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ANALYSIS CORELATION BETWEEN TOTAL ORGANIC MATTER (TOM) WITH ABUNDANCE OF VIBRIO SP. BACTERIA IN INTENSIVE CULTIVATION VANNAMEI SHRIMP (*Litopenaeus vannamei*)

Analisis Hubungan Total Organik Matter (TOM) Dengan Kelimpahan Bakteri *Vibrio sp.* Pada Budidaya Udang Vannamei (*Litopenaeus vannamei*) Secara Intensif

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ABSTRACT

Poor water quality conditions in the cultivation media can cause an increase in pathogenic bacteria such as *Vibrio* sp. which can inhibit shrimp growth. This study was conducted to analyze the relationship between Total Organic Matter (TOM) and the abundance of *Vibrio* sp. bacteria in shrimp cultivation media. The stages of the study included checking the abundance of bacteria starting from sterilization of tools, media preparation, bacterial inoculation, bacterial calculations and checking TOM using the titration method. The results showed that TOM in the cultivation media increased as the cultivation period progressed. At the beginning of the distribution, the condition of organic matter in the cultivation media showed a low value and the abundance of *Vibrio* sp. bacteria in the early stages of cultivation was also low. The highest TOM and abundance of *Vibrio* sp. bacteria occurred in DOC 91 with a total organic matter of 121.68 ppm and a TVC of 7.29 x 103 CFU/ml. while the lowest TOM and abundance of *Vibrio* sp. bacteria occurred in DOC 1 with a total organic matter of 88.48 ppm and an abundance of 2.5 x 102 Vibrio sp. CFU/ml. The correlation between TOM and the abundance of *Vibrio* sp. bacteria in the cultivation media shows a positive linear relationship. This study shows that the high content of organic matter in the cultivation media affects the abundance of *Vibrio* sp. bacteria in the cultivation media. The higher the organic matter in the cultivation media, the abundance of *Vibrio* sp. bacteria also increases.

Key words: Cultivation, Abundance, TOM, *Vibrio* sp.

ABSTRAK

Kondisi kualitas air yang buruk pada media budidaya dapat menyebabkan meningkatnya bakteri pathogen seperti bakteri *Vibrio* sp. yang dapat menghambat pertumbuhan udang.

Penelitian ini dilakukan bertujuan untuk menganalisis hubungan *Total Organic Matter (*TOM) dengan kelimpahan bakteri *Vibrio* sp. pada media budidaya udang. Tahapan penelitian meliputi pengecekan kelimpahan bakteri mulai dari sterilisasi alat – alat, persiapan media, inokulasi bakteri, perhitungan bakteri dan pengecekan TOM dengan metode titrasi. Hasil penelitian menunjukkan TOM pada media budidaya mengalami peningkatan seiring berjalannya masa budidaya. Pada awal penebaran, kondisi bahan organik pada media budidaya menunjukkan nilai yang rendah dan kelimpahan bakteri *Vibrio* sp. pada masa awal budidaya juga rendah. TOM dan kelimpahan bakteri *Vibrio* sp. tertinggi terjadi pada DOC 91 dengan total bahan organik sebesar 121,68 ppm dan TVC sebesar 7,29 x 10^3 CFU/ml. sedangkan TOM dan kelimpahan bakteri *Vibrio* sp. terendah terjadi pada DOC 1 dengan total bahan organik 88,48 ppm dan kelimpahan bakteri 2,5 x 10^2 *Vibrio* sp. CFU/ml. Korelasi hubungan antara TOM dengan kelimpahan bakteri *Vibrio* sp. pada media budidaya menunjukkan hubungan linier positif. Dalam penelitian ini menunjukkan tingginya kandungan bahan organik pada media budidaya berpengaruh terhadap kelimpahan bakteri *Vibrio* sp. pada media budidaya. Semakin tinggi bahan organik yang terdapat pada media budidaya, kelimpahan bakteri *Vibrio* sp. juga semakin mengalami peningkatan.

Kata Kunci: Budidaya, Kelimpahan, TOM, *Vibrio* sp.

INTRODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) is one of the shrimp commodities that is currently widely cultivated in all regions in Indonesia. In the 2000s, vannamei shrimp (*Litopenaeus vannamei*) began to be cultivated in Indonesia in East Java (Gunarto *et al.*, 2012). With the advantages of easy cultivation, relatively stable production, and resistance to disease, these are some of the advantages of vannamei shrimp, so that many farmers cultivate vannamei shrimp (Iskandar *et al.*, 2021). The vannamei shrimp cultivation system in Indonesia that is currently widely used is the intensive system. Intensive cultivation has the advantage of increasing productivity and efficiency of land space, as well as producing shrimp with more consistent sizes and shorter harvest times (Mustafa *et al.*, 2023). With the increasing market demand for vannamei shrimp, the intensive system is recognized as an efficient method to meet market demand (Sa'adah & Milah, 2019).

The condition of the water quality of the media in vannamei shrimp cultivation greatly influences the survival of vannamei shrimp. Poor water quality conditions can cause disease in the cultivated commodity (Kharisma, 2012). The abundance of bacteria in shrimp cultivation media is greatly influenced by the quality of the water in the cultivation media. One of the water quality parameters that affects the abundance of *Vibrio* sp. bacteria is Total Organic Matter (TOM). This is in line with Ariadi & Mujtahidah (2021) who said that the presence of organic waste in waters can trigger the growth of various types of diseases caused by bacteria. The organic matter content in the cultivation media can come from uneaten feed residue and feces that collect at the bottom of the cultivation media. One of the diseases that often attacks shrimp cultivation and causes many losses comes from vibriosis disease caused by bacteria of the genus *Vibrio* sp. (Sutiknowati, 2014). *Vibrio* sp. bacteria will be detrimental to shrimp cultivation under certain conditions. According to Idami & Nasution, (2020) *Vibrio* sp. bacteria. will be pathogenic or detrimental if the amount reaches 8.35 x 10^4 CFU/ml or more. The abundance of *Vibrio* sp. bacteria in vannamei shrimp cultivation can be said to be safe if it is in the amount of 102 or 103. Therefore, this detection needs to be done to be able to control and prevent the abundance of *Vibrio* sp. bacteria in vannamei shrimp cultivation so as not to trigger disease in shrimp and cause losses in vannamei shrimp cultivation. This study was

conducted to analyze the relationship between organic matter content in the cultivation media and the abundance of *Vibrio* sp. bacteria.

RESEARCH METHODS

Time and Place

This research activity was carried out in July - October 2024 at the research location, namely the vannamei shrimp rearing pond of CV. Jembrana Royal Vannamei, Pekutatan District, Jembrana Regency, Bali Province. The method in implementing this research uses a quantitative descriptive method. The data collection method uses primary data and secondary data. Primary data is obtained from observation, interviews and direct participation activities. Secondary data collection is obtained from literature sources in the form of related journals.

Tools

The tools used in the research of checking bacteria include autoclave for sterilization of tools used for checking bacteria. Tools used for making media, planting bacteria and checking bacterial abundance include digital scales, spatulas, 250 ml Erlenmeyer flasks, measuring cylinders, aluminum foil, electric stoves, gloves, petri dishes, micropipettes, bunsen, spread L, ovens and markers. In addition, the tools used for checking TOM include burettes, 250 ml Erlenmeyer flasks, 50ml measuring cylinders, volume pipettes, suction pipettes, electric stoves and gloves.

Materials

The materials used for checking bacteria include TCBS (Thiosulfate Citrate Bile Sucrose) agar media powder, sterile distilled water, water samples, bunsen spirt and alcohol. The materials for checking TOM include water samples, distilled water, $KMnO_4$ 0.01 N (potassium permanganate), H_2SO_4 6 N (sulfuric acid), and oxalic acid.

Research Procedures

The TOM (Total Organic Matter) checking procedure begins with taking a sample of the map water which is then taken to the laboratory for TOM checking. TOM checking is carried out once a week and the checking is carried out in the morning at 07.30 WITA. The initial step is to enter 25ml of sample and add 25ml of distilled water into the Erlenmeyer, add 10ml of KMnO4 0.01 N + 5ml of H_2SO_4 6 N, then heat on an electric stove let it boil, after 10 minutes remove from the stove then add 10ml of oxalic acid 0.01 N, titrate with KMnO4 0.01 N until the color changes to purple, then record the titration results and calculate the results obtained. For standardization and blanks, the procedure is the same but only uses 50ml of distilled water into the Erlenmeyer.

