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Chapter 5

Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture

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Abstract

Biofloc technology (BFT) is considered the new "blue revolution" in aquaculture. Such technique is based on in situ microorganism production which plays three major roles: (i) maintenance of water quality, by the uptake of nitrogen compounds generating in situ microbial protein; (ii) nutrition, increasing culture feasibility by reducing feed conversion ratio (FCR) and a decrease of feed costs; and (iii) competition with pathogens. The aggregates (bioflocs) are a rich protein-lipid natural source of food available in situ 24 hours per day due to a complex interaction between organic matter, physical substrate, and large range of microorganisms. This natural productivity plays an important role recycling nutrients and maintaining the water quality. The present chapter will discuss some insights of the role of microorganisms in BFT, main water quality parameters, the importance of the correct carbon-to-nitrogen ratio in the culture media, its calculations, and different types, as well as metagenomics of microorganisms and future perspectives.

Keywords: microbial floc, shrimp, fish, microorganisms, nitrogen compounds, metagenomics

1. Aquaculture: state of the art and challenges

In a world where more than 800 million people continue suffering from chronic malnourishment and where the global population is expected to grow by another 2 billion to reach 9.6 billion people by 2050, it is important to meet the huge challenge of feeding our planet while safeguarding its natural resources for future generations [1]. In this context, aquaculture plays a key role in eliminating hunger, promoting health, reducing poverty, as well as generating

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jobs and economic opportunities. According to FAO [1], the world food fish aquaculture production expanded at an average annual rate of 6.2% in the period 2000–2012 from 32.4 million to 66.6 million tons, in which Africa grew 11.7%, Latin America and the Caribbean 10%, Asia (excluding China) 8.2, and China 5.5. Employment in the sector has grown faster than the world's population. The sector provides jobs to tens of millions and supports the livelihoods of hundreds of millions. Fish continues to be one of the most traded food commodities worldwide. It is especially important for developing countries, sometimes worth half the total value of their traded commodities.

On the other hand, global aquaculture has yet to face some serious challenges. For instance, aquaculture has been accused of being an unsustainable activity, because of the effluents discharged to the environment which contain excess of organic matter, nitrogenous compounds, toxic metabolites, and elevated rates of chemical and biochemical oxygen demands [2]. Other serious accusations include the competition for land and water, the introduction of exotic species around the globe, the overexploitation of ocean fish stocks to obtain fishmeal and fish oil, the dispersion of pathogens, the development of antibiotic resistance genes, etc. [3, 4].

Furthermore, aquaculture has to constantly deal with other problems, such as the shortage of ingredients and their price volatility. Thus, strategies aimed to overcome these challenges are required. In this regard, the modification of physicochemical variables of the culture system to favor the proliferation of particular biotic communities has been adopted not only to improve the recirculation of nutrients (and the consequent detoxification of the system) but also to use the biomass of such biotic communities as direct food source for the cultured organisms [5]. These kinds of systems, also known as biofloc (BFT) technology systems, promise to solve some of the above challenges and revolutionize aquaculture [6].

2. Definition and applications of biofloc technology (BFT) in aquaculture

Biofloc technology (BFT) is as an environmentally friendly aquaculture technique based on in situ microorganism production. Fish and shrimp are grown in an intensive way (minimum of 300 g of biomass per square meter [7]) with zero or minimum water exchange. In addition, continuously water movement in the entirely water column is required to induce the macroaggregate (biofloc) formation. Nutrients in water (in accordance with a known carbon-to-nitrogen ratio of 12–20:1) will contribute naturally to a heterotrophic microbial community formation and stabilization. These microorganisms play three major roles: (i) maintenance of water quality, by the uptake of nitrogen compounds generating in situ microbial protein; (ii) nutrition, increasing culture feasibility by reducing feed conversion ratio (FCR) and a decrease of feed costs; and (iii) competition with pathogens.

BFT is considered the new "blue revolution" since nutrients can be continuously recycled and reused in the culture medium, benefited by the minimum or zero-water exchange. Also, the sustainable approach of such system is based on the high production of fish/shrimp in small areas. In addition, the bioflocs is a rich protein-lipid natural source of food available in situ

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24 hours per day due to a complex interaction between organic matter, physical substrate, and large range of microorganisms. This natural productivity plays an important role recycling nutrients and maintaining the water quality. The consumption of biofloc by shrimp or fish has demonstrated innumerous benefits such as improvement of growth rate, decrease of FCR, and associated costs in feed [8].

Regarding the applications, in the past years, BFT has been used in grow-out phase for tilapia [9, 10] and marine shrimp [11, 12], nursery phase [13–15], freshwater prawn culture [16, 17], broodstock formation and maturation in fish [18] and shrimp [7–19], and as aquafeed ingredient also called as "biofloc meal" [20–22]. In addition, recently BFT also has been applied in carp culture [23], catfish culture [24], and cachama culture [25].

3. Microorganisms as a tool for water quality management

3.1. Main water quality parameters in BFT

Water quality maintenance and monitoring in aquaculture are the essential practices aiming at the success of the growing cycles. Temperature, dissolved oxygen (DO), pH, salinity, solids [total suspended solids (TSS) and settling solids], alkalinity, and orthophosphate are some examples of parameters that should be continuously monitored, especially in BFT. The comprehension and understanding of water quality parameters and its interactions in BFT are crucial to the correct development and maintenance of the production cycle. For example, safety ranges of pH, DO, total ammonia nitrogen (TAN), solids, and alkalinity will lead a health growth and avoid mortalities. N:P ratio (normally using nitrate and orthophosphate values) will influence the autotrophic community that will occur in the system (e.g., chlorophytes versus cyanophytes). The same recommended water quality parameters ranges and/or normal ranges observed for tropical species (e.g., marine shrimp *Litopenaeus vannamei* and tilapia) in BFT are presented in **Table 1**.

