

Biofloc Technology: Emerging Microbial Biotechnology for the Improvement of Aquaculture Productivity

MAMDOH T. JAMAL¹, MOHAMMED BROOM¹, BANDAR A. AL-MUR², MAMDOUH AL HARBI¹,
MOHAMMED GHANDOURAH³, AHMED AL OTAIBI⁴ and MD FAZLUL HAQUE^{5*}

¹Department of Marine Biology, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

²Department of Environmental Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

³Department of Marine Chemistry, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

⁴Department of Chemistry, Faculty of Science, University of Hail, Hail, Kingdom of Saudi Arabia

⁵Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh

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Abstract

With the significant increases in the human population, global aquaculture has undergone a great increase during the last decade. The management of optimum conditions for fish production, which are entirely based on the physicochemical and biological qualities of water, plays a vital role in the prompt aquaculture growth. Therefore, focusing on research that highlights the understanding of water quality and breeding systems' stability is very important. The biofloc technology (BFT) is a system that maximizes aquaculture productivity by using microbial biotechnology to increase the efficacy and utilization of fish feeds, where toxic materials such as nitrogen components are treated and converted to a useful product, like a protein for using as supplementary feeds to the fish and crustaceans. Thus, biofloc is an excellent technology used to develop the aquaculture system under limited or zero water exchange with high fish stocking density, strong aeration, and biota. This review is highlighted on biofloc composition and mechanism of system work, especially the optimization of water quality and treatment of ammonium wastes. In addition, the advantages and disadvantages of the BFT system have been explained. Finally, the importance of contemporary research on biofloc systems as a figure of microbial biotechnology has been emphasized with arguments for developing this system for better production of aquaculture with limited natural resources of water.

Key words: biofloc, BFT, aquaculture, microbes, water quality, wastes

Introduction

The rapid growth of the human population on earth has caused a significant increase in food demand. To meet this demand, the enhancement of animal protein production, which is the main source of nutrition for human consumption, is highly required. Aquaculture is the ideal source of animal proteins, which could be produced at the lowest cost and very fast. Increasing the productivity of aquaculture in vertical and horizontal expansion leads to an excessive increase of pollutants in the surrounding environment (Ahmad et al. 2017). Biofloc technology (BFT) applications are one of the best aquaculture systems and contribute to the achievement of sustainable development and desired objectives

for a clean environment (Bossier and Ekasari 2017). Hargreaves (2013) defined the biofloc as “a mixture of algae, bacteria, protozoans and other kinds of particulate organic matter such as feces and uneaten feed in addition to some of zooplankton and nematodes, formed together to be an integrated and interdependent ecosystem”. Moreover, biofloc systems can operate with zero or low water exchange (0.5% to 1% per day) under high stocking density of fish or shrimp, assuring it to be an ideal system for saving water exchange (Hargreaves 2013).

However, fish species such as tilapia and carps, and shrimps such as pink, brine, and pacific whiteleg shrimp that are physiologically adapted to digest microbial protein obtained from biofloc consumption are usually

* Corresponding author: MF. Haque, Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh;
e-mail: drfazlul@ru.ac.bd

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suitable for biofloc systems (Emerenciano et al. 2013; Hargreaves 2013). On the contrary, biofloc systems are not suitable for fish species such as channel catfish and hybrid striped bass as well as for prawns such as giant tiger prawn and giant freshwater prawn. They cannot tolerate low water quality due to the high concentration of solids (Hargreaves 2013; Panigrahi et al. 2019a). Biofloc is generated by adding organic carbon and high aeration, which reduces toxic dissolved nitrogen in the water, where internal waste treatment processes are emphasized and encouraged (Liu et al. 2019). Although it is a potential technology, the data on operational parameters of BFT is still inadequate. Hence, there is an urgent need for more applied research on operational parameters of BFT to optimize the system, such as immunological effects, microbial associated molecular patterns production, and nutrient recycling (Bossier and Ekasari 2017). Hence, this review has been designed to highlight the application of biofloc systems and urge the development of this system to produce more aquaculture products without any pollution to the environment and with limited natural resources of water and land.