The procedure for checking bacteria begins with sterilization using an autoclave at a temperature of 121^{o}C for 15 minutes for the tools that will be used for checking bacteria (Erlenmeyer, cups, spread L, aquades media, etc.). After sterilization, the next stage is making TCBS (Thiosulfate Citrate Bile Sucrose) agar media, the agar powder is weighed then put into the Erlenmeyer, pour sterile aquades into the Erlenmeyer, cover with aluminum foil, then heat on an electric stove with occasional stirring so that the powder does not clump, after boiling the agar media is removed, then pour slowly into the media cups evenly then cool. Bacteria checking is done using the spread plate method. Water samples are taken as much as 100 ul / 1 ml using a micropipette, then the sample is put into the agar media and spread evenly. Incubation is carried out using an oven for 24 hours at a temperature of 37^{o}C, after 24 hours count the number of colonies that grow on the petri dish media.

TOM calculation:
a). Normality of KMnO4

$$V1 \times N2 = V2 \times N2$$

Description:
V1 = Volume of oxalic acid
N1 = Normality of oxalic acid
V2 = Volume of KMnO4
N2 = Normality of KMnO4
A = $\frac{0{,}01 \times 10}{\text{KMnO4 titration results}}$

$$\text{Correction factor} = \frac{A \times N \times (1/5) \times (BM\ KMnO4) \times 1000}{\text{ml sampel}}$$
$$= \frac{A \times 0{,}01 \times (1/5) \times 158 \times 1000}{25}$$
$$= A \times 12{,}64$$

b). TOM Calculation
TOM value = (Titration result – Blank) x Correction factor

Bacterial Colony Count:
a). Without dilution
Yellow = number of colonies x 10
Green = number of colonies x 10
Luminous (luminisense) = number of colonies x 10
TVC (Total Vibrio Count) = number of colonies x 10

b). With dilution
Yellow = number of colonies x 10 x Dilution factor
Green = number of colonies x 10 x Dilution factor
Luminousense = number of colonies x 10 x Dilution factor
TVC (Total Vibrio Count) = number of colonies x 10 x Dilution factor

Data Analysis

Data yang digunakan pada penelitian ini yaitu data TOM *(Total Organik Matter*) pengecekan satu minggu sekali yang disajikan berupa grafik, data kelimpahan bakteri *Vibrio* sp. pengecekan satu minggu sekali yang disajikan berupa grafik dan juga data grafik perbandingan antara TOM dan kelimpahan bakteri *Vibrio* sp.

RESULT

TOM (Total Organic Matter) Measurement

The results of TOM (Total Organic Matter) measurements in vannamei shrimp cultivation media can be seen in Figure 1. The highest TOM research results were found in DOC 91, which was 121.68 ppm, the lowest TOM was found in the initial DOC of 88.48 ppm. In the initial DOC, TOM tended to be still low, while in DOC 70 - 91 TOM increased.

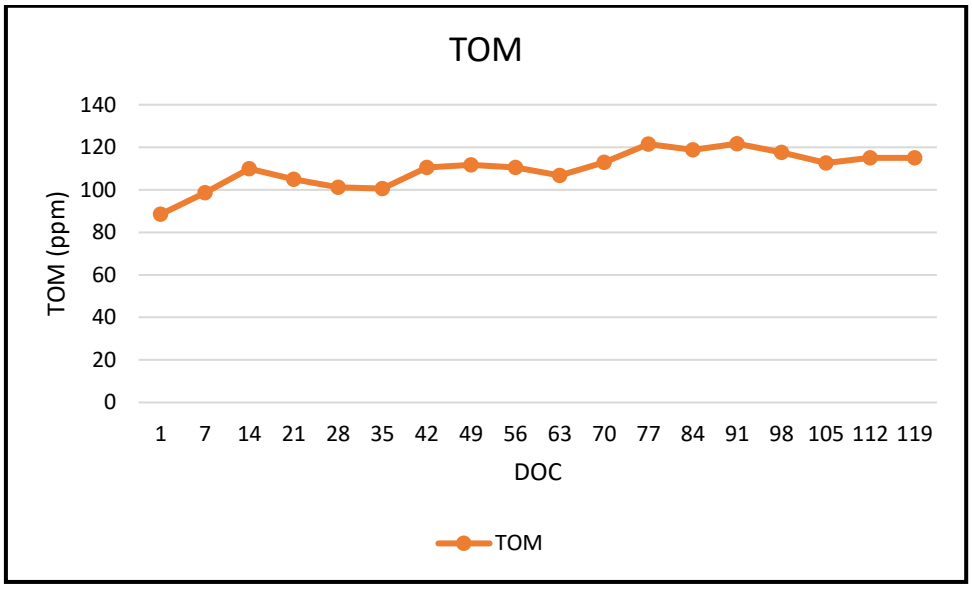

Figure 1. TOM Results in Vannamei Shrimp Cultivation Media

Total *Vibrio* Count (TVC)

The results of checking the abundance of vibrio bacteria can be seen in Figure 2. The results of checking the abundance of Vibrio sp. bacteria in the highest vannamei shrimp cultivation media were found in DOC 91, namely 7.29 x 10^3 CFU/ml, the lowest abundance of vibrio bacteria was found in the early DOC, namely 2.5 x 10^2 CFU/ml. The abundance of vibrio bacteria tends to be low in the early DOC, while in the middle to late DOC, the abundance of vibrio bacteria tends to increase.

