Chapter 12

Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry

Maurício Emerenciano, Gabriela Gaxiola and Gerard Cuzon

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53902

1. Introduction

The aquaculture industry is growing fast at a rate of ~9% per year since the 1970s [1]. However, this industry has come under scrutiny for contribution to environmental degradation and pollution. As a result, requirement for more ecologically sound management and culture practices remains fully necessary. Moreover, the expansion of aquaculture is also restricted due to land costs and by its strong dependence on fishmeal and fish oil [2,3]. Such ingredients are one of the prime constituents of feed for commercial aquaculture [4]. Feed costs represent at least 50% of the total aquaculture production costs, which is predominantly due to the cost of protein component in commercial diets [5].

Interest in closed aquaculture systems is increasing, mostly due to biosecurity, environmental and marketing advantages over conventional extensive and semi-intensive systems [6]. When water is reused, some risks such as pathogen introduction, escapement of exotic species and discharging of waste water (pollution) are reduced and even eliminated. Furthermore, because of high productivity and reduced water use, marine species can be raised at inland locations [6]. A classic example is the currently expansion of marine shrimp farms at inland location in USA, which allows local farmers market fresh never frozen shrimp in metropolitan locations with good profitability.

The environmental friendly aquaculture system called “Biofloc Technology (BFT)” is considered as an efficient alternative system since nutrients could be continuously recycled and reused. The sustainable approach of such system is based on growth of microorganism in the culture medium, benefited by the minimum or zero water exchange. These microorganisms (biofloc) has two major roles: (i) maintenance of water quality, by the uptake of nitrogen compounds generating *“in situ”* microbial protein; and (ii) nutrition, increasing culture feasibility by reducing feed conversion ratio and a decrease of feed costs.

As a closed system, BFT has primordial advantage of minimizing the release of water into rivers, lakes and estuaries containing escaped animals, nutrients, organic matter and pathogens. Also, surrounding areas are benefitted by the "vertically growth" in terms of productivity, preventing coastal or inland area destruction, induced eutrophication and natural resources losses. Drained water from ponds and tanks often contains relatively high concentrations of nitrogen and phosphorous, limiting nutrients that induce algae growth, which may cause severe eutrophication and further anaerobic conditions in natural water bodies. In BFT, minimum water discharge and reuse of water prevent environment degradation and convert such system in a real "environmentally friendly system" with a "green" approach. Minimum water exchange maintain the heat and fluctuation of temperature is prevented [7], allowing growth of tropical species in cold areas.

Currently, BFT has received alternate appellation such as ZEAH or Zero Exchange Autotrophic Heterotrophic System [8-10], active-sludge or suspended bacterial-based system [11], single-cell protein production system [12], suspended-growth systems [13] or microbial floc systems [14,15]. However, researches are trying to keep the term "BFT or Biofloc Technology" in order to establish a key reference, mainly after the book release "*Biofloc Technology – A Practical Guide Book*" in 2009 [16]. Moreover, BFT has been focus of intensive research in nutrition field as a protein source in compounded feeds. Such source is produced in a form of "biofloc meal", mainly in bioreactors [17]. In addition, the fast spread and the large number of BFT farms worldwide induced significant research effort of processes involved in BFT production systems [14].

The objective of this chapter is to review the application of Biofloc Technology (BFT) in aquaculture; and describes the utilization of biofloc biomass (also described in this chapter as "biofloc meal") as an ingredient for compounded feeds. An addition goal is to help students, researchers and industry to clarify the basic aspects of such technology, aiming to encourage further research.

2. History of BFT

According to [18], BFT was first developed in early 1970s at Ifremer-COP (French Research Institute for Exploitation of the Sea, Oceanic Center of Pacific) with different penaeid species including *Penaeus monodon*, *Fenneropenaeus merguiensis*, *Litopenaeus vannamei* and *L. stylirostris* [19,20]. Such culture system was compared with an "external rumen", but now applied for shrimp [21]. At the same period, Ralston Purina developed a system based on nitrifying bacteria while keeping shrimp in total darkness. In connection with Aquacop, such system was applied to *L. stylirostris* and *L. vannamei* both in Crystal River (USA) and Tahiti, leding considerations on benefits of biofloc for shrimp culture [22]. In 1980, a French scientific program 'Ecotron' was initiated by Ifremer to better understand such system. Several studies enabled a comprehensive approach of BFT and explained interrelationships between different compartments such as water and bacteria, as well as shrimp nutritional physiology. Also in 1980s and beginning of 1990s, Israel and USA (Waddell Mariculture Center) started R&D in BFT with tilapia and white shrimp *L. vannamei*, respectively, in which water limitation, environmental concerns and land costs were the main causative agents that promoted such research (Fig. 1).

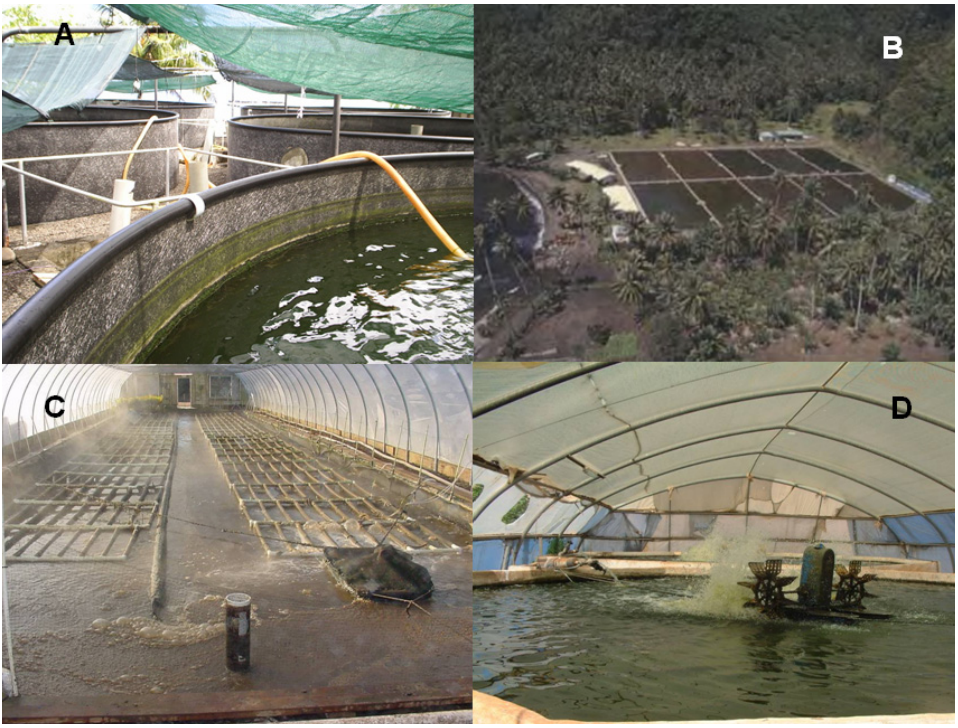


Figure 1. Biofloc technology at Ifremer, Tahiti (A), Sopomer farm, Tahiti (B), Waddell Mariculture Center (C) and Israel (D) (Photos A and B: Gerard Cuzon; C: courtesy of Wilson Wasielesky; and D: courtesy of Yoram Avnimelech)

Regarding to commercial application of BFT, in 1988 Sopomer farm in Tahiti (French Polynesia) using 1000m^2 concrete tanks and limited water exchange achieved a world record in production (20–25 ton/ha/year with two crops) [22, 23]. On the other hand, Belize Aquaculture farm or "BAL" (located at Belize, Central America), probably the most famous case of BFT commercial application in the world, produced around 11-26 ton/ha/cycle using 1.6 ha lined grow-out ponds. Much of know-how of running worldwide commercial scale BFT shrimp ponds is derived from BAL experience. In small-scale BFT greenhouse-based farms, Marvesta farm (located at Maryland, USA), probably is the well-known successful indoor BFT shrimp farm in USA, can produce around 45 ton of fresh never frozen shrimp per year using ~570 m^3 indoor race-ways [24]. Nowadays, BFT have being successfully expanded in large-scale shrimp farming in Asia, Latin and Central America, as well as in small-scale greenhouses in USA, South Korea, Brazil, Italy, China and others (Fig 2). In addition, many research centers and universities are intensifying R&D in BFT, mostly applied to key fields such as grow-out management, nutrition, BFT applied to reproduction, microbial ecology, biotechnology and economics.

Figure 2. Biofloc technology commercial-scale at BAL (A) and Malaysia (B), and pilot-scale in Mexico (C and D) (Photos A, B and D: Maurício Emerenciano; and C: courtesy of Manuel Valenzuela)

3. The role of microorganisms

The particulate organic matter and other organisms in the microbial food web have been proposed as potential food sources for aquatic animals [25]. In BFT, microorganisms present a key role in nutrition of cultured animals. The macroaggregates (biofloc) is a rich protein-lipid natural source available *"in situ"* 24 hours per day [14]. In the water column occurs a complex interaction between organic matter, physical substrate and large range of microorganisms such as phytoplankton, free and attached bacteria, aggregates of particulate organic matter and grazers, such as rotifers, ciliates and flagellates protozoa and copepods [26] (Fig 3). This natural productivity play an important role recycling nutrients and maintaining the water quality [27,28].