Development of biofloc technology as a novel microbial biotechnology

Microbial biotechnology, which deals with applying microorganisms to ensure the security, safety, and usefulness of foods, yielding high-quality products, proper human nutrition, and defense against plant and animal diseases, is still rising with different new technologies for sustainable development in the agricultural field. Nevertheless, uses of microbial biotechnology for sustainable development in aquaculture are critical because of the essential role of microorganisms in the establishment and control of ecosystem facilities, especially nutrient cycling, water quality control, and disease regulation in the culture system (Timmis et al. 2017). Although, several microbial biotechnologies have been applied or are still in the developmental pipeline to increase the productivity in aquaculture by creating the ecofriendly environment to support the growth of other aquatic organisms. These organisms are key agents of pollutant removal and recycling (Bossier and Ekasari 2017; Kabir and Aba 2019; Liu et al. 2019). BFT is one of such novel microbial biotechnologies that has been developed with an excellent ecofriendly technology not only for higher productivity but also for sustainable development (Emerenciano et al. 2017; Abakari et al. 2020a) (Table I). The scientific concepts of BFT grew concomitantly and independently by two groups of researchers. Steve Hopkins led one at the Waddell Mariculture Center, South Carolina, and the other group was led by Avnimelech in Israel (Hopkins et al. 1993; Avnimelech

et al. 1994). In both cases, the concepts of active microbial suspension and heterotrophic feed web were used to generate an intensive microbial community for degradation and assimilation of organic residues gathering in the pond in no or limited water exchange condition, which replaced the conventional external biofilter or high water exchange systems in aquaculture (Hopkins et al. 1993; Avnimelech et al. 1994). In BFT, the microbes work for regulation of water quality through the control of nitrogen, resulting in the enhancement of the microbial proteins, which functions as a source of nutrition for aquaculture species. Moreover, the microbes in BFT play a vital role in biosecurity by inhibiting the pathogenic microorganisms' growth (Emerenciano et al. 2017). For example, heterotrophic bacteria such as *Bacillus* spp. that were used as probiotics for bio-augmentation of biofloc enhanced immunity in the Indian white shrimp against pathogenic microorganisms resulting in better growth, survival, and productivity of the shrimp (Panigrahi et al. 2020). Similarly, recent improvements in biofloc-dominated, super-intensive, limited-discharge systems for raising Pacific whiteleg prawn ensure higher biosecurity from viral and bacterial disease outbreaks (Samocho et al. 2012; Prangnell et al. 2020). In a study, the polyphenols isolated from the chestnut (*Castanea sativa*) were supplemented in BFT for the culture of Nile tilapia. It resulted in an enhanced mucosal and serum immunity against pathogenic *Streptococcus agalactiae* (Van Doan et al. 2020). Likewise, Jaggery-based BFT (Jaggery – a potential source of carbon) provided an eco-friendly environment for better bacterial assimilation and nitrification, resulting in improved water quality (Elayaraja et al. 2020). Similarly, a biochar-based BFT caused improvement of water quality through active heterotrophic bacterial assimilation and nitrification, resulting in enhanced levels of NO_3^- and total nitrogen (Abakari et al. 2020b).

Biofloc composition and work mechanism

Successful fish farming is entirely dependent on the physicochemical and biological qualities of water. Consequently, water quality control is required for optimum ponds management (Sharma et al. 2018). Some studies suggest that the ratio between nitrogen (N) and carbon (C) in the water during the period of aquaculture should be controlled by implementing successful biofloc technology (Avnimelech 2009). Thus, the BFT is an integrated system (Fig. 1), and the quality of this system, which depends on the biotic composition of biofloc and the quantity of suspended solids, is checked with Imhoff cones. For checking the biotic composition of biofloc, water collected from the system is reserved in Imhoff cones for precipitation. After

Table I
Recent use of biofloc technology (BFT) in fish and prawn culture.

