Chapter 3

Biofloc, a Technical Alternative for Culturing Malaysian Prawn *Macrobrachium rosenbergii*

Carlos I. Pérez-Rostro, Jorge A. Pérez-Fuentes and
Martha P. Hernández-Vergara

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/57501

1. Introduction

Aquaculture is a major food- producing activity that is growing steadily, coupled with growing population density and land use needs of other industries. To maintain growth, aquaculture must shift to intensive or semi-intensive practices, effective and sustainable use of resources, and sustainable environmental stewardship. This often requires application of technologies that increase production efficiency and avoids competition for space and resources with other activities, such as agriculture and ranching. Aquacultural practices must be sustainable and minimally destructive to the environment, maintain quality and safety standards, and enable efficient use of space and natural resources and possibilities for expansion. Technology alternatives that reduce environmental impact and are efficient without affecting the health and growth of stock organisms must be incorporated into current practices. One option is to apply biofloc technology. Biofloc forms naturally in pond water as aggregates of nitrifying bacteria, organic material, inorganic flocculants, and suspended algae. These ingredients serve as food for the stock under cultivation and promote direct use of nitrogenous compounds in feces, urine, and food waste. Activity of nitrifying bacteria increases with addition of carbon sources and constant aeration, which maintains or significantly improves water quality during cultivation. Thus, the large volume of water required in intensive aquafarming is greatly reduced [1, 2, 3]. An example is using biofloc during cultivation of the Malaysian river prawn *Macrobrachium rosenbergii*. The approach led to major savings of water, without affecting the quality of the prawns.

1.1. What is biofloc?

Biofloc culture is a system where, after adding a carbon source and providing constant aeration, biofloc bacteria maintain water quality during cultivation of freshwater shrimp. The metabolic processes and biochemical transformations take place directly in the water column, which promotes overall balance of the system and the health of the farmed shrimp. The biofloc forms in the pond water naturally as aggregates of nitrifying bacteria, organic material, inorganic flocculants, and suspended algae. The algae serves as food for the pond stock and the bacterial promotes direct conversion nitrogenous waste to simpler compounds. The self-cycling process maintains or greatly improves the quality of the pond water during cultivation. Improvement in water quality drastically reduces the need to cycle large volumes of additional water in the farm pond system. This leads to a sustainable activity that is in balance with the environment and reduces the cost of water and feed for the pond stock [1, 2, 3] (Fig. 1).

