Development of biofloc technology to improve water quality in *Clarias batrachus* cultivation

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Abstract. Feed is one of the factors that determine success in catfish *Clarias batrachus* farming, and it contributes about 60-70% to the production costs. Furthermore, feed waste is an obstacle in cultivation that requires the application of biofloc technology. The water quality parameters observed in this study were temperature, pH, dissolved oxygen (DO), ammonia, nitrite, and nitrate. This study aims to (1) analyze several parameters that affect water quality, and (2) obtain environmentally friendly catfish cultivation technology. A completely randomized design (CRD) was adopted with five treatments and three replications. The treatments include P1 (control), P2 (biofloc), P3 (biofloc+bio balls), P4 (biofloc+ carbonation), and P5 (biofloc+bio balls+carbonation). The results showed a temperature of 26.6-28.5°C, 6.875-8.5 pH, 3.95-8.75 mg L^{-1} DO, 0.08-0.98 mg L^{-1} ammonia, 0.41-0.91 mg L^{-1} nitrite, and 9.23-11.4 mg L^{-1} nitrate. These parameters met the water quality standard. The best treatment is P5, which is a combination of biofloc, carbonation, and bio-balls. Therefore, it is feasible to be applied to catfish farming.
Key Words: bioballs, biofloc, carbonation, water quality parameters.

Introduction. Catfish *Clarias batrachus* is one of the most popular aquaculture products among Indonesians. The government has designated this species as one of the primary commodities to be developed because it is easily cultivated in areas with limited space and water resources. In addition to technical aspects, catfish has a wide consumption market with segmentation in the cultivation process. From the health aspect, 100 g of catfish contains 19.9 g of protein (Doktersehat 2019), which is only lower than snakehead fish (*Channa striata*) with 25.5 g of protein per 100 g (Solahuddin 2019).

Solid and dissolved fish waste is one of the causes of decreased water quality and susceptible to disease (Ogello et al 2021). Solid waste generally comes from feces and leftover feed not eaten by fish. It is further categorized into suspended and sedimentary solids, which settle at the bottom of the pond. Dissolved solids are a type of hazardous waste that can clog the gills and shorten the lamellae. It may result in decreased oxygen uptake and cause fish death (Hess et al 2017). This type of waste can cause an increase in the total amount of suspended and dissolved solids when left for a longer period. They can also increase the content of nitrogen compounds and cause stress in fish when decomposed.

In addition to solid waste, fish farming produces dissolved waste, which is the excretion of protein metabolism. The two most essential elements in dissolved waste, which are the critical components of fish nutrition, are nitrogen and phosphorus (Herath & Satoh 2015). However, fish generally retain 20-25% of protein, and phosphorus retention is 17-40%. The remaining protein and phosphorus are wasted as pollutants in undigested water bodies ranging from 3.6 to 37% and from 15 to 70%, respectively. The content of the excretory products ranges from 37 to 72% nitrogen and from 1 to 63% phosphorus (Deswati & Sutopo 2022). The nitrogen and phosphorus contents from the dissolved waste are mostly wasted in the form of ammonia and particulates, respectively. Ammonia is highly toxic to fish and other aquatic organisms when discharged into the environment as waste (Cai et al 2016).

The key to creating environmentally friendly, sustainable, efficient fish farming using water and feed is the biofloc technology that can minimize waste while it is being recycled into feed. This technology can also reduce the need to supply water which is currently a problem for catfish farming in urban areas. In addition to minimizing aquaculture waste, these benefits enable bioflocs to guarantee compliance with the requirements for good fish farming practices that ensure the quality and safety of products (Deswati & Sutopo 2022; Deswati et al 2022).

Deswati et al (2020a, b, c, 2021a, b, c) applied biofloc to the integration of *Oreochromis niloticus* and hydroponic plants and found that this technology can improve water quality (pH, ammonia, nitrite, nitrate, COD, BOD), heavy metals (Cu, Cd, Pb, and Zn) and macro-micro nutrients. These results are consistent with the reports of Taw et al (2008), Hari et al (2006), Ekasari (2008), Avnimelech & Kochba (2009), Kuhn et al (2009), Setiawan et al (2016), Sukendar et al (2016), and that the application of biofloc technology plays a role in improving water quality, as well as increasing biosecurity and productivity. It is also increasing feed efficiency and reducing production costs through lower feed expenses.

