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Modified biofloc technology and its effects on water quality and growth of catfish

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ABSTRACT

Biofloc-based Catfish (*Clarias batrachus*) farming allows for improving water quality, and growth. To further optimize the biofloc technology, we modified it with carbonation and bio balls to improve the C/N ratio and the performance of the floc-forming bacterial consortium. The purpose of this study is to analyze several parameters such as floc volume, BOD (biological oxygen demand), COD(chemical oxygen demand), BOD/COD, TOM (total organic matter), TDS(total dissolved suspended), orthophosphate, sulfate. SGR(specific growth rate), FE(feed efficiency), FCR(feed conversion ratio), SR (survival rate) and to obtain appropriate and environmentally friendly technology that can be applied in producing Catfish based on biofloc. In this study, a completely randomized design was used with 5 treatments and 4 replications, From the results of the study obtained: floc volume (76–88 mL L^{-1}), BOD (2.152–2.367 mg L^{-1}), COD (5.462–7.312 mg L^{-1}), BOD/COD (0.324–0.394), TOM (5.1–19.2 mg L^{-1}), TDS (318–1560 mg L^{-1}), orthophosphate (0.7–9.1 mg L^{-1}), sulfate (18.1–34.0 mg L^{-1}), SGR (0.058–0.066), FE (90.89–98.79%), FCR (0.933–1.104), SR (84–88%). In the future, the application of a combination of carbonation and bio balls in Catfish farming based on biofloc is feasible to be developed.

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Biofloc technology; modified; carbonation and bio balls; water quality; growth

Introduction

Catfish (*Clarias batrachus*) cultivation in Indonesian is growing rapidly, with national production between 2015–2018 increasing by 13.84%.[1] Waste from *C. batrachus* cultivation that is produced pollutes the environment because Clarias batrachusis a type of fish with a fairly high protein requirement for feed and the ability to retain nitrogen is only 63–66%[2] so that nitrogen waste generated in waters is 33–36% potentially pollute the environment.

Currently, the aquaculture industry faces several challenges including environmental degradation, climate change, and the spread of pathogens. In addition, feed and energy costs, as well as fish diseases contribute negatively to fish production. Aquaculture with zero or minimal water exchange can be successfully achieved with biofloc technology (BFT) (Ha.[3–5] This technology will reduce the frequency of discharge of wastewater nutrients into the environment, and will also reduce the likelihood of escape, as well as the prevalence of disease,[6,7] minimizing waste as well as recycling waste into feed,[8–10] creates environmentally friendly and sustainable fish farming,[11] and reduces the need to supply water[12] which is currently a problem for *C. batrachus* farming in urban areas.

Biofloc is a heterogeneous macro aggregate of planktonic material in the water column, which is a consortium of floc-forming bacteria, diatoms, filamentous microalgae, micro and macro invertebrates, protozoa, feces and uneaten feed. Bioflocs form the basis of food chains in aquatic ecosystems by converting them into single cell proteins (SCP). Therefore, bioflocs are responsible for early nutrient cycling processes in aquatic ecosystems.[13,14]

Heterotrophic bacteria are responsible for capturing nitrogen compounds released by fish and using them in their growth, thereby eliminating the toxicity of ammonia and nitrite.[15] For heterotrophic bacteria to grow in biofloc systems, the C/N ratio must be high (above 10) and aeration must be intense.[16] The addition of organic carbon is required to maintain a suitable C/N ratio for bacterial growth and biofloc production.[17,18] The biofloc system absorbs dissolved nitrogen compounds and improves water quality.[19–24] A high C/N ratio is required to ensure the optimal growth of heterotrophic bacteria and produce new bacterial cells.[25] In addition, it has been demonstrated that the biofloc produced in this system can also be used for shrimp and fish feed.[26–29]

Aquatic performance depends on many factors in the biofloc system. Biofloc production is influenced by many biological factors including stocking density,

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supplementary feed, and availability of natural feed.[7,26,27,29–33] Similarly, abiotic factors (NH_3, NO_2 --N, NO_3--N, BOD, COD, BOD/COD, TOM, TDS, orthophosphate, sulfate) also affect fish production in the BFT system.[9,34] The addition of carbon sources, water exchange, and addition of substrates is also important for the long-term performance of fish.[35,36]

Deswati et al[19–24] have applied biofloc to the integration of tilapia and hydroponic plants, it was found that the use of biofloc can improve water quality (pH, ammonia, nitrite, nitrate, COD, BOD), heavy metals (Cu, Cd, Pb, and Zn) and macro-micro nutrients. These findings are reinforced by research results [9,13,22,37–39] who said that the application of biofloc technology plays a role in improving water quality, increasing biosecurity, and increasing productivity. Increasing feed efficiency and reducing production costs through lower feed costs.

Furthermore, to develop biofloc technology, we have modified biofloc technology (BFT) with carbonation[40] and bio balls[19–24] in biofloc-based *C. batrachus* cultivation. Carbonation to increase the C/N ratio, and bio balls to improve the performance of a consortium of floc-forming bacteria. The aims of this study were (1) to analyze several parameters such as floc volume, BOD, COD, BOD/COD, TOM, TDS, orthophosphate, sulfate, SGR, FE, FCR, SR, and (2) to obtain appropriate and environmentally friendly technology that can be applied in producing biofloc-based *C. batrachus*.

Materials and methods

Research preparation

Biolacto bacteria breeding

Biolacto bacteria propagation is based on the procedure of Deswati et al,[19–24] 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

Carbonation was applied to increase the C/N ratio. In preparing carbonation, it is done by modifying the procedure of Ogello et al,[40] namely: (1) Prepare a bucket (capacity 100 L), carbonated rice husks, and cow dung; (2) First, the carbonated rice husks are mixed with cow dung in a ratio of 3 : 1, then moistened to 60%; (3) Lactic Acid Bacteria (LAB) which is a fermented product of rice washing and fresh cow's milk is added to the mixture and fermented for 20 days in an airtight bucket; (4) The carbonation product is put into a streaming cloth and hung in the fish pond as fertilizer. Carbon provides energy for LAB which breaks down excess feed and inorganic matter (waste) at the bottom of the pond and converts the waste into fish feed.

