BIOTIC AND BIOCHEMICAL COMPOSITION OF BIOFLOCS CULTURED IN LABORATORY CONDITIONS

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Abstract

Aquaculture serves as man's most important source of high quality protein, providing 16% of the total animal protein consumed by the world's population. But the intensive aquaculture is mainly restricted due to environmental and economic issues. To solve these problems, the present work was conducted which deals with the culture of bioflocs using molasses as carbon source in two different sets i.e. Treatment I (T-I) without the cultured species known as Ex-situ bioflocs and Treatment II (T-II) along with the cultured species known as In-situ bioflocs. Once, the bioflocs were formed their biotic and biochemical composition was done. Biotic composition revealed that bioflocs are the aggregates of heterotrophic bacteria, algae, entangled zooplankton, uneaten feed and other dead organic matter whereas biochemical composition showed that In-situ bioflocs are more protein rich incomparison to Ex-situ bioflocs. In- situ bioflocs were found to contain 34% protein, 12.6% lipids, 31% moisture and 15.2% ash whereas ex-situ bioflocs contained 31% protein, 10.5% lipids, 15.8% ash and 34% moisture. Both, these bioflocs can be used as protein rich source in aquafeed. Level of protein in both Ex-situ and In-situ biofloc system was found to be appropriate for the growth of fishes.

Keywords: Bioflocs, Cirrhinus mrigala, Imhoff cones, heterotrophic bacteria, Aquafeed.

1. Introduction

The world's population is increasing at a rapid rate and it has grown from 1.5-6.4 billion from 1900 till now and is predicted to increase to 9 billion by the year 2050. In order to satisfy the global protein demand with limited land resources there is shift towards aquatic resources. World aquaculture is growing with an annual rate of 8.9%-9.1% since 1970's. Due to slow growth in aquaculture, there exists a huge gap between production and demand. This growing demand of fish protein results in the shifting of extensive aquaculture to intensive aquaculture keeping in view the limitations of water and availability of suitable land. However,

at present the growing aquaculture industry is haunted by a number of environmental and social issues (Boyd, 1990). Aquaculture expansion by shifting from extensive system to intensive system results in few major problems:-

- Deterioration of water quality by accumulation of inorganic nitrogen species. Toxicity of nitrogenous compounds to aquatic fauna (Timmons *et al.*, 2002; Boardman *et al.*, 2004).
- 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)
- Reduction of ammonia, nitrite and nitrate by exchange and periodic replacement of water can result in resource depletion.
- Low feed utilization in case of high water exchange system (Avnimelech, 2007) where only 20-25% of protein in feed is assimilated by fish and the remaining accumulates as waste in water (Avnimelech and Ritvo, 2003).
- Reliance on high protein fish meal-based feed requires many pounds of wild fish to produce one pound of edible aquaculture product.
- Incidence of diseases causing significant economic losses (Faizullah *et al.*, 2015).

Thus, the above given problems restrict the growth of intensive aquaculture. Keeping in view the above problems and in order to make intensive aquaculture successful, Biofloc technology was developed to increase environmental and economic sustainability, which reduces inorganic nitrogen species and increases feed utilization through recycling (protein is the most important and expensive component of feed). Biofloc technology is considered as the NEW BLUE REVOLUTION in aquaculture and it works with minimum or zero water exchange and Recycling of nutrients.

By adopting this innovative technology it is possible to properly utilize the protein content present in the feed which otherwise would go waste in the intensive system and thus heavy economic loss caused by wastage of feed can be managed. Hence, biofloc technology enables intensification at a relatively reasonable investment and operating cost. The present study looks at the production of bioflocs in laboratory conditions using molasses as the carbon source and to know about its biotic and biochemical composition.

2. Material and methods

2.1 Culture of Bioflocs:

The culture of bioflocs was the pre-requisite for the experiment. The fingerlings of *Cirrhinus mrigala* were collected from the local fish farm at Gho-manhasan, Jammu which is about 14-15 kms from Jammu city and were transported from the fish farm to the departmental laboratory for the experimental work in polythene bags filled with oxygenated water. They were kept for acclimatization for 15 days in two troughs each stocked with 15 fingerlings. After acclimatization, from one of the troughs the fingerlings were removed and shifted to other tubs and same waste water containing faecal matter and uneaten feed of the fishes generated during the acclimatization was used for the culture of bioflocs (T-I/ Ex-situ biofloc system) while in other trough, bioflocs were cultured along with fingerlings of *Cirrhinus mrigala* using same water with no addition of artificial feed(T-II/ Insitu biofloc system). When level of ammonia increased in both troughs cow dung was added along with pond soil containing some pond water from the botanical garden so as to inoculate heterotrophic bacteria, nitrifying bacteria, phytoplanktons and zooplanktons.

