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EVALUATION OF WATER QUALITY, BACTERIOLOGICAL, AND PROXIMATE ANALYSIS PARAMETERS OF BIOFLOC IN TILAPIA CULTURE (*Oreochromis niloticus*)

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Abstract

This study was conducted to evaluate the water quality parameters, total bacterial load, and proximate analysis of biofloc using different feeding levels in the biofloc rearing system of Nile tilapia (*Oreochromis niloticus*). The experiment was set up fiberglass tank filled with 100 liters of fresh water in four treatments (T1, T2, T3, and T4) with two replications of each treatment. The tested treatments were namely T1 (control), T2 (normal dose of feed+floc), T3 (20%feed reduction+floc), and T4 (30% feed reduction+floc). The vital water quality parameters like temperature, pH, dissolved oxygen, ammonia, nitrite, nitrate, TDS, TSS, and alkalinity were monitored at 3-day intervals. The results for temperature and pH did not show any significant differences among all the treatments. The highest DO, ammonia, nitrite, and nitrate were observed in the control treatment (7.36, 1.78, 0.13, and 11.0 mg/L, respectively). The results for nitrite and nitrate showed highly significant differences among all the treatments. The total bacterial load was estimated, and *Bacillus spp* was found in all biofloc treatments. Proximal analysis showed differences in crude protein, crude lipids, and ash content among all the treatments, where the highest protein percentage was 41.03%, and the highest crude lipid and ash values were 12.21% and 16%, respectively. The results indicate that Biofloc technology is a sustainable way of enhancing water quality in aquaculture through microbial biomass production, which results in higher production of tilapia.

Keywords: Water quality, Biofloc technology, Bacteriological analysis, Proximate composition.

Introduction

Aquaculture output is increasing throughout the world, which has a population of about seven billion people. Bangladesh is considered one of the world's most appropriate countries for aquaculture, supplying food, nutrition, income, livelihoods, and export earnings due to its attractive resources and agroclimatic conditions (Asaduzzaman *et al*., 2008). Water quality is a critical factor in the aquaculture fish production system. Non-optimal water physicochemical characteristics (dissolved oxygen, pH, salinity, ammonia, temperature, etc.) and management variables (feeding, overcrowding, low nutritional status, etc.) can stress fish and make them susceptible to various diseases. (Ismail *et al*., 2016). The aquaculture business has faced a few major problems in recent years. Due to high amounts of organic matter, nitrogenous residues, toxic metabolites, and chemical and biochemical oxygen demands in effluents emitted to the atmosphere, aquaculture has been deemed an unsustainable industry (Martínez *et al*., 2012). Other significant allegations include competition for land and water, the introduction of exotic species around the world, overexploitation of ocean fish populations for fishmeal and fish oil, pathogen dispersal, the production of antibiotic resistance genes, and so on (Naylor *et al*., 2001). As a result, interest in closed aquaculture systems for shrimp and fish production is increasing. The risk of pollutant discharge is minimized as water is reused. This is advantageous for the preservation of natural wealth. Another benefit is that toxins and pathogens from the atmosphere

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are unlikely to affect cultured animals, particularly where biosecurity steps like source water disinfection are used (Martinez *et al*., 2017). The use of closed systems reduces the chance of animal escape, reducing the introduction of invasive pests and diseases into the natural environment. Fish farming with biofloc technology is seen as the easiest way to achieve this goal (Avnimelech, 2008; Piedrahita, 2003). Biofloc technology is an aquaculture approach that is being investigated and applied to enhance the reproductive efficiency of cultivated animals like Nile tilapia (Ekasari *et al*., 2015).

