



Biofloc-based nursery production system: heeding the call towards a sustainable shrimp culture industry in the Philippines

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Abstract

The increasing global population resulted in intense pressure on the food production sectors to meet the rise in food demand. The aquaculture industry, which is one of the major food production sectors, provides opportunities in addressing issues on malnutrition and poverty alleviation. Shrimp farming is an important sub-sector in aquaculture because shrimp are not only good sources of food, but they contribute to the national economy through export revenues. This resulted in the rapid intensification of shrimp aquaculture, which created negative issues on sustainability and environmental impacts. Hence, this necessitates an urgent need to develop aquaculture production systems that yield high productivity and profitability yet possess a low carbon footprint. Biofloc technology (BFT) fit into these criteria as this technology permits intensive culture of aquatic species, less use of resources, and improved water quality as a consequence of the production and activity of beneficial microbial biomass, which, at the same time, can be utilized as a source of feed for the growing shrimp. BFT has been shown to be successful on a commercial scale during shrimp grow-out, and recent studies have shown that this technology can be further refined and optimized for the production of shrimp during the nursery phase. This review, therefore, highlights the basics of BFT and how this technology is being optimized in the production of shrimp during the nursery phase. More specifically, this discusses the benefits of this approach in ensuring a productive yet sustainable way of producing shrimp in the context of Philippine aquaculture.

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Introduction

The world's population is expected to reach 9.6 billion by 2050 (El-Sayed, 2021). Hence, the demand for food is also increasing amidst limited natural resources such as water and land required for the continuous production of food. Aquaculture, the farming of aquatic plants and animals both in inland and coastal areas, is one of the fastest-growing food production sectors in the world. It is considered an ideal food production system because this sector is projected to produce 82 million tons of aquatic products in 2050 to meet the food demands of the growing population (FAO, 2020). This target of production is likely to be met because there is the gradual adoption of semi-intensive and intensive aquaculture practices in producing the most economically important aquaculture species. However, the intensification of aquaculture, whether involving fish or crustaceans, is an environmental concern because these intensive farms release a considerable amount of nutrient-rich wastewater back into the waterways, which could result in eutrophication. Because of these potential environmental problems, aquaculture systems are now focused on developing sustainable practices that entail efficient system designs that allow the use of fewer resources, including water, energy, and land but also reduce incidences of environmental pollution without affecting production and profitability.

The major aquaculture-producing countries are all located in Asia. China, Indonesia, India, Vietnam, the Philippines and Bangladesh account for at least 80% of the world aquaculture production (FAO, 2020). While capture fishery production did not increase since the latter part of the 1980s, aquaculture, on the other hand, has been on an upward trend. Global aquaculture production grew at an average annual rate of 5.8%, while production from capture fisheries grew by just 0.7% in 2009 until 2014 (FAO 2016). Moreover, aquaculture has paved the way for growth in the supply of fish for human consumption, with its share increasing up to 29% in 2004. In 2014, a milestone was reached when the contribution of aquaculture to the fish supply overtook that of wild-

caught fish for the first time (FAO, 2016).

Among the different species that are used for aquaculture, shrimp is the fourth most important aquaculture commodity in the Philippines in terms of the volume of production and second in terms of export value. Tiger prawn (*Penaeus monodon*) farming in the Philippines took off in the 1980s when shrimp farming systems switched from the traditional extensive culture to intensive shrimp monoculture. Due to high export demand and the high market price for tiger prawns, businessmen ventured into shrimp hatchery and grow-out operations. The shift towards these practices resulted in the increased production of shrimp, as shown by the peak level of 91,000 MT in 1994 (Palanca-Tan, 2018). Unfortunately, the occurrence of disease outbreaks in the following years led to the shut-down of most intensive shrimp farms, thereby causing a sharp decline in the production of shrimp at 41,000 MT in 1997. Since then, shrimp production has remained in the range of 35,000 to 50,000 MT. During the time when the shrimp culture industry was at its peak level of production, Western Visayas, particularly the province of Negros Occidental, was the top shrimp-producing region in the Philippines. It was at this region where an intensive culture system was carried out. By the late 1990s, Western Visayas Region was overtaken by the Central Luzon Region, where there were vast areas with extensive levels of production. Currently, the top shrimp-producing regions in the Philippines are: Central Luzon (Pampanga and Bulacan), the Zamboanga Peninsula, and in Northern Mindanao (Muegue *et al.*, 2015).

