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Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition

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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 defense mechanisms, and acts as a novel approach for health management in aquaculture by stimulating innate immune system of animals. 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, polyhydroxy acetate and polyhydroxy butyrate, could resist the growth of other pathogens, thus serves as a natural probiotic and immunostimulant. The technology is useful in maintaining optimum water quality parameters under a zero water exchange system, thus prevents eutrophication and effluent discharge into the surrounding environment. Moreover, the technology will be useful to ensure biosecurity, as there is no water exchange except sludge removal. The technology is economically viable, environmentally sustainable, and socially acceptable.

Keywords Bioflocs · C/N ratio · Immunostimulation · Nutritional composition · Carbon sources · Biosecurity · Zero water exchange systems · Sustainability

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Introduction

To support the rapidly growing human population, food production industries such as aquaculture also needs horizontal as well as vertical expansion. The rapid growth of global aquaculture industry cannot be over emphasized because environmental and economical limitations can hamper this growth. Intensification of the aquaculture activities generates an immense amount of excess organic pollutants that are likely to cause acute toxic effects and long-run environmental risks (Piedrahita 2003). The foremost common method of dealing with this problem has been the utilization of continuous replacement of the pond water through the exchange (Gutierrez-Wing and Malone 2006). The water volume required for even small to medium culture systems can reach up to several hundreds of cubic meters per day. Parenthetically 20 m^3 of water is required for the production of 1 kg shrimp (Wang 2003). A recirculating aquaculture system (RAS) is another approach for the removal of major toxic pollutants from the culture water without causing environmental concerns (Gutierrez-Wing and Malone 2006). The beneficial effect of this technology is that only 10% of the total water volume is needed to be replaced on a daily basis (Twarowska et al. 1997), but due to the high operational and maintenance cost, the adoption of RAS among the farming community especially in developing countries is low. Therefore, there was a wide search for years for a low cost, sustainable, and environment-friendly technology for large-scale adoption. Biofloc technology has gained attentions recently as a sustainable and eco-friendly method of aquaculture which controls water quality, along with the production of value added microbial proteinaceous feed for the aquatic organisms. The use of BFT systems in the marine shrimp aquaculture has been extensively studied (Emerenciano et al. 2011, 2012b, 2013b; Ray et al. 2011; Xu and Pan 2012; da Silva et al. 2013; Schveitzer et al. 2013; de Souza et al. 2014; Kumar et al. 2014). The technology is cost-effective and environment-friendly and supports sustainable aquaculture (Naylor et al. 2000; Avnimelech and Kochba 2009). The different aspects of such a system can be summarized under the following headings.

I. Biofloc technology
II. Carbon-nitrogen ratio
III. Microbial community in bioflocs
IV. Nutritional composition of bioflocs
V. Biofloc as a dietary stimulant
VI. Immuno-physiological response by bioflocs
VII. Bioflocs in aquaculture

Biofloc technology

Biofloc technology (BFT) is based on the maintenance of high levels of microbial bacterial floc in suspension using constant aeration and addition of carbohydrates to allow aerobic decomposition of the organic material (Avnimelech and Weber 1986). By adding carbohydrates, heterotrophic bacterial growth is stimulated and production of microbial proteins takes place through nitrogen uptake (Avnimelech 1999). Maintaining the C/N ratio in the aquaculture system, through the external addition of carbon source or elevated carbon level in the feed, water quality can be improved along with the production of high-quality single-cell microbial protein (Crab et al. 2012). Under such conditions, dense microorganisms develop, functioning both as bioreactor controlling water quality (Avnimelech et al. 1989) and also acts as protein food source for the fishes and shrimps (Avnimelech et al. 1994). Immobilization of toxic nitrogen species occurs much more rapidly in bioflocs because the growth rate and microbial biomass production per unit substrate of heterotrophs are 10 times higher than those of autotrophic nitrifying bacteria (Hargreaves 2006). The technology works on the basic principle of flocculation (co-culture of heterotrophic bacteria and algae) within the system (Avnimelech 2006). Biofloc technology (BFT) has been successfully implemented in aquaculture especially shrimp farming due to economical, environmental, and marketing advantages over a conventional culture system. Compared to conventional aquaculture techniques, biofloc technology provides 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 and Kochba 2009; De Schryver et al. 2008). As a startup for biofloc technology, it might be interesting to investigate the effect of adding nucleation sites to the lined ponds which include adding calculated amounts of pond bottom clay to the water at startup to stimulate floc formation. In addition, the inoculation with water from existing biofloc ponds or a prepared inoculum (20 g of pond bottom soil, 10 mg ammonium sulfate $(NH)_4SO_4$ and 200 mg of carbon sources in 1 l of water) might allow an accelerated startup. McAbee et al. (2003) and Gaona et al. (2011) suggested the use of nucleation sites such as soil or biofloc-rich water as inoculum from a prior production cycle accelerates the formation of microbial flocs in the new culture system. Compared to the productivity of various eco-friendly farming practices, the intensive farming methods with limited water exchange provide a sustainable alternative for farming intensification and biosecurity.

