Chapter

Bioflocs Technology in Freshwater Aquaculture: Variations in Carbon Sources and Carbon-to-Nitrogen Ratios

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Abstract

Aquaculture is one of the fastest food-producing sectors contributing half of the food fish destined for human consumption. Nevertheless, aquaculture production still needs to increase to fill the gap in supply and demand for fish, as the capture fisheries are stagnating over the years. Therefore, intensification of aquaculture production systems by increasing inputs such as feed has been devised as an alternative. On the other hand, intensive aquaculture has been associated with concerns related to environmental pollution in the past decades. Moreover, the increased cost of feed ingredients for aquaculture species has hampered the intensification of the sector. Therefore, alternative production systems such as biofloc technology were developed to mitigate the environmental impacts of intensive aquaculture and also to produce extra feed for cultured organisms. Due to their omnivorous feeding habit and tolerance to higher levels of suspended solids, freshwater finfishes have been the most cultured species in this system. The organic carbon sources used in the biofloc system are agricultural and industrial by-products which are cheap and readily available, making the technology economically feasible. C:N ratios of 10, 15, and 20 have been the most applied C:N ratios in the culture of freshwater aquaculture finfishes covered in this review.

Keywords: biofloc technology, C:N ratio, freshwater aquaculture, organic carbon sources

1. Introduction

Aquaculture is one of the fastest food-producing sectors in the world [1]. Without aquatic plants and non-food products, worldwide aquaculture production reached 80 million tons in 2016, with 54.1 million tons of finfish, 17.1 million tons of mollusks, 7.9 million tons of crustaceans, and 0.9 million tons of other aquatic animals [1]. On the other hand, world capture fisheries have stagnated over the last two decades, reaching 90.9 metric tons in 2016, of which 79.3 metric tons were from marine fisheries and 11.6 metric tons from inland fisheries [1]. According to the World Bank [2] report, the capture fisheries production is expected to remain stable at around 93 metric tons during the years 2010–2030.

Therefore, to meet the global demand for fish with relatively stable capture fisheries, world aquaculture production will need to produce an extra 36.25% and 75% of the current production by the years 2030 and 2050, respectively [1, 3]. Thus, intensification of aquaculture has emerged as a viable alternative to increase food fish production to fill this gap in supply. However, this intensification in the production systems demands increased inputs such as fish feed and medications, which in turn increases the environmental impacts of aquaculture and compromises its sustainability [4]. In order to preserve the environment and the natural resources and ensure the sustainability of the aquaculture sector, this expansion will need to take place in a sustainable way. To this end, sustainable production systems such as biofloc technology have been proposed and gained much interest from aquaculturists worldwide [5].

Biofloc technology is a relatively recent production system in aquaculture with a basic principle of manipulating C:N ratio of the feed and culture water to convert toxic nitrogenous wastes into the useful microbial protein (bioflocs) and help in improving water quality under zero-water exchange system [6–9]. To achieve this, a conglomeric aggregation of communities such as phytoplankton, bacteria, and living and dead particulate organic matter is stimulated by adding carbon source ingredients to the culture system [7]. Thus, the toxic nitrogenous wastes are taken by the microorganisms to produce single-cell protein and keep the water quality in an acceptable range for the species under culture. The consumption of biofloc by cultured animals has demonstrated several benefits such as improvement in growth rate [10] decrease of FCR, and associated costs in feed [11]. In addition to providing nutrition and water quality control, the bioflocs are also shown to contain bioactive compounds that enhance survival and defense mechanisms which act as a novel approach for health management in aquaculture by stimulating the innate immune system of cultured animals [6].

Although biofloc technology (BFT) was believed to be suitable for detritivorous species such as shrimp [12] and tilapia [13, 14] because of their filter-feeding behavior, recent research outputs are indicating that the technology can be applied with several more species [15]. Among them are the freshwater species carp [16] and catfish [17], in which promising results have been obtained at broodstock conditioning, nursery, and grow-out production stages.

The complexities of the carbon sources in biofloc technology range from agricultural by-products such as wheat bran, rice bran, sugar beet molasses, cassava meal, Sorghum meal, and tapioca flour to industrial products such as acetate, glycerol, cellulose, dextrose, and starch with their application dictated by availability, cost, and bacterial assimilation characteristics [18–20]. The type of carbon sources used also seems to affect the nutritional quality of the flocs [21] the microbial community structure [22], and the water quality parameters [23].

C:N ratio in the BFT plays a vital role in the incorporation of inorganic nitrogenous wastes into valuable bacterial cells that may act as a direct source of feed for cultured aquatic animals [7]. Usually, uptake of inorganic nitrogenous wastes takes place when the C:N ratio of the system is greater than 10 [24]. Thus, modification in the C:N ratio may result in a change from an autotrophic to a heterotrophic system [7]. The management of the C:N ratio in BFT is normally divided into the initial and formation phase, in which a C:N ratio of 12–20:1 is applied, and the maintenance phase, in which C:N ratio of 6:1 is applied, according to the total ammonia nitrogen (TAN) values. To this end, 10:1, 15:1, and 20:1 have been the most applied C:N ratios by either adjusting the feed formulation or adding of external organic carbon source [17, 25, 26].