3.2. The role of microorganisms in BFT aquaculture systems

Microorganisms play a key role in BFT systems. The maintenance of water quality, mainly by the control of bacterial community over autotrophic microorganisms, is achieved using a high carbon-to-nitrogen (C:N) ratio, since nitrogenous by-products can be easily taken up by heterotrophic bacteria. High carbon-to-nitrogen ratio is required to guarantee optimum heterotrophic bacteria growth, using this energy for maintenance (respiration, feeding, movement, digestion, etc.) but also for growth and to produce new bacterial cells.

The stability of zero or minimal water exchange depends on the dynamic interaction among communities of bacteria, microalgae, fungi, protozoans, nematode, rotifer, etc. that will occur naturally. Such consortia of microorganism will help on the water quality maintenance and recycling wastes to produce a high-value food. In a study with stable isotopes, Burford et al. [12] estimated a daily nitrogen retention of 18–29% into the shrimp obtained from biofloc biota, while Avnimelech and Kochba [26] found about 25% of assimilation for tilapia, using the same technique.

Parameter	Ideal and/or normal observed ranges	Observations
Dissolved oxygen (DO)	Above of 4.0 mg L^{-1} (ideal) and at least 60% of saturation	For correct fish, shrimp, microbiota respiration, and growth
Temperature	28–30° (ideal for tropical species)	Besides fish/shrimp, low temperatures (~20° C) could affect microbial development
pH	6.8–8.0	Values less than 7.0 is normal in BFT but could affect the nitrification process
Salinity	Depends on the cultured species	It is possible to generate BFT, e.g., from 0 to 50 ppt
TAN	Less than 1 mg L^{-1} (ideal)	Toxicity values are pH dependent
Nitrite	Less than 1 mg L^{-1} (ideal)	Critical parameter (difficult to control). Special attention should be done, e.g., on protein level of feed, salinity, and alkalinity
Nitrate	0.5–20 mg L^{-1}	In these ranges, generally not toxic to the cultured animals
Orthophosphate	0.5–20 mg L^{-1}	In these ranges, generally not toxic to the cultured animals
Alkalinity	More than 100 mg L^{-1}	Higher values of alkalinity will help the nitrogen assimilation by heterotrophic bacteria and nitrification process by chemoautotrophic bacteria
Settling solids (SS)	Ideal: 5–15 mL L^{-1} (shrimp), 5–20 (tilapia fingerlings) and 20–50 mL L^{-1} (juveniles and adult tilapia)	High levels of SS (measured in Imhoff cones) will contribute to the DO consumption by heterotrophic community and gill occlusion
Total suspended solids (TSS)	Less than 500 mg L^{-1}	Idem to SS

Table 1. Main water quality parameters monitored in BFT systems and its ideal and/or normal observed ranges.

Organic matter and nitrogen wastes are a huge problem in aquaculture. Phytoplankton, heterotrophic, and nitrifying bacteria have the most important role in the nitrogen and OM reutilization. Fungi, ciliate, protozoa, rotifer, copepod, and nematode complement the biofloc community, participating in the recycling of organic matter as a part of complex food webs which include the cultured species.

Mutualism and commensalism relationships occur among some group of microorganisms in BFT, e.g., bacteria-bacteria or bacteria-microalgae. In low water exchange cultures, complex biofilms are generated in which coexist heterotrophic and nitrifying bacteria. Inorganic ions are attracted to the surface of these biofilms and the solid surfaces of the substrate, promoting greater nitrification processes [27]. Some bacterial strains have a positive effect on microalgae growth not only for planktonic species but also on attached (benthic) species [28]. The extracellular polysaccharides of benthic diatoms may be used by heterotrophic organisms as carbon source [29].

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One current practice in BFT is the use of commercial bacteria consortia (probiotics). The main reasons of probiotics used in BFT are (i) help to stabilize the heterotrophic community and to compete with autotrophic microorganisms (mainly in the initial phases), (ii) help to recycling the organic matter, and (iii) control solids and TAN levels.

3.2.1. Bacteria

The heterotrophic bacteria use the organic compounds as a carbon source. This community can minimize ammonia accumulation in the water column through incorporation as bacterial biomass. Under suitable conditions (temperature, carbon:nitrogen ratio, pH, etc.), bacteria have a fast growth. Leonard et al. [30] estimated that the generation time for the free viable heterotrophic populations was around 2.5 h in laboratory conditions.

Heterotrophic bacteria utilize sugar, alcohol, and organic acids as energy source but exist in specialized species capable of decomposing cellulose, lignin, chitin, keratin, hydrocarbons, phenol, and other substances [31]. Heterotrophic bacteria are able to colonize a high diversity of environments; they are common in soil, freshwater, and saltwater. Aquatic environments are responsible to recycle high amounts of dissolved and particulate organic matter, playing one of the most important roles in the food webs [32]. In biofloc system, the heterotrophic bacteria colonize the feces, molts, dead organisms, and unconsumed food to produce bacterial biomass, which is consumed by detritivores [8]. Brown et al. [33] evaluated the biochemical compositions of seven strains of marine bacteria and reported protein content (dry weight) of 29–49%, carbohydrates 2.5–11.2, lipids 4–6%, and, additionally, the presence of all essential amino acids.

Chemoautotrophic bacterial community (i.e., nitrifying bacteria) obtains energy through oxidation of toxic nitrogen compounds. The nitrifying bacteria are naturally promoted by the presence of ammonia and nitrite as well as the accumulation of flocculated matter (used as substrate). The alkalinity consumed by these microorganisms must be replaced by different sources (i.e., sodium bicarbonate, calcium carbonate, or calcium hydroxide [34]). In laboratory conditions, the generation time of ammonia oxidizer bacteria was estimated to 25 h and nitrite oxidizer to 60 h [30].

The nitrifying bacteria thrive in a wide diversity of environments [35]. Besides the oxygen, toxic nitrogen compounds are the major concern into the biofloc systems. The main sources of ammonia are excretion of cultured organism and the decomposition of nonliving matter (dissolved and particulate). In BFT, three nitrogen conversion pathways occur for the removal of ammonia nitrogen: (a) photoautotrophic removal by algae, (b) autotrophic bacterial conversion from ammonia to nitrate, and (c) heterotrophic bacterial conversion of ammonia nitrogen directly to microbial biomass [36]. In long term, the most efficient process is the autotrophic, in which two bacterial groups are involved: (a) the ammonia-oxidizing bacteria, which obtain their energy by catabolizing unionized ammonia to nitrite, including the genera *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus*, and *Nitrosovibrio* and (b) the nitrite-oxidizing bacteria, which metabolize nitrite to nitrate, including the genera *Nitrobacter*, *Nitrococcus*, *Nitrospira*, and *Nitrospina* [37].