Several examples of biofloc applications in *C. batrachus* production have been carried out in a laboratory (Adharani et al 2016) and a field scale (Sudaryati et al 2017; Pramono et al 2018; Rizal et al 2018; Diarini et al 2019; Gaffar et al 2020). In general, the application shows the positive impact of the biofloc system on *C. batrachus* production.

The carbonation and bio balls technology were integrated into the biofloc-based *C. batrachus* farming system to develop biofloc technology. Carbonation technology increases the C/N ratio (Ogello et al 2021), and bio balls were used as substrates to optimize bacteria performance (Deswati et al 2020a, b, c, 2021a, b, c). Therefore, this study aims to (1) analyze several parameters that affect the water quality of the fish tank (FT), such as temperature, pH, dissolved oxygen (DO), ammonia, nitrite, and nitrate, as well as (2) obtain appropriate and environmentally friendly technology that can be applied in producing biofloc-based *C. batrachus*.

Material and Method

***Research time and place**.* The research was carried out from January to March 2022. The manufacture of aquaculture systems was carried out at Freshwater Aquaculture Center, Padang. Sample analysis was carried out at the Applied Analytical Laboratory of the Department of Chemistry, Andalas University, Basic and Central Laboratory of Andalas University.

Study preparation

Culture of biolacto bacteria (Lactobacillus sp.). Biolacto bacteria propagation is based on the procedure of Deswati et al (Deswati et al 2020a, b, c, 2021a, b, c), with the following steps: (1) prepared ingredients: pineapple (1 fruit), banana (3 pieces), vitamin C (3 grains), vitamin B complex (3 grains), fermented yeast (1 item), baker's yeast (1 sachet), egg yolks (3 grains), granulated sugar (1.25 kg), mashed, added with Biolacto bacteria (100 g), and stirred with other ingredients; (2) all these materials are put into gallons, added water to 90% full, tightly closed, and aerated continuously; (3) bacterial culture was carried out for 10 days and the propagation was successful if the slight fermented yeast odor with a new yellow color.

Carbonation technology. The carbonation technology increases the C/N ratio. It is manufactured by modifying the procedure of Ogello et al (2021), with the following stages: (1) prepare a 100 L airtight bucket, charcoal rice husks, and cow dung; (2) the carbonized rice husks are mixed with cow dung in a ratio of 3:1 and humidified to 60%; (3) lactic acid bacteria (LAB), which is a product of rice washing and fresh cow milk, is added to the mixture and fermented for 18 days in an airtight container; and (4) the carbonized product is placed in a filter cloth and hung in the fish pond as fertilizer. Carbon provides energy for LAB, which turns waste into fish feed by decomposing excess feed and inorganic matter at the bottom of the pond.

Adding bioballs. The bioball functions as a biological filter which is a growing medium for bacteria that play a role in the nitrification process, so bioballs can help improve water quality, especially by removing ammonia contained in water. Bacteria live by sticking to the surface of the bioball media, namely nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.); *Nitrosomonas* sp. play a role in oxidizing ammonia to nitrite, while *Nitrobacter* sp. play a role in oxidizing nitrite to nitrate. The bioball used is a round model because it has a tighter cavity, and is more effective in capturing dirt to be broken down by decomposing bacteria (Deswati et al 2020a, b, c, 2021a, b, c).

Application of biofloc system nutrition in fish ponds. The ingredients were placed in the FT with the following steps: 5 g of dolomite lime was weighed and dissolved in 5 mL of water, then kept for 30 minutes. Afterwards, 25 g of table salt was added, 20 mL of molasses in 50 mL was dissolved in water after 30 minutes, and the solution was added (Deswati et al 2021a, b, c). The C/N ratio was checked to ensure it was > 12. Molasses is added until the C/N ratio is met. Furthermore, FT ratio is C/N > 12 (Crab et al 2010).

