Ann. Anim. Sci., Vol. 24, No. 4 (2024) 983–993 *DOI: 10.2478/aoas-2023-0093*

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BIOFLOC TECHNOLOGY IN IMPROVING SHELLFISH AQUACULTURE PRODUCTION – A REVIEW

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Abstract

Biofloc technology is instrumental in improving growth performance and yield in shellfish aquaculture, while leading to enhanced water quality through maintaining the nutrients level within a safe range. More specifically, toxic nitrogenous wastes are converted into beneficial microbial biomass known as "biofloc", contributing to improve shellfish immune system. Among the various parameters affecting the efficiency of the process is the carbon source and C:N ratio. In light of these, the present work critically reviews the effects of biofloc systems on growth performance, immunity and diseases resistance in shellfish production. Moreover, it scrutinizes the microbial diversity and nutritional composition of biofloc. Then, the application of the technology in various shellfish cultures, including shrimp, freshwater prawn, crabs, crayfish, clam, and oyster, is presented. Overall, biofloc systems contribute to enhanced shellfish survival rate to the highest value of 96–100% for marine shrimp, 95–99% for freshwater prawn, 70–83% for crayfish, 83–100% for oyster, *Crassostrea* sp. and up to 2% for mud crabs larvae through substantially reducing the ammonia level in the culture (summarized in Table 1 – Table 5). Finally, the main challenges in utilizing biofloc systems, i.e., suitable aeration and mixing and microbial mechanisms involved are also explained to shed light on future research directions in the field.

Key words: biofloc system, aquaculture, shellfish, carbon source, water quality, microbial mechanism

Biofloc technology (BFT) is known as a green environmental technology that provides a good water quality condition for the aquatic species and also provides supplementary food, nutrition as well as promotes a healthy condition to the animal during the culture period (Fisher et al., 2020; Khanjani et al., 2023 a). In addition, BFT also has been identified as an eco-friendly system that promotes improvement in the water quality and the application of BFT also influences the microbial community and diversity in the biofloc system (Oliveira Costa et al., 2021; Khanjani et al., 2022 a). BFT helps reduce the use of water and helps promote nutrients recycling as well as introducing additional natural food from the aggregation of biofloc in water column (Walker et al., 2020). Apart from that, BFT also uses inorganic nitrogen from aquaculture wastewater, improves water quality and promotes beneficial microbial protein that are suitable to be consumed by the aquatic animals (Khanjani and Sharifinia, 2020). Biofloc was introduced to tackle the main issue in aquaculture where help in maintaining the water quality by taking up the nitrogen compound and convert it into a microbial protein, help in increasing the culture feasibility by reducing the feed conversion ratio (FCR) and also decrease the feed costs and also applied to compete with the pathogenic organisms (Emerenciano et al., 2016; Khanjani et al., 2023 b).

Biofloc is a new aquaculture technology that promotes a better solution to maintain water quality by recycling the nutrients and also improves the shrimp health by preventing it from the microbial infection (Kumar et al., 2020). Biofloc contains probiotic source that gives beneficial effect to aquaculture production (Jamal et al., 2020; Khanjani et al., 2023 a). *Bacillus infantis*, *Bacillus subtilis*, *Exiguobacterium profundum* and *Bacillus megaterium* isolated from biofloc was proven to successfully enhance the growth performance and genetic material of culture animal (Menaga et al., 2020). There is abundance of Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria, Planctomycetes, and Verrucomicrobia in the shrimp culture under biofloc system (Rajeev et al., 2020). Biofloc contains high probiotic microbes which help increase the number of beneficial gut microbiome in the cultured species and subsequently help improve the health and immunity system (Rajeev et al., 2020). In the biofloc system, heterotrophic bacteria were identified as the most dominant microbial community that per-

formed the bioflocculation process (Jamal et al., 2020). Biofloc was identified as a novel microbial technology that developed with an excellent eco-friendly technology for the sustainability of aquaculture production (Abakari et al., 2021). For example, the heterotrophic bacteria from *Bacillus* sp. were used as a probiotic for the bioaugmentation process of biofloc to enhance the immunity of the Pacific Whiteleg shrimp, *Litopenaeus vannamei* from disease infection and then helped promote better growth, survival and increased the productivity of cultured shrimps (Panigrahi et al., 2020).

There are two types of BFT system which is the outdoor system with the light exposed that is known as green water biofloc system dominated by autotrophic microorganisms, and the other one is the indoor system with no natural light exposure which is known as brown water biofloc system containing bacterial biomass from heterotrophic or chemotrophic microorganisms (El-Sayed, 2020). The polyculture of multi-species using biofloc technology gives much benefits to the species culture as biofloc helped improve the growth and survival of both species of Nile tilapia, *Oreochromis niloticus* and giant freshwater prawn, *Macrobrachium rosenbergii* (Hisano et al., 2020). Biofloc was identified to contain high nutritional protein content between 28 and 40% and high essential nutrition of amino acids, fatty acids, minerals and vitamins for the supplementary diet to polyculture species of *O. niloticus* with *M. rosenbergii* (Hisano et al., 2020).