3.2.2. Fungi

Fungi are a group of eukaryotic organisms that include unicellular microorganisms, such as yeasts and molds, as well as multicellular fungi. Most yeasts reproduce asexually by mitosis and many others by the asymmetric division [38]. Typically measuring 3–4 μm in diameter, they are widely distributed in freshwater and saltwater (*Candida*, *Cryptococcus*, *Rhodotorula*, and *Debaryomyces*). Some marine species live at temperatures as low as −13° and at deeps of 4000 m; some others can nearly saturate brine solutions. Seawater normally contains 10–100 yeast L^{-1}, but in estuarine environments, the number significantly increases [39]. Brown et al. [33] evaluated seven species of yeast to determine their nutritional value and found 25–37% of protein, 21–39% of carbohydrate, and 4–6% of lipid, as well as complete profile of essential amino acids. Yeasts are strictly chemoorganotrophic and require organic forms of carbon which are quite diverse and include sugars, polyols, organic and fatty acids, aliphatic alcohols, and various heterocyclic and polymeric compounds [39].

Fungi, especially yeast (chemoorganotrophic microorganisms), are also reported in biofloc. They use organic compounds as a source of energy. Carbon is obtained mostly from hexose sugars, such as glucose and fructose. In a biofloc culture of tilapia, Monroy-Dosta et al. [40] reported the presence of the yeast *Rhodotorula* sp. during the fifth week, which increases its biomass by the end of the culture period.

3.2.3. Microalgae

Photoautotrophic community (microalgae) also play an important role in the biofloc system. Microalgae assimilate mainly ammonia and nitrate to produce biomass, additionally consume carbon dioxide, and produce oxygen. The divisions of microalgae reported in biofloc cultures are Chlorophyta, Chrysophyta, and Cyanophyta. These microorganisms catch the solar energy, to produce chemical energy (carbohydrates), which is used in their metabolic process.

In biofloc cultures the microalgae can live as free cell into the water column or could form aggregates. In some cases the aggregations of chrysophytes and cyanophytes can measure up to 2 mm in diameter [41]. Their sizes are highly variable, with cells of less than 10 μm to more 50 μm [42].

Chlorophytes are green microalgae that are the most numerous and diverse in the freshwater; they can reproduce massively forming blooms, but, at difference of the cyanophytes, are nontoxic. This division presents a high plasticity and is able to colonize diverse habitats; they are spherical or oblong and may have flagella or not [43].

Chrysophytes are the most representative organisms that correspond into the Bacillariophyceae class (diatoms), which is divided in centric and pennate. The planktonic species are mainly centric; meanwhile, the pennate are commonly benthic. All centric species are marine, while most of the pennate live in freshwater [43]. In aquaculture, diatoms are considered as beneficial algae because they are a source of food and nutrients for most aquatic animals [44].

Cyanophytes are known as the most ancient photosynthetic organisms; they possess a high morphologic and structural variability. During its evolution they have developed various

ecophysiological adaptation strategies to survive in extreme environmental conditions [45]. Given its abundance in different environments, the division Cyanophyta is important for nutrient circulation, incorporating nitrogen into the food chain, which makes them primary producers or decomposers.

In aquaculture ponds, excessive concentrations of major nutrients (nitrogen and phosphorus) can lead to uncontrolled microalgae blooms, sometimes are cyanobacteria dominated which is known to produce some toxic compounds to aquatic animals, and can cause unpleasant flavors in cultured species [46]. Several authors have reported the presence of cyanobacteria in biofloc, with concentrations varying according to the biofloc type. Becerra-Dórame et al. [47] reported 2.1 × 104 cells mL^{-1} in heterotrophic biofloc, while in autotrophic, they found 3.3 × 106 cells mL^{-1}. Although cyanobacteria can become toxic or problematic, Lezama-Cervantes et al. [48] found several species of Cyanobacteria (*Nostoc* sp., *Anabaena* sp., *Phormidium* sp., *Chroococcus* sp., *Oscillatoria* sp., and *Lyngbya* sp.) in a microbial mats used to culture *L. vannamei* postlarvae and fund evidenced of active grazing by the shrimp.

Aquaculture microalgae are widely used; their nutritional characteristic has permitted to produce crustaceans, fishes, and mollusk in laboratory. Several factors can contribute to the nutritional value of microalgae, including its size and shape, digestibility, biochemical composition, and bioactive compounds as enzymes, vitamins, antioxidants, etc. Microalgae grown to late-logarithmic growth phase typically contain 30–40% protein, 10–20% lipid, and 5–15% carbohydrate; PUFAs derived from microalgae, i.e., docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA), are known to be essential for various farmed species [49].

In general, microalgae are common inhabitants in biofloc, even in a bacteria dominated (heterotrophic). Monroy-Dosta et al. [40] evaluated the microorganism composition in biofloc tilapia culture, indicated that the first microalgae appeared were chlorophytes, followed by diatoms and finally cyanobacteria, and also mentioned that diatoms achieved the highest concentration and cyanobacteria the lowest. Ray et al. [50] cultured *L. vannamei* in zero-water exchange, and differing from the previous authors, in their study the chlorophytes were dominant over the diatoms. Biofloc systems are highly dynamic; Kuang et al. [51] indicate that in nature, certain species of ciliates and rotifers have a selective consumption of microalgae, and therefore may influence their diversity. The physicochemical parameters also affect the microalgae dominance. Maicá et al. [52] observed in a *L. vannamei* biofloc culture greater abundance of chlorophytes in salinity of 2 %, while in 25 % diatoms were the most abundant.