The consumption of biofloc by shrimp or fish has demonstrated innumerous benefits such as improvement of growth rate [10], decrease of FCR and associated costs in feed [9]. Growth enhancement has been attributed to both bacterial and algae nutritional components, which up to 30% of conventional feeding ration can be lowered due to biofloc consumption in shrimp [29]. In reference [9] was reported that more than 29% of daily food consumed for *L. vannamei* could be biofloc. In tilapia, in [30] was estimated that feed utilization is higher in BFT at a rate of 20% less than conventional water-exchange systems.

Also, consumption of macroaggregates can increase nitrogen retention from added feed by 7-13% [31, 32]. In this context, BFT has driven opportunities to use alternative diets. Low protein feeds and feeds with alternative protein sources different than marine-based products (i.e. fishmeal, squid meal, etc) have been successfully applied in BFT [28, 33-35], leading "green" market opportunities.

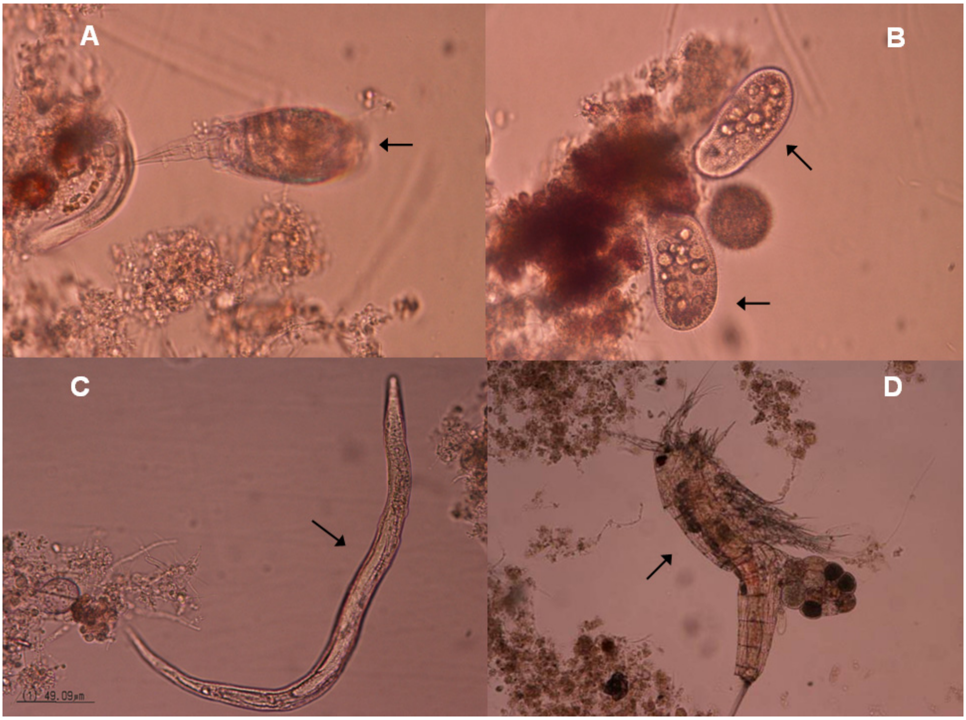


Figure 3. Grazers often observed in BFT such as flagellates protozoa (A), ciliates protozoa (B), nematodes (C) and copepods (D) (10x magnification) (Source: Maurício Emerenciano)

Regarding to maintenance of water quality, control of bacterial community over autotrophic microorganisms is achieved using a high carbon to nitrogen ratio (C:N) [30], which nitrogenous by-products can be easily taken up by heterotrophic bacteria [36]. High carbon to nitrogen ratio is required to guarantee optimum heterotrophic bacteria growth [14, 37], using this energy for maintenance (respiration, feeding, movement, digestion, etc), but also for growth and to produce new cells. High carbon concentration in water could 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 that spend about 10-15% to rise in weight [16]. Bacteria and other microorganisms act as very efficient "biochemical systems" to degrade and metabolize organic residues [36]. In other words, they recycle very efficiently nutrients in a form of organic and inorganic matter (un-consumed and non-digested feed, metabolic residues and carbon sources applied as fertilizers) into new microbial cells.

The carbon sources applied in BFT are often by-products derived from human and/or animal food industry, preferentially local available. Cheap sources of carbohydrates such as molasses, glycerol and plant meals (i.e. wheat, corn, rice, tapioca, etc) will be applied before fry/post-larvae stocking and during grow-out phase, aiming to maintain a high C:N ratio (~15-20:1) and to control N compounds peaks. Also, a mix of plant meals can be pelletized ("green-pellet") and applied into ponds [38]; or low protein diets containing high C:N ratio can also be carried out [16,33]. The carbon source serves as a substrate for operating BFT systems and production of microbial protein cells [36]. There are many considerations for its selection such as costs, local availability, biodegradability and efficiency of bacteria assimilation. In Table 1 is summarized some studies with different species and carbon source applied in BFT system.

Carbon source	Culture specie	Reference
Acetate	*Macrobrachium rosenbergii*	[39]
Cassava meal	*Penaeus monodon*	[40]
Cellulose	Tilapia	[12]
Corn flour	Hybrid bass and hybrid tilapia	[41, 42]
Dextrose	*Litopenaeus vannamei*	[43]
Glycerol and Glycerol+*Bacillus*	*M. rosenbergii*	[39]
Glucose	*M. rosenbergii*	[39]
Molasses	*L. vannamei and P. monodon*	[9, 29, 44]
Sorghum meal	Tilapia	[12]
Tapioca	*L. vannamei* and *M. rosenbergii*	[31, 45]
Wheat flour	Tilapia (*O. niloticus*)	[33]
Wheat bran + molasses	*Farfantepenaeus brasiensis, F. paulensis and F. duorarum*	[37, 46, 47]
Starch	Tilapia *O. niloticus x O. aureus* and tilapia (Mozambique)	[7, 14]

Table 1. Different carbon sources applied on BFT system (Source: adapted from [36])

Not all species are candidates to BFT. Some characteristics seems to be necessary to achieve a better growth performance such as resistance to high density, tolerance to intermediate levels of dissolved oxygen (~3-6 mg/L), settling solids in water (~10 with a maximum of 15 mL/L of "biofloc volume", measured in Imhoff cones) [38] and N-compounds, presence of filtering apparatus (i.e. tilapia), omnivorous habits and/or digestive system adaptable to better assimilate the microbial particles.

4. Applications in aquaculture

4.1. Nursery and grow-out

Nursery phase is defined as an intermediate step between hatchery-reared early postlarvae and grow-out phase [48]. Such phase presents several benefits such as optimization of farm land, increase in survival and enhanced growth performance in grow-out ponds [49-51]. BFT has been applied successfully in nursery phase in different shrimp species such as *L. vannamei* [44, 48], *P. monodon* [51], *F. paulensis* [15, 46], *F. brasiliensis* [37, 52] and *F. setiferus* [34]. The primary advantage observed is related to a better nutrition by continuous consumption of biofloc, which might positively influence grow-out performance *a posteriori* [53], but was not always the case [54]. In addition, optimization of farm facilities provided by the high stocking densities in BFT nursery phase seems to be an important advantage to achieve profitability in small farms, mainly in cold regions or when farmers are operating indoor facilities.

In [46] was observed that presence of bioflocs resulted in increases of 50% in weight and almost 80% in final biomass in *F. paulensis* early postlarval stage when compared to conventional clear-water system. This trend was observed even when postlarvae were not fed with a commercial feed (biofloc without commercial feed). In *L. vannamei* nursery in BFT conditions, references [48] and [55] reported survival rates ranging from 55.9% to 100% and 97% and 100%, respectively. In [51] was demonstrated that the addition of substrates in BFT systems increased growth and further enhanced production, while also contributing to more favorable water quality conditions. According to the same study, growth and survival was not affected by stocking density (2500 *vs* 5000 PL/m^2), therefore greater production outputs were achieved at the higher density. Furthermore, in [37] was found that *F. brasiliensis* postlarvae grow similarly with or without pelletized feed in biofloc conditions during 30-d of nursery phase, which was 40% more than conventional clear-water continuous exchange system.

