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Research Article

Optimization of Ammonia Removal in the Initial Phase of Biofloc System by RSM and Application of Maize Hydrolysate in BFT Based Culture of *Clarias gariepinus*

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Abstract

In this study, the initial phase of biofloc system was investigated and the main controllable factors were optimized by RSM. Maize hydrolysate was used as carbon source for the first time and *Bacillus subtilis* was added to a biofloc system as inoculum. Optimal condition was set at 17 of C/N ratio, 13mg/L of nitrogen concentration and 60 L/min·m^3 of aeration in practice. After scaling the optimal condition up to circular tank with volume of 30 m^3, the biofloc system was successfully matured in five days. Three groups, namely two BFT groups and one control group (traditional culture) were created. In BFT groups, starch and maize hydrolysate was used as carbon source, respectively. African catfish with initial weight of 0.667±0.03g were stocked in each group and reared for 60 days. Water quality parameters including ammonia, nitrite and BFV were improved in biofloc groups, and ammonia in maize hydrolysate group were significantly lower than starch group. Moreover, remarkable differences ($p<0.05$) were observed in performance parameters including survival rate, weight gain, feed efficiency and specific growth rate between control, starch and maize hydrolysate groups. This result indicated that maize hydrolysate was more effective carbon source than starch in biofloc system rearing catfish fingerling.

Keywords

Biofloc Technology, *Clarias gariepinus*, Response Surface Methodology, Maize Hydrolysate

1. Introduction

The increasing demand for fish and concern for protecting environment have encouraged the development of "sustainable, eco-friendly and intensive" aquaculture system. In this respect, BFT (biofloc technology) appears to be satisfactory and promising aquaculture system [14].

The principle of BFT is the microbiological removal of toxic nitrogenous compounds. i.e. ammonia assimilation by heterotrophic microorganisms [10]. In BFT, it is possible to improve water quality and reduce water and feed consumption because microorganisms could convert fish wastes into microbial biomass (biofloc) [4].

Since BFT is considered to be worthy in aquaculture, it has been widely applied to fish farming. One of the important steps for applying BFT is initial phase, that is, startup period, which is characterized by time lags and ammonia or nitrite peak. In order to shorten startup period and stabilize ammonia or nitrite concentration, several parameters should be optimized. To date, there is lack of scientific information

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about the establishment of stabilized biofloc system for a short period. Among different methods for optimization, RSM (response surface methodology) designs could be used effectively [6, 28].

A basic factor in designing a biofloc system is the species to be cultured [3, 4, 14, 15]. The suitable species for BFT are the ones that can survive in high solid concentration and poor water quality. Shrimp and tilapia are successful examples cultured in BFT system because they could not only adapt to high suspended solid concentration but also digest microbial protein [23, 29].

The African catfish of the genus *Clarias* is highly attracted by fish farmers in many countries owning to fast growth and high economic value [20]. According to [16], 62 percent of global aquaculture production in 2030 is expected to be from freshwater species, such as carp and catfish. Unlike tilapia and shrimp, catfish is not an efficient filter feeder and has limited capability to ingest biofloc from the water [13]. Some researchers reported the application of BFT to African catfish in experimental biofloc system and demonstrated that growth performance, survival, and feed utilization were higher in biofloc system than control [20]. However, most studies were conducted in a similar way with respect to commercial probiotics and molasses utilization in a laboratorial scale.

The choice of carbon source is a key to make a profit in BFT application. Commonly used carbon sources are molasses, tapioca flour, rice bran, brewery residue, cassava flour and hay because of low cost and non-toxicity. Moreover, different carbon sources have different effects on water quality, haemato-biochemical response of fish, nutritional and microbial composition of biofloc, finally effectiveness of BFT [12, 19, 24, 29, 30].

In this context, the present study aimed to determine the optimal condition for establishment of biofloc system by RSM and evaluate the effect of maize hydrolysate as carbon source in practical biofloc system rearing African catfish fingerlings.

2. Material and Methods

2.1. Biological Material

Bacillus subtilis subsp. *subtilis* 11431 was isolated by high ammonia assimilation activity from the appositional samples of pond wall in which catfish were stocked with daily water exchange over 30 days.

Amylases for liquefying and saccharifying of maize were purchased from Institute of Microbiology, Pyongyang, Democratic People's Republic of Korea.

African catfish (*Clarias gariepinus*) fingerlings were offered from Samchon Catfish Factory, Samchon, Democratic People's Republic of Korea.

2.2. Local and Establishment of Biofloc System

The experiment was carried out in a laboratory and facilities at the Central institute for fish farming of Aquaculture Ministry, Pyongyang, Democratic People's Republic of Korea.

The simulation experiment for establishing biofloc system was carried out in a series of plastic container (10 L) equipped with diffusion stones connected to air pump (Model ACO-003). Each container was filled with 8 L of water, where C/N ratio was controlled using soybean meal + ammonium chloride (1:1) as nitrogen source and maize hydrolysate as carbon source. It was inoculated with 1% of ammonia assimilation bacteria, *Bacillus subtilis* subsp. *subtilis* 11431 with density of 3×10^9 cfu/g. Water in the container was aerated continuously to grow biofloc at 25°C. On the day 5 after inoculation, TAN (total ammonia nitrogen) was measured from water sample in the system and the removal rate of ammonia was determined according to following formula.

$$\text{removal rate}(\%) = \frac{C_0 - C_t}{C_0} \quad (1)$$

where C_0 is total ammonia concentration (mg/L); C_t is ammonia concentration at t time (mg/L).