Fish/Prawn species cultured	Technology used	Effect on culture water	Effects on fish/prawn	References
Mullet (<i>Mugil liza</i>); Shrimp (<i>Litopenaeus vannamei</i>)	BFT in integrated cultivation	Modified bacterial nitrification; reduced total suspended solids	Enhanced growth of mullet, but impaired shrimp's growth	(Holanda et al. 2020)
Nile tilapia (<i>Oreochromis niloticus</i>)	Jaggery-based BFT	Enhanced bacterial assimilation and nitrification; boosted ammonia immobilization	Improved growth and survival; higher immunity to <i>A. hydrophila</i> infection; greater antioxidant capacity	(Elayaraja et al. 2020)
Nile tilapia (<i>Oreochromis niloticus</i>)	Biochar-based BFT	Reduced total suspended solids; active heterotrophic bacterial assimilation and nitrification; enhanced levels of NO_3^- and total nitrogen	No remarkable negative effects of biochar on growth and physiological performance.	(Abakari et al. 2020b)
Genetically improved Nile tilapia (<i>Oreochromis niloticus</i>)	FRP tank culture with isolated probiotic bacteria from BFT	Enrichment of probiotic <i>Bacillus infantis</i> , <i>B. subtilis</i> , <i>Exiguobacterium profundum</i> and <i>B. megaterium</i>	Enhanced growth and survival; improved immunological parameters	(Menaga et al. 2020)
The Amur minnow (<i>Rhynchocypris lagowski</i>)	BFT with differential protein	No significant change in temperature, total ammonia nitrogen, total phosphorus and NO_2^- -N; reduced pH and dissolved oxygen	Enhanced growth; boosted immune response and digestive enzymes activity; higher expression of antioxidant-related genes	(Yu et al. 2020)
Shrimp (<i>Litopenaeus vannamei</i>)	Wheat flour-based zero-water exchange BFT	Effective recovery and sustainable water quality without sodium bicarbonate; higher bacterial diversity	Affected growth performance	(Kim et al. 2020)
Shrimp (<i>Litopenaeus vannamei</i>)	Biofloc-based super intensive tank system	Low concentrations of TAN and NO_2^- -N (< 1.0 mg/l) at late stage; higher bacterial diversity including various nitrifying bacteria in biofloc	Better growth performance in outdoor conditions than in indoors	(Xu et al. 2021)
Nile tilapia (<i>Oreochromis niloticus</i>)	Chestnut polyphenols-based BFT	No data	Improved growth performance; better survival; enhanced mucosal and serum immunity against pathogenic <i>Streptococcus agalactiae</i>	(Van Doan et al. 2020)
Nile tilapia (<i>Oreochromis niloticus</i>) juveniles	BFT with prebiotics and probiotics	Reduction of nitrite concentration	Higher rate of the specific growth, weight gain and final weight; better hematological parameters	(Laice et al. 2021)
Indian major carps, e.g., rohu (<i>Labeo rohita</i>), catla (<i>Catla catla</i>), and mrigal (<i>Cirrihinus mrigala</i>)	BFT for polyculture	Maintenance of NH_4 -N, NO_2 -N and NO_3 -N in the acceptable range of water quality	Satisfactory growth performance (higher rate of specific growth)	(Deb et al. 2020)
Juvenile of Cachama blanca (<i>Piaractus brachypomus</i>)	BFT	Maintenance of the all parameters of water quality in the acceptable range except NH_4 and NO_2	Improved growth performance	(Sandoval-Vargas et al. 2020)
Bluegill (<i>Lepomis macrochirus</i>) juveniles	Corn starch or sucrose-sugar-based BFT	Lower number of human pathogens; raised ammonia level and reduced dissolved oxygen level	Reduced growth performance and higher mortality rate	(Fischer et al. 2020)

15 minutes of precipitation, we can see the aggregates of heterotrophic bacteria, algae, entangled zooplankton including protozoa, rotifers, diatoms, uneaten feed, other dead organic matter, and the suspended particles,

all of which reflect the quality of biofloc (Sharma et al. 2018). Some additives to the water can improve the BFT system; for example, the addition of carbohydrates to reduce toxic ammonia can promote the concentration