In grow-out, BFT has been also shown nutritional and zootechnical benefits. In [9] was estimated that more than 29% of the daily food intake of *L. vannamei* consisted of microbial flocs, decreasing FCR and reducing costs in feed. The reference [10] showed that juveniles of *L. vannamei* fed with 35% CP pelletized feed grew significantly better in biofloc conditions as compared to clear-water conditions. In [28] was showed that controlling the concentration of particles in super-intensive shrimp culture systems can significantly improve shrimp production and water quality. Also, the same authors demonstrated that environmentally friendly plant-based diet can produce results comparable to a fish-based feed in BFT conditions. In [56] was evaluated the stocking density in a 120d of *L. vannamei* BFT culture, reporting consistent survival of 92, 81 and 75% with 150, 300 and 450 shrimp/m^2, respectively. Moreover, the study [57] performed in a heterotrophic-based condition detected no significant difference in FCR when feeding *L. vannamei* 30% and 45% CP diets and 39% and 43% CP diets, respectively. With these results in mind, floc biomass might provide a complete source of cellular nutrition as well as various bioactive compounds even at high density. It is not known exactly how microbial flocs enhance growth. Growth might be enhanced by continuous consumption of “native protein”, protein source without previous treatment [18], which could possess a "growth factor" similar to the one investigated in squid [58]. Is well known that protein, peptides and aminoacids participate fully in synthesis of new membranes, somatic growth and immune function and biofloc can potentially provide such ingredients.

For fish and other species, BFT also has been demonstrated encouraged results. Intensive BFT *Oreochromis niloticus* tilapia culture could produce an equivalent of 155 ton/ha/crop [11]. Besides high yields, decrease of FCR and decreased of protein content in diets have also been observed. In [30] was estimated that feed utilization by tilapia is higher in BFT with a ration 20% less than conventional water exchange system. Studying the effect of BFT in juveniles tilapia, the reference [33] showed no difference in fish growth/production between 35% and 24% CP fed tanks under BFT, but both were higher than clear-water control without biofloc with 35% CP. Moreover, in [7] was investigated the effectiveness of BFT for maintaining good water quality in over-wintering ponds for tilapia. The authors concluded that BFT emerge as an alternative to overcome over-wintering problems, particularly mass mortality of fish due to low temperatures. In the study [14] was observed that biofloc consumed by fish (tilapia) may represent a very significant feed source, constituting about 50% of the regular feed ration of fish (assuming daily feeding of 2% body weight).

In *M. rosenbergii* larviculture was evaluated the effect of different carbon sources in a BFT culture conditions [39]. The authors found that using glucose or a combination of glycerol plus *Bacillus* as a carbon source in bioreactors led to higher biofloc protein content, higher n-6 fatty acids, which resulted in improved survival rates. In a study with a Brazilian endemic tropical fish species tambaqui (*Colossoma macropomum*) was observed that BFT did not improve fish growth/production as compared to clear-water conditions [59], although some water quality problems in such study remained unsolved (i.e. turbidity and nitrite). The authors showed no differences in 44% CP fed tanks under BFT and clear-water conditions, as well as 28% CP BFT. Certainly further research is needed to clarify the effect of BFT in *Colossoma macropomum*. On the other hand, *Piaractus brachypomus* or pirapitinga seems to be a candidate species to BFT [60].

4.2. Breeding

The BFT has been successfully applied for grow-out, but little is known about biofloc benefits on breeding. For example, in the shrimp industry with the global spread of viruses, the use of closed-life cycle broodstock appeared as a priority to guarantee biosecurity, avoiding vertical transmissions. Moreover, such industry places a considerable interest on penaeid breeding program, often performed in closed facilities, controlling the production plan through successive generations. These programs were frequently associated with large animals, disease resistance as well as the enhancement of reproductive performance. However, nutritional problems remain unresolved [61] and alternatives should be evaluated.

As an alternative for continuous *in situ* nutrition during the whole life-cycle, breeders raised in BFT limited or zero water exchange system are nutritional benefited by the natural productivity (biofloc) available 24 hours per day. Biofloc in a form of rich-lipid-protein source could be utilized for first stages of broodstock's gonads formation and ovary development. Furthermore, production of broodstock in BFT could be located in small areas close to hatchery facilities, preventing spread of diseases caused by shrimp transportation.

In conventional systems breeders used to be produced in large ponds at low density. However, risks associate with accumulation of organic matter, cyanobacteria blooms and fluctuations of some water quality parameters (such as temperature, DO, pH and N-compounds) remains high and could affect the shrimp health in outdoor facilities. Once the system is stable (sufficient particulate microbiota biomass measured in Imhoff cones), BFT provides stabilized parameters of water quality when performed in indoor facilities such as greenhouses, guaranteeing shrimp health.

According to studies performed with the blue shrimp *L. stylirostris* [18] and the pink shrimp *F. duorarum* broodstock [62], BFT could enhance spawning performance as compared to the conventional pond and tank-reared system, respectively (i.e. high number of eggs per spawn and high spawning activity; Fig 4). Such superior performance might be caused by better control of water quality parameters and continuous availability of food (biofloc) in a form of fatty acids protected against oxidation, vitamins, phospholipids and highly diverse "native protein", rather than conventional systems which "young" breeders are often limited to pelletized feed. These nutrients are required to early gonad formation in young breeders and subsequent ovary development. The continuous availability of nutrients could promote high nutrient storage in hepatopancreas, transferred to hemolymph and directed to ovary, resulting in a better sexual tissue formation and reproduction activity [18].

Regarding to shrimp broodstock management, one of the most important management procedures is related to control of solids and stocking density. High levels of solids negatively affect shrimp health, particularly with shrimp weight higher than 15g [47]. Settling solids or "biofloc volume" should be managed below than 15mL/L (measured in Imhoff cones) [38, 47]. Excess of particulate organic matter covered breeder's gills and could limit oxygen exchange, might resulting in mortalities.

Stocking density has to be carefully managed, mainly in sub-adult/adult phase (i.e. >15g). High density or high biomass will lead to an increase in organic matter, TSS levels and N-compounds in tanks or in ponds [63]. Moreover, physical body damages are prevented at low density, improving breeder's health. For review, a suggested stocking density is well described in [64].

For fish, no literature is available regarding BFT and application in breeders. The same trend observed in penaeid shrimp might be observed in fish. The continuous consumption of diverse microbiota (biofloc) should improve nutrients transfer, gonad formation and reproduction performance in fish. Lipid is a well-known nutrient that plays a key role in reproduction of aquatic species. In tilapia, breeders fed with crude palm oil based-feed (n-6 fatty acid rich source) presented high concentration of acid arachidonic or "ARA" (C20:4 n-6) in gonads, eggs and larvae of tilapia as compared to fish oil or linseed oil-based feeds [65]. As a result, better reproductive performance was observed in terms of higher total number of eggs per fish, larger gonad sizes, shorter latency period, inter spawning interval and higher spawning frequency. ARA is an essential fatty acid crucial in reproduction, acting as hormone precursor [66]. In the study [33] was found high ARA content in biofloc harvested in tilapia culture freshwater tanks. Bioflocs in freshwater bioreactors contained high ARA content using glucose and glycerol as a carbon source [67]. These findings suggested that biofloc (according its nutritional profile, for review see section 5.0) might positively influence the reproductive performance in fish, supplying nutrients for gonad development, possibly also enhancing larval quality *a posteriori.* BFT in tilapia broodstock could be an effective method to increase tilapia fry production and further research is need in this field.

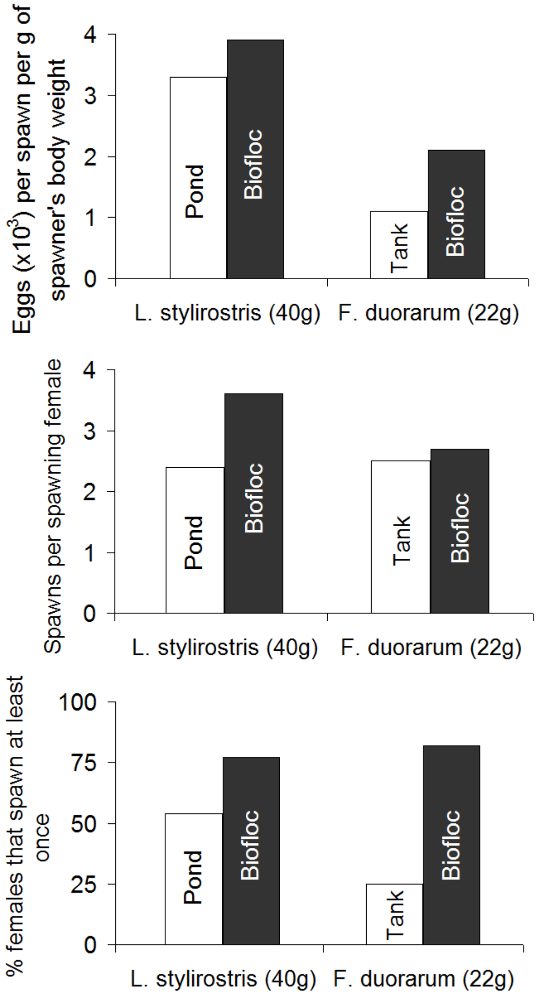


Figure 4. Spawning performance of *F. duorarum* (tank-reared vs biofloc) and *L. stylirostris* (pond-reared vs biofloc) performed in 45 and 30 days after ablation, respectively. Mean weights in parenthesis (more details in [18] and [62]).