Biofloc is fueled with the addition of organic carbon sources with high aeration to remove the toxic dissolved nitrogen from the cultured water (Liu et al., 2019). There are several carbon sources such as acetate, corn starch, glycerol, molasses and rice bran that can be used to develop the biofloc formation in the system that cultured fish, prawn, shrimp and crayfish (Dauda et al., 2019; Li et al., 2019). Carbon sources that use molasses give the higher biofloc volume (around 13.6 ml/L) as compared to other carbon sources such as maize starch that give a lower biofloc volume (5.9 ml/L) but give a better survival and growth of *M. rosenbergii* by using maize starch as carbon sources (Hosain et al., 2021). Biofloc biomass has a high crude protein content (43.9%) and is also rich in mineral contents. However, it also has a limited advantage which is the content of high excessive ash and arginine deficiency as part of the amino acids requirements for the aquatic animal's essential growth (Lunda et al., 2020).

Molasses identified as biofloc source of energy gives the best results for reducing the ammonia, nitrite and nitrate concentration as compared to tapioca and sucrose (Putra et al., 2020). In addition, biofloc system was also recognized helpful in increasing the growth rate of *L. vannamei* due to the additional supplemental nutrition of the diets coming from the planktons, bacteria and organic compound from the biofloc aggregation (Kasan et al., 2018). Other research conducted identified that the use of water discharged from biofloc system during the growing culture period does not show negative effects on the survival, production and gonad development of the cultured animal where the discharge water from the biofloc system identified contained good sources of bacterial community to the cultured animals (Gallardo-Colli et al., 2019). It was proven that the mature biofloc contains highly diverse and abundant microorganisms for food sources and also helps maintain the good water quality with lower toxic nitrogenous level of ammonia and nitrite (Krummenauer et al., 2020). Biofloc is known as an innovative and effective technology by the assimilation of microbial to treat toxic material and convert it into useful product and has been proven successful for the sustainability of environment, social and economic in aquaculture development (Jamal et al., 2020). In this review paper, the authors have identified that there is still no review paper on the effect of biofloc application towards the shellfish aquaculture including shrimp, freshwater prawns, crabs, oyster and clam for the bivalve's species and crayfish. Therefore, this review paper is focused and highlights on the biofloc application in the shellfish aquaculture combining all of the shellfish aquaculture species from the most recent study. Overall, the C:N ratio dosage applied for biofloc formation, growth performance, survival rate and type of shellfish organism's species were summarized in details, emphasizing the latest study that has been carried out. Figure 1 shows the biological process involved in the biofloc microorganism's system and the application of biofloc in shellfish aquaculture.

Biofloc applications in marine shrimp aquaculture

Biofloc was proven as a sustainable technology to help improve the shrimp aquaculture production (Khanjani et al., 2022 b). According to FAO (2020), the production of farmed shrimp and prawn has increased in a huge number from 3,400,458 metric tons in 2008 to 6,004,353 metric tons in 2018 with 76.6% increases that represent 7.3% of the total global aquaculture production within 10 years. Forty percent of the study conducted on biofloc application is directed to the shrimp farming sector where biofloc technology (BFT) was identified as a new alternative for sustainable aquaculture production following Food and Agriculture Organization (FAO) Sustainable Development Goals (SDGs) that focused on the food security (El-Sayed, 2020). The biofloc application in shrimp and prawn has received so much attention from a few years, where the study was conducted on the type of biofloc system, pathways from the ammonia removal in the BFT system, factors affecting the shrimp production in the biofloc application, nutritional value of biofloc, and also on the biofloc application as probiotic in shrimp culture (El-Sayed, 2020; Khanjani and Sharifinia, 2022 b). A higher gene expression profile of important immune gene was also observed in the biofloc system as well as expression of digestive enzyme such as trypsin, chymotrypsin, cathepsin L, cathepsin B, and alpha amylase were upregulated significantly in the biofloc system with the addition of probiotic supplementations (Panigrahi et al., 2021).