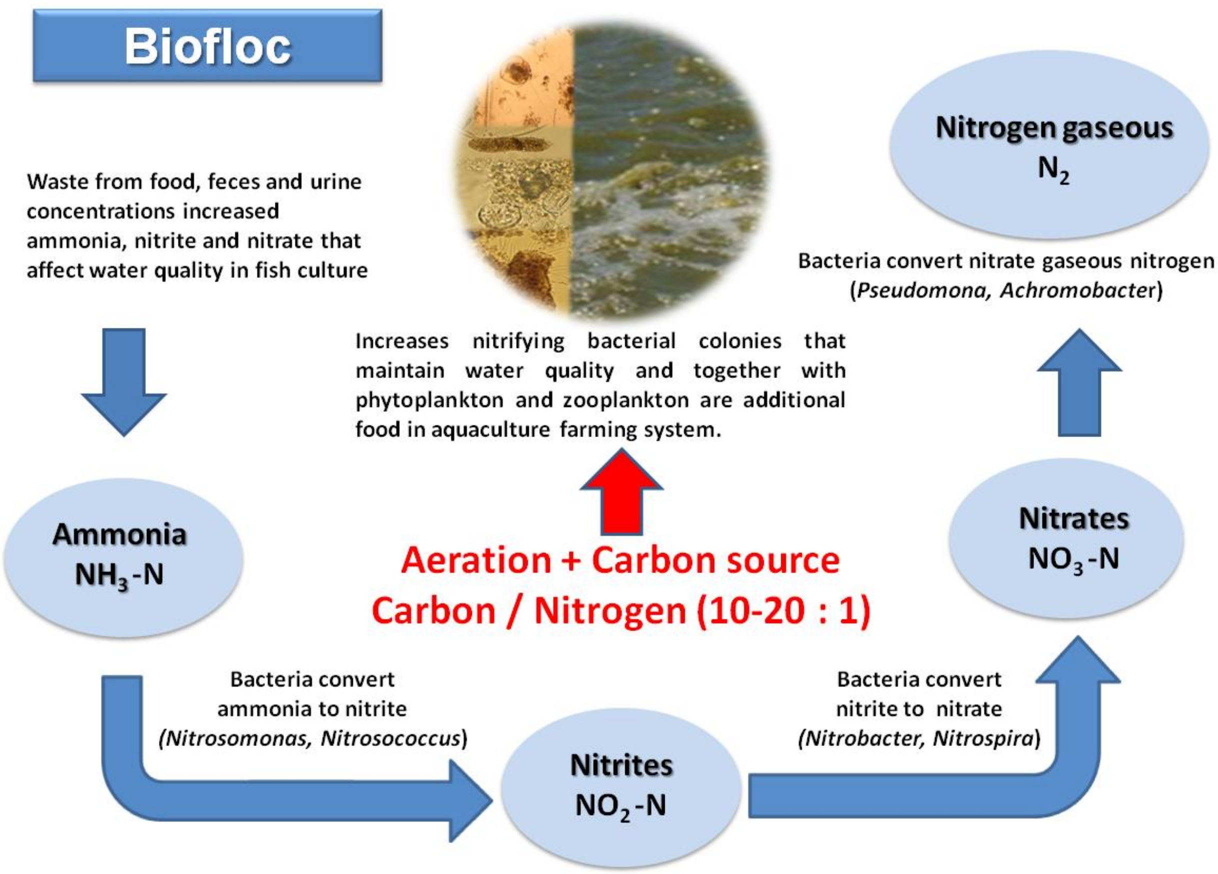


Figure 1. Biological processes in biofloc cultivation

In a biofloc system, the biological nitrification process occurs in three stages. In the first stage, bacteria of the genera *Nitrosomonas* and *Nitrosococcus* act on ammonia (NH_3/NH_4) generated by food scraps and feces and urine. The waste is oxidized to nitrite (NO_2). In the second stage, the nitrite is converted to nitrate (NO_3) by bacteria of the genera *Nitrobacter* and *Nitrospira*. The nitrate is reduced to nitrogen gas (anoxic denitrification) by bacteria mainly from the genera *Achromobacter* and *Pseudomonas* [4, 5].

Biofloc are of two main types. Classification is based on the amount and nature of organic matter and its component organisms, the latter can be bacterial or autotrophic, mainly

http://dx.doi.org/10.5772/57501

composed of algae. The importance of this is that, in both cases, the microorganisms present in the bioflocs maintain water quality because they decrease nitrogen compounds and are also nutrients for the bacterial and algae. It is important to understand that, depending on the nature of the biofloc microorganisms, their nutritional quality can vary. This affects the supply of nutrients for the stock organisms in the ponds (Fig. 2).

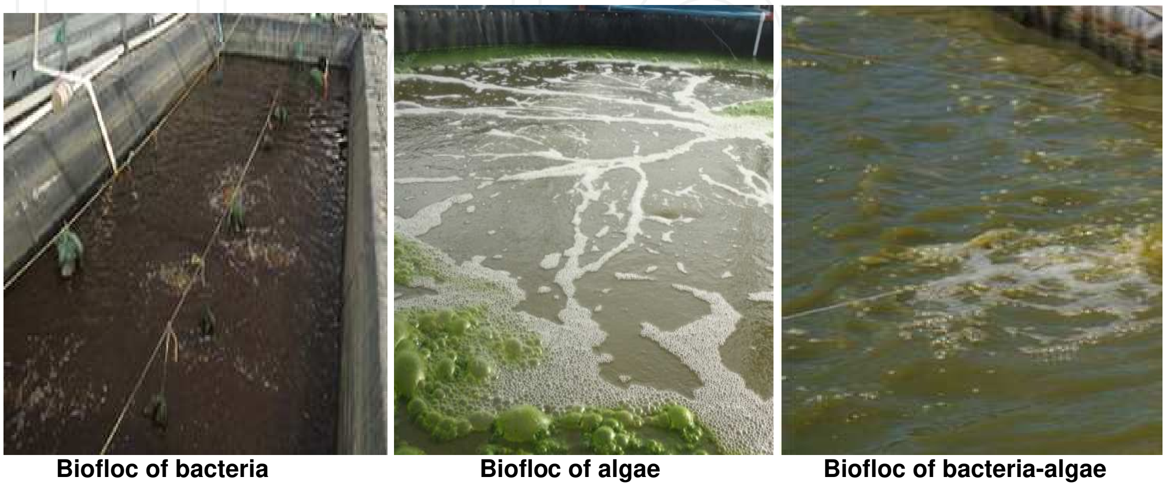


Figure 2. Types of biofloc, based on predominant species.

The microorganisms that populate biofloc systems typically inhabit natural aquatic systems. Their presence depends directly on two environmental variables: intensity of solar energy absorbed by the system and the concentration of organic matter and carbon sources that enter the system. In a biofloc system, colonies of bacteria depend mostly on organic matter present in the system for survival and proliferation, and, to a lesser degree, on the intensity of sunlight. Bacteria are also directly dependent on the system to supply constant aeration because the bacteria consume large volumes of oxygen, which is, in turn, directly related to the bacteria consuming carbon from the system. The carbon is a source of power for growth and proliferation. The concentration of carbon in the system must be maintained at a C/N ratio appropriate for maintaining reproduction of bacteria with inorganic nitrogen to a maximum concentration of 200 mg L^{-1} [6]. This level is an indicator that the system is effectively and economically controling nitrogen. High concentrations of nitrogen will usually upset the balance of the system and affect the health of stock species, especially shrimp.

In a biofloc system based on bacteria and algae, nitrogen compounds are removed as bacteria increased uptake of ammonium and better control the products of waste. This does not depend on the intensity of sunlight to run efficiently. In a microalgal biofloc system, productivity depends directly on sunlight; excess illumination can generate an excess of algae, which leads to low oxygen at midday. Hence, a system using microalgae and bacteria will be a more efficient alternative bioremediation because it is possible to maintain an efficient balance between nitrifying bacteria and algae to maintain a suitable level of nitrogen, a balanced C/N ratio, and sunlight. The diversity of live food available in ponds also increases in mixed biofloc systems, which brings benefits to the stock under cultivation. This includes reducing the amount of artificial feed necessary to meet nutrient requirements under semi-intensive and intensive pond farming. It also includes nutritients not present in synthetic diets. Not to be dismissed lightly is the great savings in costs of providing fresh water and handling organic wastes in water discharge.