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). Immobilization of inorganic nitrogen takes place when the C/N ratio of the organic matter is higher than 10 (Lancelot and Billen 1985). Thus, alteration in the C/N ratio may result in a shift from an autotrophic to a heterotrophic system (Avnimelech 1999; Browdy and Bratvold 2001). 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). By adding a carbon source (direct or indirect C-sources) to the culture medium in limited-discharge systems (changing C/N ratio), it is possible to obtain a significant enhancement of useful microbial growth and the fixation of toxic nitrogen metabolites (Ebeling et al. 2006; Hari et al. 2006; Avnimelech and Kochba 2009; Crab et al. 2010). As the C/N ratio of bacterial cells is 5:1 (Rittmann and McCarty 2001) and the conversion efficiency of bacteria is 40–60%, C/N ratio of 10 or more in the feed is required for the growth of heterotrophic microorganisms (Avnimelech 1999). The bacterial process transforms the toxic form of nitrogen (ammonia and nitrite) to one that is toxic only at high concentrations (nitrate) by the process called nitrification. 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) 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, but bacteria require about 20 units of carbon per unit of nitrogen assimilated (Avnimelech 1999). So, when C/N ratio is low in the feed, carbon becomes the limiting nutrient for the growth of heterotrophic bacterial populations in the aquaculture ponds (Asaduzzaman et al. 2009) and hence the heterotrophic bacterial population will not inflate beyond a certain point due to the limited availability of carbon in the system. 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). Different organic carbon sources (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), and C/N ratio is also widely used as a guide for analyzing the decomposition of organic matter (Alexander and Ingram 1992). The reduction of toxic nitrogenous compounds from the intensive, well-aerated systems can be achieved by the application of organic carbon sources and by altering the C/N ratio in the feed (Avnimelech 1999; Browdy and Bratvold 2001). The biofloc system maintained with C/N ratio of higher than 15–20 will be developing sufficient microbial floc to assimilate toxic nitrogenous species under intensive farming with limited discharge. Recently, a lot of work has been published in biofloc technology regarding the manipulation of C/N ratio, and also *Biofloc Technology: A Practical Guide Book* that is directed to farmers and researchers is a tremendous step forward in providing information on this technology (Avnimelech 2015).

Microbial community in bioflocs

Two functional categories of bacterial populations are primarily responsible for water quality maintenance in minimal or zero water exchange systems (intensive systems) viz., heterotrophic ammonia-assimilative and chemoautotrophic nitrifying bacteria (Ebeling et al. 2006; Hargreaves 2006). The color 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). Microbial communities formed consist of phytoplankton, bacteria, and aggregates of living and dead particulate organic matter (Hargreaves 2006). According to Ju et al. (2008), 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). 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). In conventional activated sludge systems, efficient aggregation is of principal importance, since their operational success depends heavily on good settling sludge (Bossier and Verstraete 1996). The heterotrophic bacterial population utilizes the ammonium in addition to the organic nitrogenous wastes to synthesize single-cell microbial protein (Schneider et al. 2006) which act as natural feed for shrimps (Burford et al. 2004). The sinking rate of floc aggregate will be slow at a velocity of 1 to 3 m/h when densities of the microbial biomass move slightly above 1 g wet weight/ml (Sears et al. 2006). As the bacteria move through the water column, efficient laminar regime (Reynolds envelope) which is always present around bacteria which are smaller than 100 μm interferes with the nutrient mass transfer. When the rate of substrate consumption exceeds the rate of substrate supply, mass transfer limitations take place (Simoni et al. 2001).

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). The nutritional value of bioflocs is dependent 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). 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; Burford et al. 2004; Mahanand et al. 2013). 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). Azim and Little 2008 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). Ballester et al. (2010) 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 2010a; Crab et al. 2009). Overall, bioflocs produced on glycerol gave the best results (Crab 2010a). Biofloc enhances ingestion rate, nutrient absorption, and assimilation, and provides a complete source of cellular nutrition (Tacon et al. 2002). 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. 2012a, 2014). Tilapia culture inactivated suspension ponds indicated that the fish grew well on low-protein feed (Avnimelech 1999; Milstein et al. 2001). Bioflocs are rich in proteins, vitamins, and minerals (Brown et al. 1997; Tacon et al. 2002). Ju et al. (2008) studied the amino acid profile of the biofloc and reported that bioflocs have a better essential amino acid index (0.92–0.93) with histidine and taurine as the most abundant amino acids. However, arginine and lysine were found to be the limiting amino acids in the biofloc (Avnimelech 1999).