Tilapia culture has been highly popular among fish farmers in recent years, and it is currently rated second only to carp in terms of global production, with growth expected to continue exponentially in the future (FAO, 2000). Tilapia is well-suited to biofloc systems (Avnimelech, 2007). The basic principle of this technology is to keep nitrogenous waste below harmful levels by promoting heterotrophic microbial growth and increasing feed nutrient consumption ability in cultured animals (Avnimelech, 1999; Crab *et al*., 2009; Hari *et al*., 2006), but also to include extra essential nutrients (Ju *et al*., 2008; Xu *et al*., 2012) and exogenous digestive enzymes (Xu and Pan, 2013). Another advantageous function of bioflocs has been discovered to be the immunological effects associated with the bacteria that make up the bioflocs, which provide improved non-specific defense and protection from disease infection (Kim *et al*., 2014). Biofloc technology also has some benefits over conventional fish farming, such as requiring little to no water exchange, having a lower environmental effect, including nitrogen compound recovery, bacterial biomass synthesis, and the provision of a highly nutritious complementary food (Avnimelech, 2008).

For optimum growth of a fish species as well as in formulating a healthy diet, it is important to understand the nutritional requirements of biofloc, especially for protein, lipid, and energy. Biofloc is a feed ingredient that can be ingested in situ by cultured animals. The growth and body composition of fish species have been related to dietary protein and energy levels (Crab *et al*., 2010). The use of improper nutrients will increase the cost of fish processing while also deteriorating the quality of the water (Maica *et al*., 2012). Insufficient energy in diets resulted in protein waste due to an increase in the proportion of dietary protein used for energy, and the ammonia produced would pollute the water and make it unsuitable for fish farming. However, excessive energy in diets can result in increased body lipid deposition and growth retardation due to a lack of essential nutrients for growth. Feed costs appear to be one of the major constraints to aquaculture development from an economic standpoint (Rakocy *et al*., 2004). Information on water quality, presence of bacteria, and proximate analysis of biofloc under biofloc rearing system of tilapia is limited. Thus, this study was carried out in order to determine the water quality, the presence of bacteria, and the proximate analysis of biofloc using different feeding levels in the biofloc system of tilapia (*Oreochromis niloticus*).

Materials and Methods

Experimental Design

The experiment was carried out at the Aquaculture Laboratory, Faculty of Fisheries, Sylhet Agricultural University, Sylhet, from the 18th of January to the 8th of February 2021. This study used a completely randomized design (CRD) of four levels of treatment and two replications. T1 (control), T2 (normal dose of feed+floc), T3 (20% feed reduction+floc), and T4 (30% feed reduction+floc) were the treatments that were studied. The fiberglass indoor tanks were used for the experiment, and they had been washed, dried, and filled with fresh water (100 L). Three treatments (each with two replications) were compared to one control (also with two replications). An air stone aerator was used to provide aeration. There was no water exchange in the biofloc treatment tanks, but the water was changed weekly in the control tanks.

Measurements of Water Quality Parameters

An YSI ProQuatro Multiparameter Water Quality Meter was used to measure temperature (T, °C), pH, dissolved oxygen (DO, mg/L), and electrical conductivity (EC, S/cm), total dissolved solids (TDS, ppm), and total suspended solids (TSS, ppm). The API test kit measured ammonia (NH4, mg/L), nitrate (NO3, mg/L), nitrite (NO2, mg/L), and alkalinity (mg/L) on a weekly basis. Over the period of 15 days, alkalinity and total suspended particles were assessed weekly in two replications at 3-day intervals.

Bacteriological Analysis

Water samples for bacteriological analysis were collected in a 15 ml falcon tube from the experimental tanks. All of the containers were washed with detergent and autoclaved to disinfect them before each sample set. The samples were then kept at two °C until they were analyzed. Standard tests are used to quantify total bacteria, isolate bacteria, and identify species (APHA, 1998).

Proximate Analysis of Biofloc

The standard AOAC method (1990) was used to analyze the biofloc samples' proximate composition of dry matter, ash content, crude protein, and crude fat. Culture water samples from each tank were deposited in 1-L Imhoff cones and allowed to settle for 45 minutes. The samples were then filtered through Whatman filter paper to collect concentrated floc samples. After drying the material overnight at 105°C to assess dry matter, a stable weight was achieved. The ash content was determined by incinerating the sample for five hours at 550°C in a muffle furnace. The international protein factor of 6.25 was used to calculate crude protein after measuring nitrogen using the Kjeldal method. Ether extraction and soxhlet techniques were used to evaluate crude fat.

