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Biofloc technology in aquaculture

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Abstract
Biofloc is a conglomeric aggregation of microbial communities such as phytoplankton, bacteria, and living and dead particulate organic matter. Biofloc technology involves manipulation of C/N ratio to convert toxic nitrogenous wastes into the useful microbial protein and helps in improving water quality under a zero water exchange system. It may act as a complete source of nutrition for aquatic organisms, along with some bioactive compounds that will enhance growth, survival, and defence mechanisms. Nutritionally, the floc biomass provides a complete source of nutrition as well as various bioactive compounds that are useful for improving the overall welfare indicators of aquatic organisms. Beneficial microbial bacterial floc and its derivative compounds such as organic acids, poly-hydroxy acetate and poly-hydroxy butyrate serve as a natural probiotic and immunostimulant. The biofloc technology is useful in maintaining optimum water quality parameters under a zero water exchange system, which prevents eutrophication and effluent discharge into the surrounding environment. Moreover, the technology will be useful to ensure bio security, as there is no water exchange except sludge removal. The technology is economically viable, environmentally sustainable, and socially acceptable.

Keywords: Biofloc; sustainability; carbon source; C: N ratio

1. Introduction
Aquaculture as a food-producing sector offers ample opportunities to alleviate poverty, hunger and malnutrition, generates economic growth and ensures better use of natural resources (Food and Agriculture Organization, 2017) [16]. The demand for aquatic food continues to increase and hence, expansion and intensification of aquaculture production are highly required. The main goal of aquaculture expansion must be to produce more aquaculture products without significantly increasing the usage of the basic natural resources of water and land (Avnimelech, 2009) [5]. The second goal is to develop sustainable aquaculture systems that will not damage the environment (Naylor *et al*., 2000) [29]. The third goal is to build up systems providing an equitable cost/benefit ratio to support economic and social sustainability (Avnimelech, 2009) [5]. All these three prerequisites for sustainable aquaculture development can be met by BFT or biofloc technology.

Biofloc technology (BFT) is an aquaculture system which focuses on a more efficient use of nutrient inputs with limited or zero water exchange. 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) [3]. The microbial biomass will further aggregate with other microorganisms and particles suspended in the water forming what has been called "biofloc", which eventually can be consumed in situ by the cultured animals or harvested and processed as a feed ingredient (Avnimelech 1999; Avnimelech 2007; Kuhn *et al*. 2009; Kuhn *et al*. 2010) [3, 4, 23, 24]. BFT is considered as a promising system for sustainable and environmentally friendly aquaculture system, and has been applied both at laboratory and commercial scale for various aquaculture species such as tilapia (Azim & Little 2008) [6], shrimp (Taw 2010) [35], sturgeon and snook (Serfling 2006) [33]. Biofloc technology is mainly based on the principle of waste nutrients recycling (particularly nitrogen) into microbial biomass that can be used in situ by the cultured animals or be harvested and processed into feed ingredients (Avnimelech, 2009; Kuhn *et al*., 2010) [5, 24]. The growth of heterotrophic micro biota is stimulated by steering the C/N ratio in the water through the modification of the carbohydrate content in the feed or by the addition of an external carbon source in the water (Avnimelech, 1999) [3], so that the bacteria can assimilate the waste ammonium for new biomass production. Hence, ammonium/ammonia can be maintained at a low and non-toxic concentration so that water replacement is no longer required.

2. Biofloc technology
If carbon and nitrogen are well balanced in the system, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass (Schneider *et al*., 2005) [32].