Although BFT brings several obvious advantages discussed above, challenges related to the high demand for energy for aeration, dependence on sunlight [27], and accumulation of a large amount of suspended solids limits its commercial scale application with more aquaculture species of economic importance. Considering the limitations of intensive-fed aquaculture development in Ethiopia [28], BFT can be an alternative production system with the availability of organic carbon sources and suitable fish species like tilapia, carp, and catfish. However, knowledge gap and limited power availability may be a challenge in applying the technology in the country unless alternatives are devised by the concerned stakeholders. Therefore, the objective of this review is to document the current practices of BFT in tilapia, carp, and catfish aquaculture and to reveal the variations in carbon sources and C:N ratios.

2. Overview of bioflocs technology in aquaculture

Biofloc Technology (BFT) is an environmentally friendly aquaculture system that is considered as an efficient alternative system in which nutrients and water are continuously recycled and reused. The sustainable approach of such a system is based on the growth of microorganisms in the culture medium, benefited by the minimum or zero-water exchange [8]. The main roles of microorganisms (biofloc) are maintenance of water quality by the uptake of nitrogen compounds generating in situ microbial protein [29] and increasing culture feasibility by reducing feed conversion ratio and a decrease of feed costs [9, 30]. BFT has received a variety of applications such as single-cell protein production systems, Zero exchange autotrophic heterotrophic systems (ZEAH) [11], activated-sludge or suspended bacterial-based system [31], suspended-growth systems or microbial flocs systems [32, 33]. Moreover, BFT has been the emphasis of intensive research in the aquaculture nutrition area as a protein source in compounded diets. Such source of feed ingredient is produced in the form of "biofloc meal," mainly in bioreactors [34]. In addition, the fast spread and the large number of BFT farms worldwide induced significant research efforts on processes involved in BFT production systems.

Cultured aquatic animals in the BFT system are species that are detritivorous such as shrimp and freshwater prawns [12, 21]; Emerenciano et al. [35] and omnivorous fish such as tilapia [13] because of their filter feeding behavior and tolerance to relatively high suspended solids. Recently, Catfish [36] and carp species [37] have also been cultured in the BFT system with varying degrees of success (**Table 1**).

3. Biofloc technology in freshwater aquaculture

As discussed above, in marine aquaculture, crustaceans, mainly shrimp, have been almost the only cultured organisms under BFT system [9], while freshwater finfishes and the giant freshwater Prawn (*Macrobrachium rosenbergii*) comprise the majority of the species reared under this system (**Table 1**). The freshwater finfish species cultured under BFT system (Tilapia, carp, and catfish) are among the world's top leading aquaculture species, with a share of more than half of the total landing from aquaculture in 2016 [1]. Therefore, in what follows, more emphasis has been given to those three freshwater species in detail.

Category	Species	References
Marine	Hybrid bass	Milstein et al. [38]
	Penaeus monodon	Panjaitan [39]
	Farfantepenaeus paulensis	Emerenciano et al. [35]
	Litopenaeus vanamei	Krummenauer et al. [40]
	Farfantepenaeus brasiensis	Souza et al. [41]
	Farfantepanaeus duorarum	Emerenciano et al. [12]
	Artemia franciscana	Hoa et al. [42]
Freshwater	Mozambique tilapia	Crab et al. [29]
	Guppies (*Poecilia reticulata*)	Sreedevi and Ramasubramanian [43]
	Macrobrachim rosenbergii	Crab et al. [21]
	Nile tilapia	Choo et al. [13]
	Red tilapia	Widanarni et al. [23]
	Channel catfish	Schrader et al. [44]
	African catfish	Romano et al. [36]
	Striped catfish	Duy and Ut [45]
	Rohu (*Labeo rohita*)	Kamilya et al. [46]
	Common carp	Bakhshi et al. [37]
	Piracanjuba (*Brycon orbignyanus*)	Sgnaulin et al. [47]
	GIFT tilapia	Menaga et al. [48]

Table 1.
Summary of the list of aquaculture species cultured under BFT system.

3.1 Bioflocs technology in tilapia aquaculture

Tilapia was the first suitable species for the BFT system both at the commercial scale and laboratory level as early as 1989 when Avnimelech and his colleagues investigated the efficiency of recirculated ponds in single-cell protein production and consumption with the blue tilapia (*Oreochromis aureus*). The tried treatment in the first experiment was feeding with protein-deficient feed complemented by the daily addition of cellulose and ammonium sulfate as substrates for the production of single-cell protein. In this experiment, the growth of fish receiving most of their protein as single-cell protein was lower than those fed with the commercial protein-rich feeds and higher than that of fish grown on the protein-deficient feeds alone. The crude protein and lipid contents of fish grown on the biofloc diet were similar to those of fish grown on the commercial diet. In the second experiment, sorghum meal and ammonium sulfate were used as substrates. The results showed that the growth of fish fed with single-cell protein was similar to that of fish fed conventional protein-rich feed. Microbial integration of ammonium into the carbon-rich substrate was shown to be an efficient means of dipping the level of inorganic nitrogen in the water. Finally, Avnimelech et al. [49] concluded that tilapia could eat and utilize bacterial single-cell protein, and it can be possible to replace, at least partially, expensive protein sources with cheap carbon sources.

