Biofloc-based catfish cultivation and its effect on the dynamics of water quality

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Abstract. This research aimed to monitor and maintain the dynamics and fluctuations of water quality within the optimal range and to ensure the good growth and health of catfish (*Clarias gariepinus*) in biofloc-based systems. In this study, four biofloc-based treatments for catfish cultivation in fishponds and their effect on the dynamics of water quality were studied. Treatments differ in regards to the amount of biofloc used in each one. Except for the biological oxygen demand (BOD)/chemical oxygen demand (COD) ratio, the water quality concentrations of dissolved oxygen (DO), BOD, COD, ammonia, nitrite, and nitrate in the examined water were below acceptable limits. Water quality was always dynamic and fluctuating due to human activities, natural processes, and the interaction between water and the surrounding environment. Therefore, monitoring water quality dynamics was important to ensure that water quality standards were met and necessary action could be taken if problems were found. These findings indicate that regular water quality monitoring is necessary to prevent negative impacts on fish survival and growth and that the fish is safe for human consumption.

Key Words: bio balls, BOD/COD, carbonization, fluctuations.

Introduction. Catfish (*Clarias gariepinus*) is a freshwater fish popularly consumed in various countries, including Indonesia. The rapid growth of the freshwater fisheries industry has significantly increased catfish production. However, this growth also brings new challenges in maintaining water quality in catfish farming ponds (Deswati & Sutopo 2022). *Clarias* sp. waste problems can arise due to: 1) increasing organic waste from fish manure, feed residue, and other organic particles; 2) water pollution due to improper catfish treatment; 3) the impact of increased nutrients in water due to catfish waste which can cause eutrophication, namely the excessive growth of algae and other algae. The problem can disrupt the harmony of aquatic ecosystems and harm other organisms (Singh et al 2022; Arvanitoyannis & Tserkezou 2014).

It is important to monitor and maintain the dynamics and fluctuations of water quality so that it is within optimal ranges to ensure good growth and health of catfish (Deswati et al 2023a). One way to overcome the problem of catfish waste is to apply biofloc technology (Hargreaves 2013; Avnimelech 1999). Biofloc technology is an innovative approach to aquaculture that integrates biofloc principles to increase production efficiency, reduce environmental impact, and improve water quality in aquaculture systems (Deswati et al 2021a,b,c: Deswati et al 2022a,b,c,d; Deswati et al 2023a,b,c; Emerenciano et al 2017).

Biofloc formation is a complex process that involves interactions between microorganisms, nutrients, and environmental conditions in aquaculture systems. Biofloc is an aggregate of microorganisms, including bacteria, fungi, and protozoa, which form network-shaped structures in aquaculture water (Collazos-Lasso & Arias-Castellanos 2015; Khanjani et al 2022a,b). The mechanism of biofloc formation involves the stages of initiation, accumulation, retention, and maturity. This process involves complex interactions between microorganisms, nutrients, and environmental conditions (Crab et al 2007; Deb et al 2020; Pinho & Emerenciano 2021). A deeper understanding of this mechanism can help aquaculture managers to optimize production and maintain good water quality (Zafar & Rana 2022).

Biofloc technology has been applied to an aquaponic system integrating hydroponic tilapia (*Oreochromis niloticus*) and vegetable cultivation. It turns out that the use of biofloc can improve water quality (pH, ammonia, nitrite, nitrate, biological oxygen demand, chemical oxygen demand), heavy metals (Cu, Cd, Pb, and Zn), and macro-micro nutrients (Deswati et al 2021a,b,c; Deswati et al 2022a,b,c). These findings reinforce the results of previous studies (Kuhn et al 2009; Avnimelech & Kochba 2009; Setiawan et al 2016; Sukenda et al 2016) that the application of biofloc technology can improve water quality, biosecurity, productivity, and feed efficiency and reduce FCR (feed conversion ratio). Furthermore, Deswati et al (2022d) and Deswati (2023b) have researched biofloc-based small-scale catfish farming and its impact on water quality and production. The treatments used were: A (control); B (biofloc); C (biofloc + bio balls), D (biofloc + carbonation); and E (biofloc + carbonation + bio balls). Treatment E was the best treatment and deserves to be developed further.

Therefore, this study aimed to monitor and maintain the dynamics and fluctuations of water quality within the optimal range and to ensure the good growth and health of catfish based on biofloc. For this reason, the research design was modified according to the previous studies (Deswati et al 2022d; Deswati et al 2023b), namely: treatment A (100 mL biofloc + 2 kg carbonization + 50 bio balls); B (150 mL biofloc + 2 kg carbonization + 50 bio balls); C (200 mL biofloc + 2 kg carbonization + 50 bio balls), and D (250 mL biofloc + 2 kg carbonization + 50 bio balls).

Carbonation technology was applied to increase the C/N ratio. The manufacture was carried out by modifying the Ogello et al (2021) procedure. Bioball is a biological filter that improves the performance of floc-forming microorganism consortia. The bio ball used is a spherical model because it has a denser cavity and is more effective in capturing dirt to be decomposed by decomposing bacteria (Deswati et al 2021a,b,c; Deswati et al 2022a,b,c,d).