Statistical Analysis

The experiment's data was analyzed using Minitab's General Linear Model to compare different treatments for water quality parameters. The means for nitrate, nitrite, and total suspended solids within each treatment were also compared. Significant differences for all treatments were also compared by using the Turkey's t-test at $P<0.05$.

Results & Discussion

Water Quality Parameters

Descriptive statistics of water quality parameters are shown in Tables 1 and 2. All parameters showed tolerable levels for the cultivation of tilapia in the biofloc system. The temperature in the water of all treatments was found to be very stable and in the range of 20.06-20.79^{o}C, with the lowest and highest values recorded in T4 and T3, respectively (Figure 1). There was no significant difference in temperature among all treatments (P>0.05). The pH values of all the treated water samples are found to be in the range between 7.72 and 8.07, where the lowest and highest values are from T2 and T1, respectively (Figure 2). There was no significant difference in pH among all treatments (P>0.05). According to Kubitza (2011), the optimal thermal comfort range for tilapia development is between 27ºC and 32ºC, and the pH should be held between 6.00 and 8.50.

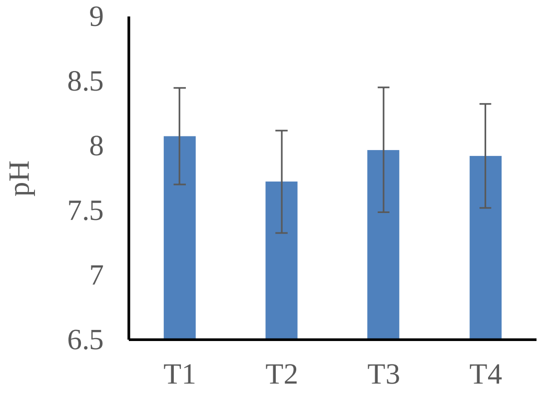

Figure 1: Temperature (ºC) in the experimental treatments

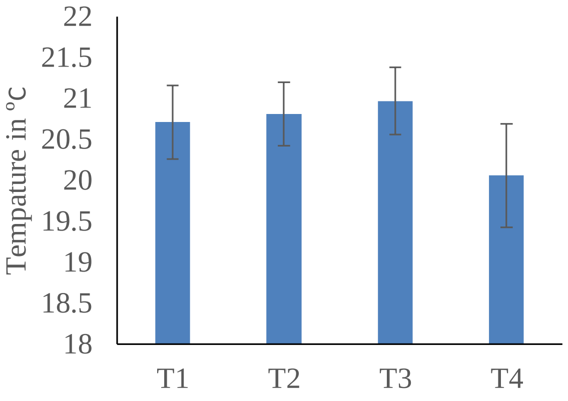

Figure 2: pH in the experimental treatments

The DO in water was found to be in the range of 5.65-7.36 mg/L in all treatments, with the highest values in T1 (control) and the lowest values in T4, respectively (Figure 3). The control treatment exhibited considerably greater dissolved oxygen concentrations ($P<0.01$) than the treatments, probably due to the lack of bacterial biomass in the bioflocs tanks. According to Avnimelech (2007), the minimum dissolved oxygen content for tilapia aquaculture in bioflocs systems should be 4 mg/L.