Molasses were added to maintain C/N ratio more than 10. After 3-4 days suspended particles appeared in both sets. These brown suspended particles are known as bioflocs. Once the biofloc started appearing in both sets they were harvested from the system using imhoff cones and their biotic composition was studied using compound microscope. For biochemical analysis, the filterate containing bioflocs was oven dried at 106˚C and processed further. The protein, lipid, moisture and ash content was determined by Standard Methods viz. Lowry et al. (1951), Folch et al. (1957), Hot air oven and Muffle furnace (AOAC, 1999) respectively.

2.2 Assessment of water quality parameters

Various water quality parameters were analysed regularly. Water temperature was recorded with the help of a mercury bulb thermometer with 1^0C. pH of the water sample was determined with the help of pH meter (Hanna). Dissolved oxygen, free CO_2, ammonia, nitrite and nitrate (APHA, 1985).

3. Results and discussion

In the culture of bioflocs pond soil and cow dung acted as the source for heterotrophic bacteria. These heterotrophic bacteria acted as driving force for reducing inorganic nitrogen and their production.

During the culture period C/N ratio was maintained above 10 by adding molasses (as carbon source) that played an important role in conversion of inorganic nitrogen into microbial biomass by heterotrophic bacteria. Molasses are also known to enhance the water quality in the pond (Burford *et al.*, 2003; Hari *et al.*, 2004; Avnimelech, 2005). Different carbon sources have also been used for

production of bioflocs but in present studies molasses were used. Burford *et al.* (2003); Hari *et al.* (2004); Samocha *et al.* (2007); Emerenciano *et al.* (2012); Widanari *et al.* (2012); Souza *et al.* (2014); Maia *et al.* (2016) and Gutierrez *et al.* (2016) too recommended molasses as carbon source for the culture of bioflocs in their studies. While maize starch was used by Liu *et al.* (2014). Tapioca, wheat, corn and sugar bagasse were used by Ahmad *et al.* (2017), sucrose by Xu and Pan (2013), dextrose and molasses by Lorenzo *et al.* (2015) and Huang *et al.* (2017), Cassava residue by Chen *et al.* (2015), soybean molasses by Santo *et al.* (2017) and jaggery by Sakkaavarthi and Sankar (2015) in their respective experimental studies. But in the present studies the preference was given to cheap, easily available and biodegradable carbon sources i.e. molasses which also resulted in:-

- Proliferation of bacteria (Ebeling *et al.*, 2006; Hari *et al.*, 2006; Avnimelech and Kochba 2009; Crab *et al.*, 2010).
- decline in the dissolved oxygen level (Tacon *et al.*, 2002; Wasielesky *et al.*, 2006; Phulia *et al.*, 2012; Emerenciano *et al.*, 2013)
- Increase in acidic conditions.
- Decline in pH (Furtado *et al.*, 2011).
- Induction of sub lethal effects on sensitive culture species (Landman *et al.*, 2005).

However, decline in pH due to addition of molasses was balanced between 7.9 to 8.3 (Table 4) by addition of sodium bicarbonate because the known ideal pH level for fish culture is between 7.5 & 8.5 and above and below this is stressful to the fishes (Santhosh and Singh, 2007). Constant aeration was also done to enhance the DO level and reduce the acidic conditions

Aeration played a very important role in the culture system:-

- ❖ It maintained the dissolved oxygen level above 5 mg/l in the culture system by diffusion from the atmosphere (Kuhn *et al.*, 2015).
- ❖ It keeps the bioflocs in suspension which otherwise would settle down and form sludge. This sludge on decomposition degrade the water quality by consumption of oxygen and release of toxic products such as nitrite, H_2S and methane, (Avnimelech and Ritvo, 2003). This accumulation was not desirable in the present culture system and so by siphoning whatever accumulates at the bottom was removed (Hopkins, 1994; Taw, 2012).

After the addition of molasses froth formation occurred which slowly disappeared within few days and resulted in the formation of brown coloured suspended particles known as bioflocs (Fig. 1, Fig. 2 and Fig. 3).

3.1 Biotic composition

The water sample from the culture system was collected in the imhoff cones and allowed to settle for 15 minutes (Fig. 4). The filterate when seen under the compound microscope showed the aggregates of heterotrophic bacteria, algae, entangled zooplankton including protozoa, rotifers, diatoms, uneaten feed and other dead organic matter (Fig. 5 and Fig. 6). These floc particles seemed to be agglutinated by a polysaccharide slime produced by bacteria (Avnimelech *et al.*, 2001).

