



# Role of Biofloc on Immuno Modulatory Responses of Cultured Aquatic Organisms

**Babitha Rani AM\***

ICAR-Central Institute of Fisheries Education, India

**\*Corresponding author:** Babitha Rani AM, ICAR-Central Institute of Fisheries Education, Rohtak Centre, Haryana, India, Email: babishibu@gmail.com

## Editorial

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**Abbreviations:** BFT: Biofloc Technology; CBC: Complete Blood Count; RAS: Ras-Related Nuclear Protein Gene; SP: Serine Proteinase Gene

## Editorial

Intensification in aquaculture is the need of the hour to meet demand of protein by the population which is increasing at an exponential rate. This intensification will lead to a threat to biosecurity and environment and antibiotics and chemotherapeutants will lead to resistant microbes or super bugs. At this point, biofloc can be a novel strategy for disease management from the perspective of antibiotic, probiotic and prebiotic application. Biofloc Technology (BFT) is a zero-water exchange technique which recycle the waste in the culture water by application of external carbon source [1]. In this method, the heterotrophic bacterial growth is promoted along with other microbes in the culture water [2]. Ammonia and its metabolites accumulate in the culture system from feed and fecal waste is assimilated by these heterotrophs and form biofloc which is nothing but microbial protein [3,4]. The microbes are capable of flocculating in the culture water and they utilize the organic substrate which include both carbon and nitrogen and they multiply in huge number in the system [5].

The effective formation of biofloc in plenty and also healthy enough to control the water quality parameters in the system. It needs to maintain the carbon: nitrogen ratio in the system with the help of external addition of carbon sources which include, molasses, jaggery, sugar, glucose, wheat flour etc., [6,7] and it is possible to obtain a significant enhancement of bacterial growth and of the fixation of toxic nitrogen metabolites [3,8-10]. The biofloc system keeps on accumulating nitrite and nitrate and they also need to

be controlled by sufficient addition of carbon source. The technology is efficient and environmentally friendly as the nutrients are recycled and reused within the system itself [11].

The advantages of the technology in aquaculture were delineated as economic and environmental sustainability, biosecurity, enhanced yield etc. [12-14]. It supports nitrogen removal even when organic matter load and biochemical oxygen demand of the system is high [9]. A few studies investigated the potential of biofloc technology in boosting immunity against the pathogens of cultured animals, though most commercially available immunostimulants are derived from the microorganism, their cell component, and their metabolites.

Biofloc as microbial aggregates normally provide proteins and nutrients to the cultured organism which in turn are well assimilated by them Avnimelech Y, et al. [9]. The microbial aggregation offers protection and stability to the flocculating organism and they will utilize the organic matter as substrate and hence have direct utilization of nutrients [4]. The biofloc not only contain bacteria, it also has algae, protozoa, detritus, dead organic particles which develop when provided with suitable aeration and sufficient amount of carbon source in the system.

Microorganisms and their cell components have been studied and applied as probiotics or immunostimulants in order to improve the innate immunity and antioxidant status of shrimp, thereby enhancing their disease resistance [15,16]. Kim SK, et al. [17] examined the effect of biofloc on growth and immune response in *L. vannamei*. The biofloc assimilate nutrient, and it colonize the gut and improve nutrient utilization by the cultured organism and hence

support overall growth and well-being of the aquatic animal. Biofloc system contains abundant number of bacteria of which cell wall consists of various components such as bacterial lipopolysaccharide, peptidoglycan and  $\beta$ -1,3-glucans, and is known as stimulating non-specific immune activity of shrimp.

Ekasari J, et al. [18] demonstrated that the application of biofloc technology leads to immune stimulation in shrimp and the actual effect depends on the type of carbon source and the amount applied in the system. Chen J, et al. [19] reported that dietary inclusion of biofloc in sea cucumber *A. japonicus* has enhanced innate immunity and disease resistance. Biocontrol effects of biofloc may be attributed to their capability to accumulate the bacterial storage compound poly- $\beta$ -hydroxybutyrate (PHB) which is reported to be having protective ability against bacterial diseases [4,20]. PHB levels in bioflocs was reported to be in the range of 0.5 and 18% of the dry matter [10]. The previous report also indicated that sufficient PHB level is present in biofloc to protect aquatic organisms under culture from disease causing pathogens [21,22]. and Anguilera-Rivera D, et al. [23] suggested that the microbial flocs can improve innate immunity in shrimp and fish as they contain carotenoids, retinoids, PHB and exo-enzymes. It is common practice in aquaculture that microbes are used as immunostimulants to enhance immunity and disease resistance in cultured organisms [24]. The biofloc ingested by the shrimp may release substances in the gastrointestinal tract that could lead to release of more haemocytes into the circulation. AHPNS (acute hepatopancreatic necrosis syndrome), with high mortality rate in shrimp was comparatively reduced when cultured with biofloc. However, the effect of innate immune system of shrimp are complicated and further study is required for accumulating more detailed knowledge.

Since biofloc technology accumulates immunostimulatory compounds exhibiting probiotic effects, immunomodulatory effects of biofloc are under serious investigations. Phenomenon like quorum sensing and molecular signaling mediated communications became a novel strategy to regulate pathogenic strains in the culture system. It is observed that biofloc in general contain more than 2000 strains of bacteria [25]. Xu, et al. recounted that biofloc played an active role in improving haematological parameters like complete blood count (CBC) and phagocytic activity of shrimp. Recently, significantly increased expression of CXC-chemokines, CC-chemokines, IL-8, and TLR7 genes were found in molasses-based biofloc groups. Menaga M, et al. [26] affirmed the importance of in situ biofloc technology in boosting the immune response of GIFT Tilapia with its upregulated immune gene expression viz., Metallothionein gene, Cathepsin L, Toll-like receptor 7, Interleukin 1 $\beta$ , TNF $\alpha$  and its role as an antimicrobial agent against *Aeromonas*

hydrophila. Panigrahi A, et al. [27] demonstrated that with optimum C/N ratio, the bacterial community and its composition can be customized for water quality control and management of health status of shrimp. They further reported four upregulated immune genes, i.e., Ras-related nuclear protein gene (RAS), serine proteinase gene (SP), prophenoloxidase Activating Enzyme (PPAE), and crustin in shrimp when cultured in biofloc. Immune stimulation may thus be an essential feature of a biofloc based culture system contributing to disease control.

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