Effect of probiotics addition on total organic matter and survival rate of catfish (*Clarias* sp.) maintenance using recirculating aquaculture system (RAS)

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Abstract. Most catfish farmers do not make efforts to manage water quality. This causes high organic matter. Therefore, water management is carried out through the Recirculating Aquaculture System (RAS). Continuous use of water in the RAS system causes filter performance to decrease, so technological improvements are needed with the addition of probiotics. The research was conducted on 22 February – 23 March 2021 at the Chemistry and Analysis Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga to determine the influence of probiotic additions on the RAS system total organic matter and the survival rate of catfish. The study was conducted experimentally using RAL by adding different doses of probiotics, namely P0 (Control), P1 (0.5 ml/L), P2 (1 ml/L), P3 (1.5 ml/L) and P4 (2 ml/L) with four replays. Data analysis using ANOVA and DMRT showed that the addition of probiotics in the RAS system has an effect (p<0.05) on total organic matter and survival rate. Organic matter was highest at P2 (46.89 mg/L) and lowest at P2 (34.07 mg/L). Survival rates were highest at P2 (85%) and lowest at P0 (71.25%).

1. Introduction
Catfish (*Clarias* sp.) is one of the freshwater fish that has been widely cultivated in Indonesia [1]. However, most catfish farmers do not make efforts to manage water such as circulation [2]. Cultivation without water management will cause residual feed and feces to accumulate at the bottom of the pond and result in high organic matter [3]. The accumulation of organic matter causes a decrease in water quality. Poor water quality causes fish to use their energy to survive, while fish that are not able to survive will die, causing low survival rates [4]. Therefore, water management efforts are needed, one of which is through the Recirculating Aquaculture System (RAS). The RAS system [5] is a culture that reuses water back through a filter. The cultivation system can minimize water turnover and maintain water quality [6]. The advantages of the RAS system depend on the effectiveness of the system in dealing with waste through the filter function [7]. Continuous use of water can cause a decrease in filter function and has been shown to cause high nitrogen and t- phosphate at 30 days of maintenance [8]. In closed recirculation systems, the organic matter content of the waters will increase so that bacteria are needed that can break down the organic matter content [9]. The addition of probiotics can be a solution to improve the RAS system because it contains bacteria.
that can help decompose food waste and feces [10]. The presence of bacteria in the waters has an active role as decomposers in the mineralization process of organic matter [11]. Bacteria can respond to chemical changes in the waters resulting in kinetic degradation of organic matter [12]. The degradation process of organic matter occurs because bacteria can metabolize organic substances through an enzymatic system to produce CO₂, H₂O, and energy [13]. Commercially, the use of probiotics has been widely carried out, especially for freshwater fish farming [14]. Provision of probiotics must be done with the appropriate dose for optimal performance [15]. Information regarding the optimal dose of probiotics for catfish culture using the Recirculating Aquaculture System (RAS) and its effect on total organic matter and survival is still little reported. On this basis, this research was conducted.

2. Methodology

2.1. Place and time

This research was conducted on February 22 – March 23, 2021, at the Anatomy-Cultivation Laboratory and Chemical-Analysis Laboratory, Faculty of Fisheries and Marine Affairs, Airlangga University, Surabaya.

2.2 Material

2.2.1 Research Equipment

The research equipment used in the study was 20 plastic tubs with a volume of 15 L for maintenance tanks, 40 plastic buckets with a volume of 10 L for reservoir tanks and filters, aerators, aeration hoses, aeration stones, and water pumps. The tools for testing total organic matter include: erlenmeyer, stirrer, burette and stand, hotplate, measuring cup, analytical balance, spatula, watch glass, volume pipette, bulb and sample bottle. While the tools for testing water quality in the form of a thermometer, pH meter, DO meter and spectrophotometer.

2.2.2 Research Material

The research material used in this study was catfish with a length of 10.58 ± 0.65 cm and a weight of 7.45 ± 1.68 grams obtained from Instalasi Budidaya Air Tawar (IBAT), Gondang, Mojokerto as 800 fish. The probiotics used are commercial probiotics with *Nitrosomonas* sp. and *Nitrobacter* sp with a density bacteria on the label 1×10¹¹ CFU/L. Filtration media form a dacron, bioball and coral. While the total organic matter testing materials included sample water, aquades, 0.01 N oxalic acid, 6 N H₂SO₄ and 0.01N KMnO₄.

2.3 Research methods

The research method in this study is an experimental method.