The two species of shrimp that are being cultivated in the Philippines include the black tiger shrimp, *Penaeus monodon* and the Pacific white shrimp, *Litopenaeus vannamei*. Black tiger shrimp is endemic in the country, while the Pacific white shrimp is an introduced species. Although shrimp production significantly declined in the 1990s due to luminescent vibriosis and white spot disease caused by *Vibrio Harveyi* and white spot syndrome virus (WSSV), respectively, the industry was able to recover due to

the close collaboration and partnerships between the public and private sectors. According to Apostol-Albaladejo (2016), the shrimp aquaculture industry in the Philippines recovered because there have been several initiatives that are directed towards improvement in culture technologies, prevention and control of infectious diseases and the mitigation of environmental impacts as a consequence of aquaculture expansion.

Biofloc technology (BFT) is a recent aquaculture technology that is geared towards a more environment-friendly approach to producing aquatic products. The benefits of using this technology include reduction in the use of water resources and effluent discharges, lesser dependence on artificial feeds and improvement in biosecurity (Avnimelech, 2007). The microbial community that is associated with biofloc systems is mainly responsible for the improvement of water quality (Choo and Caipang, 2015) and can control the proliferation of pathogenic bacteria, thus, reducing the potential spread of infectious diseases (Ekasari *et al.*, 2014). The biofloc as a source of food is available all the time and in sufficient quantity (Avnimelech, 2007); hence, this improves productivity by lowering the use of artificial feeds (Browdy *et al.*, 2001; Wasielesky *et al.*, 2006). The production of nitrogenous wastes is regulated by phytoplankton assimilation, nitrification and by the presence of heterotrophic bacteria within the culture system, such that nitrogen retention from the feed inputs is increased in the range of 7-13% (Hargreaves, 2006). The positive effects of the presence of nitrogenous wastes in the biofloc system are the following: 1) they are easily assimilated by microorganisms in the water column, 2) they serve as substrates of these microorganisms, and 3) they provide raw materials in the production of microbial protein cells (Mishra *et al.*, 2008). The production of microbial protein cells in a biofloc system is carried out successfully by regulating the amount of the carbon and nitrogen (C:N) ratio. An effective C:N ratio can regulate the levels of ammonia that are needed by the heterotrophic microorganisms as a means of preventing increased ammonia production

in the water. The use of microbial biomass is even maximized among species that possess filtering apparatus and/or a digestive system that can efficiently assimilate these biofloc particles (Hargreaves, 2006). These characteristics are well-suited in most nursery production systems of aquatic organisms. The presence of a high C:N ratio facilitates the conversion of un-ionized ammonia and other organic nitrogenous wastes into bacterial protein biomass, that can serve as food for the juvenile fish or shrimp and at the same time aids in maintaining good water quality in the rearing compartments (Avnimelech, 1999; Hargreaves, 2013).