3.2.4. Zooplankton

Protozoa is one of the most relevant microorganism groups in BFT system. They play an essential role (together with bacteria) recycling the organic matter in the system. Both groups are the "basis" of the trophic transfer of energy to the next levels. Protozoa have different body shapes (spherical, oval, and elongated) and often have one or more whip appendages called flagella or many short hair-like structures called cilia. Protozoa are abundant in many types of environments and often are found on the surfaces of submerged rocks, free living into the water column or colonizing the sediment. Ciliates are the largest group of protozoa

in nature; they eat bacteria (including cyanobacteria) and small phytoplankton. Some are carnivorous and feed on zooplankton [53].

In nature, ciliates have importance as a live food source for juvenile stages of aquatic animals including small invertebrates. Pandey et al. [54] carried out the analysis of the ciliate *Fabrea salina* to evaluate proximate and biochemical composition. The moisture, protein, fat, carbohydrate, and ash content from natural sources were 86.66%, 56.66%, 36.66%, 1%, and 4%, respectively. Gas chromatographic analysis revealed the presence of fatty acids such as oleic, palmitic, palmitoleic, linoleic, and stearic.

Ballester et al. [14] registered concentrations from 39 to 169 ciliates/mL, in postlarvae *Farfantepenaeus paulensis* biofloc culture. Maicá et al. [52] found an average concentration of 164, 64, and 29 ciliates mL^{-1} in water salinities of 2%, 4%, and 25%, respectively. Furthermore, Monroy-Dosta et al. [40] observed minimum and maximum concentrations of 13 and 39 and also noted a variation in species according to the culture age.

Rotifer belongs to the smaller group of metazoans. Most rotifers are 0.1–0.5 mm long. Their body shape varies widely between groups: they can be spherical, cylindrical, or elongated. The body can be soft or may have a firm covering called lorica. The cilia surrounding a rotifer's mouth form a circle, called a corona or wheel organ. The rapid movements of the cilia create water currents for swimming and feeding [53]. Their diets consist on microalgae, bacteria, yeast, and protozoa [55].

The rotifers are the group of organisms that probably have been largely used to replace *Artemia* as exogenous natural food in larval culture of crustaceans and fish. Campaña-Torres et al. [56] evaluated the proximal composition of the rotifer *Brachionus rotundiformis* cultured in laboratory and reported a dry content of carbohydrate of 15.9–22.7%, lipid 21.4–24.12%, protein 45.7–61.3%, and ash 4.5–4.6%.

Loureiro et al. [57] indicate that rotifers can fragment the flocs and consume bacteria. The mucilage produced by their excretions contributes to new flocs formation [58]. Ballester et al. [14] registered concentrations form 4.6 to 151 org/mL in seawater; besides, Monroy-Dosta et al. [40] reported concentrations between 28 and 96 org/mL in freshwater.

Copepods comprise two main groups: calanoids and cyclopoids. Calanoid copepods have an elongated body and the first pair of antennae is long, whereas cyclopoid have a robust body and a first pair of antennae is short. In general, both use the appendices near the head to create streams to filter or collect food. They feed on bacteria, phytoplankton, detritus, or any other organic material [53].

Farhadian et al. [59] evaluated the proximate composition of copepod *Apocyclops dengizicus* and reported protein levels between 39 and 42% and lipid between 16 and 19%, indicating that nutritional properties varied according to the microalgae used as feed.

Cladocerans posses a body covered by a transparent shell, although it may be yellowish or brown. A pair of appendages called thoracic members are inside the shell, and are important for the capture and transfer of food particles in the mouth. In general, cladocerans eat a wide

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variety of phytoplankton and suspended matter. They can greatly reduce the abundance of phytoplankton in the water column [53].

As the other zooplankton groups, the cladocerans play an important role into the natural food webs. They could supply a high amount of protein into the biofloc cultures. Berberovic [60] evaluated the elemental composition (CHN) of two *Daphnia* species, reporting the following: C, 46.1%; H, 6.5%; N, 9.7%; and ash, 23.8%, which permit to estimate a protein content of 60.6%. This group of organisms was reported in biofloc system by Emerenciano et al. [61]. Moreover, in a postlarvae culture of *L. vannamei* reared in zero-water exchange, Ferreira-Marinho et al. [62] reported *Cladocera* abundance from 0.89 to 1.16 ind mL^{-1} represented by the genus *Bosmina* (0.39–0.53 ind mL^{-1}) and *Daphnia* (0.50–0.69 ind mL^{-1}).

Nematoda is the other essential group in BFT. The body of these organisms is perfectly cylindrical, coated by a relatively thick noncellular cuticles secreted by the underlying epidermis. The cuticle is composed primarily of collagen [63]. They continuously ingest bacteria and other microbenthic organisms; almost all particles which fit into the buccal cavity are ingested, hinting at a selection mechanism based primarily on particle size. Moens and Vincx [64] proposed six major nematode feeding strategies: (a) microvores; (b) ciliate feeders, (c) deposit feeders sensu stricto, (d) epigrowth feeders, (e) facultative predators, and (f) predators.

Ray et al. [50] mentioned that nematodes are an important group in the biofloc systems, whose abundance is determined by the presence of various ciliates that serve as food. In other studies, Monroy-Dosta et al. [40] observed the appearance of nematodes around the fourth week with average of 25 org mL^{-1} with a maximum of 125 org mL^{-1}, and their abundance were correlated with the ciliates' presence. Loureiro et al. [57] reported the presence of nematodes in the stomach contents of fish grown in the biofloc and suggest that they are a rich source of live food in situ.

4. Carbon:nitrogen (C:N) ratio and its application

The management of the carbon-to-nitrogen ratio (C:N) in BFT is normally divided in two phases: (i) initial and formation phase, utilizing a carbon-to-nitrogen ratio of 12–20:1, and (ii) maintenance phase, utilizing a carbon-to-nitrogen ratio of 6:1, according to the total ammonia nitrogen (TAN) values.