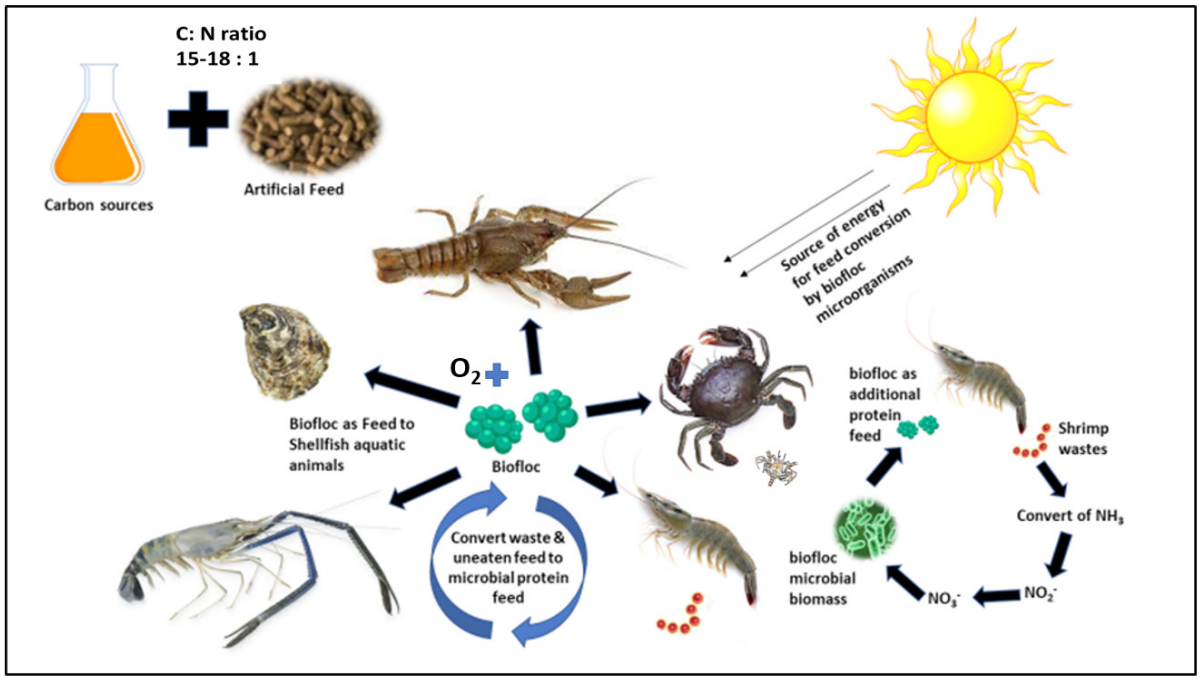


Figure 1. Process involved in the biofloc, the sources of energy from natural sunlight for feed conversion and also for the degradation process through the conversion of nitrogenous waste of ammonia, NH_3 into nitrite, NO_2^- and nitrate, NO_3^- to microbial biomass or biofloc, BFT as additional proteinaceous diets to the shellfish aquatic animals' consumption

Table 1. Details of marine shrimp species with results of biofloc applications

Species	C:N ratio	Growth performance	Survival rate	References
Pacific whiteleg shrimp (*Litopenaeus vannamei*)	12:1	8.1–10.1% BW/d	90–96%	Tinh et al. (2021)
	15:1	2.82%/d	74.6–90.95%	Khanjani et al. (2020)
	4.13	10.18–10.69 %/d SGR	89–93.33%	Lima et al. (2021)
	15:1	NA[1]	82–98%	Kasan et al. (2018)
	15:1	6.21%/d SGR	40–70%	Kumar et al. (2020)
	15:1	7.8–8.3%	96.21–99.56%	Llario et al. (2018)
	6:1	8.28 %/d SGR	89.45%	Krummenauer et al. (2020)
	12:1	NA	NA	Jiang et al. (2020)
	12.1	4.37 kg/m^3	81.47%	Khoa et al. (2020)
	6:1	0.4–1.3 g/week growth rate	12–82%	Pinto et al. (2020)
	NA	86% SGR	75%	Kasan et al. (2019)
	10:1	3.25 to 4.06 %BW/d SGR	98.77%	Manan et al. (2020)
	15:1	0.45 to 0.64 of final weight of post larvae	90–93%	Harun et al. (2019)
	NA	1.02–1.16 g final weight of post larvae	91.90–94.72%	da Silva et al. (2021)
	12:1 16:1	11.97–12.92 g/shrimp	NA	Tong et al. (2020)
	NA	NA	NA	Aguilera-Rivera et al. (2019)
	12:1 15:1 18:1	0.98–1.30 g/week	95.67–98.83%	Xu et al. (2018)
Black tiger shrimp (*Penaeus monodon*)	NA	0.58%/day daily growth and 3.528% of SGR	100%	Supriatna et al. (2020)
Green tiger shrimp *(Penaeus semisulcatus*)	15:1	i. 2.57–2.72%/d SGR (for feeding rates experiment) ii. 2.65–2.78%/d SGR (for different stocking ratio experiment)	42.22–53.33% 68.33–93.33%	Kaya et al. (2020)
Indian white shrimp (*Penaeus indicus*)	NA	Up to 4 g (DOC45) compared to control 2 g	85–91%	Panigrahi et al. (2021)
F. merguiensis				Khanjani et al. (2022 b)
F. merguiensis				Khanjani and Sharifinia (2022 b)

NA: not available.

The research conducted on the light exposure with carbon sources of molasses helped to boost up the beneficial bacteria in the biofloc system of the *L. vannamei* shrimp, meanwhile in the biofloc system without light, harmful bacteria such as *Leucothrix* sp. were identified (Jiang et al., 2020). The presence of light in biofloc production, especially in the outdoor biofloc system, is crucial for the natural food development as light is the essential factor to enhance the photosynthesis and phytoplankton bloom assimilate in the biofloc of outdoor system (El-Sayed, 2020). Thus, it can be concluded that biofloc technology is really helpful in increasing the shrimp aquaculture production, instead of maintaining the good water quality and contains higher probiotic microbes for disease prevention; the application of BFT was also identified as a potential tool in supporting the SDGs by FAO for the food security. Table 1 shows the details of the marine shrimp species with their performance when cultured with biofloc applications.