2.3.1 Research design

The research design used was Completely Randomized Design (CRD). This study used five treatments, namely P₀, P₁, P₂, P₃ and P₄ with 4 replications each, namely: P₀ = Control (without probiotics), P₁ = 0.5 ml/L, P₂ = 1 ml/L, P₃ = 1.5 ml/L and P₄ = 2 ml/L . [16] Research using probiotics containing *Nitrosomonas* sp. and *Nitrobacter* sp. on maintenance at a dose of 1.5 ml/L give the most effective results. The variables observed included independent variables: probiotic dose, dependent variable: total organic matter and catfish survival rate, and control variables: fish species, fish and pond size, feed dose, number of stocking density and filter media.

2.3.2 Work procedures

a) preparation of rearing media: starting with cleaning the aquarium using soap, then rinsing and drying to remove toxic compounds from the decomposition of organic matter [17]. Then the water was precipitated for one night before being used to precipitate particles in the water [18]. Filling of water is done after the bath is dry as much as 10L.
b) seed preparation: catfish seeds used measuring 10.58 ± 0.65 cm with a weight of 7.45 ± 1.68 g were obtained from the Instalasi Budidaya Air Tawar (IBAT), Gondang, Mojokerto. After that, the seeds were acclimatized for 1 day, and the seeds were stocked at a density of 2 fish/L. c) making RAS installations: RAS installations begin by punching holes in the inlet and outlet channels in plastic buckets and assembling PVC pipes to form a recirculation system and adding filters in the form a dacron, coral and bioball. d) rearing fish and adding probiotics: fish rearing is carried out for 30 days by feeding 5% of the total weight of the fish. Probiotics were given once a week [14] e) measurement of total organic matter using oxidimetry (Permanganometri) SNI 06-6989 22-2004 [19]. 100 ml of water sample was put into a 300 ml erlenmeyer. After that, a few drops of 0.01 N KMnO$_4$ were added to the test sample until it turned pink, but previously KMnO$_4$ was standardized according to SNI 06-6989 22-2004 [19]. Add 5 ml of 6 N sulfuric acid and heat on a hot plate to 105ºC ± 2ºC, if there is an odor of H$_2$S, the boiling is continued for a few minutes. Pipette 10 ml of 0.01 N KMnO$_4$ standard solution. Heat to boiling for 10 minutes. Pipette 10 ml standard solution of 0.01 N oxalic acid. Titrate with 0.01 N potassium permanganate until a pink color. Record the volume of KMnO$_4$ usage and calculate the organic matter content f) observation of the survival rate of fish, carried out by observing fish that died every day in the morning and evening before feeding, the results obtained were calculated using the SR formula which is a comparison of the number of fish that can survive at the end of the period with the number of fish that have survived. live early in the maintenance period.

2.3.3 Research Parameters

a) the main parameters observed were total organic matter and fish survival and b) Supporting parameters observed were water quality which included DO, pH, temperature and ammonia.

2.3.4 Data analysis

The data were analyzed using the Analysis of Variance (ANOVA) test to determine the effect of the treatment given. If the results show a significant difference, it will be continued with the Duncan's Multiple Range Test.

3. Results and Discussion

3.1. Total Organic Matter

| Treatment | Day 7 (mg/L ± SD) | Day 14 (mg/L ± SD) | Day 21 (mg/L ± SD) | Day 28 (mg/L ± SD) |
|-----------|------------------|-------------------|-------------------|-------------------|
| P0        | 44.95±2.747      | 46.95±3.781       | 42.32±2.585       | 48.84±1.410       |
| P1        | 32.86±0.674      | 30.17±1.962       | 35.08±2.474       | 46.11±2.517       |
| P2        | 31.08±2.549      | 29.10±0.763       | 39.89±0.533       | 36.22±0.855       |
| P3        | 37.48±2.931      | 42.79±1.832       | 51.72±0.987       | 55.55±1.604       |
| P4        | 39.25±2.274      | 46.82±1.389       | 44.80±2.132       | 55.28±1.604       |

Description : Different superscript values showed significantly different results (P<0.05).

Based on the results of the Analysis of Variance (ANOVA) on the 7th to 28th day of culture, it was shown that the addition of probiotics had a significant effect (p<0.05) on the total organic matter value. In Duncan's test results showed that on the 7th day of the study there was no significant difference between P1 and P2 treatments (P>0.05) but both were significantly different from P0,P3 and P4 treatments. Meanwhile, P3 and P4 treatments were not significantly different (P>0.05) against P0. On the 14th day of the study, there were no significant differences between P1 and P2 treatments (P>0.05) but both were significantly different from P0, P3 and P4. Meanwhile, the P3 treatment was significantly different (P<0.05) with the P0 and P4 treatments. On the 21st day of the study, treatment P1 was significantly different (P<0.05) with treatment P0, P2, P3 and P4. Meanwhile, treatments P0 and P2 were not significantly different, but both were significantly different (P>0.05) with treatment
On the 28th day of the study, treatment P2 was significantly different from all treatments P0, P1, P3 and P4. So, from the results of data analysis, showed that the addition of probiotics with the right dose in the recirculation system can reduce the total organic matter content. Thus, it can be seen that the addition of probiotics at a dose of 1 ml/L (P2) provides the best results for reducing the value of total organic matter in catfish rearing using a recirculation system. Average graph of total organic matter value can be seen in Figure 1.