4.3. The "natural probiotic" effect of biofloc

Biofloc can be a novel strategy for disease management in contrast to conventional approaches such as antibiotic, antifungal, probiotic and prebiotic application. The "natural probiotic" effect in BFT could act internally and/or externally against, i.e., to *Vibrio sp.* and ectoparasites, respectively. This effect is promoted by large groups of microorganisms, but mainly bacteria that is considered the first trophic level in the system.

Internally, bacteria and its synthesized compounds could act similar to organic acids and might be effective bio-control agents, also given beneficial host's microbial balance in the gut [68]. The regular addition of carbon in the water is known to select for polyhydroxyalkanoates (PHA) accumulating bacteria and other groups of bacteria that synthesize PHA granules. The microbial storage product poly-ß-hydroxybutyrate (PHB), a biodegradable polymer belonging to the polyesters class, is only one compound of a whole family of polyhydroxyalkanoates. PHB is produced by a widely variety of microorganisms such as *Bacillus sp., Alcaligenes sp., Pseudomonas sp.* from soluble organic carbon and is also involved in bacterial carbon metabolism and energy storage [68]. This polymer could comprise ~80% of the bacteria's cell dry matter and up to 16% on biofloc dry weight [69]. Different carbon sources or structures of carbon substrate will result in varying types of PHA [69].

Such granules are synthesized under conditions of physiological and nutrient stress, i.e., when an essential nutrient like nitrogen is limited in the presence of an excess carbon source [68]. When these polymers are degraded in the gut, they could have antibacterial activity similar to short chain fatty acids (SCFAs) or organic acids. The breakdown of PHA inside the gastrointestinal tract can be carried out via chemical and enzymatic hydrolysis [70].

Chemical hydrolysis can be carried out by treating the polymers with, i.e., NaOH, in which could significantly accelerate its digestibility [70]. On the other hand, enzyme hydrolysis is generally carried out by extracellular depolymerases activities which are widely distributed among bacteria and fungi, acting as a preventive or curative protector against *Vibrio sp.* infections and stimulate growth and survival of shrimp and fish larvae [69].

The working mechanism of PHAs with respect to their antibacterial activity is not well understood [68]. As they could act similarly to SCFA, some studies speculated the working mechanism by (i) reduction of pH, in which antibacterial activity increases with decreasing pH value [71]; (ii) inhibiting the growth of pathogenic bacteria by interference on cell membrane structure and membrane permeability, as well as instability of internal protons balance, lowering ATP and depletion of cellular energy [72]; and (iii) down-regulate virulence factor expression and positively influence the gut health of animals [73]. Further research is need to maximizing PHA content in bioflocs applied, i.e., for fish/shrimp feed, characterizing and analyzing their bio-control efficacy in different host-microbe systems [68].

Externally, the working mechanism of biofloc microorganisms against pathogens seems to be by competition of space, substrate and nutrients. Some essentials nutrients such as nitrogen are required by both groups (i.e. heterotrophic bacteria *vs Vibrio sp.*) limiting their growth. Inhibiting compounds excreted by BFT microorganisms, light intensity and type of carbon source also could reduce pathogens growth. Unfortunately, limited information is available on this field. In a study with fish fingerlings [74] was reported that tilapia (initial weight 0.98 ± 0.1g) reared under BFT limited water-exchange condition (FLOC) presented less ectoparasites in gills and ectoderm's mucous as compared to conventional water-exchange system (CW) after 60 days (Fig 5).

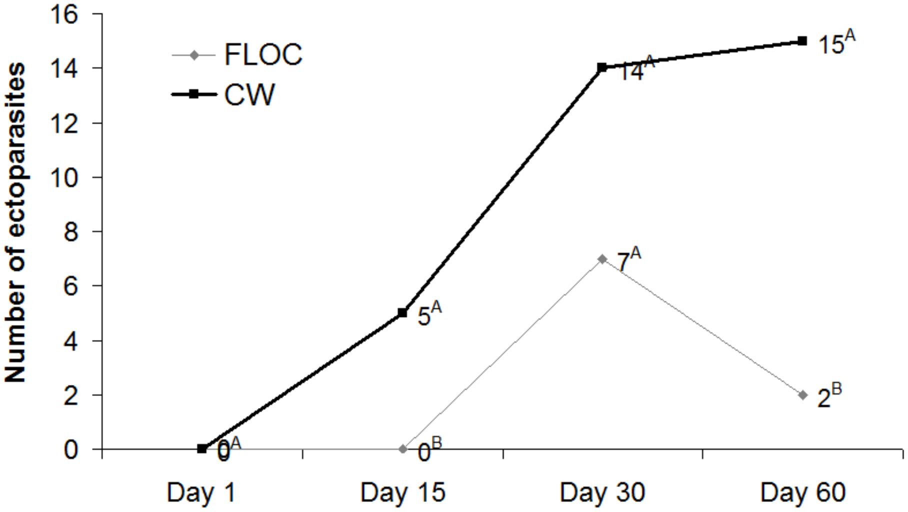


Figure 5. Number of total ectoparasites in gills and ectoderm's mucous of fry tilapia reared under BFT limited water-exchange condition (FLOC) and conventional water-exchange system (CW) after 60 days (more details in [74])

4.4. Aquaponics

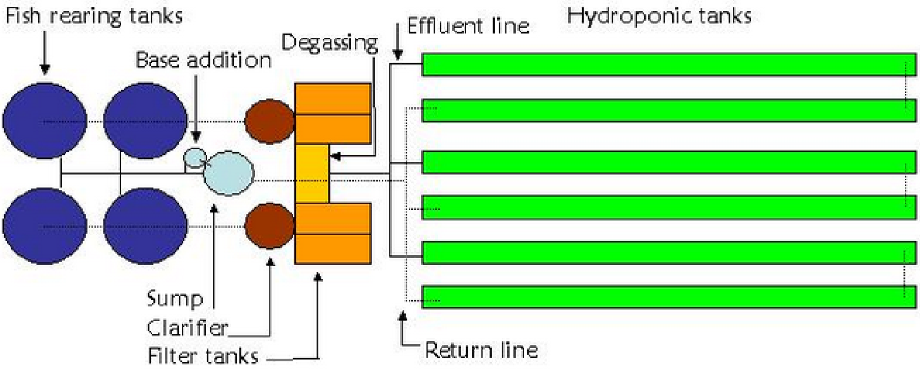
Aquaponics is a sustainable food production system that combines a traditional aquaculture with hydroponics in a symbiotic environment. The water is efficiently recirculated and reused for maximum benefits through natural biological filtration and recirculation. The waste that is excreted by aquatic species or uneaten feed is naturally converted into nitrate and other beneficial nutrients in the water. Those nutrients are then absorbed by the vegetables and fruits in a "natural fertilization way".

Aquaculture species including fish, crayfish, freshwater prawns or shrimp are usually reared in tanks and the water directed into separated race-ways of hydroponics vegetables. A worldwide well-known aquaponics system was successfully developed by University of Virgin Islands (Fig 6). Typical plants raised in aquaponics include lettuce, chard, tomato, fruits such as passion fruit, strawberry, water melon, etc.; and a large variety of spices. Size of aquaculture tanks varies according aquatic species/vegetables demand and usual shapes includes round, square or rectangular tanks.

(Source: UVI website www.uvi.edu)

Figure 6. Aquaponics system at University of Virgin Islands

Nowadays, BFT have been successfully applied in aquaponics. The presence of rich-biota (microorganisms of biofloc) and a variety of nutrients such as micro and macronutrients originated from un-eaten or non-digested feed seems to contribute in plant nutrition. A well-known example of biofloc and aquaponics interaction was also developed by UVI. However, the application of BFT in aquaponics needs particular attention, mainly on management of solid levels in water (for review, see [28]). High concentration of solids may cause excessive adhesion of microorganism on plants roots (biofilm), causing its damage, lowering oxygenation and poor growth. Filtering and settling devices are often needed (Fig 7).



(Source: UVI website www.uvi.edu)

Figure 7. Scheme of worldwide well-known UVI Aquaponics System

5. Microbial biomass application in animal food industry

The cost of diets in several animal cultures is predominantly due to the cost of protein component [75]. In the case of aquaculture, its massive expansion in the last decades has begun to face some important limitations like increasing prices of fishmeal, a raw material prime component of aquaculture diets. However, pressure caused in natural stocks (over-fishing) has depleted fishmeal production and, as a consequence, continuous increase in prices has been observed [76]. Moreover, growth of aquafeed industry (driven by an increase in fish/shrimp demand as the global population continues to growth), the competition with other animal cultures (such as swine and poultry) and differences in fishmeal quality also collaborated with increase in prices of fishmeal. The quality attributed to fishmeal includes high palatability, high content of digestible protein, highly unsaturated fatty acids (HUFA) and minerals.

In this context, alternatives should be evaluated opposing this non-optimism scenario. Aquaculture industry needs to investigate alternative source of proteins to replace less sustainable ones. Candidates of protein sources might have good digestibility, palatability, energy content, low ash content and present a well-balanced essential amino acids profile (EAA) [77].