RSM was applied to maximize ammonia removal rate of the system. In this regard, the process was modeled and optimized by considering C/N ratio, nitrogen (soybean meal + ammonium chloride, 1:1) concentration, and aeration with three levels by using a quadratic model of Box-Behnken design. The variation of ammonia removal was evaluated using quadratic polynomial model (2).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad (2)$$

where Y is response (ammonia removal rate); β_0 is the constant coefficient of intercept; β_i, β_{ii} and β_{ij} are linear, quadratic and interaction coefficients, respectively; k is the number of independent variables; X_i and X_j are the coded levels of three independent variables (C/N ratio, nitrogen concentration, aeration).

Maize hydrolysate was prepared by liquefaction and saccharification of maize powder with particle size of 20~25 mesh [9]. Organic carbon content in starch and maize hydrolysate was 6% and 42%, respectively.

2.3. Experimental Design and Biofloc System Management

The intensive farming system consisted of a series of circular tanks of $15m^3$ and $30m^3$ (volume) equipped with aeration 7.5-hp blower.

The rearing experiment was conducted in a completely randomized design with three groups according to carbon source-Starch group, Maize hydrolysate group and control (without carbon source addition) group.

Biofloc system was prepared by simulating and scaling up the optimal condition to rearing tank after sterilizing water with sodium hypochlorite and neutralizing with sodium thiosulfate in 12 hours.

In three groups, the fries with average weight of 0.667±0.03 g were stocked at a density of 2 fries/L. Fishes were fed four times daily (at 6:00, 12:00, 18:00, 24:00) at the rate of 6% of the tanks biomass with a commercial diet (35% crude protein, crude lipid 5%, crude fiber 6%, mineral mix 13%, and 13% moisture contents). In control, 70% of water was exchanged daily while no water was exchanged except replacement of evaporated water in biofloc groups. Mixing and circulation were provided with a submersible air diffuser that was placed in the center of each tank to maintain DO (dissolved oxygen) concentrations above 5.0 mg/L. Starch and maize hydrolysate were added as an additional carbon source to stimulate heterotrophic bacteria growth and to reduce TAN maintaining a C/N ratio of 6:1 when TAN concentration reached values above 1 mg/L [11, 22]. The experiments were performed for 60 days.

2.4. Water Quality and Performance Variables

The main water quality parameters such as DO, pH, and temperature were measured using a digital water checker (YSI-550 A, ASTM, Alla, France) everyday, while analysis of TAN, nitrite, nitrate (N mg/L) and alkalinity (mg/L $CaCO_3$) were carried out twice a week in accordance to [2].

Total suspended solids (biofloc volume) were also measured twice weekly using a 1000-mL Imhoff cone, by settling 1 L water sample for 30 min, following the methodology described by [2]. Afterwards, collected bioflocs was subjected to morphological observation under a binocular microscope (1 600X, Teecen, China).

Bioflocs were obtained from water by precipitation and filtration. The crude protein content was determined according to [2].

The following indices were used to assess survival and growth performance.

Survival rate (%) = (final number of fishes/initial number of fishes) × 100

Weight gain (g) = final weight of fish(g)-initial weight of fish(g)

Specific growth rate (%/d) =natural log (final weight of fish/ initial weight of fish)/experimental period(d) × 100

Feed conversion ratio = feed supply/fish biomass increase

2.5. Statistical Analysis

The establishment condition of biofloc system was optimized using the response surface methodology with Box-Behnken design. Polynomial equations were employed to generate 3D surface plots supported by Design-Expert Software. Water quality and performance parameters data were submitted to analysis of variance (one-way ANOVA) with Origin software version 9.1. All values were obtained in triplicate. Results were expressed as mean ± standard deviation.

3. Results

3.1. Optimization of Ammonia Removal in the Initial Phase of Biofloc System

RSM design-one of the most popular technique for multivariate statistic methods was applied to maximize ammonia removal rate of BFT system. The levels and range of independent variables such as C/N ratio, nitrogen concentration and aeration were given in Table 1.

Table 1. *Levels of independent variables used in the RSM.*

Independent variables	Symbol	Coded levels		
		-1	0	1
C/N ratio	A	13	15	17
nitrogen concentration (mg/L)	B	0.1	0.2	0.3
aeration (L/min·m^3)	C	50	70	90

The experimental design for optimization was given in Table 2.

Table 2. *Experimental design and values of response.*

Std	Independent variables			Response
	A	B (mg/L)	C (L/min·m^3)	Removal rate (%)
1	13.00	10.00	70.00	45.47
2	17.00	10.00	70.00	81.21
3	13.00	14.00	70.00	12.86
4	17.00	14.00	70.00	53.08
5	13.00	12.00	50.00	18.61
6	17.00	12.00	50.00	37.16
7	13.00	12.00	90.00	29.49
8	17.00	12.00	90.00	84.73
9	15.00	10.00	50.00	30.58
10	15.00	14.00	50.00	19.94
11	15.00	10.00	90.00	70.75
12	15.00	14.00	90.00	35.71
13	15.00	12.00	70.00	71.18

Std	Independent variables			Response
	A	B (mg/L)	C (L/min·m³)	Removal rate (%)
14	15.00	12.00	70.00	72.06
15	15.00	12.00	70.00	78.67
16	15.00	12.00	70.00	82.95
17	15.00	12.00	70.00	82.63

The range of ammonia removal rate was from 12.86% at C/N ratio 13, nitrogen concentration 14 mg/L, aeration 70 L/min·m³ to 84.73% at C/N 17, nitrogen concentration 12 mg/L, aeration 90 L/min·m³.