Material and Method

Biolacto bacteria breeding. Biolacto bacteria propagation was based on the procedure of Deswati et al (2021a,b,c; 2022a,b,c,d; 2023a,b,c), with the following steps: 1) preparing ingredients (1 pineapple fruit, 3 bananas, 3 grains of vitamin C, 3 grains of vitamin B complex, fermented yeast (1 unit), 1 sachet of baker's yeast, 3 egg yolks, 1.25 kg of granulated sugar), mashed and stirred together with biolacto bacteria (100 g); 2) all these materials were put into gallons, water was added until they were 90% full, they were tightly closed, and aerated continuously; 3) bacterial culture was carried out for ten days, and the propagation was successful if there was a slight fermented yeast odor with a new yellow color.

Carbonation technology. Carbonation preparation was done by modifying the procedure of Ogello et al (2021), namely:

1. Preparing a container (capacity 100 L) to carbonate rice husks, and chicken manure.
2. Carbonated rice husks were mixed with cow manure in a ratio of 3:1, then moistened to 60 %.
3. Lactic acid bacteria (LAB), a fermented product of rice washing and fresh cow's milk, was added to the mixture and fermented for 20 days in an airtight bucket.
4. The carbonation product was put into a straining cloth and hung in the fishpond as fertilizer.

Carbon provides energy for LAB and converts the waste into fish feed.

Bio balls addition. Fifty bio balls were used per fishpond (FP). The bio balls were used because of the denser cavities, and are more effective at capturing dirt to be decomposed by decomposing bacteria (Deswati et al 2021a,b,c; 2022a,b,c,d; 2023b).

Biofloc application for Clarias gariepinus production

Biofloc system nutrients in fishponds. Stages of nutrition at the beginning and per ten days are: 1) weighing 40 g of dolomite lime, dissolved in 100 mL of water and put into a fishpond (FP) (1000 L capacity) and waiting for 30 minutes; 2) 250 g of salt dissolved in water are put into the FP; 3) after 30 minutes, a mixture of molasses and water that has been boiled (ratio 1:1), cooled, is put into the FP as much as 200 mL; 4) added 100, 150, 200, or 250 mL of biolacto bacteria that have been cultured for treatments A, B, C, and D respectively.

Oxygenation and turbulence mixing. An LP-100 aerator with an output of 140 L min^{-1} helps dissolve oxygen from the air into the water. In biofloc systems, intensive turbulent mixing is required to maintain high dissolved oxygen levels and prevent solids deposition. This system requires a good aerator layout, unidirectional circulation was made, and aeration stones were permanently moved regularly to avoid the deposition of solid particles in areas with little or no current.

***Clarias gariepinus* seeds**. The *Clarias gariepinus* seeds were used at a stocking density of 300 fish per fishpond, with an average total length of 11.7 cm and weight of 7,8 g, with the criteria: fish move actively against the current and are agile, are responsive to feed, are uniform seed size, the fish's body was not deformed, the body colour is shiny and not pale, and after stocking the fish quickly spread around.

Feed management. The form of pellets used were floating pellets with a size of 2.3 – 3.0 mm. Feeding as much as 3% of the biomass weight was first done after fasting for 1 day, then the feed was given at libitum two times a day, in the morning and afternoon. The proximate composition of the feed can be seen in Table 1.

Table 1

Proximate composition of biofloc and feed used

Nutrients	*Proximate composition (%)*	
	Feed	*Biofloc*
Protein	33	37.4
Fat	5	11.48
Crude fiber	5	15.6
Ash	16.5	17.9

Water quality parameters. Water quality parameters were continuously monitored during the experiment. Analyzed parameters were dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), BOD/COD ratio, ammonia concentration, nitrite concentration and nitrate concentration. These parameters were monitored using the methodology depicted in Table 2.

Table 2

Methods used to monitor water quality parameters

No	Water quality parameters	Method used (BSN 2022)
1	DO	SNI 06-6989.14-2004: Water and wastewater - Part 14: How to test dissolved oxygen iodometrically (azide modification).
2	BOD	SNI 6989.72-2009: Water and wastewater – Part 72: How to test biochemical oxygen demand (BOD).
3	COD	SNI 6989.2-2019: Water and wastewater – Part 2: How to test chemical oxygen demand (COD) with closed reflux spectrophotometrically.
4	BOD/COD	Division of BOD by COD results from above methods.
5	Ammonia	SNI 06-0045-2006: Water and wastewater-part 30: how to test ammonia levels using phenate spectrophotometer.
6	Nitrite	SNI 06-6989.9-2004: Water and wastewater-part 9: how to test nitrite (NO_2-N) with a spectrophotometer.
7	Nitrate	SNI 6989.79-2011: Water and wastewater-part 79: a method for testing nitrite (NO_3-N) with a UV-visible spectrophotometer using cadmium reduction.

Statistical analysis. The study used a completely randomized design (CRD) consisting of four treatments and three replications. The treatments used were A (100 mL biofloc + 2 kg carbonization + 50 bio balls); B (150 mL biofloc + 2 kg carbonization + 50 bio balls); C (200 mL biofloc + 2 kg carbonization + 50 bio balls), and D (250 mL biofloc + 2 kg carbonization + 50 bio balls). The resulting data was calculated as the mean ± SD. Results from the fishponds were subjected to a one-way analysis of variance (ANOVA) at the 95% confidence level ($\alpha=0.05$) to test the significance between the mean water quality parameters values in the water. $p<0.05$ was considered significant, and where the means differed significantly, a post hoc test was performed using Tukey's Honest Significant Difference Test. All statistics were performed with SPSS software version 23.

