Journal of Academic Biology and Biology Education
Vol. 2, No. 1, June 2025, pp. 106~122
ISSN: 3062-9705, DOI: 10.37251/jouabe.v2i1.2087

Bacteria–Molasses Synergy in Heterotrophic Systems: A Sustainable Strategy for Catfish Survival and Water Quality Improvement

Muhammad Iqbal[1], Zharifa Rasul[2]
[1]Biology Study Program, Universitas Islam Negeri Syarif Hidayatullah Jakarta, Jakarta, Indonesia
[2]Mindanao State University Tawi-Tawi College of Technology and Oceanography, Bongao, Philippines

Article Info

Article history:

Received Apr 7, 2025
Revised May 7, 2025
Accepted Jun 19, 2025
Online First Jun 23, 2025

Keywords:

Catfish
Heterotrophic System
Water Quality Criteria

ABSTRACT

Purpose of the study: This study aimed to evaluate the survival of catfish (*Clarias gariepinus*) in an intensive heterotrophic culture system, focusing on the effects of the system on fish survival, growth, and health. Furthermore, this study aimed to identify environmental factors that support successful cultivation in an intensive heterotrophic system.

Methodology: This study used 12 fiber tanks with a funnel-shaped bottom and The study used 12 fiber tanks (250 L) with a density of 20 catfish (± 50 g/tail) and four treatments: feed only, feed+molasses, feed+bacteria, and feed+molasses+bacteria (heterotrophic system), each with three replications. Survival parameters and water quality (temperature, pH, DO, ammonia, nitrite, nitrate, VSS) were measured periodically. Data were analyzed using one-way ANOVA followed by a 5% Duncan test.

Main Findings: The analysis results show that the heterotrophic system produces good catfish survival and water quality that supports the growth of catfish. The heterotrophic system is able to increase the survival of catfish in intensive cultivation by up to 80–90%, reduce the concentration of ammonia to 0.98 mg/L, nitrite to 1.06 mg/L, and nitrate, thus producing water quality that is very supportive of the success of intensive cultivation. In addition, the highest volatile suspended solid value was recorded at 0.90 mg/L in the heterotrophic system.

Novelty/Originality of this study: This research presents a new approach to intensive catfish to simultaneously improve fish survival and optimize water quality. These findings expand knowledge on nitrogen waste management and provide practical strategies for the development of sustainable aquaculture technologies.

Corresponding Author:
Muhammad Iqbal,
Biology Study Program, Universitas Islam Negeri Syarif Hidayatullah Jakarta,
Jl. Ir H. Juanda No.95, Cemp. Putih, Kec. Ciputat Tim., Kota Tangerang Selatan, Banten 15412, Indonesia
Email: muhammadiqbal12@gmail.com

1. INTRODUCTION

With population growth and the increasing demand for fish products and fish consumption, aquaculture is required to increase production. Catfish is one of the most widely cultivated fishery commodities in Indonesia [1], [2]. The abiotic conditions of aquatic ecosystems play a crucial role in maintaining the sustainability of aquaculture [3], [4]. Both in nature and in ponds, catfish grow rapidly and are resistant to adverse environmental conditions [5], [6]. To achieve better results, adequate oxygenated water and pollutant-free conditions are required, along with proper cultivation practices.

Currently, catfish farming is often carried out using intensive systems. Intensification is characterized by increased fish density and supplemental feed. A recurring problem with intensive farming is the decline in water quality in the culture medium due to increased metabolite production [7], [8]. Increased fish metabolic waste ultimately leads to increased ammonia levels in the water [9], [10]. The presence of ammonia affects fish growth by reducing oxygen intake due to gill damage, increasing energy expenditure for detoxification, disrupting osmoregulation, and causing physical damage to tissues [11].

Therefore, it is necessary to manage the waste from intensive fish farming to prevent it from becoming toxic and causing fish mortality, and to increase fish survival rates. This waste management can be achieved using a heterotrophic system in intensive fish farming. A heterotrophic system is a fish farming system that utilizes heterotrophic bacteria [10], [12]. Heterotrophic bacteria convert organic nitrogen waste (ammonia, nitrite, and nitrate) into biomass [13], [14]. A heterotrophic system in intensive fish farming is necessary to maximize waste processing to achieve production with a high fish survival rate, while also reducing the pollution load of fish farming waste into the surrounding waters and resulting in a more efficient farming system and technology.

The application of a heterotrophic system in intensive catfish farming is expected to address the problem of water quality decline due to the accumulation of nitrogen compounds. The conversion of ammonia, nitrite, and nitrate by heterotrophic bacteria not only plays a role in reducing water toxicity but also produces microbial biomass that can be used as additional natural food for fish [15], [16]. Thus, this system has the dual potential of improving the quality of the farming environment while increasing feed efficiency. Another advantage of heterotrophic systems is their ability to operate sustainably without requiring large water changes, thus supporting the concept of environmentally friendly cultivation.

In addition to environmental aspects, the implementation of heterotrophic systems also impacts production parameters, particularly the survival rate of catfish in intensive culture systems. Survival rate is an important indicator of cultivation success because it reflects fish health, stress resistance, and environmental management efficiency [17], [18]. By monitoring water quality parameters such as ammonia, nitrite, nitrate, and volatile suspended solid (VSS) levels, farmers can assess the effectiveness of heterotrophic systems in maintaining optimal conditions for fish [19], [20], [21]. Therefore, this study focused on examining the relationship between the implementation of heterotrophic systems, water quality improvements, and the survival of catfish on an intensive scale.

Several studies have demonstrated that biofloc technology (heterotrophic systems) is effective in improving the survival, growth, and environmental quality of Clarias gariepinus. For example, research by Alinsangao et al. showed that the use of biofloc with various carbohydrate sources increased the survival rate by 96–98%, as well as reducing total ammonia nitrogen (TAN) and total suspended solids (TSS) levels compared to conventional systems [22]. Similarly, Salamah et al. (year not explicitly stated) noted that the addition of heterotrophic bacteria to the biofloc system increased the survival rate to ±92.7%, as well as supporting the growth rate and feed efficiency in catfish cultivation [23].

However, comprehensive analysis of environmental parameters—such as ammonia, nitrite, nitrate, and volatile suspended solids (VSS)—within a research framework that also emphasizes the survival of catfish in intensive heterotrophic culture is still very limited. Generally, previous studies only measure one or two parameters, such as TAN and TSS, without considering the entire spectrum of critical water quality. Furthermore, no publications have specifically summarized the relationship between the use of heterotrophic systems, these environmental parameters, and the survival rate of Clarias sp. in intensive culture settings. Therefore, the current study aims to fill this important gap by comprehensively evaluating fish survival while controlling ammonia, nitrite, nitrate, and VSS in heterotrophic systems.

This study aims to evaluate the survival of catfish (*Clarias gariepinus*) in intensive heterotrophic culture systems, focusing on the effects of these systems on fish survival, growth, and health. In addition, this study is intended to identify environmental factors that support successful cultivation in an intensive heterotrophic system, so that it can provide technical recommendations for cultivators in increasing the productivity and efficiency of catfish cultivation businesses.

2. RESEARCH METHOD

This research activity was conducted at the Cultivation System Laboratory of the Sukamandi Freshwater Fisheries Breeding and Cultivation Technology Research Center, Subang, West Java from July to December 2025.

2.1. Tools and materials

The tools used in this study include a 250 L round fiber tank with a funnel base, aerator, cover net, field equipment (bowls, buckets, plastic cups, plastic funnels, filters, hoses, plastic kilograms), sample bottles, funnels, dropper pipettes, measuring cups, tissue, Erlenmeyer flasks, measuring flasks, beakers, digital and

analytical scales, water quality checkers, desiccators, ovens, vacuums, micropipettes, Whatman No. 42 filter paper, furnaces, porcelain cups, and U-I500 spectrophotometers. The materials used include catfish (Clarias gariepinus) measuring 50 g/tail, Pro-vite 781 feed, molasses, commercial Minabacto bacteria, and ammonia, nitrite, and nitrate reagents.

2.2. Research Procedures

The research procedure began with the preparation of a 250-liter round fiber tank with a funnel base filled with 200 liters of water, aerated to maintain dissolved oxygen levels, and covered with a net. Inoculation of commercial Minabacto bacteria at a dose of 10^6 cfu/20 mL was carried out once at the same time as the first feeding. Catfish fry weighing ±50 g/fish were stocked at a density of 20 fish/tank after being fasted for 24 hours. Survival observations were carried out for 4 cycles (days 0 to 21), with additional fish added if the number decreased to 0% to maintain density. Pro-vite 781 floating pellet feed (crude protein) was given at 3% of the fish biomass weight, three times a day (07:00, 13:00, 16:00 WIB) for 3 weeks, with adjustments to the amount every 7 days according to biomass growth. Molasses was administered simultaneously with the morning feed, weighed according to fish weight and calculated C/N ratio, in only six treatment funnels. Bacterial inoculation was performed in only three treatment funnels at the beginning of the study.

This study employed a heterotrophic system for intensive catfish farming. The experiment was designed using a completely randomized design with four treatments and three replications. The treatment design to be implemented is as follows:

- Treatment A: Feeding without bacteria and molasses
- Treatment B: Feeding with molasses and without bacteria
- Treatment C: Feeding with bacteria and without molasses
- Treatment D: Feeding with bacteria and molasses.

In all treatments, 12 variations were obtained, which are shown in Table 1..

