



The Application of Different Types of Diffusers for African Catfish (*Clarias gariepinus*) Culture in Biofloc Systems: Effects on Growth and Water Quality

Diana Maulianawati^{1,2*} , Hendri Kiing¹, Dena Pramita Dewi³, Heni Irawati⁴ , Tri Yusuf Mardiana¹  and Muhammad Amien¹ 

¹Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Borneo Tarakan, Jl. Amal Lama No. 1, Tarakan, North Kalimantan 77115, Indonesia

²Research Centre for Tropical Nature and Waters Resources of Border Region (SEATROPS), Universitas Borneo Tarakan, Jl. Amal Lama No. 1, Tarakan, North Kalimantan 77115, Indonesia

³Laboratory of Fish Feed Nutrition, Faculty of Fisheries and Marine Science, Universitas Borneo Tarakan, Jl. Amal Lama No. 1, Tarakan, North Kalimantan 77115, Indonesia

⁴Department of Waters Resources Management, Faculty of Fisheries and Marine Science, Universitas Borneo Tarakan, Jl. Amal Lama No. 1, Tarakan, North Kalimantan 77115, Indonesia

*Correspondence :
diana.maulianawati@gmail.com

Abstract

Applying biofloc technology in the intensive and extensive culture of *Clarias gariepinus* can improve water quality and be used to feed fish. Aeration systems were a critical unit supporting biofloc and water quality. This study's objective was the assessment of various types of diffusers on the growth and water quality in a *C. gariepinus* culture. Two types of diffuser units were prepared for the experiment, there are air tube diffuser (AT) and air stone diffuser (AS). Growth parameters, water quality, and volume of biofloc were observed within 30 days. The survival rate, weight gain, average body weight, and specific growth rate of *C. gariepinus* were higher in the tanks that used air tubes (98%, 485.29 %, 7.52 g, 5.89%) than in the tanks that used air stones (92 %, 385.94 %, 5.98 g, 5.23%). The volume of biofloc ranges from 5.40-18.80 ml/L in AT tanks and 4.60-14.00 ml/L in AS tanks. There is no significant difference ($p > 0.05$) in water quality parameters and FCR value. However, using the air tube diffuser showed better results with the growth performance, survival rate, and formation of biofloc.

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Air stone, Air tube, Floc, Bacteria, Microalgae

INTRODUCTION

African catfish (*Clarias gariepinus*) is a fishery commodity with promising business potential and advantages such as fast growth, the ability to adapt to the environment, and ease of cultivation. However, the biggest challenge in catfish farming is the high feed cost, so the profit margin is relatively low. In addition, the remaining aquaculture waste from catfish produces

large amounts of nitrogen and carbon, which will impact decreasing environmental capacity (Tung *et al.*, 2021). Therefore, supporting, and efficient technology is crucial to increase the productivity of *C. gariepinus*.

Biofloc technology is a solution to environmental problems and can increase aquaculture production. The biofloc

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system is a solution to the problem of waste generated from metabolic waste and feed, where heterotrophic bacteria utilize the ammonia produced from the waste. The application of this system can increase productivity, water quality, and immune response and reduce aquaculture waste and feed use (Martínez-Córdova *et al.*, 2015). If appropriately applied, biofloc technology can minimize water replacement or even no water replacement in the cultivation system so that this technology is environmentally friendly (Dauda, 2020). Several aquaculture commodities, including *Penaeus monodon*, *Litopenaeus vannamei*, *Oreochromis niloticus*, and *C. gariepinus*, have been cultivated utilizing the biofloc technique (Schveitzer *et al.*, 2013; Widanarni *et al.*, 2012; Dauda *et al.*, 2017).