In the past years, BFT has been emerged not only as promising alternative to grow-out system, but also as a method to obtain protein for compounds diets originated from its diverse microbiota. Collected in tanks/ponds [46, 62] or produced in bioreactors [17, 39, 67] biofloc (Fig 8) is a raw material to produce “biofloc meal”. In bioreactors, biofloc production can clean up effluent waters from aquaculture facilities, converting dissolved nutrients into single-cell protein [78]. Usually, two types of bioreactors have been employed: sequencing batch reactors (SBRs) and membrane batch reactors (MBRs), both controlling ammonia, nitrite and suspended solids with great efficacy (for review of bioreactors and its employ, see Kuhn et al 2012). Moreover, excess of solids removed from culture tanks or ponds and/or concentrated into solid removal devices [28] could also be a recyclable source of biofloc for biofloc meal production. This sustainable approach of protein source is getting more attention in the aquaculture industry. The microbial particles can provide important nutrients such as protein [33, 46], lipids [10, 37], aminoacids [80] and fatty acids [33, 67, 81].

Biofloc meal (also called “single-celled” protein), added to compounded feed is currently focus of intensive research in nutrition fields [17, 78]. However, to produce this protein ingredient some processes are required such as drying, milling and storage. In this context, nutritional characteristics could be affected (by i.e. temperature during drying), which the “native” properties could be altered.

Nutritional composition of biofloc differs according to environmental condition, carbon source applied, TSS level, salinity, stocking density, light intensity, phytoplankton and bacteria communities and ratio, etc. Regarding to age of bioflocs, in “young” biofloc heterotrophic bacteria is mainly presented as compared to “old” biofloc dominated by fungi [79]. In biofloc particles, protein, lipid and ash content could vary substantially (12 to 49, 0.5 to 12.5 and 13 to 46%, respectively; Table 2). The same trend occurs with fatty acids (FA) profile. Essential FA such as linoleic acid (C18:2 n-6 or LA), linolenic acid (C18:3 n-3 or ALA), arachidonic acid (C20:4 n-6 or ARA), eicosapentanoic acid (C20:5 n-3 or EPA) and docosahexaenoic acid (C22:6 n-3 or DHA), as well as sum of n-3 and sum of n-6 differ considerably between 1.5 to 28.2, 0.04 to 3.3, 0.06 to 3.55, 0.05 to 0.5, 0.05 to 0.77, 0.4 to 4.4 and 2.0 to 27.0% of total FA. Type of carbon source, freshwater or marine water and production of biofloc biomass (in bioreactors or culture tanks) definitely influence the FA profile (Table 3 and 4). Vitamin and amino acids profile from biofloc produced in large-scale commercial bioreactors [82] in given in Table 5.

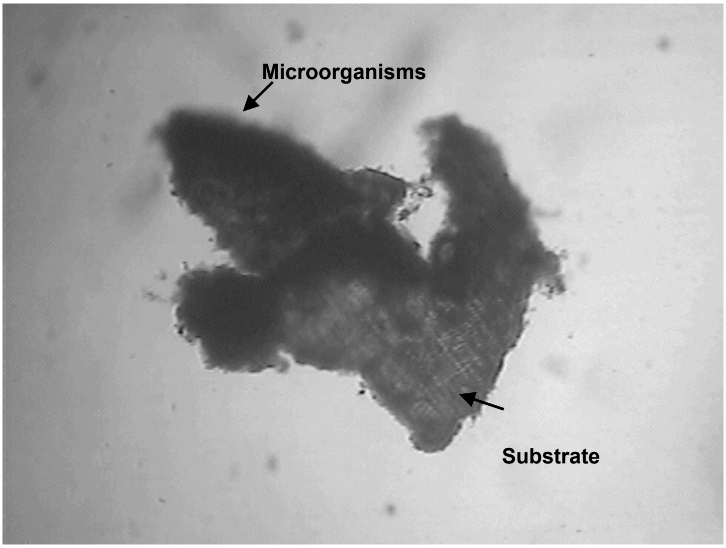


Figure 8. Biofloc particle (10x magnification) (Source: [54])

Information is still scarce about how microorganisms profile and its nutritional composition could impact animal growth. However, is already known that microorganisms in biofloc might partially replace protein content in shrimp diets, although were not always the case [10, 88]. Recent studies determined how reducing the protein content of diet would affect growth performance of shrimp reared in biofloc conditions. In the study [15] was found that at least 10% of protein content in pelletized feed can be reduced when *F. paulensis* postlarvae are raised in BFT conditions. In [89] was observed that shrimp fed with less than 25% crude protein under biofloc conditions performed similarly to shrimp raised under regular clear-water intensive culture with a 37%-protein diet. The biofloc system also delivered more consistent survival rates, especially at higher density. A low-protein biofloc meal-based pellet (25% CP) was evaluated as a replacement of conventional high-protein fishmeal diet (40% CP) for *L. vannamei* in a relatively low temperature (25°C) under biofloc conditions [35]. The results showed that is possible to replace 1/3 part of a conventional diet by alternative low-protein biofloc meal pellet without interfering survival and shrimp performance.

Crude protein (%)	Carbohydrates (%)	Lipids (%)	Crude fiber (%)	Ash (%)	Reference
43.0	-	12.5	-	26.5	[27]
31.2	-	2.6	-	28.2	[83]
12.0 - 42.0	-	2.0 - 8.0	-	22.0 - 46.0	[84]
31.1	23.6	0.5	-	44.8	[10]
26.0 - 41.9	-	1.2 - 2.3	-	18.3 - 40.7	[80]
30.4	-	1.9	12.4*	38.9	[85]
49.0	36.4	1.13	12.6	13.4	[17]
38.8	25.3	<0.1	16.2	24.7	[78]
28.8 - 43.1	-	2.1 - 3.6	8.7 - 10.4	22.1 - 42.9	[86]
30.4	29.1	0.5	0.8	39.2	[37]
18.2-29.3	22.8-29.9	0.4-0.7	1.5-3.5	43.7-51.8	[47]
18.4-26.3	20.2-35.7	0.3-0.7	2.1-3.4	34.5-41.5	[87]
28.0-30.4	18.1-22.7	0.5-0.6	3.1-3.2	35.8-39.6	[62]

*Lignin+cellulose

Table 2. Proximate analysis of biofloc particles in different studies.

Also, recent studies have been demonstrated that fishmeal in shrimp diets can be partially replaced by other protein sources under biofloc conditions or by biofloc meal. In [90] was evaluated two fishmeal replacement levels (40 and 100% of replacement) by other ingredients (soyabean meal and viscera meals) in diets for *Litopenaeus vannamei* reared in a biofloc system. The authors observed that fishmeal can be replaced in a level of 40.0% without interfering on growth performance and water quality. On the other hand, incorporating treated solids (microbial flocs) generated from tilapia effluent into shrimp feed, [91] demonstrated that shrimp performance was significantly increased as compared to untreated solids (settling basins of tilapia culture units). In [92] a trial performed in clear-water conditions detected that fishmeal can be completely replaced with soy protein concentrate and biofloc meal (obtained from super-intensive shrimp farm effluent) in 38% CP diets without adverse effects on *L. vannamei* performance. Moreover, [17] observed that biofloc produced in SBRs bioreactors using tilapia effluent and sugar as a growth media could offer an alternative protein source to shrimp feeds. Microbial floc-based diets significantly outperformed control fishmeal-based diets in terms of weight gain per week with no differences in survival.

Regarding to biofloc meal production, one bottleneck seems to be the large amount of wet biofloc biomass required to produce 1kg of dry biofloc meal. Estimative indicates that biofloc plug in 1L settling cones contained only 1.4% of dry matter [14]. The reference [17] indicated that 1 kg of microbial floc could be produced per 1.49 kg of sucrose in bioreactors. Certainly more research is needed on this field. On the other hand, other applications of biofloc meal in animal industry should be evaluated, mainly considering its nutritional profile and relatively low costs as compared to other protein sources (i.e. fishmeal) [17]. In aquaculture, biofloc meal could be included into broodstock pelletized feed, prior or after eyestalk ablation. Further research is encouraged in this field.