Adding bio ball

Bio ball functions as a biological filter which is a growing medium for bacteria that play a role in the nitrification process, so bio balls can help improve water quality, especially by removing ammonia contained in water. Bacteria live by sticking to the surface of the bio-ball media, namely nitrifying bacteria (*Nitrosomonas* sp and *Nitrobacter* sp), *Nitrosomonas* plays a role in oxidizing ammonia to nitrite, while *Nitrobacter* sp plays a role in oxidizing nitrite to nitrate. The bio ball used is a model. round because it has a tighter cavity, and is more effective in capturing dirt to be broken down by decomposing bacteria.[19–24]

Biofloc application for clarias batrachus production

Biofloc system nutrients in fish ponds

All materials have been prepared and then put into the fish tank (FT), with the following steps: (1) dolomite lime (5 g) is dissolved in 5 mL of water and allowed to stand for 30 minutes; (2) add 25 g of table salt, wait for 30 minutes, (3) dissolve 20 mL of molasses in 50 mL of water and add the solution,[19–24] (4) check the C/N ratio, and set the C/N ratio >12. If the C/N ratio is not met, add molasses as a C source,[26] considering the cheaper price of molasses from another C source.

Oxygenation and turbulence mixing

An blower is a tool that helps dissolve oxygen in the air into the pool water. In biofloc systems, intensive turbulent mixing is required to maintain high dissolved oxygen levels and prevent solids deposition. Turbulence prevents anoxic deadlocks that are lethal to cultured organisms. Therefore, the BFT system requires a good layout of the aerator, which must be moved regularly to avoid the deposition of solid particles in areas with little or no current. Biofloc systems require up to 6 mg L^{-1} h^{-1} oxygen, which translates to about 30 aerator horsepower per hectare of the pond.[3] Higher oxygen concentrations must be maintained because: (1) farmed fish and other planktonic organisms require oxygen for metabolism; and (2) bacterial populations need oxygen to degrade waste and reproduce. BFT ponds with low aeration mechanisms tend to accumulate organic waste at the bottom and facilitate anoxic conditions. The anoxic zone (also called the dead zone) is known to accumulate high ammonia. Ammonia accumulation can be harmful to fish especially during the mixing of water. Farmers must take care to avoid anoxic conditions or dead zones in the BFT system, therefore the circulation is made in the same direction and the aeration stones are moved every 3 days

Clarias batrachus seeds

The *C. batrachus* seeds used were 750 fish (stocking density of 50 fish per fish tank, total length 11.3 cm and weight 7.2 g), with the criteria: fish move actively against the current and agile, responsive to feed and others, uniform seed size, the fish's body is not deformed, the body color is shiny and not pale, and after stocking the fish are distributed quickly.[41]

Feed management

After the seeds are stocked into the pond, then the seeds have fasted for 2 days for the adaptation process to the new environment while waiting for the stomach contents to be empty. The content of fish feed used: water content max 14%, protein 21–23%, fat min 5%, fiber 5%, ash 8%, calcium 0.80–1.10%, phosphorus 0.5%, ME 3000 Kcal/kg, aflatoxin 50, amino acids (lysine 1.20%, methionine 0.45%, methionine + cystine minimum). Feeding as much as 3% of the biomass weight was first done after fasting, then the feed was given at libitum 2 times a day, in the morning and afternoon.

Biofloc nutritional requirements 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.

Research parameters

Parameters observed were: volume of biofloc, water quality, growth, and survival.

(1) The volume of the floc was measured with an Imhoff cone,[42,43] water samples were taken in the morning from a depth of 15–25 cm, then allowed to stand for approximately 20 minutes. To calculate the volume of the floc uses the following formula:

$$floc\ volume(mLL-1) = \frac{sediment\ volume}{water\ sample\ volume} \times 1000$$

(2) Water quality (BOD,[44] COD,[45] BOD/COD, TOM,[46] TDS,[47] orthophosphate,[48] dan sulfate).[49]

(3) Growth and survival : average daily growth (ADG), Average body weight (ABW), SGR (specific growth rate), FE (feed efficiency), FCR (feed conversion ratio), SR (survival rate)

(a) ADG[50]

$$\mathrm{ADG}(\mathrm{gunit}^{-1}\ \mathrm{day}^{-1}) = \frac{Final\ body\ weight\ -\ initial\ body\ weight}{duration\ of\ experiment}$$

(b) ABW[51]

$$\mathrm{ABW}(\mathrm{gunit}-1) = \frac{sample\ fish\ weight\ (g\ )}{number\ of\ fish\ samples\ (ekor\ )}$$

(c) SGR[52]

$$\mathrm{SGR}(\%) = \frac{Ln\ final\ body\ weight\ -\ Ln\ initial\ body\ weight}{duration\ of\ experiment}\ \mathrm{x100}\ \%$$

(e) FCR[52]

$$\mathrm{FCR} = \frac{Total\ feed\ consumed\ (kg\ )}{Biomassa\ (kg\ )}$$

(f) SR[52]

$$\mathrm{SR}(\%) = \frac{Final\ number\ of\ live\ fish}{Initial\ number\ of\ live\ fish\ stocked}\ x100\ \%$$

(g) FE[53]

$$\mathrm{FE}(\%) = \frac{final\ body\ weight\ -\ initial\ body\ weight}{Feed\ fed} \times\ 100\%$$

Statistical analysis

The study was conducted using a completely randomized design (CRD) [54] consisting of 5 treatments and 4 replications. The treatments that will be used are : P1 (control); P2 (biofloc); P3 (biofloc + bio balls), P4 (biofloc + carbonation); and P5 (biofloc + carbonation + bio balls). Statistical analysis of water quality parameter data using the Statistical Package for Social Sciences (SPSS, version 16.0 for windows).

Results and discussion

Mechanism of formation and maintenance of biofloc

Bioflocs are formed in the water column through complex physical, chemical, and biological interactions between organic nutrients, physical substrates, and various microorganisms such as zooplankton and phytoplankton, free and bound heterotrophic bacteria, conglomerates of particulate organic matter, grass eaters (e.g., protozoa, rotifers, flagellates, ciliates, copepods), filamentous cyanobacteria, dinoflagellates, and nematodes.[17,55–57] In this process, the main constituent of the floc matrix is the extracellular polymeric structure that forms the microbial capsule, which binds to the biofloc components.[58,59] Flocs are usually composed of polysaccharides, proteins, humic compounds, nucleic acids, and lipids, and are mainly produced as mucus layers or capsules under nitrogen restriction.[60] Under favorable conditions, the biofloc aggregates varied in size from microscopic to >1 mm, which was similar to the size of commercial fish pellets for juvenile fish. The average microbial biomass density is slightly above 1.0 g wet weight mL^{-1} of floc aggregate which makes the biofloc particles slow to sink (1–3 $m\ h^{-1}$).[61] The ability of flocculation and sinking of bioflocs is an adaptation mechanism to avoid adverse ecological impacts, such as light and stress by organisms in higher trophic chains.[58,59] In the process of sinking the biofloc sticks to the substrate and makes a mat that attracts other aquatic microorganisms.