Results and Discussion. Studies on the importance of managing water quality dynamics have provided a better understanding of the adverse impacts that can occur if water quality is not managed properly. Following are some of the water quality parameters that have been identified in biofloc-based catfish farming during this research (Table 3).

Table 3

Water quality in biofloc-based catfish farming

Parameters	*Days*	*Treatments*			
		A	*B*	*C*	*D*
DO (mg L^{-1})	10	5.1 ± 0.3^{a}	4.7 ± 0.1^{b}	3.5 ± 0.1^{c}	3.1 ± 0.1^{d}
	20	5.5 ± 0.4^{a}	4.6 ± 0.3^{b}	4.5 ± 0.1^{b}	4.1 ± 0.2^{b}
	30	6.3 ± 0.6^{a}	4.6 ± 0.1^{b}	4.0 ± 0.2^{c}	4.4 ± 0.1^{bc}
	40	3.7 ± 0.2^{a}	3.5 ± 0.3^{a}	3.7 ± 0.4^{a}	3.6 ± 0.1^{a}
	50	3.4 ± 0.4^{a}	3.7 ± 0.1^{a}	3.7 ± 0.3^{a}	3.4 ± 0.2^{a}
BOD (mg L^{-1})	10	0.450 ± 0.240^{a}	0.823 ± 0.574^{ab}	0.983 ± 0.430^{ab}	1.352 ± 0.440^{b}
	20	2.459 ± 0.000^{a}	1.058 ± 0.434^{b}	1.024 ± 0.868^{b}	1.626 ± 0.434^{ab}
	30	4.712 ± 0.607^{a}	1.124 ± 0.434^{b}	0.925 ± 0.174^{b}	2.829 ± 0.570^{c}
	40	2.165 ± 0.174^{ab}	1.729 ± 0.772^{a}	2.831 ± 0.260^{b}	2.598 ± 0.866^{b}
	50	2.598 ± 0.866^{a}	1.699 ± 0.174^{a}	2.965 ± 0.174^{b}	1.698 ± 0.173^{a}
COD (mg L^{-1})	10	5.861 ± 0.470^{a}	5.980 ± 0.571^{a}	7.637 ± 0.355^{b}	8.110 ± 0.370^{b}
	20	5.801 ± 0.370^{a}	5.998 ± 0.709^{a}	8.053 ± 0.271^{b}	8.288 ± 0.102^{b}
	30	8.229 ± 0.271^{a}	8.999 ± 0.103^{b}	10.182 ± 0.271^{c}	10.952 ± 0.271^{d}
	40	10.538 ± 0.205^{a}	9.768 ± 0.774^{ab}	9.946 ± 0.178^{a}	8.998 ± 0.674^{b}
	50	11.307 ± 0.205^{a}	11.071 ± 0.205^{ab}	10.715 ± 0.205^{bc}	10.360 ± 0.205^{c}

BOD/COD	10	0.07852 ± 0.0444^{a}	0.1323 ± 0.0868^{a}	0.1273 ± 0.0525^{a}	0.1658 ± 0.05035^{a}
	20	0.42497 ± 0.0263^{a}	0.1762 ± 0.0666^{b}	0.1294 ± 0.1099^{b}	0.19647 ± 0.05348^{b}
	30	0.5746 ± 0.0904^{a}	0.1252 ± 0.05001^{b}	0.09062 ± 0.01554^{b}	0.25757 ± 0.04636^{c}
	40	0.20526 ± 0.1264^{ab}	0.18131 ± 0.09185^{a}	0.285 ± 0.02677^{b}	0.28878 ± 0.01162^{b}
	50	0.15017 ± 0.01432^{a}	0.18062 ± 0.01753^{b}	0.27654 ± 0.01105^{c}	0.16372 ± 0.01363^{ab}
Ammonia (mg L^{-1}) <0.02	10	0.22 ± 0.02^{a}	0.22 ± 0.03^{a}	0.24 ± 0.03^{a}	0.11 ± 0.02^{b}
	20	0.24 ± 0.04^{a}	0.24 ± 0.04^{a}	0.23 ± 0.02^{a}	0.17 ± 0.05^{a}
	30	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.24 ± 0.03^{a}	0.04 ± 0.00^{b}
	40	0.32 ± 0.02^{a}	0.30 ± 0.02^{a}	0.30 ± 0.01^{a}	0.30 ± 0.01^{a}
	50	0.31 ± 0.01^{a}	0.30 ± 0.02^{a}	0.30 ± 0.03^{a}	0.30 ± 0.03^{a}
Nitrite (mg L^{-1}) <0.2	10	0.136 ± 0.011^{a}	0.190 ± 0.001^{b}	0.201 ± 0.002^{b}	0.227 ± 0.007^{c}
	20	0.063 ± 0.007^{a}	0.084 ± 0.005^{b}	0.104 ± 0.004^{c}	0.109 ± 0.003^{c}
	30	0.044 ± 0.004^{a}	0.056 ± 0.004^{b}	0.064 ± 0.003^{c}	0.066 ± 0.001^{c}
	40	0.251 ± 0.008^{a}	0.250 ± 0.006^{a}	0.176 ± 0.002^{b}	0.098 ± 0.005^{c}
	50	0.243 ± 0.017^{a}	0.110 ± 0.015^{b}	0.161 ± 0.003^{c}	0.158 ± 0.001^{c}
Nitrate (mg L^{-1}) <50	10	1.717 ± 0.096^{a}	1.851 ± 0.047^{a}	2.057 ± 0.069^{b}	2.130 ± 0.148^{b}
	20	2.476 ± 0.024^{a}	2.395 ± 0.025^{a}	3.336 ± 0.150^{b}	4.014 ± 0.180^{c}
	30	2.175 ± 1.241^{a}	3.038 ± 0.132^{a}	3.012 ± 0.132^{a}	6.041 ± 0.473^{b}
	40	8.496 ± 1.098^{a}	6.525 ± 0.451^{b}	6.535 ± 0.986^{b}	6.751 ± 0.167^{b}
	50	6.315 ± 0.128^{a}	5.379 ± 0.213^{b}	5.801 ± 0.651^{ab}	6.142 ± 0.259^{a}