Table 1. Variation of treatment for each funnel

Funnel	Treatment code	Treatment
1	B1	No Bacteria + Molasses
2	A1	No Bacteria + No Molasses
3	C2	Bacteria + No Molasses
4	D1	Bacteria + Molasses
5	C1	Bacteria + No Molasses
6	B3	No Bacteria + Molasses
7	D2	Bacteria + Molasses
8	A2	No Bacteria + No Molasses
9	A3	No Bacteria + No Molasses
10	C3	Bacteria + No Molasses
11	B2	Bacteria + No Molasses
12	D3	Bacteria + No Molasses

Observations were then conducted, starting with calculating the survival rate of the catfish. The number of fish was measured per funnel and conducted daily. Observations of fish survival began by counting the number of fish that died each day per funnel before feeding. Fish survival was measured by subtracting the initial number of fish from the final number. The survival rate of catfish and fish can be calculated using the following formula:

$$SR = \frac{Nt}{No} \times 100\% \quad ...(1)$$

Description:
SR = Survival Rate (%)
Nt = Number of catfish surviving at the end of the observation
No = Number of catfish at the beginning of the observation

Then, DO, Temperature, and pH measurements were carried out on days 0, 2, 4, 8, 12, 16, and 21 using a Water Quality Checker. Measurements were carried out every morning before feeding and molasses. Ammonia, nitrite, and nitrate measurements were carried out on days 0, 2, 4, 8, 12, 16, and 21 in the chemistry laboratory. Ammonia measurements were as follows: water samples were taken from each funnel at 6:00 a.m. before feeding and molasses. Filter the water samples using filter paper. A 5 ml water sample was placed in a test tube and then 0.2 ml of phenol solution; 0.2 ml of nitroprusside solution, and 0.5 ml of oxidant solution were

added. Allow the color to form at room temperature (22-27ºC), shake, and leave for one hour. Analysis was carried out with a spectrophotometer at a wavelength (λ) of 640 ηm. Nitrate measurements are as follows: water samples were taken from each funnel at 6:00 a.m. before feeding and molasses. Filtered the water samples using filter paper. 2 ml of water samples were put into a test tube and then added 0.4 ml of 0.5% Brusin solution.

Then carefully added 4 ml of concentrated H2SO4 solution and cooled. Analyzed with a spectrophotometer at a wavelength (λ) of 420 ηm. Nitrite measurements are as follows: water samples were taken from each funnel at 6:00 a.m. before feeding and molasses. Filter the water samples using filter paper. A total of 5 ml of water samples were put into a test tube and then added 0.1 ml of sulfinic acid, then left for 2-8 minutes. Then added 0.1 ml of NED-dihydrochloride solution and shaken. Left for 10-20 minutes will form a purplish red color. Analyzed with a spectrophotometer at a wavelength (λ) of 540 ηm.

Volatile Suspended Solid measurements were carried out on days 0, 2, 4, 8, 12, 16, and 21 in the chemistry laboratory. Water samples were taken from each funnel at 6:00 a.m. before feeding and molasses. A 100 ml water sample was filtered using a Watman 42 filter paper and vacuum. After that, the filter paper was dried in an oven at 103°C for 60 minutes, cooled in a desiccator, and weighed for dry weight (A). After that, the filter paper was put into a furnace at 550°C for 60 minutes, then cooled in a desiccator and weighed again (B). The results of weighing A and B were calculated using the following formula:

$$VSS = \frac{A - B}{Water\ sample\ volume\ (ml)} \quad ...(2)$$

Description:
A: filter weighing result after temperature 103ºC (mg)
B: filter weighing result after temperature 550ºC (mg)

2.3. Data Analysis

Parameter measurement values at the end of the study were tested using Analysis of Variance (ANOVA) to determine differences between treatments. The results of each parameter were tested using a one-way analysis of variance (ANOVA) to determine differences between treatments of feed, bacteria, and molasses on fish survival. If the ANOVA or Analysis of Variance showed that Fhit > Ftab with a significance level of 5% or 1%, further testing was carried out using the 5% Duncan test to more clearly determine the differences between treatments.

3. RESULTS AND DISCUSSION

3.1. Survival of Catfish (*Clarias sp*)

Survival is the ratio of the number of individuals surviving at the end of the culture to the number of individuals surviving at the beginning. Data from technological research on intensive catfish cultivation using a heterotrophic system with a stocking density of 50 grams for 20 individuals resulted in differences in the survival rate of catfish in each treatment. This can be seen in the four different graphic cycles observed during the observation period in the intensive heterotrophic cultivation system. Figure 2 shows the poor survival rate of the catfish from the four different cycles.

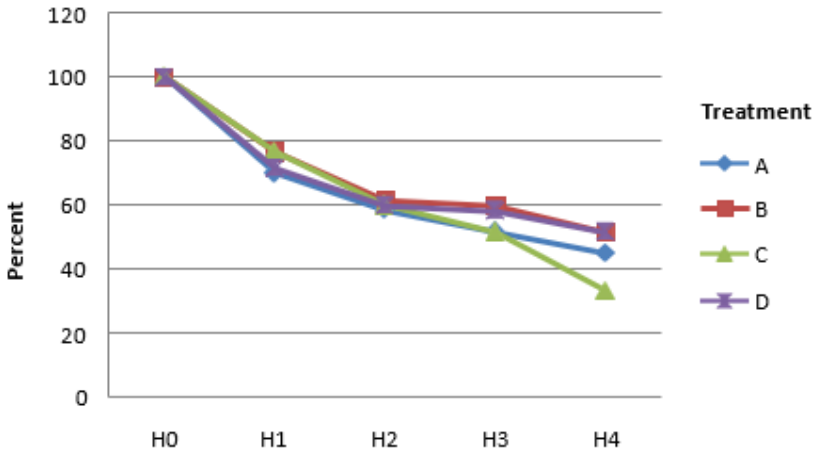

Figure 2. Survival Rate of Catfish (*Clarias sp*) Cycle 1

Fish survival rates in all treatments were less than optimal. This was evident from the beginning of the study until day 4. Fish survival rates gradually decreased to 30%. This is thought to be due to the fish adapting during this time, and high ammonia levels causing decreased survival.

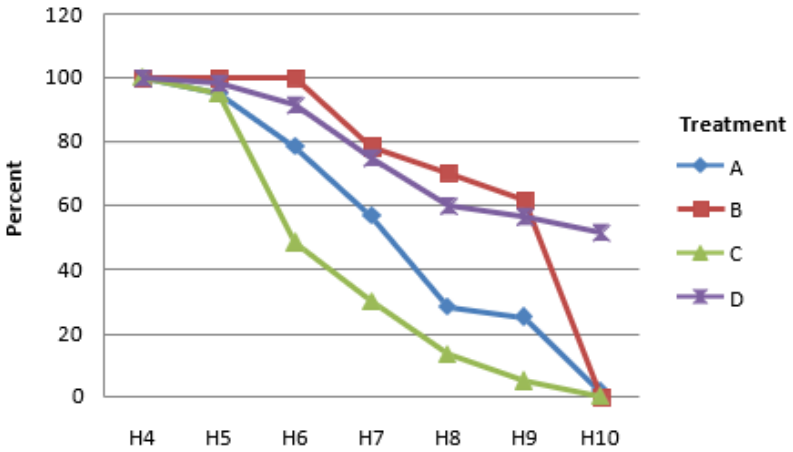

Figure 3. Survival Rate of Catfish (*Clarias sp*) Cycle 2

The results obtained on day 4 for all treatments showed a 100% fish survival rate. This was due to additional fish being added on that day, considering the conditions in cycle 1, which prevented the study from continuing. In treatment A, on days 6 and 7, the fish survival rate decreased by 80% and 60% on day 7. This was due to the nitrite level increasing by 20 mg/L on that day. Therefore, the fish survival rate decreased within a single day, then decreased again on days 8 and 10, until death occurred. In treatment A, the decline during the study was due to the large number of autotrophic bacteria inhabiting the funnel tank, which maximized the ammonia conversion process, which can produce nitrite. Two important limiting factors in intensive polyculture systems are water quality and economic aspects.

Even with excellent management, the feed produced will generate waste. [24], [25]. Of the feed given to fish, usually around 10% is wasted or uneaten, 10% is solid waste and 30% is liquid waste produced by fish, from the remaining 25% of feed is used for growth and the other 25% is used for metabolism (heat energy for biological processes). This percentage depends on the type and size of fish, activity, water temperature, and other environmental conditions. This is comparable to what has been produced by feed treatment alone without using commercial bacteria and molasses so that poor water quality is produced and can affect the survival rate of catfish *(Clarias gariepinus)*.

The analysis results for treatment B showed quite optimal results. This was evidenced by observations obtained on days 7 to 9, with a fish survival rate of 60%-80%. This was due to the role of molasses, which serves as a food source for the natural heterotrophic bacteria living in the funnel. This was due to the low ammonia levels in treatment B (2.49 mg/L) and nitrite levels on that day. Thus, treatment B was dominated by heterotrophic bacteria, although not as much as in the heterotrophic system.

The growth of heterotrophic bacteria can neutralize waste content that could potentially harm the life of catfish. Other supporting factors, such as dissolved oxygen, can still be utilized by the fish properly. Under these conditions, the water quality is still said to be relatively free of nitrogen waste, resulting in optimal catfish survival.

On the 10th day, fish survival declined to the point of death. This was due to insufficient oxygen supply due to the large amount of leftover feed and sediment that could block the air stone. This was due to high nitrogen waste caused by uneaten food waste. Furthermore, the lack of natural heterotrophic bacteria that grew unbalanced with the high levels of nitrogen waste resulted in fish mortality.