Biofloc technology is an alternative technique in which nutrients can be continuously recycled and explicitly reused nitrogen can be converted into microbial biomass which can be processed into feed ingredients for cultured fish.[10,62] Instead of destroying or storing nutrients at the bottom of the pond, these nutrients can be converted into the biomass of microscopic organisms and reused as single-cell protein (SCP). In this study, a blower was used for constant aeration and added an external carbohydrate source in the form of molasses in the fish tank water column for the development of high-level dense heterotrophic microbial flocs in suspension which can do both as a bioreactor controlling water quality and also act as a protein-based food source for catfish.

Moreover, bioflocs are irregular masses of various sizes and porosities that can be easily compressed and are highly permeable to liquids.[63,64] Different mechanisms can affect floc formation, appearance, and stability. Many organisms secrete viscous polymers (e.g., humic polymeric compounds, proteins, and polysaccharides) which act as adhesives and join cells and other particles together and form flocs.[5] Another mechanism is related to the balance between attractive forces (molecules, dipoles, hydrogen bonds) and electrostatic repulsion forces. Most organisms have a negative charge and cause mutual electrostatic repulsion. If this repulsion is reduced, then strong attractive forces can occur. This occurs when the salt concentration is high and there are polyvalent ions in the environment.[5,56] Moreover, calcium and aluminum ions stimulate the formation of stable flocs, and different organisms (e.g., algae, fungi, or bacteria) and their impurities aid in the connection and dynamics of bioflocs.[56,57,59,65] Another characteristic of biofloc is the open floc structure. An important feature of this floc causes water and chemicals to flow in/out throughout the flock, which is effective for providing nutrients and removing metabolites.[66]

Dense microbial flocs at the bottom of this pond are active phytoplankton, bacteria, aggregates of living and dead particulate organic matter, and bacterial feeders, which are inclusively defined as suspended growth system.[67] The general mechanism of biofloc formation in fish and shrimp culture systems is shown in (Figure. 1). Ammonia together with nitrogen substances will be converted into bacterial protein biomass if the C/N ratio is properly regulated in bacteria.[69] By adjusting the C/N ratio, heterotrophic microorganisms are stimulated to grow so that bacteria can assimilate waste ammonium for the production of new biomass.[70] This conversion is an additional absorbent for ammonia and contributes to the solubilization of the waste conversion. Unlike nitrifying bacteria, the rate of proliferation and production of microbial biomass per unit substrate is 10 times higher so that the fixation of inorganic nitrogen substances works faster in biofloc water and nitrogen consumption by the growth of these bacteria reduces ammonium concentrations faster than nitrification.[67] Crab et al[71] described how much organic carbon is needed to remove nitrogenous waste released from feed and manure that is not consumed in a biofloc system. For example, if fish are fed 2% of their body weight[72] then 20 g of feed will be required feed per kg of fish per day. Again, if the feed contains 25% protein, after calculation, approximately 5 g of protein will be supplied to these fish through the feed each day. Once converted to nitrogen, that means about 0.8 g of nitrogen. On the other hand, Piedrahita[11] stated that the accumulation of inorganic nitrogen from uneaten feed and fish manure was about 75% which means 0.6 g of 0.8 g of nitrogen. Finally, for microbial conversion the ratio of inorganic carbon to nitrogen, 10 will work better, and if this is followed then 6 g of carbon per kg of fish on day^{-1} may be required to produce the flock.

The average volume of flocks in treatments P2, P3, P4, and P5 were 76, 72, 84, and 88 $mL\ L^{-1}$, respectively,

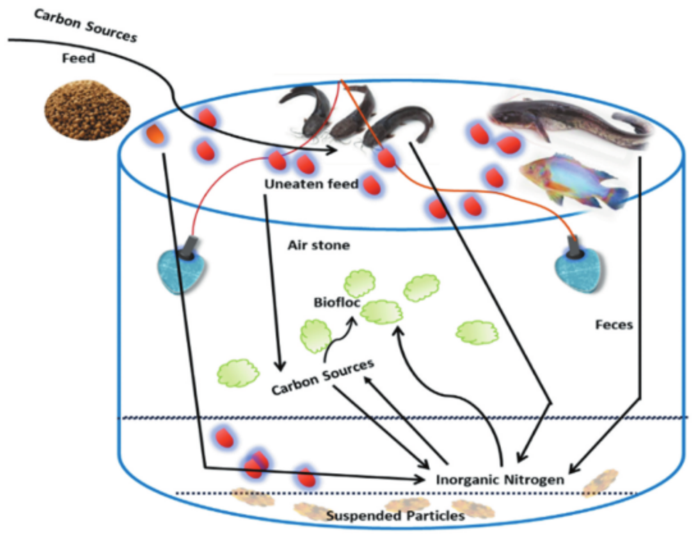

Figure 1. Mechanism of biofloc formation and maintenance in fish tanks (adapted from Zafar & Rana [68]).

while the volume of flocks for *C. batrachus* cultivation was 150 ml L^{-1} or 15% water volume.[41,73] Flock volume that is too dense causes *C. Batrachus to* be weak, less agile, and decreased appetite.[43]) In this study, biofloc monitoring is always carried out so that the C/N ratio is >12.

Daily, water quality monitoring was carried out and it was found that the volume of floc increased, but the density of bacteria at the end of the study tended to stagnate, according to Suprapto & Samtafsir[43] that the increase in floc volume was not necessarily followed by an increase in the microbial community (bacteria). Zhang et al[74] stated that what caused the increase in bacteria was the addition of a carbon source in the media. The growth of heterotrophic bacterial cells in FT aims to utilize nitrogen waste in high protein feed by providing an organic carbon source so that the C/N ratio increases.[19–24] In the P2 and P3 treatments, the floc was brown, while in the P4 and P5 treatments it was dark brown and slightly black, presumably influenced by the color of the carbonated water.