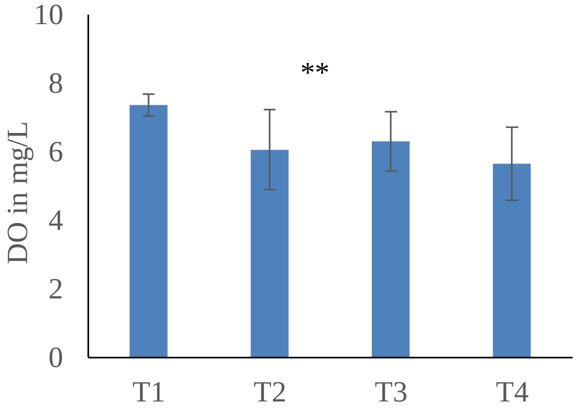

Figure 3: Dissolved Oxygen (mg/L) in the experimental treatments

The range of EC values for all treatments were recorded from 163.70-1190.40 µS/cm, where the highest and the lowest values of EC were observed in T4 and T1 (control), respectively (Figure 4). This indicates that there was very much fluctuation in the tanks of T4 with biofloc, and highly significant differences for all treatments ($P<0.001$) were observed. The ammonia concentration had mean values of 1.78, 1.08, 0.63, and 0.68 mg/L in the T1, T2, T3, and T4, treatments respectively (Figure 6). The lowest concentration of ammonia was obtained in the T3 treatment, which differed significantly from the control treatment ($P<0.05$). Ebeling et al. (2006) found that assimilation of nitrogenous compounds is closely related to floc formation and development. Miller et al. (2002) found that ammonia levels of less than 3.0 mg/L are acceptable for warm water fish farming, such as tilapia.

Nitrite is a byproduct of the nitrification and de-nitrification processes, and it is usually accumulated in intensive aquaculture systems. The concentration of nitrite varied from 0 to 0.13 mg/L in this study, with a significant difference between the control and other treatments ($P<0.001$). The nitrite level remained at low levels in biofloc treatments as these were assimilated by heterotrophic biomass, with the highest concentration in the control treatment during the experimental period. Similar to nitrite, nitrate nitrogen concentrations in both treatments remained constant, with T1 obtaining a maximum value of 11.00 mg/L (control). The nitrate concentrations remained consistent during the experiment in comparison to the biofloc technique, indicating that the biofloc culture had an effective nitrification mechanism, as previously reported by various authors (Hari et al., 2006; Avnimelech, 2009; Emerenciano et al., 2012). The amounts of nitrate in control and biofloc treatments were significantly different ($P<0.05$). The following decrease in nitrate in the BFT treatments was most likely attributable to heterotrophic bacteria immobilizing the nitrification and de-nitrification processes (Azim and Little, 2008). Also, according to Luo et al. (2013), nitrate did not accumulate in the BFT tanks, either because the nitrification process was suppressed by the high C:N ratio and utilised by heterotrophic bacteria, or because de-nitrification happened in the system.

The TDS value was found in the range of 105.9-641.0 mg/L with a highly significant difference between control and treatment ($P<0.001$) where the maximum value was in the T4 treatment, and the minimum value was in the control treatment (Figure 4). The mean concentrations of the solids agree with Avnimelech (2012), where the maximum levels for total suspended and settleable solids in fish production should be 1,000 mg/L and 100 mg/L, respectively. The TSS value in this study ranged from 0.30 to 62.5 mg/L, with a significant difference between the control and other treatments ($P<0.05$), with the T3 treatment having the highest value and the control treatment having the lowest. Increases in TSS typically impair cultured organisms' respiration (gill blockage) as well as dissolved oxygen diffusion into the water column (Crab et al., 2012). In biofloc cultures, the TSS value was within the acceptable range (200-500 mg/L) for a good effective response (Avnimelech, 2009). Suspended solids aid in the breakdown of organic matter and signify water quality improvements (Vinatea et al., 2010). TSS levels interacted inversely with changes in pH and alkalinity in the water (Furtado et al., 2011); a similar pattern was observed in this study for TSS levels. The respiration of microorganisms in the biofloc in the water column leads to carbon dioxide excretion, which lowers the pH (Gaona et al., 2011)

The alkalinity ranged from 80.0 to 230 mg/L, with the T4 treatment slightly higher (P>0.05) than the controls. This result shows that the biofloc method decreases the water's buffering capacity. To maintain alkalinity at appropriate levels, Ebeling et al. (2006) recommend a 150 mg/L alkalinity with biofloc technology and the addition of carbonates. Higher alkalinity levels aid heterotrophic bacteria in nitrogen assimilation and chemoautotrophic bacteria in nitrification.

