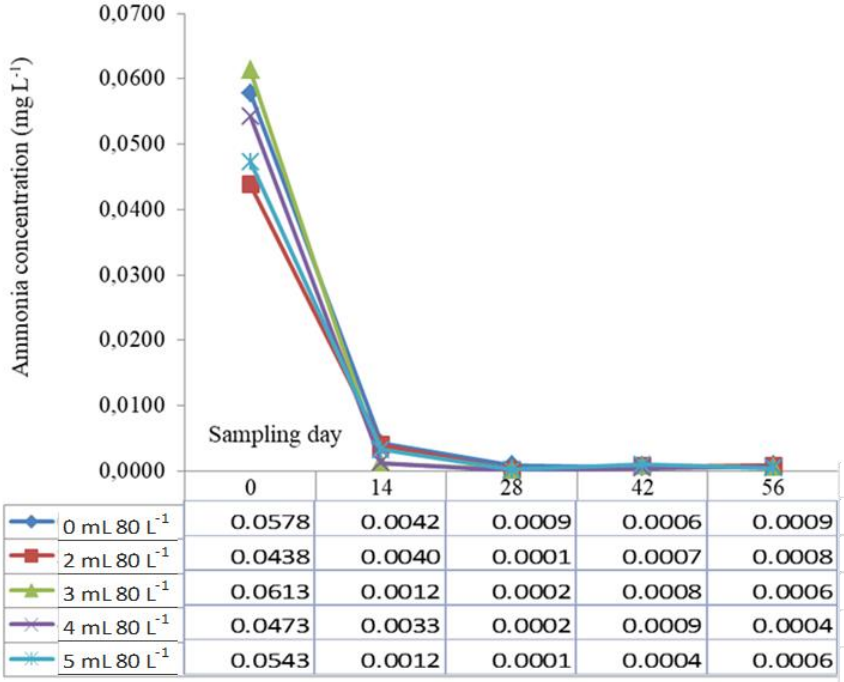
Table 3

Average temperature, pH and dissolved oxygen (DO) in all treatments during the study

Parameters measured	*Number of nitrifying bacteria added to the filter container*				
	0 mL 80 L^{-1}	*2 mL 80 L^{-1}*	*3 mL 80 L^{-1}*	*4 mL 80 L^{-1}*	*5 mL 80 L^{-1}*
Temperature (°C)	26–29.5	26–29.6	26–29.6	26–29.7	26–29.5
pH	6–7.3	6–7.4	6–7.5	6–7.5	6–7.5
DO (mg L^{-1})	4.9–5.2	5.0–5.2	5.2–5.9	5.4–5.5	5.5–5.7

Table 3 shows that the temperature, pH and dissolved oxygen (DO) were relatively the same in all treatments, because the research was conducted in a laboratory, so that the effects of weather and environment were relatively homogeneous. Giving starter bacteria with different doses on the bioball filter did not affect temperature, pH and DO on the *C. macropomum* rearing media. The range of temperature, pH and DO during the study in all treatments were appropriate for the growth of *C. macropomum*. This is in accordance with the opinion of Kordi (2010), which stated that the optimal water temperature for the growth of *C. macropomum* is 25–30°C, the dissolved oxygen value is 3-6 mg L^{-1} and the pH range 6.5-7.6.

The range of ammonia concentrations in all treatments at the beginning of the study (day 1) was relatively homogeneous and classified as still safe for the fish growth (0.0480-0.06130 mg L^{-1}), due to precipitation and aeration for 48 hours before the water was used as media. On days 14 to 56, ammonia concentrations tended to continue to decrease and the greatest decrease occurred in the administration of KP-SUPER N nitrifying bacteria of 4 mL 80 L^{-1}, so that the lowest average TAN at the end of the study was found in that treatment (0.00043 mg L^{-1}). This is because the administration of nitrifying bacteria 4 mL 80 L^{-1} provides the optimal density, so that the nitrification process becomes faster and the ammonia concentration decreases faster than in other treatments (Figure 3).