Fatty Acid	**% of total fatty acid**							
C14:0	0.10	0.60	0.80	0.45	1.43	0.69	0.61	0.43
C15:0	0.15	0.25	0.25	0.30	0.31	0.31	0.17	0.26
C16:0	2.2	17.0	26.0	15.0	6.06	8.01	6.34	8.86
C16:1	4.0	3.7	3.0	5.0	6.61	2.61	1.61	1.54
C17:0	0.05	0.4	0.5	0.2	0.20	0.23	0.14	0.68
C18:0	0.5	4.0	7.1	6.0	2.37	4.82	3.94	6.27
C18:1 n-7	1.5	3.0	1.9	2.7	3.96	1.72	2.71	4.19
C18:1 n-9	1.8	19.0	30.0	18.0	3.34	7.26	8.12	12.05
C18:2 n-6 (LA)	5.0	19.0	28.2	11.0	1.91	17.24	11.95	21.87
C18:3 n-3 (ALA)	0.04	0.5	0.45	2.0	0.23	0.99	0.20	0.21
C20:0	-	0.10	0.20	0.20	0.06	0.34	0.33	0.49
C20:1 n-9	0.05	0.10	0.15	0.10	0.25	0.20	0.06	0.02
C20:3 n-6	0.15	0.10	0.06	0.07	0.55	0.36	0.15	0.04
C20:4 n-6 (ARA)	0.7	0.3	0.15	0.20	0.77	0.87	0.17	0.06
C20:5 n-3 (EPA)	0.10	0.11	0.05	0.25	0.15	0.15	0.19	0.12
C22:6 n-3 (DHA)	0.05	-	0.07	0.05	0.18	0.06	0.18	0.10
∑ Saturated	22.08	22.99	35.35	22.45	10.76	14.85	11.53	16.99
∑ Monounsaturated	8.16	26.22	35.45	27.15	16.51	14.21	12.5	17.8
∑ n-3	0.4	0.6	0.7	0.65	1.04	2.02	0.60	0.43
∑ n-6	7.0	20.0	27.0	12.0	4.03	19.03	12.27	21.97
Type of water	freshwater	freshwater	freshwater	freshwater	freshwater	freshwater	marine	marine
Carbon source	Acetate	Glycerol	(Glycerol+ *Bacillus*)	Glucose	Glucose	Glycerol	Glucose	Glycerol
Collection	bioreactors	bioreactors	bioreactors	bioreactors	bioreactors	bioreactors	bioreactors	bioreactors
Reference	[39]				[67]			

Table 3. Fatty acid profile of biofloc (produced in experimental bioreactors) using different carbon source in marine water and freshwater

Fatty Acid	**% of total fatty acid**		
C14:0	2.02-2.48	13.8-16.1	5.4-6.2
C15:0	0.70-0.77	1.1-1.5	1.1-1.3
C16:0	17.88-19.10	45.4-53.5	48.7-49.3
C16:1	7.15-7.74	9.9-15.3	16.5-21.6
C17:0	-	0.7	0.9-1.0
C18:0	6.24-7.27	3.4-3.5	3.7-4.5
C18:1 n-7	11.05-11.28	-	-
C18:1 n-9	8.51-10.08	8.8-9.2	7.7-10.8
C18:2 n-6 9 (LA)	15.38-16.68	1.5-2.5	2.2-2.6
C18:3 n-3 (ALA)	0.65-0.73	2.0-2.3	2.2-3.3
C20:0	0.87-1.44	0.2-0.4	0.4
C20:1 n-9	0.74-0.80	0.3-0.4	0.5
C20:3 n-6	0.40-0.46	0.2	0.2
C20:4 n-6 (ARA)	3.11-3.55	0.3-0.4	0.3-0.4
C20:5 n-3 (EPA)	0.39-0.46	0.3-0.5	0.5
C22:6 n-3 (DHA)	0.74-0.77	0.2-0.4	0.3-0.4
∑ Saturated	30.2-34.92	67.6-73.0	61.5-61.9
∑ Monounsaturated	28.10-29-38	19.7-25.0	28.3-30.5
∑ n-3	1.38-1.91	2.8-3.4	3.2-4.4
∑ n-6	23.5-25.81	2.0-3.0	2.7-3.1
Type of water	freshwater	marine	marine
Carbon source	Wheat flour	molasses	molasses
Collection	Tilapia tanks	shrimp tanks	shrimp tanks
Reference	[33]	[87]	[62]

Table 4. Fatty acid profile of biofloc (collected in tanks) using different carbon source in marine water and freshwater

Amino Acids	As Fed (%)
Alanine	3.82
Arginine	3.60
Aspartic acid	6.36
Glutamic acid	8.04
Glycine	2.81
Histidine	1.46
Isoleucine	3.38
Leucine	5.06
Lysine	4.34
Methionine	1.41
Cysteine	0.55
Phenylalanine	3.29
Proline	2.77
Serine	2.82
Taurine	0.25
Threonine	3.11
Tryptophan	0.98
Tyrosine	2.83
Valine	3.52
Total	**60.4**
Vitamins	
Niacin	83.3 mg/kg
Thiamine B1	7.7 mg/kg
Riboflavin	39.0 mg/kg
Vitamin B12	12.0 mg/kg
Vitamin E	29.8 IU/kg

Table 5. Example of vitamin and amino acids profile from biofloc produced in large-scale commercial bioreactors [82].

6. Conclusions and perspectives of BFT

Biosecurity is a priority in aquaculture industry. For example, in shrimp farming, considerable impact of disease outbreaks during the past two decades greatly affected the operational management of shrimp farms worldwide [10]. Infected PLs and incoming water seem to be the main pathway for pathogen introduction. This scenario forced farmers to look for more biosecure culture practices to minimize the risk associated with exposure to pathogens [2]. Biofloc technology brings an obvious advantage of minimizing consumption and release of water, recycling *in situ* nutrients and organic matter. Furthermore, pathogens introduction is reduced, improving the farm biosecurity.

Biofloc technology will enable aquaculture grow towards an environmental friendly approach. 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 faeces and uneaten food and convert it into microbial protein. These qualities make minimal-exchange BFT system an alternative to extensive aquaculture. Microorganisms in biofloc might partially replace protein content in diets or decrease its dependence of fishmeal.

Related to biofloc meal and its perspectives, the study [17] detected initial estimates of cost for producing a metric ton of biofloc meal is approximately $400 to $1000. The same authors cited that global soymeal market varied approximately from $375 to $550/metric ton from January 2008 through May 2009. During the same time period, fishmeal varied approximately from $1000 to $1225, suggesting feasibility on replacement of either soybean and/or fish meal by biofloc meal. Moreover, generated from a process that cleans aquaculture effluents [17, 39] biofloc meal production avoids discharge of waste water and excessive damage to natural habitats [4]. This ingredient seems to be free of deleterious levels of mycotoxins, antinutritional factors and other constituents that limit its use in aquafeeds [79]. Large-scale production of biofloc meal for use in aquaculture could result in environmental benefits to marine and coastal ecosystems, as the need for wild fish as an aquafeed ingredient is reduced [79, 92].

Sensorial quality of BFT products is also an important issue. BFT may bring higher profit if fresh non-frozen shrimp/fish is sold to near-by market, mainly at inland locations. These advantages certainly should be more explored and niche markets achieved, contributing to social sustainability.

Author details

Maurício Emerenciano
Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM), Unidad Multidisciplinaria de Docencia e Investigación (UMDI), UNAM, Sisal, Yucatán, Mexico
Santa Catarina State University, Centro de Educação Superior da Região Sul (CERES), Laguna, Santa Catarina, Brazil

Gabriela Gaxiola
UMDI, Facultad de Ciencias, UNAM, Sisal, Yucatán, México

Gerard Cuzon
Ifremer (Institut Français de Recherche pour l'Exploitation de la Mer) Taravao, Tahiti, French Polynesia

Acknowledgement

The authors would like to thank CONCYTEY (Consejo de Ciencia y Tecnología del Estado de Yucatán), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES, Brazilian Ministry of Education (PhD grant number 4814061 provided to the primary author) and Consejo Nacional de Ciencia y Tecnología-CONACyT, México (grant 60824) for research support. The authors also would like to thank Wilson Wasielesky, Yoram Avnimelech and Manuel Valenzuela for photos courtesy and Miguel Arévalo, Maite Mascaró, Elsa Noreña, Santiago Capella, Adriana Paredes, Gabriela Palomino, Korynthia Aguiar, Moisés Cab, Nancy Aranda Cirerol, Concepción Burgos, Manuel Valenzuela and all staff of Programa Camarón-UMDI for their contribution towards researches performed at UMDI-UNAM cited in this chapter.

7. References

[1] Food and Agriculture Organization of the United Nations FAO (2008) Cultured aquaculture species information programme, *Penaeus vannamei* (Boone,1931). Food and Agriculture Organization of the United Nations. Available at http://www.fao.org

[2] Browdy CL, Bratvold D, Stokes AD, Mcintosh RP (2001) Perspectives on the application of closed shrimp culture systems. In: Jory ED, Browdy CL, editors. The new Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, The World Aquaculture Society, Baton Rouge, LA, USA. pp.20–34.

[3] De Schryver P, Crab R, Defoirdt T, Boon N., Verstraete W (2008) The basics of bio-flocs technology: the added value for aquaculture. Aquaculture 277:125-137.

[4] Naylor RL, Goldburg RJ, Primavera JH, Kautsk N, Beveridge MCM, Clay J, Folke C, Lubchencoi J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. Nature 405:1017-1024.

[5] Bender J, Lee R, Sheppard M, Brinkley K, Philips P, Yeboah Y, Wah RC (2004) A waste effluent treatment system based on microbial mats for black sea bass *Centropristis striata* recycled water mariculture. Aquac Eng 31:73-82.

[6] Ray A (2012) Biofloc technology for super-intensive shrimp culture. In: Avnimelech Y, editor. Biofloc Technology - a practical guide book, 2nd ed., The World Aquaculture Society, Baton Rouge, Louisiana, USA. pp. 167-188.

