AGROLAND: The Agricultural Sciences Journal
Vol. 7, No. 1 June (2020), -
P-ISSN : 2407- 7585 & E-ISSN : 2407- 7593, Published by Tadulako University

Original Research **Open Access**

ADDITION OF DIFFERENT CARBON SOURCES TO TOTAL DENSITY OF BIOFLOK BACTERIA IN THE MEDIA OF TIGER SHRIMP (*Penaeus monodon*)

Nasmia[1)], Abdul Rifai[1)]

[1)] majors of Aquaculture, Faculty of Animal husbandry and fisheries, University of Tadulako

Correspondence author's: Nasmia
Email : nasmia68@gmail.com

ABSTRACT

This research was conducted to assess different carbon sources' addition to heterotrophic bacteria's density in tiger prawn rearing media (Penaeus monodon). The research design used was a completely randomized design (CRD) consisting of 4 treatments and five replications with experimental treatments, namely: A (without giving a carbon source), B (carbon source molasses with a C/N ratio of 15: 1), C (source flour carbon with a C/N ratio of 15: 1) and D (a source of tapioca carbon with a C / N ratio of 15: 1). The test organism used was tiger shrimp PL-21 with a weight of ± 0.005 g, which was maintained with a stocking density of 100 fish / 50 liters of water for 30 days. During the maintenance of shrimp, it is given pellet type feed with a frequency of three times a day, as much as 3% of the shrimp biomass. Providing bacterial activation is carried out every ten days with organic carbon sources every day in the morning and evening. The results showed that the group of microorganisms identified as Bacillus sp. diatoms, ciliates, copepods, Paramecium sp., Synedra sp., and nematodes. Providing tapioca carbon sources resulted in the highest bacterial density (9.0 × 10⁷ CFU ml). In comparison, the highest floc volume was produced by treatment with the addition of flour carbon sources (3.65 ml/l).

Keywords: bacteria, biofloc, Penaeus monodon, TPC.

INTRODUCTION

Penaeid shrimp is a leading commodity that is being developed by the Ministry of Marine Affairs and Fisheries. One of the penaeid shrimp directed at increasing production is a tiger shrimp (*Penaeus monodon*) (Sukenda et al., 2011). The Ministry of Marine Affairs and Fisheries targets shrimp production to increase from 642,000 tons in 2017 to 700,000 tons in 2018 (KKP, 2018). The ministry's effort to increase the amount of shrimp production is by applying intensive cultivation technology. However, intensive system technology raises problems, including increasing cultivation waste (Ekasari, 2009).

Intensive system cultivation technology is applied to optimize feed use and stocking density. However, the feed that the shrimp does not use can accumulate in the water. Shrimp only use 20-30% of feed nutrients, and the rest accumulates in the water (Elfidiah, 2016). Shrimp culture waste load in the form of leftover feed, excretion, and feces can reach 39-40% at the level of feed conversion ratio (FCR) 1.69-2.14 and will continue to increase with increasing shrimp productivity (Syah et al., 2006). According to Harowitz (2000) in Sakdiah (2009), the total amount of feed (100%) given is not all used by shrimp; 17% of the feed will be used for growth; 20% is excreted through urine and feces; 48% is excreted during the molting process, and 15% are not consumed. Increasing the density of shrimp and the amount of feeding will increase the value of total ammonia nitrogen (TAN) in the culture environment (Sakdiah, 2009). Handling in handling aquaculture waste needs to be done to support the creation of aquatic organism cultivation activities that are environmentally friendly and sustainable. Biofloc technology is an alternative to solve the problem of cultivation waste because it can suppress inorganic nitrogen compounds by heterotrophic bacteria (Crab et al., 2007).

Biofloc technology uses heterotrophic bacteria to utilize organic and inorganic N in water (Schryver et al., 2008). Increasing the number of heterotrophic bacteria can reduce total ammonia-nitrogen, nitrite, and nitrate in the media on both a laboratory and field-scale (Mara, 2004). According to Ekasari (2009), heterotrophic bacteria produced at high-density function as bioreactors to control water quality, especially N's concentration, and as a protein source for the organisms being maintained.

The formation of biofloc is strongly influenced by heterotrophic bacterial populations' development in the culture medium (Usman et al., 2011). According to Crab et al. (2007), biofloc can be formed by adding an organic carbon source in the culture medium to stimulate heterotrophic bacteria's growth. Different organic carbon sources resulted in different bacterial densities, namely, 4.67 × 106 CFU/ml from carbon molasses and 2.59 × 10^6 CFU/ml from tapioca starch carbon. The difference in the level of abundance of bacteria is caused by differences in the carbohydrates structure that make up the two ingredients (Purnomo, 2012). Several carbon sources that have been used in biofloc technology applications include molasses in white leg shrimp (Dahlan et al., 2017), tapioca flour in tiger prawns (Gunarto et al., 2010), and wheat flour in tiger prawns (Megahed, 2010). The application of adding different carbon (molasses, tapioca, and flour) to the media for raising tiger prawns (Penaeus monodon) to see the three carbon sources has not existed before. Based on this description, the selection of different carbon sources in this study is expected to provide information about the types of microorganisms, including bacteria and the total plate count present in the biofloc.