According to Boyd and Tucker, [11] stated that NH3 content of 0.1 mg/l reduces growth and causes gill damage in Channel Catfish, a concentration of 0.52 mg/l reduces growth by 50%, while at a concentration of 0.97 mg/l growth will be inhibited. Ammonia can also cause decreased growth, gill hyperplasia, and hemorrhage. The results of observations of treatment C using feed and the addition of bacteria on the 6th to 10th day experienced a decrease in the survival rate of catfish until death. This is due to the very high nitrite levels in this treatment which previously occurred the process of converting ammonia to nitrite carried out by autotrophic bacteria and possibly due to the bacteria not receiving an intake of carbon sources so that the inoculation of minabacto bacteria that convert nitrogen waste materials cannot grow optimally so that their growth is inferior to autotrophic bacteria.

This is suspected to be due to the lack of a role of molasses addition to the maintenance media as stated by Avnimelech [26], Bacteria and other microorganisms utilize carbohydrates as food to produce energy and

carbon, and together with nitrogen in the water, they produce new cellular proteins. Therefore, the addition of molasses to the rearing medium promotes the growth of natural food for catfish.

Analysis results obtained in treatment D, a heterotrophic system, showed better results compared to other treatments. This was evident from the 5th to 10th day, with an average survival rate of 60-90%. This was due to the very low nitrite levels produced in treatment D.

The D treatment's performance is primarily due to the role of bacteria and molasses as carbon sources, which allow the bacteria to continue growing and converting ammonia and nitrite, which are highly toxic to fish. This is also due to water quality factors that support the performance of these heterotrophic bacteria. This demonstrates that the heterotrophic bacterial mechanism is working effectively, maintaining water quality as a result of the heterotrophic bacteria's ability to utilize nitrogenous waste from the cultivation.

The biofloc system's working mechanism is complex, with all components interacting with each other. Simply put, the performance of all organisms in a biofloc system will influence the medium in which they live, and the conditions in which they live will in turn influence their performance.

Statistical tests using analysis of variance and Duncan's test (Tables 2 and 3) are as follows:

Table 3. Analysis of Variance (ANOVA) of Fish Survival

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	166.917	3	55.639	55.639	.000
Intercept	1064.083	1	1064.083	55.639	.000
Variasi	166.000	3	55.639	55.639	.000
Error	8.000	8	1.000		
Total	1239.000	12			
Corrected Total	174.917	11			

Table 4. Duncan's Advanced Test of Fish Survival

Feed Variation, Molasses, Bacteria	N	Subset 1	Subset 2	Subset 3
1	3	6.00		
2	3	7.33	7.33	
3	3		8.67	
4	3			15.67
Sig.		.141	.141	1.000

Tables 3 and 4 show that the treatment of feed variations, bacteria, and molasses had a significant effect ($P<0.01$) on fish survival. This can be seen from the calculated F value which is smaller than the F table or sig 0.00. The Duncan test shows that there is a very significant difference in fish survival ($P<0.01$) in treatments A, B, C, and D. This can be seen from the subset where each treatment is located in a different subset..

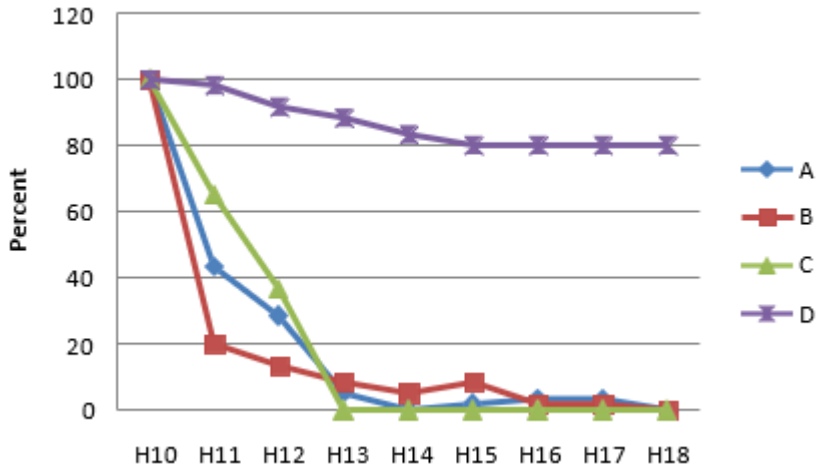

Figure 4. Survival Rate of Catfish Cycle 3

The results shown in Figure 4 indicate that on day 10 of all treatments, fish were added again, resulting in a 100% increase in the number of catfish. A very stable survival rate was found in treatment D, the heterotrophic system treatment. Furthermore, in other treatments, the survival rate was poor or continued to decline, leading to mortality.

In treatment D, from day 10 to day 18, the survival rate of catfish reached 80%-90%. This demonstrates that treatment D, using bacteria and molasses, works optimally, resulting in excellent results and low nitrite levels. This is in line with the opinion of Brune et al [27], stated that the process of heterotrophic bacterial biosynthesis takes place faster than the process of algae biosynthesis and the nitrification process, namely the generation time of 10 hours compared to 24-48 hours, thus the rapid growth of heterotrophic bacteria is able to convert ammonia and other nitrogen waste materials. The benefits of the performance of the heterotrophic system are to support the growth of cultivated fish, namely catfish and tilapia, Tacon [28] stated that biofloc is a complex mixture of microorganisms, including bacteria, microalgae, fungi, protozoa, metazoans, rotifers and gastrotrichs.

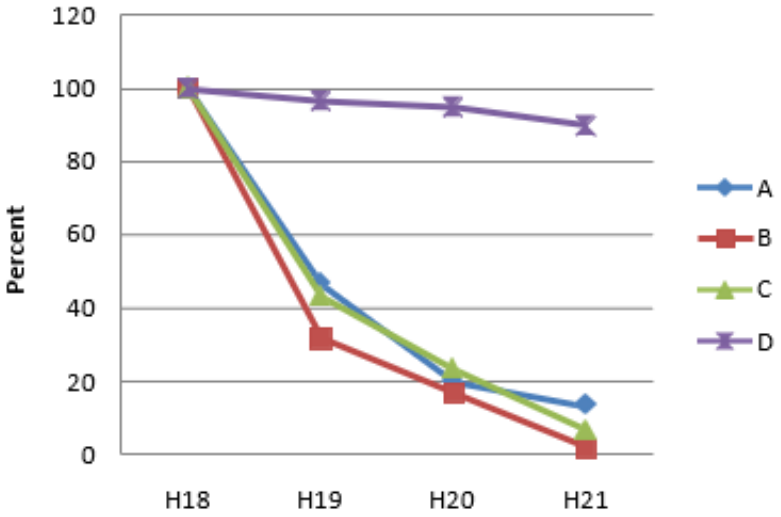

Figure 5. Survival Rate of Catfish Cycle 4.

Observations in Figure 5 show that the survival rate of catfish is not significantly different from that in Figure 4. On day 18, the survival rate increased by 100% in all treatments. This was due to the addition of more fish on that day, as the previous day saw a decline and even death in all three treatments except Treatment D.

In all three treatments except Treatment D, fish survival rates consistently decreased. This was due to the high nitrite levels in all three treatments, which were previously the result of the ammonia conversion process carried out by autotrophic bacteria in Treatments A and C. Treatment B likely lost competition with the autotrophic bacteria growing in that treatment.

Treatment D, during the fourth cycle of research, achieved excellent results, reaching 80%-90%. This is due to Treatment D's heterotrophic system, which utilizes bacteria and molasses as carbon sources, and the low nitrite levels in Treatment D. Therefore, Treatment D can be utilized in aquaculture to improve catfish survival.

3.2. Water Quality Parameters

Water quality parameters observed during the study included temperature, dissolved oxygen, pH, ammonia, nitrite, nitrate, and volatile suspended solids (VSS). VSS is a supporting factor for the survival rate of catfish (*Clarias sp*.).

3.2.1 Ammonia

Ammonia is the main metabolic waste product of fish and is often a problem in fish farming. By adding a carbon source to increase the C/N ratio in the water, optimal bacterial growth is expected to occur, allowing it to absorb ammonia compounds and convert them into bacterial biomass. Theoretically, 20 g of carbohydrates are needed to convert 1 g of ammonium [29], [30].

Ammonia is the primary form of nitrogen excretion in aquatic organisms. Other nitrogenous wastes include urea, urea, *creatine, creatinine*, amino acids, and trimethylamine oxide [31], [32]. Fish excrete significant amounts of nitrogen through their gills in the form of NH_4^+, with NH_4^+ excreted accounting for 60%–90% of total nitrogen excretion [33]. In water, ammonia exists in both un-ionized (NH3) and ionized (NH_4^+) forms. According to Heath [34], ammonia is not only found in rivers, but is also caused by the decomposition of organic materials. Figure 6 shows the differences in ammonia levels in each treatment.