Biofloc technology: definition and characteristics

The biofloc-based culture system is based on the principle of assimilating the (1) dissolved ammonia-nitrogen (TAN) that is excreted by the fish as wastes and also (2) through the breakdown of organic nitrogen from uneaten fish feeds by heterotrophic bacteria and converting them into microbial protein. This interaction that takes place in the system is shown in Fig. 1 (Avnimelech, 1999; Crab *et al.*, 2012). This type of culture system maintains optimum water quality, which is highly dependent on phytoplankton bloom, bacterial population, as well as the presence of aggregates of living and dead particulate organic matter that are present in water (Crab *et al.*, 2012). The carbon to nitrogen ratio (C:N) has a crucial role in the biofloc system (Hargreaves 2013). By maintaining an optimum C:N ratio, the proliferation of heterotrophic bacteria is efficiently regulated by providing considerable amounts of organic carbon, which is typically the limiting factor in conventional aquaculture systems (Avnimelech, 1999; Emerenciano *et al.*, 2013; Hargreaves, 2013; Luo *et al.*, 2014). If the C:N ratio is increased, this results in the rapid growth and production of heterotrophic bacteria due to higher availability of the converted microbial biomass nutrients that are required for their growth. Higher populations of these heterotrophic bacteria would mean increased assimilation of ammonia from the water and eventually converted into microbial protein biomass (Hargreaves, 2013). An effective C:N ratio must be

determined because it is this effective ratio where heterotrophic bacteria will dominate nitrifying bacteria (Michaud *et al.*, 2006). The effective C:N ratio, is thus variable and is largely dependent on both the type and quality of the organic carbon sources that are being utilized in the production of the bioflocs.

The biofloc technology (BFT) was believed to have originated in France in the early 1970s and was started by IFREMER-COP (French Research Institute for Exploitation of the Sea - Oceanic Centre of Pacific). The Center used shrimp as the main species for culture, and their approach of culturing this species was likened to an external rumen (Cuzon *et al.*, 2004). There were later initiatives on the development of biofloc technology in the USA and Israel, and the main focus of these activities was to develop an aquaculture technology using tilapia as the model species (Emerenciano *et al.*, 2013; Hargreaves, 2013). This new aquaculture technology based on biofloc aims to address issues on land and water use as well as to mitigate the negative impacts of aquaculture wastes on the environment. The first commercial applications of biofloc technology took place in shrimp farms in Tahiti and Belize, where the shrimp were reared in biofloc systems (Emerenciano *et al.*, 2013). Today, there is the global application of the BFT system in commercial aquaculture farms, with tilapia, carp and shrimp as the major species being used (Avnimelech, 2015). These species are good candidates in a BFT system because they are able to tolerate sub-optimal water quality with high suspended solids are able to obtain nutrition from the bioflocs through filter-feeding (Crab *et al.*, 2012; Hargreaves 2013). Furthermore, Crab *et al.* (2012) suggested that the choice of the aquaculture species should be those that belong to lower trophic species because they rely on an herbivorous diet. Shrimp, carp, and tilapia meet these requirements and as a result, they are being used as the species of choice in almost all BFT systems (Hargreaves, 2013). Recent studies reviewed by Emerenciano *et al.* (2013) revealed that the use of bioflocs in the culture of fish and shrimp resulted in an increment of at least 40%

in weight and 80% in total biomass upon harvest. These comparisons were made against using the clear water system in rearing shrimp and fish. Furthermore, the survival was variable ranging 55-100% regardless of the stocking density. During the grow-out phase, the use of bioflocs enables the replacement of at least 30% of the commercial feeds by the biofloc meal pellet without compromising survival and growth performance (Emerenciano *et al.*, 2013).

Biofloc systems are usually used in areas where there are limitations in the water supply. Because of these limitations, shrimp farmers utilize the minimal to zero water exchange approach in most biofloc systems (Hargreaves, 2013). Moreover, shrimp farm management concerns, especially on biosecurity, are effectively addressed in biofloc systems that limit the frequency of water exchange during the culture period. The mechanisms by which the minimal or zero water exchange in a biofloc system works are largely dependent on the dynamic interaction among the microbial populations and other biotic components that take place within the biofloc system (Avnimelech, 2015). These microbial aggregates aid in maintaining water quality and in the recycling of wastes to produce nutritious food for the cultured stock (Emerenciano *et al.*, 2013). A separate study by Ju *et al.* (2008) showed that bioflocs contain a wide array of bioactive compounds that are essential in maintaining the healthy status of the shrimp. These are due to the enhanced expressions of some immune-related enzymes in the hemocytes (Jang *et al.*, 2011) and antioxidant status (Xu and Pan, 2013). As such, BFT is perceived to be an environment-friendly culture system for shrimp.

