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Research Article

EFFECT OF TEMPERATURE ON THE PRODUCTION OF BIOFLOCS

Sharma, A., Singh, R*., Sangotra, R., Bandhana and Sharma, K. K

Department of Zoology, University of Jammu (J&K) India

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ABSTRACT

To provide adequate food for future generations, expansion and intensification of aquaculture production is required without putting larger claims on dwindling land and water resources. However, the aquaculture industry has come under scrutiny for contributing to environmental degradation. Biofloc technology is an emerging environment friendly system that degrades organic waste by microorganisms and produces microbial flocs which could be used as feed for the cultured organisms. The present work was an attempt to assess the influence of temperature on the production of bioflocs. During the experiment, bioflocs were cultured under different environmental conditions viz. indoor and outdoor. The experimental units were monitored for various physicochemical parameters along with determination of biofloc volume. It was observed that water temperature plays a key role in the formation of bioflocs. At temperature between 20-25˚C stable bioflocs were formed but as the water temperature increases bioflocs lose their floatability and sink to the bottom in the form of sludge.

INTRODUCTION

Due to the enhanced demand of seafood, the practice of fish farming has expanded rapidly to become a major global industry. It has been estimated that a five-fold increase in global aquaculture production is required for the consumption of growing population within the next five decades (FAO, 2004). To achieve this, using conventional extensive aquaculture ponds, a five-fold increase of water consumed and land occupied will be needed. The prime goals of aquaculture expansion must not only be to produce more aquacultural products without increasing the usage of basic natural resources of water and land (Avnimelech, 2009) but also to develop sustainable aquacultural system not damaging the existing environment (Naylor *et al.*, 2000) and create a system which shall provide an equitable cost/benefit ratio to support economic and social sustainability (Avnimelech, 2009). Thus, enhanced food consumption and demand for preservation of natural resources have driven aquaculture towards close-water intensive cultivation (Crab *et al.*, 2007). But the intensive aquaculture industry face few major problems such as environmental deterioration like eutrophication caused by waste water discharge from aquacultural systems containing nitrogenous compounds (ammonia, nitrite and nitrate) at high concentration (Nora'aini *et al.*, 2005), toxicity of these compounds to aquatic fauna (Timmons *et al.*,2002; Boardman *et al.*, 2004), low feed utilization in case of high water exchange system (Avnimelech, 2007) and incidence of diseases causing significant economic losses (Faizullah *et al*., 2015).

In order to overcome these limitations, a new concept is becoming very popular in recent years known as Biofloc Technology (BFT). The main principle of BFT is to recycle nutrients by maintaining a high carbon/nitrogen (C/N) ratio in the water in order to stimulate heterotrophic bacterial growth that converts ammonia into microbial biomass (Avnimelech, 1999). The microbial biomass further aggregates with other microorganisms and particles suspended in the water forming 'Biofloc', which eventually may be consumed in situ by the cultured animals or harvested and processed as a feed ingredient (Avnimelech, 1999; Avnimelech, 2007; Crab *et al.*, 2007; De Schryver *et al.*, 2008; Kuhn *et al.*, 2008; Kuhn *et al.*, 2009 and Kuhn *et al.*, 2010).

MATERIAL AND METHODS

Experimental Fish

The fingerlings of *Cyprinus carpio* were procured from the fish farm at Doomi, Akhnoor Road, Jammu and kept in tubs for 3-4 days for acclimatization. The dead fingerlings were removed from the tubs from time to time. They were then weighed accurately with the help of weighing balance and segregated according to their weight.

*Corresponding author: **Singh, R**
Department of Zoology, University of Jammu (J&K) India

Preparation and setting up of experimental tubs

The experimental units were prepared for the culture of bioflocs wherein two tubs each having a capacity of 65 L were filled with clean water and out of which; one was kept inside and other outside. A small amount of cow dung along with some pond soil from the botanical garden was added to the tubs in order to inoculate them with heterotrophic and nitrifying bacteria. Pond water was also added to expedite the development of phytoplankton and zooplankton. The acclimatized and seggregated fingerlings were counted and stocked in each of the experimental tubs in order to get the nitrogenous waste. Each tub was stocked with 10 fingerlings. They were fed twice a day at the rate of 2 % body weight with commercial diet consisting of 32% protein and 4% lipid. Aeration was provided 24 hrs throughout the experiment to ensure regular oxygen supply to the fingerlings and each experimental tub was provided with 2 air stones connected to the air pump. No water was exchanged throughout the culture period. However, water loss during evaporation was compensated by adding small quantity of water to maintain the initial level.