Studies show that biochemical compound biofloc contains high levels of protein 34.5–40.35%, lipid 3.1%, fiber 5.8%, ash 11.3% and energy 18 kJ g^{-1}. The level of protein in the biofloc depends on the crude protein in the food and the carbon source applied. Polyunsaturated fatty acids in biofloc contain 27–28%, monounsaturated fatty acids 28–29%, and 30–35% saturated fatty acids.[75] The number of bacteria in biofloc ponds can be 106–109 per mL of floc, which contains 10–30 mg of dry matter and bacteria produces 60–600 kg ha^{-1} day^{-1} protein for fish.[71] Although the biofloc has sufficient protein to sustain significant fish growth, however, supplementary feed is very important and the amount should be adjusted based on the volume of the biofloc.

Effect of different treatments on water quality

Effect of different treatments (P1, P2, P3, P4 and P5) on water quality (BOD, COD, BOD/COD, TOM, TDS, Orthophosphate, Sulfate) can be seen in Table 1.

Table 1. Value of BOD, COD, BOD/COD, TOM, TDS, orthophosphate, sulfate in fish tank water samples.

Parameter	P1	P2	P3	P4	P5
BOD	2.152 ± 0.002^{a}	$2.360 \pm 0.\ 003^{bc}$	2.358 ± 0.002^{b}	2.365 ± 0.002^{cd}	2.367 ± 0.004^{d}
COD	$5.462 \pm 0.\ 050^{a}$	6.115 ± 0.107^{b}	6.172 ± 0.049^{b}	6.375 ± 0.097^{c}	7.312 ± 0.251^{d}
BOD/COD	0.394 ± 0.003^{a}	0.386 ± 0.007^{ab}	0.382 ± 0.003^{b}	0.371 ± 0.006^{c}	0.324 ± 0.011^{d}
TOM	8.225 ± 3.286^{a}	10.925 ± 4.873^{a}	11.700 ± 5.582^{a}	12.250 ± 5.536^{a}	12.450 ± 5.719^{a}
TDS	519.625 ± 358.298^{a}	986.400 ± 339.273^{a}	1014.125 ± 314.922^{a}	929.750 ± 383.815^{a}	1083.625 ± 332.605^{a}
Ortofospat	4.068 ± 3.712^{a}	4.822 ± 3.397^{a}	5.454 ± 3.011^{a}	5.703 ± 3.116^{a}	5.691 ± 3.302^{a}
Sulfate	18.109 ± 16.963^{a}	31.838 ± 17.054^{a}	31.752 ± 21.381^{a}	29.781 ± 25.590^{a}	34.081 ± 23.413^{a}

Mean values in the same row with different letters are significantly different from each other ($P < .05$).

BOD, COD and BOD/COD

The range of BOD values obtained in water samples ranged from 2.152 to 2.367 mg L^{-1}, and statistically, the effect of treatment on BOD was significantly different ($p < .05$) (Table 1). This is presumably due to the accumulation of sources of organic matter from fish waste, uneaten feed residue, and a consortium of dead microorganisms. BOD at P5 > P4 > P3 > P2, due to the application of carbonation, bio balls, and biofloc the consortium of microorganisms needed more oxygen. The concentration of BOD in all treatments has met the permissible threshold quality standard, which is <20 mg L^{-1}.[76] This shows that the water has not been polluted so it is suitable for fish farming. The advantage of BOD is that it can be used to express the level of water pollution by organic matter. High values describe the continuous and to some extent the decomposition of biological minerals by microbes.[77]

The range of COD concentrations ranged from 5.456 to 7.296 mg L^{-1}, and statistically COD was significantly different ($p < .05$) whose value was P5 > P4 > P3 > P2 > P1 (Table 1) due to organic matter from fish feed residues, feces, and a consortium of dead microorganisms is abundant and accumulates in the waters. The COD value of all treatments was still below the predetermined quality standard, which was <20 mg L^{-1},[76] so that the water quality was suitable for *C. batrachus* cultivation. COD provides information on the level or content of oxidized organic components that may be responsible for pond pollution. The redox potential of any pond environment requires a certain amount of oxygen that can effectively oxidize the organic matter present.[77] COD is an important environmental health parameter that is used as a tool to determine the portability of pond water.

The COD test generally produces a higher oxygen demand value than the BOD test, because the pollutant is stable to biological reactions and microorganisms can be oxidized in the COD test. A high BOD value causes water quality problems but also causes a very foul odor.[78] The BOD/COD ratio indicates the biodegradability of wastewater, the higher the ratio, the lower the biodegradability of wastewater.[79]

According to Mangkoedihardjo,[80] the BOD/COD ratio is an indicator of the output impact of organic substances in water, waste, leachate, compost, and other similar materials that occur in the environment, both in the natural environment and in the man-made environment. Changes in the degree of biodegradability are indicated by an increase in the BOD/COD ratio. The ratio of BOD/COD for non-biodegradable pollutants is <0.01, while for biodegradable waste >0.1.[81] The BOD/COD ratio is divided into three zones in the waters, namely the stable zone, the biodegradable zone, and the toxic zone.[78] A good BOD/COD ratio used for cultivation and biological processes is in the biodegradable range, namely 0.2–0.5.[80] In this study, we determined the biodegradability of organic matter using the BOD/COD ratio.

According to Effendi,[82] waters that have BOD and COD values < 20 mg L^{-1} are said to be unpolluted. The BOD/COD ratio of all treatments ranges from 0.324 to 0.394 and is significantly different ($p < .05$) between P3, P4, P5 with P1 (Table 1), and is in the biodegradable range of 0.2 to 0.5,[80] which indicates that the pollutants in the waters are biodegradable. In this study, P5 is the best treatment, because the pollutants can be completely degraded.