3.1.1 BFT in tilapia reproduction

The effect of BFT on reproductive performance of aquacultured species is one of the areas of research under investigation currently, and tilapia has been a focal point of these investigations. To this end, a biofloc-based reproductive performance study of Nile tilapia broodstock was conducted by Ekasari et al. [50]. The results of the study showed higher average body weight gain in the BFT treatment, which suggests that, although more energy was allocated for reproduction, the fish grew better in the BFT environment, whereas no significant difference in fish hepatosomatic index (HSI) level was found among treatments. Furthermore, the gonadosomatic index of female brood fish in BFT seemed to increase and reached its peak at a level of 4.01% and remained relatively constant afterward at around 3%. Egg diameter in BFT treatment was found to be insignificantly different over the experimental period, while fish fecundity was constantly higher in the BFT treatments except on day 70. This is also reflected in the total fry production during the experimental period, which was 65% higher than that of the conventional clear water system. Ekasari et al. [50] concluded that, overall, a positive effect of BFT application on Nile tilapia reproductive performance was observed in their study.

A more recent study by Gallardo-Collı´ et al. [51] to evaluate the reproductive performance, organ somatic indices, and body composition of the Nile tilapia cultivated at high density reusing the water from systems with biofloc technology during a grow-out period of 14 weeks showed insignificant differences in the gonadosomatic and hepatosomatic conditions either between tilapia sexes or between the different treatments groups. The results indicated that the intensive culture of *Oreochromis niloticus* in BFT system can be conducted using reused water from biofloc systems, with no adverse effects on their reproductive capacity and gonadal development.

3.1.2 BFT in the nursery rearing of tilapia

Several research activities conducted to evaluate the BFT technology in tilapia aquaculture focus on the nursery stage of the species. This is partly attributed to the fact that fingerlings of the species have the potential to grow fast and can be easily accessed compared to brood stocks and adults. To this end, in the following paragraphs, representatives of research activities on fingerlings of tilapia are presented based on chronology.

De Araújo et al. [52] evaluated the performance of Nile tilapia fingerlings cultured in biofloc technology using different densities of *Chlorella vulgaris*. In this experiment, the water quality parameters showed insignificant differences between treatments, especially TAN and NO_2-N, which were in the range acceptable for the culture of the species. The zoo technical performance parameters were not affected by the different inoculation densities of the microalgae, attaining a final mean weight of approximately 21 g for all treatments and survival rates greater than 80%. De Araújo et al. [52] concluded that the weekly inoculation densities of the microalgae *C. vulgaris* had no influence on the growth of tilapia fingerlings cultured in a biofloc system.

In the face of all problems regarding traditional tilapia food sources and production systems, the search for sustainable alternatives has been increasing. Within this context, de Sousa et al. [53] conducted research with the aim of evaluating different inclusion levels of pizzeria by-product meal (0, 20, 40, 60, 80, and 100%) in diets for Nile tilapia (*O. niloticus*) fingerlings reared under biofloc systems. Water quality parameters and the planktonic community profile were monitored throughout the feeding trial (38 days). The results indicated that the zoo technical performance of fish fed the 20% pizzeria by-product feed was statistically nonsignificant to those fed the control feed. Feed conversion ratio was also similar between these treatments and the 40% inclusion group. de Sousa et al. [53] concluded that it is possible to successfully include up to 20% of pizzeria by-product meal in diets for Nile tilapia reared in biofloc systems, and 40% inclusion of pizzeria by-product exhibited a strategic benefit to the farms that commercialize fingerlings.

Even though studies are scarce, biofloc technology can be successfully used in tilapia fingerling commercial production with some benefits. To this end, García-Ríos et al. [51] determined the effect of BFT system on the economic feasibility parameters such as productive performance and demand of feed and water in tilapia fingerlings production using carbon sources corn flour, wheat flour, sugar, and control without carbon source addition. The water quality, productive parameters, cost of consumed food, and volume of used water were determined. At the end of the growing period, the lowest weight corresponded to the systems with wheat flour as the carbon source and the highest to systems with sugar as the carbon source and control. The FCR in control was significantly higher than in the biofloc treatments. The control exhibited the lowest protein efficiency, while the maximum was recorded in systems with sugar as a carbon source. In addition, proximate tissue composition analysis showed a crude protein content of 0.0639–0.07 g/0.1 g dry weight bases, with significant differences among treatments. The survival in the stress test was similar among treatments. To produce a set of 10,000 fingerlings, the used water in BFT was 1611–2060 gal and 6314 gal in the conventional system. The mean supplied feed in BFT was 6 kg/batch, while in the control was 10.7 kg/batch. The cost of feed and carbon source was estimated on average as 7 US$/batch in BFT and of 9 US$/batch in the control. The fingerlings cultured in corn flour and sugar treatments showed a similar zoo technical performance to the control. However, the savings in feed and culture water consumption were significant.

3.1.3 BFT in grow-out culture of tilapia

The research activities range in scope from small-scale laboratory experiments to large-scale pond cultures to justify the zoo's technical and economic feasibility of the system. Azim and Little [54] evaluated BFT in the light-limited tank culture of Nile tilapia (*O. niloticus*)in a set of two biofloc treatments and one control in indoor tanks of 250 l capacity. Two BFT treatment groups were designated with 35%, and 24% crude protein diets, and a clean water control treatment without biofloc fed 35% crude protein diet. The results indicated that, fish mortality was null and net fish production was 45 times higher in the BFT treatments than in the control confirming the utilization of biofloc by fish as food. There was no difference in fish growth performance between the 35% and 24% crude protein-fed groups under the BFT system. In addition, well-being indicators in terms of fin condition, gill histology, body proximate composition, and blood hematocrit and plasma cortisol levels were compared, and no significant differences between BFT and control treatments were observed, indicating no increased fish stress due to the presence of biofloc. Despite this fact, Azim and Little [54] concluded that, in terms of commercial viability, overall fish growth and production were poor and recommended a modified system design that would allow improved feed and biofloc utilization to be proposed.