[7] Crab R, Kochva M, Verstraete W, Avnimelech Y (2009) Bio-flocs technology application in over-wintering of tilapia. Aquac Eng 40:105-112.

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

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

[10] Wasielesky W.Jr, Atwood H, Stokes A, Browdy CL (2006) Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei*. Aquaculture 258:396–403.

[11] Rakocy JE, Bailey DS, Thoman ES, Shultz RC (2004) Intensive tank culture of tilapia with a suspended, bacterial based treatment process: new dimensions in farmed tilapia.

In: Bolivar R, Mair G, Fitzsimmons K, editors. Proceedings of the Sixth International Symposium on Tilapia in Aquaculture. pp. 584–596.

[12] Avnimelech Y, Mokady S, Schoroder GL (1989) Circulated ponds as efficient bioreactors for single cell protein production. Bamdigeh. 41:58–66.

[13] Hargreaves JA (2006) Photosynthetic suspended-growth systems in aquaculture. Aquac Eng 34:344-363.

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

[15] Ballester ELC, Abreu PC, Cavalli RO, Emerenciano M, Abreu L, Wasielesky W (2010) 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. Aquac Nut 16:163-172.

[16] Yoram Avnimelech (2012) Biofloc Technology - A Practical Guide Book, 2nd ed. The World Aquaculture Society, Baton Rouge, Louisiana, EUA. 272p.

[17] Kuhn DD, Boardman GD, Lawrence AL, Marsh L, Flick GJ (2009) Microbial flocs generated in bioreactors is a superior replacement ingredient for fishmeal or soybean meal in shrimp feed. Aquaculture 296:51–57.

[18] Emerenciano M, Cuzon G, Goguenheim J, Gaxiola G, Aquacop (2011) Floc contribution on spawning performance of blue shrimp *Litopenaeus stylirostris*. Aquac Res (published online first DOI: 10.1111/j.1365-2109.2011.03012.x)

[19] Aquacop (1975) Maturation and spawning in captivity of penaeid shrimp: *Penaeus merguiensis* de Man, *Penaeus japonicus* Bate, *Penaeus aztecus* Ives, *Metapenaeus ensis* de Haan and *Penaeus semisulcatus* de Haan. In: Avault W, Miller R, editors. Proceedings of the Sixth Annual Meeting World Mariculture Society, Lousiana State University, Baton Rouge, pp. 123–129.

[20] Sohier L (1986) Microbiologie appliquée à l'aquaculture marine intensive. pp. 119. Thèse Doctorat d'Etat, Université Aix-Marseille II Marseille, France.

[21] Cuzon G, Lawrence A, Gaxiola G, Rosas C, Guillaume J (2004) Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture 235:513–551.

[22] Rosenberry B (2010) Controlling pH in biofloc ponds. The shrimp news international. http://www.shrimpnews.com/FreeReportsFolder/phContronBioflocPonds.html. Acessed in 11/02/2012.

[23] Garen P, Aquacop (1993) Nuevos resultados en la cría intensiva de camarón *Penaeus vannamei* y *P. stylirostris*. In: Calderón JV, Sandoval VC, editors. Memorias del I Congresso Ecuatoriano de Acuicultura, Guayaquil, 18-23 Octubre 1992. Escuela Superior Politécnica del Litoral, Guayaquil, pp. 137–145.

[24] Rosenberry B (2007) Marvesta Shrimp Farms Delivers "Fresh" Shrimp to Restaurants. The shrimp news international. http://www.shrimpnews.com/FreeNewsFolder/FreeNewsBackIssues/2007BackIssues/FreeNewsApril200720.html. Acessed in 12/02/2012.

[25] Moriarty DJW (1997) The role of microorganisms in aquaculture ponds. Aquaculture 151:333–349.

[26] Ray AJ, Seaborn G, Leffler JW, Wilde SB, Lawson A, Browdy CL (2010) Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. Aquaculture 310:130–138.

[27] McIntosh D., Samocha T.M., Jones E.R., Lawrence A.L., McKee D.A., Horowitz S. & Horowitz A. (2000) The effect of a bacterial supplement on the high-density culturing of *Litopenaeus vannamei* with low-protein diet in outdoor tank system and no water exchange. Aquac Eng 21:215–227.

[28] Ray AJ, Lewis BL, Browdy CL, Leffler JW (2010) Suspended solids removal to improve shrimp (*Litopenaeus vannamei*) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. Aquaculture 299:89-98.

[29] Panjaitan P (2004). Field and laboratory study of *Penaeus monodon* culture with zero water exchange and limited water exchange model using molasses as a carbon source. Ph.D. Thesis, Charles Darwin Univ., Darwin, NT, Australia.

[30] Avnimelech Y, Kochva M, Diab S (1994) Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Bamidgeh 46:119–131.

[31] Hari B, Kurup BM, Varghese JT, Schrama JW, Verdegem MCJ (2004) Effects of carbohydrate addition on production in extensive shrimp culture systems. Aquaculture 241:179–194.

[32] Schneider O, Sereti V, Eding EH, Verreth JAJ (2005) Analysis of nutrient flows in integrated intensive aquaculture systems. Aquac Eng 32:379–401.

[33] Azim ME, Little DC (2008) The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283:29–35.

[34] Emerenciano M, Vinatea L, Gálvez AG, Shuler A, Stokes A, Venero J, Haveman J, Richardson J, Thomas B, Leffler J (2009) Effect of two different diets fish meal based and "organic" plant based diets in *Litopenaeus setiferus* earlier post-larvae culture under biofloc, green-water and clear-water conditions. CD of abstracts of World Aquaculture Society Meeting 2009, Veracruz, México.

[35] Emerenciano M, Cuzon G, López-Aguiar K, Noreña-Barroso E, Máscaro M, Gaxiola G. (2011) Biofloc meal pellet and plant-based diet as an alternative nutrition for shrimp under limited water exchange systems. CD of abstracts of World Aquaculture Society Meeting 2011, Natal, RN, Brazil.

[36] Avnimelech Y (1999) Carbon and nitrogen ratio as a control element in aquaculture systems. Aquaculture 176: 227–235.

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

[38] Taw N (2010) Biofloc technology expanding at white shrimp farms. Global Advocate may/june, 24–26 (available in http://www.gaalliance.org/mag/May_June2010.pdf)

[39] Crab R, Chielens B, Wille M, Bossier P, Verstraete W (2010) The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquac Res 41: 559-567.

[40] Avnimelech Y, Mokady S (1988) Protein biosynthesis in circulated ponds. In: Pullin RSV, Bhukaswan T, Tonguthai K, Maclean JL, editors. Proceedings of Second International Symposium on Tilapia in Aquaculture, Departament of Fisheries of Thailand and ICLARM, Manila, Philippines, pp. 301–309.

[41] Milstein A, Anvimelech Y, Zoran M, Joseph D (2001) Growth performance of hybrid bass and hybrid tilapia in conventional and active suspension intensive ponds. Bamidgeh 53:147–157.

[42] Asaduzzaman M, Rahman MM, Azim ME, Islam MA, Wahab MA, Verdegem MCJ, Verreth JAJ (2010). Effects of C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds. Aquaculture 306: 127–136.

[43] Suita SM (2009) O uso da Dextrose como fonte de carbono no desenvolvimento de bioflocos e desempenho do camarão-branco (*Litopenaeus vannamei*) cultivado em sistema sem renovação de água. Tese de mestrado. Universidade Federal do Rio Grande, Rio Grande do Sul, Brasil.

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

[45] Asaduzzaman M, Wahab MA, Verdegem MCJ, Huque S, Salam MA, Azim ME (2008) C/N ratio control and substrate addition for periphyton development jointly enhance freshwater prawn *Macrobrachium rosenbergii* production in ponds. Aquaculture 280, 117–123.

[46] Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W (2011b) Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance. Aquac Int 19:891-901.

[47] Emerenciano M, Cuzon G, Paredes A, Gaxiola G (2012) Biofloc technology applied to intensive broodstock farming of pink shrimp *Farfantepenaeus duorarum* (Part I): grow-out, water quality, microorganisms profile and proximate analysis of biofloc. Aquac Res (*submitted*)

[48] Mishra JK, Samocha TM, Patnaik S, Speed M, Gandy RL, Ali A (2008) Performance of an intensive nursery system for the Pacific white shrimp, *Litopenaeus vannamei*, under limited discharge condition. Aquac Eng 38:2–15.

[49] Apud FD, Primavera JH, Torres PL (1983) Farming of Prawns and Shrimps. Extension Manual (5), SEAFDEC Aquaculture Department, Iloilo, Philippines. 67pp.

[50] Sandifer PA, Stokes AD, Hopkins JS (1991) Further intensification of pond shrimp culture in South Carolina. In: Sandifer PA, editor. Shrimp Culture in North America and the Caribbean, World Aquaculture Society, Baton Rouge, LA, USA. pp.84–95.

[51] Arnold SJ, Coman FE, Jackson CJ, Groves SA (2009) High-intensity, zero water-exchange production of juvenile tiger shrimp, *Penaeus monodon*: An evaluation of artificial substrates and stocking density. Aquaculture 293:42-48.