1.2. Source of energy for bacteria and algae in biofloc

The microorganisms that form the biofloc and process nitrogen compounds that pollute fish pond water need a source of energy for metabolism. In aquatic biofloc systems, there are three likely energy sources, depending on the nature of the organisms present in the biofloc system (bacteria–algae aggregations). Most important is sunlight, which is the main source of energy for phototrophic microorganisms, such as algae and vascular plants. Solar reception can be controlled or semi-controlled to support the needs of the biofloc crop and promote any type of biofloc system. The second source of energy is the forms of inorganic compounds that are used by the microorganisms that oxidize reduced forms of simple compounds, especially nitrogen to obtain energy. In fish farming, by metabolizing organic nitrogen and ammonia, nitrogen is oxidized to nitrite and nitrate. The third source of energy is organic compounds that are transformed by microorganisms that derive energy from the metabolic oxidation of organic carbon and transform it to carbon dioxide.

Both chemothrophic and phototrophic microorganisms naturally consume and deplete nitrogen concentrations in the water because of the relatively large quantity of energy sources that are present, but also because this is an indispensable function of the microorganisms. Transformed energy is used to synthesis proteins from the nitrogen sources.

Systems for cleaning and wastewater bioremediation, using microalgae and bacteria, is a widely known technology; however, in aquaculture systems, they should be used with caution because, with microalgae, the efficiency of the system depends directly on solar energy and intensity, which in open systems can be a risk because there is no control over productivity [7, 8]. An excess of primary production leads to constant consumption of oxygen during the night. On cloudy days, productivity will reduce water quality.

Biofloc or nitrifying colonies of bacteria in aquaculture requires incorporation of additional carbon sources into the system to adequately reproduce biofloc and maintain high density because carbohydrates in the system may be insufficient. Some of the main sources of carbon that can be used in aquaculture crops are: glycerol and sodium acetate, sugar, tapioca flour, wheat flour, and molasses [9, 10, 11, 12, 13, 14]. Use depends directly on the local costs of these products. For a biofloc system to operate efficiently, it is best to maintain a C/N ratio between 10:1 and 20:1 [15, 1, 16, 17, 10]. The amount of carbon depends on several factors, including: water quality, physiology and growing body density of the stock, quality and quantity of food to be cultivated, and solubility of the carbon source. The carbon additive must be continuously monitored to ensure that the system is functioning properly.

1.3. Ecological importance of using biofloc in aquaculture

Biofloc technology provides more efficient and sustainable aquaculture by reducing environmental impacts. One major advantage is reducing the volume of water required by the system during cultivation. Biofloc in the cultivation system uses the initial water volume throughout

http://dx.doi.org/10.5772/57501

the production cycle and needs additional water only to replace water lost by evaporation, leakage, or to remove organic material during production. The biofloc microorganisms serve as natural food, depending on the eating habits of the stock species. This will reduce consumption of artificial food and lead to more efficient conversion of food. Biofloc is more than a supplemental source of nutrients in aquatic systems. It brings economic benefits during production and enables more efficient use of resources, given that the main source of protein during production is fish meal. Fish meal often comes from overharvesting of fisheries. Considering the rapid growth of pond farming, biofloc can directly contribute to reduced pressure on fisheries.

1.4. Physical-chemical parameters of water in the biofloc

Efficient operations with biofloc aquaculture systems depend on maintaining water physicochemical parameters within the range of tolerance of cultivated stock because this affects yield per unit volume. This is important because biofloc pond farming is a form of simple and complete synthetic ecosystems, based on three components that interact in the same space: (1) Stock of one or more commercial species; (2) Microalgae interact and function as biofiltrates that also have oxygen demand and, like commercial stock, produce metabolites; and (3) Bacteria responsible for transforming nitrogenous metabolites that are used by the planktonic microalgae. The purpose of a complex pond ecosystem with a biofloc cultivation system is the comprehensive use of energy and biotransformed products that maintain water quality and provide natural nutrients that promote the health and quality of a commercial animal crop without negative impacts on adjacent water bodies.

Water quality in aquatic systems is directly related to biological and chemical processes that occur in the aquatic environment and depend on several factors.

1.4.1. Dissolved oxygen

Oxygen in aquatic systems should be >5 mg L–1. In a biofloc pond system, the bacteria and algae that form the biofloc also have oxygen demand, so competition can occur in the pond. It is recommended that dissolved oxygen be maintained at 7–8 mg L–1 to ensure proper functioning of the system.

1.4.2. pH

pH should range from 6.5 to 9, depending on the cultivated stock. Aslo, pH <6 and >8.5 usually affects the efficiency of the biofloc components, as well as growth and survival of the cultivated stock. In a biofloc system, pH varies during the day as concentration of carbon dioxide build up from the respiration of the stock. We recommend a range of pH 7.0–8.5, which favors functioning of biological cycles in the system. To maintain the pH balance, low pH can be adjusted with calcium hydroxide, potassium hydroxide, sodium carbonate, or sodium bicarbonate. High pH can be adjusted with carbonic acid, hydrochloric acid, sulfuric acid, phosphoric acid, or their salts.

1.4.3. Dissolved solids and volatiles

Bacteria depend on suspended solids as a substrate for adhesion and as a source of energy from carbon. We maintained concentrations of suspended matter in the range of 250–450 mg L–1, which ensures efficient bacterial activity. An excess of suspended matter can affect breathing processes in the stock species, lead to stress, or, in extreme cases, lead to death by clogging gills. Cultivation of Litopenaeus vannamei, in one biofloc system contained 453.0 ± 50.0 mg L^{-1} total suspended solids and 256.0 ± 106 mg L–1 volatile solids, which improved shrimp production and provided efficient exchange of oxygen [18].