Lima et al. [55] evaluated the water quality and the growth performance of Nile tilapia cultured in bioflocs system with different stocking densities. A 128 days experiment was conducted with an initial weight of 123.0 ± 0.6 g stocked in twelve 800 L circular tanks in a completely randomized design with three densities of 15, 30, and 45 fish/m^3

and four replicates. The result showed that there was a significant effect of the different densities on the level of dissolved oxygen, with the lowest concentration of 3.97 mg/L) for the highest tested density of 45 fish/m^3. The total ammonia showed a statistical difference between the density of 15 fish/m^3 and the others. The nitrite also showed a significant difference between the density of 15 and 45 fish/m^3, but both at a directly proportional relationship with the increasing of stocking density, showing higher average concentrations of 2.56 and 3.26 mg/L (NH_3 + NH_4 and NO_2, respectively), in 45 density fish/m^3. The growth performance in the 45 fish/m^3 density showed the best results, with a yield of 16.6 kg/m^3, with a significant difference between treatments. Survival was higher than 90% for the whole three tested densities. Lima et al. [55] concluded that, bioflocs technology could be employed in intensive culture of Nile tilapia in the grow-out phase, using stocking densities up to 45 fish/m^3.

Madyod et al. [56] investigated the efficacy of dried bioflocs supplemented with immune stimulants as β-glucan and nucleotide on the mortality rate and relative percent survival (RPS) of tilapia infected with *Flavobacterium columnare*, VETSV01. The results of the study demonstrated that feeding tilapia with commercial feed in combination with dried biofloc with or without supplementation of 2 immunostimulants could lead to 100% survival rate and 100% relative percent survival rate, and it was statistically significant compared to the control group. Madyod et al. [56] conclude that biofloc product supplemented with betaglucan or nucleotide could effectively stimulate immunity against *F. columnare*, and such highly effective performance is viewed as a promising potential product to be further developed and practically employed for the aquaculture industry in the future.

Martins et al. [57] evaluated the effects of heterotrophic and mature biofloc systems on yield, water quality, slurry production, and water bacterial community composition, recovery of nutrients, and fish health in *Litopenaeus vannamei* and *O. niloticus* integrated culture through a 53 days experiment. The results showed that shrimp growth performance was unaltered, but fish cultured in the heterotrophic treatment showed better values for all growth performance variables assessed. TAN and NO_2-N were lower in the mature biofloc system, while the total slurry generated in the heterotrophic system was higher than in the mature biofloc system, resulting in greater sludge generated per animal biomass. The bacterial community range was reduced in the heterotrophic treatment, and the relative abundance of *Vibrionaceae* was reduced. The assimilation of nitrogen and phosphorus was higher in the heterotrophic treatment, and fish health was not affected by the biofloc system. Martins et al. [57] concluded that the biofloc system favored fish growth in the integrated culture system, maintaining water quality appropriate for growing organisms and healthy fish.

3.2 Bioflocs technology in catfish aquaculture

The consumption of bioflocs, and in turn, the ability to promote animal growth, is largely based on the fish's ability to collect and consume these particles. Due to this fact, suspension-feeding fish have been thought to be better adapted to consume smaller bioflocs than carnivorous species, such as catfish [36]. Nevertheless, in a series of experiments, it has been shown that catfish juveniles greatly benefited from biofloc-based systems, which may help produce better quality and more disease-resistant stock. Benefits of biofloc technology (BFT) to this carnivorous species largely depend on the carbon source [18] and the ratio to nitrogen [25]. Therefore, in the following sections, the available research on the different production stages of catfish aquaculture are presented.

3.2.1 BFT in catfish reproduction

The development of catfish aquaculture is constrained by the limited supply of good quality and quantity fingerlings [58]. Catfish fingerlings rearing, in particular, has been constrained by the seasonal spawning behavior and low reproductive success of the brood stock, and the low survival rate of larvae [59]. To this end, few studies have been conducted to evaluate the effects of BFT in the reproduction performance of the species.

Nadio [60] evaluated the effects of biofloc technology on the reproductive performance of *Clarias gariepinus* females, especially during their re-maturation period. The BFT treatments were differentiated by temperature: biofloc at 25°C (BFT + 25), biofloc at 28°C (BFT + 28), and biofloc at 31°C (BFT + 31). The results indicated that females reared in the biofloc system at 31°C achieved better reproductive performance in terms of higher eggs production per spawning (127×10^3) per kg female, shorter re-maturation period (80% of females reached maturation within 3 weeks), higher gonadal-somatic index (GSI ≥ 19%) as compared with the control with lower eggs production per spawning (57.3×10^3) per kg female, longer re-maturation period (20% of females reached maturity within 6 weeks) and lowest gonadal-somatic index (GSI ≤ 10%) respectively. On the other hand, no significant difference among the BFT treatments was observed. Nadio [60] concluded that the better reproductive performance shown by females reared in BFT system justified the application of this technology in *C. gariepinus* broodstock management.