Consumption and regeneration of bioflocs can increase feed utilization efficiency of the microbial population by recycling feed residues and/or recovery of some fraction of excreted nutrients (Hargreaves 2006). The microorganism not only removes excess nutrient but also improves growth rate, feed conversion ratio, and weight gain in shrimps and tilapia (Burford et al. 2004; Wasielesky et al. 2006). Although bioflocs meet nutritional standards, nutritional properties and the ability to maintain water quality in the BFT system depend on the carbon source used to produce the flocs. Different carbon sources are not only used to manipulate the

C/N ratio and stimulate the specific bacteria, protozoa, and algae but also to influence the microbial composition and community organization of the bioflocs (Crab et al. 2009; Crab 2010a). *L. vannamei* production rates and water quality were maintained without water exchange using a biofloc system supplemented with dextrose or molasses (Antonio et al. 2015). According to Avnimelech (2007) and Emerenciano et al. (2013b), the bioflocs are rich in natural protein and lipid and hence serve as natural in situ food for culture organisms while at the same times act as bio-control to the system by treating the feeding waste and reducing ammonium concentrations (Crab et al. 2007; De Schryver et al. 2008; Hargreaves 2013) thereby maintaining the water quality. However, further research should focus on the use of cheap and fermented non-conventional agro-industrial residues as carbon sources to upgrade wastes as healthy feeds and to find means of fish meal replacement for the aquatic organisms.

Bioflocs as dietary stimulant

Bioflocs or its attached microorganisms could exert a positive effect on the digestive enzyme activity of shrimp (Xu and Pan 2012). 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); 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). 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. 2013a). According to the original definition, probiotics are "organisms and substances which contribute to intestinal microbial balance". Fuller (1989) revised the definition as "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Therefore, several terms such as "friendly", "beneficial", or "healthy" bacteria are also commonly used to describe probiotics. Presently, research is going on in the field of probiotic biofloc (bio-engineered biofloc) in which beneficial probiotic bacterial strains are added to the biofloc to increase the bio-control over the pathogenic microbes and to improve the quorum-sensing ability of beneficial bacterial population present in the floc, as probiotic-based biofloc showed a bio-control effect towards the pathogenic *vibrio* species (Crab 2010a). Bioactive compounds present in the floc cultures were considered to be effective enhancers of growth and immunity in fishes and shrimps (Burford et al. 2004; Ju et al. 2008; Linan-Cabello et al. 2002). In biofloc-based rearing units, excess nitrogen and carbon sources will lead to the development of mixed cultures which accumulate polyhydroxy butyrate (PHB) (Salehizadeh and Van Loosdrecht 2004). When the bacterial cell death or lysis happens in the culture system, there will be degradation of PHB through the activity of extracellular PHB depolymerase enzymes which are widely found in the microbes (Jendrossek and Handrick 2002). The activity of extracellular PHB depolymerase enzymes results in the release of 3-hydroxyl butyrate into the surrounding environment (Trainer and Charles 2006). PHB can act as a prebiotic for the cultured organisms. Prebiotics also known as immunosaccharides are indigestible food ingredients that selectively and beneficially affect the host by stimulating the growth of one or a limited number of bacteria in the colon thus changing the composition of gut microflora. The carbohydrates added to the BFT can also act as a source of prebiotics to the cultured organisms and could modulate the gut microbial population which is beneficial to improve the health status of the cultured organisms. Further research should be directed to know the role and activity of immunostimulatory and bioactive compounds present in the biofloc, since the technology deals with bacteria and bacterial products.