1.4.4. Turbidity

In aquaculture systems, transparency is directly affected by the amount of organic and inorganic matter in suspension (suspended solids, phytoplankton, zooplankton, and bacteria). Turbidity is measured with a turbidimeter or nephelometer, which uses a beam of light passing through a water sample. In aquaculture, a Secchi disk is frequently used because turbidity is measured by the depth when the disk cannot be seen. Solar heating of the water is also affected by

http://dx.doi.org/10.5772/57501

TDS meter

transparency or turbidity. Using the Secchi disk, turbidity of 35–40 cm is acceptable. Turbidity produced by plankton in pond water should be >30 cm [29]. Higher cocentrations of plankton can increase oxygen demand of the fish stock during the night, when the same plankton community that contributes to the turbidity and dissolved oxygen during the day competes with the fish stock at night. Low oxygen not only damages the stock, but also affects the biofloc bacteria and plankton. Oxygen demand may increase up to 300% overnight. A simple method for maintaining the concentration of suspended matter at optimal levels is by sedimentation, using tanks with conical bottoms to remove solid waste in the recycling systems [19].

Secchi Disk

1.4.5. Temperature

Temperature is one of the most influential parameters in fish pond systems because it affects the metabolic rate of cold-blooded fish and microorganisms, oxygen consumption, pH, and concentrations of ionized and un-ionized ammonia during cultivation. The temperature range will depend on the stock species and the bacteria adapted to the system temperature, as well as environmental and seasonal variations. This is important because biofloc systems are more efficient when water temperature is between 28 and 30 °C. [20] Reports that nitrifying bacteria can support a range from 8–30 °C, but efficiency is reduced by 50% at 16 °C and by 80% at 10 °C.

Termometer

1.4.6. Total Ammona-nitrogen

Total Ammona-nitrogen is the excretion product of feces and urine of fish, uneaten food and matter in descomposition, phytoplankton and zooplankton. Ammonia-nitrogen toxicity on aquatic organisms has been attributed to ammonia or non-ionized ammonia (NH3) (gaseous), while the ionized ammonia or ammonium ion (NH4) is considered not significantly toxic or less toxic [42].

The reaction that occurs is as follows.

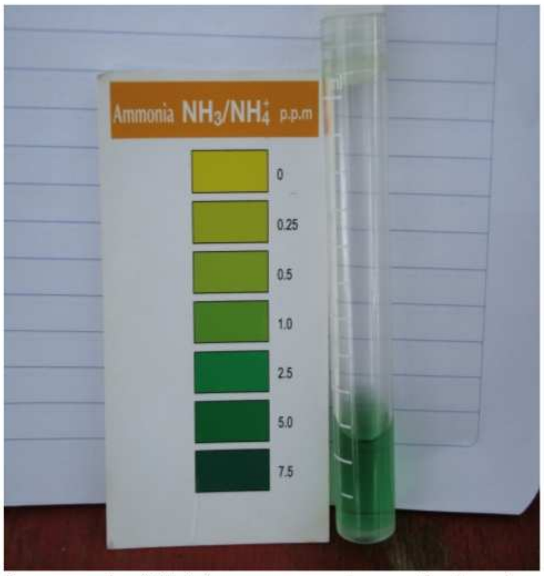
$NH_3 + H_2O \rightarrow$	**$NH_4OH \rightarrow$**	**$NH^+{}_4 + OH^-$**
Nonionized form	Its rate of conjugation	Ionized form
Nontoxic form	with water depends of pH	Nontoxic form.

Ammonia-nitrogen toxicity in the unionized form (NH3), increases with a low oxygen concentration, high pH (alkaline) and a high temperature. With a low pH (acid) is less toxic. A high concentration of ammonia-nitrogen in the water has effects on the cultured organisms, causing blockage of the metabolism, affecting the balance of salts in the osmoregulation, which produces gill internal organ damage, immunosuppression and susceptibility to diseases, reduced growth and survival. In cultured crustacean as Litopenaeus vannamei, ammonia-nitrogen concentrations should be less than 1.2 and 6.5 mg / L in post-larvae and juveniles [36, 37]. The recommended concentrations less than 1.5 mg / L in cultures with biofloc.

1.4.7. Nitrite-nitrogen

They are a vital parameter for its high toxicity and for being a pollutant. The transformation process to ammonia-nitrogen to nitrite-nitrogen and their toxicity form depends on the amount of chlorides, temperature and oxygen concentration in the water. The main toxic effects of NO2 are those who have a direct effect of transport of oxygen, oxidation of important compounds and tissue damage. Nitrite-nitrogen in the larvae of M. rosenbergii tolerate concentrations of 2 mg / L, increasing their tolerance as they grow, and can support up to 16 mg / L of nitrate-nitrogen, however reduce its growth rate and culd cause their mortality [38]. Recommending nitrite-nitrogen concentration less than 2 mg / L in cultures with biofloc.

