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RESEARCH ARTICLE

Biofloc the Nutritional Enriched Feed for IMC Culture in India**Sudhansu Shekhar Mahanand¹ and Dibyajyoti Sahoo²**

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ABSTRACT

World population is growing rapidly, the food production industry needs to be developed. As the aquaculture industry intensively develops, its environmental impact increases and its strong dependency on fishmeal in the diet, the use of biofloc technology can reduce these problems. The basic principle of the biofloc technology is the retention of waste and its conversion to biofloc as a natural food within the culture system. Now a days biofloc technology has become very popular in aquaculture sector. The concept of floc formation in activated sludge systems can be used for application in biofloc technology. There are so many factors which influence the formation of biofloc. Microbes are manipulated in BFT systems in order to control and reduce toxic inorganic nitrogen concentrations. Extensive development of microbial biomass is an integral part of this process. Microbial proteins in BFT systems should be considered only as a supplement of feed proteins. Many researchers revealed that net protein uptake by the shrimp from biofloc was equivalent to 25 to 50 per cent of conventional protein feeding. Biofloc contains about 30 per cent crude protein along with other essential minerals. Essential fatty acid also found in biofloc which is suitable for dietary requirement of Indian Major Carp (IMC). Therefore, it appears the biofloc can be successfully used as fish feed particularly for herbivorous and omnivorous species. Microorganisms in biofloc might partially replace protein content in diets or decrease its dependence of fishmeal. Biofloc technology provides a more sustainable approach with minimal water exchange along with reduce feed intake and transform it in to a low-cost sustainable technology for sustainable aquaculture development.

Key words: Biofloc technology; Indian Major Carp (IMC); Microbial protein; Fish feed.

Aquaculture is currently the most rapidly growing animal food-producing sector globally. India stands second in terms of aquaculture production in the world with 10.79 million metric tons from 6.9 million hectares of freshwater area and 1.24 million hectares of brackish water area (NFDB, 2019). India's sea food export was valued at US\$ 4.7 billion (MPEDA, 2016-17). As the aquaculture industry intensively develops, its environmental impact increases. Due to rapid environmental change, the diversification of aquaculture sector towards sustainable production has become the most fretful issue (Sakib et al., 2020). Discharges from aquaculture deteriorate the receiving environment. Further the need for fishmeal and fish oil for fish feed production also increases. One of the common solutions used

to remove the excessive nitrogen is to frequently exchange and replace the pond water. This approach is limited for environmental regulations. A high surface area with immobilized nitrifying biomass enables a high nitrifying capacity in a controlled environment. One problem associated with bio-filtration is the involvement of high cost. An additional strategy is presently getting more attention is the removal of ammonia from the water through its assimilation into microbial proteins by the addition of carbonaceous materials to the system. If properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation. Another important aspect of this process is the potential utilization of microbial protein as a source of feed protein for fish or shrimp. This process is popularly known as biofloc

technology. The biofloc is a unique ecosystem of rich and potent particles suspended in relatively poor water (*Avnimelech, 1999*). The basic principle of the biofloc technology (BFT) is the retention of waste and its conversion to biofloc as a natural food within the culture system.

METHODOLOG

Four numbers of tanks of individual capacity 1000 L was utilised for producing biofloc using aquaculture effluent from a nearby carp culture pond. Periodically wheat flour containing approximately 50% organic carbon was added at the rate of 20 times the concentration of total ammonia nitrogen (TAN) concentration in the tank water to maintain a C:N ratio of 10 for optimum production of biofloc (*Avnimelech, 1999*). Concentrated floc samples were collected from the biofloc tanks using a muslin cloth and dried in an oven at 102°C until constant weight and then preserved in a refrigerator for proximate analysis following *AOAC (1990)* procedure. For ash content, a known amount of dry sample were burnt in a muffle furnace at 550°C for 4 h and the ash cooled and weighed. The crude protein content was determined by the Kjeldahl method. Lipid content was determined with Soxhlet apparatus. The energy content was determined by a bomb calorimeter. The C:N ratios were determined using a CHN analyzer (Perkin Elmer 2400 Series II). To determine the fatty acid composition, the freeze dried samples were extracted for total lipids, according to *Bligh and Dyer (1959)*. Total lipids were subjected to transmethylation, using methanol containing 1% concentrated sulphuric acid, according to *Christee (1982)*. Crude methyl esters thus obtained were purified by thin layer chromatography (TLC), following the method of *Mangold (1969)*. The purified fatty acid methyl esters were analysed by gas liquid chromatography (GLC) using a BPX-70 megabore column (30 mts × 0.53 mm, supplied by SGE Australia). A Chemito Toshniwal (India) Gas Chromatograph, Model GC 1000, equipped with Flame Ionization Detector (FID) was used. Peaks of fatty acid methyl esters were identified following the method of *Ackman and Burgher (1965)*. Quantifications were done by the "Clarity Lite" (Data Apex Ltd., The Czeck Republic) software.