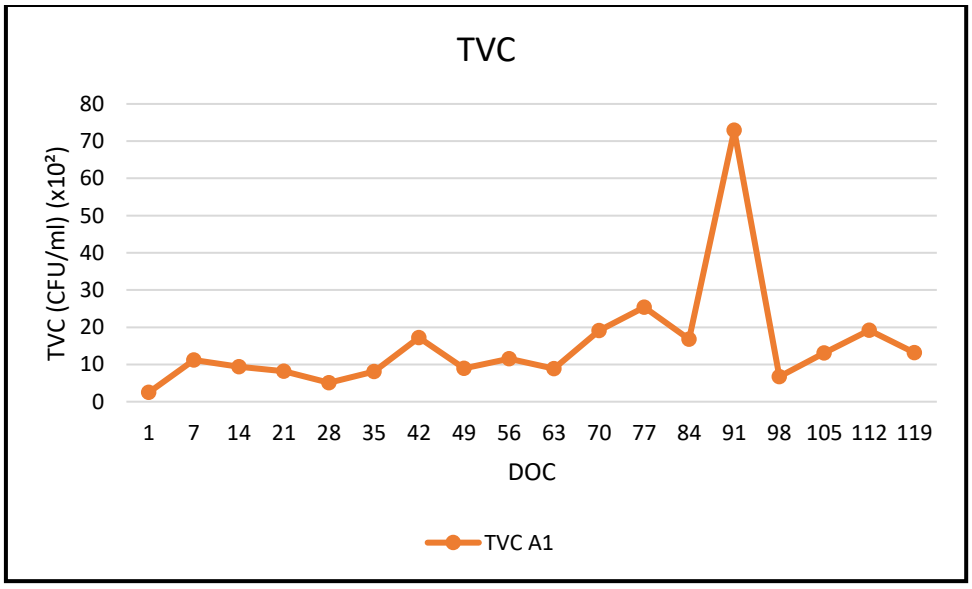

Figure 2. Total Vibrio Count (TVC) in Vannamei Shrimp Cultivation Media

Correlation of Total Organic Matter (TOM) with Abundance of *Vibrio* sp. bacteria.

The results of the correlation study of TOM with *Vibrio* sp. bacteria can be seen in Figure 3. The results of the study of the relationship between TOM and the abundance of vibrio bacteria showed comparable/unidirectional results where the amount of TOM had a significant

effect on the abundance of *Vibrio* sp. bacteria in the cultivation medium. *Vibrio* sp. bacteria tend to grow rapidly in high TOM conditions, while in low TOM conditions, the abundance of *vibrio* bacteria tends to be small. In Figure 3, the highest TVC occurred in DOC 91, which was 7.29 x 10^3 CFU/ml, the highest TOM also occurred in DOC 91, which was 121.68 mg/l. While the lowest TVC occurred in DOC 1, which was 2.5 x 10^2 CFU/ml, the lowest TOM was also found in DOC 1, which was 88.48 mg/l. In the early DOC, the TOM conditions in the cultivation medium were still relatively low and the TVC was also low, in the middle to late DOC, the TOM conditions tended to increase so that the TVC also increased.