Stirring and aeration. Stirring and aeration must be performed during maintenance to avoid overhauling plankton bodies due to high ammonia content and low oxygen. Furthermore, stirring is required to keep the floc suspended in the water (Hargreaves 2006). The aeration and air conveying systems are usually combined to achieve aeration and circulation for indoor biofloc systems. Higher oxygen concentrations must be maintained because: (1) cultured fish and other planktonic organisms require oxygen for metabolism, and (2) bacterial populations need oxygen to degrade waste and reproduce.

Seed selection and sowing. The selected catfish seeds are based on SNI, 2000, namely they have active movement against the current and agile, the response to feed is excellent and are responsive to stimuli, the size of the seed must be uniform; there are no defects; and no spots on the seed body, body color shiny and not dull (pale), and once stocked in the rearing pond will quickly spread. The catfish seeds used were 750 fish (stocking density of 50 fish per fish tank, total length 11.3 cm and weight 7.2 g).

Feed management. After the seeds are stocked into the pond, they are fasted for two days for the adaptation process to the new environment while waiting for the stomach contents to be empty. The first feeding after fasting was 3% of biomass weight given twice daily, in the morning and afternoon.

Feeding biofloc nutrition per week. The stages of providing nutrition per week are (1) weighing 4 g of dolomite lime, dissolved in 10 mL of water (taken the soluble) and put into FT (100 L capacity), waiting 30 minutes; (2) weighing 25 g of salt dissolved in water (taken the soluble), put into the FT; (3) wait 30 minutes, a mixture of molasses and water that has been boiled (ratio 1:1), cooled, put into the FT as much as 20 mL; (4) added 100 mL of bacteria that have been cultured are added.

Study parameters. The parameters observed include temperature, pH, DO, ammonia, nitrite, and nitrate.

Experiment design. The study was conducted using a completely randomized design (CRD) consisting of 5 treatments and three replications. The treatments that were used were: P1 (control); P2 (biofloc); P3 (biofloc + bioballs), P4 (biofloc + carbonization); and P5 (biofloc + carbonization + bioballs). Statistical analysis of water quality parameter data using CRD was done with the Statistical Package for Social Sciences (SPSS, version 16.0 for Windows).

Results and Discussion

Temperature. The optimal temperature to maintain flock stability ranges from 26 to 32°C (Azim & Little 2008). However, higher temperatures (> 35°C) may cause a decrease in floc stability due to increased fish manure (Kuhn et al 2010). Temperature fluctuations also affect biofloc-based fish culture media (Azim & Little 2008). High oxygen solubility occurs at low temperatures due to low bacterial activity (Harvey et al 2011) the process of protein formation slows down (Simon & Azam 1989). In the present study temperature fluctuations were under normal conditions in the range of 26.6-28.5°C, which the fish could tolerate and had better bacterial activity, as shown in Figure 1. Statistically, it showed that each treatment was not significantly different ($p > 0.05$) concerning the temperature value and had met the quality standards of 22-32^{o}C (Sommerville et al 2014), and of 20-30^{o}C (Emerenciano et al 2017; Santhosh & Singh 2007).

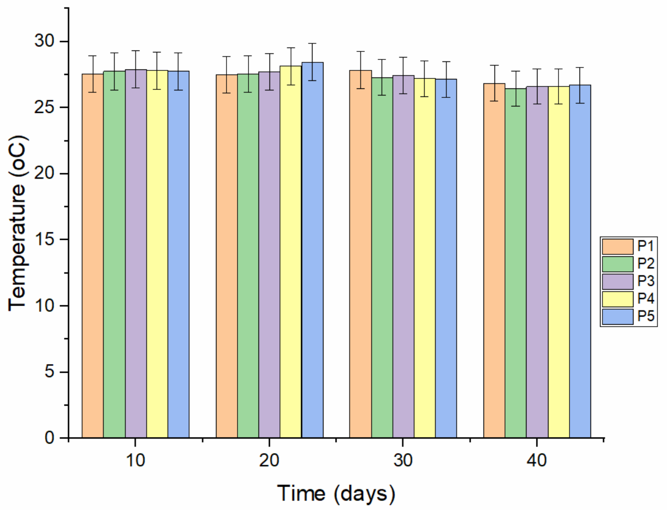

Figure 1. Temperature (^{o}C) at different times (days).