Mineral content of the samples were analysed by wet-acid digested using a mixture of nitric acid, perchloric acid and sulphuric acid (3:2:1). The amount of minerals in the digested samples was determined

by Atomic Absorption spectrophotometer (Analyst 700 Perkin Elmer) following the method described by *Sreeramaiah et al. (2007)*.

RESULTS AND DISCUSSION

The use of biofloc as a feed for aquaculture species: Microbes are manipulated in BFT systems in order to control and reduce toxic inorganic nitrogen concentrations. Extensive development of microbial biomass is an integral part of this process. Suspended biofloc in intensive limited water exchange ponds amount to high protein feed equivalent. Research and commercial scale results showed that for certain fish, mostly shrimp (different species) and tilapia, the suspended bioflocs are utilized and replace feeds. Composition of biofloc was found to contain many components required by fish. The potential and importance of such information are existing. Suspended flocs from commercial shrimp ponds in bft systems were analyzed and found to higher contain protein levels compared to the commercial feed, used in the ponds. Floc amino acid levels contain adequate lysine and arginine which can meet shrimp nutritional requirement. Analysis of minerals indicates that microbial flocs are rich in phosphorus as high source of other minerals which is essential for both shrimp and fish. Microbial proteins in BFT systems should be considered only as a supplement of feed proteins. BFT systems are based on using microbial proteins as a partial source of protein. In addition, the bioflocs contain algae and fungi as well as significant amounts of grazers, such as protozoa and zooplankton, possibly affecting the percentage of nucleic acids in the overall crude protein pool. Many researchers revealed that net protein uptake by the shrimp from biofloc were equivalent to 25 to 50 per cent of conventional protein feeding. Inclusion of processed biofloc from tilapia ponds into shrimp feed, at levels of about 8 to 16 per cent, raised shrimp yields by 160 per cent over those obtained with commercial diets. It has to be noticed that all known feed components were kept equal in the different commercial and biofloc containing diets. *Mahanand et al. (2012)* conducted a feeding trial using different diets by mixing fish feed with biofloc in dry as well as wet forms at various proportions to evaluate the growth parameters of rohu in a light-limited indoor culture system. The results of these studies indicated the growth parameters of rohu were maximum when fish feed and biofloc in wet form were used in 1:1 ratio. Further *Mahanand*

et al. (2013) studied and demonstrated the potential of biofloc technology in the culture of rohu, *Labeo rohita*. The nutritional quality of biofloc was found to be suitable for IMC culture.

Nutritional quality of biofloc as fish feed for IMC culture : Indian Major Carp (IMC) such as rohu, catla, mrigal is the most preferred species for farmers in India. The proximate composition, mineral content, energy content and C:N ratio of biofloc produce from wheat flour as a carbohydrate sources. Many researchers worked on the dietary protein requirements of IMC feeding on dry formulated feeds (*Singh et al., 2006*). The studies reported that about 30 per cent crude protein is sufficient for obtaining optimum growth of carp. The biofloc quality in terms of fish nutrition contained 35 per cent protein, 1 per cent lipid, 15 per cent fibre, 15 per cent ash and 19 kJg⁻¹ energy (on dry matter basis) harvested from wheat flour (Table 1). Thus, the biofloc is certainly appropriate for IMC culture except high fiber and ash content as high fibre content results in the decrease of the quantity of the usable nutrient in the diet (*De Silva and Anderson, 1995*). The crude protein level of 35 per cent in biofloc was consistent with levels reported by *Tacon et al. (2002)* and *Wasielesky et al. (2006)*, but less than the 43 per cent and 49 per cent crude protein described by *Jory (2001)*. Recently *Emerenciano et*

al. (2012) studied the nutritional quality of biofloc and found 30 per cent crude protein on it.