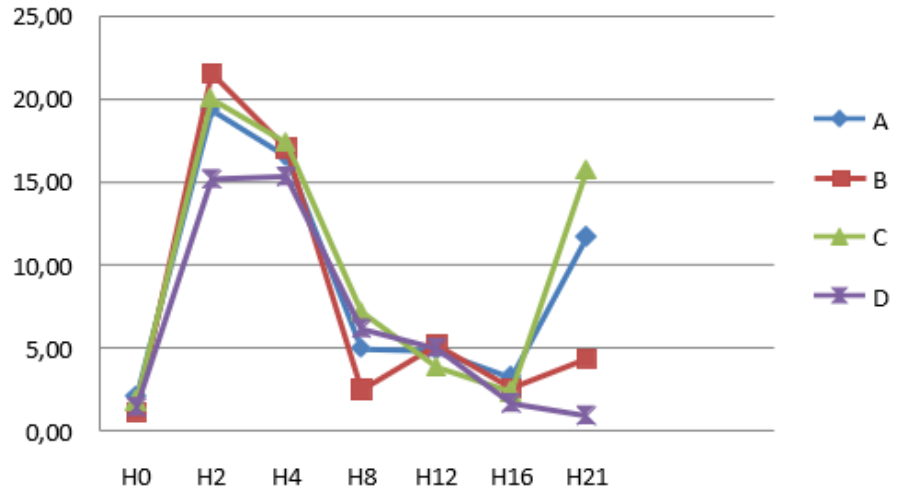

Figure 6. Ammonia Parameters During the Study

Observations of water quality parameters, such as ammonia, throughout the study revealed significant differences. On days 2 and 4 in treatment A, ammonia levels increased by 19.28 mg/L. This is thought to be due to the lack of autotrophic bacterial activity, resulting in high ammonia levels. This was also due to the lack of water changes, resulting in high nitrogenous waste, such as ammonia, in this treatment. The large amount of uneaten or wasted feed and fish excretions contributed to the cloudy and dirty condition of the tank.

The decrease in ammonia levels occurred, in part, due to microbial utilization. Ammonia utilization can occur in three main processes: photoautotrophic algal biosynthesis, which produces algal biomass; heterotrophic bacterial biosynthesis, which produces bacterial biomass; and chemoautotrophic nitrification, which produces nitrite compounds, which are then converted into nitrate [27]. Furthermore, on days 8 to 16, ammonia levels decreased by 4.95 mg/L. This was due to the activity of autotrophic bacteria, and an increase occurred on day 21, presumably due to the high levels of leftover feed and fish excretion on that day.

In treatment B, ammonia levels increased on days 2 and 4. This was suspected to be due to the small number of natural heterotrophic bacteria utilizing molasses as a carbon source, therefore ammonia levels increased by 21.20 mg/L on that day. In contrast, on day 8 to day 21, there was a decrease in ammonia of 2.49 mg/L, because on that day the heterotrophic bacteria had utilized carbon sources optimally so that ammonia levels could be suppressed as little as possible.

In treatment C, on days 2 and 4, ammonia levels increased by 17.37 mg/L to 19.99 mg/L. This was thought to be due to the lack of autotrophic bacterial activity, allowing the latter to convert ammonia to nitrite and then nitrate. On days 8, 12, and 16, ammonia levels decreased by 7.20 mg/L to 2.36 mg/L, thought to be due to autotrophic bacterial activity. On day 21, ammonia levels increased again to 15.70 mg/L, thought to be due to increased fish metabolism and feed waste, which can cause ammonia levels to rise.

In treatment D with a heterotrophic system, ammonia levels increased on days 2 and 4 by 15.19 mg/L, this is suspected to be due to the accumulation of nitrogen waste and the presence of leftover feed produced by fish and the presence of heterotrophic bacteria which are still in the adaptation phase or the heterotrophic bacterial population has not grown well and there is no water change during the study which causes an increase in ammonia Montoya and Velasco [35], Furthermore, on days 8 to 21, ammonia levels decreased very well by 0.98 mg/L-6.19 mg/L, this is due to the activity of heterotrophic bacteria, so that ammonia levels can be suppressed as little as possible with a heterotrophic system and then converted into biomass.

The potential for increased ammonia in this culture system is influenced by pH and temperature. At low water pH (acidic), ammonia tends to be more in the NH_4^+, form, while at high water pH (alkaline), ammonia tends to be more in the NH_3 form. At low water temperatures, ammonia tends to be more in the NH_4^+ form, while at high water temperatures, ammonia tends to be more in the NH_3^+ form.

According to Boyd [11] an NH_3 sebesar 0,1 mg/l content of 0.1 mg/L reduces growth and causes gill damage in *Channel Catfish*. A concentration of 0.52 mg/L reduces growth by 50%, while at a concentration of 0.97 mg/L growth is inhibited. Ammonia can also cause decreased growth, gill hyperplasia, and hemorrhage. High ammonia can cause changes in blood-brain barrier function, disrupt amino acid transport, and disrupt blood circulation. $NH4^+$ can disrupt the ion exchange mechanism in the central nervous system by displacing K^+ ions.

In the presence of organic matter, the microbial processes occurring in the water will be dominated by heterotrophic bacteria, which absorb ammonium into bacterial biomass more quickly than nitrifying bacteria, which are classified as autotrophic. Heterotrophic bacteria can absorb up to 50% of the amount of dissolved

ammonium in water. Some heterotrophic microorganisms have also been reported to be able to oxidize ammonia or organic nitrogen into nitrite or nitrate [36], [37].

3.2.2 Nitrite

Nitrite compounds are the result of ammonia breakdown in the first stage of the nitrification process, carried out primarily by the bacteria *Nitrosomonas sp.* In natural waters, nitrite (NO_2) is usually found in very small amounts, less than nitrate, because it is unstable in the presence of oxygen. Nitrite is an intermediate form between ammonia and nitrate (nitrification), and between nitrate and hydrogen gas (denitrification). Denitrification occurs anaerobically. The nitrite value can be seen in Figure 7 below.

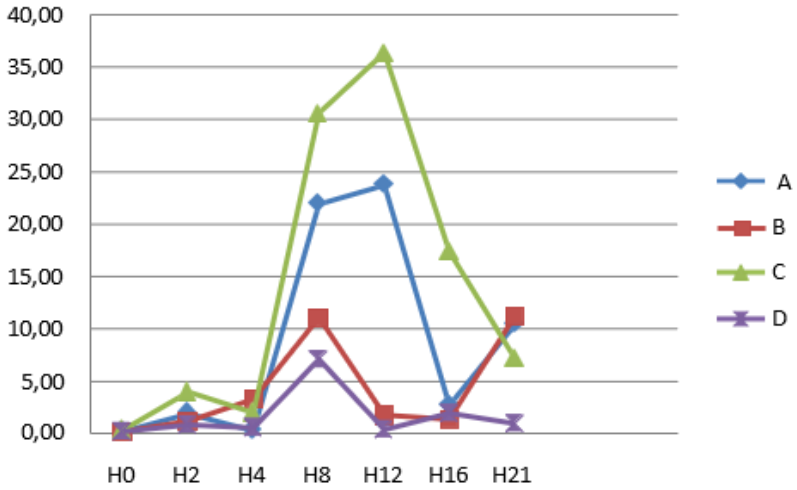

Figure 7. Nitrite Parameters During the Study

Observations revealed that each treatment exhibiting high nitrite levels was found in treatment C, which used bacteria without a carbon source in the form of molasses, reaching 30 mg/L-35 mg/L. This was due to the lack of heterotrophic bacteria and the absence of a carbon source for heterotrophic bacteria to develop. Therefore, in this treatment, heterotrophic bacteria lost out to autotrophic bacteria in growth. Therefore, the bacterial inoculation given in this treatment was unable to change nitrite in catfish cultivation, based on survival.

These high nitrite levels were found on days 8, 12, and 16. However, in heterotrophic systems where there is an increase in the C/N ratio through the addition of a carbon source, the nitrification process will be hampered by the faster heterotrophic process [35].

The highest nitrite figures were also obtained in treatment A, namely treatment using only feed without the role of bacteria and molasses of 22 mg / L -24 mg / L on days 8 and 12, this is also thought to be due to the activity of autotrophic bacteria in converting ammonia into nitrite which can endanger the survival of fish. On day 16, there was a decrease in nitrite levels of 2.79 mg / L, this was because on that day there was a decrease in ammonia so that autotrophic bacteria did not convert much ammonia into nitrite and then there was an increase in nitrite again on day 21 of 10.45 mg / L, this was thought to be because on that day there was a fairly high increase in ammonia so that autotrophic bacteria used a lot of ammonia and converted it into nitrite.

According to Ebeling and Timmons [38], the threshold value for nitrite cultivation is less than 1 mg/L. The mechanism of nitrite toxicity is its effect on oxygen transport in the blood and tissue damage. The accumulation of nitrite in the tank is thought to be due to an imbalance between the rate of conversion from nitrite to nitrate and from ammonia to nitrite. Nitrite ions formed in the water are absorbed into the blood and enter erythrocytes, then oxidize the Fe^{2+} (ferrous) ions in hemoglobin (Hb) and convert them to Fe^{3+} (ferric) ions, resulting in the formation of MetHb. This MetHb is no longer capable of carrying oxygen to the tissues, resulting in a lack of oxygen in the blood (hypoxia) and possible oxygen deficiency in these tissues [11].

If the change from Hb to MetHb reaches 20%-30% of the normal Hb value, hypoxia will occur, namely a lack of oxygen in the blood of fish suffering from poisoning so that their blood is no longer able to carry oxygen. If this situation continues, and the change in Hb to MetHb reaches 80%-90% of normal Hb, then a condition occurs that can cause poisoning for fish.

In other treatments, nitrite values were not significantly different from those in treatments A and C. For example, in treatment B, which used molasses as a carbon source, nitrite values were low on days 0, 2, and 4. This was due to the presence of fewer autotrophic bacteria than heterotrophic bacteria in that treatment, and the small amount of ammonia produced, resulting in competition between heterotrophic and autotrophic bacteria for ammonia conversion. The high sugar content of molasses can be utilized in aquaculture systems as a carbon source [39], [40].