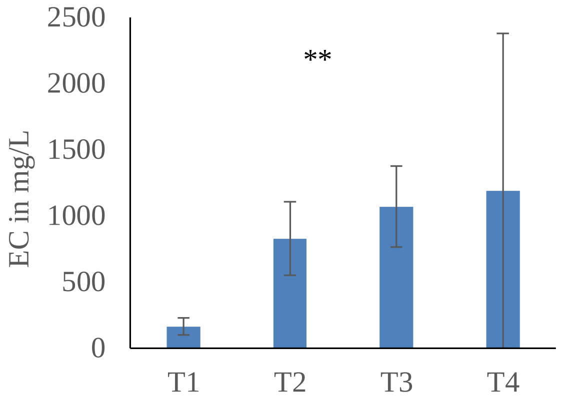

Figure 4: Electrical conductivity (mg/L) in the experimental treatments

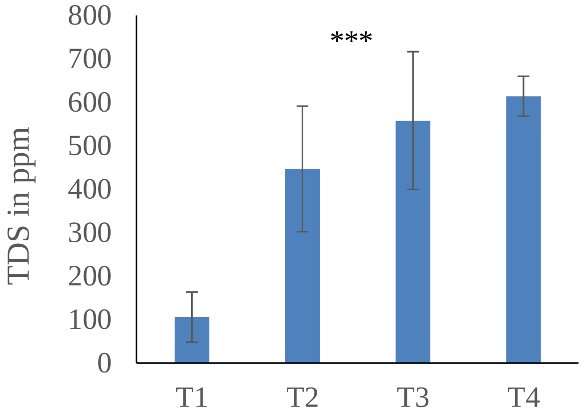

Figure 5: TDS (ppm) in the experimental treatments

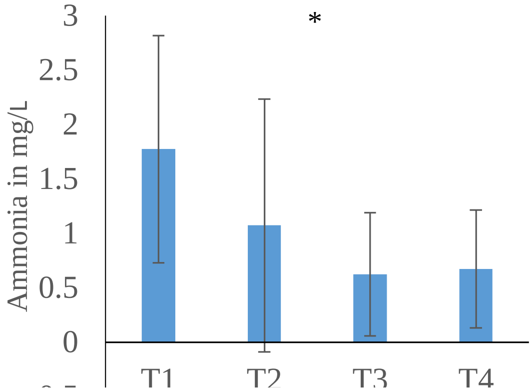

Figure 6: Ammonia (mg/L) in the experimental treatments

Table 1: Water quality parameters performed in all treatments

Sample	Temp. (°C)	pH	DO (mg/L)	EC (µS/cm)	TDS (ppm)	Ammonia (mg/L)
T1	20.71± 0.45	8.07± 0.37	7.36± 0.32^a	163.70± 63.90^c	105.90± 57.70^c	1.78± 1.04^a

T2	0.81± 0.39	7.72± 0.39	6.06± 1.17^{b}	827.50± 277.10^{b}	446.40± 144.30^{b}	1.08± 1.16^{ab}
T3	20.97± 0.45	7.96± 0.4827	6.31± 0.87^{ab}	1069.90± 306.60^{ab}	557.70± 158.80^{ab}	0.63± 0.57^{b}
T4	20.06± 0.63	7.92± 0.40	5.65± 1.07^{b}	1190.40± 91.60^{a}	641.00± 46.30^{a}	0.68± 0.54^{b}
P value	0.37	0.23	0.002	0.001	0.001	0.02

Table 2: Nitrate, nitrite, total suspended solid (TSS) and alkalinity of different treatments

Sample name	Nitrite	Nitrate	TSS	Alkalinity
T1	0.13^{a}	11.00^{a}	0.30^{c}	80^{b}
T2	0b	0b	20.00^{b}	200^{a}
T3	0b	0b	62.50^{a}	185^{a}
T4	0b	0b	30.00^{b}	230^{a}
P value	0.001	0.004	0.046	0.022