http://dx.doi.org/10.5772/57501

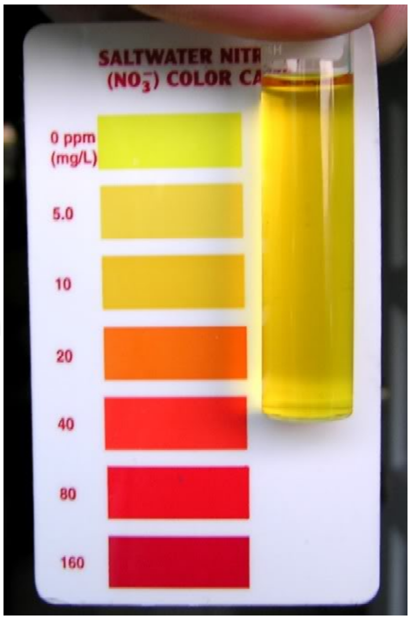


Ammonia (NH_3) measure by colorimetry

Nitrites (NO_2) measure by colorimetry

1.4.8. Nitrate-nitrogen

Nitrate-nitrogen is the end product of aerobic nitrification [32], are considered the less toxic inorganic nitrogen compounds, but can be a potential problem when its levels increase and accumulate. The toxicity of these compounds is due to its effects on osmoregulation and possibly on oxygen transport [40]. For the specie M. rosenbergii, nitrate concentrations in brackish water must be less than 20 mg / L [41]. Recommending that nitrate concentrations should not exceed 10.0 mg / L in biofloc culture.



1.5. Economic benefits of crops biofloc

The economic advantages of biofloc systems to traditional pond farming are generally reflected in the profit margin, based largely on savings in feed, faster growth rates, and increased biomass during cultivation, which is related to high survival rates. However, biofloc systems have increased operating costs of the aeration system, which can be 10–40%, depending on the concentration of oxygen in ponds have to be maintained at 7–8 mg L–1, costs for the carbon source added to the system. Despite the foregoing, [21] reports savings of 14% in a shrimp biofloc system compared to traditional methods.

In our laboratory in one study, Malaysian prawn Macrobrachium rosenbergii raised in a biofloc system achieved a 13.27% saving in operating expenses. In a second study, tilapia Oreochromis niloticus raised in a biofloc system achieved a 12.90% savings in operating expenses compared with the costs in traditional pond farming of both species. The major savings in our study led to less pumping time for maintaining water quality in the system, an increase in survival from 10 to 30%, and an increase in final biomass average content from 20 to 45%. [22] report that, Nile tilapia (Oreochromis niloticus), net production was 45% higher in the biofloc tanks than in tanks without biofloc, where there was also a significant improvement in feed conversion. [23] indicates that the cultivation of Litopenaeus vannamei in biofloc systems led to a 30% decrease in the use of a commercial feed. In tilapia cultivation, producers can expect to reduce commercial feed by up to 20% [24]. Natural food produced by microalgae and bacteria in biofloc systems have high nutritional value.

2. Biofloc cultivation of Malaysian prawn *Macrobrachium rosenbergii*

Biofloc technology has been successfully applied mainly in shrimp farming [9]. Despite the positive results, few fish farms use this technique [25, 26]. The benefits associated with the

http://dx.doi.org/10.5772/57501

production of aquatic organisms under biofloc technology are apparent, so it is necessary to develop cultivation with omnivorous species in a scheme of sustainability and ecological balance to obtain the best performance with the least environmental impact.

Among economically important crustaceans in aquaculture having omnivorous eating habits, the Malaysian prawn (*Macrobrachium rosenbergii*) has successfully adapted to farming conditions, thanks to their physical endurance, fast growth, and high survival rate. This species is widely distributed in tropical and subtropical areas and, compared with similar wild shrimp [27], are a suitable candidate for biofloc practices. Despite this, there are few attempts to cultivate this shrimp in a biofloc system, making it difficult to validate the technology for application on commercial farms.

At the laboratory facilities of the breeding and production technology institute at Boca del Rio, Veracruz, Mexico, cultivation of Malaysian prawns was undertaked for six months in rectangular ponds 10 m × 2 m × 1.20 m high, with a capacity of 20 m^3, which were inside a shadehouse with shade cloth providing 90% reduction of sunlight. During cultivation, a continuous air supply was provided by a 2 hp blower connected to a 1.5 inch PVC pipe at the bottom of the ponds. Placed in the pond were four clay bricks per m^2 with 3 holes in each one. These served as dens for the prawns.The study measured the growth performance of the prawns under two conditions: biofloc shrimp farming and traditional farming, including standard water exchanges in the latter treatment. During the study the prawns were fed twice daily (9:00 and 18:00 h) with a commercial shrimp diet (El Pedregal Silver Cup with 35% protein), by an estimated 20% of the initial biomass for the first month of cultivation. Subsequently adjusted percentage monthly food supplied in connection with the consumption and increased biomass (Table 1). To promote training and biofloc production, molasses added daily diluted in water as a carbon source in ponds, in a ratio of 20:1 C: N, according to the recommendations of [10], considering feed rate.

Month	% Biomass (biofloc)	% Biomass (traditional)	Food per day (g)	Molasses per day (g)
Start	20	20	3.78	7.42
1	5	7.54	30.81	60.38
2	5	6.13	92.00	180.33
3	5	7.67	237.59	465.67
4	3	3.18	167.75	328.79
5	3	3.19	228.33	447.52

Table 1. Percentage of biomass per month to provide same amount of food in two treatments.

2.1. Physicochemical parameters of water during cultivation

During cultivation, physicochemical parameters were similar among treatments and within the tolerance range for growing Malaysian shrimp [28]. The average temperature was 25.90 ± 0.78 °C, dissolved oxygen 5.8 ± 0.55 mg L^{-1}, pH 8.77 ± 0.18. Transparency in both treatments was within the range recommended by [29] for aquaculture crops (minimum visibility of 30 to 40 cm). If turbidity is greater, there is a substantial increase in oxygen demand.