On days 8 and 21, nitrite levels increased. This is suspected to be due to the presence of a sufficient number of autotrophic bacteria on those days, resulting in a further increase in nitrite. With the abundance of autotrophic bacteria, the naturally occurring heterotrophic bacteria are less able to oxidize ammonia into cell biomass. In the treatment with a heterotrophic system, nitrite levels can be suppressed by the role of bacteria and molasses, although the suppression of nitrite levels does not follow the threshold of nitrite values of less than 1 mg / L, this is due to the large number of heterotrophic bacteria in converting ammonia into cell biomass and autotrophic bacteria in the treatment are less able to convert ammonia with heterotrophic bacteria. This has proven that the heterotrophic system can inhibit or minimize nitrite levels as little as possible compared to other treatments.

3.2.3 Nitrate

Nitrate (NO_3) is the primary form of nitrogen in natural waters and a key nutrient for plants and algae. Nitrate nitrogen is highly soluble in water and is stable because it results from the complete oxidation of nitrogen compounds in water. Nitrate levels in unpolluted waters are typically higher than ammonium. Nitrate is stable in water.

Nitrate is not toxic to aquatic organisms. According to Ebeling and Michael [38], a good nitrate level for aquaculture environments is around 0-400 mg/L. This can be seen in Figure 8, which shows the nitrate levels for each treatment.

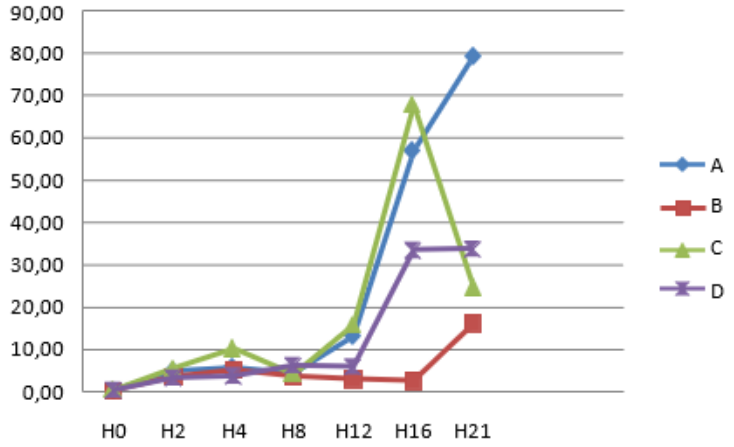

Figure 8. Nitrate Parameters During the Study

The results for each treatment showed that high nitrate levels were found in Treatment A, which used feed alone without the use of bacteria and molasses, reaching 56.94 mg/L-79.23 mg/L on days 16 and 21. This was due to the conversion of nitrite to nitrate by autotrophic bacteria. From days 0 to 12, nitrate levels decreased. This was due to the low activity of autotrophic bacteria in converting nitrite to nitrate, resulting in a decrease in nitrate levels. It is assumed that autotrophic bacteria were still oxidizing ammonia to nitrite.

Treatment B, which used molasses as a carbon source, showed low nitrate levels throughout the study from days 0 to 21. This is assumed to be due to the presence of naturally occurring heterotrophic bacteria growing well in the funnel, resulting in a decrease in nitrate levels in this treatment. In treatment C, high nitrate levels were obtained on day 16 to day 21. This was no different from treatment A. The high nitrate levels on that day were due to the process of converting ammonia into nitrite carried out by autotrophic bacteria. By converting high nitrite levels, high nitrate levels were also produced. On day 0 to day 12, there was a decrease in nitrate levels. It is assumed that on that day there was a decrease in autotrophic bacteria so that with the low growth of autotrophic bacteria, the work of these bacteria in oxidizing ammonia into nitrite and then into nitrate became low.

In treatment D, using bacterial inoculation and molasses as a carbon source, nitrate levels decreased from day 0 to day 12 due to the activity of heterotrophic bacteria converting ammonia into cell biomass. This activity of these bacteria reduced nitrate levels in treatment D. However, on days 16 and 21, nitrate levels increased by 33.50 mg/L to 33.83 mg/L. This was due to the presence of other heterotrophic bacteria converting ammonia to nitrite and oxidizing nitrite to nitrate. However, the activity of heterotrophic bacteria in converting ammonia and nitrite was very low.

Nitrate compounds are the end result of a chemoautotrophic bacteriological process, namely nitrification bacteria. In this process, ammonia is first converted to nitrite by *Nitrosomonas sp.* Nitrite is then converted to nitrate by *Nitrococcus sp.* [35].

3.2.4 Volatile Suspended Solids (VSS)

Volatile Suspended Solids (VSS) is the amount of organic solids suspended in water. Organic solids are solids that burn at 550°C after being dried at 103°C and are retained on the filter.

Volatile Suspended Solids levels can be a key indicator of floc quality; the higher the levels in the water, the higher the biofloc quality. The higher the levels, the higher the quality of the feed, which is not utilized by the catfish. This can be seen in Figure 9, which shows the volatile suspended solids values for each treatment.

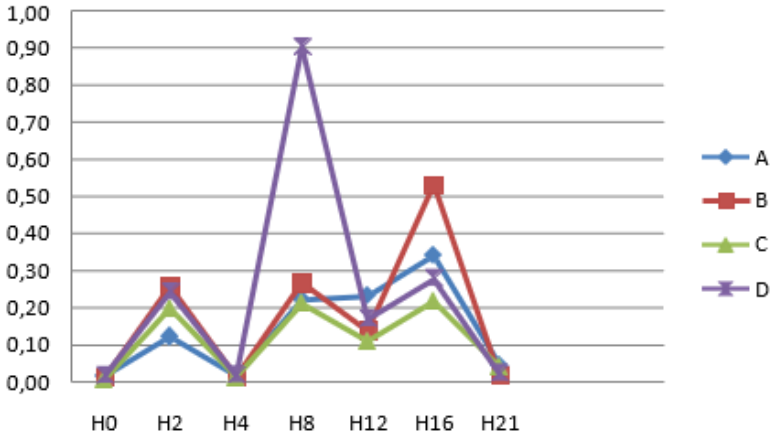

Figure 9. Volatile Suspended Solid (VSS) Parameters During the Study

The average level of Volatile Suspended Solids (VSS) in a 200 L fiber tank with a stocking density of 20 fish per 50 grams. The highest value was found in treatment D, using bacteria and molasses at 0.90 mg/L on day 8 in the heterotrophic system. This resulted in a high survival rate for catfish, and it is assumed that the bacterial population was in the log phase, or growth phase, on that day.

On days 0, 2, 4, and 12, the volatile suspended solids decreased. This is suspected to be due to the bacteria not yet maximizing their utilization of molasses as a carbon source or the bacteria being in the adaptation phase, resulting in slow growth. On day 21, the volatile suspended solids decreased, due to a lack of carbon sources in that treatment, leading to competition among heterotrophic bacteria. A relatively high volatile suspended solids value was also found in treatment B, at 0.53 mg/L, although the high volatile suspended solids value was not found as in treatment D, the heterotrophic system. This is suspected because the addition of molasses to the culture medium is expected to reduce nitrogen waste and increase fish growth, thus increasing production.

The relatively high volatile suspended solids value in treatment B is likely due to the role of the naturally occurring heterotrophic bacteria in the tank, although growth of these bacteria is not as rapid as in the heterotrophic system. Heterotrophic bacteria utilize organic carbon as an energy source, correlated with nitrogen, which is used for protein synthesis to produce new cell material [41], [42], [43].

On day 0, the volatile suspended solids value decreased because the naturally occurring heterotrophic bacteria living in the funnel were not yet maximally utilizing the carbon source, or because they were in the adaptation phase to the environment. On days 2 and 4, volatile suspended solids values increased. This is suspected to be due to heterotrophic bacteria utilizing the carbon source as nutrition, resulting in an increase in the bacterial population in treatment B.

On day 4, a decrease occurred in treatment B. This is suspected to be due to the bacteria returning to the adaptation phase or a lack of molasses as a carbon source. The decrease in volatile suspended solids values is likely due to the low dissolved oxygen value of 0.73 on day 4. According to Schneider et al. [44], a minimum dissolved oxygen level of 2 mg/L is required to support optimal heterotrophic processes. On day 21, volatile suspended solids values decreased again in treatment B. This is suspected to be due to the heterotrophic bacteria being in the death phase or the lack of molasses as a carbon source, resulting in competition among heterotrophic bacteria for the use of molasses.

In treatment C, with bacterial inoculation without a carbon source in the form of molasses, a decrease occurred on days 0 and 4. This is suspected because on those days the minabacto bacteria inoculation was in the adaptation phase or the absence of a carbon source as a growth stimulant for the minabacto bacteria inoculation, or the low ability of the minabacto bacteria inoculation to survive with the natural bacteria present in that treatment.

On days 2, 8, and 16, there was an increase in the volatile suspended solid value. This increase was because on those days the inoculated bacteria were in the growth phase, so it was assumed that the autotrophic

bacteria had begun to grow well. On day 21, there was another decrease, this was because on that day the bacteria were in the death phase..

3.2.5 Temperature

Temperature is the most important parameter in fish farming, as it regulates both physical and chemical processes within a body of water. Water temperature affects oxygen solubility, substrate composition, turbidity, and the rate of chemical reactions. This is demonstrated in Figure 10, which shows that temperature values for all treatments are still considered normal for catfish (*Clarias sp*.) growth.

