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-Review-

# Nutritional importance for aquaculture and ecological function of microorganisms that make up Biofloc, a review

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**Abstract:** The objective of this review is to provide an overview of the nutritional importance and ecological function of microorganisms in Biofloc systems. For the elaboration of this review different databases were consulted, principally, PubMed, Scopus, Web of Science and Scielo. First, it is described the interactions between bacteria and phytoplankton in Biofloc, which are important for maintenance of water quality, because they are involved in transformation of organic matter and a better removal of contaminant residues such as nitrogenous compounds. Also, these organisms are important for aquatic ecosystems because they are an essential component of trophic nets in activity and biomass quantity, contributing to regeneration of nutrients and interacting with many organisms serving as base of food chain. Subsequently, mention is made of relevance of zooplankton that develops in Biofloc culture systems, because they represent a key role in animal's nutrition in culture and have proven benefits in growth rates, in food conversion factor and reduction in costs associated to commercial food.

**Keywords**: Heterotrophic bacteria, phytoplankton, aquaculture nutrition, zooplankton

## Introduction

Nowadays, aquaculture production systems have been transformed to solve different activity problematics, as use of large quantities of water, contamination of tributaries discharge and dependence in feeding meal in formulated diets (Hernández-Barraza *et al.,* 2009). Regarding to alimentation, it is important to point out that in production units, large quantities of formulated food of high price are used, which represents 40% of production costs (FAO, 2005). In addition, in most cases, commercial diets do not cover all nutritional requirements of species or include protein contents superior to necessary and cause low digestibility, palatability, and water stability, that up to 60% of food that is supplied it is not used by organisms in culture and when it decompose it compromises water quality (Martínez-Córdova *et al*., 2002; Tacon *et al*., 2004).

Because of this, one production systems that has attracted attention in recent years is Biofloc technology (BFT), which consist in generation of microbial flocs, using an external carbon source, which is available in water and used by bacteria as energy source, that through of redox process they produce less complex chemical forms and nitrogen from food wastes, that are used for proteins synthesis (Azam *et al*., 1983), which results in a micro trophic net, where several ecological relations are carried out (commensalism, competition, predation among others), in a community constituted by bacteria, microalgae, ciliates, rotifers, crustaceans and nematodes which are available whole day as natural food for cultured species (Avnimelech and Kochba, 2009; Emerenciano *et al*., 2013). Different authors agree that there are some advantages of the Biofloc system compared to conventional farming systems. These advantages are: lower investment costs, good use and maintenance of water quality during cultivation, pathogen control, high crop densities and benefits associated with the feeding of cultivated organisms, as well as reduced costs associated with it (Avnimelech, 2009; Crab *et al*., 2012; Hargreaves, 2013).

This cultivation technique allows to work with a much smaller investment than the traditional systems,

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because bio filters, pumping, solids filtration and water disinfection are not used and without considerable water inputs throughout the crop, thus providing an advantage over the use of water resources, being a more economical alternative, reducing water treatment costs by up to 30% (Crab *et al.,* 2012).

It is recognized that the normal operation of the ponds may include the replacement of water (usually 10% per day) as a method to control the quality of the liquid. In contrast Biofloc systems can operate with a low water exchange rate of 0.5 to 1% per day, while maintaining sufficient aeration to maintain suspended flocs, reduce pumping costs, preserve nutrients in tanks and the volume of the effluents is reduced (Gaona *et al.,* 2011; Ray *et al.,* 2011), with the use of this type of systems reduces the discharge of pollutants and diseases to the wild, on the other hand working with high crop densities reduces the ecological footprint of cropping systems, thus contributing to the conservation of ecosystems (Krummeanuer *et al.,* 2011). About the maintenance of water quality, this is mediated by the bacterial community and this is achieved by using a high carbon to nitrogen (C:N) ratio to ensure the best growth of heterotrophic bacteria by applying an external carbon source. The heterotrophic bacteria are responsible for capturing nitrogenous compounds released by the fish and use them in their growth, thus eliminating ammonium and nitrite toxicity (Azim and Little, 2008; Nootong *et al.,* 2011).