By adding carbohydrates to the pond, heterotrophic bacterial growth is stimulated and nitrogen uptake through the production of microbial proteins takes place (Avnimelech, 1999) [3]. Biofloc technology is a technique of enhancing water quality through the addition of extra carbon to the aquaculture system, through an external carbon source or elevated carbon content of the feed. This promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves, 2006). The suspended growth in ponds consists of phytoplankton, bacteria, aggregates of living and dead particulate organic matter, and grazers of the bacteria (Hargreaves, 2006) [17]. Typical flocs are irregular by shape, have a broad distribution of particle size, are fine, easily compressible, highly porous (up to more than 99% porosity) and are permeable to fluids (Chu and Lee, 2004) [8]. The biofloc technology makes it possible to minimize water exchange and water usage in aquaculture systems by maintaining adequate water quality within the culture unit, while producing low cost bioflocs rich in protein, which in turn can serve as a feed for aquatic organisms (Crab, 2010) [9]. As compared to the conventional water treatment technologies used in Aquaculture, biofloc technology provides a more economical alternative (decrease of water treatment expenses up to 30%), and additionally, a potential gain on feed expenses (the efficiency of protein utilization is twice as high in biofloc technology systems when compared to conventional ponds), making it a low-cost sustainable constituent to future aquaculture development (Avnimelech, 2009) [5]. Biofloc technology is a robust & economical technique which is also easy in operation. One important aspect of the technology to consider is the high concentration of total suspended solids present in the pond water. Suitable aeration and mixing needs to be sustained in order to keep particles in suspension and intervention through either water exchange or drainage of sludge might be needed when suspended solids concentrations become too high (Avnimelech, 2009) [5]. Although it is a critical aspect of biofloc technology, detailed knowledge about selection and placement of aerators is still insufficient.

The strength of the biofloc technology lies in its ‘cradle to cradle’ concept as described by McDonough and Braungart (2002) [27], in which the term waste in fact does not exist. Translated in biofloc terms, ‘waste’ nitrogen generated by uneaten feed and excreta from the cultured organisms is converted into proteinaceous feed available for the same organisms. Instead of ‘downcycling’, a phenomenon often found in an attempt to recycle, the technique actually ‘upcycles’ through closing the nutrient loop. Hence, the water exchange can be decreased without deterioration of water quality and, consequently, the total amount of nutrients discharged into adjacent water bodies may be decreased (Lezama-Cervantes and Paniagua-Michel, 2010) [26]. In this context, biofloc technology can also be used in the specific case of maintaining appropriate water temperature, good water quality and high fish survival in low/no water exchange, greenhouse ponds to overcome periods of lower temperature during winter. Besides winter periods, it is important to be aware of the fact that future impacts of climate change on fisheries and aquaculture are still poorly understood and colder periods might be a grave issue to deal with in the future. The key to minimizing possible negative impacts of climate change on aquaculture and maximizing opportunities will be through understanding and promoting a wide range of inventive adaptive new technologies, such as the biofloc technology combined with greenhouse ponds.

2.1 Carbon- Nitrogen Ratio

Carbon-nitrogen ratio (C/N) in the aquatic environment plays an important role in the immobilization of toxic inorganic nitrogen compounds into useful bacterial cells (single-cell protein) that may act as a direct source of food for the cultured organisms (Avnimelech 1999) [3]. Immobilization of inorganic nitrogen takes place when the C/N ratio of the organic matter is higher than 10 (Lancelot and Billen 1985) [25]. Thus, alteration in the C/N ratio may result in a shift from an autotrophic to a heterotrophic system (Avnimelech 1999) [3]. Once a mature biofloc community is established, TAN and NO_2-N concentrations can be effectively controlled by either heterotrophic assimilation or autotrophic nitrification that helps to maintain their concentrations at acceptable ranges for the cultured organisms even at higher stocking densities (Xu *et al*. 2016) [37]. By adding a carbon source (direct or indirect C-sources) to the culture medium in limited-discharge systems, thus changing C/N ratio, it is possible to obtain a significant enhancement of useful microbial growth and the fixation of toxic nitrogen metabolites (Crab *et al*. 2010) [9]. The BFT being zero water exchange system thus tends to accumulate the nitrate in the long run, and hence usually the nitrate level in biofloc systems increases as the culture progresses. Kuhn *et al*. (2009) [23] observed that carbon supplementation enhanced the removal rates of TAN at 26% per hour compared to 1% per hour in a control system. The C/N ratio of around 10 is maintained in most of the feeds used in semi-intensive aquaculture ponds. The C/N ratio in an aquaculture system can be increased by adding different locally available cheap carbon sources (agricultural by-products) and also by the reduction of protein content in the feed (Avnimelech 1999; Hargreaves 2006) [3, 17]. Different organic carbon sources (like glucose, cassava, molasses, wheat, corn, sugar bagasse, sorghum meal, etc.) are used to enhance production and to improve the nutrient dynamics through altered C/N ratio in shrimp culture (Avnimelech 1999) [3], and C/N ratio is also widely used as a guide for analyzing the decomposition of organic matter. The biofloc system maintained with C/N ratio of higher than 15–20 develops sufficient microbial floc to assimilate toxic nitrogenous species under intensive farming with limited discharge.