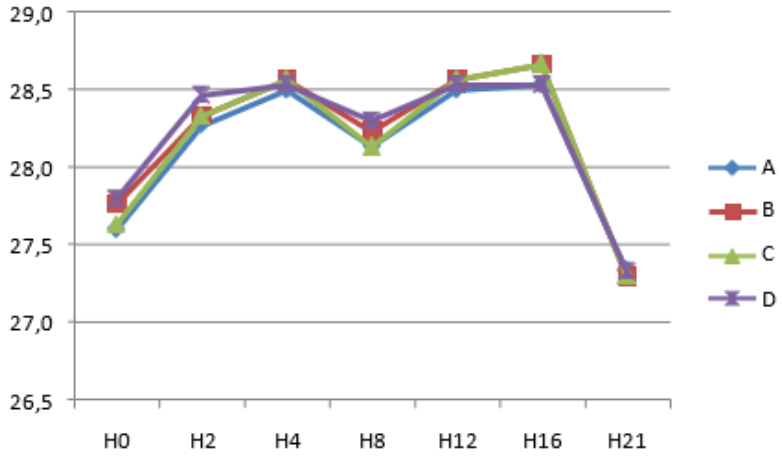

Figure 10. Temperature Quality Parameters During the Research

From the first day of observation until the final day, all treatments showed temperatures still considered normal for catfish growth, namely 27.5 to 28.5°C. This proves that the ideal temperature for catfish growth is between 27 and 29°C. Similarly, stated that the optimal water temperature for freshwater fish ranges from 24 to 30°C in subtropical areas and 26 to 32°C in tropical areas.

At temperatures above 32°C, catfish fry begin to lose their appetite and the digestive process is disrupted [45], [46]. This disruption is due to decreased digestive enzyme activity due to denaturation [47], [48]. Sudden temperature changes can cause stress and subsequent death of catfish, therefore, a stable temperature is expected throughout the rearing period . Temperature affects dissolved oxygen levels, and oxygen is inversely proportional to temperature. This means that higher temperatures reduce oxygen solubility. The higher the water temperature, the higher the metabolic rate. The vertical distribution of temperature affects water viscosity.

3.2.6 pH

pH is a measurement of hydrogen ion activity in water, indicating the balance between acidity and alkalinity [49]. In general, most aquatic biota are sensitive to changes in pH, and almost all prefer a pH of 7-8.5. The pH value significantly influences the biochemical processes occurring in a body of water. For example, the nitrification process will stop when the water pH is low. This can be seen in Figure 11, which shows the pH values for each different treatment.

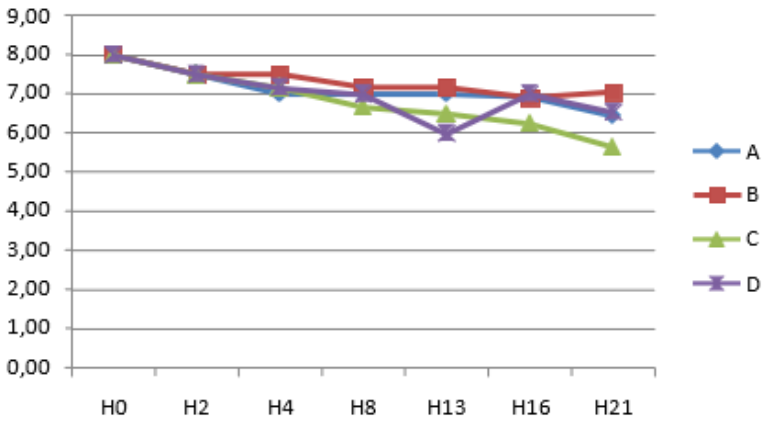

Figure 11. pH Parameters During the Research

In general, the pH values measured in this study were still within the optimal range for most fish and other aquatic biota. The optimal pH range for fish is 6.5-8.5 [50], [51]. Meanwhile, heterotrophic bacteria, such as *Bacillus sp*., actively grow at a pH of 5.5-8.5.

In each treatment, the pH fluctuated, and a decrease in the pH range from the normal range was only found in treatment C, which used bacteria but did not use molasses as a carbon source, at 5.64. This is likely due to the addition of fish biomass in the funnel. With increased biomass, respiration activity increases, resulting in increased oxygen consumption. With increased oxygen consumption, the amount of carbon dioxide released increases. This condition shifts the reaction to the formation of H+ ions, causing the pH to drop.

At a low pH, dissolved oxygen content decreases. Furthermore, the pH range is still considered normal and good for fish survival. A pH below 4 and above 11 will kill fish, while a pH of 9.5 will disrupt catfish development.

3.2.7 Dissolved Oxygen (DO)

The main source of oxygen in a body of water comes from a diffusion process from the free air and the results of photosynthesis of organisms living in the water [52], [53], the speed of oxygen diffusion from the air depends on several factors, such as water turbidity, temperature, salinity, movement of water and air masses. Data regarding dissolved oxygen can be seen in Figure 12 below which shows the dissolved oxygen parameters for each treatment.

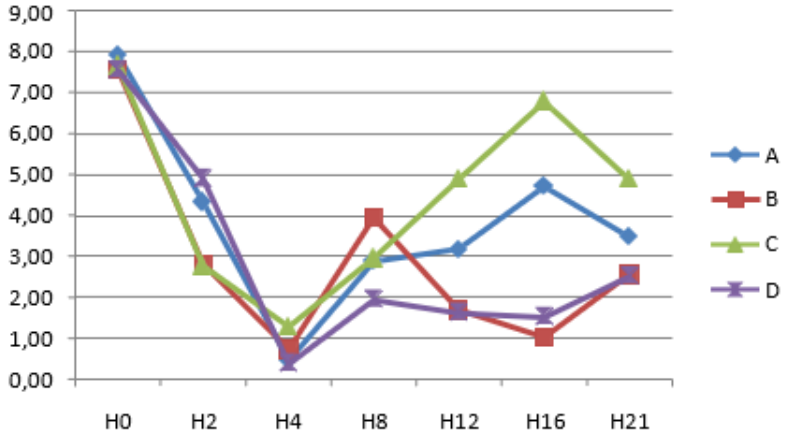

Figure 12. Dissolved Oxygen Parameters During the Study

The results of the analysis of dissolved oxygen levels across the different treatments fluctuated significantly. Aeration in each catfish tank was insufficient to maintain optimal dissolved oxygen levels. Low dissolved oxygen levels do not support the optimal functioning of the heterotrophic system. According to Schneider et al. [44], a minimum dissolved oxygen level of 2 mg/L is required to support optimal heterotrophic processes. For most fish, adequate dissolved oxygen levels are also required to meet their respiratory needs. In a study using a stocking density of 20 fish per 50 grams of water, catfish, which have the ability to utilize oxygen from additional aeration, were used.

Data shows that the dissolved oxygen range in each treatment is still considered quite ideal, ranging from 3-8 mg/L. This is because the water quality is still good and not heavily polluted with nitrogen waste levels that can cause toxicity to fish survival. Likewise, the role of aeration in each funnel is useful for continuously supplying oxygen. On the 4th and 16th days, there was a decrease in dissolved oxygen in all treatments. This is because the aeration stone on that day was blocked by dirt or leftover feed deposits, so that dissolved oxygen could not be supplied smoothly or due to damage to the equipment and calibration errors. In general, African catfish live normally at a dissolved oxygen concentration of 4 mg/liter. If the oxygen supply is below 20% of the normal requirement, African catfish will become weak and can cause death [54], [55]. The dissolved oxygen that supports fish growth and production is more than 3 ppm. This is in line with the opinion of that dissolved oxygen levels in a 200 L tank decrease due to the presence of pollutants that can consume oxygen [56].

These pollutants include organic and inorganic materials derived from fish waste and leftover food, as well as the large amount of sediment at the bottom of the tank, which blocks the airstone, preventing aeration from supplying sufficient oxygen. Dissolved oxygen levels should not be less than 1.7 mg/L for 8 hours, with a saturation level of at least 70%.

Furthermore, the heterotrophic process of bacterial biosynthesis occurs more quickly than that of algae or nitrification, approximately 10 hours compared to 24-48 hours. According to Schneider et al. [44], to support the optimal heterotrophic process, a minimum dissolved oxygen level of 2 mg/L is required. The heterotrophic production coefficient is 1500% (15 times) greater than that of autotrophic bacteria, so that the need for oxygen is

reduced, excessive oxygen reduction in the water causes the water condition to become anaerobic, low dissolved oxygen conditions do not support the heterotrophic system to run optimally.

The results of this study confirm previous findings showing that the addition of simple carbon sources—such as glycerol or molasses—in biofloc systems significantly improves fish survival and nitrogen removal efficiency. For example, Dauda et al. [57] reported that the use of glycerol as a carbon source in the cultivation of Clarias gariepinus fingerlings not only improved nitrogen removal but also increased survival rates significantly above the control group. Furthermore, a review by Yu et al. [58] confirmed that biofloc systems improve production performance, including survival rates and fish physiological health. Other studies also explain that simple carbon sources disperse more quickly in water and are more quickly utilized by heterotrophic bacteria to form flocs that help reduce ammonia compared to complex carbon sources [59]. Overall, your findings on water quality management and high survival rates (80–90%) through the application of bacteria and molasses at high densities in Clarias sp. not only strengthen existing empirical evidence but also provide new contributions regarding dosage optimization and practical approaches in intensive biofloc systems.

This research presents a novelty through the application of a heterotrophic system based on the addition of bacteria and molasses in intensive cultivation of catfish (*Clarias sp.*) which is directly compared with conventional systems and other treatment variations. The results show that the combination of bacteria and molasses is able to significantly reduce ammonia and nitrite levels, improve water quality, and maintain catfish survival rates up to 80–90% in the research cycle, which has not been widely reported at high stocking densities with a biofloc system in small-scale containers. This approach proves the efficiency of converting nitrogen waste into biomass through a heterotrophic mechanism that is faster than the autotrophic nitrification process, while optimizing the use of carbon sources from molasses as natural microbial feed.