pH. The pH values obtained in water samples varied between 6.875 and 7.75, and statistically, it showed that each treatment was not significantly different ($p > 0.05$) These values indicate a slightly basic or alkaline pH. These values are included in the recommended values for freshwater fish farming. The pH value provides information about the number of heavy metals that may be present in the pond water medium. These findings are consistent with previous studies, namely: pH 6.7-9.8 (Santhosh & Singh 2007), 6-9 (GRRI No. 22/2021), 6.3-8.5 (Ray et al 2011), and 6.8-8.0 (Emerenciano et al 2017). Furthermore, Sommerville et al (2014) recommend the best performance for nitrifying bacteria a pH of 6.0-8.5, and Furtado et al (2011) recommend 7-8 for significant effects on the metabolism of heterotrophic bacteria.

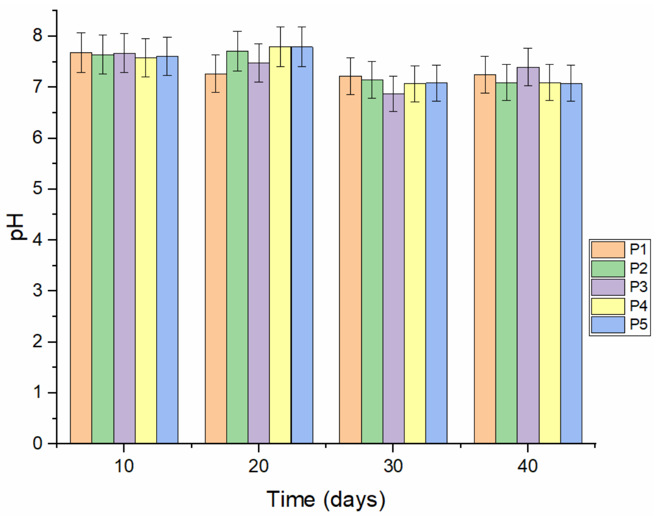

Figure 2. pH at different times (days).

Dissolved oxygen (DO). DO plays an important role because microbial metabolic activity requires continuous and sufficient oxygen to decompose organic matter (Green & McEntire 2017). Furthermore, bioflocs, small media with large amounts of organic matter, require a continuous water supply to decompose the organic matter (Liu et al 2018).

The dissolved oxygen content of the examined water samples from the FT varied from 3.95 to 8.75 mg L^{-1} (Figure 3), and this DO concentration was very suitable for the development of biofloc-based catfish culture. Statistically, it showed that each treatment was significantly different ($p < 0.05$) on the optimal DO value and met the water quality standard interval, namely: 4-5 mg L^{-1} (Martinez-Cordova et al 2015), > 4 mg L^{-1} (Emerinciano et al 2017), > 3 mg L^{-1} (GRRI No. 22 2021). The DO concentration remained high for the first 20 days, then DO decreased (Figure 3) which may be due to the addition of molasses to maintain the C/N ratio > 12 so that the consortium of microorganisms could grow and develop rapidly. The decrease in DO levels in different treatments was due to differences in the number of consortia of floc-forming microorganisms. This is useful for obtaining information about the level of bacterial activity, photosynthetic characteristics, nutrition, and the ability of fish to survive in an aquatic environment. Temperature is the main factor that determines the amount of dissolved oxygen in the water. High temperatures reduce DO and increase microbial growth (Deswati et al 2021d).

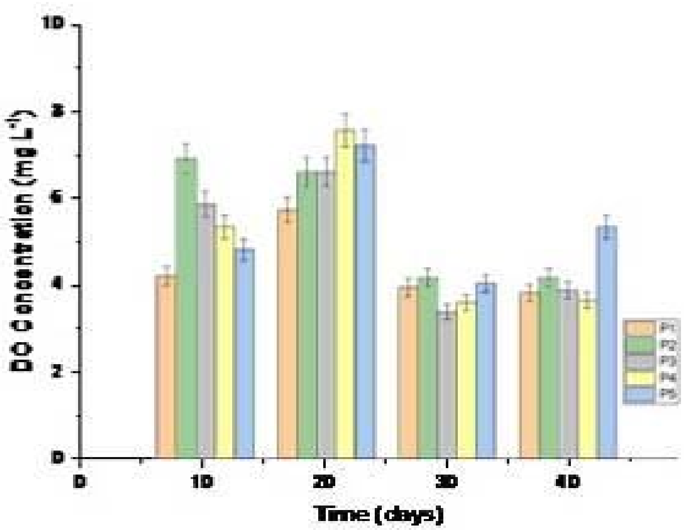

Figure 3. DO concentration (mg L^{-1}) at different times (days).