The duration of start-up depends on a wide range of factors, including temperature, nutrient scheduling, and pre-seeding of the system with appropriate microbes. Perfect protocols for biofloc systems have not been standardized, and many operators have developed their own techniques through hard-won experience [17]. In addition, high level of ammonia or nitrite can be found in un-matured system, which negatively affects fish, especially in the case of larvae, fry and fingerling. So in BFT application it is important to establish vigorously-working system for a short period ready to rear fish. In this point, research on the inoculum and exploration of start-up phase is of great significance. It is considered as a general rule that biofloc samples originated from an acclimated biofloc system or soil sample is used as inoculum for accelerating the development of biofloc system [1, 5, 27]. However, this might negatively influence a biosecurity of the system [17]. It is also possible to add a number of commercial or homemade microbial consortia. In some cases, probiotics were used as inoculum [20]. It can be said that such methods are based on adaptation of microorganism to the environment but not highly efficient and aboriginal microorganisms. On the other hand, [25] reported that Japanese eels could be reared effectively by biofloc technology with exogenous bacteria inoculum isolated from fish pond. So we also chose *Bacillus subtilis* subsp. *subtilis* 11431, which was isolated from the catfish pond and screened with the ability to efficiently remove ammonia, as inoculum for biofloc system.

Though inoculum, time, and temperature were fixed on constant, ammonia removal of the system changed with three factors. By applying a multiple regression analysis to the experimental data, the quadratic model for ammonia removal rate of the system could be expressed as Eq (3).

$$\text{Ammonia removal rate (\%)} = -1511.89563+77.72125\times C/N+115.51900\times \text{nitrogen concentration}+6.44909\times \text{aeration}+2.29312\times C/N \times \text{nitrogen concentration}+0.028\times C/N\times \text{aeration}-0.15250\times \text{nitrogen concentration}\times \text{aeration}-3.26131\times (C/N)^2-5.48881\times \text{nitrogen concentration}^2-0.040744\times \text{aeration}^2 \quad (3)$$

Table 3. *Analysis of variance (ANOVA) of response for ammonia removal rate.*

Source	Sum of Squares	Df	Mean Square	F-value	p-value	Remarks
Model	10635.307	9	1181.701	52.805	< 0.0001	significant
A-C/N	2803.133	1	2803.133	125.259	< 0.0001	significant
B-Nitrogen	1635.634	1	1635.634	73.089	< 0.0001	significant
C-Aeration	1415.652	1	1415.652	63.259	< 0.0001	significant
AB	336.539	1	336.539	15.038	0.0061	significant
AC	5.018	1	5.018	0.224	0.6503	not significant
BC	148.840	1	148.840	6.651	0.0365	significant
A^2	716.541	1	716.541	32.019	0.0008	significant
B^2	2029.613	1	2029.613	90.694	< 0.0001	significant
C^2	1118.386	1	1118.386	49.975	0.0002	significant
Residual	156.651	7	22.379			
Lack of Fit	29.727	3	9.909	0.312	0.8169	not significant
Pure Error	126.924	4	31.731			
Cor Total	10791.958	16				

R^2=0.9855, Adj R^2=0.9668, Pred R^2=0.9376, Adeq Precision=19.203

Table 3 summarized the results of the ANOVA for response surface quadratic model simulating ammonia removal of the system.

The Model F-value of 52.80 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this study, A, B, C, AB, BC, A^2, B^2, C^2 are significant model terms. The adjusted R^2 value of 0.9855 was scarcely different from the predicted R^2 of 0.9376 and the Adeq Precision of 19.203 is much bigger than four, indicating the experimental data was sufficiently satisfactory to navigate the design space [6].

Figure 1 showed the surface plots of three dimensional responses for the ammonia removal of the biofloc system, indicating the relations between the variables.

C/N ratio is a key to ammonia removal because ammonia is instantly assimilated by heterotrophic bacteria with high C/N ratio. From the stoichiometry, 15.17 g of carbohydrates are required per 1 g of N assimilation by heterotrophic bacteria [11]. However, in this study RSM spotted 16.93 of C/N ratio as optimum in the range from 13 to 17 and revised to 17 considering practical application, which was similar to [31]. Adding excess carbohydrates to biofloc system may negatively affect the operation by wasting carbon source, producing high level of TSS and delaying the development of nitrification process for ammonia removal [8, 21]. Feed mixture and ammonium salt, in general, are used organic and inorganic nitrogen source [1]. In this study organic and inorganic nitrogen source were used together as 1:1 ratio, and total nitrogen concentration was an important factor on ammonia removal (Table 3 and Figure 1). Aeration affected significantly to ammonia removal of the system and also interacted with C/N ratio (Table 3 and Figure 1). At the level above 60 L/min·m^3, the removal rate rather decreased though without changes in dissolved oxygen, which could be interpreted by influence of aeration on mixing density. Mixing density is the equilibrium between the rate of aggregation and breakage. At higher mixing density, the average floc size decreases due to the increased floc breakage [7]. Particle size of bioflocs has been reported to interfere with the nitrification process. Rather than the particle size or floc abundance, the rupture of the floc structure could affect the distribution and activity of nitrifying bacteria [27].

As shown in Figure 1a, the best ammonia removal was obtained within C/N range from 16 to 17 and at 13 mg/L of nitrogen concentration. The interaction between C/N and aeration on ammonia removal was given in Figure 1b. Ammonia removal remarkably increased as C/N ratio goes up till 16.5 and aeration reached to about 62 L/min·m^3.

Figure 1c was 3D surface plot showing the dependency of ammonia removal on nitrogen concentration and aeration. When the amount of aeration reached to 62 L/min·m^3, the ammonia removal rate became higher while it decreased at nitrogen concentration above 13 mg/L. With numerical optimization by Design-Expert software, the generated optimal condition was C/N ratio 16.93, nitrogen concentration 12.92 mg/L, aeration 58.89 L/min·m^3.