The results of this study have implications for the development of environmentally friendly fish farming technologies that can increase productivity through heterotrophic water quality management. These findings can serve as a reference for fish farmers to integrate bacteria and organic carbon sources such as molasses to reduce nitrogen pollutants and minimize fish mortality, especially at high densities. However, this study is limited by its laboratory-scale experiments, relatively short maintenance duration, and the inability to evaluate economic aspects in depth. Therefore, further research at a field scale and with longer maintenance periods is needed to test the consistency of the results and the feasibility of its commercial application.

4. CONCLUSION

This study concludes that the heterotrophic system with the addition of bacteria and molasses (Treatment D) is the most effective method for intensive catfish (Clarias sp.) culture, as it maintained the highest survival rate (80–90%) and stabilized water quality by reducing ammonia and nitrite accumulation. In contrast, treatments without balanced bacterial and carbon supplementation showed poor survival due to nitrogen toxicity and unstable conditions. These findings highlight that integrating heterotrophic bacteria with molasses is a practical and sustainable strategy to improve fish survival, enhance water quality, and support efficient aquaculture practices. For further development, it is recommended to conduct further research on the growth and development of catfish with a longer period of time in an intensive heterotrophic-based cultivation system.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to all parties who have contributed to the completion of this research. Appreciation is extended to the laboratory staff and technical team for their assistance in sample preparation and data collection, as well as to colleagues who provided valuable input during the study. Sincere thanks are also given to the institution for providing facilities and support, and to all individuals whose contributions, directly or indirectly, made this research possible.

REFERENCES

[1] H. Kartikaningsih, Yahya, D. Setijawati, and H. I. Fathoni, "The behavior of catfish traders on the food safety perspective in Malang Markets, Indonesia," *Biodiversitas*, vol. 23, no. 5, pp. 2270–2275, 2022, doi: 10.13057/biodiv/d230504.

[2] S. Supriyadi, K. I. Abdillah, and M. Primyastanto, "Risk analysis of catfish cultivation (Pangasius hypophthalmus) business in Gondosuli Village, Gondang, Tulungagung," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1036, no. 1, 2022, doi: 10.1088/1755-1315/1036/1/012025.

[3] R. Abisha, K. K. Krishnani, K. Sukhdhane, A. K. Verma, M. Brahmane, and N. K. Chadha, "Sustainable development of climate-resilient aquaculture and culture-based fisheries through adaptation of abiotic stresses: a review," *J. Water Clim. Chang.*, vol. 13, no. 7, pp. 2671–2689, 2022, doi: 10.2166/wcc.2022.045.

[4] I. A. Bhat, M. A. Rather, I. Ahmad, I. Ahmad, I. N. Mir, and Hussna, "Impact of shifting abiotic factors in aquaculture on fish breeding and reproduction: a review," *Blue Biotechnol.*, vol. 2, no. 1, 2025, doi: 10.1186/s44315-025-00027-9.

[5] H. Wang *et al.*, "Transcriptome analysis revealed potential mechanisms of channel catfish growth advantage over blue catfish in a tank culture environment," *Front. Genet.*, vol. 15, no. April, pp. 1–11, 2024, doi:

10.3389/fgene.2024.1341555.
[6] E. Gisbert *et al.*, *Development, nutrition, and rearing practices of relevant catfish species (Siluriformes) at early stages*, vol. 14, no. 1. 2022. doi: 10.1111/raq.12586.
[7] B. Raza, Z. Zheng, and W. Yang, "A review on biofloc system technology, history, types, and future economical perceptions in aquaculture," *Animals*, vol. 14, no. 10, 2024, doi: 10.3390/ani14101489.
[8] J. Day *et al.*, "A review of 50 years of study of hydrology, wetland dynamics, aquatic metabolism, water quality and trophic status, and nutrient biogeochemistry in the Barataria Basin, Mississippi Delta—System functioning, human impacts and restoration approaches," *Water*, vol. 13, no. 5, p. 642, Feb. 2021, doi: 10.3390/w13050642.
[9] S. P. Lall and S. J. Kaushik, "Nutrition and metabolism of minerals in fish," *Animals*, vol. 11, no. 9, pp. 1–41, 2021, doi: 10.3390/ani11092711.
[10] M. Takvam, C. M. Wood, H. Kryvi, and T. O. Nilsen, "Role of the kidneys in acid-base regulation and ammonia excretion in freshwater and seawater fish: implications for nephrocalcinosis," *Front. Physiol.*, vol. 14, no. June, pp. 1–18, 2023, doi: 10.3389/fphys.2023.1226068.
[11] C. E. Boyd and C. S. Tucker, *Pond Aquaculture Water Quality Management*. Boston, MA: Springer US, 1998. doi: 10.1007/978-1-4615-5407-3.
[12] A. B. Kusunur *et al.*, "Tetracycline resistance potential of heterotrophic bacteria isolated from freshwater fin-fish aquaculture system," *J. Appl. Microbiol.*, vol. 134, no. 4, Apr. 2023, doi: 10.1093/jambio/lxad060.
[13] S. Duan, Y. Zhang, and S. Zheng, "Heterotrophic nitrifying bacteria in wastewater biological nitrogen removal systems: A review," *Crit. Rev. Environ. Sci. Technol.*, vol. 52, no. 13, pp. 2302–2338, Jul. 2022, doi: 10.1080/10643389.2021.1877976.
[14] Y. Ma *et al.*, "Research on nitrogen transformation pathways of a thermophilic heterotrophic nitrifying bacterial consortium GW7," *Front. Microbiol.*, vol. 16, no. June, 2025, doi: 10.3389/fmicb.2025.1578865.
[15] A. Chamoli, A. Bhambri, S. K. Karn, and V. Raj, "Ammonia, nitrite transformations and their fixation by different biological and chemical agents," *Chem. Ecol.*, vol. 40, no. 2, pp. 166–199, Feb. 2024, doi: 10.1080/02757540.2023.2300780.
[16] C. Wang *et al.*, "Improvement of fish production and water quality in a recirculating aquaculture pond enhanced with bacteria-microalgae association," *Aquaculture*, vol. 547, p. 737420, 2022, doi: 10.1016/j.aquaculture.2021.737420.
[17] G. Kaur *et al.*, "Recent advancements in deep learning fameworks for precision fish farming opportunities, challenges, and applications," *J. Food Qual.*, vol. 2023, pp. 1–11, Feb. 2023, doi: 10.1155/2023/4399512.
[18] M. Dara, P. Carbonara, C. La Corte, D. Parrinello, M. Cammarata, and M. G. Parisi, "Fish welfare in aquaculture: Physiological and immunological activities for diets, social and spatial stress on Mediterranean aqua cultured species," *Fishes*, vol. 8, no. 8, 2023, doi: 10.3390/fishes8080414.
[19] P. Lindholm-Lehto, "Water quality monitoring in recirculating aquaculture systems," *Aquac. Fish Fish.*, vol. 3, no. 2, pp. 113–131, 2023, doi: 10.1002/aff2.102.
[20] S. K. Nagothu, P. Bindu Sri, G. Anitha, S. Vincent, and O. P. Kumar, "Advancing aquaculture: fuzzy logic-based water quality monitoring and maintenance system for precision aquaculture," *Aquac. Int.*, vol. 33, no. 1, pp. 1–21, 2025, doi: 10.1007/s10499-024-01701-2.
[21] H. Brandão, Í. V. Xavier, G. K. K. Santana, H. J. K. Santana, D. Krummenauer, and W. Wasielesky, "Heterotrophic versus mixed BFT system: impacts on water use, suspended solids production and growth performance of Litopenaeus vannamei," *Aquac. Eng.*, vol. 95, p. 102194, 2021, doi: 10.1016/j.aquaeng.2021.102194.
[22] A. M. Alinsangao, L. B. Igano, and P. A. M. Flores, "Efficiency of biofloc system on the growth and survival of African catfish (Clarias gariepinus) fingerlings," *J. Agric. Res. Dev. Ext. Technol.*, vol. 1, no. 1, pp. 10–20, 2019, [Online]. Available: https://jardet.usm.edu.ph/index.php/jardet/article/view/2
[23] Salamah, N. Bambang Priyo Utomo, M. Yuhana, and Widanarni, "Kinerja pertumbuhan ikan lele dumbo, Clarias gariepinus Burchel 1822 yang dikultur pada sistem berbasis bioflok dengan penambahan sel bakteri heterotrofik [Growth performance of African catfish, Clarias gariepinus Burchel 1822 cultured in a biofloc-based ," *J. Iktiologi Indones.*, vol. 15, no. 2, pp. 155–164, 2014.
[24] P. C. Nath *et al.*, "Valorization of food waste as animal feed: a step towards sustainable food waste management and circular bioeconomy," *Animals*, vol. 13, no. 8, pp. 1–23, 2023, doi: 10.3390/ani13081366.
[25] G. C. Shurson, "'What a waste'-can we improve sustainability of food animal production systems by recycling food waste streams into animal feed in an era of health, climate, and economic crises?," *Sustain.*, vol. 12, no. 17, 2020, doi: 10.3390/su12177071.
[26] Y. Avnimelech, M. Kochva, and S. Diab, "Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio," *Isr. J. Aquac.*, vol. 46, no. 3, pp. 119–131, 1994.
[27] D. E. Brune, G. Schwartz, A. G. Eversole, J. A. Collier, and T. E. Schwedler, "Intensification of pond aquaculture and high rate photosynthetic systems," *Aquac. Eng.*, vol. 28, no. 1, pp. 65–86, 2003, doi: 10.1016/S0144-8609(03)00025-6.
[28] A. G. J. Tacon, "Thematic review of feeds and feed management practices in shrimp aquaculture," *Intellect. Prop.*, pp. 1–38, 2002, [Online]. Available: http://library.enaca.org/Shrimp/Case/Thematic/FinalFeed.pdf
[29] P. Bossier and J. Ekasari, "Biofloc technology application in aquaculture to support sustainable development goals," *Microb. Biotechnol.*, vol. 10, no. 5, pp. 1012–1016, 2017, doi: 10.1111/1751-7915.12836.
[30] C. Li, Z. Ge, L. Dai, and Y. Chen, "Integrated application of biofloc technology in aquaculture: a review," *Water (Switzerland)*, vol. 17, no. 14, 2025, doi: 10.3390/w17142107.
[31] T. Lovell, *Nutrition and Feeding of Fish*. Boston, MA: Springer US, 1998. doi: 10.1007/978-1-4615-4909-3.
[32] P. Pallerla *et al.*, "Evaluation of amino acids and other related metabolites levels in end-stage renal disease (ESRD) patients on hemodialysis by LC/MS/MS and GC/MS," *Anal. Bioanal. Chem.*, vol. 415, no. 26, pp. 6491–6509, 2023, doi: 10.1007/s00216-023-04926-x.
[33] R. R. Stickney, *Principles of warmwater aquaculture.* John Wiley & Sons.