![Graph of average total organic matter value](image)

Based on the results of the Analysis of Variance (ANOVA) on the 7th to 28th day of the study, it was shown that the addition of probiotics to the recirculation system had a significant effect (p<0.05) on the total organic matter value. Figure 1. shows that there was a fluctuation in total organic matter during maintenance. The results of observations of total organic matter in the control treatment (P0) showed a relatively stable value with an average value of 45.76 mg/L. This is because probiotics are not given, so that the degradation of organic matter only relies on natural bacteria that are in the rearing tank. However, the P0 treatment showed a decrease on the 21st day presumably due to mass mortality that affected the total organic matter value. This is in accordance with the statement Putra et al [20] that the content of organic matter in the waters is closely related to the metabolism of domesticated fish. So that when a water is reduced to living organisms, the organic matter will also decrease.

The increase in total organic matter occurred significantly in the P3 treatment on the 7th to 28th day of maintenance. Meanwhile, in the P4 treatment there was an increase during maintenance, except on the 21st day it decreased. In the P3 treatment, the average value of the high organic matter was 46.89 mg/L, followed by P4 46.54 mg/L. The high value of total organic matter in the P3 and P4 treatments was thought to be due to the abundance of bacteria in the rearing tank. Total organic matter is closely related to bacterial abundance [21]. This statement is in line with the research of Putra et al [22] that the greater the abundance of bacteria, the higher the value of organic matter in the waters. Thus, increasing the dose of 1.

Meanwhile, the average value of total organic matter on day 7 to day 28 in P2 treatment showed results that tended to be low. The average value of total organic matter in treatment P2 was 34.07 mg/L, followed by treatment P1 was 36.06 mg/L. This value is included in the acceptable range of waters with a threshold of 40 mg/L [23]. This shows that with the addition of probiotics, the content of *Nitrosomonas* sp. and *Nitrobacter* sp. with a dose of 0.5 – 1 ml/L is quite effective to reduce the value of total organic matter in catfish rearing system recirculation.
Hartini [24] stated that the role of probiotics in water is to maintain the stability of organic matter accumulated through the bioremediation process. Taufik [25] stated that the *Nitrosomonas* sp. and *Nitrobacter* sp. is a remediation bacteria that can convert ammonia into nitrite which is used for the decomposition of organic matter so that it is not harmful to fish. In the study of Primashita [14] the bacteria *Bacillus* sp. and *Nitrosomas* sp. can work effectively to decompose organic matter so that the water becomes stable.

### 3.2 Catfish Survival Rate

| Treatment (dose) | Survival Rate (SR ± SD) |
|------------------|-------------------------|
| P0 (0 ml/L)      | 71.25\(a\) ± 4.787     |
| P1 (0.5 ml/L)    | 80.00\(b\) ± 4.082     |
| P2 (1 ml/L)      | 85.00\(b\) ± 4.082     |
| P3 (1.5 ml/L)    | 81.25\(b\) ± 4.787     |
| P4 (2 ml/L)      | 77.5\(ab\) ± 6.455     |

Description : Different superscript values showed significantly different results (P<0.05).

Based on the calculation of Analysis of Variance (ANOVA) showed that the provision of probiotics in catfish rearing using the Recirculating Aquaculture System (RAS) had a significant effect (P<0.05) on the survival of catfish. Based on the calculation results Advanced Duncan test (Duncan's Multiple Range Test) showed that P0 was not significantly different (P>0.05) against P4 but significantly different (P<0.05) against P1, P2 and P3. This shows that the addition of the right dose of probiotics can increase the survival of catfish reared in a recirculation system. The graph of the average survival rate of catfish can be seen in Figure 2.

![Figure 2. Graph of the average survival value of catfish](image)

The survival rate of fish is the ratio of the number of fish that can survive during maintenance [26]. Survival can be used to determine the ability and tolerance of fish in terms of maintaining their lives [27]. Based on the results of the Analysis of Variance (ANOVA) showed that the addition of probiotics had a significant effect (p<0.05) on the survival of catfish. Based on Figure 2. It can be seen that in the treatment of P1, P2, P3 and P4 mortality occurred, but not as much as in P0. Mortality in the P0 treatment occurred due to a decrease in water quality and was not supported by the addition of probiotics so that the fish had a low survival rate. Probiotics in the waters can maintain the immunity
of cultured organisms. Thus, waters with the addition of probiotics cause cultured organisms to be healthier and have an immune response to pathogens [28]. This is reinforced by the research of Suwoyo and Mangampa [29] that with the addition of probiotics can increase immunity which causes a higher survival rate.