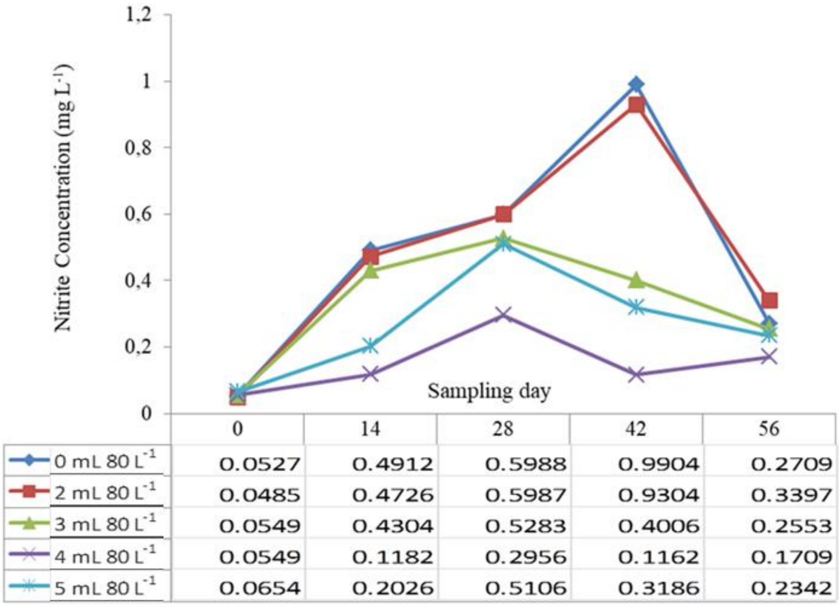
	0	14	28	42	56
0 mL 80 L^{-1}	0.0578	0.0042	0.0009	0.0006	0.0009
2 mL 80 L^{-1}	0.0438	0.0040	0.0001	0.0007	0.0008
3 mL 80 L^{-1}	0.0613	0.0012	0.0002	0.0008	0.0006
4 mL 80 L^{-1}	0.0473	0.0033	0.0002	0.0009	0.0004
5 mL 80 L^{-1}	0.0543	0.0012	0.0001	0.0004	0.0006

Figure 3. Average concentration of ammonia in all treatments during the study.

The average ammonia concentrations in all treatments during the study (0.00043–0.06130 mg L^{-1}) was still in the range that could support the growth and survival of *C. macropomum*, in line with the statement of Royan et al (2019) who reported that lethal concentrations (LC50) of TAN ranged from 1.10 to 22.8 mg L^{-1} for invertebrates and from 0.56 to 2.37 mg L^{-1} for fish, within 24-96 hours after exposure. Based on this, it was concluded that the administration of nitrifying bacteria as starter bacteria in bioballs could reduce the concentration of ammonia in the culture media. The administration of nitrifying bacteria KP-SUPER N 4 mL 80 L^{-1} gave the best reduction in ammonia concentration.

The range of nitrite (NO_2) in the administration of KP-SUPER N 0 mL 80 L^{-1} and 2 mL 80 L^{-1} was relatively not different, but significantly different from the treatments of 3 mL 80 L^{-1}, 4 mL 80 L^{-1} and 5 mL 80 L^{-1}, from day 14 to day 42. On day 56, the nitrite concentration was relatively the same in all treatments. The lowest average nitrite concentration was found in the 4 mL 80 L^{-1} treatment. This was because the bacterial density was in the optimal range to accelerate the process of converting nitrite to nitrate (Figure 4). The highest nitrite content in the culture media was found on day 42, for all treatments, except for 3 mL 80 L^{-1}, where the peak occurred on day 28, then it decreased until day 56 suggesting that the density of nitrifying bacteria accelerated the decomposition of nitrite into nitrate optimally. According to Wahyuningsih & Gitarama (2020), the decomposition of organic matter (food residue and feces) will produce ammonia, which will then diffuse from the sediment to the water column. Bacteria will

convert ammonia into nitrite and then into harmless nitrate, through a nitrification process. Nitrite is a temporary form before turning into nitrate. Nitrite is toxic to fish: the concentration level of 16 mg L^{-1} is the lethal dose, 1-5 mg L^{-1} is already dangerous and the safety limit is maximum 1 mg L^{-1} (Siikavuopio & Saether 2006).



