**A Major Step Towards the Sustainability in Aquaculture Practices Through Biofloc Culture Technology**

**---------------------------------------------------------------------------------------------------------------------**

**Shubham Banchhod 1, Subhalaxmi Rath 2\*, Karun Kant Yadava3, Shailendra Relekar3and Jayappa Koli 3**

1ICAR- Central Institute of Freshwater Aquaculture, Bhubaneswar-751002, Odisha, India

2College of Fisheries (OUAT), Rangeilunda-760007, Odisha, India

3College of Fisheries (Dr.BSKKV), Ratnagiri, Maharashtra (415629), India

\*Corresponding author: rathsubhalaxmi2018@gmail.com

**------------------------------------------------------------------------------------------------------------------INTRODUCTION**

 Aquaculture is considered as a fastest growing industry in the present scenario of India. It has been proved that the Indian fisheries industry (Both capture and culture) grew at an average growth rate of 4.35 %, quantity production of 2.4 million tons in 1980 to 14.1million tons in 2019-20. It has been seen that the states like Andhra Pradesh, Tamilnadu, Odisha, West Bengal contribute more than 50% of the fish production of India, But the other hand due to high demand of food with the growing population facilitates the farmers towards the intensive aquaculture practice instead of extensive or semi-intensive, which affects the sustainability of natural resources like land, water, which are the main components of aquaculture. The high thing of aquaculture expansion must be to produce further products of aquaculture without significantly adding the operation introductory natural sources of water and land (Avnimelech, 2009). In order to avoid the unavailability of natural resources in future, there is a technology has been developed which is called as the closed re-circulatory aquaculture systems (RAS). The second goal is to develop sustainable aquaculture systems that will not damage the environment (Naylor et.al 2009). The third aim is to make up systems furnishing an indifferent benefit rate to support profitable and social sustainability. All these three prerequisites for sustainable cultivation can be met by biofloc technology. The biofloc: Defined as macro-aggregates of diatoms, macro-algae, fecal pellets, exoskeleton, remains of dead organisms, bacteria, Protista and invertebrates (Debbarma, R., Biswas, P & Singh S. K. (2021). Biofloc technology is a type of RAS in which the suspended solids are managed in the culture system through the microbial community instead of removing by different process and utilises the excess nitrogen waste coming from the uneaten feed and faces of the cultured species.

**COMPOSTIONS OF BIOFLOC:**

 Mainly the biofloc is the aggregation of algae, bacteria, protozoans with some particulate organic matter like the faces and uneaten feed. Each floc held together by the mucus secreted by the bacteria and bounded by the filaments microorganisms present in it (Held by the electrostatic attraction). There is also the presence of some grazer of floc like some zooplanktons and nematodes. The size of floc in green water BFT is around 50-200 microns.

**TYPES OF BIOFLOC TECHNOLOGY-**

Mainly the biofloc technology is divided into two types.

1. **The culture unit, which is exposed to the natural light**- The outdoor lined ponds or tanks for shrimp or tilapia, lined raceways for shrimp culture in green house. There is a complete mixture of algal, bacterial process concentrated water such as green water biofloc system.

2**. The indoor culture unit, which isn’t exposed**- Closed indoor systems are operated as “Brown-Water” biofloc system, where only bacteria control the water quality.

 There is the necessity of continuous vigorous aeration to make the solids suspended in the water column otherwise the biofloc settled out of suspension and form piles that consume the nearby DO2.These anaerobic zones release the hydrogen sulphide, methane and ammonia that are highly toxic to the fish as such as shrimp culture. In the biofloc culture unit the water respiration rate is 60% more than the other traditional methods, so there is the requirement to continue the aeration system to balance the DO2 level. The solids can be removed by periodic flushing, pumping sludge from pond centre that may be the paddle wheel aerator So creating turbulence in small tanks or raceways is relatively much easier than large outdoor culture units.