In the beginning of culture period, high carbon-to-nitrogen ratio (12–20:1) in water is a key factor to promote and stabilize the heterotrophic community in BFT [8]. High carbon concentration will induce the nitrogenous by-product assimilation by heterotrophic bacteria and also will supersede the carbon assimilatory capacity of algae, contributing to bacteria growth. Aerobic microorganisms are efficient in converting feed to new cell material (40–60% of conversion efficiency), rather than higher organisms (e.g., micro-herbivores, micro-carnivores, and deposit feeders) that spend about 10–15% to rise in weight. The system is considered "mature" (~30 to 50 days) when SS reaches at least 5 mL/L (measured using Imhoff cones) and TAN and nitrite peaks already occurred. To accelerate the water "maturation" (biofloc

equilibrium), an inoculum of a previous BFT culture can be used once sanitary conditions are satisfactory.

It is important to note that as long as the production cycles advance, nitrifying (chemoautotrophic) bacteria play a major role in N-compound control. In addition, suspended particles or solids (bioflocs) also will be increasing over time. With this information in mind, carbon addition could be reduced (or even stopped), preventing the excess of solids (bioflocs) in the cultured system that will lead an excessive DO consumption [65] and shrimp/fish gill occlusions [66].

For the maintenance phase, the monitoring of TAN values is an important tool for water quality maintenance. When values of TAN are higher than 1.0 mg L^{-1}, external carbon source application is recommended with a C:N ratio of 6:1 [36]. In such phase, the use of monosaccharide and oligosaccharide carbohydrate-rich types (e.g., molasses and other sugars) is recommended due to the faster bacteria assimilation and consequently TAN reduction. Same examples of C:N calculations for the phase I and phase II are presented as followed. For both examples, the carbon content of the feed will be considered 50% (based on dry matter). For the carbon source, molasses was chosen and its content in such case is also 50%. It is important to note that the carbon content will change according to the dry matter composition and type of carbon source. In a practical way, dry matter of the feed will be 90%. Fish and shrimp assimilation will be considered 35 and 20%, respectively.

Example 1 (initial and formation phase using a C:N ratio of 20:1) in a tilapia culture tank that receives 4 kg of feed (30% of crude protein) per day.

Calculation 1 (C:N content in the feed)

C: 4 kg of feed × 0.9 (90% dry matter) × 0.7 (30% of fish assimilation or 70% of waste that remains in water)/2 (carbon content of the feed is ~50% based on dry matter) = 1260 g of C

N: 4 kg of feed × 0.9 (90% dry matter) × 0.7 (30% of fish assimilation or 70% of waste that remains in water) × 0.3 (30% crude protein content of feed)/6.25 (constant) = 121 g of N. **The results indicated a ~10:1 C:N ratio of feed**.

Calculation 2 (adjusting the C:N ratio)

If I want a C:N ratio of 20:1, 121 g of N in feed × 20 = I need 2420 g of C. But I already have 1260 g of C (calculated in feed). So 2420 g–1260 g of C = I really need 1160 g of C.

If the molasses has 50% of carbon content (based on dry matter), 1 kg of molasses represents 500 g of carbon. So, 1160 g of carbon requirement will represent **2320 g (or 2.3 kg) of molasses (applied daily until biofloc maturation)**.

Example 2 (maintenance phase and C:N ratio of 6:1) in a *L. vannamei* culture tank (30 m^3) that indicates 2.0 mg L^{-1} TAN values.

Calculation 1 (TAN in water)

For 2.0 mg L^{-1} of TAN in a 30 m^3 tank = 0.002 g × 30,000 L = 60 g of TAN

Calculation 2 (adjusting the C:N ratio)

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If I want a C:N ratio of 6:1, 60 g of TAN in water × 6 = I need 360 g of C. If my molasses has 50% of carbon content (based on dry matter), 1 kg of molasses represents 500 g of carbon. So, 360 g of carbon requirement will represent 720 g (or 0.72 kg) of molasses (one application and checked after 2–3 days).

5. Economics and types of carbon sources

The carbon sources applied in BFT are often by-products derived from human and/or animal food industry, preferentially cheap and local available. Cheap sources of carbohydrates such as molasses, glycerol, and plant meals (i.e., wheat, corn, rice, tapioca, etc.) will be applied before the fry/postlarvae stocking (fertilization protocols) and during grow-out phase, aiming to (i) provide food for the first stages of growth and (ii) to maintain a high C:N ratio and to control N-compound peaks in the culture tanks, respectively [67].

Depending of the carbon source chosen, organic fertilization could be considered as an important item of The production costs. Local available sources should be tested, but bacteria assimilation's characteristics will certainly need to take into account. Monosaccharide and oligosaccharide simple carbohydrate-rich types (e.g., glucose, sucrose-rich sugars, etc.) versus polysaccharide complex-rich types (e.g., starch and cellulose) will lead different bacteria assimilations, nutritional value, and growth. Crab et al. [16] evaluated the effect of different carbon sources for *Macrobrachium rosenbergii* postlarvae. Besides the price, different sources will lead diverse nutritional value of the flocs. The authors observed that when using glycerol as compared to glucose and acetate, higher values of n-6 PUFA were observed.

For each phase (initial and formation phase or maintenance phase), different sources should be chosen according to the price and purpose. For example, dextrose (high purified sugar) versus molasses; refined sugar versus grains by-products, etc. Grains and tubercles contain high levels of carbon (carbohydrates), as polysaccharides. Some grains used as carbon sources additionally contain protein and lipids. García-Ríos [68] compared three carbon sources into the BFT tilapia fry culture and found that corn meal contains 11.79% of protein and 2.8% lipid; meanwhile, wheat has 15.5% of protein and 3.73% lipid. The unrefined sugar (monosaccharide) without protein and lipid promoted the best growth and the highest protein content into the tilapia tissue. It is possible that the chemical structure of sugar presented a high bioavailability to heterotrophic bacteria, hence, fast increase of bacterial biomass.

6. Groups and metagenomics

Phytoplankton, free and attached bacteria, aggregates of particulate organic matter and grazers, such as rotifers, ciliates and flagellates, and protozoa and copepods are common groups of microorganisms in BFT. As the use, identification and study of microbes in aquaculture have become a usual practice in the last decade [69]. For a long time, techniques based on

culture media were used as the main strategy to know the microbial composition of biotic communities, including biofilm and BFT; however, this was a very superficial approach considering that >80% of the bacteria thriving in any environment are readily culturable or unculturable at all [70]. The overcome of culture-independent techniques such as denaturing gradient gel electrophoresis (DGGE) but particularly high-throughput sequencing (next-generation sequencing) increased the depth and coverage of studies aiming to study the microbial diversity of these kinds of conglomerates [71, 72].