Total organic matter (TOM) dan total dissolved solid (TDS)

The TOM values in this study varied from 5.1 to 19.2 mg L^{-1} and tended to increase with the length of maintenance time (Figure. 2). Statistically, the effect of treatment P2, P3, P4 and P5 on the concentration of TOM was significantly different ($p < .05$) (Table 1). This is to the findings of Putra et al[83] that the rest of the feed and fish waste will accumulate in the form of dissolved organic matter. The accumulation of organic matter at the bottom of the water can affect the availability of oxygen in the water. If oxygen demand exceeds availability it can result in anoxic conditions. [84] In addition, the consumption of oxygen by microbes during the oxidation of organic matter will reduce the availability of oxygen in the waters.[85]

Furthermore, for the process of decomposition of dissolved organic matter, bacteria need oxygen both in the ammonification and nitrification processes.[86] In the ammonification process, organic matter consisting of polysaccharides, polypeptides, fats, and amino acids[82,83] is converted into ammonia.[78] In this process, ammonified bacteria involve various enzymes, such as proteinase, peptidase, chitinase, chitobiase, lysozyme, endonuclease, exonuclease, urease, and deaminase,[87] then ammonia is converted into nitrate through a series of nitrification processes by *Nitrosomonas* and *Nitrobacter bacteria* under aerobic conditions.[88]

TOM is a parameter that provides information about the properties and characteristics of the particle and its ability to withstand complex compounds. The higher the TOM value, the implication that it can hold more minerals on itself, thereby increasing the heavy metal content in sediment or soil (Arfiati et al., 2020).[89]

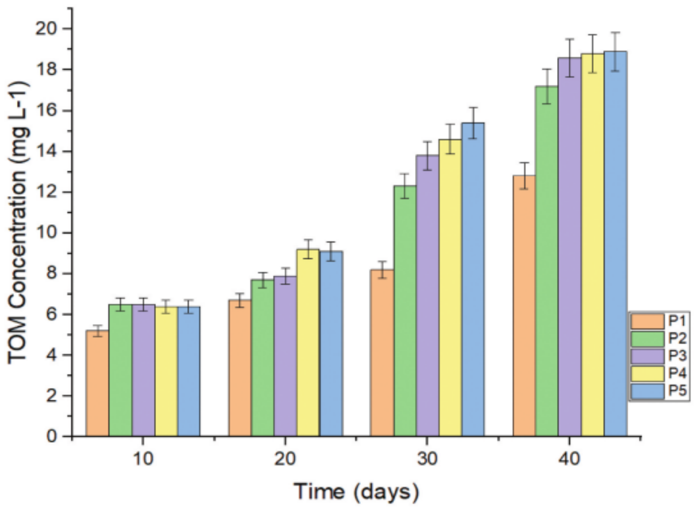

Figure 2. TOM concentration (mg L $^{-1}$) in different time (days). **Description**: P1 (control); P2 (biofloc); P3 (biofloc + bioball), P4 (biofloc + carbonation); and P5 (biofloc + carbonation + bioball).

TDS in water samples varied between 318 and 1560 mg L $^{-1}$ (Figure. 3). The TDS value at P3 and P5 is higher than the recommended value for freshwater fish farming, which is 1000 mg L $^{-1}$.[76] Statistically, different treatments had a significant effect ($p < .05$) on the TDS value, successively the TDS value was at P5>P3>P2>P4>P1 (Table 1). It can be explained that the increasing value may be a consequence of the redox potential of the water system,[90] and the organic and inorganic materials present in the solution.[91] The level of pollution affecting water can be related to the concentration of TDS present. TDS is a good indicator of water quality because it affects the taste, color, and smell of water and also increases the ability of light to penetrate. An increase in TDS levels makes the water unfit for fish farming, causing a decrease in photosynthetic capacity and a water temperature rise (NRCC, 2011).[92]

From day 0–30 organic matter tends to increase, but after day 30 the mineralization process is not fast as

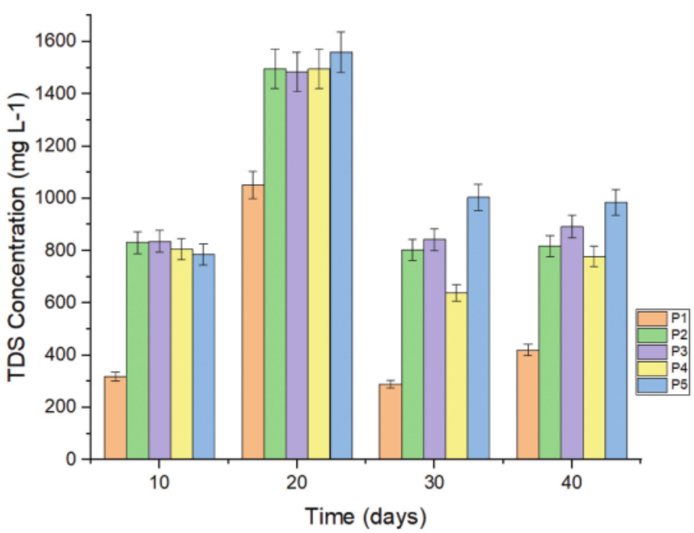

Figure 3. TDS concentration (mg L $^{-1}$) in different time (days). **Description** : P1 (control); P2 (biofloc); P3 (biofloc + bioball), P4 (biofloc + carbonation); and P5 (biofloc + carbonation + bioball).

indicated by stagnant TDS values (Figure. 3). This is presumably because the decrease in the conversion of organic matter to inorganic occurs due to the buildup of leftover feed and fish waste that settles at the bottom of the *C. batrachus* pond.[93–95] More organic matter in the waters is in dissolved form (TDS) than in suspended or colloidal form (TSS, total suspended solvent). This condition must be watched out for by *C. batrachus* cultivators considering that the TDS value describes the amount of dissolved solids in the culture pond.[89]

TDS analysis is carried out to determine the amount of dissolved organic and inorganic substances in water.[96] High and low TDS does not describe water quality specifically because it is only an indicator to determine water quality in general,[97] presumably due to a large number of ions or compounds that affect TDS such as ammonia, nitrite, and nitrate.[98] However, TDS is closely related to the process of decomposition of organic matter into inorganic in *C. batrachus* culture.[99]

Orthophosphate concentration

The orthophosphate concentrations in the water samples ranged between 0.7 and 9.1 mg L^{-1} (Figure. 4), and met the quality standards, namely: (0.5–20) mg L^{-1}.[100] Statistically showed that there was an effect of treatment on orthophosphate reduction ($p < .05$), namely P5>P4>P3>P2>P1 (Table 1). Sources of orthophosphate in pond water samples may come from the degeneration of organic matter, fish feces, uneaten fish feed, and other sources. The result of increased phosphate in the air or aquatic environment is an overgrowth of algae, which results in eutrophication, especially in lakes and stationary air. This will eventually lead to the shallowness of the lake, thus causing most of the basic species that require a certain level of depth to inhabit or settle in any aquatic environment.