The C:N ratio application for BFT

Ninety percent survival rate of *L. vannamei* shrimp was identified in the biofloc treatment which applied corn starch and molasses as the carbon sources using C:N ratio of 12:1 (Tinh et al., 2021; Khanjani and Sharifinia, 2022 a). They also identified that specific growth performance was higher and the total ammonia nitrogen (TAN) was in lower range in the corn starch treatment as compared to the molasses treatment for the biofloc application in both treatments (Tinh et al., 2021). The C:N ratio at 12:1 gives the optimum water quality and good performance to *L. vannamei* shrimps (Xu et al., 2018). Different carbon sources like molasses, starch and wheat flour were found to give a similar growth performance result during culture of *L. vannamei* post larvae but meanwhile helped promote a better feed conversion ratio (FCR), around 0.89 to 1.76, to the shrimp that cultured in biofloc system (Khanjani et al., 2019). Feeding of commercial diet that supplemented with biofloc at salinity 32 ppt in a zero-water exchange system showed the better performance of the juvenile *L. vannamei* compared to the growth performance in 10 ppt treatment (Khanjani et al., 2020).

Isolated biofloc bacteria inoculum added during early stocking helped promote a good water quality and increase the shrimp, *L. vannamei* biomass which subsequently helped optimize the shrimp survival and production (Kasan et al., 2018). Biofloc system with C:N ratio at 15:1 helped improved the water quality and contributed beneficial nutritional diets to the shrimp, meanwhile the biofloc system also promoted a better survival rate and increased immunity of the shrimp upon challenge with *Vibrio parahaemolyticus* AHPND strain (Kumar et al., 2020). It can be concluded that the C:N ratio 12–15:1 is successful in controlling the water quality and helps promote better performance to shrimp culture and prevents from pathogenic microorganisms as well.

Effect of biofloc on immunity and disease resistance

The biofloc system also significantly helped improve the growth performance of *L. vannamei* against AHPND from *V. parahaemolyticus* strain (Kumar et al., 2020; Khanjani et al., 2023 c). The application of *Bacillus amyloliquefaciens* as probiotic for *L. vannamei* in the biofloc system did not show a significant effect on the water quality and shrimp growth but showed significant improvements to the shrimp immune system and it can be concluded that *B. amyloliquefaciens* give greater immune system to the shrimp from the pathogens infestation through the biofloc system application (Llario et al., 2018). Supplementation of probiotic bacterial strains of *Marinilactibacillus piezotolerans* and *Novoshingobium* sp. in the biofloc system helps promote healthy shrimp and significantly improves the water quality as well as benefits the shrimp in terms of growth performance, survival rate, microbial community and also the immunity of the cultured shrimp, *P. indicus* (Panigrahi et al., 2021).

The addition of light and carbon sources in altering the microbial community in biofloc identified that the carbon addition increases the bacterial domination but not its diversity where denitrifying bacteria *Paracoccus* sp. was identified in the system provided with light and carbon sources, meanwhile harmful bacteria *Leucothrix* sp. were identified in the biofloc system without light provided with addition of carbon sources (Jiang et al., 2020). Through the Spearman correlation analysis, the abundance of nitrifying bacteria was positively correlated with the nitrite, nitrate and total organic carbon concentrations (Jiang et al., 2020). A high stocking density and low light exposure give negative impact on the shrimp, with low survival rate and biomass to the shrimp production (Khoa et al., 2020). The mixture of microalgae and bacteria in the biofloc system is more appropriated to the benefit and development of the shrimp's culture farming (Khoa et al., 2020). Shrimp biofloc system that was exposed to the natural light showed better performance towards the photosynthetic microorganisms' growth as compared to the indoor biofloc system (Fleckenstein et al., 2019). The algae in the biofloc system help in converting nutrient waste into protein, fatty acid and bioactive compounds to be consumed by the shrimp and then help enhance the shrimp growth and disease resistance (Fleckenstein et al., 2019).