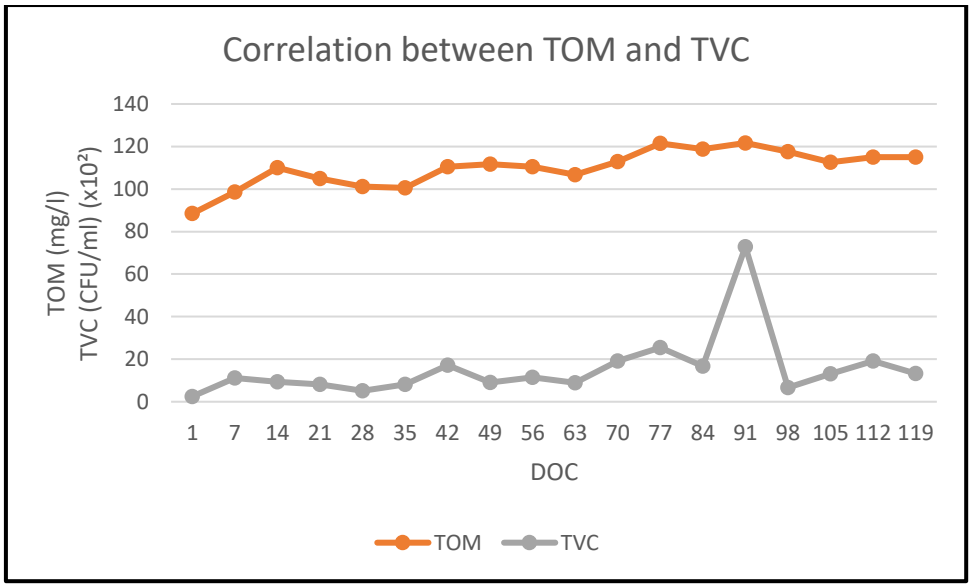

Figure 3. Correlation between TOM and TVC

DISCUSSION

Total Organic Matter (TOM) Level

Total organic matter is a water quality parameter in shrimp farming related to the amount of organic matter accumulated from uneaten feed residue, feces, dead plankton and metabolic products. According to Manengkey (2007) TOM is the total amount of organic matter originating from a collection of organic compounds in the cultivation waters that undergo a decomposition process. High organic matter in shrimp farming media can reduce water quality and interfere with shrimp growth.

From the results of measuring TOM levels, it can be seen that TOM levels increase with the age of shrimp farming. Arfiati *et al*., (2019), total organic matter in shrimp farming media will increase over time. According to SNI - 8008 2014, total organic matter in vannamei shrimp farming is optimal in the range of 80 - 120 ppm. In my research, it was recorded that at the early age of stocking, the organic matter content in the cultivation media was still relatively low, ranging from 88 - 110 mg / l, in the middle to late DOC the organic matter content in the cultivation media increased by 111 - 121 mg / l. In the early DOC the total organic matter contained in the cultivation media was still relatively low because, in the early DOC the accumulation of organic matter in the form of feces and shrimp feed residue was still not too much. The highest TOM was found in DOC 91 at 121.68 mg / l where the organic matter content in this cultivation media exceeded the maximum threshold of the company and SNI, which was 120 ppm. In addition to DOC 91, in DOC 77 the TOM also exceeded the optimal limit, which was 121.47 mg / l. The increase in organic matter in the cultivation media is thought to have occurred due to uneaten feed residue mixed with feces accumulating in the

central part which was not immediately handled, causing an accumulation of organic matter in the cultivation media. Kamal *et al*., (2017), stated that the increase in organic matter will increase along with the increasing shrimp cultivation period and shrimp biomass, along with the increasing cultivation period, feeding increases resulting in an increase in organic matter from metabolic waste, feces, and uneaten feed waste. Control of organic matter in the cultivation medium is carried out by circulating water as much as 5-10% to reduce the presence of organic matter deposits in the cultivation medium. In addition to changing the water, reducing the amount of feed is also carried out as a form of control. This is in accordance with Tahe, (2008) who stated that reducing feed is one of the control methods carried out to suppress the rate of metabolism and accumulation of feed waste.

Abundance of Vibrio Bacteria

The abundance of *vibrio* bacteria in the cultivation media is closely related to the water quality in the cultivation media. This is in line with Madusari *et al.*, (2022), who stated that the abundance of vibrio bacteria in shrimp cultivation media increased and decreased due to the dynamics of the dynamic aquaculture ecosystem. Excessive abundance of vibrio bacteria in the cultivation media can cause disease in the shrimp being cultivated. According to Kharisma (2012) less than optimal physical (temperature) and chemical (pH, Salinity, DO and TOM) water quality parameters can cause an increase in the number of *Vibrio* sp. bacteria in the cultivation media.