In most aquaculture systems that incorporate BFT, the choice of the organic carbon source is dependent on the proximity and availability of a cheap carbon source (Emerenciano *et al.*, 2013). There are various sources of organic carbon, including wheat bran (Emerenciano *et al.*, 2011), molasses (Burford *et al.*, 2004;), glucose (Crab *et al.*, 2010), cellulose (Avnimelech *et al.*, 1989), cassava meal (Chen *et al.*,

2015), sorghum meal (López-Elías *et al.*, 2015), sweet potato flour (Caipang *et al.*, 2015), wheat flour (Azim and Little, 2008) and corn/maize meal (Asaduzzaman *et al.*, 2010) that has been tested both experimentally and validated in the field.

These carbon sources were found to be effective in producing and maintaining biofloc volume and density during the grow-out phase. Table 1 shows the various biofloc starters that are used in shrimp culture. Moreover, Crab *et al.* (2012) pointed out that

there are several factors that will necessitate the implementation of the BFT in aquaculture (Crab *et al.*, 2012). First, the availability of water is becoming a limiting factor in the development of aquaculture. Second, in some countries, the release of wastewaters from aquaculture sites is prohibited. Third, the regular occurrence of infectious disease outbreaks in some aquaculture farms resulted in the development of strict biosecurity measures, which include the significant reduction in the rates of water exchange (Avnimelech, 2015).

Table 1. Sources of organic carbon that are utilized during production and maintenance of bioflocs in shrimp culture.

Source of organic carbon	Cultured species
Acetate	<i>Macrobrachium rosenbergii</i>
Cassava meal	<i>Penaeus monodon</i>
Cassava flour	<i>M. rosenbergii</i> ; <i>Litopenaeus vannamei</i>
Dextrose	<i>L. vannamei</i>
Glucose	<i>M. rosenbergii</i>
Glycerol	<i>M. rosenbergii</i>
Molasses	<i>L. vannamei</i> ; <i>P. monodon</i>
Wheat bran and molasses	<i>Farfantepenaeus brasiliensis</i> ; <i>F. paulensis</i>

The above data were taken from Caipang *et al.* (2015) and Rathore *et al.* (2016).

There are many benefits of using a biofloc-based system of growing fish and shrimp (Rathore *et al.*, 2016). These advantages encompass the breeding, nursery and grow-out production of shrimp and some species of fish. The biofloc system is highly compatible with the aquaponics system and these two aquaculture innovations can be combined together as one functional unit in the cultivation of either fish or shrimp together with vegetables. Because bioflocs are aggregates of a number of microorganisms, these can also serve as good sources of natural probiotics in the culture system (Avnimelech, 1999).

The species that are good candidates to be cultured using the biofloc system should be able to withstand sub-optimal water quality with high suspended solids and must have the filter-feeding ability in order to maximize the nutritional benefits from bioflocs (Hargreaves, 2013). Furthermore, Crab *et al.* (2012) suggested that the choice of the aquaculture species

should be those that are on the lower trophic levels, which normally exhibit an herbivorous diet.

Impacts on nursery production of shrimp

The shrimp nursery phase is the intermediate phase between shrimp post-larvae (PL) and the grow-out stages (Mishra *et al.*, 2008). The nursery systems in shrimp aquaculture provide several benefits such as optimization of farmland, increased survival, and enhanced growth performance of the stock in grow-out ponds (Arnold *et al.*, 2009; Emerenciano *et al.*, 2012). Previous studies, mainly with white leg shrimp, have documented the several advantages of having nurseries in the shrimp farm (Samocha *et al.*, 2007; Mishra *et al.*, 2008).