The culture of *L. vannamei* shrimp with biofloc using artificial salinized freshwater has found out that water quality and ionic balance showed no difference among treatments and did not reduce the nutritional quality of the cultured shrimp (Pinto et al., 2020). The combination of 35% crude protein and biofloc density at 10 ml/L gives the best results of 100% survival, total growth rate about 17.21 g and 0.974 feed conversion ratio (FCR) (Supriatna et al., 2020). The floc density and protein level give good results to the growth performance and feed utilization of the cultured black tiger shrimp, *Penaeus monodon* under biofloc system where the effect on the biofloc as feed source to *P. monodon* has never been assessed from previous study (Supriatna et al., 2020). Biofloc volume and shrimp biomass showed a significant effect with the aeration units and with the use of pellet as nitrogen type (Harun et al., 2019). The biofloc application successfully

improved the shrimp performance from the additional natural diets supplied to the shrimp (Manan et al., 2020). The application of biofloc boost-up bacteria to the post larvae shrimp culture showed that the biofloc started to form as early as 10 days and the biofloc formed effectively promotes good water quality and helps optimize the shrimp survival and production in a very different way (Kasan et al., 2019). Thus, it can be concluded that the biofloc technology identified helps in improving the shrimp immune system, helps in preventing from pathogenic infestation in the first place, as well as contains higher nutritional value of proteins for the better shrimp growth performance. It was also identified that at 10 ml/L density biofloc could promote the best survival rate of 100% of the shrimp culture.

Nutritional contents of biofloc

The addition of live feed such as diatom, *Navicula* sp. and rotifer, *Branchious plicatilis* enhanced the growth performance of the shrimp post-larvae in the biofloc system as the diatom, *Navicula* sp. addition to biofloc system was able to reduce the tissue damage of the shrimp challenged with white spot syndrome virus (WSSV) (Da Silva et al., 2021). It was also identified that 70% feeding level at C:N ratio 12:1 of biofloc system provides enough nutrition to maintain the normal health of the *L. vannamei* cultured shrimp (Tong et al., 2020). Thus, it can be concluded that the biofloc technology is rich in natural food sources that contain highly nutritious value for the development and growth performance of the shrimp and in the meantime promotes health benefits to the cultured shrimp as well.

Microbial diversity of biofloc

The microbial dynamic in the biofloc helps in preventing the development of bacteria disease produced by *Vibrio* sp. where the appearance of *Shewanella* sp. helped control the vibriosis outbreak and reduced the severe lesion in shrimp as well as contributed to the low colony forming unit (CFU) number of bacteria isolated from the shrimp hepatopancreas (Aguilera-Rivera et al., 2019). The biofloc application towards *Penaeus semisulcatus* for the first time showed that the application of biofloc to the shrimps resulted in better growth performance and higher survival rates, when 6% body weight/day feeding and stocking density of 20 shrimps/0.24 m^2 in the biofloc system were applied (Kaya et al., 2020). Study conducted on the bacterial population from the biofloc shrimp pond of *Penaeus vannamei* identified that there were varieties of bacterial species from the biofloc that could be identified such as *Exiguobacterium aestuarii*, *E. profundum*, *E. aurantiacum*, *Bacillus pumilus*, *B. velezensis*, *B. cereus*, *B. safensis*, *B. subtilis*, *Vibrio diazotrophicus*, *V. diabolicus*, *V. natriegens*, *V. harveyi*, *Cobetia marina*, *Acinetobacter junii*, *Pseudoalteromonas* sp. and bacteria from species *Rheinheimera aquimaris* were dominated by *Exiguobacterium* spp. (Manan et al., 2022). Thus, it can be concluded that biofloc contains diversity of microbes or bacteria from beneficial group such as *Bacillus* sp. that are helpful in the prevention of disease and also some of the bacteria also identified help in reducing the colony of pathogenic vibriosis in the shrimp culture tank and consequently help promote better growth to the shrimps as well.

Biofloc applications in freshwater prawn aquaculture

Research on different carbon sources to giant freshwater prawn, *Macrobrachium rosenbergii* identified that the treatment using maize starch as carbon sources for biofloc has enhanced the survival, growth and promoted a better FCR and net income of *M. rosenbergii* production. The survival rate reached up to 88.6% in maize starch treatments as compared to molasses with around 68.3% of survival rate. The specific growth rate (SGR) was identified to range between 6.02 and 6.98%/day with highest SGR identified from maize starch treatment (Hosain et al., 2021). It is highly potential to apply biofloc in culturing of Amazon river prawn, *Macrobrachium amazonicum*, but the actual nutritional diet and management techniques have to be improved for this species to make it more successful to be cultured with biofloc technology (Jatoba et al., 2020).

Research conducted on the monoculture, polyculture and recirculation aquaculture system (RAS) using mixed culture of tilapia and giant freshwater prawn in biofloc system found out that both monoculture and polyculture system improved the prawn's growth and survival rate using biofloc application (Hisano et al., 2020). The prawn achieved weight gains of 65.4% compared to the prawn cultured in the RAS system from the biofloc consumption as additional feed and it was suggested that polyculture of Nile tilapia, *O. niloticus* with giant freshwater prawn, *M. rosenbergii* with biofloc can be promoted as an eco-friendly aquaculture model for the production (Hisano et al., 2020). It is potential to culture *M. rosenbergii* in the biofloc system as it contains high microorganism load such as rotifer as additional diets to the prawn (Ballester et al., 2017).