**NUTRITIVE VALUE OF BIOFLOC**

 One of the major challenges facing aquaculture producers is the high cost of aquaculture feeds. Protein levels and adequate amino acid balance are critical in aquaculture feeds due to their essential role in maintaining the growth and the general wellbeing of aquatic organisms. However, these nutrients are an expensive component of the feeds and hence influence their market price. In tilapia, for example, feeding can account for 50% of the operational costs and could even reach higher levels with high-protein diets and/or inadequate protein (Hisano *et al*., 2020). However, this could be mitigated by feeding tilapia on alternative feed sources such as phytoplankton, zooplankton, and algae, whose nutritive content would enhance the growth, survival, and production of fish (Narimbi *et al.,* 2018). (Avnimelech *et al*., 2009) found that tilapia can uptake 240 mg N kg−1 of biofloc, which is equivalent to 25% of the protein in fish diets. Moreover, bioflocs can contain

Dry weight protein -25-50%

Fat content-0.5-5%

Limiting amino-acids-Methionine and lysine

Vitamins and minerals are present specially phosphorus

Thus it is providing an alternative feed source to the reared aquatic species

It’s worth noting that the nutritive value of bioflocs is mainly dependent on the microbial community that encompasses it and, as mentioned in the former section, certain factors viz. carbon sources and C:N rate influence the biochemical composition of bioflocs. Example of, (Moreno-Arias et al, 2018), reported that the fatty and amino acid composition of both biofloc and shrimp cultured in BFT systems depends on the composition of the feed used. The use of plant-based protein diets in the feed is more acceptable for biofloc systems and is considered to be sustainable and eco-friendly. This is because their use reduces the release of phosphorous and nitrogenous wastes in the aquatic ecosystem as well as the dependency on overexploited marine source (Mugwanya *et al*., 2021).

Presently, further studies in the field of probiotic and prebiotic bioflocs are going on. Probiotics are salutary microbes that are moreover added or naturally developed in the BFT system to stimulate the immunity for the reared species against biotic and abiotic stress. Several salutary microorganisms, such as those from the Bacillaceae family, have been earlier identified and isolated from the shrimp culture BFT system (Ferreira *et al*., 2017).

**MICROORGANISMS IN BIOFLOC CULTURE SYSTEMS**

 Microorganisms are an important member of any aquaculture system. The microorganism in the BFT (Biofloc Technology) system are also crucial, diverse and therefore have different roles. The significance of biofloc organisms (BFOs) in BFT, the factors impacting their population compositions, the impact of BFOs on water quality, and uses as food source for cultivated aquatic species. Common in the BFT system generally include photoautotrophic (e.g. microalgae), chemoautotrophic (e.g. nitrifying bacteria), and heterotrophic organisms, including fungi, protozoans, ciliates, and zooplankton (e.g. rotifers, copepods, and nematodes). Factors such as salinity, carbon source type, carbon-nitrogen ratio, aeration, light, stocking density, and total suspended solids act on the density, quality and diversity of BFOs (Khanjani *et al.,* 2022). Various microorganism shows different functional characteristics and perform three main functions: (i) help to improve the water quality by removing inorganic nitrogen compounds (bioaccumulation, bio-assimilation, nitrification, and de-nitrification); (ii) function as a supplementary food source, and (iii) create probiotic properties; key roles for any aquatic farming system.The heterotrophic bacteria, which is the nitrogen conversion agent fascinates like other nitrogen conversion mechanisms such as nitrites (Ekasari, 2014) take Floc as substrates and help in Sludge degradation (Debbarma, R., Biswas, P & Singh S. K. (2021), the heterotrophic bacteria involve in the nutrient recycling by taking inorganic phosphorus (Kirhman, 1994) which reduces the discharged phosphorus with enhancing the availability of nutrients. Come to phototropic, they help in nitrogen uptake (Khanjani *et al*., 2022), and de-nitrifications (It depends on the environmental conditions).

 **MONITORING & CONTROL OF THE GROWTH OF BIOFLOC DEVELOPMENT:**

 The proportion and predominance of microorganisms helps in interaction of different biotic and abiotic factors, exhibiting an ecological succession over time. Practical way for monitoring of this bio floc system is by colour of the medium. First stage is predominantly photoautotrophic (up to 3 weeks) after addition of carbohydrate Heterotrophic dominance occurs. 6–8 weeks after the stabilization, nitrifying bacteria also found in that biofloc system (Debbarma, R., Biswas, P & Singh S. K. 2021).

**THE SPECIES, WHICH CAN BE CULTURED IN BFT:**

 The probiotic effect of bioflocs has been demonstrated, either in situ or added into the diets of various aquatic animals in BFT systems, enhancing their growth performance and wellbeing. The most suitable aquaculture species that can benefit from these probiotic effects on aquaculture practice include omnivorous animals with a high ability to consume flocs and tolerate high levels of suspended solids. Thus, tilapia (*O. niloticus*) (Luo *et al*., 2014) and white shrimp (Xu & Pan, 2014) are the most typical animals suitable for BFT systems. However, other aquacultural animals have been successfully cultivated in biofloc systems in recent years, including *Macrobrachium rosenbergii*, common carp, African catfish, Golden crucian carp (*Carassius auratus*) and Indian major carp, among others. so it is proved that there is a possibility to culture some species, those who can tolerate some suspended solids in the water column with zero water exchange.