Biofloc technology can significantly contribute to improving growth rates, survival, feed efficiency, health status, and profitability of different shrimp species during the juvenile and grow-out phases (El-

Sayed, 2021). Bioflocs have been effectively used as natural food or supplemental ingredients during the juvenile stage of the Pacific white shrimp *L. vannamei* (Yun *et al.*, 2017; Panigrahi *et al.*, 2018). In an experiment in tanks that were stocked with shrimp postlarvae at a density of 1,200 PLs per m², the ammonia concentration was significantly lower in the biofloc tanks supplemented with molasses (Serra *et al.*, 2015) than the non-biofloc tanks. In terms of the optimum stocking density and whether shrimps that are grown in the nurseries have the ability to exhibit compensatory growth when transferred to the rearing units, Wasielesky *et al.* (2013) demonstrated that increased stocking density during the nursery phase affected the growth and survival of the white leg shrimp postlarvae in a biofloc system. Rapid growth

in nursery tanks is attained at a stocking density of 1,500 shrimp per m². However, if there are limitations in the area of the farm, nursery tanks or ponds can be stocked up to 4,500 shrimp per m² without negatively compromising survival rates. Their results also indicate that the biofloc system can contribute to achieving full compensatory growth of shrimp within 20 days at a stocking density of 300 shrimp per m² during the grow-out phase. As to the immune responses of shrimp, Ekasari *et al.* (2014) demonstrated that bioflocs have positive effects on the immune response of whiteleg shrimp by inducing a higher resistance against viral challenge. Moreover, the effect on the immune system of the shrimp was independent of the type of biofloc starters that were used.