The biofloc system is distinguished by its high productivity, continuous utilization of mechanical aerators, minimal requirement for water renewal, and extensive capacity for water reuse throughout several production cycles (Hargreaves, 2013). The biofloc system is considered a technological innovation because, in its formation, leftover feed, feces, bacteria, phytoplankton, zooplankton, filaments, and organic compounds form floc aggregates and can be a source of protein for fish. The biofloc system also improves water quality due to the biodegradation of wastes such as ammonia and nitrites (Emerenciano *et al.*, 2013).

Aeration is the most critical aspect of biofloc cultivation. The movement and circulation of water created by the aeration system in the tank are one of the physical variables influencing the development of biofloc. The turbulence generated by the aeration unit will influence biofloc aggregation and degradation (Crab *et al.*, 2012). Since biofloc is created by the aggregation of microorganisms, biofloc will break easily; hence, it prevents floc formation (Chaignon *et al.*, 2002). Therefore, proper installation of aeration units can assist in establishing and maintaining

biofloc in culture systems. The biofloc aggregation and dissolution rates can be modified using various aeration units. The aeration units often used in biofloc cultivation systems are blowers and diffusers. The diffuser that is commonly used is an air stone or disc diffuser. However, this diffuser does not produce appropriate water movement at the bottom of the tank, so the mixing process only occurs evenly. This study's objective was to assess various types of diffusers on the growth and water quality in a *C. gariepinus* culture using a biofloc system.

METHODOLOGY

Ethical Approval

There are no animals harmed or improperly treated during this research. The test animals in this study were treated properly according to the optimal environment and there was no addition of harmful bacteria or toxic material.

Place and Time

This research was conducted from January to February 2022 in Mini Hatchery, Faculty of Fisheries and Marine Sciences, Borneo Tarakan University. Water quality parameters were carried out in the Water Quality Laboratory, Faculty of Fisheries and Marine Sciences, Borneo Tarakan University.

Research Materials

Aerator (Yamano LP110, Japan), analytical balance (OHAUS, China), pH meter (Lutron, USA), DO Meter (Thermo Fisher Scientific, USA), spectrophotometer (PG Instrument, UK), Micropipette (Socorex, Germany), Imhoff cone (VitLab, Germany), molasses, chlorine, dolomite, salts, probiotic (EM4, Indonesia), HDPE tank (Type A20, Korea), air tube, air stone.

Research Design

The experiment consists of two treatments and five replications. There were two treatments to provide dissolved oxygen. Treatment one used air stones

(AS), and treatment two used air tubes (AT) distributed at the bottom of the tanks to increase turbulence and suspension of the particulate material. Each tank was stocked with fifty *C. gariepinus* (6-8 cm) (Affandi *et al.*, 2022).

Work Procedure

Tank Preparation

The HDPE tank was used as pond fish (1.0 x 0.5 x 0.5 m). Before use, the water quality was measured. The tank was washed and disinfected with chlorine (5%). After two days of disinfection, the tank was filled with 200 l of fresh water. This method was based on previous work (Dauda *et al.*, 2017). The layout of the pond is shown in Figure 1.

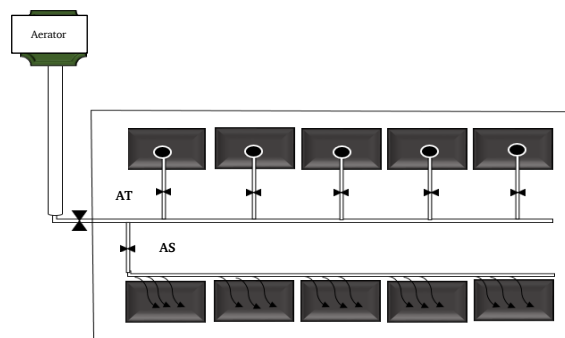


Figure 1. The arrangement of the biofloc system for *C. gariepinus* culture (AT=Air Tube; AS= Air Stone).