MATERIALS AND METHODS

Test Organism

The test organism was the tiger prawn (Penaeus monodon) PL-21 with a weight of ± 0.005 g. Shrimp are reared with a stocking density of 100 heads/container (2 heads/liter of water) (Muliani et al., 2011). The seeds used from the BPBAP Takalar Hatchery.

Research design

The study was designed using a completely randomized design with four treatments and five replications to obtain 20 experimental units. The treatments used were:

A: Without giving a carbon source
B: Providing a carbon molasses source
C: Providing a carbon source for wheat flour
D: Providing a carbon source for tapioca flour

Work procedures

The containers used in this study were 20 units of the bucket (container for rearing organisms) with 80 liters and a tray (bioreactor) with a capacity of 5 liters of 4 units. Each rearing container is filled using 50 liters of seawater.

Shrimp are kept for 30 days with a stocking density of 100 birds/container. The feeding frequency is carried out three times a day at 08.00, 16.00, and 24.00 WITA with a feed dose of 3% of the shrimp biomass (Lante et al., 2015). Shrimp sampling is done every seven days.

The application is made by mixing several materials such as carbon, BIO-S probiotic with a bacterial concentration of 2×10 CFU/g, and seawater into the reactor container. All the ingredients are then stirred until they are homogeneous and let stand for 12 hours under aerated conditions.

The dosage used for each material is a source of carbon (molasses, flour, and tapioca) according to the calculation of C/N 15: 1, probiotics as much as 250 mg/container (0.005 g/l) (Zulfahmi, 2017) and seawater as much as 1000 ml/container reactor.

The addition of daily carbon sources is carried out every day with a ratio of C / N ratio of 15:1. The amount of carbon added to support the growth process of heterotrophic bacteria in each treatment was calculated using the formula (Avnimelech, 1999):

$$\Delta CH = \frac{(P \times \%\,NP \times \%\,N) \times (C/N)}{\%C \times E}$$

ΔCH : Amount of carbon added
C/N : The required C / N ratio
% C : The carbon content of a carbon source added
E : Microbial conversion efficiency
P : The amount of feed given
% NP : N content in the feed
% N : N content released by the shrimp body

Observation of the floc profile on the maintenance medium was carried out at the end of the study by observing the biofloc sample directly under a microscope with a magnification of 100-400×, and the resulting floc images were recorded using a digital camera.

The measurement of the bacterial population was carried out every ten days. The total bacteria test is carried out when the bacteria enter the exponential phase by taking a 1 ml water sample. Furthermore, doing serial dilutions 10^{-1} to 10^{-6}, the results of dilution 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were grown in duplicate plate count agar (PCA) medium and incubated at 28-30 °C for 24 hours. The growing population was determined in the colony-forming unit (CFU), which is calculated using the formula (Indonesian National Standard Agency, 2015):

$$N = \frac{\sum C}{n \; x \; (p)}$$

N : Number of colonies (CFU / ml)
∑C : Number of colonies on all of the plates calculated
n : Number of cups
(*p*) : Dilution factor

Floc volume measurements were carried out once every ten days by taking a 1000 ml water sample, then depositing it for 30 minutes in an *imhoff* cone tube. The volume of the floc that settles is recorded and then calculated using the formula (Apriani, 2015):

$$\text{Floc volume} = \frac{\text{Sediment volume}}{\text{Water sample volume}} \times 1000 \text{ ml}$$

Data analysis

The data obtained during the study were processed using Microsoft Excel 2010 and analyzed descriptively. The results are presented in tabulated and graphic form.

RESULTS AND DISCUSSION

Flok Profile

Flocculation is a part of the biofloc formation process. The floc that had formed was observed using a microscope with magnifications of 100x and 400×. Under the microscope, all treatments using a carbon source showed a clear attraction between the detritus with bacteria (Figure 1a) and the nematode and the surrounding organic matter (Figure 1b). Presumably, because of the ability of the bacteria Bacillus sp. can bind particles to form biofloc and the polymers that make up the carbon source used contain viscous polysaccharides and monosaccharides, causing carbon associations with other microorganisms to bind to one another. According to Gunarto and Suwoyo (2011), one of the bacteria that can form biofloc is Bacillus sp. It can synthesize polyhydroxy alkanoate (PHA) compounds poly beta-hydroxybutyrate, which functions as a polymer bond between floc-forming substances. Suazo (2006) states that polymer components are the main means of aggregating individual bacterial cells to form floc particles. Biopolymers are generally charged with negative ions, which are essential structural components of bonding agents. cations, ionic strength, and the number of particles present in the maintenance medium.

Microscopic examination showed that the biofloc was composed of several microorganisms: *Bacillus* sp., Ciliata, Nematodes, *Paramecium* sp., Copepoda, Diatoms, and *Synedra* sp. The observation results are listed in Table 1 and Figure 2.