Ammonia concentration. The ammonia concentrations ranged from 0.0043 to 1.6405 mg L^{-1}, tended to decrease, as shown in Figure 4, and still met the water quality standard that was set at < 0.5 mg L^{-1} (GRRI-22/2021), except P1 (1 .6405 mg L^{-1}). This is presumably due to the ability of bioflocs to improve water quality (Azim & Little 2008; Deswati et al 2022), which are found in P2, P3, P4 and P5. Another cause is the presence of carbonation which can increase the C/N ratio and bio balls which can improve the performance of microorganisms.

The results showed that the best treatment to reduce ammonia concentration is P5, presumably due to a consortium of biofloc bacteria combined with bio balls and carbonation. Bio balls used a bacterial consortium as a substrate, according to the results of Deswati et al (2020a, b, c, 2021a, b, c). Furthermore, carbonation was used to increase the C/N ratio (Ogello et al 2021). Carbon from rice husks is used as energy for LAB, decomposing excess feed and inorganic materials at the bottom of the FT and converting the waste into fish feed. LAB can produce compounds such as lactic acid, hydrogen peroxide, diacetyl, acetaldehyde, and bacteriocin. The antibacterial properties of this compound inhibit the growth of pathogenic microbes (Ringo & Gatesoupe 1998; Gatesoupe 1999). Some studies argued that LAB is an inhabitant of the digestive tract in fish (Nikoskelainen et al 2001), which does not cause side effects (Ringo et al 2002). Meanwhile, *Lactobacillus* is a Gram-positive genus of bacteria from LAB because of its ability to form a thick layer of peptidoglycan. It is facultative or microaerophilic anaerobes that can live in environments with low oxygen content. In addition to producing lactic acid, these bacteria can also produce toxic compounds, namely hydrogen peroxide and bacteriocins, often used to kill pathogenic and spoilage microbes (Deswati & Sutopo 2022; Deswati et al 2022).

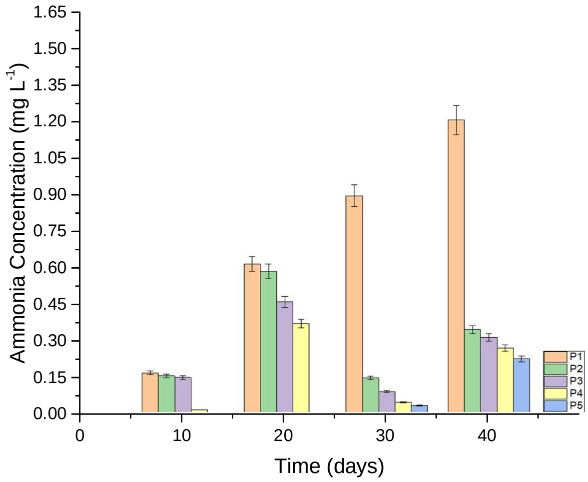

Figure 4. Ammonia concentration (mg L^{-1}) at different times (days).

Bacillus bacteria can live in various habitats and decompose complex into simpler compounds. These bacteria partake in the decomposition process of organic compounds because of their amylolytic, proteolytic, lipolytic, antibiotic, and cellulolytic properties, which have great potential to be developed in the biotechnology industry (Hatmanti 2000). According to Waluyo (2007), deamination can be used to decompose ammonia by microbes. Deamination is the process of disassembling protein into amino acids, then into ammonia and several other substances by bacteria producing the enzyme urease. With this enzyme, urea can be broken down into ammonium carbonate, while ammonium carbonate is easily decomposed into ammonia, carbon dioxide and water. Gianfreda & Bollag (1996) also emphasized that urease is the only catalytic enzyme in the hydrolysis of urea. George et al (2011) reported that the genus *Bacillus* is a microbe from a group of bacteria that can synthesize urease sufficiently in waters. Therefore, it is suspected that the presence of several bacteria from the genus *Bacillus* contained in the consortium optimizes the ammonia reshuffle process. According to Van Wyk & Avnimelech (2007), *Bacillus* sp. can reduce excess ammonia to form protein, thereby removing toxic substances and simultaneously forming nutrients.