Regarding the control of pathogens, Crab *et al.,* (2010) mention that in this type of culture systems, bacteria with probiotic potential are developed, this can be due to the fact that, in the feces, part of the intestinal microbiota is released, and being in a nutrient medium, it is possible that the cultivated species take advantage of the benefits that these microorganisms provide, some of these benefits are: increase in the immune response against infectious processes and better assimilation of nutrients by the cultivated species, which leads to their greater survival and growth, likewise, probiotic bacteria participate in a process of competitive exclusion because they generate a hostile environment to pathogenic bacteria by the excision of exoenzymes and polymers that release in the aquatic environment (Monroy-Dosta *et al.,* 2015).

As for the benefits associated with food, these can be attributed to the fact that microbial aggregates can provide important nutrients such as carbohydrates, proteins, amino acids, fatty acids and minerals. Therefore, microorganisms associated with biofloc play a key role in nutrition of animals in cultivation since they are a rich natural source of protein-lipid "in situ" available 24 hours a day, with the above, it is known that the potential feed gain with this technology is 10 to 20% due to that the costs per feeding decrease between 40 to 50% (Hargreaves, 2013), the nutritional quality of Biofloc is considered good for the cultivation of aquatic organisms since it has been reported that it can contain between 25 and 50% of protein and up to 0.5 to 15% of fats, however, this content may vary in relation To the carbon source used to promote Biofloc, the planktonic community that develops in the system and the cultivated species (Ekasari *et al.,* 2014b; Emerenciano *et al.,* 2013). Despite many benefits, the Biofloc system also has some disadvantages to take into account, for example, the need for a start-up period, the investment required for an aeration system to ensure the maintenance of suspended solids, cyanobacteria blooms and the accumulation of nitrogen compounds, as well as another limitation to increase the use of this type of crops is that, being a system with zero water change, the water becomes cloudy so there is a resistance on the part of the farmers for a misconception that the crop water must always be clean (Hargreaves, 2013).

It has been documented the need of use of live food organisms in of aquatic production, mainly in larvae stages, because they provide higher diversity of nutrients, are easy to assimilate and more attractive for cultured species (Wasielesky *et al.,* 2006). Even though, in last years, studies have been published about positive effect in growth of fish and crustaceans cultured in Biofloc, few studies have been made to characterize developed planktonic communities, their importance as food and ecological function in production productive systems. Because of this, it must be made a review that allows to have a broader picture of planktonic groups that develop in Biofloc culture systems, their importance as natural food for cultured species and the ecological role they have in the system.

## Biofloc system characteristics

Biofloc is an intensive production system in aquaculture, which can overcome difficulties of activity, such as increase in biomass per volume of water and use of less water, overcoming paradigms of sustainability (Avnimelech, 2009). The term “floc” or “Biofloc” can be defined as flocculation of organic matter present in medium, resulting in a high concentration of biomass in form of particles or bioflocs (Cuzon *et al.,* 2004; Emerenciano *et al.,* 2011). This term applies to a compound consisting of 60 to 70% of organic matter, which includes an heterogeneous mix of microorganisms (fungi, algae, bacteria, protozoans, and rotifers) and 30 to 40% of inorganic matter such as colloids, organic polymers, cations and death cells (Fig. 1) (Chu and Lee, 2004).