Note: Average ± SD (n=3) with different superscript letters indicate significant differences ($p<0.05$).

Dissolved oxygen (DO). Based on Figure 1a, the dissolved oxygen (DO) trend decreased on days 10, 20, and 30 (all treatments). This trend was related to the increase in the volume of biofloc successively across treatments (100, 150, 200, and 250 mL) resulting in increased demand for oxygen by the consortium of microorganisms in biofloc so that DO decreased. Floc density refers to the number of organisms living together in each area or volume. Floc density can have a significant impact on water quality in aquatic environments. The existence of dense populations can lead to an increase in organic waste, ammonia, nitrate, and phosphate in the water. This condition can decrease water quality, including increased turbidity, decreased DO levels, and changes in pH. High levels of pollution can cause stress to organisms, damage to water quality, and even death (Deswati et al 2023b). Statistically, treatments A and B were significantly different ($p<0.05$), and the addition of 100 mL Biofloc was the best treatment (Table 3 and Figure 1a). On days 40-50, the addition of biofloc had no effect ($p>0.05$) on DO, indicating a stagnant trend. This result showed that the addition of 100, 150, 200, or 250 mL of Biofloc did not affect DO.

Biofloc needs dissolved oxygen because biofloc was formed under aerobic conditions, so it requires sufficient oxygen (Deswati et al 2021a,b,c; 2022a,b,c,d; 2023b). An aerator is a tool that helps to dissolve oxygen from the air into the water. Intense turbulent mixing was required to maintain high dissolved oxygen levels and prevent solids deposition. Biofloc is formed through a floc coagulation process in which organic particles and microorganisms are trapped in a matrix of microscopic air bubbles.

These air bubbles are generated by sufficient aeration in the biofloc system. When air bubbles rise to the surface of the water, flocs are also lifted and form solid mud on the surface (Deswati et al 2023a; Zafar & Rana 2022). In this study DO concentrations were always dynamic and fluctuated in the interval between 3.1 ± 0.1 and 6.3 ± 0.6 mg L^{-1} and were still within the ideal interval, namely > 3 mg L^{-1} (GRRI 2021). This is in line with the findings of Deswati et al (2022c), that the DO in biofloc-based catfish farming was 5.5-7.7 mg L^{-1}, while the DO concentration of ±3 mg L^{-1} was produced without using biofloc (Deswati et al 2019).

Biological oxygen demand (BOD). Table 3 and Figure 1b show that the biological oxygen demand (BOD) values from day 10-20 tended to increase, fluctuate, they are dynamic. The highest increase occurred on day 20 (treatment A), and statistically, treatment A was significantly different ($p<0.05$) from the other treatments. This condition was presumably due to the accumulation of organic matter sources from fish waste, uneaten feed residue, and dead microorganisms. The same thing happened on day 30 (treatment A). On days 40-50, BOD concentration tended to decrease, except on day 50 (treatment C). This decrease in BOD was related to the addition of 100, 150, 200, and 250 mL of biofloc, where the BOD value decreased with increasing biofloc. The high concentration of biofloc can reduce the BOD value. The BOD value increased due to the oxygen needs of fish, the decomposition of nitrifying bacteria, and the activity of a consortium of microorganisms in biofloc (Deswati et al 2023b).

This condition indicated that the nitrifying bacteria were already working according to the principles of aquaculture, even though the concentration of biofloc was given every ten days. In biofloc-based fish farming, the fish feeding is done by mixing biofloc and the fish feed so that an oxidation process occurs in fishponds, namely the conversion of ammonia to nitrite. Adding biofloc aims to speed up the process of converting nitrite to nitrate so that BOD increases (Deswati et al 2022c). Adding dolomite lime is one way to reduce BOD concentrations in ponds (Poonam et al 2013). Statistically, treatment C had the highest BOD and significantly differed ($p<0.05$) from the other treatments. In contrast, treatments A, B, and D were not significantly different ($p>0.05$).

