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Biofloc technology as a promising tool to improve aquaculture production

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Abstract

Given that the expansion of the aquaculture industry is associated with increased environmental impact and its strong dependence on fishmeal in the diet, the use of biofloc technology can reduce these problems. This technology absorbs inorganic nitrogen from aquaculture wastewater and improves water quality as well as produces microbial protein that is directly used as a suitable feed supplement for aquatic animals. Furthermore, this technology reduces the feed conversion ratio and subsequent production cost. Biofloc is available to aquatic animals throughout the day and provides the necessary nutrients, fatty acids and minerals. Biofloc along with formulated diets provides a complete food chain for aquatic animals' growth and thus improves growth performance. Since feed constitutes a major part of the aquaculture cost, having accurate information about biofloc system in aquatic animals' nutrition can be beneficial and helpful. Compared to traditional aquaculture techniques, biofloc technology provides a more sustainable approach with minimal water exchange along with reduced feed intake and transforms it into a low-cost sustainable technology for sustainable aquaculture development. Overall, this study highlights the significance of developing biofloc technology to improve aquaculture production and could be an alternative system for cultivation of important commercial species in aquaculture.

Key words: aquatic species, biofloc, carbon substrate, microbial interactions, nutrition, water quality management.

Introduction

Due to the rapid growth of population worldwide, the food production industries such as aquaculture industry needs to be well developed. The growth of the aquaculture industry must take into account environmental, economic and social conditions and somehow consider the factors of sustainable development in the expansion of this industry. The intensity of aquaculture activities, especially in coastal areas, increases the load of organic matter in the water and will have environmental risks in the long-term period (Piedrahita 2003; Sharifinia *et al.* 2018, 2019). In recent decades, recirculating systems have been developed that are in the direction of sustainable aquaculture development and including an appropriate approach to control aquaculture wastewater (Gutierrez-Wing & Malone 2006). In this system, 10% of the total volume of water is replaced daily (Twarowska *et al.* 1997), but due to the high operating and maintenance costs, adoption of the recirculating system among farmers, especially in developing countries, is low (Badiola *et al.* 2012; Ahmad *et al.* 2017; Karimanzira *et al.* 2017). Therefore, the need for a low-cost, sustainable and environmentally friendly technology that is acceptable to farmers and can be used on a large scale is evident and noteworthy.

Biofloc system, also called as biofloc technology (BFT), has recently attracted great attention as a cost-effective, sustainable and environmentally friendly (as it is basically zero water exchange and artificial feeding ratio are reduced) way that improves water quality as well as produces microbial protein for aquatic species (Avnimelech & Kochba 2009; Ekasari & Maryam 2012; Sgnaulin *et al.* 2018; Dinda *et al.* 2019; Gao *et al.* 2019). Biofloc system due to maintain water quality, improving feed conversion ratio (FCR), application of low-protein diets, reducing costs of production and replacing conventional high-cost feeds with alternative protein sources (Wasielesky *et al.* 2006; Ballester *et al.* 2010; Emerenciano *et al.* 2013a; Ahmad *et al.* 2017; Sgnaulin *et al.* 2018; Khanjani *et al.* 2019a) have received great attention in recent decades. The use of this system in shrimp farming and to some extent in finfish culture farming has been extensively studied (Emerenciano *et al.* 2011, 2012a, 2013a; Ekasari & Maryam 2012; Xu & Pan 2012; Monroy-Dosta *et al.* 2013; Caldini *et al.* 2015; Khanjani *et al.* 2016, 2017, 2019a; Najdegerami *et al.* 2016; Abbaszadeh *et al.* 2019a,b; Ebrahimi *et al.* 2020).

Production in biofloc system in the large scale aquaculture can have environmental benefits in marine and coastal ecosystems, and aquaculture wastewater and its environmental effects can be controlled by replacing soybean or fish meal with floc compounds in aquatic nutrition. Brito *et al.* (2016) and Bossier and Ekasari (2017) reported that culturing in a biofloc system can contribute to removal of total ammonia nitrogen (TAN) and nitrite, reducing water utilization and waste generation, decrease in Vibrio density, improve feed utilization efficiency and increased body-bound crude protein. Furthermore, studies by Khatoon *et al.* (2016) and Kamilya *et al.* (2017) have suggested that enhance of growth performance in a biofloc system could be attributed to presence of microbial floc and maintaining water quality by this system. Ekasari (2014) reported that biofloc systems can improve net productivity by 8–43% in comparison with non-biofloc systems such as conventional and recirculating aquaculture system. More importantly, to minimize environmental issues in aquaculture industry the biofloc nutrient-rich waste can be used as a feed in BFT. Therefore, the aim of this article is to (i) review the studies that have been done on the development and application of biofloc technology in aquaculture industry; (ii) intends to make this information available for an international audience and give an overview of the current status on biofloc technology; (iii) intends to highlight the significance of developing biofloc technology for improve aquaculture production as an alternative system for cultivation of important commercial species in aquaculture; and (iv) make recommendations for management of biofloc system in aquaculture.

Biofloc technology

Biofloc technology is a technique of enhancing water quality by adding extra external carbon sources in accordance with high level of aeration to produce high levels of microbial bacterial floc in aquaculture system (Crab *et al.* 2012; Ahmad *et al.* 2017). Maintaining a carbon-to-nitrogen ratio above 10 is essential in this system by adding carbon-containing organic materials such as molasses, wheat flour, starch or reducing the protein level of the feed to increase the activity of heterotrophic bacteria (Crab *et al.* 2012; Khanjani *et al.* 2017, 2019a). Under such circumstances, the production of microbial proteins takes place and thereby improves water quality as well as serve as sources of dietary protein for fish and shrimp (Crab *et al.* 2012). The uptake rate of inorganic nitrogen compounds by heterotrophic bacteria is higher than denitrifying bacteria; therefore, the growth rate and production of microbial biomass per unit substrate in heterotrophic bacteria is 10 times higher (Hargreaves 2006). Therefore, if there are sufficient organic carbon sources, immobilization of ammonia by heterotrophic bacteria usually takes place rapidly in bioflocs during hours or days (Hargreaves 2006).