Experimental Fish Stocking and Management

Visually healthy and disease-free *C. gariepinus* was collected from a local hatchery. Commercial food was used as a diet and contained protein (38%), fat (2%), fiber (2%), and ash (13%). Fish were fed two times a day at 3% of body weight. EM-4 was distributed to each tank at two-night intervals. Fish were observed daily to estimate survival rate and measured the growth at ten nightly intervals (Sumitro *et al.*, 2022).

Monitoring Water Quality

Water quality observations include pH and temperature collected daily using a pH meter and thermometer. Dissolved

Biofloc Culture Production

The production of biofloc required a constant supply of air, which was provided by an air stone and air tube. Before starting the trial, 200 L fresh water, 10 mg/L dolomite, 10 mg/L salt, and 2 mg/L molasses were added to a tank. The tank was covered and kept for 24 hours. After 24 h, 2 mg/L EM-4 (containing *Lactobacillus* sp., yeast, *Actinomycetes*, and *Streptomyces*) was added to each tank. EM-4 was added every day until the floc volume increased. The Imhoff Cone was used to calculate floc volume (FV). This method was based on previous work with modification (Dauda *et al.*, 2017).

oxygen (DO) was measured by a digital DO meter. Ammonia, nitrite, and nitrate were collected at a three-nightly interlude and analyzed spectrophotometrically following the APHA (2017). Imhoff Cone measured the floc volume (1000 mL), and the standardized sedimentation time was 30 min.

Growth Parameters

C. gariepinus were harvested after 30 days of treatment to evaluate specific growth rate (SGR), survival rate (SR), final weight (FW), final length (FL), average body weight, weight gain, and food conversion ratio (FCR) (Aftabuddin *et al.*, 2020).

$$\text{SGR (\%)} = \frac{\ln W_t - \ln W_0}{t} \times 100$$

Where:

SGR = Specific Growth Rate
Wt = final weight (g)
Wo = Initial weight (g)
t = time of experiments

$$SR (\%) = \frac{N_t}{N_o} \times 100$$

Where:

SR = Survival rate (%)
Nt = the number of fish lives
No = the Initial number of total fish
ABW = Wt – Wo

Where:

ABW = Average body weight (g)
Wt = final weight (g)
Wo = Initial weight (g)

$$\text{Weight gain (\%)} = \frac{W_t - W_o}{t} \times 100$$

$$\text{Feed Conversion Ratio} = \frac{\text{feed applied (g)}}{\text{live weight gain (g)}}$$

Data Analysis

All collected data were analyzed using SPSS 21 software. The T-test examines

the difference in mean between two samples at a significance level of 5%.

RESULTS AND DISCUSSION

Growth Performance

The fish performance after 30 days of culture is shown in Table 1. Final weight, ABW, and SGR were projected to evaluate the *C. gariepinus* growth performance. The growth of *C. gariepinus* in tanks that used air tubes was better than that obtained in those that used air stones. SR was higher in tanks that used air tubes than in tanks that used air stones. There was no statistically significant difference in the FCR across treatments. T-test analysis showed no significant differences ($P > 0.05$) in the final weight, ABW, SGR, SR, and FCR among the treatments.

Table 1. *C. gariepinus* growth parameters in biofloc treatments with different diffusers (means ± SE).

Parameters	Air Stone	Air Tube
Initial weight (g)	1.55 ± 0.00	1.55 ± 0.00
Final weight (g)	7.53 ± 1.20	9.07 ± 0.32
Initial length (cm)	6.00 ± 0.00	6.00 ± 0.00
Final length (cm)	10.50 ± 0.35	11.00 ± 0.00
ABW (g)	5.98 ± 2.54	7.52 ± 2.79
Weight gain (%)	385.94 ± 77.51	485.29 ± 20.68
SGR (%)	5.23 ± 0.60	5.89 ± 0.12
SR (%)	92.00 ± 1.10	98.00 ± 0.30
FCR	0.76 ± 0.13	0.76 ± 0.05

Final weight, final length, ABW, and SGR varied between the treatments (Table 1). The air tube aided (AT tanks) in the growth performance and survival of *C. gariepinus* were higher than in the AS tanks. The biofloc system's impact on *C. gariepinus* growth and survival depends on several variables. Several studies have reported that culture using biofloc can enhance the survival and growth of *C. gariepinus* compared to extensive systems (Dauda *et al.*, 2017). Bacteria, exoskeletons, food, diatoms, feces remnants, macroalgae, invertebrates (Jatobá *et al.*, 2014), and other microorganisms make up

the diverse mixture known as biofloc (Hargreaves, 2006).