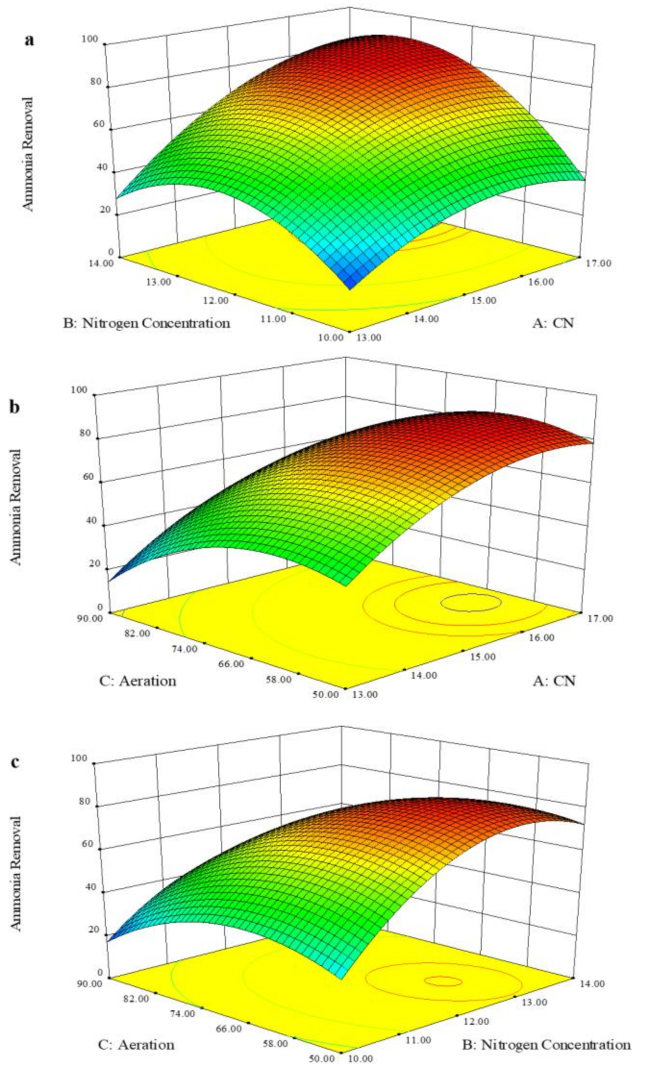

Figure 1. *Response surface plots showing the effects of independent variables on the ammonia removal (a: C/N-nitrogen concentration, b: nitrogen concentration-aeration, c: C/N-aeration).*

This is the first report of fixing C/N ratio, the concentration of nitrogen source, and aeration in initial phase by RSM optimization, and also applying maize hydrolysate for carbon source in biofloc system. It was reported that when rearing Pacific white leg shrimp in biofloc system, water became slimy with pungent smell in the group of fishmeal, air stone and no inoculating [18], demonstrating the importance of setting proper condition for fertilizing biofloc system.

The practical condition for establishing biofloc system was fixed at 17 of C/N ratio, 13 mg/L of nitrogen concentration, and 60 L/min·m^3 of aeration (Table 4). The response of the model was in ideally agreement with ammonia removal of the system in practical optimized condition. It showed the developed model was considerably accurate in predicting the ammonia removal rate of biofloc system.

Table 4. *Experimental and predicted values at optimized condition.*

Optimized experimental condition			Predicted values	Experimental values
C/N ratio	Nitrogen (mg/L)	Aeration (L/min·m^3)		
17	13	60	92.95	93.29±0.25

After optimization, the biofloc system in small vessel was scaled up to circular tank with volume of 30 m^3. Changes in water quality parameters of the system were recorded daily (Table 5).

Table 5. *Changes of water quality parameters during initial phase of BFT tank with volume of 30 m^3.*

№	water quality parameters	days(d)						
		1	2	3	4	5	6	7
1	TAN (mg/L)	0	0.3	4.8	1.2	0.08	0	0
2	NO_2—N (mg/L)	0	0.2	0.4	0.1	0.1	0.1	0.1
4	NO_3—N (mg/L)	0	0	0	0.1	0.5	0.5	0.5
5	Bubble	—	+	++	+++	++	++	++
6	Color	Cream-colored		Light brown			Brown	
7	DO (mg/L)	6~7						
8	Alkalinity (mg/L as $CaCO_3$)	100~200						
9	pH	7.5~8						
10	Temperature	27~29°C						
11	SS (mL/L)	8.2~9.7						

Water quality parameters kept stable with the exception of some fluctuations at the beginning. At 5th day, ammonia and nitrite parameters decreased to the level under 0.1 mg/L, which was safe enough for catfish fingerlings. It could be seen that the simulated model in small scale using RSM worked well in practical tank system. The completed biofloc system was light brown in color and bubbled over, and SS ranged from 8.2 mL/L to 9.7 mL/L.

The morphological observation of formed bioflcs by microscopic examination was shown in Figure 2.

As seen in Figure 2, bioflocs were highly porous and had a broad distribution of particle size. In addition, various kinds of microorganism including protozoa such as *Paramoecium caudatum* were observed.

It was interesting that various kinds of microorganisms were generated naturally and multiplied sharply in short period despite of adding only *Bacillus* inoculum. In this study, biofloc tanks were situated in light-limited indoor system, so no algae were observed, but heterotrophic microorganisms were dominated. As biofloc system is open to environment, the existence of various kinds of microorganisms is inevitable, but the important thing is the balance of microbial consortia capable of sustaining the rearing system without water exchange. In this sense, plankton such as protozoa can contribute to the nutritional value of biofloc and nutrition cycle of the system in spite of consuming heterotrophic bacteria.