The results of the observations on the types of microorganisms obtained were not much different in all treatments, both in the control treatment and carbon sources (Table 1). The slight differences regarding the types of microorganisms in all treatments were caused by limiting factors for each of these microorganisms so that the communities encountered were microorganisms that could withstand changes and environmental conditions. According to Sulistiowati et al. (2016), the factors that influence microorganisms' presence in waters are strongly influenced by abiotic and biotic factors. Abiotic factors include water temperature, conductivity, current, turbidity, light, pH, salinity, Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD). Biotic factors include competition for food and interactions between other organisms. This study shows that the provision of carbon sources only serves as a food source and cannot affect the diversity of these microorganisms, increasing the number of microorganism populations when viewed from the volume of floc formed.

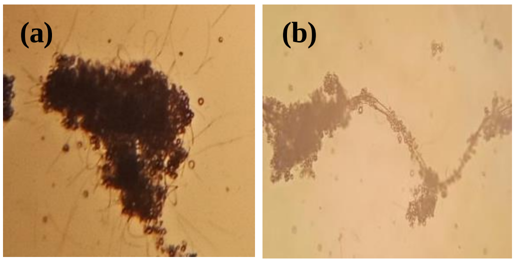

Figure 1. Biofloc under the microscope: (a) biofloc particles flocculate with detritus and bacteria; (b) flocculating biofloc with nematodes (magnification 100×)

Table 1. Biofloc Constituent Microorganisms.

Microorganisms	Treatments			
	(A) Control	(B) Molasses	(C) Wheat	(D) Tapioca
Bacillus sp	+	+	+	+
Ciliata	+	+	+	+
Nematoda	+	+	+	+
Paramecium sp.	+	+	+	+
Copepoda	-	-	+	+
Diatom	+	+	+	+
Synedra sp.	-	+	-	-

Note: (+) = presence; (-) = absence

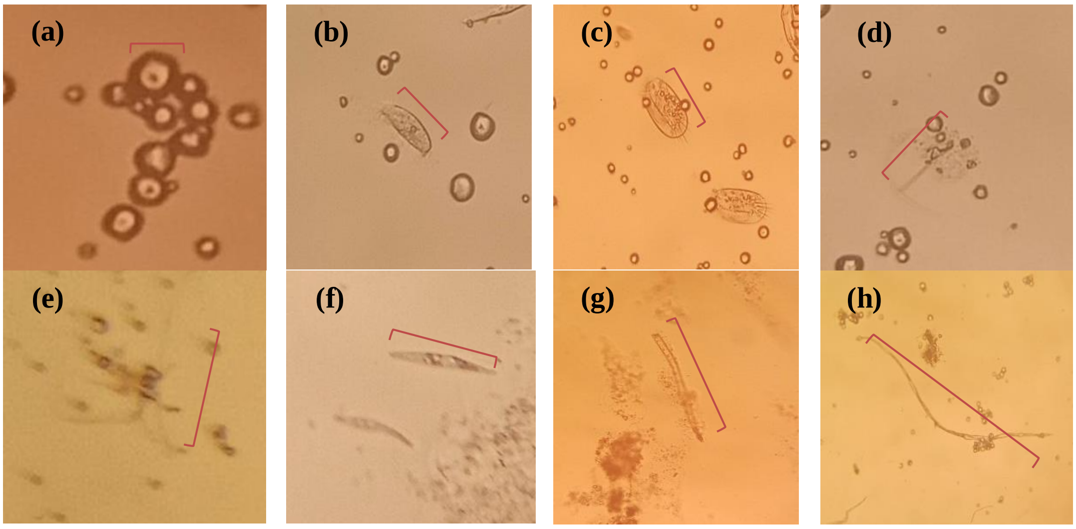

Figure 2. Microorganisms identified in the flock: (a) diatoms; (b and c) ciliates; (d and e) copepods; (f) *Paramecium* sp.; (g) *Synedra* sp. (h) nematodes (magnification 400×)

Based on these results, bacteria and other groups of microorganisms such as diatoms, ciliates, copepods, *Paramecium* sp., *Synedra* sp. and nematodes are a collection of biofloc constituent microorganisms in this study. Bacteria, diatoms, nematodes, *Paramecium* sp., and *ciliata* were the most frequently encountered microorganisms in all treatments. The presence of these microorganisms has its respective benefits and functions.

While microalgae or phytoplankton were not found in all treatments, this is thought to be influenced by the research location conditions, which is in the room not to get enough light. The lack of penetration of light entering the aquatic environment is a limiting factor for microalgae in carrying out the photosynthesis process. According to Handayani and Ariyanti (2012), photosynthesis is needed by microalgae to produce their food.

Apart from being influenced by light factors, it is suspected that the addition of organic carbon sources in the cultivation medium can suppress microalgae growth. Microbes will utilize organic carbon sources to increase their population to become natural competitors by microalgae, where this phenomenon is precisely like the flow in the food chain. Some microorganisms such as ciliates and copepods will use microalgae as their food source and the nematodes that occupy the top of the microscopic organisms' food chain. Directly proportional to the research results by Burford et al. (2004), who showed that the addition of carbon sources to the cultivation system could increase microbial growth than microalgae. According to Nasution (2009), microbes are generally heterotrophic, so they cannot produce organic compounds from inorganic compounds themselves for food. For their survival, microbes are highly dependent on organic compounds from other organisms such as phytoplankton for food.