Culture of bioflocs

After few days when the ammonia level in the tubs increased, locally available cheap carbon source, i.e. molasses was added into the system to manipulate the C/N ratio and initiate the formation of bioflocs. The amount of organic carbon source to be added was calculated based on the methodology proposed by Avnimelech (1999).

As the molasses contained 50% carbon, hence 20 g molasses were used for each 1 g of Total Ammonia Nitrogen (TAN) in order to maintain the C/N ratio above 10. Pre-weighed molasses were mixed in a glass beaker with the water taken from the corresponding culture tub and poured directly to the water column. After the addition of molasses and continuous aeration, froth appeared within 2-3 days. During the subsequent days, froth disappeared and small suspended particles started appearing in the water column. A drop of suspension when observed under the microscope revealed clusters of algae, protozoans like paramecium, diatoms and zooplanktons along with the colonies of bacteria.

Assessment of Water Quality Parameters

Water samples were collected and monitored for various physicochemical parameters, viz. air and water temperature, pH, DO, free CO_2, ammonia, nitrites and nitrates. Air and water temperature was measured twice a day in the morning and evening using mercury bulb thermometer. pH was monitored once daily with the help of a portable field pH meter (Hanna). DO and free CO_2 were analysed thrice a week by Winkler's method and Titrimetric method (APHA, 1985) respectively. The concentration of ammonia, nitrites and nitrates were determined twice a week as per the standard methods APHA (1985). Floc volume was measured once a week starting from Week IV using Imhoff cones (Eaton *et al.*, 1995).

Table 1 Weekly variations in physico-chemical parameters in indoor conditions (Treatment I).

Duration / Parameters	Week I	Week II	Week III	Week IV	Week V	Week VI	Week VII	Week VIII	Mean ± SD
Air Temperature (˚C)	21.9	21.1	23.5	25.9	28.1	28.1	28.7	29.8	25.9 ± 3.3
Water Temperature (˚C)	20.5	22.4	23.3	24.8	26.6	26.7	27.9	28.1	25.0 ± 2.8
pH	8.2	8.2	7.9	7.1	7.1	7.2	6.9	6.9	7.4 ± 0.56
Dissolved Oxygen (mg/l)	6.6	7.3	6.8	4.5	5.3	5.5	4.9	6.1	5.9 ± 1.0
Free Carbon Dioxide (mg/l)	9.0	12.0	17.3	25.3	18.6	18.0	27.3	14.0	17.7 ± 6.2
Floc Volume (ml/l)	-	-	-	20.0	16.3	14.0	5.0	3.0	11.7 ± 7.3

Table 2 Weekly variations in physico-chemical parameters in outdoor conditions (Treatment II).

Duration / Parameters	Week I	Week II	Week III	Week IV	Week V	Week VI	Week VII	Week VIII	Mean ± SD
Air Temperature (˚C)	20.6	21.1	22.6	26.4	28.0	28.0	31.8	34.8	26.7 ± 5.1
Water Temperature (˚C)	20.7	23.0	24.0	26.9	27.8	28.5	30.2	30.2	26.4 ± 3.5
pH	8.1	8.2	8.0	7.0	7.0	7.0	7.1	6.9	7.4 ± 0.57
Dissolved Oxygen (mg/l)	6.2	6.9	6.8	5.3	5.2	5.1	4.3	5.3	5.6 ± 1.0
Free Carbon Dioxide (mg/l)	12.0	16.6	18.6	25.3	16.0	23.3	34.0	23.3	21.1 ± 6.9
Floc Volume (ml/l)	-	-	-	19.0	13.8	10.0	2.5	2.0	9.5 ± 7.3

Statistical analysis

The data was analysed by statistical approaches like standard deviation (SD) and Pearson correlation coefficient (r).

