Effectiveness of Bacillus subtilis Bacteria as a Total Organic Matter Reducer in Catfish Pond (Clariasgariepinus) Cultivation

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Abstract

Catfish cultivation produces aquaculture wastes containing organic matter. Organic material is a collection of complex organic compounds that have undergone a process of decomposition by decomposers, both in the form of humus and mineralized inorganic compounds. To reduce the water of organic material in catfish (Clariasgariepinus) maintenance media and aquaculture ponds, a technology for cultivation management are needed, one of these technologies is bioremediation using Bacillus subtilis as bioremediation. This study aims to determine and analyze the potential of Bacillus subtilis in reducing levels of TOM (Total Organic Matter) in vitro and in vivo. This research was conducted in February 2019 at the UB MIPA FMIPA Laboratory (in vitro) and in the catfish pond UPT PTPBP2KP Kepanjen (in vivo). The study used a factorial complete randomized design (Factorial RAL) with the addition of Bacillus subtilisfour treatments (K = Control, D1 = 100 ml, D2 = 10 ml and D3 = 1 ml) at 24th, 48th, 72nd, 96th and 120thhours, consisting of three replications. The results obtained from this study indicate that TOM concentrations in treatments D1, D2 and D3 were lower than controls. This shows that Bacillus subtilis can reduce TOM from catfish ponds. The best treatment was found in W5D2 treatment as bioremediation of catfish organic pond water with the remaining TOM 16.23% with an efficiency value of 84%. The results of this study were also supported by measurements of water quality namely dissolved oxygen ranging from 1.83 - 3.50 mg/L, pH ranged from 6.8 to 7.6, temperatures ranged from 27^{0}C - 28^{0}C and ammonia ranged from 0.29 -2.90 mg/L.

Keywords — *Bioremediation*, *Bacillus subtilis*, *TOM*, *DO*, *pH*, *Temperature*, *Ammonia*.

I. INTRODUCTION

The intensification of catfish cultivation has resulted in the use of artificial feed rich in increasing organic matter so that the fish growth is getting bigger. Pond water produced by the cultivation system will also be higher. Intensive aquaculture pond water comes from the accumulation of organic residues derived from an inedible feed, excretion of ammonia, feces and feed particles [1] According to [2], there was an increase in the total organic matter by 318.88% from the inlet to the outlets in the sangkuriang catfish pond (*Clariasgariepinus*) at UPT PTPBP2KP Kepanjen.

The use of microorganisms that have been chosen to be grown in certain organic materials in an effort to reduce the levels of pollutants is known as Bioremediation. When the bioremediation process takes place, the enzymes produced by microorganisms will modify the structure of toxic organic materials to be not complex so that they become non-toxic and dangerous metabolites [3].

According to [4] *Bacillus cereus* bacteria can reduce organic matter in reclaimed pond water which can reduce levels of BOD 20,000 mg/L to <20 mg/L in 12 days at optimum aerobic conditions and incubate for 60 hours at 28^{0}C. While COD levels decreased from 127 mg/L to 36 mg/L. Based on the description above, the author will analyse the effectiveness of *Bacillus subtilis* bacteria as a bioremediation agent in reducing the total levels of organic matter in catfish farming ponds.

II. RESEARCH METHODS

This research was conducted in February 2019 using the experimental method at the MIPA FMIPA University of Brawijaya Laboratory (*in vitro*) and in UPT PTPBP2KP Kepanjen catfish ponds (*in vivo*), using a density of *Bacillus subtilis* bacteria of 3.12 x 10^8 CFU/mL. The study design used a Completely Randomized Design Group with a dose factor of *Bacillus subtilis* bacteria at D1 (10^7 CFU/mL), D2 (10^6 CFU/mL) and D3 (10^5 CFU/mL) and Control. The test time factor includes observations at the beginning of the experiment, 24th hour, 48th hour, 72nd hour, 96th hour and 120th hour after incubation is carried out.

A. Equipment Preparation

The equipment used in this study was sterilized in order to avoid contamination. The first step is to wash the equipment using detergent and then rinse with water. The equipment is dried and covered with aluminum foil to protect it from contamination from outside influences and put into an autoclave. The autoclave is set at 121°C for 15 minutes.

B. *Observation Parameters*

1. *Total Organic Matter (TOM)*

Analysis of total organic matter is calculated using the following formula:

$$\text{TOM (mg/L)} = \frac{(x-y)\times 31{,}6\times 0{,}001\times 1000}{mL}$$

Information:

x = mL of titrant for the sample water

y = mL titrant for aquades (blank)

31,6 =1/5 from BM $KMnO_4$ because every mole of $KMnO_4$ releases 5 oxygen in this reaction

0,01 = normality of $KMnO_4$

2. *Water Quality Measurement*

Dissolved oxygen measurement using a tool namely DO meter. PH measurement using a pH meter. Temperature is measured using a Thermometer. Ammonia levels were measured using a spectrophotometer. The measurement of each treatment was carried out at 24th, 48th, 72nd, 96th and 120th hours.