Sulfate concentration

Sulfate concentrations in water samples were found to be between 13.1–34.0 mg L^{-1} and tended to increase (Figure. 5). The values obtained in the water samples were lower than the standard required for freshwater aquaculture, the limit being <300 mg L^{-1}.[76] Elevated levels of sulfate in water beyond standard requirements are associated with several physiological disorders or diseases such as lack of water in the human system and gastrointestinal irritation.[37] The presence of sulfate in water is always associated with some metal cations (Pb and Fe) and anions (PO_4^{3-}). Therefore, the presence of sulfate in water is an indication of the presence of Pb and Fe salts in the medium.[38]

The results of statistical analysis showed that all treatments were significantly different ($p < .05$) in decreasing sulfate concentration, and the best treatment was P1 (control), it was suspected that high orthophosphate values at P2, P3, P4 and P5 were closely related to sulfide (H_2S).[101] Under aerobic conditions, hydrogen sulfide is oxidized by *Thiobacillus bacteria* to sulfate so that it does

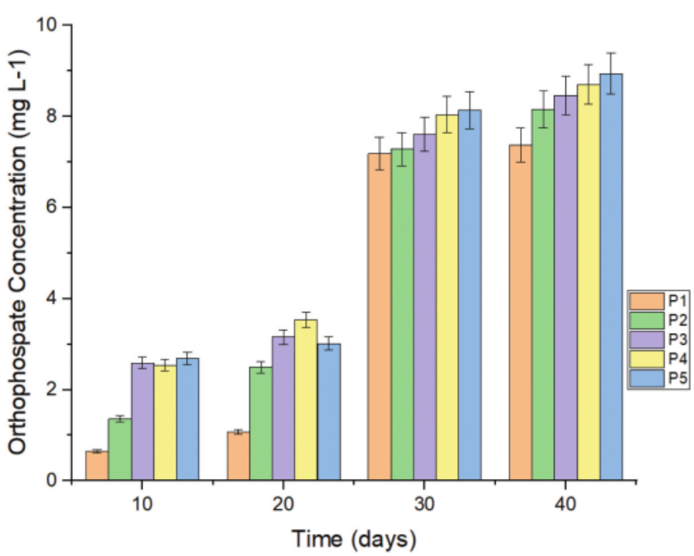

Figure 4. Orthophosphate concentration (mg L^{-1}) at different times (days). **Description**:P1 (control); P2 (biofloc); P3 (biofloc + bioball), P4 (biofloc + carbonation); and P5 (biofloc + carbonation + bioball).

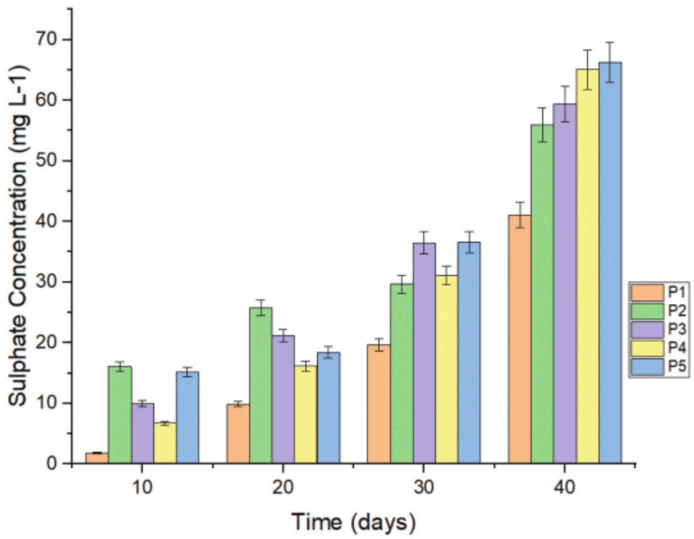

Figure 5. Sulfate concentration (mg L $^{-1}$) at different times (days). **Description**:P1 (control); P2 (biofloc); P3 (biofloc + bioball), P4 (biofloc + carbonation); and P5 (biofloc + carbonation + bioball).

not harm *C. batrachus*. Sulfur is an important element for fish and bacteria found in biofloc-based *C. batrachus* farming systems, especially as sulfate ions. Under aerobic conditions, sulfur decomposes to sulfide and is oxidized to sulfate.[22]

Effect of different treatments on growth and survival

Effect of several different treatments on ADG, ABW, SGR, FE, FCR, and SR can be seen in Table 2.

Specific growth rate (SGR)

Based on Table 2, the average daily growth (ADG) and average body weight (ABW) of Clarias batrachus treated were P1 (control), P2 (biofloc), and P3 (biofloc + bio ball), P4 (biofloc + carbonation), P5 (biofloc + carbonation + bio ball) tend to increase ADG (67.50–84,67) g - unit $^{-1}$ day $^{-1}$ and ABW (46.50–53.10) g unit $^{-1}$. The same trend with the specific growth rate (SGR) (0.058–0.066)%.

In addition to artificial feed, *C. batrachus* also take advantage of supplementary feed that is high in protein and available at any time, namely floc so that fish growth increases.[39,102] The growth of *C. batrachusis* thought to be due to the supply of energy contained in the feed, namely the energy in the feed consumed exceeds the energy needed by the fish, so that the excess energy is used for growth. This is to the opinion of (Handajani & Widodo[103] that fish growth depends on the quality of the feed provided so that ADG and ABW increase.

Statistically, the treatment had a significant effect ($p < .05$) on SGR of *C. Batrachus* (Table 2). SGR (P2, P3, P4, P5) was higher than P1 (control), presumably because: (1) the consortium of floc-forming bacteria produced polyhydroxy butyrate (PHB) which was beneficial in digestion and fatty acid metabolism,[104] (2) the activity of *Lactobacillus* sp bacteria which can produce lactic acid from sugar and other carbohydrates. According to Arief[105] *Lactobacillus* converts carbohydrates into lactic acid, then lactic acid can create a lower pH atmosphere. Under acidic conditions, *Lactobacillus* can inhibit pathogenic bacteria and spoilage

Table 2. Growth dan survival of *Clarias batrachus* based on biofloc.