RESULTS AND DISCUSSION

Air and Water Temperature

Air and water temperature remained low in indoor conditions and high in outdoor conditions, throughout the experimental period (Table 1 and 2). Water temperature closely followed air temperature and a steady increase in water temperature was observed in both the conditions from first to last week of the culture period (April and May). However, a comparative analysis revealed higher water temperature in outdoor conditions and lower in indoor conditions which may be attributed to relatively more heat exchange in the shallow water of outdoor conditions as compared to indoor conditions. The earlier workers have already suggested such phenomenon of heat exchange (Kant and Raina, 1990; Sawhney, 2008 and Shinde *et al.*, 2011).

pH

In both the treatments pH showed variation from alkaline to acidic range and remained constant (alkaline) for the first 3 week followed by a slight decline due to addition of molasses for rest of the period, which was corrected by the addition of sodium bicarbonate ($NaHCO_3$) that provided physical and chemical water quality conditions favourable for biofloc development (Table 1 and 2). This drop in pH may be due to enhanced decomposition, respiration by microorganisms present in biofloc resulting in the release of carbon dioxide and hydrogen ions produced and released in water column during nitrification process.

DO and Free CO_2

DO concentration remained high for the first 3 weeks in both the experimental setup, thereafter; it showed a decline (Table 1 and 2). Such variations in DO may be attributable to the addition of molasses in the culture water. However, the overall DO concentration was found to be slightly higher in indoor conditions than in outdoor conditions and may be ascribed to high water temperature in outdoor conditions. The observations falls in line with the findings of Kalff (2000) who postulated that oxygen solubility in water has an inverse relationship with water temperature. Moreover, during the whole experimental period of eight weeks free CO_2 level remained high (Table 1 and 2) but was within the desirable limits for fish culture as suggested by Boyd and Lichtkoppler (1979), who were of the view that most fish species can survive in waters containing up to 60 mg/l carbon dioxide, provided DO concentrations are high and many others have also suggested that fishes can survive at the maximum and minimum value of free CO_2 recorded presently. However, the lowest concentration of free CO_2 was observed in Week I and highest in Week VII in indoor as well as outdoor conditions due to high DO concentration during this period, justifying inverse relationship between the two.

Ammonia, nitrite and nitrate

Sequential accumulation of ammonia followed by nitrite occured in the culture water for the first three weeks in both the Treatment sets (Fig. 1 and 2). But the ammonia concentration was maintained below the toxic level of 1.0 mg/l and this level of ammonia supported the production of bioflocs as the microorganisms use this form of nitrogen for their biomass production along with the provided carbon source (molasses). It has been well documented that NH_3 concentration beyond this is highly toxic to aquatic organisms (Meade., 1985; Santhosh and Singh., 2007). With the addition of molasses from fourth week onwards, the ammonia concentration showed a decreasing and increasing pattern. Concentration of nitrite followed a similar pattern as that of ammonia. Inspite of the similarity in concentration profiles, nitrite accumulated at a slower rate in the first three weeks of the culture period. Conversely, nitrate became predominant and continued to accumulate in the culture water during the proceeding weeks (Fig 1 and 2). It is the safest form of nitrogen in the aquatic system for the growth of biota therein. In the present experiment, this significant amount of nitrogen accumulation in the form of nitrate clearly reflected the activity of microorganisms/ nitrifying bacteria of the bioflocs and confirmed the important role of nitrification process in the culture water. However, it was observed that the concentration of ammonia, nitrite and nitrate was high in outdoor conditions.