This technology works based on the principle of flocculation or co-culture of heterotrophic bacteria and algae within the system (Avnimelech 2006; Ahmad *et al.* 2017). Emerenciano *et al.* (2017) stated that biological interaction can takes place between some group of microorganisms in biofloc system such as bacteria-microalgae. Some strains of bacteria can have a positive effect on growth of pelagic and benthic microalgae species (Fukami *et al.* 1997). The extracellular carbohydrates of benthic microalgae (diatoms) may be used as carbon source by heterotrophic microorganisms (Bruckner *et al.* 2008). Biofloc technology because of economic, environmental and social benefits has been successfully applied in aquaculture, especially shrimp farms. Compared to traditional aquaculture techniques, BFT technology provides a more sustainable approach with minimal water exchange along with reduced feed intake and transforms it into a low-cost sustainable technology for sustainable aquaculture development (De Schryver *et al.* 2008; Avnimelech & Kochba 2009). Taking advantage of this system can reduce water exchange and increase density and biosecurity. For sustainable intensive aquaculture, farm biosecurity and biofloc technology need to be considered as two major factors. BFT provides higher degree of biosecurity by limited water exchange, higher environmental control, biological and physical (indoors) barriers against pathogens and enhances immune system. Ju *et al.* (2008) biofloc has bioactive compounds that contribute for a healthy status of cultured prawns. Also, expressions of certain hemocytes enzymes related to immune system (Jang *et al.* 2011) and antioxidants status (Xu & Pan 2013) enhanced in *Litopenaeus vannamei* reared in biofloc system. Furthermore, BFT showed positive impacts on the immune response to higher resistance against infectious myonecrosis virus (IMNV) challenge (Ekasari 2014) and *Vibrio* (Liu *et al.* 2017). Therefore, with respect to the present issues it appears that biofloc technology to be the solution.

Biofloc formation

To create biofloc, the tanks were firstly filled with water then a certain amount of nitrogenous material (aquatic feed and urea fertilizer) was added in order to supply the nitrogen then carbonate organic materials (such as molasses, wheat flour, starch) about 0.7 of diet were distributed on the surface of the tanks in order to provide carbon. Clay is also added to the microbial reservoirs to help forming the microbial mass after softening and passing through the sieve (53-μm-sized particles or less pass with mesh number of 270). Adding clay at the beginning and during biofloc formation is suitable for further mass continuity. In addition, the use of farm wastewater containing nitrogenous wastes is helpful as an inoculum. 20 g of clay, 10 mg of ammonium sulphate and 200 mg of carbonaceous organic matter such as molasses stimulate biofloc formation in one litre of water. Various studies have shown that the use of clay and water rich in a biofloc production cycle as the primary inoculum improves microbial mass formation in the new culture system (Gaona *et al.* 2011; Zemor *et al.* 2019).

After providing the necessary elements in the system, in order to activate the activity of bacteria in water the aeration was conducted. The presence of carbonated organic matter has made heterotrophic bacteria more active than other bacteria, which these bacteria remove nitrogen and carbon from water by absorption process, and produce microbial biomass, as well as, other organisms in the water attach to them to feed on the microbial biomass and form biofloc (Khanjani *et al.* 2017, 2019a). During the biofloc formation period, algae first develop and then foam form, and eventually, the development of a brown state indicates the presence and activity of heterotrophic bacteria. During the experimental period, when there are aquatic animals in the tanks, the physicochemical parameters (temperature, oxygen, pH, alkalinity, total nitrogen, ammonium, nitrite and nitrate) should be measured and subsequently appropriate responses as outlined below should be adopted quickly (Avnimelech 2009; Khanjani *et al.* 2015):

- If ammonia levels were high: adding carbohydrates to the tank and reducing protein in the feed
- If the nitrite level is high: checking for low-oxygen areas, sludge collection, putting aerators and adding carbon
- If the microbial biomass is low: adding carbohydrates
- If the volume of biofloc is too high: excretion of waste material and some of the bioflocs

The amount of settled solids in the BFT culture tanks (using Imhoff cone; measured in 15–20 min to be well deposited), and total suspended solid (TSS) must also be measured to better management of the system (Avnimelech & Kochba 2009).

Factors such as the amount of salinity and type of carbon affect the rate and duration of biofloc formation (Khanjani *et al.* 2017, 2019a). Maicá *et al.* (2012) found that increasing salinity increases the density of biofloc formation, and the type of carbon source also affects the quality of the flocs, so that saltier waters promote better formation of stable bioflocs. Furthermore, addition of simple carbonaceous organic matter such as molasses to the aquaculture system without water exchange leads to improved water quality and faster growth of heterotrophic bacteria compared to complex carbohydrates like wheat flour. Figure 1a,b shows the formed and condensed of wet and dry biofloc, respectively, that can be used by aquatic animals, as well as Figure 1c shows how the heterotrophic bacteria are interconnected in a chained and porous manner. The open and porous structure is characteristic of biofloc micrographs, which allows water and chemicals to flow throughout the floc and is effective in supplying nutrients and eliminating the metabolites from in and out of biomass in the microbial mass (Khanjani *et al.* 2015, 2016, 2019b).

Mechanism of microbial cell bonding

The massing of microbial communities is a complex process involving the physical, chemical and biological processes in its formation (De Schryver *et al.* 2008). There are several mechanisms that influence the formation, appearance and stability of the microbial mass. Many organisms repel polymeric compounds of humic, proteins and polysaccharides that cover their outer surface; these slimy polymers act as adhesives and integrate other cells and particles to form a biofloc. Another mechanism is the balance between the forces of gravity (molecular, dipole, hydrogen bond) and electrostatic repulsion forces. Most organisms are negatively charged and cause counter electrostatic repulsion. If this repulsion reduced, then strong gravity forces can occur; this is the case when the salt concentration is high and multivalent ions are present in the environment (Avnimelech 2009). Calcium and aluminium ions stimulate the formation of stable flocs and algae, fungi or bacteria organisms can help to bonding between the components of different flocs (De Schryver *et al.* 2008; Avnimelech 2009). Another advantage of the biofloc in terms of biofloc formation is that the presence of common groups of microorganisms in BFT. Fungi, ciliate, protozoa, rotifer, copepod and nematode complement the biofloc community play an essential role in recycling of organic matter in the BFT system. Ciliates are the largest group of protozoa in nature; they eat bacteria (including cyanobacteria) and small phytoplankton (Emerenciano *et al.* 2017). High porosity of biofloc causes relatively low mass density, which keeps the bioflocs suspended in water and reduces sedimentation rate (Avnimelech 2009).