**CARBON–NITROGEN RATIO:**

 In the aquatic environment, the carbon–nitrogen ratio plays a important role in the curb and conversion of toxic inorganic N compounds into beneficial microbial biomass that might act as a source of food for the reared species. C/N ratio can be achieved through modification of the carbohydrate content in the feed or the addition of an external carbon source in the rearing water so that microbes can assimilate waste ammonium for microbial biomass production (Azaduzzaman *et al.,* 2010) recommended that the Increasing the C/N ratio from 10 to 20 significantly increased the bio volume of [phytoplankton](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/phytoplankton), crustaceans and rotifers in the water column by 15%, 6% and 11%, respectively. The bio volume of periphyton was 50% higher in treatment CN20 compared to treatment CN10. Increasing the C/N ratio from 10 to 20 raised the bio volume of total heterotrophic bacteria (THB) in the water column (70%), deposition (36%) and periphyton (40%). (Pérez-Fuentes et al.2016) setup that, under high-density cultivation of *O. niloticus* in a BFT system, C/N ratios exceeding 15:1 increases the production of dissolved salts and settled biomass, which directly impacted the growth performance of fish. The authors recommended a C/N ratio of 10:1 as the optimum condition for the production of *O. niloticus* reared under similar conditions. In another study, (Silva *et al*., 2017) also found poor water quality (high TSS, turbidity, alkalinity, and settleable solids) at a C/N ratio of 20:1, which affected the growth performance of *O. niloticus*. Similar results have been reported in *Clarias gariepinus*. However, (Yu *et al*., 2020, Haghparast *et al.,* 2020, and Wang *et al*., 2015) reported better growth performance and immunity in carp at high C/N ratios (20:1 and/or 25:1) cultured in BFT. The discrepancy in results could be attributed to the difference in species and the source of organic carbon. C: N ratio from 10 to 20 is recommended for biofloc based system according to species preferences. The thing of the present exploration is to quantify the single and combined effects of C: N ratio manipulation and substrate addition on fish production and sustainability in aquaculture.

The efficient establishment of flocs by different carbon sources mainly depends on their carbon content and speed of degradation, it would say that certain carbon sources are more efficient in promoting floc formation than others. Generally, simple sugars such as molasses are degraded faster than complex sugars such as cassava starch, leading to improved water quality, as indicated by lower concentrations of ammonia and a higher growth rate of beneficial microbial biomass (El-Sayed *et al*., 2021). Khanjani *et al.,* (2021a) found that simple sources of carbon (starch, molasses) led to advance densities of heterotrophic bacteria compared to complex sources (barley, flour, corn). Molasses are the most extensively used carbon sources in BFT systems during larval, nursery, and grow-out stages due to their effectiveness in improving water quality for the sustainable production of aquatic species. (Mugwanya *et al*., 2021).

Factors determining carbon addition are

* Protein level present in feed
* Ease of availability and cost
* Carbon content of the source
* Production efficiency

Eg. 1.49g of sucrose can produce 1g of floc

**CONCLUSION**

 From the above descriptions it can be concluded that BFT can be an environment friendly culture practice which can be a major step towards maintain the concept of sustainability. Despite the increasing admiration of BFT systems, further exploration is demanded to optimize the functional parameters (such as energy requirements) with lower costs of product for the system in order to insure that they’re practicable and acceptable for small-scale aqua culturist and those from developing countries. Energy alternatives such as solar power, gas, and wind turbines should be considered. Moreover, for this system to produce a further sustainable impact in production, further studies on forbearance situations regarding the water quality of cultivable species reared in BFT under marketable settings are required since reference situations used for the product of certain species are deduced from water exchange or clear water systems.