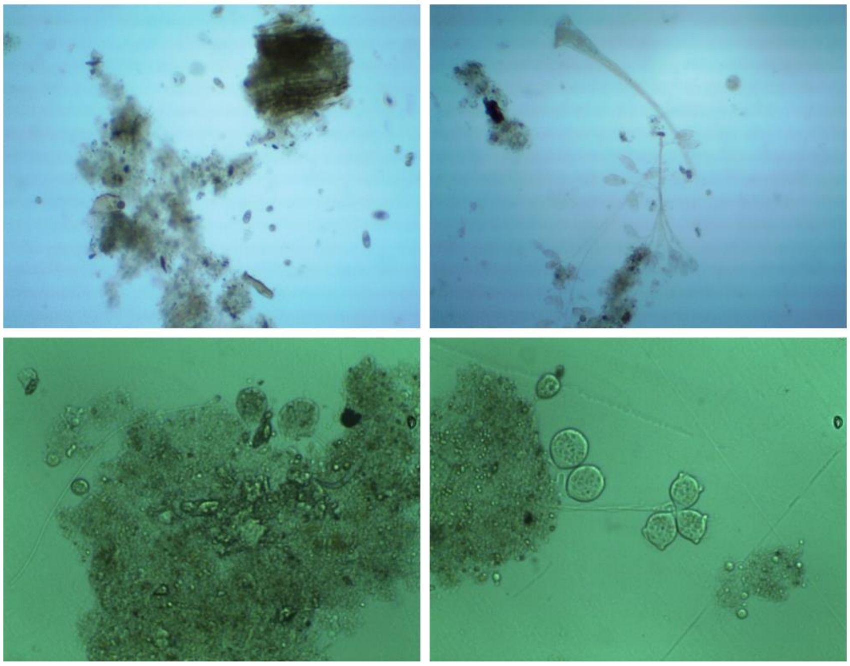

Figure 2. Morphological observation of bioflcs.

3.2. Water Quality in Biofloc System During Catfish Rearing

Temperature, dissolved oxygen, pH during catfish rearing ranged within acceptable level for aquaculture practice. No significant differences (p>0.05) were observed between control and biofloc groups (Table 6).

Table 6. Mean±standard deviation of Temperature, DO, alkalinity, and pH in control and BFT groups during 60 days of catfish rearing.

WQ parameter	Control	starch	maize hydrolysate	p value
Temperature (°C)	27.7±0.6	27.7±0.3	27.5±0.5	0.8717
Dissolved oxygen (mg/L)	5.9±0.2	5.7±0.2	5.6±0.3	0.12327
Alkalinity (mg/L as $CaCO_3$)	141.7±12.6	133.3±24.7	130.0±34.6	0.85141
pH	8.1±0.2	7.9±0.4	7.9±0.4	0.85435

There were no significant differences (p>0.05) between the treatments.

Since alkalinity decreased below 50 mg/L at about 30 days in biofloc groups, sodium bicarbonate was added to keep alkalinity above 130 mg/L and to prevent pH from decreasing below 7.0.

Inorganic nitrogen concentration (ammonia and nitrite) and BFV were analyzed for the determination of bioremediation efficiency by biofloc (Figure 3).

The analysis of water quality indicated TAN and nitrite were kept much more safely and desirably for fishes in biofloc system than control. As shown in Figure 3, TAN in control gradually accumulated, reached the maximum level on day 10(0.8 mg/L) and maintained high level for almost 10 days before falling and keeping under 0.5 mg/L while keeping below 0.4 mg/L in biofloc groups. However, there was significant difference between starch and maize hydrolysate groups.