Parameter	P1	P2	P3	P4	P5
ADG	67.50 ± 0.65^a	70.29 ± 0.67^b	73.14 ± 1.04^c	81.06 ± 0.66^d	84.67 ± 1.10^e
ABW	46.50 ± 0.74^a	48.90±.87^b	50.10 ± 1.17^b	52.10 ± 1.73^c	53.10 ± 0.88^c
SGR	0.058 ± 0.006^a	0.059 ± 0.004^a	0.064 ± 0.003^a	0.063 ± 0.006^a	0.066 ± 0.008^a
FE	90.89 ± 1.08^a	95.22 ± 1.27^b	95.66 ± 0.80^b	95.66 ± 1.62^b	98.79 ± 0.56^c
FCR	1.104 ± 0.003^a	0.951 ± 0.007^b	0.951 ± 0.002^b	0.942 ± 0.001^c	0.933 ± 0.002^d
SR	84.00 ± 1.40^a	86.00 ± 0.54^b	88.00 ± 0.94^c	88.00 ± 0.84^c	88.00 ± 0.55^c

Mean values in the same row with different letters are significantly different from each other ($P < .05$).

bacteria.[106] The acidic atmosphere in the intestines will increase the secretion of proteolytic enzymes (digestibility of feed) in the digestive tract to remodel proteins into amino acids which are then absorbed more quickly by the intestines. This statement is by the findings of Gatesoupe [107] that the activity of bacteria in the digestive tract will change rapidly if there are microbes that enter through feed or water which causes changes in the balance of bacteria that already exist in the intestine (digestive tract) with incoming bacteria. The balance between fish digestive tract bacteria causes probiotic bacteria to be antagonistic to pathogenic bacteria so that the fish digestive tract is better at digesting and absorbing feed nutrients.

SGR at P3 was higher than P2 and P1 (Table 2), presumably due to the biofloc factor and the addition of 10 units of bio balls per fish tank (FT) which served as a substrate for the biofloc bacterial consortium, and this study is supported by the findings of Deswati et al[19–24] which states that the use of bio balls as a substrate can improve the performance of bacteria.

SGR of P4 was higher than P3, P2, and P1 (Table 2), presumably due to biofloc and carbonation factors. The use of carbonation is based on the findings Ogello et al-[40] to increase the C/N ratio. The carbon used comes from rice husks as energy for Lactic Acid Bacteria (LAB) which decompose fish feed waste, feces, and dead floc into fish feed. These bacteria can produce compounds that have antibacterial properties so that they can inhibit the growth of pathogenic microbes.[107] LAB are a group of gram-positive bacteria that do not form spores and can ferment carbohydrates to produce lactic acid. Based on taxonomy, there are about 20 genera of bacteria that belong to LAB. Some LABs that are often used in food processing are *Aerococcus, Bifidobacterium, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus*, and *Weissella*. Examples of food products made using LAB assistance are yogurt, cheese, butter, sour cream, and other fermented products. In food processing, LAB can protect from contamination by pathogenic bacteria, improve nutrition, and have the potential to have a positive impact on human health.[41]

Some experts argue that Lactic Acid Bacteria (LAB) is an inhabitant of the digestive tract in fish that does not cause side effects.[108] The bacteria used in this study were *Lactobacillus* which is a genus of bacteria from LAB and is g-positive because of its ability to form a thick layer of peptidoglycan. *Lactobacillus* are facultative or microaerophilic anaerobes that can live in environments with low oxygen concentrations. In addition to producing lactic acid, *Lactobacillus bacteria* are also capable of producing hydrogen peroxide and bacteriocins. Both of these compounds are toxic, so they are often used to kill pathogenic and spoilage microbes.

SGR of P5 was higher than other treatments, presumably due to the resultant use of biofloc, bio balls, and carbonation. Another factor according to Putri et al[109] is due to the use of protein in the optimal feed digestion process for the growth and maintenance of the body of catfish.

The results of a study on the growth performance of *C. batrachus* similar to those of biofloc-based tilapia culture (Fuentes et al[110]) showed that, with a 20% reduction in feed dose, the growth of biofloc-based fish was comparable to that of control ponds fed 100% feed. These findings indicate that a 10–20% reduction in feed can be replaced by consuming biofloc, as evidenced by the presence of plankton in the fish intestines. A study evaluating the nutrient abundance of bioflocs was carried out by Anand et al[8] who reported that the main nutrients in dry biofloc materials were protein (24.30%) and essential fatty acids such as palmitic (46.54%), cis-vaccenic (15.37%), linoleic (10.67%), and oleic (9.19%).

Feed Efficiency (FE)

Feed efficiency (FE) is the ratio value between weight gain and feed consumed expressed in percent. The higher the feed efficiency value, the better the response of fish to feed with fast fish growth.[111] The factors that influence the high and low efficiency of the feed are the type of nutrient source and the amount of each component of the nutrient source in the feed. The amount and quality of feed given to fish affect the growth of fish.

Statistically, the treatment had a significant effect ($p < .05$) on FE of *C. Batrachus* (Table 2). The highest feed efficiency in *C. batrachus*, namely treatment P5, followed by treatment P4, P3, P2, and P1. The results of the study showed that the use of biofloc (P2, P3, P4, P5) was able to increase the efficiency of the utilization of *C. batrachus* feed compared to without the use of biofloc because biofloc-based *C. batrachus* cultivation utilizes the feed provided and utilizes floc which is rich in nutrients with floc protein ranging from 34.5–40.35%.