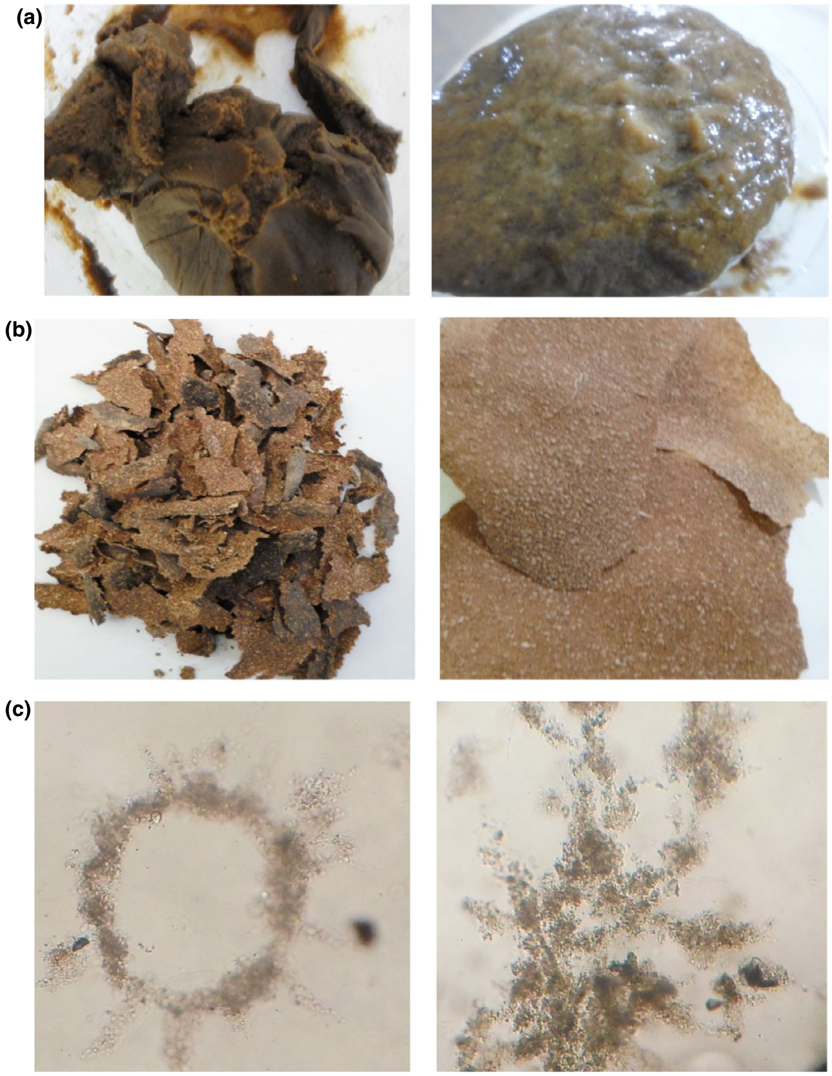

Figure 1 (a) Wet biofloc, (b) dry biofloc and (c) produced floc in rearing tanks of pacific white shrimp, bioflocs are porous and has a lot of bacteria that were connected like a chain (Khanjani 2015, 2019b).

Microbial communities in biofloc

Biofloc can be defined as a set of organic matter in a culture environment that forms at a high density as suspended particles (Cuzon *et al*. 2004; Emerenciano *et al*. 2012b, 2013a) and includes compounds of organic materials (60–70%), heterogeneous combination of microorganisms (i.e. fungi, algae, bacteria, protozoa, rotifer, nematode) and inorganic substances (30–40%) such as colloids, organic polymers, binary ions, salts and dead cells (Chu & Lee 2004). Bacterial communities are primarily responsible for maintaining water quality in the zero water exchange system by relying on heterotrophic and nitrifying chemoautotrophic bacteria (Ebeling *et al*. 2006; Hargreaves 2006). In BFT systems, the heterotrophic bacteria community are able to colonize dead organisms, moults, unconsumed food and faeces to produce bacterial biomass. This community use the organic compounds as a carbon source and can reduce accumulation of ammonia by incorporation as bacterial biomass in the water column that are a main component of biofloc (Emerenciano *et al*. 2017). During biofloc formation, the colour changes from green to brown as progress for the development of bacteria due to transition from algal to bacterial in a BFT system. Avnimelech (2007) suggested that the number of bacteria in the ponds containing biofloc can be between 10^6 and 10^9 colonies per ml, with each ml of biofloc containing 10–30 mg of dry matter. Khanjani (2015) and Khanjani *et al*. (2019a) stated that the 3.36×10^7 number of heterotrophic bacteria per ml can indicate the maturation of a biofloc system.

The different groups of organisms present in the biofloc depend on various factors such as the type of carbon source, salinity level and cultured species (Ray *et al.* 2010). Ju *et al.* (2008) reported that bioflocs collected from Pacific white shrimp tanks consist of 24.6% phytoplankton (often diatoms such as *Thalassiosira*, *Chaetoceros* and *Navicula*), 3% biomass of bacteria (two-thirds Gram-negative and one-third Gram-positive), some protozoa communities (98% flagellates, 1.5% rotifer and 0.5% amoeba), 32.2% detritus and 39.25% ash. Also, Yunos *et al.* (2017) showed that the biofloc structure contained of 29% microalgae, 35% bacteria, 24% fungi and 12% zooplankton.

Khanjani *et al.* (2016) quantified the amount of different organisms in the biofloc system based on methods applied by Thompson *et al.* (2002) and Emerenciano *et al.* (2012b) and found that biofloc compounds comprised 73.47% of flagellum and ciliated protozoa, 20.53% of microalgae, 3.33% of nematodes and 2.67% of other suspended organisms. Also, biofloc sizes of 20–437.5 μm were observed. Zhao *et al.* (2012) stated that dominant species of bacteria that exist in bioflocs include *Proteobacteria*, *Bacillus* and *Actinobacteria* and there are also some other minor species of bacteria such as the genus *Roseobacter* and *Cytophaga*.