Table 3: Total bacterial count of each sample

Sample	Total Bacteria Count (CFU /mL)
T1	3.50×10^{5}
T2	1.89×10^{6}
T3	2.08×10^{6}
T4	2.24×10^{6}

Table 4: Proximate parameters of biofloc collected from tanks of different treatment

Composition (% DM)	Crude protein	Crude lipid	Ash
T1 (Commercial feed)	37.00%	8.00%	16.00%
T2	38.02%	11.45%	9.09%
T3	40.25%	12.21%	10.87%

T4	41.03%	10.89%	11.23%

Bacteriological Analyses

For each sample, the Total Bacteria Count (TBC) was determined, and it was observed that the microbial load ratios differed greatly. The range of TBC was 3.50×10^5 CFU/mL to 2.24×10^6 CFU/mL. From the comparative analysis, the TBC (CFU/mL) was higher in the T4 treatment and lower in the control treatment due to the absence of microbial biomass and the making of water changes at intervals. By metabolizing organic wastes from feces and non-consumed food, and decreasing nitrogenous compounds, particularly hazardous forms, biofloc-associated microbes serve a crucial role in maintaining water quality for fish production (Wang *et al*., 2016; Suita *et al*., 2015). In this biofloc research, *Bacillus spp.*, *Lactobacillus spp.*, *Pseudomonas spp.*, *Aeromonas spp.*, *Rhodococcus spp.*, and other gram-positive and gram-negative bacteria were identified. Bacillus spp. may be used as a natural probiotic source or for water biocontrol in super intensive aquaculture systems (Ferreira *et al*., 2017). Bacillus sp. has been shown to reduce nitrites, nitrates, and ammonia levels in fish culture in previous studies (Lalloo *et al*., 2005). Other authors have observed the same phenomena with B. subtilis and B. licheniformis, and have related these results to bacterial bioaccumulation, bio assimilation, and nitrification (Kim *et al*., 2005). The bacteria in the biofloc treatment use organic compounds (feces, molts, dead animals, and unconsumed food) as a carbon source, which helps to reduce ammonia accumulation in the water tank by generating microbial biomass (Avnimelech, 2015). Bacteria, which play one of the most significant roles in food chains by recycling enormous amounts of liquid and particulate organic matter, grow rapidly under the appropriate circumstances (temperature, carbon: nitrogen ratio, pH, etc.) (Glazer and Nikaido, 2007)

Proximate Composition

Proximal analysis results of different types of treatment are shown in Table 5. A Proximal study of biofloc revealed differences in nutritional quality between all treatments, with the highest percentage of protein obtained with T4 (40.03%) and the lowest percentage of protein obtained with T2 (38.02%). In the case of crude, the values were recorded in the range of 8.00-12.21%, where the highest value of crude lipid was recorded in T3 treatments and the lowest value was recorded in T1 (control), respectively. The ash content for all treatments was recorded in the range of 9.09 to 16.00%, whereas the highest value of ash content was 16.00% in T1 and the lowest value of ash content was 9.09% in T2, respectively. Depending on the species produced, environmental variables, feeding habits, culture length, and the presence of certain microorganisms, the proximate composition of biofloc varies (Ulloa *et al*., 2020). According to Azim and Little (2008), bioflocs with more than 38% crude protein, 3% lipids, 6% fiber, and 12% ash on a dry matter basis are suitable for tilapia production. The biofloc created in the study was shown to give good nourishment to the fish. The bioflocs from the current experiment had an appropriate level of crude protein and crude lipid, according to their proximate compositions. The biofloc was able to offer adequate food content for the tilapia's growth.

Biofloc technology has a number of advantages, including improved water quality and nutritional content. We conclude that biofloc provides a healthy, nutritious source of protein, fat, and carbs for cultured tilapias based on the proximate composition of biofloc obtained by feed reduction. These findings suggest that biofloc systems could be a long-term solution. Future BFT studies will look at growth efficiency, plankton diversity in the culture tank, the amino acid and fatty acid composition of the biofloc, and the impact of biofloc related bacteria on tilapia digestive function.

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