III. RESULT AND DISCUSSION

A. *Result of TOM Analysis*

Addition of Bacillus subtilis bacteria to catfish pond water media resulted in a decrease in TOM concentration. At the time of testing the 24th, 48th, 72nd, 96th and 120thhours there was a significant decrease in TOM concentration. Total organic matter decreases because the added Bacillus subtilis bacteria can decompose organic matter in pond water samples.

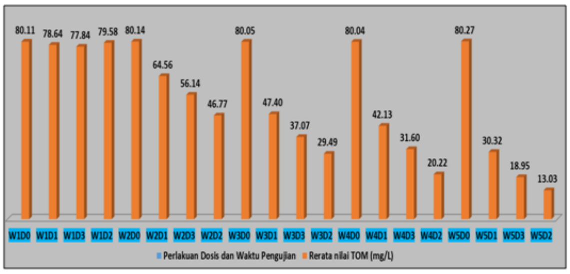

Fig.1 TOM remaining for each treatment

The average level of TOM at 24th hour has the highest value in each treatment which is ± 52.61 mg/L. The average level of TOM levels decreases in time. At 120th hours, the highest average TOM level is in treatment D1 of 30.32 mg/L, and the smallest in treatment D2 of 13.03 mg/L. According to Afu (2005), the quality standard for safe TOM concentration is ≤ 30 mg/L. The remaining TOM showed a fairly good decrease, namely the remaining 16.23% with an efficiency of 84% found at the 120th hour of treatment D2 (10^6 CFU/mL) can be seen in Figure 1.

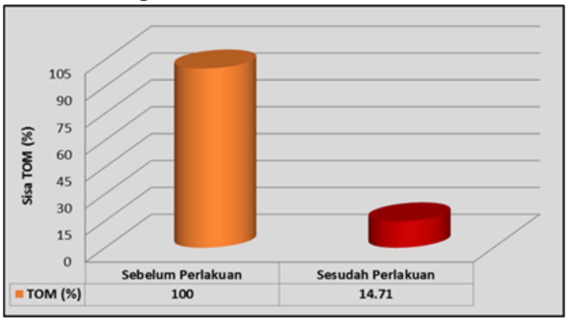

Fig.2 The lowest dose of TOM concentration (in vivo)

The effectiveness of the bioremediation test from the best bacterial dose, namely the dose of *Bacillus subtilis* 10^6 CFU/mL at 120th hours was then applied to the media of catfish maintenance at UPT PTPB Kepanjen. Based on the results of these tests it is known that the initial TOM testing in catfish ponds was 86.58 mg/L, but after being applied with the best dose the results of in vitro TOM concentrations decreased to 12.74 mg/L with a decrease in efficiency of 85.29%, so the remaining TOM is 14.71%. The concentration of decreasing TOM value in catfish farming pond can be seen in Figure 2.

B. *Results of Dissolved Oxygen (DO)Analysis*

DO or dissolved oxygen is the amount of oxygen dissolved in water originating from the results of photosynthesis or adsorption from the air. Dissolved oxygen plays an important role in intensive cultivation processes that utilize bioremediation technology. This is because the activity of short microorganisms in the composition of organic matter requires sufficient oxygen. This statement is also supported by the measured dissolved oxygen parameters at the time of the study. Most DOs measured during the study were less than 5 mg/L. This indicates that the oxygen requirements of decomposing microorganisms in decomposing organic matter are quite high.

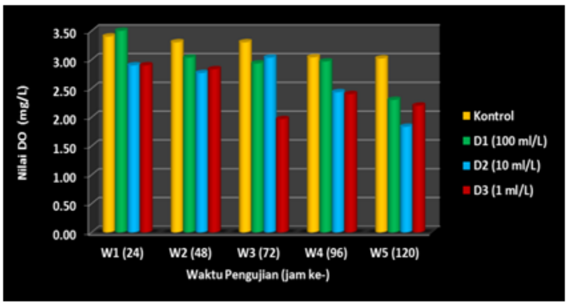

Fig.3 Average fluctuations in dissolved oxygen concentration

Low oxygen is generally followed by increasing ammonia and carbon dioxide in water which causes the nitrification process to be inhibited. Oxygen removal at the bottom of the waters is mostly due to the decomposition of organic matter that requires dissolved oxygen. [5] added, that the high content of TOM can cause low levels of dissolved oxygen in the waters. This is evident from the results of research on dissolved oxygen at 1.83 - 3.50 mg/L, where this value is below the specified threshold of> 5 mg/L (SNI 01-6483.4-2000). The low value of dissolved oxygen is caused by an oxidation process which in reaction uses a large amount of oxygen.