Monroy-Dosta *et al.* (2013) reported that a great number of heterotrophic microbial communities related to bioflocs include genera such as the *Sphingomonas* (*Sphingomonas paucimobilis*), *Pseudomonas* (*Pseudomonas luteola*, *Pseudomonas mendocina*), *Bacillus*, *Micrococcus*, *Nitrospira*, *Nitrobacter* and yeast Rhodotorula sp. These microorganisms are suitable for maintaining water quality and the physiological health of organisms in culture (Monroy-Dosta *et al.* 2013). In the biofloc production system, the development of microbial groups can vary depending on the species, environmental conditions, amount of feed and especially the carbon source (Monroy-Dosta *et al.* 2013). Gutiérrez *et al.* (2016) investigated the cultivation of *Puntius conchonius* fish in a biofloc system and found that the genera *Aeromonas* and *Vibrio* were dominant biofloc-related bacteria in the first weeks of bacterial cultivation period. At week 12, the concentration of these bacteria was very low in the culture medium, but in the last two weeks, the concentration of *Bacillus subtilis* and yeast *Rhodotorulla* increased, which showed probiotic properties. Therefore, the biofloc system could be considered as a microbial ecological sequence model. Bacteria in the biofloc system consume organic matter and nitrogen compounds for growth and development. The ability to adhere to suspended particles and surfaces as well as the use of organic matter are important physiological properties of bacteria in a biofloc. Microorganisms play a major role in natural aquatic resources, and the intensity of solar energy, organic matter density and added carbon sources affect their activity. In the biofloc system, bacteria are more dependent on organic matter and strong aeration to maintained C: N ratio (Pérez-Rostro *et al.* 2014).

Nutritional value of biofloc

Bioflocs have a dynamic nutritional value (Ekasari *et al.* 2010) and can be used as a complete aquatic food source, as well as being able to supply bioactive compounds (Ahmad *et al.* 2017). Therefore, analysing the factors affecting nutritional value of biofloc such as aquatic nutrition priority, their ability to ingest and digest microbial protein, and biofloc density in water are of interest (Hargreaves 2006). The single-cell protein formed by heterotrophic bacteria through uptake of inorganic N can be used as a food source for cultured aquatic species such as shrimp, tilapia and carp (Burford *et al.* 2004; Monroy-Dosta *et al.* 2013; Khanjani *et al.* 2019a; Ebrahimi *et al.* 2020). The nutritional value of bioflocs for a particular organism depends on its particle size, digestibility and biochemical compounds. Ekasari *et al.* (2014) reported that flocs with particle size of > 100 and < 48 μm due to higher nutritional value and N recoveries are more favourable for shrimp *L. vannamei*, red tilapia *Oreochromis niloticus* and mussel *Perna viridis*. They also found that with particle size of > 100 μm contained highest levels of protein and lipid, whereas the flocs < 48 μm rich in essential amino acids.

The growth performance of Pacific white shrimp increases in the presence of biofloc (Khanjani *et al.* 2016). Improving growth performance of aquatic species in the biofloc system is related to the simultaneous presence of biofloc production with the artificial diet, which is a natural food supplemented with a formulated diet, forms a complex food chain that the aquatic animal uses. Khanjani *et al.* (2015) investigated the effects of BFT on feed conversion ratio (FCR) and recorded the FCR values 1.52 and 1.2–1.29 in clear water and BFT treatments, respectively. Khanjani (2015) reported that the presence of biofloc can lead to decrease in FCR (1.20–1.29) and increase in feed efficiency (78.61–84.26%) compared to clear water treatment (1.52 for FCR and 66.81% for feed efficiency). In terms of quality, based on the dry matter biofloc contains 38% protein, 3% lipid, 6% fibre, 12% ash and 19 kJ g^{-1} energy (Azim & Little 2008). They reported that the quality of biofloc is independent of the feed quality used for biofloc production. Ballester *et al.* (2010) found that bioflocs contained 30.4% crude protein, 4.7% crude fat, 8.3% fibre, 39.2% ash and 29.1% nitrogen-free extract based on dry matter. Wheat bran and molasses's were used as sources of carbohydrates in this study. Brown *et al.* (1996) assessed the nutritional value of seven species of yeast and reported protein content of 25–37%, carbohydrates 21–39%, lipids 4–6%, and, additionally, the presence of all essential amino acids. Yeasts are chemoorganotrophic microorganisms that reported in the biofloc and used organic compounds of carbon as a source of energy, which are quite diverse and include sugars, polyols, organic and fatty acids, aliphatic alcohols, various heterocyclic and polymeric compounds (Emerenciano *et al*. 2017). Ekasari *et al*. (2010) reported that bioflocs with glycerol as carbon source contained higher total $n-6$ PUFAs than those with glucose. This suggested that the nutritional value of bioflocs can be dynamic and microbiota is likely to affect the nutritional value of bioflocs.

Khanjani *et al*. (2017) were used different carbon sources including molasses, wheat flour, starch and their mixtures for biofloc formation and found different nutritional value results of the bioflocs grown on each carbonaceous material. They suggested that changing in carbon sources alters the nutritional composition and quality of bioflocs. Tacon *et al*. (2002) stated that biofloc enhances ingestion and digestion of feed and provides a complete food source for cultured aquatic organisms. The presence of biofloc in the diet of brood-stocks of *L. vannamei* (Emerenciano *et al*. 2013b) and *Litopenaeus stylirostris* results in improved reproductive performance and egg quality (Cardona *et al*. 2016). Emerenciano *et al*. (2013b) found that *L. vannamei* females fed with fresh food under biofloc conditions achieved better eggs production (higher number per spawn), less unfertilized spawns, spawned more quickly and showed higher levels of highly unsaturated fatty acids (HUFA) in eggs. They suggested that the enhanced reproductive performance and egg quality of brood-stocks fed with fresh food under biofloc conditions can be explained by their high HUFA content (are essential nutrients for gonad development) and the contribution of biofloc particulates as the source of essential nutrients for reproduction including antioxidant and essential lipids. Hoa *et al*. (2009) reported that a large fraction of HUFA often observed in natural fresh food items such as squid and mussels. Cardona *et al*. (2016) reported that higher spawning rate and frequency as well as higher gonado-somatic index and number of spawned eggs in shrimp brood-stocks maintained in biofloc system compared to clear water might be explained by the dietary supplement obtained from natural productivity during BFT rearing and the contribution of biofloc particulates as a source of dietary glutathione and lipids, particularly essential phospholipids and HUFAs for shrimps.