The application of probiotic in the biofloc system cultured with *M. rosenbergii* prawn identified that addition of probiotic increased the prawn survival which *B. subtilis* colonized in the hepatopancreas of the prawn and *B. licheniformis* dominated in the biofloc water column (Frozza et al., 2021). Probiotic bacteria help increase the concentration of enzyme where the high enzymatic activity contributes to significant increase for the amino acids, proteins, carbohydrates and lipids digestion and subsequently gives good influence to the prawn feed conversion, survival as well as to production (Sudha et al., 2019). A research conducted on Chilean river shrimp, *Cryhiops caementarius* identified that higher survival was obtained from biofloc system, about 93%, with 5.96% higher growth level as compared to clear water around 87% survival rate (Walker et al., 2020). The use of probiotics to the freshwater prawn cultured in biofloc

system showed that it helped give beneficial effects to the prawn growth, survival, helped improve the immune system and disease resistance as well as helped sustain a good water quality (Cienfuegos-Martínez et al., 2020).

The application of probiotic species such as *Lactobacillus sporogenes*, *Bacillus subtilis* and *Saccharomyces cerevisiae* gives significant effect to the post-larvae growth and enzymatic production of protease, amylase and lipase in the *M. rosenbergii* post-larvae culture (Cienfuegos-Martínez et al., 2020). The FCR was lower in the prawn cultured in biofloc tank as compared to the prawn cultured in RAS (Nguyen et al., 2019). The replacement of fishmeal with brewer yeast in the biofloc and RAS system did not give significant effect on the growth and survival of the prawn except in 60% increased brewer yeast identified with increment on the prawn growth (Nguyen et al., 2019). The different stocking density of 50, 100, 150, 200 and 250 individual/m^2 does not give significant effect on the weight and length of the prawn under 60 days culture period, where 50 individuals/m^2 were identified as recommended stocking density for culturing *M. rosenbergii* in the biofloc system with produced higher survival rate of prawn (Negrini et al., 2017).

Prawn in biofloc treatment produced antioxidant and antibacterial response during the pathogenic challenge test of *Aeromonas hydrophila*, resulting in higher production of survived prawn (Miao et al., 2020). Research conducted on different carbon sources effect on settling velocity of biofloc shows slow settling velocity when using molasse (0.1044 cm/second) as compared when using chancaca (0.1131 cm/second) as carbon sources (Mendez et al., 2021). The survival of prawn decreased from 89% to 28% as nitrate concentration increased from 0 to 36 mg/L (Dutra et al., 2020). The nitrate concentration at 4.5 mg/L gives negative effects to the post larvae *M. rosenbergii* prawn culture (Dutra et al., 2020). The application of biofloc technology in the culture of freshwater prawn, *M. rosenbergii* was developed where the BFT was identified to help in promoting the shrimp growth performance, survival rate, help improve the immune system and prevent from disease as well as help in promoting a good water quality and save environment to the culture prawn. Table 2 shows the freshwater prawn species with the detailed results obtained from the biofloc applications.

Biofloc applications in crab aquaculture

Research conducted on the application of biofloc water system, green water and RAS system on the survival and growth of mud crab, *Scylla paramamosain* larvae from zoea to megalopa stages identified that the highest survival and good quality of larvae reached to megalopa stage was from the green water system as compared to other system (Ong et al., 2019). The growth performance of the larvae was not affected by the systems used, where the ammonia level also did not show significant difference among the system (Ong et al., 2019). The biofloc water was identified to provide natural food sources through the floc particle that comes from the bacteria, protozoa, phytoplankton, zooplankton, nematodes, ciliates and rotifer as diets (Manan et al., 2017) such as the compositions of live feed found in the green water system (Ong et al., 2019). The results found out that the most suitable for rearing the crab larvae is through the green water system as compared to RAS and biofloc system (Ong et al., 2019). The application of different biofloc sedimentable solids to improve the water quality and survival rate of mud crab, *S. paramamosain* larvae found out that sedimentable solids of biofloc at 2 mL/L were suitable for crab larvae culture until megalopa stages (Kasan et al., 2021). The sedimentable solids in biofloc help maintain the nutrients level and water quality as well as help enhance the survival rate and development of the crab larvae (Kasan et al., 2021). The highest survival rate of mud crab crablet, *Scylla olivacea* was achieved when fed with both commercial pellet and biofloc pellet, with about 30% survival rate of the crablets culture. The application of the biofloc in pellet feed successfully promoted a good crablet performance to optimize the crab survival rate and also helped in sustaining a good water quality condition (Kasan et al., 2022). Table 3 shows the details of the biofloc application on crab species.