From the results of the research conducted, the abundance of *vibrio* bacteria in the cultivation media is still within standard limits. The lowest abundance of *vibrio* bacteria was found in DOC 1, which was 2.5 x 10^2 CFU / ml, this is thought to be because at the beginning of the distribution, the quality of the cultivation water was still maintained, so that pathogenic bacteria such as *Vibrio* sp. were still in small numbers. In DOC 7, the abundance of *Vibrio* sp. bacteria increased, which was 1.12 x 10^3 CFU / ml, then in the next DOC, namely DOC 14 to 28, the abundance of vibrio bacteria decreased successively. The decrease in the abundance of vibrio bacteria can occur due to the control of water quality by suppressing pathogenic bacteria using probiotics, in addition, temperature, pH, salinity and TOM are also controlled which can affect the abundance of vibrio bacteria in the cultivation media. The use of probiotics in cultivation waters has several functions including improving water quality, suppressing the growth of pathogenic bacteria, improving digestion and feed efficiency, improving the shrimp immune system and accelerating shrimp growth. This is in accordance with the opinion of Munaeni *et al.* (2023), who said that the bacteria contained in probiotics can function as decomposers and utilize organic compounds from dissolved feed residues and dirt from cultivated organisms so that they can improve the quality of cultivated water. Probiotics contain bacteria, namely the *Bacillus* sp. and *LactoBacillus* sp. According to Kuebutornye (2020) the combination of *Lactobacillus* sp. and *Bacillus subtillus* is able to produce bacteria that are effective in inhibiting the growth of pathogenic bacteria such as *Vibrio* sp..

The abundance of vibrio bacteria experienced a fairly high increase in DOC 70 - 91, the highest abundance of vibrio bacteria occurred in DOC 91 as much as 7.29 x 10^3 CFU / ml. The high vibrio bacteria occurred allegedly due to the high organic matter in the cultivation media, the TOM level in DOC 91 reached 121.68 ppm. The high organic matter in the cultivation media can occur because the water change is not carried out immediately, thus triggering the development of *Vibrio* sp. (Mangampa, 2015).

Correlation of TOM and Abundance of Vibrio Bacteria

From the results of the study, the relationship between Total Organic Matter (TOM) and the abundance of vibrio bacteria in the cultivation media showed a positive linear relationship

where the abundance of *Vibrio* sp. bacteria in the cultivation media was followed by the amount of organic matter that increased in the cultivation media. The large amount of organic matter from uneaten feed residues, feces that settle at the bottom of the media can have a negative impact on the cultivation media (Liu *et al.*, 2021). Widigdo *et al.*, (2020) stated that the high content of organic matter in vannamei shrimp ponds can cause a high population of pathogenic bacteria such as *Vibrio* sp.. Yunarty *et al*., (2024) said that bacteria use the abundance of organic matter contained in the cultivation media as a source of substrate for their growth and development. Organic compounds containing nutrients such as carbon, nitrogen, phosphorus and minerals are utilized by *Vibrio* sp. bacteria for their growth. As the cultivation period increases, the abundance of organic matter in the cultivation media will also increase if no control is carried out. This increase in organic matter can trigger an increasing abundance of vibrio bacteria if control and prevention are not carried out. Pathogenic bacteria Vibrio sp. will thrive in cultivation media with organic matter content >90 mg/L (Handayani *et al.*, 2023). An increase in the abundance of vibrio bacteria in the cultivation media that exceeds the optimal limit can cause vibriosis disease and harm cultivation activities. The abundance of Vibrio sp. bacteria in the cultivation media can be controlled by administering probiotics to suppress the growth of pathogenic bacteria, in addition, water circulation is carried out to improve the quality of water in the cultivation media so that the organic matter content does not continue to increase as the cultivation period increases.

CONCLUSION

Based on the results of the study, the abundance of Vibrio sp. bacteria in the vannamei shrimp farming ponds of CV. Jembrana Royal Vannamei is still within the optimum limit. The highest abundance of vibrio bacteria occurred in DOC 91 as much as 7.29 x 10^3 CFU/ml, while the lowest abundance of vibrio bacteria occurred in DOC 1, namely 2.5 x 10^2 CFU/ml. This is directly proportional to the abundance of organic matter in the cultivation media, the highest TOM value was found in DOC 91, namely 121.68 ppm, while the lowest TOM occurred in DOC 1, namely 88.48 ppm. In the initial DOC, the organic matter content in the cultivation media was relatively low and the abundance of vibrio bacteria was also relatively low. The abundance of organic matter continued to increase as the cultivation age increased, and the abundance of vibrio bacteria also increased as the abundance of organic matter in the cultivation media that was not immediately handled. This shows that the increase in organic matter in shrimp culture media is one of the causes of the increase in the abundance of vibrio bacteria in the culture media.

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