2.2 Microbial community in Bioflocs

There are two functional categories of bacterial populations primarily responsible for water quality maintenance in minimal or zero water exchange systems (intensive systems) *viz*., heterotrophic ammonia-assimilative and chemoautotrophic nitrifying bacteria (Hargreaves 2006) [17]. The colour changes from green to brown which takes place as the culture progresses due to the transition from a mostly algal-dominant to a bacterial biofloc-dominant system. The number of bacteria in biofloc ponds can be between 10^6 and 10^9/ml of floc plug which contains between 10 and 30 mg dry matter making the pond a biotechnological industry (Avnimelech 2007) [4]. The microbial communities formed consist of phytoplankton, bacteria, and aggregates of living and dead particulate organic matter (Hargreaves 2006) [17]. According to Ju *et al*. [21], bioflocs collected from *Litopenaeus vannamei* tanks contained 24.6% phytoplankton (dominated by diatoms like *Thalassiosira*, *Chaetoceros*, and *Navicula*), 3% bacterial biomass (two thirds was gram-negative and one third gram-positive), a small amount of protozoan community (98% flagellates, 1.5% rotifers, and 0.5% amoeba), and 33.2% detritus, and the remaining quantity was ash (39.25%). Only

2–20% of the organic fraction of sludge flocs is believed to be living (microbial cells) while the rest is of total organic matter (60–70%) and total inorganic matter (30–40%) (Wilen *et al*. 2003) [36]. Dominant bacterial species that are present in the bioflocs include Proteobacterium, *Bacillus* species, and Actinobacterium. Besides this, there are some other minor bacterial species such as *Roseobacter* sp. and *Cytophaga* sp. (Zhao *et al*. 2012) [39].

2.3 Nutritional composition of Bioflocs

Nutritionally, the floc biomass could provide a complete source of nutrition as well as various bioactive compounds (Akiyama *et al*. 1992) [1]. The nutritional value of bioflocs depends on several factors such as food preferences by the animal, their ability to ingest and digest microbial protein, and the floc density in the water (Hargreaves 2006) [17]. The single-cell protein formed by heterotrophic bacterial population through uptake of inorganic N can be utilized as a source of food for cultured animals like shrimps, tilapia, and carps (Rahmatullah and Beveridge 1993) [31]. In terms of quality, biofloc contains 38% protein, 3% lipid, 6% fiber, 12% ash, and 19 kJ/g energy (on dry matter basis) (Azim and Little 2008) [6]. Azim and Little 2008 [6] observed 50% crude protein, 2.5% crude lipid, 4% fiber, 7% ash, and 22 kJ g^{-1} energy and reported that the quality of biofloc is independent of the quality of feed used for biofloc production (35 and 22% crude protein). However, Ballester *et al*. (2010) [7] reported that bioflocs contain 30.4% crude protein, 4.7% crude lipid, 8.3% fiber, 39.2% ash, and 29.1% nitrogen free extract on dry matter basis when wheat bran and molasses were used as carbohydrates sources. Thus, the change in the carbon source changes the nutritional composition and quality index of the flocs. Besides these characteristics, the type of carbon source also influences the palatability and digestibility of the cultured organisms (Crab 2010 Crab *et al*. 2009) [9, 10]. Overall, bioflocs produced on glycerol give the best results (Crab 2010) [9].

3. Application of Biofloc technology in Aquaculture

The Biofloc technology enhances the production and productivity in fish culture, by its contribution to the supply of good quality fish juveniles, the latter being one of the most important inputs in the production systems. Biofloc technology could support the supply of good quality seeds by improving the reproductive performance of aquaculture animals and by enhancing the larval immunity and robustness (Emerenciano *et al*., 2013) [13]. The application of biofloc technology in grow out systems of some aquaculture species could improve net productivity by 8–43%, relative to the non-biofloc control (traditional with water exchange, clear water system or recirculating aquaculture system) (Ekasari, 2014) [11].