Biofloc applications in crayfish aquaculture

A research conducted on crayfish, *Procambarus clarkii* for 60 days has identified that the biofloc technology helps to promote better growth performance and the protein demands of the crayfish which is rich in fatty acids contents (Li et al., 2021). By using the recycling biofloc waste to be fed to the red swamp crayfish, *P. clarkii* for 9 weeks it was found that the biofloc biomass can increase the growth at 33 to 66% using the feed formulation (Lunda et al., 2020). Arginine was identified as the most less amino acid content in the biofloc biomass dietary for the part of protein supplementation to the crayfish and also identified no critical bioaccumulation of heavy metal in the biofloc biomass feed and the heavy metal contents in the biofloc biomass is below the critical pollution limit (Lunda et al., 2020). Biofloc technology helps promote the health of the crayfish, *P. clarkii* for its immune system and antioxidant enzymatic activity and also promotes better weight gain and growth rate compared to control group (Li et al., 2019). Results of the study also proved that the biofloc technology is more effective than fed with commercial diets alone for the farming of the juvenile crayfish (Li et al., 2019). It was also identified that 500 to 600 mg/L of flocculation of biofloc biomass could fulfill the feeding demand of the juvenile crayfish, *P. clarkii* (Li et al., 2018). Thus, it can be concluded that the application of biofloc in culturing the crayfish was successful in promoting a better growth of the crayfish due to richness of protein and fatty acid contents as well as helped increase the crayfish immune system and antioxidant enzyme activity in a mean way. Details of the biofloc application used in crayfish are summarized in Table 4.

Table 2. Details of freshwater prawn species with results obtained from biofloc applications

Species	C:N ratio	Growth performance	Survival rate	References
Amazon river prawn, *Macrobrachium amazonicum*	10:1	0.29 g/day weekly weight gain and 0.61%/day of SGR	77.67%	Jatoba et al. (2020)
Giant freshwater prawn, *M. rosenbergii*	15:1	SGR range 6.02–6.89 %/day	56.3%–88.6%	Hosain et al. (2021)
	12:1	0.43 g weight gain	87%	Hisano et al. (2020)
	NA	1.21%/day	76.81%	Ballester et al. (2017)
	NA	0.63 g weight gain	95–99%	Frozza et al. (2021)
	NA	0.036–0.019 g weight from toxicity test of NO_3^-	Reduction from 89% to 28% From toxicity test of NO_3^-	Dutra et al. (2020)
	20:1	–	–	Cienfuegos-Martínez et al. (2020)
	20:1	1.42%/day (BFT vs RAS) and 1.30–1.41%/day different level of yeast	65.8% (BFT vs. RAS) and 65%–72.5% for different level of yeast	Nguyen et al. (2019)
	NA	3.20–3.47% SGR	39–73%	Negrini et al. (2017)
	10:1	2.39–2.45% SGR	91.79%–93.85%	Miao et al. (2020)
Chilean river shrimp, *Cryphiops caementarius*	15:1	N/A	N/A	Mendez et al. (2021)
Chilean river shrimp, *Cryphiops caementarius*	10:1	5.96% growth rate	93%	Walker et al. (2020)

NA: not available.

Table 3. The details on the application of biofloc to the crab culture

Species	C:N ratio	Growth performance	Survival rate	References
Mud crab larvae, *Scylla paramamosain*	15:1	3.37 mg of wet weight, 4.71 mm of body length of megalopa	Up to 2%	Ong et al. (2019)
Mud crab larvae, *Scylla paramamosain*	10:1	Enhanced the larvae development to megalopa stages	2%	Kasan et al. (2021)
Mud crab, *Scylla olivacea*	NA	Highest survival when crablet fed with commercial pellet and biofloc pellet	30%	Kasan et al. (2022)

NA: not available.

Table 4. The details of crayfish with the result of biofloc application on the species

Species	C:N ratio	Growth performance	Survival rate	References
Red swamp crayfish, *Procambarus clarkii*	6:1 to 15:1	6–44 mg/day weight gain of stage 3 juveniles	70–83%	Lunda et al. (2020)
Red swamp crayfish, *Procambarus clarkii*	15:1	2.71%/day of SGR for juvenile	66.88%	Li et al. (2019)
	NA	N/A	N/A	Li et al. (2018)
	15:1	4.04%/day SGR	69.32%	Li et al. (2021)

NA: not available.

Table 5. The details of oyster species with the results obtained using biofloc applications

Species	C:N ratio	Growth performance	Survival rate	References
Crassostrea sp.	4.13	17.65–20.50% cumulative growth	83.33–93.94%	Lima et al. (2021)
Crassostrea gasar	N/A	N/A	100%	Oliveira Costa et al. (2021)

Biofloc application in clam and oyster culture

Biofloc application in clam culture system is still scanty. There is only one study conducted by Brito et al. (2018) that applied clams, *Anomalocardia brasiliana* to assess the bioremediation of the effluents in the biofloc culture of shrimp juveniles and found out that salinity at 25 ppt helps the *A. brasiliana* clam to consume and subsequently reduce the wastes solids and increases the nitrification process from 72 to 96 hours. This study also indicates that the clam, *A. brasiliana* can be used as a bioremediatory and contributes to reduce the effluent in shrimp culture treatments (Brito et al., 2018).