Feed utilization efficiency is enhanced, and a significant percentage of the nutritional need is met by bio flocs produced from recycling feed leftovers and fecal excrements (Crab *et al.*, 2010). In addition, there was an increase in the digestive enzyme production of *C. gariepinus* due to the microorganisms that attached to biofloc (Moss *et al.*, 2007). In part, increased levels of digestive enzymes in the fish's digestive tissues contributed to better digestion and absorption of the feed, which increased the fish's growth

performance and feed utilization. (Xu and Pan, 2012).

In the present study, the weight gain, SGR, and SR of *C. gariepinus* were higher than in the previous report of *C. gariepinus* reared in biofloc systems with different carbon sources and aeration design (Dauda *et al.*, 2017; Sumitro *et al.* 2020). AT treatment it also resulted in better growth performance and survival rate than AS treatment. This observation may support the hypothesis that using diffuser hoses with smaller micropores than air stones for aeration needs produces a smaller bubble diameter than diffuser stones. The bubble diameter with a smaller size can increase the surface of the water, which coincides with the air, facilitating the process of air diffusion into the pond and increasing oxygen solubility (Navisa *et al.*, 2014). The air tube produced consistent water movement and circulation, which in turn helped to foster the creation of biofloc. An air tube, as opposed to an air stone, will produce smaller and more manageable bubbles in situations with more sophisticated water flow and circulation. Because of this, a more favorable environment was produced for the biofloc to connect and clump (Chaignon *et al.*, 2002).

In contrast, the air stone units caused unequal water movement and emitted bubbles of varied sizes. The suspended biofloc was ultimately dissolved due to the vigorous water movement, which affected the synthesis of biofloc. The biofloc that had made its way to the bottom of the tank and begun concentrating there started absorbing the dissolved oxygen. Consequently, there was a lack of oxygen in the water at the bottom of the tank (Choo and Caipang, 2015), which degraded the water quality. This present

study is consistent with the conclusion of Hidayat *et al.* (2016) that using microporous pipes can increase survival in catfish culture by 97.22%, while aeration stones only increase survival by 33.33%.

The FCR value from AT and AS tanks is 0.76. Sumitro *et al.* (2020) reported FCR values of 0.8 for *C. gariepinus* in the biofloc system based on a different design of the aeration system. In this study, different diffusers did not significantly differ in the FCR value. This means both diffusers have an excellent function in producing floc as a source of use for fish. *C. gariepinus* obtained a natural feed source from flocks apart from a commercial feed. Floc contains various microalgae and bacteria, which are known to increase feed absorption to be more optimal and efficient (Khanjani and Sharifinia, 2020). Different microbial communities in the biofloc system contribute proteins, lipids, amino acids, and fatty acids (Emerenciano *et al.*, 2011). Probiotic bacteria have been proven could prevent disease infectious on *C. gariepinus* (Maulianawati *et al.*, 2022). As a result, The FCR value of the shrimp can be significantly lowered using the biofloc method. In addition, the biofloc raises the amount of natural feed available for the shrimp, which improves their overall output performance, which is especially significant during the nursery and growth phases.

Floc Volume

Floc-forming bacteria will decompose organic matter from the pond's leftover feed, fish waste, and dead biota Floc volume is a value to describe the abundance of biofloc-forming organisms. Floc density for 30 days can be seen in Table 2.