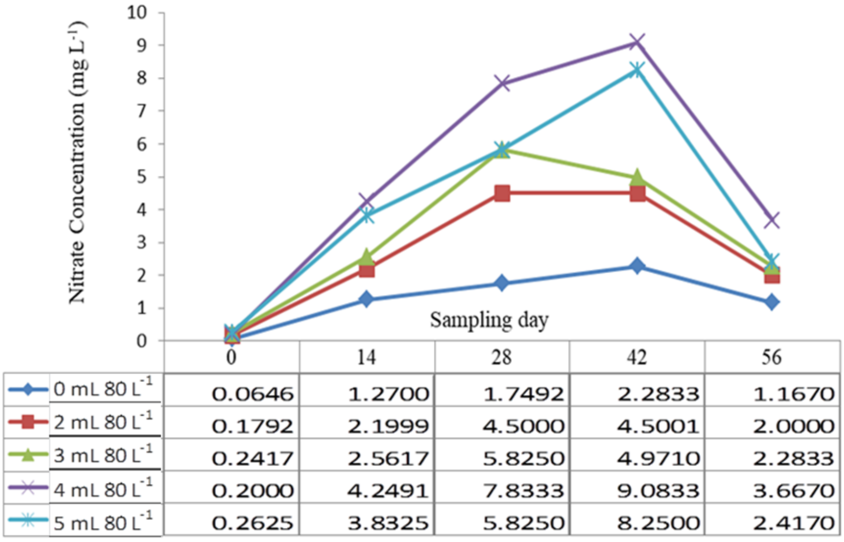
	0	14	28	42	56
◆ 0 mL 80 L^{-1}	0.0527	0.4912	0.5988	0.9904	0.2709
■ 2 mL 80 L^{-1}	0.0485	0.4726	0.5987	0.9304	0.3397
▲ 3 mL 80 L^{-1}	0.0549	0.4304	0.5283	0.4006	0.2553
× 4 mL 80 L^{-1}	0.0549	0.1182	0.2956	0.1162	0.1709
✳ 5 mL 80 L^{-1}	0.0654	0.2026	0.5106	0.3186	0.2342

Figure 4. The content of nitrite (NO_2) in all treatments during the study.

In Figure 4, the highest nitrite content in the culture media was found on day 42, for all treatments, except for 3 mL 80 L^{-1}, where the peak occurred on day 28, then it decreased until day 56 suggesting that the density of nitrifying bacteria accelerated the decomposition of nitrite into nitrate optimally. According to Wahyuningsih & Gitarama (2020), the decomposition of organic matter (food residue and feces) will produce ammonia, which will then diffuse from the sediment to the water column. Bacteria will convert ammonia into nitrite and then into harmless nitrate, through a nitrification process. Nitrite is a temporary form before turning into nitrate. Nitrite is toxic to fish: the concentration level of 16 mg L^{-1} is the lethal dose, 1-5 mg L^{-1} is already dangerous and the safety limit is maximum 1 mg L^{-1} (Siikavuopio & Saether 2006).

As observed from Figure 5, the range of nitrate in treatments 0, 2, 3, 4 and 5 mL 80 L^{-1} on day 1 was relatively the same, then it continued to increase until its peak on day 42 for treatments 0 and 2 mL 80 L^{-1}, while for 34 and 5 mL/80 L the highest concentration of nitrate occurred on day 28. This was due to the highest bacterial density in treatments 3, 4 and 5 mL 80 L^{-1} compared to the other treatments, so that the conversion of nitrite to nitrate took place more quickly. The 4 mL 80 L^{-1} treatment, whose nitrate concentration was 9.0833 mg L^{-1} (Figure 5), was significantly different from the other treatments. The addition of starter bacteria containing *Nitrosomonas* and *Nitrobacter* bacteria accelerates the decomposition process of ammonia into nitrate which is not harmful to fish.