The biofloc contained essential fatty acids for the fish. There were 21.11 per cent polyunsaturated, 35.63 per cent monounsaturated, and 43.1 per cent saturated fatty acids present in the biofloc sample which is essential for the fish (Table 2). *Tacon (1990)* reported that about 1 per cent of 18:2 ω 6 and 18:3 ω 3 dietary essential fatty acids are required for common carp. In the biofloc samples those values were 12 per cent and 4.4 per cent. Therefore, it appears the

Table 2. Fatty acid composition (% lipid) of biofloc produce from wheat flour as a carbohydrate source

Components	Biofloc
12:0	0.3
13:0	0.2
13:1	0.3
14:0	2.9
14:1	4.5
15:0	2.3
15:1	0.9
16:0	29.3
16:1	7.9
16:2	2.0
17:0	0.2
17:1	0.3
18:0	4.6
18:1 ω 9	20.8
18:2 ω 6	12.7
18:3 ω 6	0.6
18:3 ω 3	4.4
20:0	0.2
20:1 ω 9	0.5
20:3 ω 6	0.3
20:4 ω 6	0.1
22:0	1.9
20:4 ω 3	-
22:1 ω 11	0.4
20:5 ω 3	0.4
21:5 ω 3	0.3
22:5 ω 6	0.01
24:0	1.2
24:1	0.03
22:6 ω 3	0.3
Total - ω 3	5.4
Total - ω 6	13.71
Total PUFA	21.11
Total saturates	43.1
Total monoenes	35.63
Total lipid per cent	4.22

The ω values represent the methyl end chain from the centre of double bond furthest from the carboxyl end.

Table 1. Mean (\pm SD) proximate composition, mineral content, energy content and C:N ratio of biofloc produce from wheat flour as a carbohydrate source

Composition (per centDM)	Biofloc
Moisture	4.1 \pm 0.10
Crude protein	35.40 \pm 0.63
Crude fiber	15.03 \pm 0.37
Crude lipid	1.1 \pm 0.26
Ash	15.38 \pm 0.35
Nitrogen free extract	28.99 \pm 0.26
Energy (kJ g ⁻¹)	18.78 \pm 0.19
C:N ratio	7 \pm 0.48
Phosphorous	1.40 \pm 0.59
Potassium	0.69 \pm 0.21
Calcium	1.78 \pm 0.90
Magnesium	0.29 \pm 0.09
Sodium	2.51 \pm 1.28
Manganese (mg kg ⁻¹)	28.12 \pm 12.01
Iron (mg kg ⁻¹)	295.158 \pm 68.58
Copper (mg kg ⁻¹)	26.84 \pm 25.14
Zinc (mg kg ⁻¹)	310.50 \pm 188.06
Boron (mg kg ⁻¹)	27.73 \pm 13.26

Nitrogen free extract = 100 – (ash + crude protein + moisture + crude lipid + crude fibre)

developed biofloc can be successfully used as fish feed particularly for herbivorous and omnivorous species.

CONCLUSION

Biosecurity is a priority in aquaculture industry. For example, in shrimp farming, considerable impact of disease outbreaks during the past two decades greatly affected the operational management of shrimp farms worldwide. This scenario forced farmers to look for more biosecure culture practices to minimize the risk associated with exposure to pathogens. Biofloc technology brings an obvious advantage of minimizing consumption and release of water, recycling *in situ* nutrients and organic matter. BFT may bring higher profit if fresh non-frozen shrimp/fish is sold to near-by market, mainly at inland locations. These advantages certainly should be more explored and niche markets achieved, contributing to social sustainability. Researchers are challenged to further develop this technique and farmers to implement it in their future aquaculture systems. Its further development, fine-tuning and implementation will need further research and development to make this technique a keystone of future sustainable aquaculture.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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