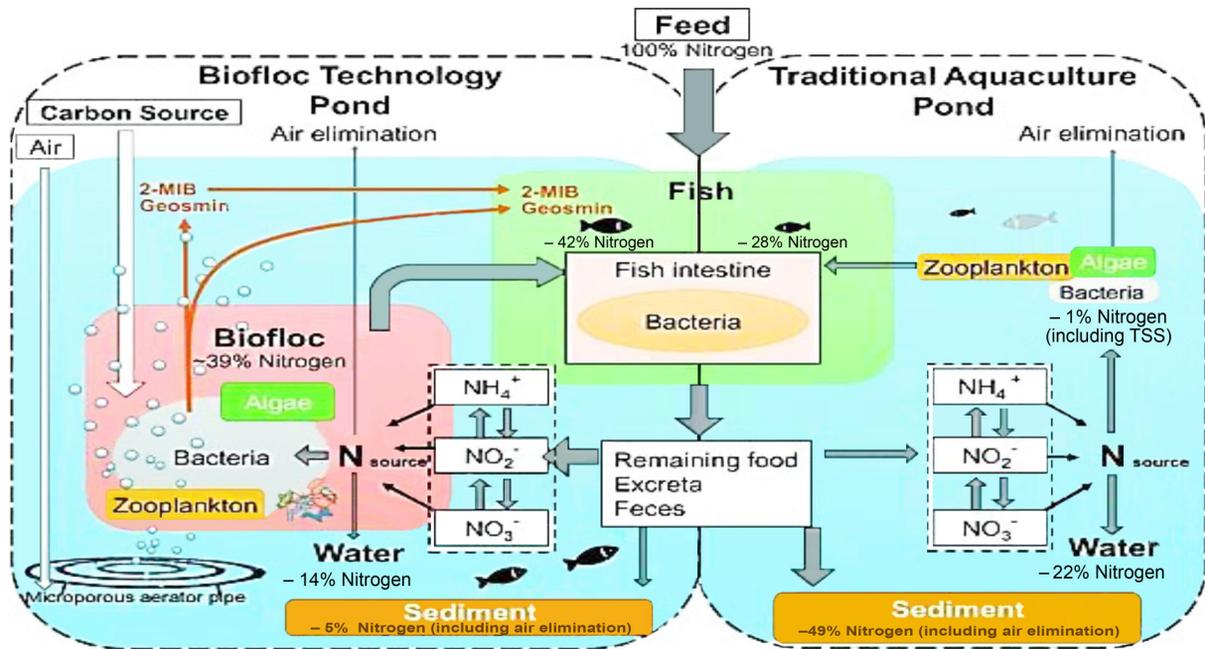


Fig. 1. Role of microbial communities in biofloc technology (BFT) to improved water quality and fish yield in freshwater indoor and outdoor pond aquaculture (Liu et al. 2019).

of microorganisms within the biofloc as well as multiplication of heterotrophic bacteria to enhance the production of proteins and to reduce the growth of pathogenic strains (Panigrahi et al. 2019b).

Biofloc is the best system for water utilization and waste treatment

Fish and shrimp ponds contain a high load of nutrients because of the vast amount of feeds. Almost 50–70% of feeds is in the water or in the sediment, thus resulting in water quality deterioration due to the imbalance of carbon and nitrogen in the culture pond. In such fish and shrimp ponds, biofloc technology can improve water quality by balancing carbon and nitrogen in the aquaculture system by photosynthesis and nitrification processes (Crab et al. 2012). It was reported that the optimum ratio of carbon and nitrogen in biofloc could help to maintain the water quality to raise Pacific white leg shrimp in lower salinity for inland culture. In turn, it decreases the possibility of land pollution by the release of saline wastewater from the shrimp pond (Kumar et al. 2019). Furthermore, the biofloc working on enhancement of the physicochemical parameters of water to the optimum range is essential for the proper growth of fishes and shrimp (Sharma et al. 2018). A study reported that mullet (*Mugil liza*) was able to reduce total suspended solids originating from shrimp (*Litopenaeus vannamei*) culture in a BFT system by consumption of solids, but their culture in the same tank caused the decreased growth of shrimp

(Holanda et al. 2020). Moreover, in the combined culture of mullet and shrimp, the nitrifying bacterial community could be modified by applying biofloc inoculum (Holanda et al. 2020). Another study revealed that the bacterial community structure in culture water and digestive tracts of shrimp *L. vannamei* were depended on the environmental factors in the wheat flour-based BFT system. Moreover, the BFT system showed the capacity for adequate recovery and sustainable water quality management without any supplementation of sodium bicarbonate (Kim et al. 2021).