C. *Results of pH analysis*

One of the factors that influence bacterial activity in decomposing organic matter is pH. Optimum pH for the decomposition of organic matter between 5-8. The results of the initial measurements in this study of pond water pH were 7.2. Based on Figure 5, the pH value of each treatment in catfish pond water experienced an increase during the decomposition process occurred by Bacillus subtilis bacteria. The increase in pH from acid to neutral in catfish pond water, estimated by the activity of Bacillus subtilis. The decomposition process runs perfectly when the pH value is close to 7. As for one of the characteristics of the decomposition of organic matter, among others, produces gas that smells like ammonia (NH_3) [6].

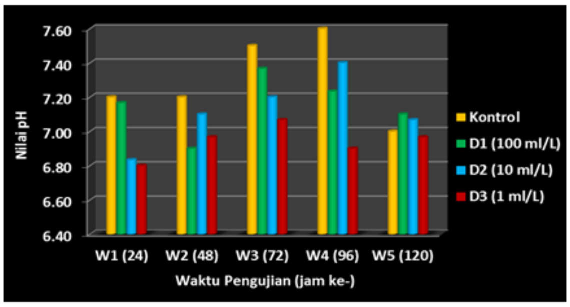

Fig.4 Average fluctuations in the concentration of pH values

The decline in TOM value turns out to be in line with the rise in pH value, because of the reduced number of H^+ ions produced from the oxidation process, so that the pH value does not decrease. This is evident from the results of pH measurements during the study resulting in a pH value of almost close to 7 which is 6.8 - 7.6. The pH value of each treatment is still at the specified threshold of 6.5 - 8.5 (SNI 01-6483.4-2000).

D. *Results of Temperature Analysis*

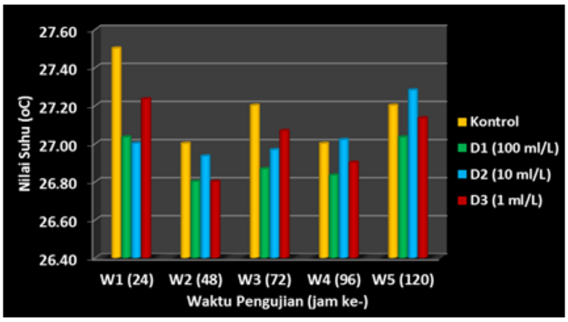

Fig. 5 Average fluctuations in temperature values

Water temperature can affect water life indirectly through its influence on the solubility of oxygen in the water. The higher the water temperature, the lower the solubility of oxygen in the water. The higher the water temperature, the higher the metabolic rate of aquaculture biota, which means the greater the oxygen consumption. The water temperature during treatment shows an average rate of 27^0C - 28^0C. The relationship between temperature and TOM is inversely proportional, that is, each parameter temperature rises, it can be seen that the TOM value will decrease as well as vice versa when the pH and DO values fall then the TOM value will also rise.

E. *Results of Ammonia Analysis*

Based on the results of the observation, the water-ammonia levels of the media in this study ranged from 0.29-2.9 mg/L. Ammonia levels decrease every time. The lowest ammonia concentration occurred at D1 (10^7 CFU/mL) at 96 hours. The highest concentration was at D3 (10^5 CFU/mL) at 72^{nd}hours. The low ammonia seen in the curve at the 96^{th} hour was thought to be due to bacteria that had not started optimal in overhauling organic matter becomes simpler.

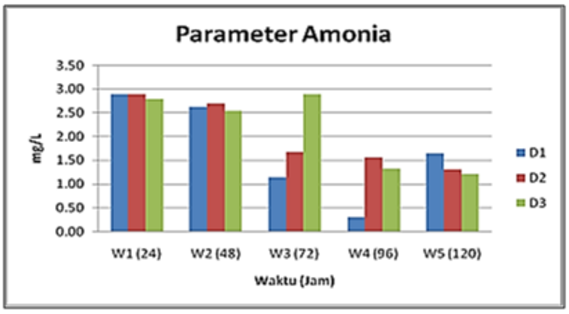

Fig.6 Average fluctuations in the concentration of Ammonia values

Ammonia levels decrease in each treatment because of the nitrification process which converts ammonia to nitrite and nitrate. This is in accordance with [7], which states that heterotrophic bacteria in the waters will usually utilize *uneaten feed*, feces, and other organic materials as sources of organic material to be converted into inorganic ammonia. According to [8], probiotic microorganisms can oxidize ammonia. Photosynthetic bacteria also use ammonia as a source of nitrogen for the decomposition of organic matter and its growth.

IV. CONCLUSIONS

1. *Bacillus subtilis* bacteria can reduce TOM levels from 80.26 mg/L to 13.03 mg/L, remaining 16.23% with an efficiency reduction of 83.8%. The best treatment was found at the dose of *Bacillus subtilis* bacteria 10^6 CFU/mL at 120 hours.
2. In the study, obtained the value of water quality ie dissolved oxygen ranged from 1.83 to 3.50 mg/L, pH ranged from 6.8 to 7.6, temperatures ranged from 27^0C -28^0C and ammonia ranged from 0.29 -2.90 mg/L. The overall parameters influence the process of reforming organic matter in catfish ponds.

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