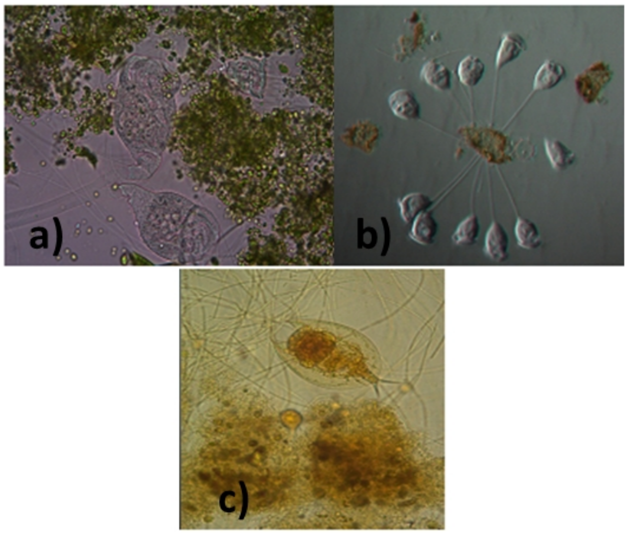


**Fig. 1: Biofloc observed with an optical microscope a) Microalgae and *Vorticella* sp. (40x); b) *Vorticella* adhered to a floc (10x); c) Rotifer of the genus *Lecane* and Cyanobacteria species. (10x) (Own source)**

BFT has been used on different aquaculture species. Table 1 summarizes some studies and show the different carbon sources that have been used in these, besides briefly explain the objectives of the study and the main results

## Diversity of microorganism associated to Biofloc

The diversity of organisms that make up bioflocs are, bacteria, microalgae, yeast, rotifers, ciliates, protozoans, nematodes, and crustaceans (Monroy *et al.,* 2013; Collazos and Arias, 2015), this biodiversity of species, depends on carbon source and cultured specie (Ray *et al*., 2010).

In these bioflocs, both autotrophic and heterotrophic activities occur, also aerobic and anaerobic processes from which key interactions emerge for water quality maintenance, as control of nitrogenous compounds (Ray *et al*. 2010; Ebeling *et al*., 2006). Microorganisms are an essential part of aquatic ecosystems because of their place in trophic webs in marine and freshwater environments both in activity and biomass quantity, contributing to nutrient regeneration and interacting with a wide range of organisms (Monroy *et al*., 2015). Three main groups of microorganisms associated to Biofloc are recognized, these are bacteria, phytoplankton, and zooplankton.

**Tab. 1: Summary of different studies carried out with biofloc technology.**

| Species | CS | Objective | Main result | Ref. |
|---|---|---|---|---|
| *Macrobrachium. rosenbergii* | Acetate, glycerol | Evaluate effect of different carbon sources on the nutritional value of bioflocs, as feed. | They suggest that the choice of the carbon source used for biofloc culture influences its nutritional value. | 1 |
| *Oreochromis. niloticus* | Molasses | Estimate the effect of producing and culturing nile tilapia larvae in Biofloc. | Indicated that Biofloc positively affects nile tilapia larvae performance, in terms of larval growth performance and robustness. | 2 |
| *Litopenaeus. vannamei* | Molasses | They tested the effect of three concentrations of bioflocs in the culture, water quality and performance of shrimp. | They mention that intermediate levels of bioflocs (TSS between 400 and 600 mg L−1) may be suitable to superintensive culture and suggest that ammonia and nitrite in T400-600 and T800-1000 tanks were mainly controlled by nitrifying bacteria, which provided greater stability of these parameters and of dissolved oxygen. | 3 |
| *Penaeus. monodon* | Wheat flour | Suitability of biofloc whit diferents leves of inclusion of dried biofloc, as dietary supplement in shrimp feed | The study demonstrates thatdietary supplementation of biofloc at 4–8% level had beneficial effects on growth performance and digestive enzyme activities. | 4 |
| *Marsupenaeus japonicus* | Sucrose | Investigated the effectiveness of bioflocs technology for maintaining good water quality, supplying feed nutrition and inhibiting potential pathogens. | BFT offers the possibility to simultaneously maintain a good water quality and produce additional food for shrimp, besides microbes detected in bioflocs could be useful in resisting disease in high-intensive shrimp farming systems. | 5 |

**Tab. 1: Continued**

| Species | CS | Objective | Main result | Ref. |
|---|---|---|---|---|
| *Labeo rohita* | Molasses | They investigated the effect of biofloc system, on inorganic nitrogen control, growth and immunological response of rohu. | They report an increase in the growth and welfare of the fish cultivated, which can be attributed to the *in situ* maintenance of water quality within the biofloc system and the presence of microbial flocs. | 6 |

CS = Carbon sourse employed
**Ref.**: 1) Crab *et al*. (2010), 2) Ekasari *et al. (*2015), 3) Schveitzer *et al. (*2013), 4) Anand *et al*. (2004), 5) Zhao *et al.* (2012), 6) Kamilya *et al*. (2017)

## Bacteria

Bacteria and unicellular fungi are important for aquaculture production systems because they act in a positive way in organic matter transformation, removal of contaminant compounds and as a source of microbial biomass available for larger organisms (De Scryver *et al*., 2008).