Metagenomics is therefore a relative recent genomics subdiscipline that has emerged as a promising scientific tool to analyze the complex genomes contained within microbial communities. However, its use is not yet common in some agro-industrial disciplines such as aquaculture. The reason of this relies in the high cost of this technology; however, prices have significantly decreased during the last decade, and now it is possible for individual laboratories to perform metagenomics studies using high-throughput sequencing.

The study of microbial diversity can be studied with the highest resolution so far, for instance, ribosomal genes such as 16S and 23S have been used as a targeted loci approach for diversity studies of prokaryotic communities. Herein, universal genes are used for the amplification of particular hypervariable regions of these genes [4]; these hypervariable regions contain elements to differentiate organisms. In this regard, this technology offers the possibility to reveal most of the bacteria thriving in any biofloc biomass. However, current sequencing technologies can cover only a fraction (~600 bp) of the ribosomal genes used for taxonomic classification, which means that only 2 or 3 of the 10 hypervariable regions of the 16S gene can be used for this classification. Researchers have made efforts to elucidate whose sequences are the most information richness [73]; however, there is still a loss of information contained on the regions that cannot be covered by these sequencing platforms.

Novel technologies such as single-molecule real-time (SMRT) sequencing have been developed [74]. This particular technology has advantages such as the generation of long reads and high accuracy. Long reads could be useful for sequencing not only ribosomal genes but also larger fragments of DNA, which would serve for multilocus classification. Whether the price for doing metagenomics of bacteria is still high, it is expected to decrease along the following years.

In spite of the incomplete coverage of ribosomal genes by most of the current high-throughput sequencing platforms, the amplification of two or three hypervariable regions of these genes is still a very useful tool to know most of the bacteria contained in biofilms and biofloc [72] and inclusively to detect novel species, study dynamic population patterns, probiotic activity, etc. Furthermore, metagenomics based on single-gene surveys and random shotgun studies of all accessible genes in any environment could be two useful approaches to study the biological activity and communications of these complex bacterial networks. Whether the use of BFT systems is now a reality and promises to be revolutionary strategy, their biology studied through novel genomic tools is still to provide mass information of these biotic communities.

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7. Conclusions and perspectives

Biofloc technology will enable aquaculture grow toward an environmentally friendly approach and biosecurity. Consumption of microorganisms in BFT reduces FCR and consequently costs in feed. Also, microbial community is able to rapidly utilize dissolved nitrogen leached from shrimp/fish feces and uneaten food and convert it into microbial protein, maintaining the water quality. The physical, chemical, and biological interactions that occur into the biofloc systems are complex; further studies can elucidate specific phenomena and their possible applications to other biotechnological fields.

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References

[1] FAO (Food and Agriculture Organization of the United Nations). The State of World Fisheries and Aquaculture 2014. Opportunities and Challenges. 2014; p. 223. Available at http://www.fao.org

[2] Martínez-Porchas M, Martinez-Córdova LR. World aquaculture: environmental impacts and troubleshooting alternatives. The Scientific World Journal. 2012;389623:1–9.

[3] Naylor RL, Williams SL, Strong DR. Aquaculture – A gateway for exotic species. Science. 2001;294(5547):1655–1656.

[4] Martínez-Porchas M, Vargas-Albores F. Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. Reviews in Aquaculture. 2015 (online published first 6 July 2015).

[5] Martínez-Córdova LR, Emerenciano M, Miranda-Baeza A, Martínez-Porchas M. Microbial-based systems for aquaculture of fish and shrimp: an updated review. Reviews in Aquaculture. 2015;7(2):131–148.

[6] Martínez-Córdova LR, Martínez-Porchas M, Emerenciano M, Miranda-Baeza A, Gollas-Galván T. From microbes to fish the next revolution in food production. Critical Reviews in Biotechnology. 2016; Early Online: 1–9.

[7] Emerenciano M, Cuzon G, Goguenheim J, Gaxiola G, Aquacop. Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*. Aquaculture Research. 2012a;44:75–85.

[8] Avnimelech Y. Biofloc Technology – A Practical Guide Book. 3rd ed. The World Aquaculture Society, Baton Rouge, Louisiana, United States. 2015.

[9] Avnimelech Y. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture. 2007;264:140–147.

[10] Crab R, Kochva M, Verstraete W, Avnimelech Y. Bio-flocs technology application in over-wintering of tilapia. Aquacultural Engineering. 2009;40:105–112.

[11] Burford MA, Thompson PJ, McIntosh RP, Bauman RH, Pearson DC. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture. 2003;219:393–411.

[12] Burford MA, Thompson PJ, McIntosh RP, Bauman RH, Pearson DC. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. Aquaculture. 2004;232:525–537.

[13] Samocha TM, Patnaik S, Speed M, Ali AM, Burger JM, Almeida RV, Ayub Z, Harisanto M, Horowitz A, Brock DL. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. Aquacultural Engineering. 2007;36:184–191.

[14] Ballester ELC, Abreu PC, Cavalli RO, Emerenciano M, Abreu L, Wasielesky W. Effect of practical diets with different protein levels on the performance of Farfantepenaeus paulensis juveniles nursed in a zero exchange suspended microbial flocs intensive system. Aquaculture Nutrition. 2010;16:163–172.

[15] Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). Aquaculture Research. 2012b;43:447–457.

[16] Crab R, Chielens B, Wille M, Bossier P, Verstraete W. The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquaculture Research. 2010;41:559–567.

[17] Pérez-Fuentes JA, Pérez-Rostro CI, Hernández-Vergara MP. Pond-reared Malaysian prawn *Macrobrachium rosenbergii* with the biofloc system. Aquaculture. 2013;400–401:105–110.