The average concentration of ammonia during the study remained at 0.1 mg L^{-1}, N-nitrite was 0.5 mg L^{-1}, and N-nitrate was 10 mg L^{-1} in both treatments, concentrations below what is considered toxic. Stability of the parameters in the biofloc system results from bacterial activity, which according to several authors, transform bacteria metabolites to the advantage of the shrimp because they are nutrients, as well as prevent accumulation of toxic products in the production system [1, 10]. By nitrification, where ammonia-nitrogen (N-NH_3/N-NH_4) is transformed by oxidation to nitrite-nitrogen (N-NO_2) by bacteria of the genera Nitrosomonas and Nitrosococcus, and others. Nitrite-nitrogen is converted to nitrate-nitrogen (N-NO_3) for nitrite-oxidizing bacteria of the genera *Nitrobacter* and *Nitrospira*. Ultimately, nitrate-nitrogen is reduced to nitrogen gas (denitrification) by bacteria of the genera *Pseudomonas* and *Achromobacter* and others [4, 5]. Unlike biofloc systems, water quality in traditional systems is maintained by continuous dilution of metabolites by influx of fresh water.

2.2. Response variables

Survival of the prawns in the two contrasting treatments, at the end of the study, was similar (85%) and is largely attributed to maintaining water quality. In the biofloc system, there was an increase of the contact surface for bacteria, which allows increased prawn density, compared to the traditional density (8–10 org m^{-2}) and dens that increased the area of protection during molting (Pineda, 2005). High survival further suggests that the biofloc does not affect the health of the prawn. Prawns grown in the biofloc system reached a higher weight (15.17 ± 8.2 g) than prawns rose by the traditional method (12.57 ± 7.89 g; Fig. 3).

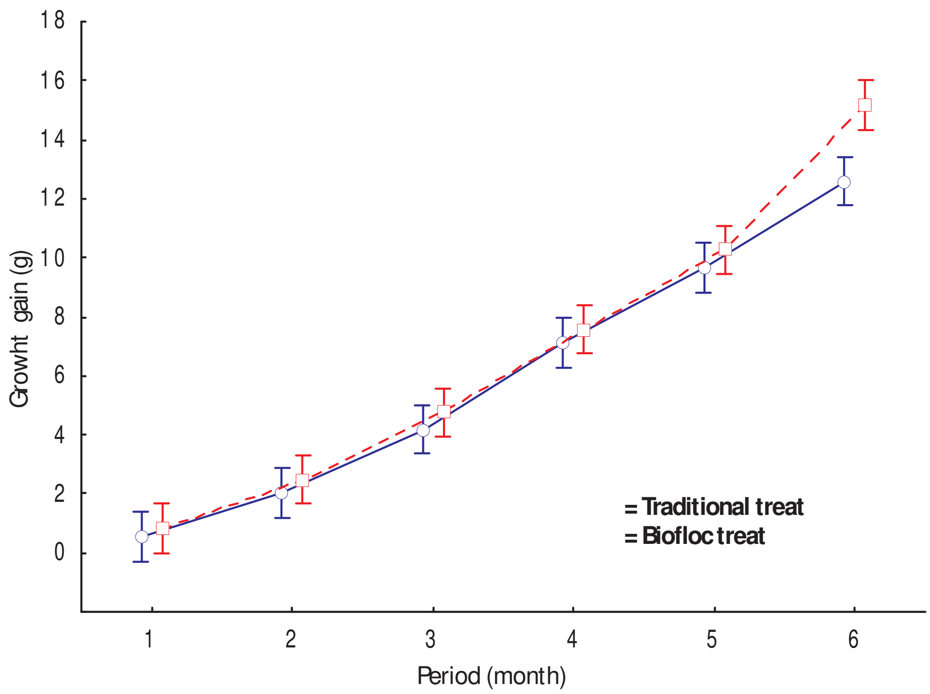


Figure 3. Weight gain of prawns raised under biofloc conditions (red) and traditional conditions (blue). The latter required large inputs of fresh water.

http://dx.doi.org/10.5772/57501

[30] raised Malaysian prawns for 182 days at a density of 10 prawns m^{-2}, obtaining an average weight gain similar to our biofloc system; however, we raised 37 prawns m^{-2}, and used organic and inorganic fertilized during they study. Prawns in biofloc showed a feed conversion rate that was significantly lower (2.27 ± 0.99), compared to traditional cultivation (2.74 ± 0.91), indicating that the biofloc system with the increased contact area and holes, can increase production of shrimp, along with a saving in consumption of commercial feed, which according to [24] comes from biofloc microorganisms that have high nutritional value and promote growth because the microorganisms contain up to 49% protein [31].

2.3. Saving water during biofloc cultivation

One of the major advantages of biofloc systems is reducing the volume of water required for maintaining good water quality. The biofloc system only recycles the water. It does not replace water with fresh water. Only losses from evaporation need replacement. In traditional treatments, 30% of the water is replaced every third day and 60% every two weeks (Table 2) to maintain water quality during cultivation.

Cultivation with biofloc		Traditional cultivation	
Initial fill	20 m^3	Initial fill	20 m^3
6 top-offs at 20%	24 m^3	84 replacements at 30%	504 m^3
		11 replacements at 60%	132 m^3
Total	**44 m^3**	**Total**	**656m^3**

Table 2. Water consumption in prawn cultivation.

Additional water to maintain biofloc water quality was 24 m3, while water to maintain traditional cultivation was 636 m3. The biofloc system saved about 96% of the water needed to maintain nontoxic conditions during production. An additional large saving in electrical expenses was achieved, estimated at about 96% during production time. This is similar to the findings of [1, 2, 3].