Immuno-physiological response by bioflocs

The knowledge of immunology has diversified greatly due to the variety of fish that are farmed and the inclusion of species such as zebra fish to the animal model repertoire in comparative immunological studies (Van Der Sar et al. 2004; Yoder et al. 2002). The immune system of fish acts as a crossroad between innate and adaptive immune responses and is hardened to the environment and the poikilothermic nature of the fish (Tort et al. 2003). The immune responses initiated by injury or by pathogenic invasion will entail phagocytosis and inflammatory processes (Corbel 1975) assisted by non-specific immune cells such as macrophages, neutrophils, and non-specific cytotoxic cells. When bacterial load increases in the adjacent water, they stimulate the release of high molecular weight glycoproteins from fish skin mucus (van der Marel et al. 2010). A number of humoral factors are released by the fishes, once they are exposed to the pathogens, such as cytokines, anti-proteases, peroxidases, lysozymes, etc. Among them, lysozyme is a preferred marker of the immune response. Lysozyme acts as an anti-inflammatory and antiviral agent, besides its high potential for bactericidal or bacteriolytic activity against pathogenic gram-positive and gram-negative bacteria (Saurabh and Sahoo 2008). Recently, researchers have hypothesized possibilities of immunostimulatory features of the bioflocs leading to enhancement of the immunity and antioxidant status of shrimps and fishes to provide broad-based resistance towards many infections (Crab et al. 2012; Xu and Pan 2013; Ahmad et al. 2016). The heterotrophic microbial biomass is suspected to have a controlling effect on pathogenic bacteria (Michaud et al. 2006). Ju et al. (2008) reported that floc carotenoids have been shown to provide essential nutritional and many bioactive physiological functions in animal tissue, including stimulating the animal immune system. The disruption of quorum-sensing, bacterial cell-to-cell communication system is a new strategy to control bacterial infection in aquaculture. Interestingly, a similar phenomenon was observed in bioflocs grown on glycerol against *Vibrio harveyi* in *Artemia franciscana* culture (Crab et al. 2010); it was also speculated that microbial flocs contain many strains of probiotics bacteria (Bairagi et al. 2002).

Most of the existing immunostimulants available are a group of live and synthetic compounds derived from bacteria and bacterial products and also extracts from plants and animals (Wang et al. 2008). Since biofloc technology deals with the bacterial environment, it might also contain some immunostimulatory compounds that are beneficial for the health of cultured organisms. 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 the shrimp, thereby enhancing their disease resistance (Ninawe and Selvin 2009; Smith et al. 2003; Vazquez et al. 2009). Even though bioflocs have been confirmed as being the richest source of natural microorganisms and bioactive compounds, little effort was made to study its effect on the physiological health of cultured shrimp, particularly concerning immune and antioxidant defense systems. Jang et al. (2011) found that the expression of a prophenol oxidase-activating enzyme (lvPPAE1) in hemocytes of *L. vannamei* was enhanced significantly when shrimp were reared in a biofloc for a long term. More recently, Becerra-Dorame et al. (2014) 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) and then modulate the immune system of the host whether as viable microbes or microbial components (Jang et al. 2011). Therefore, in order to improve the welfare of fishes in aquaculture, further work needs to be carried out in the exact nature of humoral innate or cellular immune response and to determine the protective biofloc life of bacteria. Finally, this paper proposes a long-term perspective of bioflocs that can be used as a strategic control of diseases in aquaculture.

Bioflocs in aquaculture: future prospectus

A number of beneficial features are associated with biofloc technology along with 10–20% potential feed gain as estimated by application of biofloc technology (Crab et al. 2007; De Schryver et al. 2008) to nitrogen recovery from the culture system. This increase was based on the internal recirculation of nutrients through the formation of new microbial biomass, which was subsequently grazed by the fish (Avnimelech 2006). The advantages of the technology in aquaculture has been well documented which includes low feed and water input (economical), less risk of pathogen introduction and diseases, more biosecurity, increased growth and survival, and hence increased crop yield (Otoshi et al. 2009; Crab et al. 2009; Samocha et al. 1998, 2007; Krummenauer et al. 2011; Perez-Fuentes et al. 2013). It also lowered the feed conversion rate by utilizing the in situ natural feed and has small footprints, hence reducing environmental impacts (Krummenauer et al. 2014). It is also robust, easy to operate, and economically feasible (Crab et al. 2012). The zero water exchange system has advantages of maintaining temperature and heat fluctuations (Crab et al. 2009). It supports nitrogen removal even when organic matter and biochemical oxygen demand of the system is high (Avnimelech 2015). It will be important for the future BFT to understand the microbial mechanisms involved in the process of flocculation viz., quorum sensing and controlling effect on pathogenic microbes. BFT also improves sustainability and biosecurity and development of high-intensity grow out systems with no water discharge over the entire crop cycle. It can also serve as a cheap and effective immunostimulant for the cultured organisms, so more research should focus on the optimal way to manage the BFT in the culture ponds.

Conclusion

The scarcity of water, growing demand for protein food, and conflict for land usage for the expansion of aquacultural practices have become a major problem at the global level. To cater to the growing demand for animal protein, intensive aquaculture is one of the major options. But intensification of aquaculture practices will generate a lot of effluents which will damage the aquatic environment. Moreover, the intensification will lead to heavy dependence on fish meal which is a scarce commodity, disease outbreaks in the cultured organisms, environmental degradation, and socioeconomic conflicts. In an attempt to minimize the impact of the environmental, health, and economic problems associated with aquaculture, BFT has become increasingly popular as a sustainable alternative for intensification (Avnimelech 1999, 2006; Browdy et al. 2001; Crab et al. 2007; De Schryver et al. 2008). The requirements for sustainable and eco-friendly aquaculture development can be fulfilled by the use of biofloc technology.

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