Overall, the BOD value is always dynamic and fluctuating and is still at an ideal interval of ≤ 6 mg L^{-1} (GRRI 2021). This value indicates that the waters were not polluted, so they were suitable for fish farming. The advantage of BOD is that it can be used to express the level of water pollution by organic matter. The high value describes the continuous decomposition of biological minerals and, to some extent, by microbes (Simeon et al 2019).

Chemical oxygen demand (COD). Table 3 and Figure 1c show that day 10-50 chemical oxygen demand (COD) values tend to increase, fluctuate, they are dynamic. The increase in COD value was due to the large amount of organic matter originating from leftover feed and fish waste that had accumulated in the pond. On days 10-20, COD was lower than on days 30-50, presumably due to the large amount of organic matter degraded into inorganic matter. Nitrifying bacteria that convert organic substances into inorganic substances are needed to oxidize organic substances in the pond (Hlordzi et al 2020). The increase in COD value again occurred on days 30-50. The higher the COD concentration, the higher the organic and inorganic compounds number is in the water, so more oxygen was needed to oxidize organic and inorganic compounds (Deswati et al 2022b). This data was closely related to biofloc nutrition and dolomite lime given every ten days.

COD values of all treatments and sampling times were situated at ideal intervals, namely <40 mg L^{-1} (GRRI 2021), so the water quality was feasible for *Clarias gariepinus* cultivation. The COD value provides information about the level or content of oxidized organic components which may be responsible for pond pollution. The redox potential of each pond environment requires a certain amount of oxygen which effectively oxidizes existing organic matter (Simeon et al 2019).

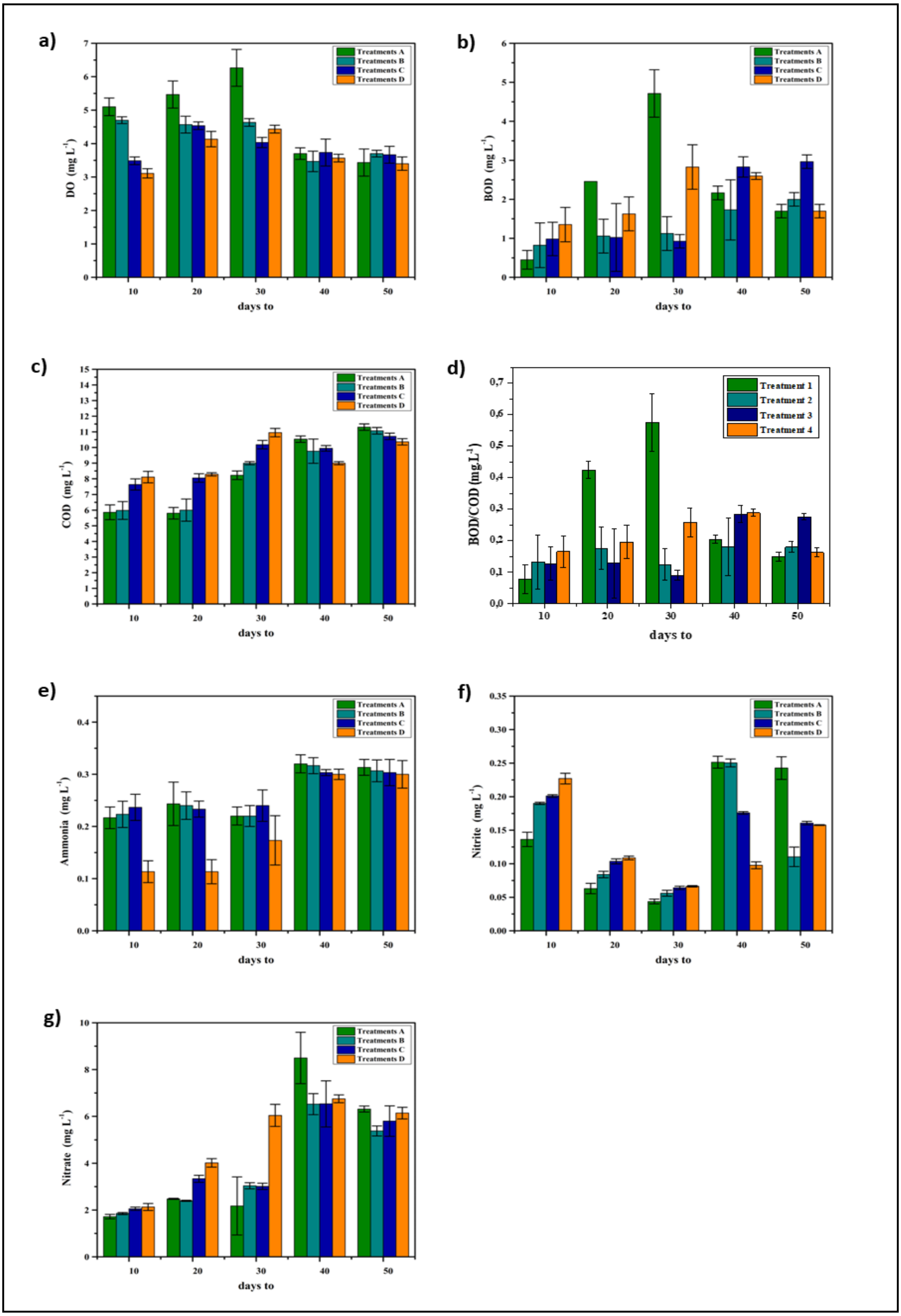

Figure 1. Water quality parameters in biofloc-based catfish ponds: a) DO, b) BOD, c) COD, d) BOD/COD ratio; e) ammonia, f) nitrite, g) nitrate.