In BFT, heterotrophic bacteria are the most common microbial community members forming the structure of biofloc. Simultaneously, the chemoautotrophic nitrifiers are fewer in number than other types of bacteria, which in turn leads to less efflux of nitrogen to the ecosystem of the pond. Also, actinobacteria stimulate the formation of biofloc and could be necessary for secondary benefits of protecting against fish pathogens. However, they may upsurge the accumulation of off-flavor in the fish flesh and water (Liu et al. 2019). Some additives like glucose, starch, and glycerol are preferred as carbon sources to cultivate the biofloc outdoor, and these carbon sources are not only affecting the microbial community structure and composition but also affect the number of pathogenic bacteria, for example, *Vibrio* spp. (Wei et al. 2020). Many studies have revealed that heterotrophic microbes are often induced to assimilate total ammonia nitrogen, resulting in the accumulation of comparatively higher nitrate levels in a culture system of biofloc technology (Azim et al. 2008; Nootong et al. 2011; Chen et al. 2019).

Table II

The key factors of water quality checked in BFT systems and its optimal and/or normal detected ranges (Emerenciano et al. 2017).

Factors	Normal and/or ideal detected ranges	Remarks
Temperature	28–30° (usually perfect for species in tropical region)	In addition to shrimp and fish, microbial growth might be affected by low temperatures (~20°C)
pH	pH 6.8 to 8.0	Optimum pH values in BFT are less than pH 7.0 but these might disturb the process of nitrification
Dissolved oxygen (DO)	Optimum level is above of 4.0 mg/l and as a minimum 60% of saturation	For precise growth and respiration of fish, shrimp and microbiota
TAN	Depend on pH, optimum level is less than 1 mg/l in pH ≤ 7.0	pH could play a vital role on toxicity values
Salinity	Optimum range relied on the cultured fish/shrimp species	It is promising to produce BFT, e.g., from 0 to 50 ppt
Alkalinity	Ideal level is greater than 100 mg/l	Greater values of alkalinity aid in assimilation of nitrogen by heterotrophic bacteria as well as assist in process of nitrification by chemoautotrophic bacteria
Nitrite	Optimum level is less than 1 mg/l	One of the critical factors (hard to regulate). Distinct attention to be required
Orthophosphate	Optimum range from 0.5 mg/l to 20 mg/l	In these ranges, usually nontoxic to the cultured fish and shrimp
Nitrate	Optimum range from 0.5 mg/l to 20 mg/l	The same as orthophosphate
Total suspended solids (TSS)	Ideal level is less than 500 mg/l	As measured in Imhoff cones, the excessive levels of TSS contributes to the DO intake by gill occlusion and heterotrophic community
Settling solids (SS)	Optimum range relied on the cultured fish/shrimp species. 5–20 ml/l for tilapia fingerlings, 5–15 ml/l for shrimp, and 20–50 ml/l for adult and juveniles tilapia	The same as TSS

Moreover, nitrifying bacteria in biofloc, such as ammonia oxidizers, oxidize ammonia to nitrite, and then nitrite oxidizers, oxidize nitrite to nitrate (Chen et al. 2006). Both nitrite and nitrate can damage aquatic animals' gill tissue, resulting in problems with respiration and/or higher mortality (Lin and Chen 2001; Kuhn et al. 2010b). Hence, some factors such as temperature, salinity, alkalinity, pH, dissolved oxygen, settling solids, total suspended solids, and orthophosphate have to be monitored continuously and practically in biofloc technology as shown in Table II (Emerenciano et al. 2017). Better water quality in BFT results from a complex interaction among different water parameters. Hence, the demand for more research in this field is increasing day by day to better understand this complex interaction of parameter of water quality to develop the aquaculture production methods.