Heterotrophic bacteria, is the most important bacterial group in Biofloc system their growth is involved in a better removal of contaminant compounds, through their metabolism. These bacteria release many inorganic compounds to environment that can be used by other organisms and produce exo-enzymes which decompose diverse compounds as cellulose, lignin, keratin, and other natural molecules that are hard to transform. The processes of denitrification and decomposition will not be carried out so effectively without the involvement of heterotrophic microorganisms (Avnimelech, 1999), these microbial groups reduce impact through removal of ammoniacal nitrogen by nitrification process that consists in successive oxidation of ammonia first to nitrite and finally to nitrate. In Biofloc systems, transformation of toxic nitrogenous compounds is more efficient, because this process is carried out by bacteria of *Bacillus* and *Pseudomonas* genders (Monroy *et al.,* 2015). Also, presence of organic matter as carbon, inhibits denitrification by autotrophs, so they grow up slowly compared to heterotrophic, that increase their population faster in water with high content of organic matter and high oxygen concentration, so they can transform ammoniacal nitrogen into low toxicity compounds; even some of them, transform this compound to produce microbial biomass and allow generation of diverse protozoans in a short time (days) compared to conventional systems (Ebeling *et al*., 2006). Studies where they have a more specific characterization on bacterial genus or species in Biofloc are few, some of them are resumed in Table 2. In addition to its ecological function, these bacteria can be used as unicellular or microbial protein (SCP or Single Cell Protein) and can provide important percentages of protein (40-80% of raw protein in dry basis, depending on specie) (Tacon, 2013), and therefore they can be considered as a supplement for fish and shirmps larvae (Palmerin *et al*., 2012).

**Tab. 2: Description of bacterial groups reported in Biofloc systems by different authors.**

| Marine or freshwater specie employed | Reported bacterial group | Ref. |
|---|---|---|
| Reported the different bacterial groups that can be found in Biofloc | Indicated the presence of genus such as *Pseudomonas, Bacillus, Alteromonas* and *Micrococcus, Cellulomonas, Clostridium* | 1 |
| *Marsupenaeus japonicus* | They report predominant bacterial communities, such as Proteobacterium, Actinobacterium*, Bacillus sp.*, *Roseobacter sp.*and *Chytiphaga* sp. | 2 |
| *Litopenaeus stylrostris* | Reported bacterial phyla such as *Proteobacteria, Bacteroidetes, Cyanobacteria, Planctomycetes* and *Verrumibrobia* | 3 |
| *Oreochromis niloticus* | Reported genus of probiotic and degradative bacteria: *Lactobacillus Burkholderia, Lactococcus, Saccharomyces, and Bacillus* among others | 4 |
| Employed fresh pond water to inoculate the tank with natural microorganisms | Reported the presence of different phyla: *Firmicutes,* Protobacteria, Actinobacteria, Acidobacteria | 5 |
| *Litopenaeus vannamei* | Reported mainly pathogen species such as: *Vibrio rotiferianus, Photobacterium jeanii, Photobacterium damselae,* Pseudoalteromonas spongiae, *Vibrio mytil* | 6 |

**Ref.:** 1) Monroy *et al.* (2015), 2) Zhao *et al.* (2012) , 3) Cardona *et al.* (2016) , 4) Maya *et al. (*2016), 5) Wei *et al.* (2016) , 6) Luis-Villaseñor *et al. (*2015)

## Phytoplankton

Another important group in Biofloc systems is phytoplankton, microalgae use ammonia, and compounds as nitrite and nitrate for construction of proteins and sugars, and they also provide oxygen during day time. Diverse phytoplankton species, like diatoms, are nutritive and can improve the production of cultured species as prawn culture, due to its contributions of essential amino acids and highly unsaturated fatty acids (Moss, 2002).