BOD/COD ratio. The BOD/COD ratio indicates the impact of releasing organic matter in water, waste, leachate, compost, and other similar materials that occur in the environment, both in the natural environment and in humans. Artificial environment changes in the degree of biodegradability are indicated by an increase in the BOD/COD ratio (Mangkoedihardjo 2010). The BOD/COD ratio for non-biodegradable pollutants is < 0.01, while for biodegradable waste, it is > 0.1 (Koch et al 2002). The BOD/COD ratio in waters is divided into three zones, namely the stable zone, the biodegradable zone, and the toxic zone (Samudro & Mangkoedihardjo 2010). The ratio of BOD/COD that is good for cultivation and biological processes is in the biodegradable range, namely 0.2 – 0.5 (Mangkoedihardjo 2010). In this study, we determined the biodegradability of organic matter using the BOD/COD ratio.

According to Effendi (2003), waters with BOD and COD values <20 mg L^{-1} are said to be unpolluted. The BOD/COD ratio, which was in the biodegradable range of 0.2 to 0.5 (Mangkoedihardjo 2010), was treatment A (day 40), treatment C (days 40 and 50) and treatment D (days 30 and 40) (Table 3). which indicates that the pollutants in the fishponds are biodegradable. In this study, the BOD/COD ratio was very volatile and dynamic (Figure 1d), and treatment B with the addition of 150 mL of biofloc showed that the waters were polluted. The organic matter was not completely degraded. Treatment C (day 50) was the best treatment because the pollutants can be completely degraded. Statistically, it was significantly different ($p<0.05$) from the other treatments. Generally, the BOD/COD ratio was very volatile and dynamic and was not at an ideal interval of 0.2-0.5 (Mangkoedihardjo 2010).

Ammonia (NH_3). Based on Table 3 and Figure 1e, on days 10-40, the ammonia (NH_3) concentration tends to increase, presumably due to an increase in faeces, uneaten fish feed, and dead microorganisms in biofloc. On days 10, 20, and 30, the highest concentrations of ammonia occurred in treatments A, B, and C. Statistically, the three were not significantly different ($p>0.05$) and significantly different from treatment D. This was related to the addition of different concentrations of biofloc, respectively concentrations of 100, 150, 200, and 250 mL for treatments A, B, C, and D. The results obtained showed that the addition of 250 mL of biofloc had a significant effect ($p<0.05$) on the reduction of ammonia. Ammonia that fish have absorbed can be excreted by fish through gills and other epithelium as nitrogenous waste. Previous studies have shown that crustaceans can withstand high levels of ammonia through an ammonia excretion strategy to maintain normal cellular function (Leone et al 2017; Hans et al 2018). Gills and antennae glands are important organs involved in the excretory process of crustaceans. Nearly 60–95% of the total nitrogen is excreted through the gills (Weihrauch et al 2009; Fregoso-López et al 2018). Kormanik and Cameron (1981) stated that the excretion of ammonia in the blue crab *Callinectes sapidus* occurs mainly through NH_3 diffusion. However, other researchers have shown that ammonia excretion in the beach crab *Carcinus maenas* is partly in ammonium (NH_4^+) (Siebers et al 1985).

High concentrations of ammonia can cause a decrease in catfish survival. Fish that are exposed to ammonia for a long time have a higher mortality rate than those that are not exposed to ammonia. Ammonia can also inhibit the growth of catfish. Fish exposed to ammonia tend to have slower growth and lower body weight compared to fish living in an environment free of ammonia. Ammonia toxicity can also affect the physiological functions of catfish, including disorders of the respiratory system, disorders of the nervous system, and decreased immune response. Mechanism of elimination of ammonia, some fish species can convert ammonia into less toxic compounds, such as urea or amino acids. This process is known as neurogenesis or monogenesis. This process helps reduce the ammonia load in the fish's body.

On days 30-40, the ammonia concentration tended to increase with increasing addition of biofloc. On day 40, statistically, all treatments were not significantly different ($p>0.05$), as well as on day 50. This data shows that the ammonia concentration remained high with the addition of biofloc. There are several ways to control high concentrations of ammonia in fishponds. The two main ones are the uptake of ammonium by phytoplankton and the oxidation of ammonia to nitrate by nitrifying bacteria. Ammonia is a gas that can diffuse into the air when the pH is raised, and diffusion is the loss of small amounts of ammonia from the pond on windy days and in very aerated ponds. Of course, the escaping water removes the ammonia, and small amounts of ammonium may be adsorbed at the cation exchange sites at the bottom of the soil. The most important is to use feed containing no more protein than necessary, feed conservatively, avoid having uneaten feed, achieve a low feed conversion ratio, and prevent low dissolved oxygen concentrations. Fish feed better when not stressed by low dissolved oxygen concentrations, and nitrification is inhibited at dissolved oxygen concentrations < 3 mg L^{-1}.