Biofloc technology (BFT) has been successfully implemented in aquaculture especially shrimp farming owing to economical, environmental, and marketing advantages over the conventional culture system. Compared to conventional aquaculture techniques, biofloc technology provides a more economical alternative and sustainable technique in terms of minimal water exchange and reduced feed input making it a low-cost sustainable technology for sustainable future aquaculture development (Avnimelech 2009) [5]. Several studies were performed on the use of bioflocs as an in situ produced feed and they indicate that bioflocs can be taken up by aquaculture species and uptake depends on the species and feeding traits, animal size, floc size and floc density (Avnimelech, 2009; Crab, 2010) [5, 9]. *In situ* utilization of microbial flocs generated in biofloc systems by some aquaculture organisms as well as the utilization of processed bioflocs as a feed ingredient has been well documented (Kuhn *et al*., 2009, 2010;) [23, 24]. Ju *et al*. (2008) [21] demonstrated that the concentrations of free amino acids such as alanine, glutamate, arginine and glycine, which are known attractants in shrimp diet (Nunes *et al*., 2006) are present in bioflocs. Levels in bioflocs were found to be comparable to that of the shrimp commercial diet suggesting that bioflocs are likely to be recognized as food particles by some aquaculture organisms. Xu and Pan (2012) [38] found that bioflocs or its attached microorganisms could exert a positive effect on the digestive enzyme activity of shrimp. Inclusion of bioflocs in the diet at BFT 75% results in improved growth performances and digestive enzyme activity of the common carp (Najdegerami *et al*. 2016) [28]; also biofloc as a dietary supplement at a 4% level in shrimp feed can enhance the growth and digestive enzyme activities in *P. monodon* (Anand *et al*. 2014) [2]. Furthermore, biofloc technology application in larviculture (at least to some species which can handle particles in suspension) may provide easily accessible food source for the larvae outside the regular feeding moments, thus minimizing possible negative social interaction during feeding (Ekasari *et al*., 2015) [12].

According to Tacon *et al*., [34] biofloc enhances ingestion rate, nutrient absorption, and assimilation, and provides a complete source of cellular nutrition. Broodstock diets fortified with biofloc supplementation improve reproductive performance in terms of fecundity, spawning, and egg biochemical composition in *Farfantepenaeus duorarum* and L. *vannamei* (Emerenciano *et al*. 2014) [14]. Bioflocs have been recently projected as a possible novel strategy for disease management with the “natural probiotic effect” in contrast to conventional approaches such as antibiotic, antifungal, and external probiotic and prebiotic application (Emerenciano *et al*. 2013) [13]. Tilapia culture in activated suspension ponds indicated that the fish grew well on low-protein feed (Avnimelech 1999) [3]. Jesús Becerra-Dorame *et al*. (2014) [19] reported that *L. vannamei* reared in biofloc-based systems showed improved physiological performance as indicated by selected hemolymph parameters including superoxide dismutase activity. Most probably, some active microorganisms enter a shrimp body continuously along with the process of ingesting biofloc (Johnson *et al*. 2008) [20] and then modulate the immune system of the host whether as viable microbes or microbial components (Jang *et al*. 2011) [18].

No technique is without drawbacks and also biofloc technique is prone to obstacles. A major obstacle is to convince farmers to implement the technique, since the concept of biofloc technology goes in against common wisdom that water in the pond has to be clear (Avnimelech, 2009) [5]. On the other hand, several factors promote the implementation of the technique. Firstly, water has become scarce or expensive to an extent of limiting aquaculture development. Secondly, the release of polluted effluents into the environment is prohibited in most countries. Thirdly, severe outbreaks of infectious diseases led to more stringent biosecurity measures, such as reducing water exchange rates (Avnimelech, 2009) [5]. Experience regarding biofloc technology and technical knowledge about the technique needs to be transferred to the farmers in a clear, practical and straightforward way, not forgetting to emphasize the economic benefits of this technique.

4. Conclusion

As the human population continues to grow, food production industries such as Aquaculture will need to expand as well. In order to preserve the environment and the natural resources, this expansion will need to take place in a sustainable way. Biofloc technology enhances water quality in aquaculture by balancing carbon and nitrogen in the system. A variety of beneficial features have been ascribed to biofloc technology, from water quality control to in situ feed production and others. Biofloc technology offers aquaculture a sustainable tool to simultaneously address its environmental, social and economical issues concurrent with its growth. This technology could result in higher productivity with less impact to the environment. Furthermore, biofloc systems may be developed and performed in integration with other food production systems; thus promoting productive integrated systems aiming at producing more food and feed from the same area of land with fewer input. Researchers are challenged to further develop this technique and farmers to implement it in their future aquaculture systems. The further development, fine-tuning and implementation of this technology will need further research and development from the present and future generation of researchers, farmers and consumers to make this technique a keystone of future sustainable aquaculture.

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