Genus and species reported by various authors are; *Scenedesmus quadricauda, Coelastrum sp., Pediastrum dúplex, Cyclotella sp, Navicula sp, Synedra sp, Fragilaria sp, Fragilaria, Orthoseira, Rhabdonema, Ulothrix, Skeletonema, Cylindrotheca, Hemiaulus, Phymatodocis, Ulothrix, Cyanobacteria Chlorophyta, Bacillariophyta, Euglenophyta* and *Dinophyta*, (Ferreira and Otavio 2014; Castro *et al.,* 2014; Monroy *et al.,* 2013; Brito *et al.,* 2013).

Nutritional value of microalgae, is related to the environment in which they develop and inorganic compounds they use for growth. The composition of carbohydrates, regarding to specific sugars, can considerably variate between algae species. Generally, glucose is found in higher concentrations, followed by galactose, mannose, and ribose. Nutritional values of some microalgae species are shown in the Table 3. According to Fernandez-Reiriz (1989), green microalgae have low concentrations of monounsaturated fatty acids and high concentrations of polyunsaturated that are of great importance for growth of fish and crustaceans.

**Tab. 3: Nutritional values of micro algae according to the authors cited.**

| Species | Protein | Lipids | Carbohydrate | Ref. |
|---|---|---|---|---|
| Freshwater *Scenedesmus* | 24.1 | 2.47 | n/a | 1 |
| Marine *Dunaliella* | 38.3-52.8 | 7.7-10.8 | 8.4-12.7 | 2 |
| Marine *Chlorella* sp. | 16.6-35.8 | 13.6-15.3 | 22.6-54.5 | 3 |
| Marine *P. lutheri* | 16.02-52.35 | 12.39-37.83 | 15.63-53.10 | 4 |

Values represented in percentage, n/a = Not available

**Ref.:** 1) Andrade *et al.* (2009), 2) Suárez *et al*. (2007), 3) Paes *et al.* (2015), 4) Fernandez-Reiriz, (1989)

Therefore, today different microalgae are specifically used in aquaculture, taking into consideration their nutritive inputs and its ecological importance because they are base of all aquatic food chains (Muller, 2000). There is an important microalgae-bacteria relation as determinant of groups develop in aquatic environments, in such a way the increment of carbon-transforming heterotrophic bacteria, make possible an increase of diatoms in system and restrict other groups like cyanobacteria, that are potentially harmful in aquaculture systems because they can form superficial layers that adhere to fish gills causing mortality, also some genus as *Anabaena* sp. and *Ocillatoria* sp. have capacity to produce toxic metabolites that can increase in case of exponential growth (Monroy *et al.,* 2013; Massaut and Ortiz, 2003).

## Zooplankton

In aquaculture production systems with Biofloc, diverse planktonic groups develop in a natural way, such as rotifers, protozoans (ciliates and flagellates), crustaceans and nematodes which play an important role in nutrient recycle, maintenance of water quality and in nutrition of cultured animals (Emerenciano *et al.,* 2013). Consumption of zooplankton present in Biofloc has shown countless benefits, such as improvement of growth rate, increase in food conversion factor and benefits costs associated to balanced food (Hargreaves, 2013). Proximal composition of some planktonic species that are found in bioflocs, indicate that rotifers can contain between 54 and 60% of raw protein, while cladocerans 50-68% and copepods 70-71%, regarding lipid values in rotifers, they contain from 3.9 to 13.2% depending on specie, cladocerans have between 1 and 2.9% while copepods can contain up to 2.6% of lipids (Ray *et al*., 2010), while nematodes are 76% water and 24% dry matter; 40% of dry matter is protein and 20% fat, remaining 40% correspond to nitrogen-free extract and other macronutrients (Figueroa, 2009).