Biofloc is a significant food source in the recirculating aquaculture system (RAS)

RAS is a modern aquaculture system that could be applied to nurture fish fingerlings in a biosecurity environment to assist in the production schemes; neverthe-

less, it is still obscure what kind of RAS could be the best for such activities. A comparative study on the role of RAS and BFT on the growth of *Tinca tinca* fry revealed that the microbial community in RAS and BFT was different and the microbial diversity was relatively higher in the RAS than in the BFT (Vinatea et al. 2018). In another study, the performance of the BFT versus the RAS on Nile tilapia, *Oreochromis niloticus* in monoculture and polyculture with giant freshwater prawn, *Macrobrachium rosenbergii* was evaluated. It was reported that the BFT offers better growth performance for *O. niloticus* in monoculture and in polyculture with *M. rosenbergii* compared to RAS (Hisano et al. 2019). Thus, both RAS and BFT systems play a significant role optimizing the growth performance of aquatic animals resulting in the reduction of their production expenses (Fleckenstein et al. 2018). Nevertheless, fossil fuel-based RAS increases both the operational cost and the bad environmental impact because of its high energy requirements (Badiola et al. 2018). The development of renewable energy-based, more efficient RAS and its integration with other systems could be a favorable choice. Hence, integrating RAS and biofloc systems in aquaculture would be promising to enhance their positive features through combined effects. Moreover, biofloc itself is a protein-rich

live natural feed formed as a result of the conversion of unused feed and excreta in an aquaculture system upon exposure to sunlight. In a biofloc system, the loose matrix of mucus, which is secreted by various microbes, assists in linking each floc together, which is bound by electrostatic attraction or filamentous microbes form a large floc. The large flocs, which are easily consumed by fish can be observed by the bare eyes, but the maximum flocs are minute in size ranging from 45 to 250 microns. Some technical studies reported that some aquaculture animals preferred the consumption of microbial biofloc generated in BFT systems instead of consumption of processed biofloc as a feed component (Kuhn et al. 2009; Kuhn et al. 2010a; Anand et al. 2014; Bossier and Ekasari 2017). Moreover, BFT application in larvae culture may provide an easily accessible food source for the larvae of Nile tilapia (*O. niloticus*) between different feeding times and reduce the possible harmful competition during feeding (Ekasari et al. 2015). Similarly, a marked improvement in the growth of the juveniles of Nile tilapia was observed when they were reared with biofloc and compared with the others in control without biofloc (Durigon et al. 2020). Thus, the better growth performance of the aquaculture animals is resulted from the adequate nutritional value of biofloc, especially for high content of proteins and fat as their dry weight ranges from 25–50% and 0.5–15%, respectively. It is also a good source of vitamins and minerals, particularly phosphorous (Verster 2017). Because of good nutritional quality, the dried biofloc is proposed as an ingredient to replace the fishmeal or soybean partially in the feed for aquaculture; but the availability and diversity of dried biofloc are limited. Moreover, biofloc is also a probiotic source and has a probiotic-like beneficial effect in aquaculture production (Ferreira et al. 2015; Vikaspedia 2019; Jamal et al. 2020). Altogether, in an integrated system of BFT and RAS, excessive feeds which are used for the rapid growth of cultured animals would be converted more efficiently into microbial biomass, which in turn can be consumed as food by the animals resulting in less pollution of water (Choo and Caipang 2015).

Biofloc is a safe, integrated aquaculture system for higher productivity

The high-density fish and shrimp culture usually demand a waste treatment infrastructure, and biofloc is the best waste treatment system. It was revealed that BFT systems could be efficiently applied to upsurge fish production by establishing an encouraging relationship between biofloc and fish density, which showed a higher capacity to recycle nitrogenous waste at high culture densities (4 million fishes per ha) (Park et al. 2017). Moreover, BFT systems are suitable for achiev-