Ekasari et al. [61] evaluated the effects of biofloc technology application on African catfish *(C. gariepinus)* broodstock reproductive performance and the quality of eggs and larvae. Biofloc and conventional clear water systems were compared for the reproductive performance of c, while the larvae produced by the brood stocks in both systems were subsequently assessed by larval starvation tolerance and growth tests. The results showed that the gonadosomatic index and fecundity of female brood stocks in both treatments were generally comparable, except on day 122 when the relative fecundity of the control brood stock was 26% lower than that of the biofloc brood stock. In addition, the embryonic development rate of eggs spawned by biofloc brood stocks was higher than the control brood stock. The survival in starvation test and growth tests were remarkably improved in the larvae produced by biofloc brood stocks. Ekasari et al. [61] concluded that rearing African catfish broodstock in biofloc systems significantly affected the embryonic development rate and the larval quality. Improvements in survival and growth were observed in the larvae reared in biofloc systems.

3.2.2 BFT in the nursery rearing of catfish

The rearing in biofloc system may be beneficial for rearing carnivorous fish larvae, especially in the early stages of culture when larvae possess feeding habits that include consumption of debris [62]. In addition, due to the high rate of cannibalistic nature, larval culture of such species might give better survival when implemented in turbid environments with low light levels [63], which could be another benefit of the biofloc system. However, tolerance of species to different concentrations of total suspended solids still needs to be investigated. TSS is the variable used to quantify the level of biofloc in the cultivation, and although Avnimelech [64] recommended values between 200 and 400 mg/L for tilapia culture, the author reported that the optimal concentrations for growing fish are not well known yet.

Poli et al. [65] conducted a 21-day experiment to evaluate the application of biofloc technology in South American catfish larvae (*Rhamdia quelen*) grown at different concentrations of biofloc. *R. quelen* larvae were grown in biofloc systems developed from the intensive culture of *O. niloticus*. Three biofloc concentrations, expressed as TSS were tested: up to 200 mg/L, between 400 and 600 mg/L, and between 800 and 1000 mg/L. In the control, the larvae were cultured in clear water from a recirculation system. The results showed that, even though the increase in TSS concentrations was not associated with the best growth of *R. quelen*, the performance of the larvae was better in the TSS level of 200 mg/L compared to 400–600 and 800–1000 mg/L. Poli et al. [65] concluded that *R. quelen* larvae could be grown in a biofloc system with TSS concentrations up to 1000 mg/L, but the best growth was registered in tanks with a lower percentage of total suspended solids which is <200 mg/L.

Hapsari [66] evaluated the effect of molasses addition on African catfish (*C. gariepinus*) fingerlings growth and feed conversion rate when the biofloc was fermented for 24 h using molasses, fish meal, and grains. The results showed that molasses treatment on the application of bioflocs showed the highest growth and the lowest feed conversion rate. FCR of African catfish in fermented biofloc was lower by almost half compared to the control and unfermented biofloc treatments. Hapsari [66] concluded that biofloc could be used as the alternative feed and that molasses treatment in fermenting biofloc can increase the nutritional values and hence increase the growth of African catfish fingerlings.

Putra et al. [67] evaluated the growth performance and feed utilization of African catfish (*C. gariepinus)* fingerlings fed a commercial diet and reared in the biofloc system enriched with probiotics where the frequency of probiotic application into the cultured system was the objective of the experiment. The results of the experiment showed that growth performance, survival, and feed utilization of African catfish were higher in the treatment with the addition of probiotics at 5-day intervals over a period of 60 days culture.

Soedibya et al. [68] determined the effect of high stocking densities on the growth performance of African catfish fingerlings in biofloc system with stocking densities of 1000 fingerlings/m^3, 1500 fingerlings/m^3), 2000 fingerings/m^3), and 2500 fingerlings/m^3). The results showed a significantly different effect against the value of hepatosomatic index, absolute growth, and daily growth rate, while specific growth rate showed insignificant difference. The treatment with the stocking density of 1500 fingerlings/m3 showed the best results than the other densities in absolute growth rate and daily growth rate. These findings demonstrate the role of biofloc technology in catfish aquaculture.

3.2.3 BFT in grow-out culture of catfish

Schrader et al. [44] determined the development and composition of phytoplankton communities and related off-flavor problems in outdoor biofloc production systems of channel catfish. In this study, water and fish flesh were analyzed for quantities of geosmin and 2-methylisoborneol as the common off-flavor compounds. The development and composition of phytoplankton in each culture tank was also observed. In addition, water and biofloc samples were assessed for the microbial sources of geosmin and 2-methylisoborneol within the culture vessels. The results of the study indicated that phytoplankton biomass, as determined by concentrations of chlorophyll a in the water, gradually increased in all culture vessels over time. In addition, a positive correlation between cumulative feed addition and chlorophyll a concentration was reported. Although geosmin and 2-methylisoborneol were present in the culture water of each tank during most of the study, levels were typically low, and only one tank yielded catfish with geosmin and 2-methylisoborneol in their muscle at levels high enough to be designated as off-flavor. A positive correlation between feed addition and 2-methylisoborneol concentrations in the water of culture tanks indicates a greater potential for 2-methylisoborneol -related off-flavor problems at high feed application rates.