Bacterial Population

The number of bacterial colonies in the tiger shrimp culture medium (Penaeus monodon) was calculated using the total plate count (TPC) method. TPC measurements were carried out every ten days interval according to the bacterial activation schedule. The average total number of bacteria from the effect of carbon sources is shown in Table 2.

The results of TPC measurement showed that the highest bacterial abundance was produced by tapioca treatment, while the lowest bacterial abundance was found in molasses treatment. The growth pattern of bacteria in all treatments fluctuated. The number of bacterial colonies in all treatments at H-0 to H-10 tended to increase. The increase in the number of colonies showed that bacteria could use organic matter to increase their population. Furthermore, when entering H-20, the total bacteria in the control treatment and molasses decreased, except for the flour and tapioca treatment, which increased significantly. The total bacteria in all treatments resulted in a density ranging from 10^5-10^7 at the end of the study. A decrease in organic matter can cause a decrease in the number of bacterial colonies. The utilization of organic material is carried out by converting organic material by bacteria to form new bacterial cells. According to Apriadi (2008), the formation of new bacteria can lead to competition between bacteria for food. Bacteria that cannot compete will lack food and eventually die. These dead bacteria will then settle on the bottom of the waters or accumulate with organic matter in these waters.

It can be seen that the total bacteria in the control treatment is higher than the treatment with the addition of molasses (Table 2). The total bacteria in the control treatment can exceed the molasses treatment, presumably due to the availability of naturally available organic material. This happened because the probiotics that were also given to the control treatment could take advantage of the accumulation of organic matter from leftover feed and shrimp feces in the culture medium. According to Putro et al. (2014), organic matter in the cultivation environment may be available accidentally due to cultivation activities during the maintenance period. This type of organic material can come from unfeed pellets and biological activity (feces). Thus most of the protein in the feed will be wasted as organic material (Paena et al., 2017). The accumulation of organic matter was thought to be used by bacteria to increase their control treatment population.

Table 2. Mean Total Bacteria in Each Treatment

Treatment	Total Bacteria/10 days (CFU/ml)				Mean
	0	10	20	30	
(A) Control	$3{,}5 \times 10^6$	$9{,}3 \times 10^6$	$7{,}2 \times 10^5$	$2{,}4 \times 10^7$	$9{,}4 \times 10^6$
(B) Mollases	$6{,}5 \times 10^5$	$9{,}5 \times 10^6$	$1{,}9 \times 10^5$	$8{,}1 \times 10^5$	$2{,}7 \times 10^6$
(C) Wheat	$6{,}6 \times 10^5$	$2{,}6 \times 10^7$	$6{,}9 \times 10^7$	$1{,}1 \times 10^7$	$2{,}6 \times 10^7$
(D) Tapioca	$4{,}9 \times 10^5$	$4{,}1 \times 10^7$	$2{,}8 \times 10^8$	$3{,}1 \times 10^7$	$9{,}0 \times 10^7$

Furthermore, the low level of bacteria in the molasses treatment compared to the control treatment is thought to be influenced by the carbon molasses' carbohydrate structure. Molasses is a simple organic carbon from glucose, which is composed of monosaccharides. The glucose content is thought to cause the lack of bacterial populations in the molasses treatment. According to Leepel et al. (2009), the higher the concentration of glucose added to the media will increase bacterial colonies' growth. In contrast to Mahae et al. (2011), simple sugar or glucose has antibacterial compounds (bactericides) that can inhibit bacterial growth. Bacteria from the genus Bacillus added to this study are a group of Gram-positive bacteria. According to Hatmanti (2000), the bacteria Bacillus sp. classified into Gram-positive heterotrophic bacteria. The minimum inhibitory level (MIC) test results on glucose have a more significant potential for Gram-positive bacteria (Purnama, 2013).

These results indicate that the number of heterotrophic bacteria can be increased with the addition of organic carbon sources and the highest is produced by complex carbon from tapioca. Organic carbon that is successfully utilized can stimulate and maximize bacterial growth. The same results were shown by the research of Sukendar et al. (2016) that the use of tapioca carbon sources produced the highest total bacteria (10^7 CFU / ml) compared to other carbon sources. The addition of a carbon source to the biofloc media was able to increase the total bacteria. The resulting increase can reach 10^6-10^7 (Kim et al., 2014).

Although tapioca treatment produced the highest bacterial population average, all treatments' average value also showed good results to support shrimp farming activities. According to Avnimelech (2009), a good flock is composed of a large number of bacterial populations ($\geq 10^6$ CFU / ml), while a less good flock has a low bacterial population ($\leq 10^3$ CFU / ml).

Another factor influencing bacterial abundance is the carbohydrate structure of the carbon source used. The type of carbon used in this research is a group of simple carbon (molasses) composed of monosaccharides and complex carbon (flour and tapioca) which are composed of polysaccharides from starch, agarose and cellulose. According to Azhar (2013), carbohydrates from simple carbon are easier to use by bacteria as an energy source, while bacteria can also utilize complex carbon, but it takes a longer time to utilize the breakdown process. Generally, simplification of complex carbon so that it can be utilized can be pursued more quickly if using the right type of bacteria. Suitable bacteria can speed up the process of breaking down complex compounds to become more straightforward.