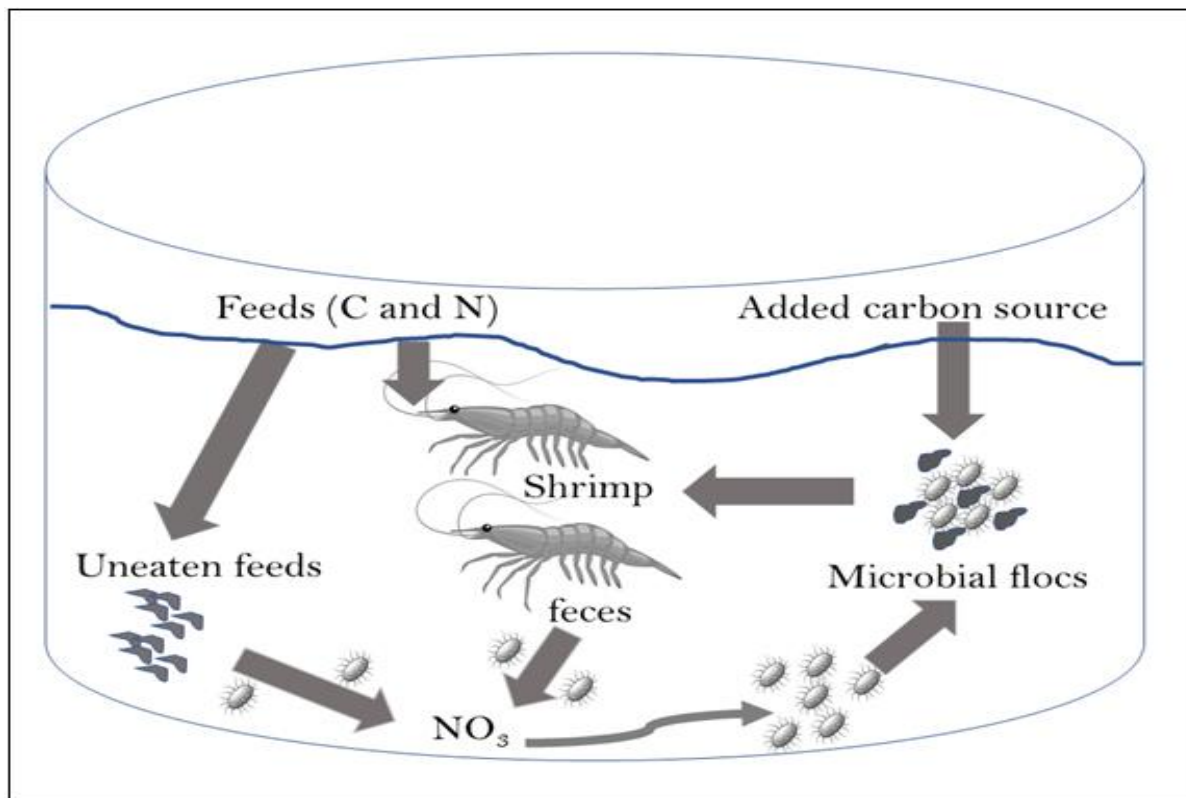


Fig. 1. Mechanism of the biofloc technology in a shrimp aquaculture system. Adapted from Avnimelech (1999).

In the Philippines, some studies on the positive effects of biofloc in the culture of white leg shrimp have been investigated. For example, Barcenal *et al.* (2015) isolated *Micrococcus luteus* from the biofloc culture system and this bacterial species possessed potent anti-Vibrio activity. In another study, Cadiz *et al.* (2016) showed that shrimp tanks using the BFT

had higher total *Vibrio* counts than the non-BFT tanks, indicating a favourable medium that supported bacterial growth. Their study also demonstrated that the numbers of pathogenic *Vibri*os were lesser in this culture system than in control. In both studies, the use of biofloc system provided significant benefits to the shrimp during the grow-out phase.

Future perspectives

The preceding studies clearly indicate the benefits of incorporating BFT during various phases in rearing shrimp. Biofloc technology will enable aquaculture to grow towards an environmental-friendly approach. Consumption of microorganisms in BFT by the growing shrimp reduces feed conversion ratio (FCR) and consequently costs in feed. The microorganisms that are associated with bioflocs can partially replace protein content in diets or decrease the dependence on fishmeal during the nursery phase. Moreover, the microbial community is able to effectively utilize dissolved nitrogen wastes from shrimp and uneaten food and convert them into microbial protein. These qualities make the BFT system an important add-on during the temporary rearing of shrimp postlarvae in nursery tanks. As such, future studies are necessary to optimize nursery production of shrimp using BFT and how would incorporation of this feature in the shrimp grow-out production affects productivity and biosecurity.

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References

Apostol-Albaladejo MAG. 2016. Status of acute hepatopancreatic necrosis disease (AHPND) of cultured shrimps in the Philippines. In Addressing Acute Hepatopancreatic Necrosis Disease (AHPND) and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia:

Proceedings of the ASEAN Regional Technical Consultation on EMS/AHPND and Other Transboundary Diseases for Improved Aquatic Animal Health in Southeast Asia, 22-24 February 2016, Makati City, Philippines (pp. 65-72). Aquaculture Department, Southeast Asian Fisheries Development Center.

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.
<https://doi.org/10.1016/j.aquaculture.2009.03.049>.

Asaduzzaman M, Wahab MA, Verdegem MCJ, Adhikary RK, Rahman SMS, Azim ME. 2010. Effects of carbohydrate source for maintaining a high C:N ratio and fish driven re-suspension on pond ecology and production in periphyton-based freshwater prawn culture systems. *Aquaculture* **301**, 37–46.
<https://doi.org/10.1016/j.aquaculture.2010.01.025>.

Avnimelech Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* **176**, 227–235.
[https://doi.org/10.1016/S0044-8486\(99\)00085-X](https://doi.org/10.1016/S0044-8486(99)00085-X).

Avnimelech Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture* **264 (1-4)**, 140-147.
<https://doi.org/10.1016/j.aquaculture.2006.11.025>.

Avnimelech Y. 2015. *Biofloc Technology. A Practical Guidebook*, 3rd edn. The World Aquaculture Society, Baton Rouge, LA.