[34] A. G. Heath, *Water Pollution and Fish Physiology*. CRC Press, 2018. doi: 10.1201/9780203718896.
[35] M. Ruby and Velasco Mario, "Role of bacteria on nutritional and management strategies in aquaculture systems - Responsible Seafood Advocate," *Glob. Advocate*, pp. 35–36, 2000, [Online]. Available: https://www.globalseafood.org/advocate/role-of-bacteria-on-nutritional-and-management-strategies-in-aquaculture-systems/
[36] M. Wang, Y. Wu, J. Zhu, C. Wang, Y. Zhu, and Q. Tian, "The new developments made in the autotrophic and heterotrophic ammonia oxidation," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 178, no. 1, pp. 8–13, 2018, doi: 10.1088/1755-1315/178/1/012016.
[37] W. Fu *et al.*, "Advances in research into and applications of heterotrophic nitrifying and aerobic denitrifying microorganisms," *Front. Environ. Sci.*, vol. 10, no. July, pp. 1–16, 2022, doi: 10.3389/fenvs.2022.887093.
[38] J. M. Ebeling and M. B. Timmons, "Recirculating Aquaculture Systems," in *Aquaculture Production Systems*, Wiley, 2012, pp. 245–277. doi: 10.1002/9781118250105.ch11.
[39] A. F. Rocha *et al.*, "Water quality and juvenile development of mullet Mugil liza in a biofloc system with an additional carbon source: Dextrose, liquid molasses or rice bran?," *Aquac. Res.*, vol. 53, no. 3, pp. 870–883, Feb. 2022, doi: 10.1111/are.15628.
[40] W. Ren *et al.*, "Tropical cellulolytic bacteria: potential utilization of sugarcane bagasse as low-cost carbon source in aquaculture," *Front. Microbiol.*, vol. 12, no. October, pp. 1–11, 2021, doi: 10.3389/fmicb.2021.745853.
[41] W. Wang *et al.*, "Microbial protein production under mixotrophic mode: ammonium assimilation pathway and C/N ratio optimization," *J. Environ. Chem. Eng.*, vol. 12, no. 1, p. 111727, 2024, doi: 10.1016/j.jece.2023.111727.
[42] T. K. Marella, R. Bhattacharjya, and A. Tiwari, "Impact of organic carbon acquisition on growth and functional biomolecule production in diatoms," *Microb. Cell Fact.*, vol. 20, no. 1, pp. 1–13, 2021, doi: 10.1186/s12934-021-01627-x.
[43] Y. Cai *et al.*, "Effects of C/N ratio on the growth and protein accumulation of heterotrophic Chlorella in broken rice hydrolysate," *Biotechnol. Biofuels Bioprod.*, vol. 15, no. 1, pp. 1–11, 2022, doi: 10.1186/s13068-022-02204-z.
[44] O. Schneider, V. Sereti, M. A. M. Machiels, E. H. Eding, and J. A. J. Verreth, "The potential of producing heterotrophic bacteria biomass on aquaculture waste," *Water Res.*, vol. 40, no. 14, pp. 2684–2694, 2006, doi: 10.1016/j.watres.2006.05.008.
[45] S. M. Kasihmuddin, Z. C. Cob, N. M. Noor, and S. K. Das, "Effect of different temperature variations on the physiological state of catfish species: a systematic review," *Fish Physiol. Biochem.*, vol. 50, no. 2, pp. 413–434, 2024, doi: 10.1007/s10695-024-01323-8.
[46] S. M. Kasihmuddin, M. A. Ghaffar, and S. K. Das, "Rising temperature effects on growth and gastric emptying time of freshwater african catfish (Clarias gariepinus) fingerlings," *Animals*, vol. 11, no. 12, 2021, doi: 10.3390/ani11123497.
[47] K. Kut, G. Bartosz, and I. Sadowska-Bartosz, "Denaturation and digestion increase the antioxidant capacity of proteins," *Processes*, vol. 11, no. 5, pp. 1–11, 2023, doi: 10.3390/pr11051362.
[48] B. Wang, Y. P. Timilsena, E. Blanch, and B. Adhikari, "Lactoferrin: structure, function, denaturation and digestion," *Crit. Rev. Food Sci. Nutr.*, vol. 59, no. 4, pp. 580–596, 2019, doi: 10.1080/10408398.2017.1381583.
[49] A. Goyal and M. T. M. Koper, "The interrelated effect of cations and electrolyte pH on the hydrogen evolution reaction on gold electrodes in alkaline media," *Angew. Chemie - Int. Ed.*, vol. 60, no. 24, pp. 13452–13462, 2021, doi: 10.1002/anie.202102803.
[50] D. Makararpong, M. Ketcham, T. Ganokratanaa, N. Chumuang, W. Yimyam, and P. Pramkeaw, "Development of a system for measuring pH and oxygen levels in water using Internet of Things (IoT) technology," in *2025 IEEE International Conference on Cybernetics and Innovations (ICCI)*, 2025, pp. 1–7. doi: 10.1109/ICCI64209.2025.10987306.
[51] A. Mariu, A. M. M. Chatha, S. Naz, M. F. Khan, W. Safdar, and I. Ashraf, "Effect of temperature, pH, salinity and dissolved oxygen on fishes," *J. Zool. Syst.*, vol. 1, no. 2, pp. 1–12, 2023, doi: 10.56946/jzs.v1i2.198.
[52] J. A. Raven and M. Lavoie, "Movement of Aquatic Oxygenic Photosynthetic Organisms BT - Progress in Botany Vol. 83," U. Lüttge, F. M. Cánovas, M.-C. Risueño, C. Leuschner, and H. Pretzsch, Eds., Cham: Springer International Publishing, 2023, pp. 315–343. doi: 10.1007/124_2021_55.
[53] V. Singh, R. K. Srivastava, and A. K. Bhatt, "Dissolved Oxygen and Water Quality BT - Battling Air and Water Pollution: Protecting Our Planet's Vital Resources," V. Singh, R. K. Srivastava, and A. K. Bhatt, Eds., Singapore: Springer Nature Singapore, 2025, pp. 111–120. doi: 10.1007/978-981-96-4375-2_8.
[54] M. A. O. Dawood, A. E. Noreldin, and H. Sewilam, "Blood biochemical variables, antioxidative status, and histological features of intestinal, gill, and liver tissues of African catfish (Clarias gariepinus) exposed to high salinity and high-temperature stress," *Environ. Sci. Pollut. Res.*, vol. 29, no. 37, pp. 56357–56369, 2022, doi: 10.1007/s11356-022-19702-0.
[55] S. Langi, S. Maulu, O. J. Hasimuna, V. Kaleinasho Kapula, and M. Tjipute, "Nutritional requirements and effect of culture conditions on the performance of the African catfish (Clarias gariepinus): a review," *Cogent Food Agric.*, vol. 10, no. 1, p., 2024, doi: 10.1080/23311932.2024.2302642.
[56] E. Beltrán-Flores, S. Tayar, P. Blánquez, and M. Sarrà, "Effect of dissolved oxygen on the degradation activity and consumption capacity of white-rot fungi," *J. Water Process Eng.*, vol. 55, p. 104105, 2023, doi: 10.1016/j.jwpe.2023.104105.
[57] A. B. Dauda *et al.*, "Different carbon sources affects biofloc volume, water quality and the survival and physiology of African catfish Clarias gariepinus fingerlings reared in an intensive biofloc technology system," *Fish. Sci.*, vol. 83, no. 6, pp. 1037–1048, Nov. 2017, doi: 10.1007/s12562-017-1144-7.
[58] Y.-B. Yu, J.-H. Choi, J.-H. Lee, A.-H. Jo, K. M. Lee, and J.-H. Kim, "Biofloc technology in fish aquaculture: a review," *Antioxidants*, vol. 12, no. 2, p. 398, Feb. 2023, doi: 10.3390/antiox12020398.
[59] S. Chakrapani *et al.*, "Utilization of complex carbon sources on biofloc system and its influence on the microbial

composition, growth, digestive enzyme activity of pacific white shrimp, Penaeus vannamei culture," *Turkish J. Fish. Aquat. Sci.*, vol. 22, no. 4, 2021, doi: 10.4194/trjfas18813.