Channel catfish have been cultured successfully in an outdoor BFT system. Outdoor BFT culture systems in the tropics are conducted yearly, whereas the channel catfish studies were conducted only during the growing season, and biofloc production systems were harvested and kept vacant for the winter. If an outdoor BFT culture is to be implemented by farmers in temperate areas, data gaps associated with system and fish performance over the winter must be addressed. To this end, Green [69] conducted a study to evaluate the performance of a temperate-zone channel catfish biofloc technology production system during winter. Culture waters from a recently completed biofloc production experiment that contained low and high total suspended solids were retained for the study. Three 16 m^3 tanks per water type each were stocked with market-sized channel catfish from the same experiment for a 38 weeks period. Green [69] reported that mean chlorophyll *a* concentrations were similar in both treatments during the first 55 days, after which treatments deviated and chlorophyll *a* concentration increased linearly in the low total suspended solids treatment. Ammonia from ammonium chloride spikes) added on three occasions during the experiment was transformed completely by algal uptake and nitrification. Ammonia bioconversion rate was linearly related to mean water temperature in the high total suspended solids and low solids treatments. Catfish survival through the winter was high in biofloc tanks and did not differ significantly between treatments. Net fish yield did not differ significantly between treatments. Green [69] concluded that having an active biofloc in the spring obviates the start-up time required to establish a new, fully functional biofloc and the associated TAN and nitrite spikes in winter.

More recently, Hastuti and Subandiyono [70] observed the hematological parameters of catfish (*C. gariepinus*) cultivated with a density of 1000 individuals/m^3 using biofloc technology. With an explorative method, samples were taken from *C. gariepinus* cultivated with applications of biofloc technology. The measured variables were: total bilirubin, direct bilirubin, indirect bilirubin, and blood glucose. Calculated blood cells are red blood cells, white blood cells, hemoglobin, hematocrit, and platelets. The results showed total bilirubin and indirect bilirubin were in normal ranges, while direct bilirubin values increased by 0.3 mg/dL at week 8. According to the results, *C. gariepinus* shows a stress response with an indicator of high blood glucose from 114 to 188 mg/dL. Water quality was in optimal conditions which are in accordance with the *C. gariepinus* requirements, with a survival rate value of 96%.

3.3 Bioflocs technology in carp aquaculture

Another freshwater group of fish gaining recent attention to be cultured in the BFT system is the family Cyprinidae in which few attempts have been made to evaluate the performance of the system in the different species of the family [16, 46, 71]. This group of fishes exhibits suspension-feeding behavior and better tolerance to higher concentrations of suspended solids which are the most important attributes for the successful cultivation of a species in the BFT system [36]. Applications of BFT on the reproductive performance of carp have never been reported so far. Therefore, in the following sections, available research outputs are presented on the nursery and grow-out production cycles of different carp species.

3.3.1 BFT in the nursery rearing of Carp

The first study on BFT application on carp was undertaken by Nadjigerami et al. [16] in a 30-days experiment to investigate the effects of partial replacement of daily feeding intake with biofloc on the growth performances, digestive enzymes activity, and liver histology of the common carp *(Cyprinus carpio)* fingerlings. Two hundred and eight healthy fingerlings were randomly distributed in 12 tanks (30 L) at a density of 25.4 kg/m^3 and fed experimental treatments (100% daily feeding rate as a control, biofloc +75% daily feeding rate, biofloc +50% daily feeding rate, biofloc +25% daily feeding rate). The results indicated that the highest weight gain was observed in the fish-fed biofloc and 75% feeding rate and control, which varied significantly from those fed biofloc and 25%. The treatment with biofloc and 7% feeding rate enhanced total protease and pepsin activity compared with biofloc and 25% feeding rate and biofloc and 50% feeding rate. Insignificant difference was observed between the treatments of lipase, amylase, and alkaline phosphatase activity. In the liver, histological variations were found in the treatments, and feeding the fish with biofloc at 75% rate significantly improved hepatocellular quantification and qualification than the other treatments. Nadjigerami et al. [16] concluded that the biofloc improved growth performances, digestive enzyme activity, and liver condition of the common carp fingerlings when 25% of the daily feeding rate was replaced with one carbohydrate such as molasses in zero-water exchange system.

Sarker [72] evaluated the comparative efficacy of biofloc and feed-based common carp *(C. carpio,* L.) production systems with special reference to environmental health. The findings of the study indicated that biofloc alone was not sufficient for the growth of the test fish under a supplementary feeding regime. However, the mortality rate of the test fish was highest in biofloc alone treatment. It was clear that biofloc improved the performance of the supplementary feed both in terms of feed conversion rate and feed conversion efficiency. In spite of less absolute weight gain with feeding at 3% body weight/day because of the most promising feed conversion rate and less nutrient loading, the system demonstrated to be economically and ecologically more sustainable in the presence of bioflocs. Biofloc not only supported the test fish nutritionally but supported the planktonic productivity as well. Moreover, it also favored phosphate mineralization in the soil phase. Himaja [71] evaluated the growth performance of *Catla catla* using prepared biofloc meals incorporated into the diet with 20, 30, and 40 percent levels. The biofloc was developed in four experimental indoor cement tanks at the carbon-to-nitrogen ratio of 15:1 in the indoor tanks. Experimental diets were prepared using indoor and outdoor dry biofloc meals by replacing fish meals at different levels, while the control diet was prepared without biofloc. The results of the study revealed that *C. catla* fed with a control diet exhibited higher growth. Similarly, the growth performance of *C. catla* in 40% was more or less the same. Whereas the outdoor biofloc meal incorporated diets had better FCR than other treatments next to control and 40% feed replacement. Himaja [71] concluded that 20% indoor biofloc meal incorporated diet performed similarly to the control diet and had higher performance than that of the commercial carp diet. Hence fish meal could be replaced in the carp diet successfully by 20% of either indoor or outdoor biofloc meal.