In biofloc-based catfish culture systems, ammonia is controlled to some extent by nitrification. However, carbohydrate sources are often used to promote biofloc formation through bacterial growth due to the high ammonia concentration in these systems. The bacteria remove the ammonia for use in producing new bacterial cells. A study conducted by Hargreaves and Kucuk (2001) on three fish species exposed to daily cycles of pH and temperature as observed in ponds revealed that exposure to relatively high concentrations of ammonia for several hours each day was not harmful. In addition, ponds often contain 2 to 5 mg L^{-1} of total ammonia nitrogen, but adverse effects on fish are rarely seen. The author has never seen dead fish in aquaculture ponds which could be associated with high ammonia concentrations. However, the author is not implying that high ammonia concentrations do not often stress fish. The result is that high ammonia concentrations in aquaculture tend to stress fish but rarely kill them. Shrimp stay at the bottom of ponds with lower pH and temperature. However, in well-mixed and aerated ponds, the pH and temperature near the bottom are usually the same as in surface water.

Ammonia absorption by fish is influenced by several factors that can affect their level of tolerance to this substance. Several factors affect ammonia uptake by fish, including: 1) each type of fish has a different tolerance level to ammonia. Some types of fish can tolerate high levels of ammonia, while others are more sensitive to it; 2) larger fish tend to have a higher tolerance level of ammonia compared to smaller fish; larger fish have a greater capacity to remove ammonia through mechanisms such as efficient renal filtration; 3) water quality is very important in affecting the uptake of ammonia by fish. High levels of ammonia in the water will cause greater absorption of ammonia by fish. Water quality parameters such as pH, temperature, dissolved oxygen, and turbidity can affect the absorption rate of ammonia; 4) the pH of water affects the ionization state of ammonia. Ammonia is more toxic in the form of un-ionized ammonia (NH_3) than in the ionized form of ammonium (NH_4^+). The balance between NH_3 and NH_4^+ depends on the pH of the water. At a higher pH, the amount of NH_3 will increase, thereby increasing the uptake of ammonia by fish; 5) the duration of fish exposure to ammonia can also affect its absorption rate. Long-term exposure to high ammonia levels can reduce the uptake rate of ammonia by fish because it can trigger adaptation mechanisms and physiological changes in fish (Ip et al 1992; Wilkie 1997). In catfish ponds based on biofloc, the concentration of NH_3 fluctuates daily due to photosynthesis (increasing pH) and respiration (reducing pH).

Conversely, during the rainy season, the ambient temperature decreases, causing bacterial activity and the nitrification process to run slowly. As a result, the amount of ammonia in the environment increases (Titiresmi & Sopiah 2006). Overall, in this study, ammonia concentration is always dynamic and fluctuating but still within the ideal interval of <0.5 mg L^{-1} (GRRI 2021).

Nitrite (NO_2^-). Based on Table 3 and Figure 1f (day 10), nitrite concentrations tended to increase in all treatments; the highest increase occurred in treatment D>C>B>A. This data was due to the different concentrations of biofloc, 250, 200, 150, and 100 mL for treatments D, C, B, and A, respectively. Statistically, treatment D was significantly different ($p<0.05$) from the other treatments. The same thing happened on days 20 and 30, but treatment D was not significantly different ($p>0.05$) from the other treatments. The study conducted by Wang et al (2015) found that adding nitrite to fish feed significantly increased fish growth. This research was conducted on carp (*Cyprinus* *carpio*). It showed that the provision of nitrite in the feed can increase the body weight, body length, and growth rate of fish. Nitrite can play an important role in maintaining water quality in aquaculture systems. Nitrite plays a role in the nitrogen cycle, where ammonia produced by fish metabolism is converted into nitrite by nitrifying bacteria. Research conducted by Heimer (1975) showed that nitrites are effectively converted to safer compounds in the nitrification process, thereby reducing the risk of toxic ammonia accumulation in fishpond water. Research by Deswati et al (2022a) shows that catfish cultivated on a biofloc basis can improve water quality. Deswati et al (2023b) showed that cultivated catfish on a biofloc basis can grow fast, which is characterized by specific growth rate (0.058 – 0.066), feed efficiency (90.89–98.79%), feed conversion ratio (0.933–1.104) and survival rate (84–88%).

On day 40, nitrite concentration in all treatments tended to decrease; the highest value of nitrite concentration in a row was treatment A>B>C>D. This data shows that increasing the concentration of biofloc affects by decreasing the concentration of nitrite; the greater the concentration of Biofloc, the more significant the decrease in nitrite concentration is. Statistically, treatments A and B were not significantly different ($p>0.05$) in terms of decreasing nitrite concentration and significantly different ($p<0.05$) from treatments C and D. The mechanism by which nitrites enter the fish's body can involve several pathways, including through the breathing system and the food they consume. The study by Mansour et al (2021) on *Oreochromis niloticus* showed that gills are the main route for the absorption of nitrite in freshwater fish. Nitrite diffuses through the gill membranes and enters the fish's blood circulation. This study shows that the rate of uptake of nitrite through the gills depends on the concentration of nitrite in the aquatic environment and the time of exposure of the fish. Fish can also accumulate nitrites through the food they eat. The study conducted by Zeitoun et al (2016) on *O. niloticus* showed that feed containing high nitrites can cause an increase in nitrite concentrations in fish tissue. Nitrite-contaminated nutrients can be a major source of nitrite exposure for fish in aquaculture systems. Several studies have also shown that fish can absorb nitrites through their skin and mucus layers. A study by Eddy and Williams (1987) on marine fish showed that fish skin and mucus layers can absorb nitrite from the aquatic environment. However, the rate of uptake of nitrites via this pathway may be less significant than that for uptake via gills and food.