[52] Souza DM, Suita SM, Leite FPL, Romano LA, Wasielesky W, Ballester ELC (2011). The use of probiotics during the nursery rearing of the pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817) in a zero exchange system. Aquac Res (published online first doi:10.1111/j.1365-2109.2011.02992.x)

[53] AQUACOP, Le Moullac G, Damez D (1991) Modélisation de la résistance au chocs de salinité des postlarves de Penaeus vannamei. Aquat Living Resour 4:169–173

[54] Emerenciano MGC, Wasielesky W, Soares RB, Ballester EC, Cavalli RO, Izeppi EM (2007) Crescimento e sobrevivêcia do camarão-rosa *Farfantepenaeus paulensis* na fase de berçário em meio heterotrófico. Acta Scientiarum Biological Sciences 29, 1–7.

[55] Cohen J, Samocha TM, Fox JM, Gandy RL, Lawrence AL (2005) Characterization of water quality factors during intensive raceway production of juvenile *L. vannamei* using limited discharge and biosecure management tools. Aquac Eng 32:425–442.

[56] Krummenauer D, Peixoto S, Cavalli RO, Poersch LH, Wasielesky W (2011) Superintensive culture of white shrimp, *Litopenaeus vannamei*, in a biofloc technology system in southern brazil at different stocking densities. J. World Aquacult. Soc. 42:726-733.

[57] Samocha TM, Patnaik S, Gandy RL(2004) Heterotrophic intensification of pond shrimp production. Book of abstract of Fifth International Conference on Recirculating Aquaculture, 22– 25 July 2004, Roanoke, Virginia, USA.

[58] Cruz-Ricque LE, Guillaume J, Cuzon G (1987) Squid protein effect on growth of four penaeid shrimp. J. World Aquacult. Soc. 18: 209–217.

[59] Itani AL, Neto ETA, Silva SL, Araújo ML, Lima AF, Barbosa JM (2010) Efeito do sistema heterotrófico no crescimento do Tambaqui (Colossoma macropomum). X JORNADA DE ENSINO, PESQUISA E EXTENSÃO – JEPEX 2010 – UFRPE: Recife.

[60] Poleo G, Aranbarrio JV, Mendoza L, Romero O (2011) Cultivo de cachama blanca en altas densidades y en dos sistemas cerrados. Pesq. Agropec. Bras. 46: 429-437

[61] Wouters R, Lavens P, Nieto J, Sorgeloos P (2001a) Penaeid shrimp broodstock nutrition: an updated review on research and development. Aquaculture 202:1–21.

[62] Emerenciano M, Cuzon G, Arévalo M, Gaxiola G (2012) Biofloc technology applied to intensive broodstock farming of pink shrimp *Farfantepenaeus duorarum* (Part II): spawning performance, biochemical composition and fatty acid profile. Aquac Res (submitted)

[63] Vinatea L, Gálvez AO, Browdy CL, Stokes A, Venero J, Haveman J, Lewis BL, Lawson A, Shuler A, Leffler JW (2010) Photosynthesis, water respiration and growth

performance of *Litopenaeus vannamei* in a super-intensive raceway culture with zero water exchange: Interaction of water quality variables. Aquac Eng 42:17–24.

[64] Emerenciano M, Gaxiola G, Cuzon G (2012). Biofloc Technology applied to shrimp broodstock. In: Avnimelech Y, editor. Biofloc Technology - apractical guide book, 2nd ed., The World Aquaculture Society, Baton Rouge, Louisiana, USA. pp. 217-230.

[65] Ng W, Wang Y (2011) Inclusion of crude palm oil in the broodstock diets of female Nile tilapia, *Oreochromis niloticus*, resulted in enhanced reproductive performance compared to broodfish fed diets with added fish oil or linseed oil. Aquaculture 314:122–131.

[66] Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev Aquac 1:71-124.

[67] Ekasari J, Crab R, Verstraete W (2010) Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. HAYATI Journal of Biosciences 17:125-130.

[68] Sinha AK, Baruah K, Bossier P (2008) Horizon Scanning: the potential use of biofloc as an anti-infective strategy in aquaculture – an overview. Aquac Health Int 13:8-10.

[69] De Schryver P, Boon N, Verstraete W, Bossier P. (2012) The biology and biotechnology behind bioflocs. In: Avnimelech Y, editor. Biofloc Technology - a practical guide book, 2nd ed., The World Aquaculture Society, Baton Rouge, Louisiana, USA. pp. 217-230.

[70] Yu J, Plackett D, Chen LXL (2005). Kinetics and mechanism of the monomeric products from abiotic hydrolysis of poly [(R) -3-hydroxybutyrate] under acidic and alkaline conditions. Polym Degrad Stabil 89:289-299.

[71] Ricke SC (2003). Perspectives on the use of organic acids and shortchain fatty acids as antimicrobials. Poult Sci 82:632-639.

[72] Russel JB (1992). Another explanation for the toxicity of fermentationacids at low pH: anion accumulation versus uncoupling. J Appl Bacteriol 73:363-370.

[73] Teitelbaum JE, Walker WA (2002) Nutritional impact of pre and pro-biotics as protective gastrointestinal organisms. Annu Rev Nutr 22:107-138.

[74] Emerenciano M, Avnimelech Y, Gonzalez R, Leon ATD, Cuzon G, Gaxiola G (2009) Effect of bio-floc technology (BFT) in ectoparasite control in Nile tilapia Oreochromis niloticus culture. CD of Abstracts of World Aquaculture Society Meeting 2009, Veracruz, Veracruz, Mexico.

[75] Tacon AGJ (1990) Standard methods for the nutrition and feeding of farmed fish and shrimp, Washington DC, Argent Laboratories Press, 454 pp.

[76] Tacon AGJ (1997) Feeding tomorrow’s fish: keys for sustainability. In: Tacon A, Basurco B, editors. Feeding tomorrow’s fish. Proceedings of the workshop of the CIHEAM Network on Technology of Aquaculture in the Mediterranean (TECAM), jointly organized by CIHEAM, FAO and IEO Mazarron, Spain, 24-26 June 1996, CIHEAM, Apodo, Spain, pp. 11-33.

[77] Hardy RW (2010) Utilization of plant proteins in fish diets effects of global demand and supplies of fishmeal. Aquac Res 41:770-776.

[78] Kuhn DD, Lawrence AL, Boardman GD, Patnaik S, Marsh L, Flick GJ (2010) Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303:28–33.

[79] Kuhn DD, Lawrence A (2012) Ex-situ biofloc technology. In: Avnimelech Y, editor. Biofloc Technology - a practical guide book, 2nd ed., The World Aquaculture Society, Baton Rouge, Louisiana, USA. pp. 217-230.

[80] Ju ZY, Forster I, Conquest L, Dominy W, Kuo WC, Horgen FD (2008) Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. Aquac Res 39:118-133.

[81] Izquierdo M, Forster I, Divakaran S, Conquest L, Decamp O, Tacon A (2006). Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp *Litopenaeus vannamei*. Aquac Nut 12:192–202.

[82] Logan AJ, Lawrence A, Dominy W, Tacon AGJ 2010. Single-cell proteins from food by-products provide protein in aquafeed. Global Advocate 13:56-57.

[83] Tacon AGJ, Cody JJ, Conquest LD, Divakaran S, Forster IP, Decamp OE (2002) Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquac Nut 8:121–137.

[84] Soares R, Jackson C, Coman F, Preston N (2004) Nutritional composition of flocculated material in experimental zero-exchange system for *Penaeus monodon*. In: Proceedings of Australian Aquaculture, 2004, WAS, Sydney p.89

[85] Ju ZY, Forster I, Conquest L, Dominy W (2008) Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. Aquac Nut 14:533–543.

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

[87] Emerenciano M, Cuzon G, Arévalo M, Miquelajauregui MM, Gaxiola G (2012) Effect of short-term fresh food supplementation on reproductive performance, biochemical composition and fatty acid profile of Litopenaeus vannamei (Boone) reared under biofloc conditions. Aquac Int (submitted).

[88] McIntosh D, Samocha TM, Jones ER, Lawrence AL, Horowitz S, Horowitz A (2001) Effects of two commercially available low protein diets (21% and 31%) on water sediment quality, and on the production of *Litopenaeus vannamei* in an outdoor tank system with limited water discharge. Aquac Eng 25:69–82.

[89] Nunes AJP, Castro LF, Sabry-Neto H (2010) Microbial flocs spare protein in white shrimp diets. Global Advocate 10:28-30.

[90] Scopel RB, Schveitzer R, Seiffert WQ, Pierri V, Arantes RF, Vieira FN, Vinatea LA (2011) Substituição da farinha de peixe em dietas para camarões marinhos cultivados em sistema bioflocos. Pesq Agropec Bras 46: 928-934

[91] Kuhn DD, Boardman GD, Craig SR, Flick GJ, Mclean E (2008) Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, *Litopenaeus vannamei*, in recirculating aquaculture systems. J. World Aquacult. Soc. 39:72–82.

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