3.2 Biochemical composition of Bioflocs

Biochemical composition of bioflocs revealed variations in protein, lipid, moisture and ash content in both In-situ and Ex-situ biofloc culture system. In- situ bioflos were found to contain 34% protein, 12.6% lipids, 31% moisture and 15.2% ash whereas ex-situ bioflocs contained 31% protein, 10.5% lipids, 15.8% ash and 34% moisture (Table 1; Fig. 7). Higher protein and lipid content was observed in T-II as compared to T-I with low moisture and ash content. This may be due to continual production and utilisation of inorganic nitrogen in In-situ biofloc system as fingerlings were reared along with the culture of bioflocs while in Ex-situ biofloc system the inorganic nitrogen was immobilized into microbial biomass with no or very little production due to decomposition of sludge only as this system did not have fingerlings. Continual removal of sludge resulted in very less production of inorganic nitrogen as compared to its utilization. So the source of inorganic nitrogen was added from time to time in ex-situ biofloc culture system for continual production of bioflocs. This may affect protein content of Ex-situ biofloc system. Slight variations in protein level was seen in both the systems but their protein level was found to be optimum (31-34%) for the growth of fingerlings.

4. Conclusion

The present studies revealed that bioflocs cultured in-situ (T-II), the protein content was high and in the same culture system where bioflocs and fingerlings were reared together. In this system, the inorganic nitrogen from the faecal waste water was properly utilized by the bacteria to form bioflocs. In-situ biofloc culture system is best in comparison to Ex-situ biofloc culture system due to following reasons:-

- Continuous production of bioflocs in the system. The heterotrophic bacterial population also utilize the ammonium in addition to the organic nitrogenous wastes to synthesize single cell microbial protein (Schneider *et al.*, 2006) which also act as natural feed for fish (Burford *et al.*, 2004).
- Proper harnessing of biofloc by fingerlings as both bioflocs and fingerlings were cultured in same unit.
- High nutritional value of bioflocs on which the fingerlings completely rely.

- Constant availability of food in the form of bioflocs.
- Fingerlings were fed until apparent satiation (Silva *et al.*, 2015).
- Adequate supply of bioflocs.
- In-situ maintenance of water quality and microbial flocs within the system are responsible for the enhancing the growth and welfare of the cultured fish (Kamilya, 2017).

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Table 1:- Biochemical composition of in-situ and ex-situ bioflocs.

Biochemical components	In-situ bioflocs	Ex-situ bioflocs
Moisture	31%	34%
Protein	34%	31%
Lipid	12.6%	10.5%
Ash	15.2%	15.8%

Table 2:- Variations in the physicochemical parameters in Ex-situ and In-situ biofloc system.

TREATMENTS / PARAMETERS	EX-SITU BIOFLOC SYSTEM	IN-SITU BIOFLOC SYSTEM
Temperature (°C)	15.1-18.2	15.3-18.3
pH	8.0-8.3	7.9-8.2
Dissolved Oxygen (mg/l)	4.5-6.7	4.4-6.5
Free Carbon dioxide (mg/l)	6.67-9.67	7.0-11.33
Ammonia (mg/l)	0.016-0.040	0.014-0.033
Nitrite (mg/l)	0.005-0.035	0.003-0.033
Nitrate (mg/l)	0.003-0.062	0.003-0.031

Fig. 1:- Froth formation after the addition of molasses during biofloc culture.

Fig. 2:- Intense froth formation

Fig. 3:- Suspended bioflocs in water sample taken in beaker.

Fig. 4:- Imhoff cones showing floc volume.

Fig. 5:- Microscopic view of bioflocs showing Heterotrophic bacteria and algae.

Fig.6 :- Bioflocs with entangled rotifer, diatoms and protozoan

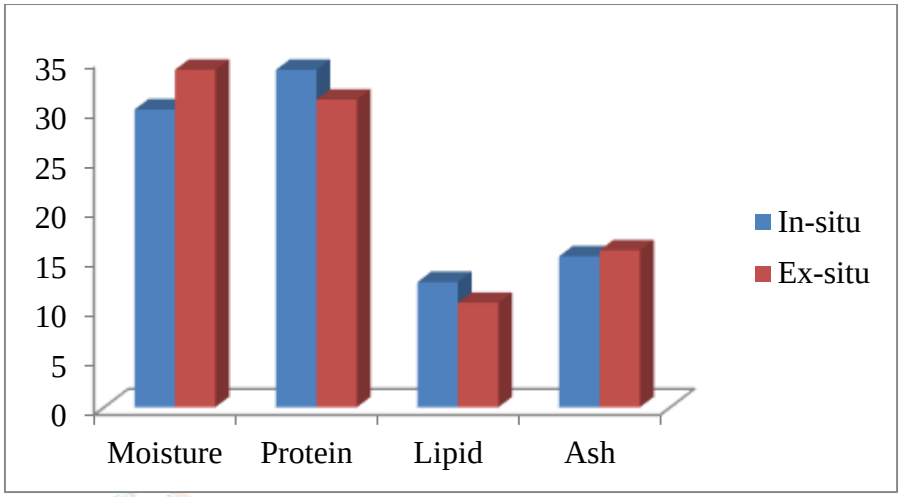

Fig 7:- Biochemical composition of in-situ and ex-situ bioflocs.