The results of the analysis of variance showed that the use of biofloc had a significant effect ($P < .05$) on the efficiency of feed utilization in fish. Based on the results, the highest feed utilization efficiency value was obtained in the P5 treatment of 98.79%, and the lowest was obtained in the P1 treatment of 90.89%, presumably due to bacteria originating from the *Bacillus* sp role in helping to increase the digestibility of feed. According to Deswati et al[73] and Deswati & Sutopo,[41] *Bacillus* sp bacteria can reduce the number of pathogenic bacteria in the digestive tract and increase feed absorption by increasing the concentration of protease enzymes in the digestive tract, where protease enzymes are biocatalysts for these reactions. protein breaker. Srihartati & Sukirno[112] digestibility is directly proportional to feed efficiency, so if the digestibility of fish is high, then the value of feed efficiency is also high. Furthermore, Irianto[113] stated that bacteria in the digestive tract can produce digestive enzymes such as proteases and amylase. The number of secreted enzymes increased, presumably due to the support of biofloc, bio balls, and carbonation, so that the amount of digested feed also increased. The efficiency of the P5 treatment feed was 98.79%, indicating that the fish could utilize the feed optimally, and the feed was absorbed into the body through blood circulation and converted the feed into meat. The feed efficiency value is in a good range. This is by the opinion of Ahmadi[114] that the feed is said to be good if the feed efficiency value is more than 50% or even close to 100%. With the increase in the value of feed efficiency, the level of effectiveness of the feed given to the fish is getting better, because by giving a little feed, the weight of the fish is better. In addition to the feed provided, floc can also be used as a natural food source so that the utilization of feed by fish is more optimal and efficient.[115]

Feed Conversion Ratio (FCR)

In this study, the protein floc ranged from 34.5 to 40.35%, which was higher than the findings of Suprapto & Samtafsir,[43] which was 29.2–34.3%. The higher the protein value of the flock, the better the quality of the flock because the floc is a source of additional nutritional food for *C. batrachus*.

Statistically, the treatment had a significant effect ($p < .05$) on FCR of *C. Batrachus* (Table 2). The best feed FCR for *C. Batrachus* was treatment P5, P4, P3, P2 and P1 respectively. Based on the results of the study, the FCR of biofloc-based *C. batrachus* ranged from (0.933–0.951) better than the control FCR (1.104), it was suspected that *C. batrachus* could optimally utilize pellets and flocks of feed, so that the feed was absorbed and converted into meat. This is in accordance with the opinion of Kaya et al[116] that FCR is closely related to feed quality, the lower the FCR value, the more efficient the fish in utilizing the feed they consume for growth, so that the body weight of the fish increases, it is suspected that the feed is digested by the fish optimally.

The FCR in this study was 0.933–0.951 better than the findings of Putri et al.[109] namely 1.48 and Irsyad et al-[117] which is 1.25. This difference in FCR is thought to be due to different nutrient absorption in each species, age, size and stocking density of fish. According to Barrows & Hardy[118] FCR value is influenced by feed protein. Protein feed that is in accordance with the nutritional needs of fish results in more efficient feeding, besides that it is influenced by the amount of feed given, with the less feed given, the more efficient the feeding.

The efficiency of the use of feed shows the value of feed that can change into an increase in fish body weight. Feed efficiency can be seen from several factors, one of which is the FCR value. According to Kaya et al[116] that the best level of feed use efficiency will be achieved at the lowest FCR value, where in this treatment the condition of feed quality is better than other treatments. The condition of good feed quality results in more energy obtained in *C. batrachus* for growth, so that fish with less feed are expected to increase their growth rate.

Survival Rate (SR)

The results of the study showed that the P1 treatment had a survival rate of 84%, presumably because the feed provided could make a good contribution to the survival of catfish. Furthermore, the use of bioflocs in P2, P3, P4 and P5 treatments could increase survival by 86, 88, 88 and 88%, respectively. This study is in accordance with the opinion of Nizar[119] that probiotic microbes are safe and relatively beneficial microbes in the digestive tract.

Statistically, the treatment had a significant effect ($p < .05$) on SR of *C. Batrachus* (Table 2). The high survival of *C. Batrachus* is thought to be due to: (1) the consortium of floc-forming bacteria can increase genes related to immunity,[120] and can act as an immunostimulant for fish,[121,122] (3) the availability of feed that is by the needs and good water quality management is carried out using a biofloc system. This is in accordance with the opinion of Fitria[123] which states that the survival of fish is largely determined by the availability of good food and good water quality management. According to Trisnawati et al[124] feed availability and environmental quality such as temperature, dissolved oxygen, pH, and ammonia content can affect the survival of organisms. Biofloc technology is commonly used to control water quality and as an additional feed source. With biofloc technology, nitrogenous waste produced by cultured organisms is converted into bacterial biomass (which contains protein) which can be utilized by cultured organisms.[59]

Based on the description above, biofloc-based *C. batrachus* farming has several advantages, including: (1) floc can be used as fish feed to reduce feed costs and produce lower FCR; (2) floc can be used as available nutrients at any time, to accelerate the weight of the fish being reared; (3) Heterotrophic bacteria convert nitrogen compounds released by fish and used for growth, eliminate ammonia and nitrite toxicity, and utilize toxic nitrogenous materials as substrates to improve water quality; (4) does not require water exchange, so that less water input is required, minimizes the entry of animal pathogens through water and increases biosecurity in fish farming; (5) enhance immunity-related genes; (6) produces polyhydroxy butyrate (PHB) which is beneficial in the digestion and metabolism of fatty acids; (7) improve fish survival because of bacteria in biofloc act as an immunostimulant for fish.

Conclusion

It can be concluded that modifying the biofloc technology have several advantages, including low FCR, flocs can be used as nutrients that are available at any time, improve water quality, increase biosecurity in *C. Batrachus* farming, increase survival because bacteria in biofloc act as immunostimulants and increase *C. batrachus* production. The study results obtained: floc volume (76–88 mL L^{-1}), BOD (2.152–2.367 mg L^{-1}), COD (5.462–7.312 mg L^{-1}), BOD/COD (0.324–0.394), TOM (5.1–19.2 mg L^{-1}), TDS (318–1560 mg L^{-1}), orthophosphate (0.7–9.1 mg L^{-1}), sulfate (18.1–34.0 mg L^{-1}), SGR (0.058–0.066), FE (90.89–98.79%), FCR (0.933–1.104), SR (84–88%), and has met the water quality standards for *C. batrachus* farming. The combination of biofloc, carbonation, and bio balls (treatment P5) is the best treatment, and deserves to be developed and widely applied for *C. batrachus* farming.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Statement of Novelty

Here we research : In this paper reports (1) to analyze several parameters such as floc volume, BOD, COD, BOD/COD, TOM, TDS, orthophosphate, sulfate, SGR, FE, FCR, SR, and (2) to obtain appropriate and environmentally friendly technology that can be applied in producing biofloc-based catfish. Biofloc system cultivation allows it to be applied to improve the water quality, growth, and survival rate of catfish. To further optimize the biofloc technology, we modified biofloc with carbonation and bio balls to improve the C/N ratio and the performance of the floc-forming bacterial consortium.

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