3.3.2 BFT in the grow-out culture of carp

Unlike tilapia and catfish culture, applications of BFT in the grow-out of the different carp species are scarce, although the group constitutes potential species for the technology. Nevertheless, few attempts have been made and are presented herewith.

Sasmal et al. [73] conducted a trial of six months period to investigate the growth and production of common carp in fresh water Biofloc System in India. Three rectangular cemented tanks (5000 liter capacity) were used for this purpose. Probiotic was used for developing beneficial bacterial colonies and controlling ammonia in the confined water system. The results indicated that flocs volume ranged between 12 and 47 ml/1000 liter water sample while the average yield was recorded at 218 kg/tank after a period of 6 months from stocking, and FCR was found to be 0.9. The other important parameters recorded were an average pH value 7.7, dissolved oxygen 5.9 ppm, TDS 454 ppm, and C:N ratio 12:1. Sasmal et al. [73] concluded that the biofloc system in freshwater aquaculture improves growth performances of the common carp in almost zero-water exchange system.

4. Variations in carbon source and C:N ratios in freshwater biofloc aquaculture systems

The carbon sources applied in BFT can be classified broadly in two, that is, by-products derived from agricultural activities such as wheat bran, molasses, tapioca flour, sorghum meal, etc., and industrial products such as glycerol, acetate, glucose, and starch (**Table 2**). The addition of these carbon sources in the culture system or in the feed is aimed at maintaining a high C:N ratio, preferably above 10:1 to control nitrogenous compounds peaks. Also, a mix of plant meals can be pelletized and applied into ponds [81], or low protein diets containing high C:N ratio can also be used [15, 64]. The carbon source serves as a substrate for operating BFT systems and the production of microbial protein cells [7].

There are many considerations for the selection of carbon sources such as costs, local availability, biodegradability, and efficiency of bacteria assimilation. The organic carbon source of choice to a large degree determines the composition of the flocs produced. As an example, it was reported by Ekasari et al. [82] that, Bioflocs with glycerol as a carbon source had higher total n-6 PUFAs than that of glucose, while there was no effect of carbon source on crude protein, lipid, and total n-3 PUFAs contents of the bioflocs.

Later on, Dauda et al. [18] compared different carbon sources, that is, sucrose, glycerol, and rice bran, in a carbon-to-nitrogen ratio of 15:1 in a biofloc-based African catfish culture system. The results of the experiment indicated that glycerol significantly increased total biofloc production, and both the sucrose and glycerol treatments generally had lower nitrogenous waste levels compared to the control. Liver histopathology of fish in the rice bran biofloc treatment showed substantial vacuolation and less glycogen, while the highest was in fish from the glycerol treatment. Fish growth was not affected among the treatments, but mortality was lowest in the glycerol treatment. Dauda et al. [18] concluded that rice bran appears unsuitable for *C. gariepinus*, likely due to being a slower-releasing carbon source. Instead, they recommended glycerol based on significantly higher biofloc production and subsequently improved water quality and survival of *C. gariepinus* during their experiment.

Another study by Deng et al. [19] indicated that BFT systems with plant cellulose and plant cellulose + tapioca starch treatment groups had a higher total bacterial diversity and greater microbial richness than those with no plant cellulose treatment groups (tapioca starch alone and the conventional clear water systems).

Species	Carbon sources	C/N ratios	References
Tilapia	Cellulose and sorghum meal	15	Avnimelech et al. [49]
	Wheat flour	8.4, 11.2	Azim and little [54]
	Molasses	15	Caldini et al. [74]; Ekasari et al. [50]
	Poly-β-hydroxybutyric acid	—	Zhang et al. [75]
	Molasses	15, 30	Cavalcante et al. [76]
	Sugar, molasses, and cassava starch	10, 20	Silva et al. [77]
	Molasses	6	Alvarenga et al. [78]
	Sugar, liquid molasses, and powder molasses	15	Lima et al. [55]
	Sugar, corn flour, and wheat flour	12	García-Ríos et al. [14]
	Distillery Spent wash	10	Menaga et al. [48]
Catfish	Molasses	10, 15, 20, 25, 30	Bakar et al. [25]
	Molasses	10	Ekasari et al. [61]
	Sugar	20	Hastuti and Subandiyono [70]
	Molasses + *Bacillus* spp.	—	Putra et al. [67]
	Sucrose, glycerol, rice bran	15	Dauda et al. [18]
	Glycerol	10, 15, 20	Dauda et al. [17]
	Tapioca flour	10	Fauji et al. [79]
	Rice bran + *Bacillus* spp.	15	Romano et al. [36]
Carp	Beet molasses (24% carbon)	20	Najdegerami et al. [16]
	Boiled rice water mixed with molasses	—	Sarker [72]
	Molasses	15	Kamilya et al. [46]
	Molasses, sugar, and cornstarch	20	Bakhshi et al. [37]
	Coffee, moringa, macroalgae, and yucca	—	Castro et al. [80]

Table 2.
Summary of carbon sources and carbon to nitrogen ratios in freshwater aquaculture.