Consumption and recycling of biofloc in the culture system increase feed efficiency (Hargreaves 2006). Microorganisms and their associated microbial communities not only remove excess waste but also increase growth rate, feed efficiency and weight gain in farmed species (Burford *et al*. 2004; Wasielesky *et al*. 2006; Khanjani *et al*. 2016, 2017). The nutritional properties of biofloc and their ability to maintain water quality in the BFT system depend on the type of carbon source used for biofloc production (Khanjani *et al*. 2017). Various carbon sources influence on the carbon/nitrogen ratio, increased activity of heterotrophic bacteria, protozoa, algae as well as microbial compounds and social structures in the bioflocs (Crab *et al*. 2010, 2012). Furthermore, carbonaceous organic matters such as dextrose and molasses in the biofloc system increases the production of Pacific white shrimp (de Lorenzo *et al*. 2015). Table 1 presents the various studies conducted on different aquatic species by adding different carbon sources.

Numerous studies have reported that biofloc provides essential nutrients such as protein, lipid, essential fatty acids, minerals, vitamins, carotenoids and digestive enzymes to facilitate digestion and consequently improves nutritional status (Crab *et al*. 2012; Xu & Pan 2012; Xu *et al*. 2012; Khanjani *et al*. 2016; Abbaszadeh *et al*. 2019a;

Table 1 Studies with different species and carbon sources used in biofloc production system

Reference	Carbon sources	Species
Crab *et al*. (2010)	Acetate	*Macrobrachium rosenbergii*
Suita (2009)	Dextrose	*Litopenaeus vannamei*
Crab *et al*. (2010)	Glucose	*M. rosenbergii*
Burford *et al*. (2004)	Molasses	*L. vannamei* and *P. monodon*
Asaduzzaman *et al*. (2008)	Starch	*L. vannamei* and *M. rosenberg*
Emerenciano *et al*. (2012a,b)	Wheat flour + Molasses	*Farfantepenaeus brasiensis*, *F. duorarum*
Avnimelech (2009)	Cellulose	Tilapia
Azim and Little (2008)	Wheat flour	Tilapia (*O. niloticus*)
Emerenciano *et al*. (2011)	Wheat bran + Molasses	*F. paulensis*
Emerenciano *et al*. (2012a,b)	Wheat bran + Molasses	*F. brasiliensis*
Wang *et al*. (2016)	Wheat bran	*Litopenaeus vannamei*
Serra *et al*. (2015)	Molasses + dextrose + rice flour	*Litopenaeus vannamei*
Khanjani *et al*. (2017)	Molasses + wheat flour + Starch	*Litopenaeus vannamei*
Abbaszadeh *et al*. (2019a,b)	Molasses + palm sap	*Litopenaeus vannamei*
Mirzakhani *et al*. (2019)	Wheat flour and Molasses	Tilapia (*O. niloticus*)

Ahmad *et al*. 2019). Ahmad *et al*. (2019) investigated the effects of different organic carbon sources on haematological indices, digestive and metabolic enzyme activities of *Labeo rohita* fingerlings rearing under biofloc conditions. They reported that activities of amylase and protease enzymes in the whole intestine were significantly higher in tapioca biofloc system while lipase activity did not show significant differences in biofloc systems with different carbon sources. De *et al*. (2015) stated that nutrient digestibility is positively associated with activity of digestive enzyme and higher activities of digestive enzymes in biofloc systems can improved utilization of macromolecules (Ahmad *et al*. 2019). Ahmad *et al*. (2019) suggested that these changes may occur due to compositional characteristic of the carbon sources used. Mahanand *et al*. (2013) noted that the nutrient content of the bioflocs indicates that the bioflocs can be successfully used to feed herbivorous and omnivorous fish and achieve optimal growth. Previous studies have shown that using bioflocs in the zero water exchange system significantly contribute to the growth and production of tilapia (Avnimelech 2007; Azim & Little 2008). Furthermore, the nutritional value of bioflocs extracted from different studies is shown in Table 2.

Impact of biofloc on growth performance, immune system and activity of digestive enzymes

Biofloc and its attached microorganisms have a positive effect on the activity of digestive enzymes in aquatic species (Xu & Pan 2012; Ahmad *et al*. 2019). Inclusion of biofloc in the diet resulted in about 75% improvement in growth performance and digestive enzyme activity in common carp (Najdegerami *et al*. 2016). Also, biofloc as a dietary supplement at 4% level in feeding of tiger shrimp increases growth and activities of digestive enzyme (Anand *et al*. 2014). Bioflocs have recently been recognized as a new strategy for controlling and reducing pathogens as antibiotics and antifungals that exhibit probiotic activity (Emerenciano *et al*. 2013a). Biological compounds in biofloc are contributing factors affecting the growth and immunity of fish and shrimp (Burford *et al*. 2004; Ju *et al*. 2008). Activity of serum lysozyme (LSZ) is an important factor in the fish immune defence, which plays an important role in the antibacterial activity against Gram-positive and Gram-negative bacteria (Saurabh & Sahoo 2008). Long *et al*. (2015) reported that biofloc can enhance the immune response of tilapia to a certain extent. Ju *et al*. (2008) and Crab *et al*. (2012) noted that bioflocs can provide abundant natural microbes, immunostimulatory and bioactive compounds such as carotenoids and soluble vitamins that could stimulate the immune response in cultured fish. Furthermore, biofloc played a positive role in utilization of feed and activities of digestive enzyme in cultured fish, which may improve the assimilation of dietary bioactive substances from the feed and then exerted an immune-stimulating effect on the fish (Xu & Pan 2012; Long *et al*. 2015; Promthale *et al*. 2019).