A research conducted on the multitrophic biofloc application to determine the performance on shrimp, *L. vannamei* and oyster, *Crassostrea* sp. has found out that the oyster survival was higher than 83% with stocking density of 100 oysters/m^2 and 200 oysters/m^2 than in 300 oysters/m^2 and subsequently also increased in terms of total shell length and total weight (Lima et al., 2021). The oysters also have a continuous growth during culture period from 3.21 cm to 3.82 cm with average 18.91% of mean growth and the total suspended solid (TSS) and settleable solid (SS) were lower in the multitrophic treatment as compared to the shrimp monoculture tank (Lima et al., 2021). The biofloc as alternative diet for *Crassostrea gigas* has shown that there is no significant effect from the biofloc application on promoting the growth of the bivalve. The addition of algae with the biofloc also gives no effect on the measured growth parameter, dry weight, shell area and shell height but biofloc can potentially be applied as a partial feed additional for the oyster culture. The final survival rate of the *C. gigas* was around 53.7 to 59.6%. Other research conducted on the oyster, *Crassostrea gasar* did not show significant effect on the reduction of biofloc TSS (Oliveira Costa et al., 2021). This might be due to the prey selective of *C. gasar* by the discovery of dominance of the flagellate organisms in the stomach of the oysters which makes them selective feeding for the type of microorganism present in the biofloc as diet (Oliveira Costa et al., 2021). It can be concluded that there is still limited study conducted on the application of biofloc with culturing of oyster, thus further study should be conducted in order to help promote better growth of the bivalve species with the application of BFT. Table 5 shows the details of oyster species with the results obtained using biofloc applications.

Challenges in biofloc technology

The larger biofloc contain more beneficial bacteria such as Rhodobacteraceae and Flavobacteriaceae, however, the smaller biofloc identified contains potential pathogenic bacteria from group Vibrionaceae, Mycoplasmataceae and Pseudoalteromonadaceae (Huang et al., 2020).

On the top of that, other main problem of biofloc based system is this system identified less stable than clearwater system and also being difficult to be maintain for long term implementation in large pond systems (Romano, 2021). Meanwhile, the critical aspect of biofloc technology is the details knowledge on the selection and placement of the aerators. The suitable aeration and mixing are important to be sustained in the biofloc system to keep the particle in suspension and exchange or drainage of the sludge is needed if the solid concentration is too high in the system (Avnimelech, 2009).

In addition, the microbial population in the biofloc technology need a good and better understanding on how the microscopic mechanisms involve in the bio flocculation and also the BFT should be supported by the probiotic organisms in the microbial community in the biofloc system (Ogello et al., 2021).

It can be concluded that event though biofloc technology promotes many benefits to the culture organism and toward promoting an optimum water quality levels, it's also identified has in own weakness and further research should be conducted by the researchers to overcome this problems or issue in the futures.

Conclusions and future perspectives

The application of biofloc technology in shellfish aquaculture including marine shrimp, freshwater prawn, crayfish, crab, clam and oyster cultures gives significant effect to the performance and water quality of the cultured species. Apart from improving the growth and survival of the culture species, biofloc technology also helped increase the immune system and prevented the culture animals from the pathogenic infection. There is still scanty study conducted on the biofloc application in crabs, clam and also in the oyster culture. Research study conducted on the application of biofloc in other shellfish species such as lobster, crabs and green mussel could be carried out in the future to identify the significant effect from the biofloc application to these aquaculture species for more understanding of biofloc application towards shellfish aquaculture species. Other than that, the main issue in BFT application such as the suitable aeration and mixing system, the maintenance of the biofloc in long term in a large pond system and also on the microbial mechanisms in the biofloc should be tackled on for the good and better understanding of the BFT technology and also for the successes of the BFT operation system in the future.

Acknowledgements

This review paper is part of the research conducted on the effects of biofloc application in Indoor Multi-Techno Aquaculture System (IMTAS) towards sustainable shellfish aquaculture at the Institute of Tropical Aquaculture and Fisheries (AKUATROP), University Malaysia Terengganu (UMT). All authors would like to acknowledge the main funder, Ministry of Higher Education, Malaysia under Higher Institution Centre of Excellence (HICoE), AKUATROP, UMT, Vot number [Vot No. 63933, JPT.S (BPKI) 2000/016/018/015 Jld.3 (23)] for research equipment and facilities. Authors would also

like to acknowledge all the writers who involved in ideas and reviews contributed along the publication process.

Authors contribution

Hidayah Manan: wrote the original manuscript, conceptual and conduct on the revision of manuscript; Nor Azman Kasan: comments on critical parts of the manuscript and approved on the final draft of manuscript; Mhd Ikhwanuddin: comments on critical parts of the manuscript; Amyra Suryatie Kamaruzzan: comments on critical parts of the manuscript; Adnan Amin-Safwan: comments on critical parts of the manuscript; Siti Jalilah Mohamad: comments on critical parts of the manuscript; Fazlan Fauzan: comments on critical parts of the manuscript; Ashraf Suloma: comments on critical parts of the manuscript, conceptual and approved on final draft of manuscript**.**

Conflict of interest

The authors declare no conflict of interest amongst the authors.

Ethical approval

All applicable international, national and institutional guidelines for the care and use of animals were followed by the authors.

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Received: 18 V 2023
Accepted: 22 IX 2023