**REFERENCES**

1. Asaduzzaman, M., Rahman, M. M., Azim, M. E., Islam, M. A., Wahab, M. A., Verdegem, M. C. J., & Verreth, J. A. J., Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds. *Aquaculture*, *306*(1- 4) ,2010, pp 127-136.
2. Avnimelech, Y.; Kochba, M. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using 15N tracing. *Aquaculture* **2009**, *287*, pp 163–168.
3. Avnimelech, Y. *Biofloc technology: a practical guidebook*. World Aquaculture Society.,2009.
4. Debbarma R , Biswas P & Singh S. K. An integrated biomarker approach to assess the welfare status of Ompok bimaculatus (Pabda) in biofloc system with altered C/N ratio and subjected to acute ammonia stress. *Aquaculture*, *545*, 2021, pp.737184.
5. Ekasari, J. *Biofloc technology as an integral approach to enhance production and ecological performance of aquaculture* (Doctoral dissertation, Ghent University), 2014.
6. El-Sayed, A.F.M. Use of biofloc technology in shrimp aquaculture: A comprehensive review, with emphasis on the last decade. *Rev. Aquac.* **2021**, *13*, pp 676–705.
7. Ferreira, M.G.; Melo, F.; Lima, J.V.; Andrade, H.A.; Severi, W.; Correia, E.S. Bioremediation and biocontrol of commercial probiotic in marine shrimp culture with biofloc. *Lat. Am. J. Aquat. Res.* **2017**, *45*, pp 167–176.
8. Hisano, H.; Parisi, J.; Cardoso, I.L.; Ferri, G.H. and Ferreira, M.F. Dietary protein reduction for Nile tilapia fingerlings reared in biofloc technology. *J. World Aquac. Soc.* **2020**, *51*,pp 452–462.
9. Haghparast, M.M.; Alishahi, M.; Ghorbanpour, M.; Shahriari, A. Evaluation of hemato-immunological parameters and stress indicators of common carp (*Cyprinus carpio*) in different C/N ratio of biofloc system. *Aquac. Int.* **2020**, *28*, pp 2191–2206.
10. Kirchman, D. L. The uptake of inorganic nutrients by heterotrophic bacteria. *Microbial Ecology*, *28*, 1994,pp 255-271.
11. Khanjani, M. H., Mohammadi, A., & Emerenciano, M. G. C. Microorganisms in biofloc aquaculture system. *Aquaculture Reports*, *26*,2022, 101-103.
12. Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L., & Tan, H. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (Oreochromis niloticus) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture*,2014, *422*, pp 1-7.
13. Mugwanya, M., Dawood, M. A., Kimera, F., & Sewilam, H. Biofloc systems for sustainable production of economically important aquatic species: A review. *Sustainability*, *13*(13),2021, 7255.
14. Moreno-Arias, A.; López-Elías, J.A.; Martínez-Córdova, L.R.; Ramírez-Suárez, J.C.; Carvallo-Ruiz, M.G.; García-Sánchez, G.; Lugo- Sánchez, M.E.; Miranda-Baeza, A. Effect of fishmeal replacement with a vegetable protein mixture on the amino acid and fatty acid profiles of diets, biofloc and shrimp cultured in BFT system. *Aquaculture* **2018**, *48,* pp 53–62.
15. Narimbi, J. Mazumder, D.; Sammut, J. Stable isotope analysis to quantify contributions of supplementary feed in Nile Tilapia *Oreochromis niloticus* (GIFT strain) aquaculture. *Aquac. Res.* **2018**, *49*, pp 1866–1874.
16. Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C., Clay, J. & Troell, M. Effect of aquaculture on world fish supplies. *Nature*, *405*(6790), 2000, pp 1017-1024.
17. Pérez-Fuentes, J.A.; Hernández-Vergara, M.; Pérez-Rostro, C.I.; Fogel, I. C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture* **2016**, *452*,pp 247–251.
18. Silva, U.L.; Falcon, D.R.; Pessôa, M.N.D.C.; Correia, E.D.S. Carbon sources and C:N ratios on water quality for Nile tilapia farming in biofloc system. *Rev. Caatinga* **2017**, *30*, pp 1017–1027.
19. Wang, G.; Yu, E.; Xie, J.; Yu, D.; Li, Z.; Luo, W.; Qiu, L.; Zheng, Z. Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. *Aquaculture* **2015**, *443*,pp 98–104.
20. Xu, W. J., & Pan, L. Q. Dietary protein level and C/N ratio manipulation in zero‐exchange culture of Litopenaeus vannamei: Evaluation of inorganic nitrogen control, biofloc composition and shrimp performance. *Aquaculture Research*, *45*(11),2014, pp 1842- 1851.
21. Yu, Z.; Li, L.; Zhu, R.; Li, M.; Duan, J.; Wang, J.Y.; Liu, Y.H.; Wu, L.F. Monitoring of growth, digestive enzyme activity, immune response and water quality parameters of Golden crucian carp (*Carassius auratus*) in zero-water exchange tanks of biofloc systems. *Aquac. Rep.* **2020**, *16*, 100283.