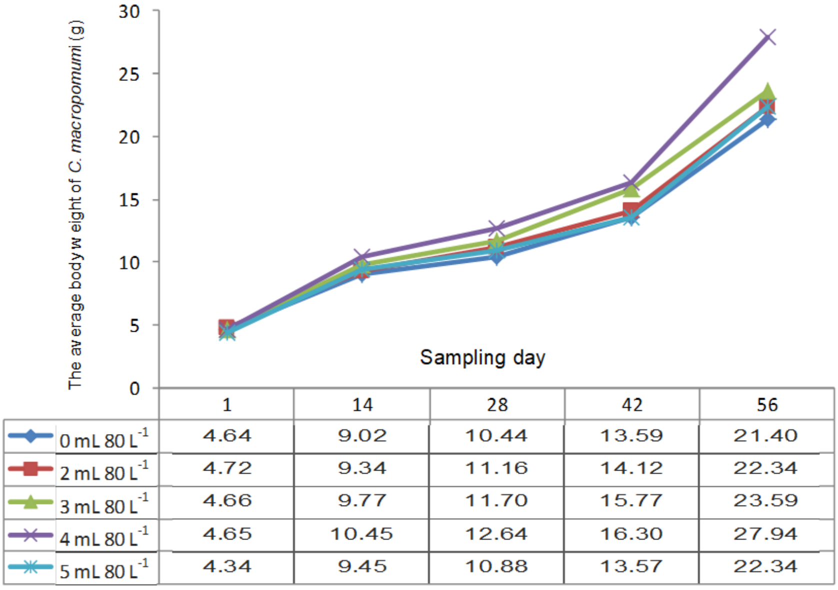
The range of nitrate in all treatments was good, in line with the opinion of Effendi (2014), who stated that a nitrate content of 0–1 mg L^{-1} was included in the oligotrophic (low) category, and 1–5 mg L^{-1} was included in the mesotrophic (moderate) category. According to Ulqodry et al (2010), a normal nitrate content in marine waters generally ranges from 0.01–50 mg L^{-1}. Furthermore, Hartoko (2010) reported that algae, especially phytoplankton, can grow optimally at a nitrate content of 0.009–3.5 mg L^{-1}. Nitrate levels obtained during the study are still in accordance with the quality standards that refer to the Government Regulation no. 82 of 2001, which sets the maximum threshold at 20 mg L^{-1}. Widayat (2010) stated that nitrate compounds resulting from the nitrification process are utilized by aquatic organisms and plants in the biosynthesis process which will produce organic nitrogen.



	0	14	28	42	56
0 mL 80 L^{-1}	0.0646	1.2700	1.7492	2.2833	1.1670
2 mL 80 L^{-1}	0.1792	2.1999	4.5000	4.5001	2.0000
3 mL 80 L^{-1}	0.2417	2.5617	5.8250	4.9710	2.2833
4 mL 80 L^{-1}	0.2000	4.2491	7.8333	9.0833	3.6670
5 mL 80 L^{-1}	0.2625	3.8325	5.8250	8.2500	2.4170

Figure 5. Fluctuations in nitrate (NO_3) concentration in all treatments during the study.

Growth performance of C. macropomum. Fish growth can occur if the maintenance is carried out by providing nutritional feed that exceeds the need for the maintenance of live fish. The quality of the maintenance media that is suitable for fish life will be able to supply feed for the production of the energy necessary for the maintenance of fish life. The provision of nitrifying bacteria on the bioball filter is intended to improve the quality of the rearing media so that it can support fish growth and development. The average absolute weight growth of *C. macropomum* is presented in Figure 6.