Albumin and globulin are among the most important proteins in plasma and increasing the levels of proteins can lead to strong innate immune status (Rao *et al*. 2006). Mansour and Esteban (2017) found that the immune status of fish reared in biofloc conditions is stronger than that in clear water and also reported that the levels of these proteins, total immunoglobulin, myeloperoxidase and lysozyme noticeably increased in fish reared under biofloc conditions. Myeloperoxidase and lysozyme are immune enzymes involved in defence against bacterial infection. In fish, lysozyme is made by leucocytes and leads to bacterial cell wall lysis, therefore stimulating and triggering the complement system and phagocytosis of different pathogens (Cecchini *et al*. 2000; Mansour & Esteban 2017). Moreover, myeloperoxidase expressed and stored in neutrophils and

Table 2 Nutritional value of bioflocs obtained from different studies

Reference	Crude protein%	Carbohydrate%	Lipid%	Crude fibre%	Ash%
Wasielesky *et al*. (2006)	31.1	23.6	0.50	–	44.8
Ju *et al*. (2008)	30.4	–	1.9	12.4	38.9
Kuhn *et al*. (2009)	49	36.4	1.13	12.6	13.4
Kuhn *et al*. (2010)	38.8	25.3	0.1>	16.2	24.7
Maicá *et al*. (2012)	28.8–43.1	–	2.1–3.6	8.7–10.4	22.1–42.9
Emerenciano *et al*. (2012a,b)	30.4	29.1	0.5	0.8	39.2
Emerenciano *et al*. (2012a,b)	18.2–29.3	22.8–22.9	0.4–0.7	1.5–3.5	43.7–51.8
Emerenciano *et al*. (2012a,b)	18.4–26.3	20.2–35.7	0.3–0.7	2.1–3.4	34.5–41.54
Emerenciano *et al*. (2012a,b)	28–30.4	18.1–22.7	0.5–0.6	3.1–3.2	35.8–39.6
Khanjani *et al*. (2017)	27.43	–	0.86	–	39.83
Khanjani *et al*. (2017)	23.8	–	1.14	–	21.81
Khanjani *et al*. (2017)	30.73	–	2.18	–	29.97
Abbaszadeh *et al*. (2019a,b)	–	–	0.5–0.8	6.8–8.9	7.5–9.3

plays a role in respiratory burst through peroxide to produce hypochlorous acid (Dalmo *et al.* 1997). The effects of biofloc as immune-stimulants appeared to be carbon source-dependent and the improvement observed in the immunological factors may be due to some probiotic microorganisms such as *Bacillus* and *Lactobacillus* present in BFT system (Anand *et al.* 2014; Ahmad *et al.* 2016; Zhao *et al.* 2016). Panigrahi *et al.* (2019) suggested that biofloc can improve the shrimp immune status by presence of the microbial cell wall in the biofloc, containing of peptidoglycans, lipopolysaccharides and glucans, which were known to have a potential to activate the immune response in shrimps by triggering the major non-specific defence mechanism (Labbe & Little 2009; Rao *et al.* 2010; Panigrahi *et al.* 2018).

Bioflocs have various compounds include microbial protein (Ballester *et al.* 2010; Hargreaves 2013) organic polymer (PHB: Poly-β-hydroxybutyrate) created by bacteria (De Schryver *et al.* 2010), microalgae, protozoa, nematodes (Azim & Little 2008), copepods and rotifers (Ray *et al.* 2010). PHB is a biodegradable polymer with several benefits including helping to improve digestibility in the intestine, increase unsaturated fatty acids and improve growth in fish and shrimp (Crab *et al.* 2007; Emerenciano *et al.* 2013a). Polyhydroxybutyrate acts as a probiotic for aquatic species and can, therefore, adjust the gut microbial population, which is useful for improving aquatic health (Trainer & Charles 2006).

In the Khanjani (2015) study, feed conversion ratios of 1.51 and 0.98–1.27 were obtained in control (non-biofloc) and biofloc treatments, respectively, and it was found that biofloc could account for about 30% of daily food intake Pacific white shrimp. Megahed and Mohamed (2014) found that dietary protein levels could be reduced from 45% to 25% in the presence of biofloc without affecting the growth of *Fenneropenaeus indicus*. Biofloc biochemical compounds can provide important nutrients such as protein, fat and minerals to shrimp. Various studies have shown that biofloc can make amino acids, fatty acids and minerals accessible for shrimps (Izquierdo *et al.* 2006; Emerenciano *et al.* 2012b; Toledo *et al.* 2016). The presence of biofloc in shrimp rearing tanks improves the feed conversion ratio (Wasielesky *et al.* 2006; Abbaszadeh *et al.* 2019b), enhances feed efficiency (Xu & Pan 2012), improves growth performance (Megahed & Mohamed 2014; Khanjani *et al.* 2016), reduces feed cost (Xu & Pan 2012; Xu *et al.* 2012), increases digestive enzyme activity (Xu & Pan 2012) and impacts on biochemical composition of shrimp body (Izquierdo *et al.* 2006; Ju *et al.* 2008; Xu & Pan 2012; Khanjani *et al.* 2017). Additionally, several studies revealed that bioflocs may contribute in production of exogenous microbial enzymes like proteases (Arnold *et al.* 2009; Xu & Pan 2012; Zhang *et al.* 2016) and moreover induce the generation of endogenous digestive enzymes (Xu & Pan 2014; Najdegerami *et al.* 2016; Mirzakhani *et al.* 2019) facilitating the digestion and absorption of feed nutrients.