Table 2. The volume of floc during 30 d of treatments (means ± SE).

Treatment	Floc Volume (ml/L)		
	Day 10	Day 20	Day 30
Air tube	5.40 ± 1.14	8.80 ± 0.83	18.80 ± 1.78
Air stone	4.60 ± 1.34	8.40 ± 1.51	14.00 ± 2.00

This study showed that all treatments developed biofloc formation as early as ten days. The floc volume of AT tanks was significantly higher than that of the AS tanks. Furthermore, the floc volume gradually grew during the experiment, and the color changed to brown. At day 30, the maximal floc volume was 18.80 ± 1.78 mL/L. This value was statistically better than the other treatments ($P < 0.05$). In addition, the biofloc volume in HD and SD tanks rapidly increased between day ten and day 20 from 5.40 ± 1.14 mL/L to 8.80 ± 0.83 mL/L, 4.60 ± 1.34 mL/L to 8.40 ± 1.52 mL/L and respectively (Table 3). Also, from day 20 to day 30, the biofloc volume tended to increase rapidly to 8.80 ± 0.83 mL/L to 18.80 ± 1.78 mL/L in HD tanks and 8.40 ± 1.52 mL/L to 14.00 ± 2.00 mL/L in SD tanks.

Biofloc micrographs include an open and porous structure that facilitates the transport of water and chemicals throughout the floc, hence facilitating the uptake of nutrients and the excretion of metabolites in the microbial mass (Khanjani *et al.* 2020). The rate and duration of the generation of biofloc are influenced by several parameters, including salinity and carbon (Khanjani *et al.* 2017). The formation density of biofloc increases with increasing salinity, and the quality of the flocs is also influenced by the kind of carbon source, so saltier waters support better development of stable biofloc. They also found that in contrast to the introduction of complex carbohydrates like wheat flour, introducing simple carbonaceous organic matter like molasses into the aquaculture system

improved water quality and accelerated the development of heterotrophic bacteria.

In this research, all treatments used similar carbon sources. The difference in floc volume between treatments is suggested to be due to differences in the type of diffuser used. Floc volume formation is also affected by water movement. The movement of water generated by the air stone only affects the surface, causing the agitation at the bottom of the pond to be reduced. Particles in BFT must be continuously mixed to keep the biofloc floating, allowing the animals to consume them without their settling and decaying. When more particles are in the air, more oxygen and energy are needed to maintain them suspended biochemically (Minaz and Kubilay, 2021; Lim *et al.*, 2021). Overall, both treatments produced the appropriate volume of flocs for the needs.

Water Quality Parameters

Water quality is an essential factor in fish farming as a living medium for fish. Besides, the source and quantity must be adequate, and the water used must comply with fish needs. The primary organisms that form flocs are bacteria. Bacteria can improve water quality, even in conditions polluted with organic pollutant compounds such as DDT (Purnomo *et al.*, 2019; Maulianawati *et al.*, 2021). Observations made during the study included temperature, pH, DO, ammonia, nitrite, and nitrate. The average value of the measurement results of water quality parameters can be seen in Table 3.

Table 3. Water quality parameters during 30 days of the production cycle.

Parameters	Treatment	
	Air Stone	Air Tube
Temperature (°C)	30–32	30–32
pH	6.89–8.72	7.2–8.60
DO (mg/L)	4.30–4.82	4.3–5.71
Total Ammonia (mg/L)	0.00–0.85	0.00–1.50
Nitrite (mg/L)	0.019–2.00	0.023–2.00
Nitrate (mg/L)	0.011–4.00	0.004–4.00

The optimum temperature for the growth of *C. gariepinus* is 28-30°C. The high temperature during rearing is caused by the rearing pond being in an open area, but the catfish can still tolerate these conditions. The temperature that *C. gariepinus* can tolerate is in the range of 22-34°C. In general, the temperature in the rearing medium still fulfills the requirements to support the life of *C. gariepinus*.