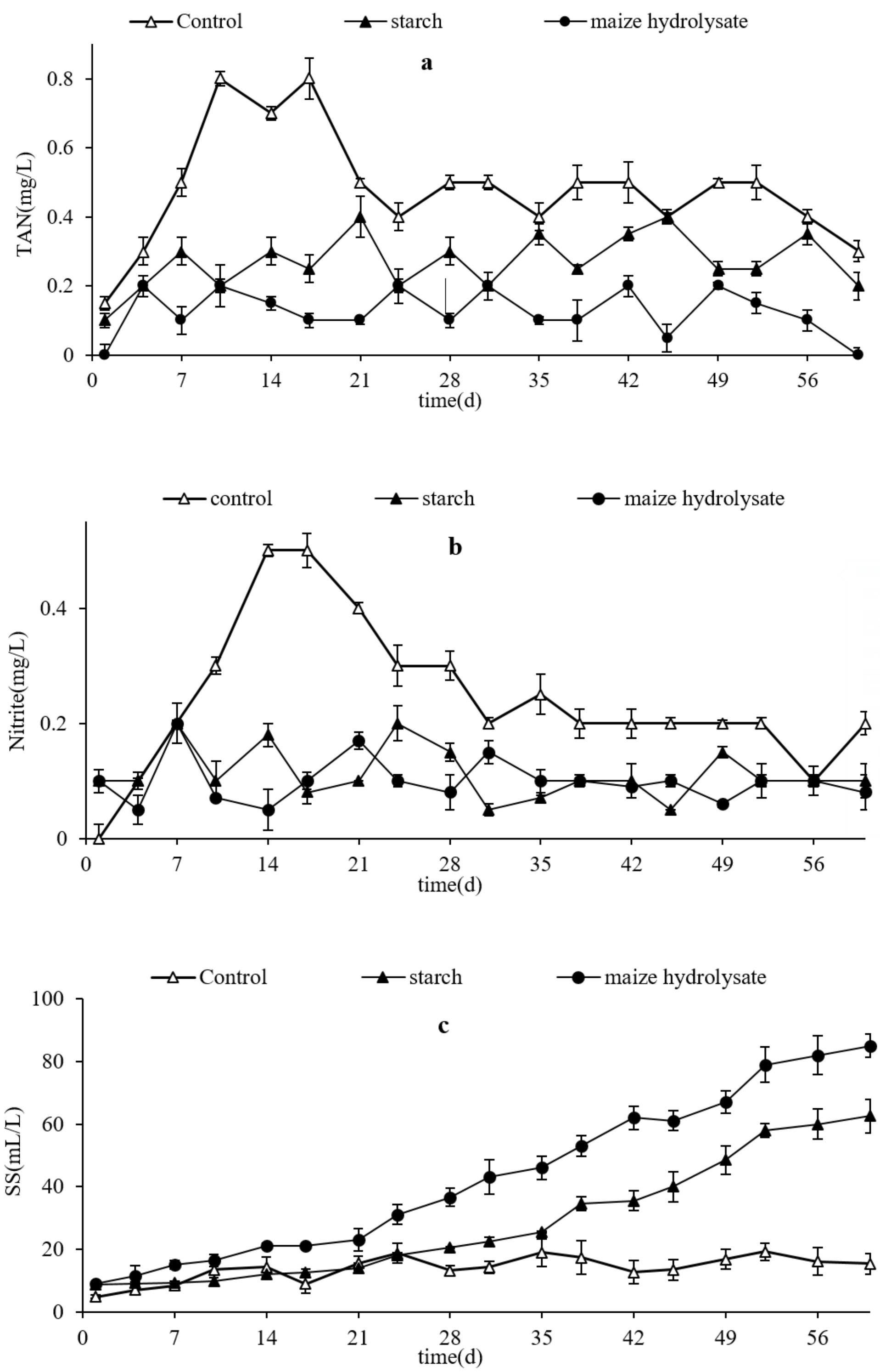

Figure 3*. Ammonia, nitrite, and BFV in control and BFT groups during 60 days of catfish rearing (a: ammonia, b: nitrite, c: BFV).*

The accumulation peak of TAN was followed by nitrite rise extending for 5 days with the mean nitrite 0.5 mg/L in control, in which both TAN and nitrite were controlled simultaneously under safe level after 30 days. No significant difference in nitrite level was detected between starch and maize hydrolysate. Biofloc formation, measured as total suspended solids, showed a startling difference between control receiving feed only and treatments receiving feed and maize hydrolysate as carbon source (Figure 3). Biofloc concentration remained relatively constant under 20 mL/L with mean value of 13.78 mL/L in control while total suspended solids in BFT groups increased continuously with the experimental period in treatments and significant difference was observed between starch and maize hydrolysate.

The type of carbohydrates used as organic carbon source are various, but glucose, acetate, glycerol, starch and agricultural by-product are typical examples [1, 24, 26, 29, 30]. However, maize hydrolysate was applied in this study, which had never been reported before. It was stated simple sugars, such as sucrose, resulted in a faster ammonia removal, while more complex carbohydrates such as rice bran required more time for decomposition into simple sugars, thereby resulting in slower ammonia removal, so fermentation of the complex carbon sources prior to BFT application could be an alternative solution and interesting subject for the use of complex or fibrous carbon sources [12]. With adding maize hydrolysate as carbon source to ensure C/N ratio of 6:1 only when TAN concentration mounted up to 1 mg/L, ammonia and nitrite were kept under 0.2 mg/L. In addition, lower ammonia concentration were observed in maize hydrolysate group compared to starch group (Figure 3).

3.3. Crude Protein of Biofloc and Growth Performance of African Catfish Fingerlings in Biofloc System

The crude protein of biofloc was analyzed (Figure 4).

As shown in Figure 4, crude protein content of biofloc was two and half folds higher than control, indicating bioflocs of the system had plenty of microbial protein sufficient for fish feed. But the difference of crude protein between starch and maize hydrolysate groups was still non-significant.

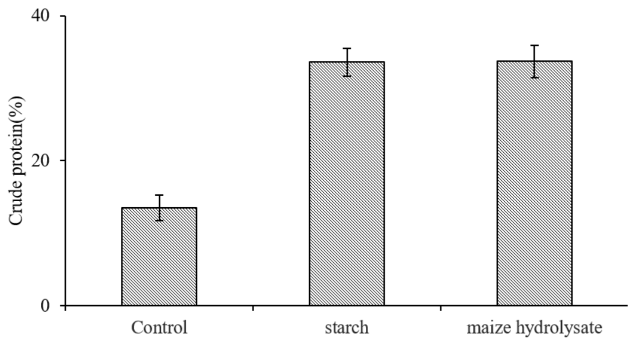

Figure 4. *Crude protein of biofloc in control and BFT groups.*

Table 7 showed the growth performance of catfish fingerlings in control and biofloc groups.

Remarkable differences were observed in performance parameters including survival rate, weight gain, feed efficiency, and specific growth rate, which were significantly improved in treatments.

Table 7. *The survival rate, growth performance, and feed utilization of African catfish fingerlings in control and BFT groups.*

Variable	control	starch	maize hydrolysate	p value
Survival (%)	76.02±2.73^{a}	85.63±2.54^{b}	92.36±2.41^{c}	6.99073E-4
Weight gain (g)	16.88±0.35^{a}	19.29±0.61^{b}	20.64±0.35^{c}	1.52118E-4
Feed conversion ratio	1.11±0.04^{a}	0.86±0.05^{b}	0.74±0.02^{c}	6.36601E-5
Specific growth rate (%)	5.38±0.03^{a}	5.61±0.05^{b}	5.72±0.03^{c}	1.35649E-4

Different letters in the rows indicate significant differences ($p<0.05$)

In control group, survival rate of catfish fingerlings was 76.02% with regular and instant disinfection by using calcium hypochlorite and copper sulfate. In contrast to control, the survival rate was raised to 85.63% in starch biofloc and 92.36% in maize hydrolysate biofloc without any disinfection. Weight gain and specific growth rate were significantly higher ($p<0.05$) in biofloc groups than control, which was opposite to feed conversion ratio. Moreover, performance parameters differed statistically between starch and maize hydrolysate group, which indicated maize hydrolysate could be more effective than starch as carbon source in biofloc based fish farming.