Essential fatty acids, carotenoids, free amino acids, chlorophylls (Ju *et al.* 2008), trace minerals (Tacon *et al.* 2002) and vitamin C (Crab *et al.* 2012) are considered as the bioactive compounds of bioflocs which improve antioxidant status, growth and reproduction in aquatic species. Khanjani (2015) reported that weight gain, length, percentage of body weight gain, growth rate and specific growth rate showed better performance in treatments with biofloc compared to control (no biofloc). Yun *et al.* (2016) stated that when juvenile Pacific white shrimp were cultured in BFT tanks, the dietary protein content could be reduced by up to 10% without affecting growth performance, body composition and hemolymph properties. In zero water exchange systems, improved growth performance is due to the presence of a suitable substrate for the growth and attachment of microorganisms, ultimately, creating a colony and biofloc, together with commercial diets, provide a complete food chain for shrimp growth. Studies have shown that the presence of biofloc improves the digestive system of shrimp, increases growth by 15% and decreases food conversion ratio by 40% (Wasielesky *et al.* 2006). The presence of biofloc-dependent microorganisms in the biofloc treatments results in improving growth performance compared to the clear water treatment (Thompson *et al.* 2002). Biofloc consumption in zero water exchange treatments decreased feed conversion ratio (1.39–1.03) and increased growth rate (0.39–1.25 g week^{-1}; Wasielesky *et al.* 2006). The findings from studies on growth performance in fish and shrimp indicated that species reared under biofloc conditions significantly improves growth parameters compared to non-biofloc systems. These improvements could be due to beneficial microbiota dominance in gut and consequent improvement of digestive function (Zhou *et al.* 2013; Wang *et al.* 2018).

Economic aspects

The main factors affecting the growth and development of the aquaculture industry are environmental protection and feed cost (Avnimelech 2009). Reducing production costs and more profitability are considered as important goals in the aquaculture industry. Growth rate and feed conversion ratio play an important role in aquaculture costs, which are improved in biofloc system compared to conventional system and profitability is also better in the biofloc treatments (Khanjani 2015). Better feed recycling, improved feed conversion ratio, increased specific growth rate and survival rate are key components of aquaculture management costs. Hanson *et al.* (2009) found that biological parameters such as survival rate are effective factors in cost return and profitability. Increasing stocking density (up to 20%) and growth rate enhances profitability by 57% and 45%, respectively (Browdy *et al.* 2001). On the other hand, a 20% reduction in feed costs has a significant impact on profitability. Specifically, investing in aquaculture bases such as eggs, larvae and feed increases survival rate, growth rate and stocking density, which has a positive effect on cost return. In the biofloc system, it is more profitable than the clean water system due to the reduction of commercial feed consumption and the use of biofloc and consequently lower food prices. Production of one kilogram of green tiger shrimp (*Penaeus semisulcatus*) and tilapia using biofloc was associated with 33% (Megahed 2010) and 10% reduction in cost (De Schryver & Verstraete 2009), which depends on the species, diet, amount of consumed biofloc and price of carbohydrates. The biofloc system eliminates the cost of organic and inorganic fertilizers and only covers the cost of carbon source. Reducing the cultivation period, increasing growth rate and survival per cent has made the biofloc system more useful than the clear water system (Sontakke & Haridas 2018). Furthermore, biofloc systems provide more economical benefits such as reducing expenses of water treatment by 30%, and additionally, the efficiency of protein utilization is twice as high in biofloc technology systems in compared with conventional water treatment technologies (De Schryver *et al.* 2008; Avnimelech 2009). Conventional technologies to manage and remove nitrogen compounds are based on either earthen treatment systems, or a combination of solids removal and nitrification reactors (Crab *et al.* 2007).

Application of BFT technology for sustainable aquaculture

In recent years, numerous studies have been carried out on the application of new technologies in aquaculture (multi-species aquaculture, hybrid aquaculture, recirculation system, aquaponics and more recently BFT technology) to increase production. Due to lack of flawless technology, it is more difficult to justify BFT technology and persuade the farmers to set it up than conventional methods. On the other hand, droughts, scarcity and expensive water for the development of aquaculture, the destructive effects of aquaculture effluents on the environment, pollutions and the spread of infectious diseases and consequently attention to the farm biosecurity have resulted that the amount of water exchange in the farms is minimized (Avnimelech 2009). The successful experiences of BFT technology as well as the economic benefits of this technology need to be taught to farmers in a practical way. One of the most important parts in launching BFT technology in aquaculture is monitoring and evaluation of ponds. Water quality monitoring including determining and stabilizing the total concentration of suspended solids, settling solids, the number of aerators, their type and location in the ponds are important (De Schryver *et al.* 2008). Future research should focus on the role and importance of BFT technology to persuade the farmer to launch this technique. Consumers also need to be encouraged to buy organic aquaculture products from BFT ponds. Recycling faeces and converting it into aquatic feed may cause consumers to refrain from buying such products. However, as the population grows, aquaculture strategies are needed to conserve wild fish stocks and control the price of edible fish (Jiang 2010). An increase in population, followed by a shortage of seafood and a pressure on fish stocks, is driving up fish prices (Péron *et al.* 2010). In contrast to the fisheries strategic plans, it is important to preserve fish stocks and reduce fish prices and increase commercial fish stocks. Therefore, BFT technology can reduce the pressure on aquatics stocks and improve social welfare by reducing the cost of fish production, which is beneficial to both the farmer and the consumer. The consumer wants the guarantee that the fish produced is not harmful to its health, respect to ethical and social considerations. BFT technology has been successful in this field. Many researchers are looking to integrate this technology with other aquaculture methods to control water quality and its effects.

Combining the BFT system with the presence of periphyton (Asaduzzaman *et al.* 2008), combining heterotrophic and autotrophic communities in the culture system to control environmental factors (Avnimelech 2009), the use of nitrification, denitrification and anaerobic oxidation of ammonia for nitrogen removal have been investigated (Kumar & Lin 2010). The results of these researchers showed that these technologies with low energy consumption have the potential to control harmful compounds in aquaculture systems. Other researchers have investigated the use of the BFT system for multi-species culture such as tilapia with vegetables, shrimp with microalgae, mussels and seaweed, which have achieved positive results (Kuhn *et al.* 2009). BFT technology can be combined with multi-species ponds to improve water quality, access to natural food, better nutrition performance, growth and production (Rahman *et al.* 2008). BFT technology shows that aquaculture is a sustainable tool that emphasizes environmental, social and economic issues as it develops. Researchers are working to further develop this method and persuading farmers to implement the system principally in new-generation aquaculture. The development of this method requires careful adjustment and implementation so that researchers, farmers and consumers need more research and information to build a platform for this system, which is the basis of sustainable aquaculture.