3. Conclusion

The potential of biofloc technology applied to shrimp farming to promote good aquaculture practices is manifold resource sustainability and environmental care and in reduction in energy consumption. This is important if we expect to maintain current growth rates of aquaculture. Aquaculture is now competing for space and water with other food-producing activities, so that properly designed and improved systems to maintain high biological load in a relatively small space is essential. Intensive biofloc system is a strategy that will promote the growth of aquaculture [32, 33].

Expanding systems of semi-intensive and intensive production aquatic animals will ead to increasing volumes of waste nitrogen and solids that foul the water [35]. Therefore, reducing effluents and effluent pollution to near zero can only benefit the downstream quality of water in rivers, estuaries, lagoons, and nearshore environments [35].

While closed recirculation systems increase the costs of installation of equipment and operation of a farm (pumps, clarifiers, biological filters), nitrifying bacteria to maintain water quality and reduce environmental impact of biofloc systems lead to a large increase the density of the fish and shrimp and their final biomass, which more than compensates for the initial investment. In a closed, recirculating system, the biological treatment is within the water. Despite being efficient, recirculating systems require auxiliary equipment (pumps, filters, settlers) that increase installation costs, may limit production volumes, and increases operating costs resulting from continous pumping during the crop cycle.

Biofloc, as a culture, is a closed system that works in the same cultivations tanks to largely natural maintainance of the quality of water. In turn, the environmental impact is greatly reduced. Another advantage of biofloc systems is that the naturally occurring organisms in the system are used as complementary food, which reduces consumption of commercial feed, which usually contain products from marine fisheries. This helps to reduce the pressure on fisheries to provide ingredients for diets used in aquaculture.

Author details

Carlos I. Pérez-Rostro[1], Jorge A. Pérez-Fuentes[1] and Martha P. Hernández-Vergara[2]

1 Laboratory of Genetic Improvement and Aquaculture Production, Technological Institute of Boca del Río, Veracruz, Mexico

2 Laboratory of Native Crustacean Aquaculture, Technological Institute of Boca del Río, Boca del Río, Veracruz, Mexico

References

[1] Avnimelech Y., Lacher M. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 1999; 176: 227–235.

[2] Crab R., Defoirdt T., Bossier P., Verstraete W. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 2007; 270: 1-14.

[3] Schryver P., Crab R., Defoirdt T., Boon N., Verstraete W. The basics of bio-flocs technology: The added valuefor aquaculture. Aquaculture 2008; 277: 125-137.

[4] Schramm A., D. de Beer A., Van den Heuvel S., Ottengraf S. Microscale distribution of populations and activities of Nitrosospira and Nitrospira spp. along a macroscale

gradient in a nitrifying bioreactor: quantification by in situ hybridization and the use of microsensors. Applied and Environmental Microbiology 199; 65: 3690-3696.

[5] Ferrer, J., Seco A. Tratamientos Biológicos de Aguas Residuales. Editorial UPV. Valencia, Spain, 2007. Pp.184.

[6] Shan H., Obbard J. Ammonia removal from prawn aquaculture water using immobilized nitrifying bacteria. Appl Microbiol Biotechnol 2001; 57:791–798.

[7] Avnimelech Y., Kochva M., Diab S. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Israel Journal of Aquaculture- Bamidgeh 1994; 46: 119–131.

[8] Chamberlain G., Avnimalech Y., Mcintosh RP., Velasco M. Advantages of aerated microbial reuse systems with balanced C/N. In: Nutrient transformation and water quality benefits. Global Aquaculture Allliance Advocate 2001; 4: 53–56.

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

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

[11] Kurup BM., Prajiht KK. Application of biofloc technology in the semi intensive culture system of giant prawn *Macrobrachium rosenbergii*. World Aquaculture Society: Aquaculture 2010 San Diego, California 2010. https://www.was.org/.../meetings/SessionAbstracts.aspx

[12] Megahed, ME. The Effect of Microbial Biofloc on Water Quality, Survival and Growth of the Green Tiger Shrimp (*Penaeus Semisulcatus*) Fed with Different crude Protein Levels. Arabian Journal of the Aquaculture 2010; Vol.5 No. 2, p. 119-142.

[13] Emerenciano M., Ballester ELC., Cavalli RO., Wasielesky, W. Biofloc technology application as a food source in a limited water exchanger nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817) 2011. Aquaculture Research,doi: 10.1111/j.1365-2109.2011.02848.x

[14] 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.

[15] Goldman JC., Caro D.A., Dennett MR. Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnology and Oceanography 1987; 32 (6), 1239–1252.

[16] Avnimelech Y., Panjaitan P. Effects of carbon:nitrogen ratio control on water quality and shrimp growth in zero water exchange microcosms. Abstracts. World Aquaculture, Florence, Italy; 2006.

[17] Hargreaves JA. Photosynthetic suspended-growth systems in aquaculture. Aquac. Eng. 2006; 34: 344–363.

[18] Ray JA., Lewis LB., Browdy LC., Leffler WJ. Suspended solids removal to improve shrimp (Litopenaeus vannamei) production and an evaluation of a plant-based feed in minimal-exchange, superintensive culture systems. Aquaculture 2010; 299:89-98.

[19] Rakocy JE. Tank Culture of Tilapia. Southern Regional Aquaculture Center (SRAC) 1989. Publication Number 282. Texas Agricultural Extension Service, Texas A&M University, College Station, Texas, USA. Pp. 4.