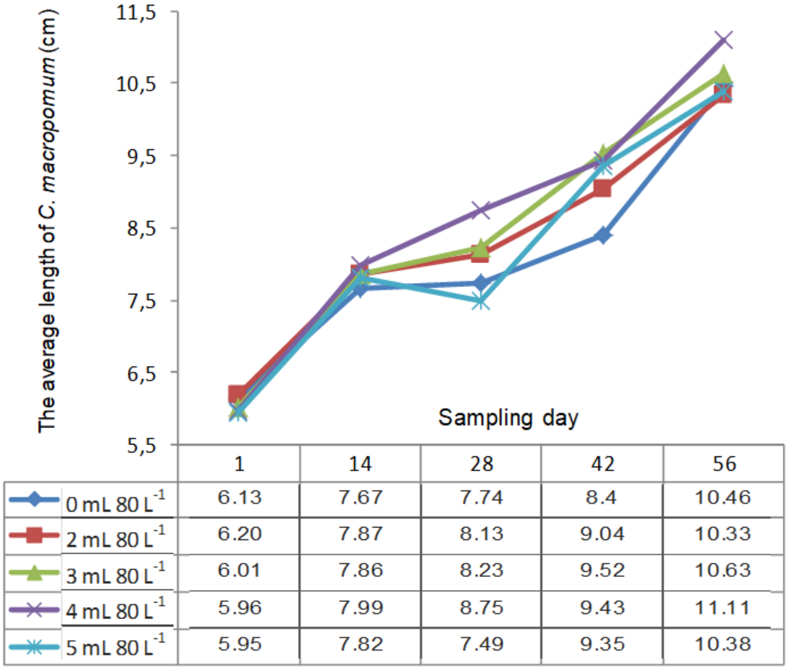


	1	14	28	42	56
0 mL 80 L^{-1}	4.64	9.02	10.44	13.59	21.40
2 mL 80 L^{-1}	4.72	9.34	11.16	14.12	22.34
3 mL 80 L^{-1}	4.66	9.77	11.70	15.77	23.59
4 mL 80 L^{-1}	4.65	10.45	12.64	16.30	27.94
5 mL 80 L^{-1}	4.34	9.45	10.88	13.57	22.34

Figure 6. Average growth of *Colossoma macropomum* in all treatments during the study.

Figure 6 shows the average weight of *C. macropomum* increased from day 1 to day 28, then from day 28 to day 56 it increased significantly. The highest average weight growth was found in the 4 mL 80 L^{-1} treatment and the lowest at 0 mL L^{-1}. The average weight of *C. macropomum* in the filter given the nitrifying bacteria KP-SUPER N was higher than without the nitrifying bacteria. This is because KP-SUPER N bacteria, as starter bacteria in the bioball filter, serves to help accelerating the process of decomposition of toxic ammonia into nitrate which is a source of nutrients, thus maintaining water quality within a good range for fish growth. A good water quality will be able to maintain the appetite and survival of the fish. By reducing the fish's energy to maintain its body, more energy can be used for growth.

The shape of growth of the average length of *C. macropomum* was similar to the shape of growth of the absolute weight, which was higher with KP-SUPER N nitrifying bacteria into the bioballs than without. The average length growth of *C. macropomum* in all treatments during the study can be seen in Figure 7. The growth of the average length of *C. macropomum* experienced a different increase in each treatment. The increase in the length of *C. macropomum* from day 1 to day 14 was not that significant and it was relatively the same in all treatments. The length of *C. macropomum* increased significantly from day 14 to day 56. Administration of nitrifying bacteria KP-SUPER N on bioballs resulted in a higher average length of *C. macropomum* compared to control (0 mL 80 L^{-1}). The highest length growth rate, of 11.1 cm, was found in the 4 mL 80 L^{-1} treatment. According to Said (2002), the analysis of the relationship between the length and weight of a fish population (by predicting the weight of a type of fish from its length) is useful for determining the biomass of the fish population.



	1	14	28	42	56
0 mL 80 L^{-1}	6.13	7.67	7.74	8.4	10.46
2 mL 80 L^{-1}	6.20	7.87	8.13	9.04	10.33
3 mL 80 L^{-1}	6.01	7.86	8.23	9.52	10.63
4 mL 80 L^{-1}	5.96	7.99	8.75	9.43	11.11
5 mL 80 L^{-1}	5.95	7.82	7.49	9.35	10.38

Figure 7. Average length growth of *Colossoma macropomum* in all treatments during the study.

Growth is influenced by several factors, namely internal and external factors, internal factors include heredity, disease resistance and ability to utilize food, while external factors include physical, chemical and biological characteristics of waters and food. The application of KP-SUPER N bacteria to the bioball filter is intended to improve the quality of the physical, chemical and biological parameters of the culture media. Improving water quality will reduce the use of energy from feed for the body maintenance, leaving more energy for growth. The measurement results for the absolute growth weight, absolute growth length, specific growth rate, feed efficiency, feed conversion and survival rate of *C. macropomum* in all treatments, during the study, are presented in Table 4.