Maize hydrolysate was proved to be more effective and convenient as carbon source for biofloc system than starch since they could be easily and cheaply made from maize using enzymatic hydrolysis, quickly absorbed by microorganism to improve ammonia removal ability of the system.

It was indicated that bioflocs could be used as an *in situ* produced feed and biofloc uptake depended on the species and feeding traits, animal size, floc size and floc density [10]. It was well known that giant freshwater prawn (*Macrobrachium rosenbergii*), white leg shrimp (*Litopenaeus vannamei*) and tilapia (*Oreochromis niloticus*) were all able to take up bioflocs and profit from this additional protein source, but the nutritional contribution of biofloc on the catfish performance was limited and varied. A higher growth performance in biofloc group was observed in our research. It might be due to consumption of biofloc by catfish fingerlings as additional nutrition. In addition to nutritional contribution, performance elevation might be accomplished by reduction of various stress including water exchange, disinfection and management. According to [13], the embryonic development rate of eggs produced by brood stocks in biofloc group was higher than control, and the survival in starvation tolerance test and growth tests were notably improved.

Further researches are necessary to investigate the effects of maize hydrolysate on intestinal morphology, immune system, hematological parameters of African catfish and microbial diversity in biofloc system.

4. Conclusion

When applying RSM design to preparation for biofloc system used *Bacillus subtilis* as inoculum and maize hydrolysate as carbon source, the biofloc systems with volume of 30 m^3 were successfully matured in five days and growth performances of catfish fingerlings were significantly improved in biofloc system. And maize hydrolysate was proved to be more effective and convenient as carbon source than starch for biofloc system rearing African catfish.

This study will provide fundamental data to prepare optimized biofloc system and to rear catfish fingerlings by BFT. Further research is necessary to study intestinal morphology, immune system, and hematological parameters of African catfish rearing in biofloc system.

Abbreviations

RSM	Response Surface Methodology
BFT	Biofloc Technology
DO	Dissolved Oxygen
TAN	Total Ammonia Nitrogen

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Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Ahmad, I., Leya, T., Saharan, N., Majeedkutty, B. R. A., Rathore, G., Gora, A. H., Bhat, I. A., Verma, A. K., 2019. Carbon sources affect water quality and haemato-biochemical responses of *Labeo rohita* in zero-water exchange biofloc system, Aquaculture Research 00, 1–9. wileyonlinelibrary.com/journal/are

[2] APHA, 2012. Standard Methods for the Examination of Water and Wastewater, twenty-second ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington D.C, USA.

[3] Alvarenga, É. R., Alves, G. F. O., Fernandes, A. F. A., Costa, G. R., Silva, M. A., Teixeira, E. A., Turra, E. M., 2018. Moderate salinities enhance growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings in the biofloc system. Aquac. Res. 49, 2919–2926. https://doi.org/10.1111/are.13728

[4] Azim, M. E., Little, D. C., 2008. The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283, 29–35. https://doi.org/10.1016/j.aquaculture.2008.06. 036.

[5] Bartholomew W. G., Kevin K. S., Peter W. P., 2014. Effect of stocking biomass on solids, phytoplankton communities, common off-flavors, and production parameters in a channel catfish biofloc technology production system, Aquaculture Research 45, 1442–1458. https://doi.org/10.1111/are.12096

[6] Birhanu, Y., Leta, S., 2021. Application of response surface methodology to optimize removal efficiency of water turbidity by low-cost natural coagulant (*Odaracha soil*) from Saketa District, Ethiopia, Results in Chemistry 3, 100108. https://doi.org/10.1016/j.rechem.2021.100108

[7] Chaignon, V., Lartiges, B. S., El Samrani, A., Mustin, C., 2002. Evolution of size distribution and transfer of mineral particles between flocs in activated sludges: an insight into floc exchange dynamics. Water Res. 36(3), 676–684.

[8] Chen, X., Luo, G., Menga, H., Tan, H., 2019. Effect of the particle size on the ammonia removal rate and the bacterial community composition of bioflocs, Aquacultural Engineering 86, 102001, 1-8. https://doi.org/10.1016/j.aquaeng.2019.102001

[9] Cristina, C. H., Esther, P. C., Sergio, O. S. S., 2009. Production of bioethanol from steam-flaked sorghum and maize, Journal of Cereal Science 50, 131–137.

[10] De Schryver, P., Crab, R., Defoirdt, T., Boon, N., Verstraete, W., 2008. The basics of bioflocs technology: the benefit for aquaculture. Aquaculture 277, 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019

[11] Ebeling, J. M., Timmons, M. B., Bisogni, J. J., 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture 257, 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019

[12] Ekasari, J., Azhar M. H., Surawidjaja, E. H., Nuryati, S., De Schryver, P., Bossier, P., 2014. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources, Fish & Shellfish Immunology 41, 332-339. http://dx.doi.org/10.1016/j.fsi.2014.09.004

[13] Ekasari, J., Suprayudi, M. A., Wiyoto, W., Hazanah, R. F. H., Lenggara, G. S., Sulistiani, R., Alkahfi, M., Zairin, M., 2016. Biofloc technology application in African catfish fingerling production: The effects on the reproductive performance of broodstock and the quality of eggs and larvae. Aquaculture 464, 349–356. https://doi.org/10.1016/j.aquaculture.2016.07.013

[14] Emerenciano, M., Cuzon, G., Paredes, A., Gaxiola, G., 2013. Evaluation of biofloc technology in pink shrimp *Farfantepenaeus duorarum* culture: growth performance, water quality, microorganisms profile and proximate analysis of biofloc. Aquac. Int. 21, 1381–1394. https://link.springer.com/content/pdf/10.1007%2Fs10499-013-9640- y.pdf

[15] Emerenciano, M. G. C., Gaxiola, G., Cuzon, G., 2015. Biofloc technology applied to shrimp broodstock. In: Avnimelech, Y. (Ed.), Biofloc Technology – A Practical Guide Book, third ed. The World Aquaculture Society, Baton Rouge, Louisiana, 215–228.