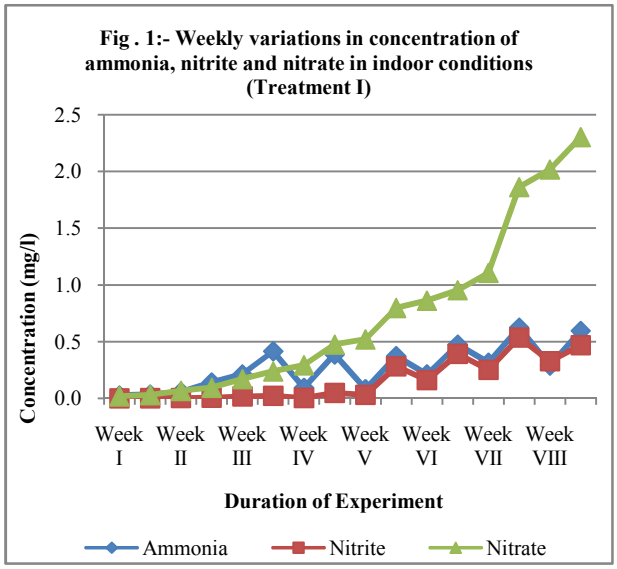

Fig . 1:- Weekly variations in concentration of ammonia, nitrite and nitrate in indoor conditions (Treatment I)

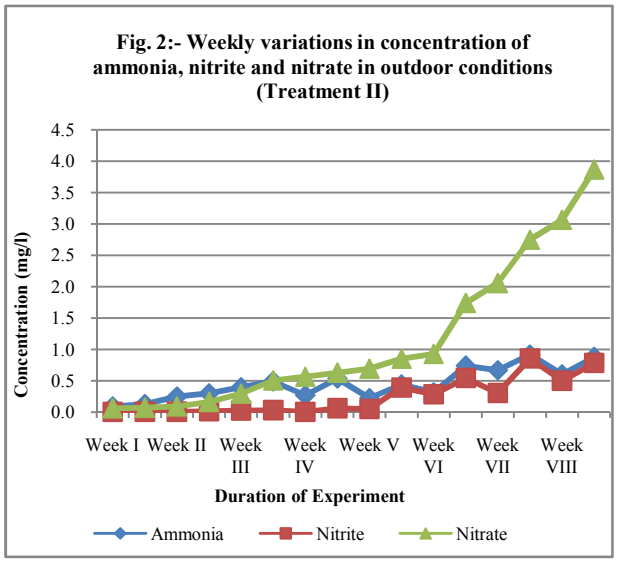

Fig. 2:- Weekly variations in concentration of ammonia, nitrite and nitrate in outdoor conditions (Treatment II)

Floc volume

Floc volume was higher in indoor conditions (Table 1) than outdoor conditions (Table 2), due to slightly lower water temperature in indoor conditions that provided floatability to the bioflocs causing lesser sludge formation. These findings were in line with the findings of Krishna and Van Loosdrecht (1999) who documented that temperature ranging from 30 – 35 ˚C result in bulking of flocs because of the excessive production of extracellular polysaccharides, this may lead to a loss of floatability and sinking of flocs to the bottom. They further stated that at intermediate water temperature (20-25 ˚C) flocs are stable and have intermediate floc volume index. The Pearson correlation coefficient (r) revealed negative correlation between the water temperature and the floc volume in both the treatments.

Water colour

During the present experimental period, it was observed that the colour of the water was different in both indoor as well as outdoor tub. The biofloc tub kept outside under natural light was having green coloured water (Fig. 3) in contrast to the one kept in indoor conditions which was having brown coloured water (Fig. 4).

Fig 3 Outdoor tub

Fig 4 Indoor tub

This difference in the water colour may be due to their direct exposure to sunlight in outdoor conditions and absence of sunlight in indoor conditions. The tub kept outside was having more algal content which imparted green colour to the water as compared to the one kept inside. This fact is already well explained by Hargreaves (2013), who emphasized a complex mixture of algal and bacterial processes control water quality in the system exposed to outdoor light, also called 'green water' biofloc system. However, biofloc system that are not exposed to outdoor light are instead installed indoor, operate as 'brown water' system where only bacterial processes control the water quality in the system. This has been further substantiated by Perez-Rostro *et al.* (2014) and Choo *et al.* (2015) who also recorded similar observations.

CONCLUSION

It can be concluded that Biofloc system supports the repeated cycling of waste nitrogen between dissolved ammonia and bacterial biomass and reduces the environmental risk of polluted water discharged from culture systems. Water temperature played major role in the production and stability of the bioflocs. Stable flocs i.e. 20-25ml/l were formed at intermediate water temperature (20-25˚C). However, at high temperature i.e. 30-35˚C floc volume was low due to loss of floatability and sinking of flocs to the bottom, causing more sludge formation.

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