The P2 treatment with the addition of a probiotic dose of 1 ml/L showed the highest survival rate of 85%, followed by P1 (85%), P3 (81.25%), P4 (71.25%) and the lowest was P0 (77.5%). It is proven that with the addition of a dose of probiotics can increase the survival rate of catfish reared in the RAS system. Hartini [25] stated that the administration of probiotics containing *Nitrosomonas* sp. and *Nitrobacter* sp. once a week can help provide ideal water ecosystem conditions. This is also in line with Primashita's research [14] that the maintenance of catfish using aquaponics with the addition of probiotics can increase the survival rate.

In the study of Rosmawati and Muarif [30] in rearing catfish with a recirculation system with a density of 20 fish/L resulted in a 66% survival rate. Meanwhile, the results of Hasibuan's research [31] on catfish rearing with the addition of probiotics resulted in a survival rate of 77.78%. This shows that the addition of probiotics with the right dose in the recirculation system gives better results on the survival of catfish.

3.3 Water quality

| Treatment | Parameter       | Temperature (°C) | DO (mg/L)  | pH     | Ammonia (mg/L) |
|-----------|----------------|------------------|------------|--------|----------------|
| P0        |                | 28.44-29.03      | 3.65-3.81  | 7.81-7.88 | 0.18-0.32      |
| P1        |                | 28.42-28.79      | 3.88-3.96  | 7.68-7.79 | 0.17-0.33      |
| P2        |                | 28.00-28.83      | 3.96-4.29  | 7.64-7.74 | 0.17-0.29      |
| P3        |                | 28.64-29.11      | 2.00-2.51  | 7.86-8.22 | 0.19-0.37      |
| P4        |                | 28.49-29.07      | 2.40-2.82  | 7.81-8.10 | 0.19-0.35      |
| SNI 6484.3 (2014) |                | 25-30            | >3         | 6.5-8     | <0.1           |

Table 3 shows the value of water quality during the study. Water quality is an important factor that supports the success of an aquaculture business [32]. Water quality management in this study was carried out using a recirculation system with filters in the form of dacron, coral, and also bioball. In addition, water quality control is carried out by adding commercial probiotics with different doses. It is intended that the bacteria in probiotics can grow perfectly on the bioball [33]. The water quality parameters measured were temperature, pH, dissolved oxygen and ammonia. Temperature is a factor that affects the growth and survival of fish. The temperature obtained ranged from 28ºC-29.11ºC. The temperature value is included in the normal limits according to SNI 6484.3 [34] with the standard 25ºC-30ºC. Based on total organic matter testing, showed an increase in the total organic matter content along with the increase in temperature in the culture water. This is in line with Arfiati's research [35] that organic matter is strongly influenced by temperature.

Meanwhile, dissolved oxygen (DO) in this study tends to fluctuate. The highest dissolved oxygen value was in the P2 treatment with a value of 4.29 mg/L. While the P0 treatment was 3.81 mg/L and P1 was 3.96 mg/L. This figure is included in the reasonable limit of cultivation according to SNI 6484.3 [34] with a threshold of >3 mg/L. Meanwhile, in the P3 treatment the average dissolved oxygen value was 2.51 mg/L and in the P4 treatment it was 2.82 mg/L. The value of dissolved oxygen in the P3 and P4 treatments was not good because it was less than the standard that had been set. This is because the P3 and P4 treatments have high organic matter content. This statement is reinforced by Arfiati et al [35] that if the amount of organic matter is excessive, the amount of dissolved oxygen will decrease.

The degree of acidity (pH) has a sensitivity of H+ ions will result in an acidic pH and vice versa if there is a shortage of H+ ions, the pH value will be alkaline [36]. According to SNI 6484.3 [34] the optimal pH range for catfish farming is 6.5-8. In fish rearing using a recirculation system, the average
pH value ranges from 7.64-8.22. This shows that the pH value m for catfish cultivation. The ammonia measurement showed that the range of ammonia in all treatments ranged from 0.17 to 0.37 mg/L with the highest ammonia content in the P3 treatment of 0.37 mg/L. According to SNI 6484.3 [34], the range of ammonia that can be tolerated by catfish is less than 0.1 mg/L. This means that the ammonia condition during the study was in a state that exceeded the threshold.

4. Conclusion
Based on the results of the research that has been carried out, it can be concluded that the addition of probiotics in catfish rearing using the Recirculating Aquaculture System has a significant effect (p <0.05) on the total organic matter content and the survival rate of fish. The addition of a dose of 1ml/L probiotics can reduce organic matter up to 34.07 mg/L and increase survival by 85%.

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