[18] Ekasari J, Zairin M, Putri DU, Sari NP, Surawidjaja EH, Bossier P. Biofloc-based reproductive performance of Nile tilapia *Oreochromis niloticus* L. broodstock. Aquaculture Research. 2015;46:509–512.

[19] Emerenciano M, Cuzon G, Arevalo M, Mascaró M, Gaxiola G. Effect of short-term fresh food supplementation on reproductive performance, biochemical composition, and fatty acid profile of *Litopenaeus vannamei* (Boone) reared under biofloc conditions. Aquaculture International. 2013a;21:987–1007.

[20] Kuhn D, Boardman G, Lawrence A, Marsh L, Flick G. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture. 2009;296:51–57.

[21] Bauer W, Prentice-Hernandez C, Tesser MB, Wasielesky W, Poersch LHS. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 2012;342–343:112–116.

[22] Neto HS, Santaella ST, Nunes AJP. Bioavailability of crude protein and lipid from biofloc meals produced in an activated sludge system for white shrimp, *Litopenaeus vannamei.* Brazilian Journal of Animal Science. 2015;44(8):269–275.

[23] Najdegerami EH, Bakhshi F, Lakani FB. Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. Fish Physiology Biochemistry. 2016;42(2):457–465.

[24] Yusuf MW, Utomo NBP, Yuhana M, Widanarni. Growth performance of catfish (*Clarias gariepinus*) in biofloc-based super intensive culture added with *Bacillus* sp. Journal of Fisheries and Aquatic Science. 2015;10(6):523–532.

[25] Poleo G, Aranbarrio JV, Mendoza L, Romero O. Cultivo de cachama blanca en altas dens- idades y en dos sistemas cerrados. Brazilian Journal of Agricultural Research (PAB). 2011;46(4):429–437.

[26] Avnimelech Y, Kochba M. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using 15 N tracing. Aquaculture. 2009;287(1):163–168.

[27] Timmons, M.B., J.M. Ebeling, F.W. Wheaton, S. T. Summerfelt, and B.J. Vinci. 2002. Recirculating Aquaculture Systems, 2nd edition. Northeastern Regional Aquaculture Center. Publication No. 01-002. Cayuga Aqua Ventures. Ithaca, NY. 769 pp.

[28] Fukami K, Nishijima T, Ishida Y. Stimulative and inhibitory effects of bacteria on the growth of microalgae. Hydrobiologia. 1997;358:185–191.

[29] Bruckner CG, Bahulikar R, Rahalkar M, Schink B, Kroth PG. Bacteria associated with benthic diatoms from Lake Constance: phylogeny and influences on diatom growth

and secretion of extracellular polymeric substances. Applied and Environmental Microbiology. 2008;74(24):7740–7749.

[30] Leonard N, Blancheton JP, Guiraud JP. Populations of heterotrophic bacteria in an experimental recirculating aquaculture system. Aquacultural Engineering. 2000;22(1):109–120.

[31] Glazer AN, Nikaido H. Microbial Biotechnology: fundamentals of Applied Microbiology. Cambridge University Press, Cambridge, New York, USA, 2007.

[32] Van FB, Meyer-Reil LA. Biomass and metabolic activity of heterotrophic marine bacteria. In: Advances in Microbial Ecology. Plenum Press, New York, USA. 1982; pp. 111–170.

[33] Brown MR, Barrett SM, Volkman JK, Nearhos SP, Nell JA, Allan GL. Biochemical composition of new yeasts and bacteria evaluated as food for bivalve aquaculture. Aquaculture. 1996;143(3):341–360.

[34] Furtado PS, Poersch LH, Wasielesky W. Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture. 2011;321(1):130–135.

[35] Koops HP, Pommerening-Röser A. Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiology Ecology. 2001;37(1):1–9.

[36] Ebeling JM, Timmons MB, Bisogni JJ. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture. 2006;257(1):346–358.

[37] Hagopian DS, Riley JG. A closer look at the bacteriology of nitrification. Aquacultural Engineering. 1998;18:223–244.

[38] Hartwell LH, Culotti J, Pringle JR, Reid BJ. Genetic control of the cell division cycle in yeast. Science. 1974;183:46–51.

[39] Walker GM. Yeast physiology and biotechnology. John Wiley & Sons. Chichester, UK,1998.

[40] Monroy-Dosta MDC, Lara-Andrade D, Castro-Mejía J, Castro-Mejía G, Emerenciano M. Composición y abundancia de comunidades microbianas asociadas al biofloc en un cultivo de tilapia. Journal of Marine Biology and Oceanography (RBMO) 2013;48(3):511–520.

[41] Bowling L. Freshwater phytoplankton: diversity and biology, In: Suthers IM, Rissik D (Eds.). Plankton: A Guide to Their Ecology and Monitoring for Water Quality. CSIRO Publishing. Melbourne, Australia, 2009; pp. 115–139.

[42] Decamp O, Conquest L, Cody J, Forster I, Tacon AG. Effect of shrimp stocking density on size-fractionated phytoplankton and ecological groups of ciliated protozoa within zero-water exchange shrimp culture systems. Journal of the World Aquaculture Society. 2007;38(3):395–406.

[43] Graham LE, Wilcox LW. Algae. New Jersey: Prentice-Hall. 2000.

[44] García N, López-Elías JA, Miranda A, Huerta MMPN, García A. Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases. Latin American Journal of Aquatic Research. 2012;40(2):435.

[45] López-Rodas V, Maneiro E, Costas E. Adaptation of cyanobacteria and microalgae to extreme environmental changes derived from anthropogenic pollution. Limnetica. 2006;25(1–2):403–410.

[46] Pearl HW, Tucker CS. Ecology of blue-green-algae in aquaculture ponds. Journal of the World Aquaculture Society. 1995;26:109–131.

[47] Becerra-Dórame MJ, Martínez-Córdova LR, Martínez-Porchas M, Lopez-Elías JA. Evaluation of autotrophic and heterotrophic microcosm-based systems on the production response of *Litopenaeus vannamei* intensively nursed without Artemia and with zero water exchange. Israeli Journal of Aquaculture – Bamidgeh. 2011;63:1–7.