Management aspects

It is important to control the amount of suspended solids (TSS) in BFT tanks, which is associated with dissolved oxygen, carbon dioxide, pH and inorganic nitrogen compounds (Ray *et al*. 2010), and it also prevents gill clogging. Excessive increase in suspended solids results in reduced oxygen, increased carbon dioxide and pH, and the system loses its performance in a short time (less than one hour). As the flocs develop, the density of filamentous bacteria increases unpredictably, creating a 'filamentous bulking' state that impairs TSS control and interferes with shrimp gills and leads to death (Fig. 2). The major challenge with the biofloc system is the control of TSS. Its concentration should be around 500 mg L^{-1} may reach up to 1000 mg L^{-1} (Avnimelech 2009). Therefore, the level of turbidity increased and the visibility reduced, so FCR will rise and production will decrease. Poor growth performance and FCR as a result of high TSS concentration (more than 500 mg L^{-1}) in tilapia have been reported (Azim & Little 2008). The same result has also been stated about growth performance of shrimp (Furtado *et al*. 2011).

Vibrio bacteria accumulate in shrimp BFT systems and can cause pathogenicity by changing the system conditions (low or high concentrations of suspended solids). In most recirculated aquaculture systems, especially BFT systems, nutrients and minerals (especially metals) accumulate in the water. In shrimp ponds with limited water exchange, nitrate can accumulate up to several hundred mg L^{-1}, which reduces shrimp food intake at one level; in marine systems, maintaining a nitrate concentration of about 50 mg L^{-1} is an effective way to reduce the production of high volumes of toxic hydrogen sulphide. In this regard, water quality management, bacterial control and pollution in the BFT system are indispensable that should be taken into consideration.

Monitoring of the following parameters is important (Avnimelech 2009):

- Oxygen, reduce number of aerators if the level of dissolved oxygen is high. However, if the level of dissolved oxygen is less than 4 mg L^{-1}, some aerators should be added.
- TAN. Low level of TAN (<0.5 mg L^{-1}) means that the system works properly. If TAN increases, the amount of carbon should be added.
- NO_2. Nitrite negatively impacts tilapia. An increase in nitrite may be an indication of the presence of anaerobic areas. If the level of nitrite increases the sludge piles may exist in the pond, so the place of aerators should be changed.
- Floc volume (FV) determination using Imhoff cones is easy and cheap. FV should be in the range of 5–50 mL L^{-1}. Carbohydrates should be added if the level of FV is low and sludge should be removed if its level is more than 50.

Concluding remarks and future directions

Water resources constraint, increasing demand for seafood consumption and land resources constraint to expand aquaculture activities are identified as major problems. To respond to the growing demand for animal protein, intensive aquaculture is one of the main options. The needs for sustainable development and the development of environmentally

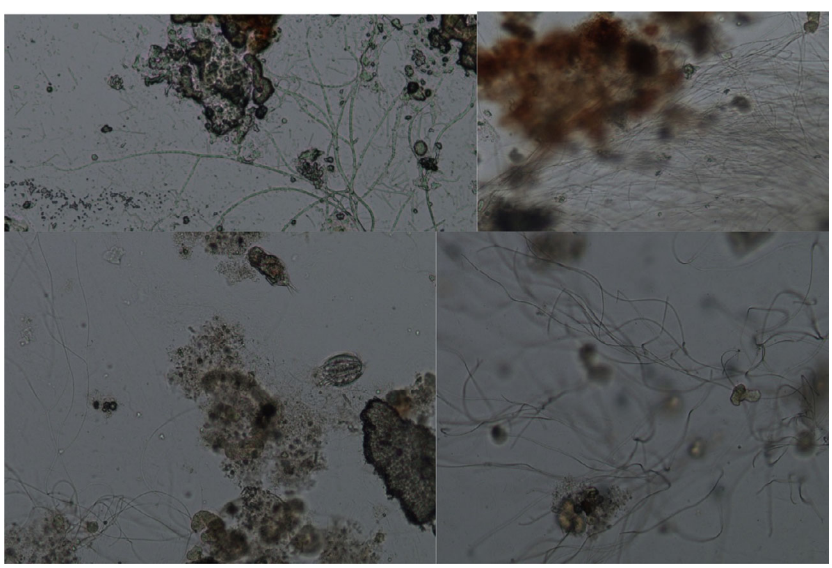

Figure 2 Filamentous bacteria in biofloc system (Khanjani 2015; 2019b).

friendly aquaculture can be achieved through the use of biofloc technology. The use of biofloc in aquatics nutrition is important as it provides aquatics supplementation needs and can be a good substitute for fish meal in the aquatics diet. Bioflocs contain microbial protein, organic polyhydroxybutyrate polymer as well as bacteria containing peptidoglycan and lipopolysaccharides in their cell walls, which, together with commercial diets, formulate a complete food chain for aquatics growth and thus improve growth performance. Studies have shown that the presence of biofloc in the zero water exchange system leads to improved water quality and better growth performance of western white shrimp, which these particles are used by shrimp. Commercial pellets may not provide all the required nutrients for growth of shrimp, some of the nutrients come from the biofloc in the rearing tank. Therefore, biofloc can be used as a food supplement for aquatic animals. Biofloc is produced as a rich food source in the culture system that is available for aquatics 24 h a day. Benefits of the biofloc production system include lower feed intake, reduced water exchange, increased biosecurity, and reduced risk of pathogens, increased growth, and survival and thus increased productivity and production. Also, the use of natural food in the same culture area has increased and the commercial diets are less used, which results in reduced environmental impacts of wastewater. Due to the nutritional value of bioflocs, applying them damply at the same culture area as well as the use of dried biofloc and its addition to the aquatic diet will effectively reduce feed costs and subsequently reduce production costs. Standardization of methods, techniques and equipment for pond construction, stocking management and harvesting in BFT aquaculture systems is required. It is necessary to conduct a detailed study for the construction of BFT ponds; circular ponds with central conical outlet and slope towards the centre are appropriate. Water flow velocity and water disruption in circular ponds are better, and the slope towards the centre causes the excess waste to move outward and discharge easily, and the conical outlet also helps to accumulate excess waste. At the specified time, open the outlet to remove the waste depending on the stocking density, rearing species, size of the fish, etc. Put two pieces at the outlet of the six-inch pipe; the first of which is lower than the surface of the pond water, and by removing the upper part of the pipe, the wastewater will be removed, which the first piece will be inserted again, after the waste has been removed.

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