During the study, the pH values obtained ranged from 6.89 to 8.72. Water conditions with an appropriate pH for an individual fish will accelerate the growth rate of that individual because pH is closely related to metabolic processes. *C. gariepinus* farming is best done at a water pH ranging from 6-9. The optimal pH for applying biofloc technology is 7.5–8.7.

The results of dissolved oxygen measurements showed fluctuating results in all treatments. Dissolved oxygen (DO) in the rearing medium during the study ranged from 4.30 to 5.71 mg/L. The optimal oxygen concentration for *C. gariepinus* should not be less than 3 mg/L. However, dissolved oxygen produced in tanks with air tubes is better than in tanks with air stones. Microporous pipes create finer air bubbles and significantly more surface area than aeration stones. Small bubble diameters can increase the surface area of water that coincides with air, facilitating air-to-water diffusion and increasing oxygen solubility. A sufficient amount of oxygen in the water will accelerate the oxidation process of ammonia (Navisa *et al.*, 2014).

The content of ammonia in the rearing medium during the study ranged from 0.25–3.0 mg/L. The measurement results for ammonia (NH₃) levels at the start of stocking were 0.23 mg/L and 0.25 mg/L for the AT and AS tanks. At the end of the study were 0.85 and 1.5 mg/L. The ammonia content in each treatment during the study showed unsatisfactory results for *C. gariepinus*, because the optimum concentration of ammonia for *C. gariepinus* growth was <0.025 mg/L. Excess

ammonia can cause high oxygen consumption in water and be susceptible to gill damage (Irawati *et al.*, 2020) because oxygen is required to oxidize ammonia to nitrite and nitrate.

The results of nitrite measurements in all treatments during the study ranged from 0.019-2.0 mg/L. The nitrite value obtained is relatively high and not optimal for the growth of *C. gariepinus*. The high nitrites content can cause cultivated fish poisoning (Rejito, 2019). Besides that, it can also cause a decrease in the oxygen binding capacity of hemoglobin, causing brown blood disease, slowing fish movements, being unable to move and respond to stimulants, and appearing a yellow color on the gills. Sumitro *et al.* (2020) said that the optimal nitrite tolerance value in intensive catfish farming based on biofloc technology is 0.02-0.5 mg/L.

Sumitro *et al.* (2020) state that the optimal nitrate value in intensive catfish farming based on biofloc technology is 0.3-0.9 mg/L. The nitrate obtained from the measurement results during the study did not differ much from the nitrite. In comparison, the measurement results ranged from 0.004-4.0 mg/L. The nitrates value obtained is relatively high, so it is not optimal for the growth of *C. gariepinus*. The high nitrites content can cause cultivated fish poisoning using the biofloc system.

The temperature, DO, and pH in all treatments still fulfill the requirements to support the life of *C. gariepinus*. However, ammonia, nitrite, and nitrate in this study were different from the results obtained by Adharani *et al.* (2016) who conducted water quality management in raising catfish (*Clarias* sp.) using biofloc technology and concluded that the biofloc system effectively reduced ammonia, nitrite, and nitrate concentrations. This condition is caused by not changing the water during the maintenance period. However, the concentrations of ammonia, nitrite, and nitrate did not affect *C. gariepinus* productivity and survival rate.

CONCLUSION

The present study concluded that biofloc systems using air tubes showed better growth performance regarding final weight, average body weight, weight gain, and survival rate. Biofloc with air tube significantly increased the floc's volume and specific growth rate volume.

CONFLICT OF INTEREST

There is no conflict of interest in this manuscript between all authors upon writing and publishing this manuscript.

AUTHOR CONTRIBUTION

Diana Maulianawati: First author, writing and analysis of the research result, Hendri Kiing and Dena Pramita Dewi: contributing to preparation and data collection, Heni Irawati: Data analysis, Muhammad Amien: writing articles.

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