[16] FAO Food and Agriculture Organization of the United Nations, 2020. The state of world fisheries and aquaculture. Sustainability in action. Food and Agriculture Organization of the United Nations. Rome.

[17] Hargreaves, J. A., 2013. Biofloc Production Systems for Aquaculture. Southern regional Aquaculture Center, United States Department of Agriculture, National Institute of Food and Agriculture, Auburn.

[18] Harun, A. A. C., Mohammad, N. A. H., Ikhwanuddin M., Jauhari, I., Sohaili, J., Kasan, N. A., 2019. Effect of different aeration units, nitrogen types and inoculum on biofloc formation for improvement of Pacific Whiteleg shrimp production, Egyptian Journal of Aquatic Research 45, 287–292. https://doi.org/10.1016/j.ejar.2019.07.001

[19] Nootong, K., Pavasant, P., 2011. Effects of Organic Carbon Addition in Controlling Inorganic Nitrogen Concentrations in a Biofloc System, JOURNAL OF THE WORLD AQUACULTURE SOCIETY 42(3), 339-346.

[20] Putra, I., Rusliadi, R., Fauzi, M., Tang, U. M., Muchlisin, Z. A., 2017. Growth performance and feed utilization of African catfish fed a commercial diet and reared in the *Clarias gariepinus* biofloc system enhanced with probiotic. F1000Research 6(1545), 1-9, https://doi.org/610.12688/f1000research.12438.1

[21] Robles-Porchas, G. R., Gollas-Galván, T., Martínez-Porchas, M., Martínez-Cordova, L. R., Miranda-Baeza, A., Vargas-Albores, F., 2020. The nitrification process for nitrogen removal in biofloc system aquaculture. Rev. Aquacult. Online Version. https://doi.org/10.1111/raq.12431

[22] Serra, E. P., Gaona, C. A. P., Furtado, P. S., Poersch, L. H., Wasielesky, J. W., 2015. Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. Aquacult. Int. 23, 1325–1339. http://dx.doi.org/10.1007/s10499-015-9887-6

[23] Sgnaulin, T., Durigon, E. G., Pinho, S. M., Jerônimo, G. T., Lopes, D. L. A., Emerenciano, M. G. C., 2020. Nutrition of Genetically Improved Farmed Tilapia (GIFT) in biofloc technology system: optimization of digestible protein and digestible energy levels during nursery phase. Aquaculture 521, 734998. https://doi.org/10.1016/j.aquaculture.2020.734998

[24] Silva, U. L., Falcon, D. R., Pessôa, M. N. C., Correia, E. S., 2017. Carbon sources and c: n ratios on water quality for Nile tilapia farming in biofloc system. Rev. Caatinga 30, 1017–1027. https://doi.org/10.1590/1983-21252017v30n423rc

[25] Sin C., Ri Y., Kim C., Rim S., Rim S., Kim H., 2024. Addition Ammonia Assimilation Bacteria to a Biofloc System for Japanese Eel (*Anguilla japonica*) Farming, Comparison of Growth Performance and Water Quality, Advances in Bioscience and Bioengineering 12(1), 14-18. http://www.sciencepublishinggroup.com/j/abb

[26] Souza, D. M., Suita, S. M., Romano, L. A., Wasielesky Jr., W., Ballester, E. L. C., 2014. Use of molasses as a carbon source during the nursery rearing of *Farfantepenaeus brasiliensis* (Latreille, 1817) in a biofloc technology system. Aquac. Res. 45, 270–277. https://doi.org/10.1111/j.1365-2109.2012.03223.x.

[27] Souza, J., Cardozo, A., Wasielesky, W., Abreu, P. C., 2019. Does the biofloc size matter to the nitrification process in Biofloc Technology (BFT) systems. Aquaculture 500, 443–450. https://doi.org/10.1016/j.aquaculture.2018.10.051

[28] Su, T., Gao, W., Xing, X., Lan, X., Song, Y., 2021. Optimization for simultaneous removal of NH_3-N and COD from coking wastewater via a three dimensional electrode system with coal-based electrode materials by RSM method, Green Processing and Synthesis 10, 756–767. https://doi.org/10.1515/gps-2021-0072

[29] Vilani, F. G., Schveitzer, R., Arantes, R. F., Vieira, F. N., Santo, C. M. E., Seiffert, W. Q., 2016. Strategies for water preparation in a biofloc system: effects of carbon source and fertilization dose on water quality and shrimp performance. Aquac. Eng. 74, 70–75. https://doi.org/10.1016/j.aquaeng.2016.06.002.

[30] Wei, Y., Liao, S., Wang, A., 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs, Aquaculture 465, 88–93. http://dx.doi.org/10.1016/j.aquaculture.2016.08.040

[31] Zhao Z., X. Qiyou, Luo L., Wang C., Li J., Wang L., 2014. Effect of feed C/N ratio promoted bioflocs on water quality and production performance of bottom and filter feeder carp in minimum-water exchanged pond polyculture system, Aquaculture 434, 442–448. http://dx.doi.org/10.1016/j.aquaculture.2014.09.006