Avnimelech Y, Mokady S, Schroeder GL. 1989. Circulated ponds as efficient bioreactors for single-cell protein production. *Israel Journal of Aquaculture, Bamidgheh* **41**, 58-66.

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. <https://doi.org/10.1016/j.aquaculture.2008.06.036>.

Barcenal ARB, Traifalgar RFM, Corre Jr VL. 2015. Anti-Vibrio harveyi property of Micrococcus luteus isolated from rearing water under biofloc technology culture system. *Current Research in Bacteriology* **8(2)**, 26-33.

Browdy CL, Bratvold D, Stokesland AD, McIntosh P. 2001. Perspectives on the application of closed shrimp culture systems. In: Browdy CL, Jory DE (eds) *New Wave, Proceedings of the Special Session on Sustainable Shrimp Farming*, p 20–34. World aqua. Soc., Baton Rouge, LA.

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. [https://doi.org/10.1016/S0044-8486\(03\)00541-6](https://doi.org/10.1016/S0044-8486(03)00541-6).

Cadiz RE, Traifalgar RFM, Sanares RC, Andrino-Felarca KGS, Corre Jr VL. 2016. Comparative efficacies of tilapia green water and biofloc technology (BFT) in suppressing population growth of green Vibrios and *Vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei*. *Aquaculture, Aquarium, Conservation & Legislation* **9(2)**, 195-203.

Caipang CMA, Choo HX, Bai Z, Huang H and Lay-yag CM. 2015. Viability of sweet potato flour as carbon source for the production of biofloc in freshwater culture of tilapia, *Oreochromis* sp. *International Aquatic Research* **7**, 329-336. <https://doi.org/10.1007/s40071-015-0117-7>.

Chen QL, Zhang RJ, Wang YH. 2015. The Effect of biofloc technology on nursery system of *Litopenaeus vannamei*. *Applied Mechanics and Materials* **737**, 358–361.

Choo HX, Caipang CMA. 2015. Biofloc technology (BFT) and its application towards improved production in freshwater tilapia culture. *Aquaculture, Aquarium, Conservation & Legislation* **8**, 362-366.

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. *Aquaculture Research* **41**, 559–567. <https://doi.org/10.1111/j.1365-2109.2009.02353.x>.

Crab R, Defoirdt T, Bossier P, Verstraete W. 2012. Biofloc technology in aquaculture: beneficial effects and future challenges. *Aquaculture* **356**, 351-356. <https://doi.org/10.1016/j.aquaculture.2012.04.046>.

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. <https://doi.org/10.1016/j.aquaculture.2003.12.022>.

Ekasari J, Angela D, Waluyo SH, Bachtiar T, Surawidjaja EH, Bossier P. 2014. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture* **426–427**, 105–111. <https://doi.org/10.1016/j.aquaculture.2014.01.023>.

El-Sayed AFM. 2021. Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. *Reviews in Aquaculture* **13(1)**, 676-705. <https://doi.org/10.1111/raq.12494>.