[20] Gerardi M. Nitrification and denitrification in the activated sludge process 2002; Wiley-Interscience. Nueva York. Estados Unidos.

[21] Hanson TR., Posadas BC., Samocha, T.M., Stokes AD., Losordo TM., Browdy, C.L. Economic factors critical to the profitability of suer-intensive biofloc recirculating shrimp production system for marine shrimp Litopeneus vannamei. Pages 267-283 in C.L. Browndy and D.E. Jory, eds. The resing Tide,Proceedings of the Special Session on Sustainable Shrimp Farmin, World Aquaculture 2009.The World Aquaculture Society, Baton Rouge, Louisiana.

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

[23] Avnimelech Y. Biofloc Tecnology – A practical guide book, 2nd edition. The World Aquaculture Society, Baton Rouge, LA; 2012.

[24] Ekasari J., Crab, R., Verstraete W. Primary Nutritional Content of Bio-flocs Cultured with Different Organic Carbon Sources and Salinity. *HAYATI Journal of Biosciences 2010*; 17(3):125-130.

[25] Milstein A., Avnimelech Y., Zoran, M., Josep D. Growth performance of hybrid bass and hybrid tilapia in conventional and active suspension intensive ponds. Israeli Journal of Aquaculture – Bamidgeh 2001; 53 (3–4): 147-157.

[26] Serfling SA. Microbial flocs. Natural treatment method supports freshwater, June 2006, marine species in recirculating systems. Global Aquaculture Advocate 2006; 34–36.

[27] Martínez-Córdova LR., Porchas-Cornejo MA., Villarreal-Colmenares H., Calderon-Perez JA. Winter culture of yellow leg shrimp *Penaeus californiensis* in aerated ponds with low water exchange. *Journal of the World Aquaculture Society* 1998; 29(1): 120-124.

[28] New, M.B., Singholka, S. Cultivo de camarón de agua dulce. Manual para el cultivo de *Macrobrachium rosenbergii*. FAO, Doc. Tec. Pesca 1984; (225): 118 pp.

[29] Arboleda-Obregón DA. Limnología aplicada a la acuicultura. Revista Electrónica de Veterinaria 2006, Vol. VII, No. 11. http://www.veterinaria.org/revistas/redvet/n111106.html. 23/05/2010.

[30] García-Rodríguez J., Granados-Ramírez JG., Quiroz-Castelán H., Molina-Astudillo FI., Díaz-Vargas M. Utilización de fertilizantes y desechos agrícolas para el crecimiento del langostino *Macrobrachium rosenbergii* (de man), en estanques rústicos. REDVET Revista electrónica de Veterinaria 2005; Vol. VI, Núm. 8, agosto-sin mes, pp. 1-10.

[31] Kuhn DD., Boardman GD., Lawrence AL., Marsh L., Flick-Junior GJ. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture 2009; 296:51-57.

[32] Twarowska JG., Westerman PW., Losordo TM. Water treatment and waste characterization of an intensive recirculating fish production system. Aquacult. Eng. 1997; 16: 133–147.

[33] Thoman ES., Ingal ED., Davis DA., Arnold CR. A nitrogen budget for a closed, recirculating mariculture system. Aquacultural Engeneering 2001; 24: 195-211.

[34] Goddar S. Feed Management in Intensive Aquaculture. Chapman & Hall,N.J.;1996. p. 189.

[35] Páez-Osuna F. Retos y perspectivas de la camaronicultura en la zona costera. Revista Latinoamericana de Recursos Naturales 2005; 1: 21-31.

[36] Frías-Espericueta MG., Harfush-Meléndez M., Osuna-López JI., Páez-Osuna F. Acute toxicity of ammonia to juvenile shrimp *Penaeus vannamei* Boone. Bulletin of Environmental Contamination and Toxicology 1999; 62: 646-652.

[37] Frías-Espericueta MG., Harfush-Meléndez M., Páez-Osuna F.Effects of ammonia on mortality and feeding of postlarvae shrimp *Litopenaeus vannamei*. Bulletin ofEnvironmental Contamination and Toxicology 2000; 65: 98-103.

[38] Mallasen M., Valenti WC. Effect of nitrite on larval development of giant river prawn Macrobrachium rosenbergii Centro Avançado do Pescado Continental, Instituto de Pesca, Caixa Postal 1052, 15025-970, São José do Rio Preto, SP, Brazilb São Paulo State University, Aquaculture Center (CAUNESP) and College of Agriculture and Veterinarian Sciences,14884-900, Jaboticabal, SP, Brazil. Aquaculture 2006; 261:1292–1298. http://www.caunesp.unesp.br/publicacoes/artigos/valenti/RP_MALLASEN_Effect%20of%20nitrite%20on%20larval.pdf

[39] Pierce RH., Weeks JM. Nitrate toxicity to five species of marine fish. Journal of the World Aquaculture Society 1993; 24: 105-107. Colt JE., Armstrong DA. Nitrogen toxicity to crustaceans, fish and molluscs. Proceedings of the Bio-Engineering Symposi-

um for Fish Culture. Fish Culture Section of the American Fisheries Society 1981; (FCS Publ. 1): 34-47.

[40] New MB. Farming freshwater prawns: a manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). FAO Fisheries Technical Paper No. 428. FAO, Rome, Italy, 2002. 212p.

[41] Emerson K, Russo RC., Lund RE., Thurston RV. (1975). Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Research Board of Canada 1975; 32: 2379-2383.