The application of nitrifying bacteria to the bioball filter affected the growth of absolute growth weight, absolute growth length, specific growth rate, feed efficiency and feed conversion of *C. macropomum*, but had no effect on its survival. During the study, the survival of *C. macropomum* was 100% in all treatments, due to the use of a recirculation system, so that the water quality could be maintained within the appropriate range for the life of *C. macropomum.*

Table 4

Growth in absolute growth weight, absolute growth length, specific growth rate, feed efficiency, feed conversion and survival of *Colossoma macropomum* in all treatments during the study

Parameters	*Addition of KP-SUPER N nitrifying bacteria*				
	0 mL 80 L^{-1}	*2 mL 80 L^{-1}*	*3 mL 80 L^{-1}*	*4 mL 80 L^{-1}*	*5 mL 80 L^{-1}*
Wm (g)	16.76 ± 0.15^{a}	17.62 ± 0.56^{ab}	18.93 ± 0.81^{b}	23.29 ± 1.41^{c}	18.00 ± 0.41^{ab}
Lm (cm)	4.33 ± 0.24^{ab}	4.12 ± 0.22^{a}	4.61 ± 0.14^{b}	5.14 ± 0.05^{c}	4.42 ± 0.06^{ab}
LPS (%)	3.04 ± 0.02^{a}	3.08 ± 0.01^{ab}	3.13 ± 0.06^{b}	3.30 ± 0.06^{c}	3.08 ± 0.02^{ab}
EP (%)	79.46 ± 1.20^{a}	79.96 ± 1.83^{a}	80.05 ± 4.70^{a}	93.83 ± 5.72^{b}	84.20 ± 2.83^{a}
FCR	1.26 ± 0.02^{b}	1.25 ± 0.03^{b}	1.25 ± 0.05^{b}	1.07 ± 0.06^{a}	1.19 ± 0.04^{b}
SR (%)	100	100	100	100	100

Wm-absolute weight growth; Lm-absolute length growth; LPS-specific growth rate; EP-feed efficiency; FCR-feed conversion; SR–survival.

The results of ANOVA showed that absolute weight growth, absolute length, specific growth rate, feed efficiency and feed conversion of *C. macropomum* were significantly different for the administration of KP-SUPER N nitrifying bacteria of 4 mL 80 L^{-1}, than in other treatments. This is because the number of nitrifying bacteria added to the bioball filter in this treatment was able to provide an optimal living medium for bacteria, characterized by the lowest ammonia levels and the highest nitrate concentrations, compared to other treatments. The low value of ammonia in the rearing media can increase the appetite of *C. macropomum*, while the high concentration of nitrate can stimulate the growth of plankton in the media. This higher availability of natural food for the cultured fish determined a faster weight growth to than in other treatments. This is in line with the opinion of Azhari & Tomasoa (2018), who stated that the well maintained water quality of fish rearing media can increase fish appetite, reducing the stress level and supporting an optimal growth.

Conclusions. The results showed that the best treatment was the application of KP-SUPER N nitrifying bacteria at a concentration of 4 mL 80 L^{-1} to the bioball filter of *C. macropomum* rearing media, generating a bacterial density of 1.857±0.022 X 108 CFU mL^{-1}, with a nitrogen absorption efficiency of 91.71±5.48%, for a temperature ranging from 26–29.7^{0}C, a pH of 6–7.5, a DO of 5.4–5.5 mg L^{-1}, determining levels of ammonia between 0.00011 and 0.05430 mg L^{-1}, nitrite levels between 0.0549 and 0.2956 mg L^{-1} and nitrate levels between 0.2000 and 9.0833 mg L^{-1}, and consequently an absolute weight growth of 23.29±1.41 g, an absolute length growth of 5.14±0.05 cm, a specific growth rate of 3.30±0.06%, a feed efficiency of 93.83±5.72%, a feed conversion rate of 1.07±0.06 and a survival rate of 100%. It can be concluded that applying KP-SUPER N nitrifying bacteria in the bioball filter positively affects the growth performance of *C. macropomum.*

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