Heterotrophic bacteria from the genus Bacillus added in this study are thought to be able to hydrolyze complex compounds to become more straightforward so that bacteria can be used more quickly to increase their population. According to Zahidah and Shovitri (2013), Bacillus bacteria isolates have amylolytic, cellulolytic, and proteolytic properties with indices of 0.93, 1.95, and 1.39, respectively. Based on its characteristics, it is confirmed that these types of bacteria are able to play a faster role in the degradation process of organic waste by hydrolyzing complex compounds into simple compounds with their enzymes (Syamsudin et al., 2008).

Probiotics in the maintenance medium contain the bacteria *Baccilus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus* which have many benefits for the organism and the culture environment. *B. subtillis*, *B. licheniformis* and *B. pumilus* bacteria have a positive role in cultivated organisms, including increasing growth, survival, digestibility, increasing the immune system and improving water quality through bioremediation processes. The application of probiotics containing *B. subtilis* and *B. licheniformis* to trout produces fast growth and is resistant to disease (Bagheri et al., 2008).

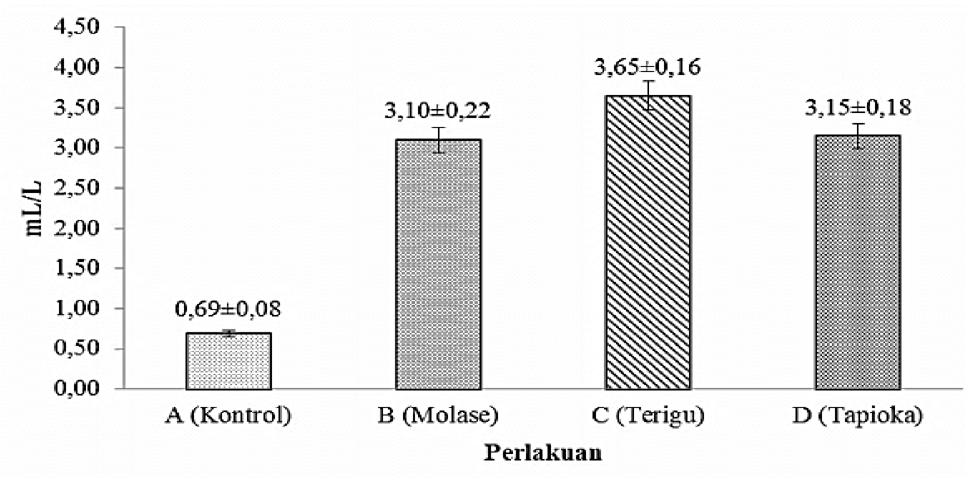

Figure 3. Floc volume in the tiger prawn rearing medium (*Penaeus monodon*): (a) control; (b) molasses; (c) flour and (d) tapioca.

Flock Volume

Floc volume was measured based on the activation time of the bacteria at intervals of 10 days. Controls such as watercolor and flock suspension are routinely carried out to see changes in the maintenance medium's water conditions. The average results of the observed biofloc volume observed for 30 days are shown in Figure 3.

The highest average was produced by flour treatment, and the lowest was in the control treatment. This study shows that the provision of organic carbon sources affects floc volume during the maintenance period. It is suspected that the high volume of floc produced by the treatment of flour is due to the carbon complex from the flour being utilized more quickly by Bacillus bacteria, thus forming and producing more floc. According to Chamberlain (2001), the use of simple carbon sources has the advantage of being easily absorbed and utilized by bacteria in adsorbing nitrogen contained in cultivation media, while the use of complex carbon sources has the advantage of being able to provide particles that can be used as a place for bacteria to stick, but in utilization takes a longer time. The utilization of complex carbon compounds can be pursued more quickly if using suitable bacteria. According to Hatmanti (2000), the bacteria Bacillus sp. has amylolytic, cellulolytic, and proteolytic enzymes, which can degrade complex organic such as protein and starch cellulose and hydrocarbons. These enzymes can accelerate the process of decomposing organic matter, increase the productivity of flocks, and improve cultivated species' digestive systems (Chamberlain, 2001).

According to Wijaya et al. (2016), the abundance of floc microorganisms can be determined through the volume of floc formed. The floc's total volume indicates the abundance of bacteria, which are the main constituent components in the biofloc. Apriani (2015) added that the high value of floc volume could be a sign that bacteria are forming better floc. This statement is inversely proportional to the TPC acquisition value (total plate count) during the observation with the highest average number of TPC produced by tapioca treatment (Table 2).

The tapioca treatment resulted in a lower floc volume (3.15 ml/l) than the flour treatment (3.65 ml/l), but produced the highest total bacteria. This could occur because the shrimp that consumed biofloc from tapioca treatment was higher than the flour treatment. This leaves a smaller number of flocks. This estimation was proven by the absolute average growth rate (0.154 g / head) and the addition of daily weight (0.005 g / head) produced by shrimp in tapioca treatment, which was higher than the other carbon treatments. According to Purnomo (2012), the high population of heterotrophic bacteria in biofloc can form better microbial proteins to support cultivated organisms' growth.