ing aquaculture with an intensive aeration, low or no exchange of water, higher stocking densities and organic input, heavy mixing and an additional advantage of the generation of an additional feed source for some species well-suited for these conditions. However, BFT was developed to secure fish rearing ponds from diseases caused by the pathogens available in incoming water (Hargreaves 2013). It was reported that Jaggery-based BFT enhanced the immunity of Nile tilapia against the pathogenic *Aeromonas hydrophila* infection through upregulation of various immune-cells, immune-related genes and enzymes, and antioxidant capacity. Thus, Jaggery-based BFT was capable of increasing the growth performances and the survival rate of Nile tilapia (Elayaraja et al. 2020). Another study revealed that the probiotic bacteria viz. *Bacillus infantis*, *Bacillus subtilis*, *Exiguobacterium profundum* and *Bacillus megaterium* isolated from BFT were able to enhance growth performance and survival rate of genetically improved Nile tilapia while cultured in 500 l FRP tank. However, *B. subtilis* and *B. megaterium* showed better antioxidant and immunological capacity than the remaining two strains (Menaga et al. 2020). Likewise, the intensive cultivation of Nile tilapia can be established by using water discharged from biofloc systems during the growing period without negative effects on fish survival and productive performance (Gallardo-Collí et al. 2019). The discharge water from the BFT system could be a good source of a healthy microbial community. It was reported that the beneficial microorganisms, which were liable for nitrogen conversion, showed the diverse and dense growth in the water column of intensive, minimal-exchange production systems. Moreover, a part of the beneficial microbiota was linked with particles of biofloc, and the density of these particles could be regulated to some degree to increase production (Ray et al. 2011). A study revealed that the aquaculture performance of the shrimp *Marsupenaeus japonicus* in a high-intensive, zero exchange farming system was improved by biofloc technology, and this high performance might be related to the biodiversity of microbial flora (Zhao et al. 2012). Furthermore, biofloc particle size has played a vital role in some aquaculture species' nutritional quality and productivity. It was demonstrated that the combination of BFT with an integrated culture system resulted in lower suspended solids, higher biomass production, enhanced total feed efficiency, and higher nitrogen and phosphorus recovery (Ekasari 2014).

Advantages and strengths of biofloc technology

BFT has many benefits that distinguish it from many other advanced fish farming systems, such as an eco-friendly culture system with zero drain water after cul-

ture. BFT is dominated over other systems because of its higher capacity for reduction of environmental impact, improvement of effective use of land and water, maintenance of the suitable quality of water with minimal water usage and exchange within the rearing ponds, and production of protein-rich biofloc as a supplementary feed for aquatic organisms (Reddy 2019). Likewise, Arias-Moscoso et al. (2018) stated that biofloc could ensure clean water and higher biosecurity by reduction of wastewater pollution as well as lowering the risk of introduction and spread of diseases and pathogens. Moreover, BFT enhances survival rate, growth performance, and feed conversion in fish culture systems, urging its application as a more economical alternative to other culture systems. Because of the generation of biofloc as non-costly and adequate feed, BFT also reduces the need to utilize protein-rich feed resulting in the cost-effective production of cultured animals. Thus, BFT also reduces the burden on capturing fisheries to market cheaper food fish and trash fish for the fishmeal industry (Reddy 2019).

Disadvantages and weaknesses of biofloc technology

There are many weaknesses in the biofloc system, such as the urgent need for high aeration and water movement, which leads to the increased energy requirement for mixing and aeration. Furthermore, several disadvantages are also reported for BFT, such as reduced response time because of consumption of dissolved oxygen in the water and higher respiration rates, potential pollution from nitrate accumulation, and the requirement of start-up period and alkalinity supplementation (Reddy 2019). Moreover, the performance of BFT may be inconsistent and dependent on seasonal variation of the daylight period for sunlight-exposed systems (Reddy 2019). Finally, BFT has sown frequent requirements of maintenance, which sometimes may be too expensive.

Conclusions

Biofloc technology is an innovative and effective microbial biotechnology in which toxic materials such as nitrogen components can be treated and converted to a useful product like proteins, which can be used by the fish and crustaceans as supplementary feeds. It is an excellent technique to develop the aquaculture system under limited or zero water exchange with high fish stocking density, vigorous aeration, and biota formed by biofloc. BFT presented aquaculture as a sustainable tool for the environmental, social, and economic issues

that synchronize with its growth. So this technique needs to be developed by more researches to keep up and maintain the aquaculture systems in the future.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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