## Nutritional contributions of Biofloc

The carbon source is the main input for Biofloc system operation, so selection of this source can cause variation in nutritional value and taxonomic composition of bioflocs (Crab *et al*., 2012). Maicá *et al*. (2012) using molasses as carbon source reported protein values of 28.7 to 43.1% and lipids between 2.11 and 3.62% in bioflocs used to culture *L. vannamei*. In tilapia cultures using wheat flour protein levels of 38% and lipid values between 3.16 and 3.23% (Azim and Little, 2008). Emerenciano *et al*. (2011), reported values of protein of 30.4%, carbohydrates of 29.1% and lipids of 0.5% in a system adding molasses; Crab *et al*. (2009), used acetate, glycerol and glucose as carbon source finding values between 42 and 58.9% of protein in dry weight in their different treatments; López Tarín (2011) made a tilapia culture in closed system, partially substituting formulated food with bioflocs with molasses, and found that their composition presented acceptable values of proteins and lipids. Other evidences of nutritional contributions that Bioflocs can contribute are shown in Table 4.

**Tab. 4: Bromatological composition based in bioflocs dry matter and compared with commercial food for fattening stage.**

| Carbon source | RP (%) | Carb (%) | Lipids (%) | RF (%) | Ashes (%) | Ref. |
|---|---|---|---|---|---|---|
| Starch | 31.5 | n/a | 85.2 | n/a | 12.4 | 1 |
| Saccharose | 49 | 36.4 | 1.13 | 12.6 | 13.4 | 2 |
| Unrefined granulated sugar | 23.7-25.4 | 32.2-39.1 | 2.6-3.5 | n/a | 33.0-40.4 | 3 |
| Molasses with cornmeal | 23.5-32.3 | n/a | 2.9- 5.33 | n/a | 20 - 36 | 4 |
| - | 25-35 | n/a | 5-7 | 3-6 | 5-12 | 5 |
| - | 30-35 | n/a | 5-7 | 4 | 11-12 | 6 |

RP= Raw protein, Carb= Carbohydrates, FB= Raw Fiber, n/a= Not available

**Ref.:** 1) Wei *et al.* (2016), 2) Kuhn *et al.* (2009), 3) López *et al.* (2014), 4) Wang *et al.* (2016), 5) Commercial food for tilapia (Alimentos del Pedregal®), 6) Commercial food for shrimp (Alimentos del Pedregal®)

Benefits of these nutritional inputs are reflected in higher growth rates, survival, improvement in food conversion factors and general well, being in cultured species. Regarding this, Azim and Little (2008) in a Nile Tilapia *Oreochromis niloticus* culture, in Biofloc system, reported a production 45% higher in this system compared to traditional system. Sierra-De la Rosa *et al*. (2009), presented good growth rates (500 g/226 days; 2.1 g/day), survival of 70% and better food conversion factor (1.5) in a study with tilapia.

## Conclusions

Nutritional quality contributed by microorganisms associated to Biofloc is comparable and/or superior regarding to commercial food in terms of protein and fats, also they contribute and adequate content of carbohydrates and ashes for its use as food in aquaculture, bioflocs are also good vitamin and mineral sources, specially of phosphorus, calcium, and magnesium (Crab *et al*., 2011; Hargreaves, 2013; Emerenciano *et al*., 2013). On other hand, diverse investigators (Maya *et al.,* 2016; Azim and Little, 2008) coincide that Biofloc technology application, improves different aspects like higher growth rates, increased survival, better food conversion and decrease in diseases. Also, there are important environmental benefits, because of amount of used water reduction during culture cycle, because microorganisms regulate water quality present in Biofloc which transform organic wastes, favoring environmental conservation (Avnimelech, 2009; Wasieliesky *et al.,* 2006; Ekasari *et al.,* 2014a). Nutritional and ecological role that microorganisms present in Biofloc develop is important, nevertheless, is still unknown in its totality, so it is suggested to carry out more studies that characterize species diversity, both bacterial and planktonic that can be present in Biofloc systems and that help to explain interactions that are carried out in this type of aquaculture farming systems.

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