One way to prove the abundance of bacteria in the culture medium apart from TPC measurement is to measure volatile suspended solid (VSS). According to Azhar (2013), the VSS value is related to the total number of bacteria present in the waters. The increase in VSS content can indicate the rate of conversion of organic nitrogen waste by bacteria. Heterotrophic bacteria will utilize organic carbon sources to assimilate inorganic nitrogen into organic nitrogen in the form of bacterial biomass. The increase in bacterial growth will also increase the VSS value in the culture medium. The highest VSS yield was directly proportional to the statement by tapioca treatment as much as 656 mg/l.

It can be seen that the floc formed did not only occur in the treatment with the addition of a carbon source, but also in the control treatment, although in a small amount. This can occur because the balance of the ratio of carbon and nitrogen is thought to have formed naturally in the rearing media due to the accumulation of leftover feed and shrimp feces. According to Apriani (2015), during the maintenance period of aquatic organisms, the balance of carbon and nitrogen ratio can naturally form in the culture medium. The C/N ratio that occurs naturally in pond waters is ≤12 (Maharani, 2012).

Based on the average value of the flocks in all treatments, the flocks produced in this study were classified as low due to the relatively short maintenance period of 30 days. However, the maintenance period of 30 days gave positive results on the formation of biofloc. According to Avnimelech (2009), the volume of biofloc with a range of 1-10 mg / L is low, a range of 10-20 mg / L is moderate, and ≥20 mg / L is high. The ideal value of flock volume for shrimp is 5-15 mL / L. Mcintosh (2000) in Gunarto et al. (2012) stated that it takes a long time to form biofloc in a culture system. The overall change from intensive ponds with autotrophic systems to heterotrophs takes ≥2 months with a long maintenance period of 9-10 weeks.

Apart from observing floc volume, routine control of floc suspension changes and the watercolor was also carriexd out to add information about the description of biofloc formation. Visually, the biofloc began to form less than 24 hours after the addition of carbon. It is suspected that the floc encountered was the initial stage of utilizing carbon sources and accumulation of food residue by bacteria. This shows the foaming conditions on the water surface. According to Gunarto et al. (2012), the development of floc can be indicated by discovering foam on the surface of the water when it enters the transition period.

Furthermore, an overall change in the floc suspension condition occurred on day 10, where the resulting floc was small and evenly distributed in the water column. When entering the 20th day, the color of the water changes to brown in the treatment using organic carbon (molasses, flour and tapioca treatment). This continued until the end of the study. According to Ombong and Salindeho (2016), the biofloc medium water will be green if the floc is dominated by algae, while if bacteria dominate the floc, the water will be brownish. Gunarto et al. (2012) added that the difference in the color of the water produced in the biofloc system is caused by differences in the dominance of the phytoplankton components that make up the biofloc. The dominance of chlorophyll pigments (primary pigments) will change the color of the water to green and carotenoids from bacteria (secondary pigments) to brown.

CONCLUSIONS

Based on the results obtained in the study, it can be concluded that:

1. The types of microorganisms obtained in the biofloc in this study are Bacillus sp., Ciliata, Nematodes, Paramecium sp., Copepoda, Diatoms, and Synedra sp., And the main constituents are bacteria.
2. The provision of different carbon sources results in a comparison to the total plate count (TPC) in the tiger prawn rearing media. Treatment of tapioca carbon sources produced the highest TPC of 9.0×10^7 CFU / mL, and the lowest was in the molasses treatment of 2.7×10^6.

REFERENCES

Apriadi. T. Kombinasi Bakteri dan Tumbuhan Air sebagai Bioremediator dalam Mereduksi Kandungan Bahan Organik Limbah Kantin. Skripsi. Fakutas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor. 2008. Bogor.

Apriani. I. Produksi Benih Ikan Patin (*Pangasianodon hypophthalmus*) dengan Penambahan Sumber Karbon Berbeda pada Sistem Budidaya Berbasis Bioflok. Sekolah Pasca Sarjana. Institut Pertanian Bogor. 2015 Bogor.

Azhar, M. H. Peranan Sumber Karbon Eksternal yang Berbeda dalam Pembentukan Bioflok dan Pengaruhnya Terhadap Kualitas Air Serta Produksi Pada Sistem Budidaya Udang Vaname *Litopenaeus vannamei*. Thesis. Sekolah Pasca Sarjana. Institute Pertanian Bogor. 2013. Bogor.

Avnimelech, Y., Carbon/Nitrogen Ratio as A Control Element in Aquaculture Systems. Aquaculture 1999, 176: 227-235.

Avnimelech, Y., Nitrogen Control and Protein Recycling: Activated Suspension Ponds. Global Aquaculture Alliance: 2009, 24, 227-238

Badan Standar Nasional Indonesia. Penentuan Angka Lempeng Total (ALT) pada Produk Perikanan. 2015. Jakarta.

Bagheri, T., Hedayati, S.A., Yavari, V., Alizade, M., & Farzanfar, A. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turk. *J. Fish. Aquat. Sci.*,2008, 8: 43-48.