[48] Lezama-Cervantes C, Paniagua-Michel J. Effects of constructed microbial mats on water quality and performance of *Litopenaeus vannamei* post-larvae. Aquacultural Engineering. 2010;42(2):75–81.

[49] Brown MR. Nutritional value and use of microalgae in aquaculture. Avances en Nutrición Acuícola VI. Abstract of VI Internacional Simposium of Aquaculture Nutrition. 2002;3:281–292.

[50] Ray JA, Seaborn G, Leffler WJ, Wilde BS, Lawson A, Browdy LC. Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. Aquaculture. 2010;310:130–138.

[51] Kuang Q, Bi Y, Xia Y, Hu Z. Phytoplankton community and algal growth potential in Taipinghu reservoir, Anhui Province, China. Lakes & Reservoirs: Research & Management. 2004;9:119–124.

[52] Maicá PF, Borba MR, Wasielesky W. Effect of low salinity on microbial floc composition and performance of *Litopenaeus vannamei* (Boone) juveniles reared in a zerowater-exchange super-intensive system. Aquaculture Research. 2012;43:361–370.

[53] Kobayashi T, Shiel R, King JA, Miskiewicz GA. Freshwater zooplankton: diversity and biology In: Suthers, IM, & Rissik, D (Eds.). Plankton: A Guide to Their Ecology and Monitoring for Water Quality. CSIRO Publishing. Melbourne, Australia, 2009; pp.157–179.

[54] Pandey BD, Yeragi SG, Pal AK. Nutritional value of a heterotrichous ciliate, Fabrea salina with emphasis on its fatty acid profile. Asian Australasian Journal of Animal Sciences. 2004;17(7):995–999.

[55] Ben-Amotz AD, Fishler R, Schneller A. Chemical composition of dietary species of marine unicellular algae and rotifers with emphasis on fatty acids. Marine Biology. 1987;95(1):31–36.

[56] Campaña-Torres A, Martínez-Córdova LR, Martínez-Porchas M, López-Elías JA, Porchas-Cornejo MA. Productive response of *Nannochloropsis oculata*, cultured in dif-

ferent media and their efficiency as food for the rotifer *Brachionus rotundiformis*. Phyton. 2012;81:45–50.

[57] Loureiro KC, Wilson WJ, Abreu PC. Utilização de protozoários, rotíferos e nematódeos como alimento vivo para camarões cultivados no sistema BFT. Atlantica. 2012;34(1):5–12.

[58] Pérez AJD. Aplicación y evaluación de un reactor de contactores biológicos rotativos (RBC o biodiscos), a escala de laboratorio como tratamiento de los lixiviados generados en el relleno sanitario de la Pradera. Master's degree thesis (Urban Engineering) Engineering Faculty. University of Medellín. 2010; pp. 259.

[59] Farhadian O, MdYuso F, Mohamed S. Nutritional values of *Apocyclops dengizicus* (Copepoda: Cyclopoida) fed *Chaetocerous calcitrans* and *Tetraselmis tetrathele*. Aquaculture Research. 2009;40:74–82.

[60] Berberovic R. Elemental composition of two coexisting Daphnia species during the seasonal course of population development in Lake Constance. Oecologia. 1990;84(3):340–350.

[61] Emerenciano M, Gaxiola G, Cuzon G. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. INTECH open science_ open minds. 2013b;Cap 12:301–327. http://dx.doi.org/10.5772/53902.

[62] Ferreira-Marinho Y, Otavio-Brito L, Silva CVF, Santos IGS, Olivera-Gàlvez A. Effect of addition of *Navicula* sp. on plankton composition and postlarvae growth of *Litopenaeus vannamei* reared in culture tanks with zero water exchange. Latin American Journal of Aquatic Research. 2014;42(3):427.

[63] Johnstone IL. The cuticle of the nematode *Caenorhabditis elegans*: a complex collagen structure. Bioessays. 1994;16(3):171–178.

[64] Moens T, Vincx M. Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom. 1997;77(01):211–227.

[65] Vinatea L, Gálvez AO, Browdy CL, Stokes A, Venero J, Haveman J, Lewis BL, Lawson A, Shuler A, Leffler JW. Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: interaction of water quality variables. Aquacultural Engineering. 2010;42:17–24.

[66] Schveitzer R, Arantes R, Costódio PF, Espírito Santo CM, Arana LV, Seiffert WQ, Andreatta ER. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. Aquacultural Engineering. 2013;56:59–70.

[67] Avnimelech Y. Carbon nitrogen ratio as a control element in aquaculture systems. Aquaculture. 1999;176:227–235.

[68] García-Ríos L. Crecimiento, sobrevivencia y calidad de crías de Tilapia del Nilo (Oreochromis niloticus) cultivadas en biofloc con diferentes fuentes de carbono. Master's degree thesis. Sonora State University, Navojoa, Sonora, México. 2015;42 p.

[69] Moriarty DJW. The role of microorganisms in aquaculture ponds. Aquaculture. 1997;151:333–349.

[70] Streit WR, Schmitz RA. Metagenomics the key to the uncultured microbes. Current Opinion in Microbiology. 2004;7:492–498.

[71] Xia S, Wang F, Fu Y, Yang D, Ma X. Biodiversity analysis of microbial community in the chem-bioflocculation treatment process. Biotechnology and Bioengineering. 2005;89(6):656–659.

[72] Martínez-Córdova LR, Martínez-Porchas M, Porchas-Cornejo MA, Gollas-Galván T, Scheuren-Acevedo S, Arvayo MA, López-Torres MA. Bacterial diversity studied by next-generation sequencing in a mature phototrophic Navicula sp-based biofilm promoted into a shrimp culture system. Aquaculture Research. 2016 (online published first 16 April 2016).

[73] Soergel DA, Dey N, Knight R, Brenner SE. Selection of primers for optimal taxonomic classification of environmental 16S rRNA gene sequences. The ISME Journal. 2012;6(7):1440–1444.

[74] Roberts RJ, Carneiro MO, Schatz MC. The advantages of SMRT sequencing. Genome Biology. 2013;14(7):405.

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