On day 50, the nitrite concentration is dynamic, fluctuating, and tends to decrease. The high concentration of nitrite from treatment A is because the addition of biofloc concentration had no effect ($p>0.05$) on the decrease in nitrite concentration. Nitrite exposure in fish can harm their health and performance. The study conducted by Grosell et al (2002) investigated the impact of nitrite toxicity on freshwater fish. This study found that exposure to nitrite in high concentrations can cause disturbances in the respiratory system and oxygen transport in fish, resulting in hypoxia or lack of oxygen in the fish's body. The study conducted by Zhang et al (2020) evaluated the effect of nitrite on the immune system of goldfish (*Cyprinus carpio*). This research shows that exposure to nitrites in water can inhibit the activity of immune cells and impair the immune response of fish, which can increase the susceptibility of fish to disease and infection. The study conducted by Monsees et al (2017) investigated the interaction between nitrite and stress conditions in *O. niloticus*. This research found that exposure to nitrites in combination with stressful conditions can increase the negative impacts on fish health, including weight loss, impaired immunity, and organ damage. It should be noted that concentrations of nitrites that are potentially harmful to fish can vary depending on fish species, water temperature, pH, and other environmental factors. In general, the concentration of nitrite is always dynamic and fluctuating. However, it is still in the ideal concentration of < 1 mg L^{-1}.

Nitrates (NO_3^-). Nitrate is the final component of the nitrogen cycle in water. High nitrate levels in water can lead to algae overgrowth and other potential water quality problems. Based on Table 3 and Figure 1g, day 10-40 nitrate concentrations tended to increase in all treatments. This condition was presumably due to the ammonification and nitrification processes (Webster & Lim 2002). Ammonification occurs when bacteria and microorganisms decompose food scraps, fish waste, and other organic materials in the pond. During this process, organic nitrogen compounds are converted into ammonia (NH_3) or ammonium (NH_4^+). During nitrification, ammonia or ammonium produced from ammonification is then oxidized by nitrifying bacteria to become nitrite (NO_2^-) and then nitrate (NO_3^-). This process consists of two steps: oxidation of ammonia to nitrite by *Nitrosomonas* spp. bacteria and nitrite to nitrate by *Nitrobacter* spp. bacteria.

The highest increase was in treatment D (days 20 and 30); it was suspected that adding 250 mL of biofloc significantly affected the increase in nitrate concentration and was due to the nitrification process. Increased concentrations of nitrate in the aquatic environment can be toxic to fish. Nitrates that are too high can interfere with the function of the fish's respiratory system, inhibit the binding of oxygen in the blood, and cause hypoxia (lack of oxygen). The results are induced stress, decreased growth, organ damage, and even fish death. Increased concentrations of nitrate in water can also affect fish reproduction. Excessive nitrates can interfere with reproductive processes such as sperm and egg production, embryo development, and survival of fish larvae. This condition can reduce the reproductive success of fish and threaten population sustainability. High nitrates in water can also increase the risk of disease infection in fish. Excess nitrates can weaken fish's immune systems, making them more susceptible to bacterial infections, parasites, and other diseases. This excess can decrease health and reduce success in fish farming (Richards 1983; Li et al 2021).

On days 40-50, nitrate concentration tends to decrease and fluctuate; one of the causes of the decrease is the nitrate uptake by phytoplankton. Aquatic plants in ponds, such as algae and water plants, use nitrate as a source of nitrogen for their growth. These plants absorb nitrate through their roots and use it in the process of photosynthesis and the synthesis of other important substances. Nitrates can enter the fish's body through several mechanisms, especially through the water where the fish live and the food they consume. Kefford et al (2004) stated that fish can absorb nitrate through the gills by passive diffusion, where high nitrate concentrations outside the fish body will diffuse into the fish body where the concentration is lower. Fish can also eat food containing nitrates derived from algae and other aquatic plants. In our study, nitrate concentrations were always dynamic and fluctuating, but were still at ideal intervals, namely <50 mg L^{-1}.

Conclusions. With the exception of BOD/COD ratio, which was excessively high and harmful to aquatic life, it can be considered that the use of biofloc systems in catfish (*Clarias gariepinus*) farming allowed the concentration of dissolved oxygen, biological oxygen demand, chemical oxygen demand, ammonia, nitrite, and nitrate to be maximized while still being below the permitted levels. Water quality dynamics and variations over time were influenced by a number of elements, including human activities, natural processes, and interactions between water and the environment. In order to make sure that water quality standards were met and that required actions could be done if problems were discovered, it was crucial to monitor the dynamics and changes in water quality. Regular monitoring of water quality is necessary to prevent catfish toxicity and make it safe for human consumption.

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