Burford, M. A., Thompson, P. J., Mcintosh, R. P., Bauman, R. H dan Pearson, D. C. The Contribution Of Flocculated Material to Shrimp (*Litopenaeus vannamei*) Nutrition in A High-Intensity, Zero-Exchange System. Jurnal Aquaculture. 2004 Vol. 232: 525-537.

Chamberlain, G. Advantages of Aeerated Microbial Reuse Syster with Balanced C:N. Jurnal Global Aquaculture Alliance: 2001 22-24.

Crab. R., Avnimelech. Y., Defoirdt. T., Bossier. P dan Vestraete. W. Nitrogen Removal Techniques in Aquaculture for a Sustainable Production. Jurnal Aquaculture. 2007 Vol. 270: 352-356.

Dahlan. J., Hamzah. M dan Kurnia. A. Pertumbuhan Udang Vaname *(Litopenaeus vannamei)* yang Dikultur pada Sistem Bioflok dengan Penambahan Probiotik. Jurnal Sains dan Inovasi Perikanan. 2017 Vol 1 (1): 19-27.

Ekasari. J. Teknologi Biotlok: Teori dan Aplikasi dalam Perikanan Budidaya Sistem Intensif. Jurnal Akuakultur Indonnesia. 2009 Vol. 8 (2): 117-126.

Elfidiah. Study Kasus Optimalisasi Tambak Udang dari Pencemaran Amoniak (NH3) dengan Metode Bioremedasi. Jurnal Distilasi. 2016 Vol. 1 (1): 57-61.

Gunarto., Muliana dan Mansyur. A. 2010. Pengaruh Aplikasi Sumber C- Karbohidrat (Tepung Tapioka) dan Fermentasi Probiotik pada Budidaya Udang Windu, *Penaeus Monodon* Pola Intensif di Tambak. Jurnal Riset Akuakultur. 2010 Vol. 5 (3): 393-409.

Gunarto dan Suwoyo, H. Produksi Bioflok dan Nilai Nutrisinya Dalam Skala Laboratorium. 2011, Prosiding Teknologi Akuakultur.

Gunarto, Suwoyo, H. S dan Tampangajio, B. R., Budidaya Udang Vaname Pola Intensif dengan Sistem Bioflok di Tambak. Jurnal Riset Akuakultur. 2012 Vol. 7 (3): 393-405.

Handayani. N. A dan Ariyanti. D. Potensi Mikroalga sebagai Sumber Biomasa dan Pengembangan Produk Turunannya. Jurnal Teknik. 2012 Vol. 3 (22): 58-65.

Hatmanti. A. Pengenalan *Bacillus* sp. Jurnal Oseana. 2000 Vol 25 (1): 31-41.

Kementrian Kelautan dan Perikanan (KKP). Menuju *Transformasi* Budaya Maritim. 2018, Buku Laut Kita. kkp.go.id

Kim S.K., Pang Z, Seo H.C., Cho Y.R., Samocha T. Effect of bioflocs on growth and immune activity of pasific white shrimp, Litopenaeus vannamei postlarvae. Aqua- culture Research, 2014 45(2): 362-371.

Lante, S., Usman dan Laining, A., 2015. Pengaruh Kadar Protein Pakan Terhadap Pertumbuhan dan Sintasan Udang Windu, *Penaeus monodon* Fab.Transveksi. Jurnal Perikanan. 2015 Vol. 18 (1): 10-17.

Leepel, L. A., Hidayat, R., Puspitawati, R dan Bahtiar, B. M. Efek Penambahan Glukosa pada Saburoud Dextrose Broth Terhadap Pertumbuhan *Candida Albicans*(Uji *In Vitro*). Jurnal Indonesia Dentistry. 2009 Vol. 16 (1): 58-63.

Mahae, N., Chalat, C dan Muhamud, P. Antioxidant and Antimicrobial Properties Of Chitosan-Sugar Complex. Jurnal International Food Research 2011 Vol. 18 (4): 1543-1551.

Maharani. F. Aplikasi Teknologi Bioflok Pada Pemeliharaan Benih Ikan Nila (*Oreochromis niloticus*). Thesis. Program Pascasarjana. Universitas Terbuka. 2012, Jakarta.

Mara, D., 2004. Domestic waste water treatment in developing countries. Earthscan. 2004, UK. 293p.

Megahed. M. E. The Effect of Microbial Biofloc on Water Quality, Survival and Growth of the Green Tiger Shrimp (*Penaeus Semisulcatus*) Fed with Different crude Protein Levels. Jurnal Arabian Aquaculture Society. 2010 Vol. 5 (2): 119-143.

Muliani., Nurbaya dan Madeali. I. Teknik Aplikasi Bakteri Probiotik pada Pemeliharaan Udang Windu (*Penaeus monodon*) di Laboratorium. Jurnal Riset Akuakultur. 2011 Vol. 6 (1).

Nasution, A. Analisis Ekologi Ikan Kurau, *Eleutheronema tetradactylum* (Shaw, 1804) pada Perairan Laut Bengkalis, Propinsi Riau. Tesis.Fakultas Matematika dan Ilmu Pengetahuan Alam, Program Studi Ilmu Kelautan, Universitas Indonesia. 2009, Depok.