Ammonia concentrations at P2, P3, P4 and P5 were lower than P1, as shown in Figure 4. This is presumably due to the addition of molasses to grow heterotrophic bacteria, which effectively removed ammonia-N (Avnimelech 1999). However, the decomposition of algae and bacterial cells (Avnimelech 2012) and the reduction of the nitrification process due to the lack of carbohydrate supplementation kill the bacteria due to insufficient carbon (Van Wyk 2004). Furthermore, the increase in temperature, which causes agglomeration of sludge and forms an anaerobic zone (Somerville et al 2014), as well as the release of ammonia along with other toxic compounds (Hargreaves 2013) may be the reasons for the spike in its concentration.

Ammonia concentrations at P2, P3, P4, and P5 were lower than P1 (Figure 4), presumably due to the addition of molasses for the growth of heterotrophic bacteria which effectively removed N-ammonia (Avnimelech 1999). However, the lack of carbohydrates can cause the decomposition process of algae and dead bacterial cells to decrease (Avnimelech 2012) and the nitrification process also decreases (Van Wyk 2004). The decreased temperature causes agglomeration of sludge which forms an anaerobic zone (Sommerville et al 2014) by releasing ammonia along with other toxic compounds (Hargreaves 2013).

Nitrite concentration. A nitrite is a form of nitrogen compound which can cause death in fish at a level of 1.0 mg L^{-1}. It results from the decomposition process of organics by microorganisms in water. Nitrite usually does not cause water quality problems in FT because of the rapid conversion of nitrite to nitrate (Gómez et al 2019) and presumably due to aeration and vigorous stirring (Deswati & Sutopo 2022). The results of the statistical test of the best treatment for the concentration of nitrite is the P5 treatment. Nitrite reform or the nitrification process by nitrifying bacteria by oxidizing ammonia to nitrite and nitrate, while nitrate reform or denitrification process by denitrifying bacteria by reducing nitrate to nitrite with low oxygen levels (Effendi 2003). *B. megaterium* is an aerobic bacterium and its ability as a nitrifying bacterium (Heylen & Keltjen 2012; Peela et al 2015); but can also live in low oxygen conditions and its ability to assist in the denitrification process (Otari & Ghosh 2009; Peela et al 2015). At 20 and 40 day sampling, the nitrite concentration tends to decrease due to the conversion of nitrite to nitrate during nitrification as suggested by Sang et al (2020) and Kim et al (2008). However, the increase may be due to the oxidation of ammonia to nitrite.

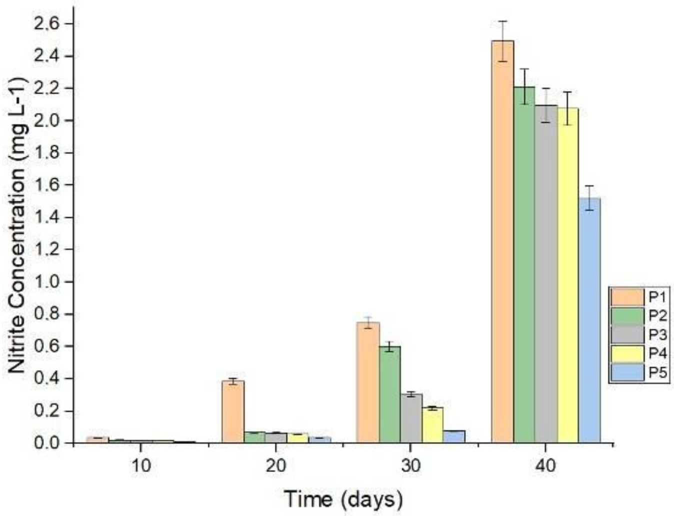

Figure 5. Nitrite concentration (mg L^{-1}) at different times (days).