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

Emerenciano M, Ballester ELC, Cavalli RO,

- Wasiolesky W.** 2012. Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817). *Aquaculture Research* **43**, 447–457. <https://doi.org/10.1111/j.1365-2109.2011.02848.x>.
- Emerenciano M, Gaxiola G, Cuzon G.** 2013. Biofloc technology (BFT): a review for aquaculture application and animal food industry. *Biomass now-cultivation and utilization*, pp.301-328.
- FAO.** 2016. The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all. FAO, Rome. p 200.
- FAO.** 2020. Global Aquaculture Production 1950–2018. <http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en>.
- Hargreaves JA.** 2006. Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering* **34**, 344–363. <https://doi.org/10.1016/j.aquaeng.2005.08.009>.
- Hargreaves JA.** 2013. Biofloc production systems for aquaculture. Stoneville, MS: Southern Regional Aquaculture Center.
- Jang IK, Pang Z, Yu J, Kim SK, Seo HC, Cho YR.** 2011. Selectively enhanced expression of prophenoloxidase activating enzyme 1 (PPAE1) at a bacteria clearance site in the white shrimp, *Litopenaeus vannamei*. *BMC immunology* **12(1)**, 1–11.
- 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. *Aquaculture Research* **39**, 118–133. <https://doi.org/10.1111/j.1365-2109.2007.01856.x>.
- López-Eliás JA, Moreno-Arias A, Miranda-Baeza A.** 2015. Proximate composition of bioflocs in culture systems containing hybrid Red tilapia fed diets with varying levels of vegetable meal inclusion. *North American Journal of Aquaculture* **77**, 102–109.
- Luo GZ, Gao Q, Wang CH, Liu WC, Sun DC, Li L, Tan HX.** 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture* **422-423**, 1-7. <https://doi.org/10.1016/j.aquaculture.2013.11.023>.
- Michaud L, Blancheton JP, Bruni V, Piedrahita R.** 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aquaculture Engineering* **34**, 224–233. <https://doi.org/10.1016/j.aquaeng.2005.07.005>.
- 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. *Aquacultural Engineering* **38**, 2-15. <https://doi.org/10.1016/j.aquaeng.2007.10.003>.
- Muegue MSF, Caipang CMA, Geduspan JS.** 2015. Current status of shrimp aquaculture in the Philippines. In: Caipang CMA, Bacano-Maningas MBI and Fagutao FF (eds) *Biotechnological Advances in Shrimp Health Management in the Philippines*, Research Signpost, Kerala, India, p 1-17.
- Palanca-Tan R.** 2018. Aquaculture, poverty and environment in the philippines. *The Journal of Social, Political, and Economic Studies* **43**, 294-315.
- Panigrahi A, Saranya C, Sundaram M, Kannan SV, Das RR, Kumar RS.** 2018. Carbon: nitrogen (C: N) ratio level variation influences microbial community of the system and growth as well as immunity of shrimp (*Litopenaeus vannamei*) in biofloc based culture system. *Fish & Shellfish Immunology* **81**, 329–337. <https://doi.org/10.1016/j.fsi.2018.07.035>.

- Rathore SS, Yusufzai SI and Katira NN.** 2016. Biofloc technology – the futuristic technology for improving the ecological and economic sustainability of aquaculture. *Life Sciences International Research Journal* **3**, 29-32.
- Samocha TM, Patnaik S, Speed M, Ali AM, Burger JM, Almeida RV.** 2007. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquacultural Engineering* **36**, 184–191.
<https://doi.org/10.1016/j.aquaeng.2006.10.004>.
- Serra FP, Gaona CA, Furtado PS, Poersch LH, Wasielesky W.** 2015. Use of different carbon sources for the biofloc system adopted during the nursery and grow-out culture of *Litopenaeus vannamei*. *Aquaculture International* **23**, 1325–1339.
<https://doi.org/10.1007/s10499-015-9887-6>.
- Wasielesky W, 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.
<https://doi.org/10.1016/j.aquaculture.2006.04.030>.
- Wasielesky W, Froes C, Foes G, Krummenauer D, Lara G, Poersch L.** 2013. Nursery of *Litopenaeus vannamei* reared in a biofloc system: the effect of stocking densities and compensatory growth. *Journal of Shellfish Research* **32**, 799–806.
<https://doi.org/10.2983/035.032.0323>.
- Xu WJ, Pan LQ.** 2013. Enhancement of immune response and antioxidant status of *Litopenaeus vannamei* juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. *Aquaculture* **412**, 117-124.
<https://doi.org/10.1016/j.aquaculture.2013.07.017>.
- Yun H, Shahkar E, Hamidoghli A, Lee S, Won S, Bai SC.** 2017. Evaluation of dietary soybean meal as fish meal replacer for juvenile whiteleg shrimp, *Litopenaeus vannamei* reared in biofloc system. *International Aquatic Research* **9(1)**, 11-24.
<https://doi.org/10.1007/s40071-017-0152-7>