Ombong, F dan Salindeho, I. Aplikasi teknologi bioflok (BFT) pada kultur ikan nila (*Orechromis niloticus*). Jurnal Budidaya Perairan. 2016 Vol. 4 (2): 16-25.

Paena, M., Suhaimi, R dan Undu, M. C. Karakteristik Sedimen Perairan Sekitar Tambak Udang Intensif Saat Musim Hujan di Teluk Punduh Kabupaten Pesawaran Provinsi Lampung. Jurnal Ilmu dan Teknologi Kelautan Tropis. 2017 Vol. 9 (1): 221-234.

Purnama, W. Aktivitas Antibakteri Glukosa Terhadap Bakteri *Staphylococcus aureus, Pseudomonas aeruginosa,Bacillus subtilis,* dan *Escherichia coli.* Skripsi. Fakultas Farmasi, Universitas Muhammadiyah Surakarta, 2013, Surakarta.

Purnomo. P. D.Pengaruh Penambahan Karbohidrat pada Media Pemeliharaan Terhadap Produksi Budidaya Intensif Nila (*Oreochromis niloticus*). Jurnal Manajement dan Teknologi Akuakultur. 2012 Vol. 1 (1): 161-179.

Putro, S. P., Febria, I. J dan Muhammad, F. Comparative Study of Characteristicsof Sediment and Water Qualityin Aquaculture Farming Systems Area with Coastal Area Adjacent to Industrial Activities. Jurnal Sains dan Mtematika. 2014 Vol. 22 (3): 79-83.

Sakdiah. M. Pemanfaatan Limbah Nitrogen Udang Vaname (*Litopenaeusvannamei*) Oleh Rumput Laut (*Gracilaria verrucosa*) pada Sistem Budidaya Polikultur. Tesis. Sekolah Pasca Sarjana. Institut Pertanian Bogor.2009, Bogor.

Schryver. P. D., Crab. R., Defoirdt. T., Boon. N dan Verstraete. W. The Basics Of Bio-Flocs Technology: The Added Value for Aquaculture. Jurnal Aquaculture. 2008 Vol. 277: 125-137.

Suazo, F. J. C. Efect Of Reactor Feeding Pattern On Performance Of An Activated Sludge SBR. Thesis. Faculty Of The Virginia Polytechnic Institute, 2006, Blacksburg, Virginia.

Sukenda., Nuryati. S dan Sari. I. R. Pemberian Meniran *Phyllanthus niruri* untuk Pencegahan Infeksi IMNV (*infectious myonecrosis virus*) pada Udang Vaname *Litopenaeus vannamei*. Jurnal Akuakultur Indonesia. 2011 Vol. 10 (2): 192-202.

Sukendar. W., Widanarni dan Setiawati. M. Respons Imun dan Kinerja Pertumbuhan

Ikan Lele, *Clarias gariepinus* (Burchell 1822) pada Budidaya Sistem Bioflok dengan Sumber Karbon Berbeda Serta Diinfeksi *Aeromonas hydrophila*. Jurnal Iktiologi Indonesia. 2016 Vol. 16 (3) : 309-232.

Sulistiowati, D., Tanjung, R dan Lantang, D. Keragaman dan Kelimpahan Plankton Sebagai Bioindikator Kualitas Lingkungan di Perairan Pantai Jayapura. Jurnal Biologi Papua. 2016 Vol. 8 (2): 79-96.

Syah. R., Suwoyo. H. S., Undu. M. C dan Makmur. Pendugaan Nutrient Budget Tambak Intensif Udang *Litopenaeus vannamei*. Jurnal Riset Akuakultur. 2006 Vol. 1 (2): 181-202.

Syamsudin., Purwati, S dan Taufick, A. T, Efektivitas Aplikasi Enzim dalam Sistem Lumpur Aktif pada Pengolahan Air Limbah PULP dan Kertas. Jurnal Biology Science. 2008 Vol. 43 (2): 83-92.

Usman., Harris. E., Jusadi. D., Supriyanto. E dan Yuhana. M. Penumbuhan Bioflok dalam Media Budidaya Ikan Bandeng. Jurnal Riset Akuakultur. 2011 Vol. 6 (1): 41-50.

Wijaya, M., Rostika, T dan Andriani, Y. Pengaruh Pemberian C/N Rasio Berbeda Terhadap Pembentukan Bioflok dan Pertumbuhan Ikan Lele Dumbo (*Clarias gariepinus*). Jurnal Perikanan Kelautan. 2016 Vol. 8 (1): 41-47.

Zahidah, D dan Shovitri, M., Isolasi, Karakterisasi dan Potensi Bakteri Aerob sebagai Pendegradasi Limbah Organik. Jurnal Sains dan Seni Pomits. 2013 Vol. 2 (1): 12-15.

Zulfahmi. I. Pengaruh Padat Tebar Berbeda Terhadap Pertumbuhan Benih Udang Windu (*Penaeus Monodon* Fabricius, 1798) yang dipelihara pada Media Bioflok. Jurnal Pendidikan Sains. 2017 Vol. 6 (1): 62-66.