The nitrite content of the water samples during the study varied from 0.0107-2.4931 mg L-1 (Figure 5), and nitrite on day 0-30, except that P1 was very suitable for the development of catfish culture, which was < 1 mg L-1 (GRRI-22/2021; Emericiano et al 2017). This can be explained that the use of biofloc can improve water quality, and improve the performance of nitrifying bacteria and a consortium of microorganisms that convert nitrite to nitrate. Furthermore, on the 40th day, the nitrite content increased dramatically and exceeded the permissible threshold.

Nitrate concentration. Nitrate is a nitrogen component in water and the main nutrient for plant and algal growth. According to Hendrawati et al (2008), nitrogen in the form of nitrate is very soluble in water and stable. Furthermore, the performance of microorganisms is influenced by temperature and pH. The nitrification process is influenced by temperature, which impacts enzyme activity. Increasing temperature will cause a decrease in the enzyme's pH, and digestive enzymes can easily destroy the raw materials that come from the food consumed. Meanwhile, higher temperature results in an increased enzymatic process or metabolism, thereby causing faster decomposition of a material (ammonia or nitrite) (Deswati et al 2020c, 2021d).

The nitrate value ranged from 3.6909 to 16.6055 mg L^{-1} and met the quality standard of < 400 mg L^{-1} (Somerville et al 2014), 20 mg L^{-1} (GRRI-22/2021), 0.5-20 mg L^{-1} (Emerenciano et al 2017). The statistical analysis showed that each treatment was significantly different ($p < 0.05$) with respect to the concentration of nitrate values, as shown in Figure 6. Furthermore, P2, P3, P4 and P5 had higher nitrate concentrations than P1, reflecting the activity of nitrifying microorganisms in bioflocs. Due to the important role of nitrification in culture water, several studies suggested nitrate accumulation in biofloc systems (Azim & Little 2008; Hargreaves 2013; Kim et al 2008).

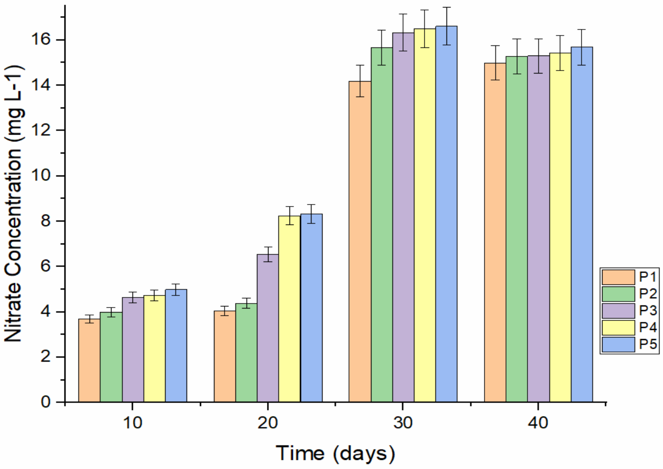

Figure 6. Nitrate concentration (mg L^{-1}) at different times (days).

Conclusions. Carbonation and bio balls technology was integrated into the biofloc-based Clarias batrachus farming system to develop biofloc technology. Carbonation technology increases the C/N ratio, and bio-balls are used as substrates to optimize the performance of bacteria. This study aimed to (1) analyze several parameters that affect pond water quality, such as temperature, pH, DO, ammonia, nitrite, and nitrate; as well as to (2) obtain appropriate and environmentally friendly technology that can be applied in producing *Clarias batrachus*. The results showed a temperature ranging from 26.6 to 28.5°C; pH 6.875-7.75; DO 3.95-8.75 mg L^{-1}; ammonia 0.0043-1.6405 mg L^{-1}; nitrite 0.0107-2.4931 mg L^{-1}; nitrate 3.6909-16.6055 mg L^{-1} which met the water quality standard for freshwater fish farming. The best treatment was P5, which combined biofloc, carbonation, and bio balls and is feasible to be applied to *Clarias batrachus* farming.

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Conflict of interest. The authors declare that there is no conflict of interest.

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