El-Husseiny et al. [20] evaluated the effect of different carbon sources on biofloc conditions and tilapia performance. Biofloc treatments with five different organic carbon simple sources (glucose and molasses) and complex sources (starch, wheat bran, and cellulose) were conducted in the presence of control (clear water). The results of the experiment showed that no significant differences were noticed among different carbon sources concerning tilapia growth performance. Complex carbon sources represented in wheat bran and cellulose showed less fluctuation in the values of ammonium and nitrite during the experimental period than the other carbon sources. The precipitated biofloc from both wheat bran and cellulose showed the highest fat content. In terms of heterotrophic bacteria production, plankton count, and biofloc nutritional content, cellulose appears to be the better choice. El-Husseiny et al. [20] concluded that, from nutritional and economic points of view, using agricultural by-products with high cellulose content as a carbon source in biofloc system is more reasonable.

The natural condition of the aquaculture system, which is rich in inorganic nitrogen, could not sustain BFT due to limitation of carbon. Thus, additional carbon is required to obtain a suitable C:N ratio for the effective formation of bioflocs complex. To this end, Bakar et al. [25] conducted an experiment on the determination of optimum carbon-to-nitrogen ratio by varying the amount of carbon introduced into the system using five different C:N ratios in remediating the aquaculture system culturing *C. gariepinus* to an acceptable water quality levels. Bioflocs density and water quality parameters were monitored throughout the treatment period. The results of the experiment showed that the highest elimination of ammonia was achieved with the formation of 92 mL/L of bioflocs concentration at an optimum carbon-to-nitrogen ratio of 15. The results showed that the application of BFT can possibly provide a sustainable and low-maintenance treatment of aquaculture systems.

Pérez-Fuentes et al. [26] also evaluated the effects of different C:N ratios (10, 12.5, 15, 17.5 and 20) on the growth performance of juvenile tilapia *(O. niloticus)* rose under biofloc cultivation using molasses as a carbon source. The results indicated that survival in all treatments with biofloc was similar and significantly higher (94.60 ± 2.03%) than in the control treatment (84.96 ± 1.53%). The 10 and 15 carbon-to-nitrogen ratio treatments had similar weight gains but not the 12.5 and 17.5 carbon-to-nitrogen treatments. Total production in the 10 ratio treatment was the highest, with slightly declining production. Water quality remained similar in all biofloc treatments, but lower concentrations of nitrogenous compounds occurred in the 10 carbon-to-nitrogen ratio treatments. Pérez-Fuentes et al. [26] concluded that a 10:1 carbon-to-nitrogen ratio provides good survival and growth of tilapia with no water exchange.

A study by Dauda et al. [17] on the effects of increasing glycerol loading rates to create carbon-to-nitrogen ratios of 0, 10, 15, and 20 on the biofloc formation, biochemical composition, and water quality, as well as the growth performance, feeding efficiency, enzyme activities, and liver glycogen levels of African catfish *(C. gariepinus)*. After 6 weeks, all fish were measured for growth; ten fishes per replicate were used for additional analysis, while ten fishes per replicate were later challenged with the bacterial pathogen, *Aeromonas hydrophila*. The results indicated that biofloc volume was significantly higher at carbon-to-nitrogen ratio of 20, but flocs biomass was significantly higher at carbon-to-nitrogen ratio 15. Dissolved oxygen was significantly lower at carbon-to-nitrogen of 20 while TAN was significantly higher in the control than the biofloc treatments. Survival, growth, and feed utilization efficiency were similar among treatments. Fish muscle cholesterol, lipid peroxidation, blood triglyceride, and blood cholesterol levels were all significantly lower in the biofloc treatments, but liver glycogen level was significantly higher in the carbon-to-nitrogen ratio of 15 treatment. Chymotrypsin activities were significantly higher in the biofloc systems while trypsin was not different among the treatments. After challenging the catfish to *A. hydrophila*, survival was significantly higher in the C:N ratio of 15 and 20 groups, which was accompanied with less histopathological liver damage compared to those in the control or C:N ratio 10 treatments. Overall, Dauda et al. [17] concluded that in a glycerol-based biofloc system, a C:N ratio of 15 led to the best balance of better water quality, nutritive value of *C. gariepinus* as well as their resistance to *A. hydrophila*.

5. Prospects and challenges of biofloc technology for aquaculture in Ethiopia

As discussed in the sections above, the BFT system proved to be a viable alternative to RAS and other conventional aquaculture systems for the major freshwater species in terms of zoo technical performances [7]. This was also partly true for the socioeconomic performances at commercial production levels, especially for tilapia aquaculture. As an illustration, Avnimelech [83] estimated that feed rations in biofloc tilapia systems could be lowered by at least 20% from conventional system levels, which can significantly reduce the cost of production. In another success story in Malawi at Chambo fisheries, from an economic perspective, results indicated a 50% feed cost reduction when compared to feeding fish on conventional 32% crude protein level feeds raised in a recirculation aquaculture system [84]. The advantages of biofloc technology include a significant reduction in the final farm-gate production cost of raising tilapias to about $1.30/kg in Malawi in 2016. A broad economic study based on data gathered at Chambo fisheries showed biofloc farms to conceivably produce tilapia at about 60% lower cost than large-scale cage culture, 34% less than RAS, and 8.5% less than green water pond farming, supposing all farms are located in or near Lake Malawi [84].

Despite this fact, using the BFT displayed some problems to cultured aquatic animals practically. Organic carbon must be supplemented to the culture water to sustain a carbon-to-nitrogen ratio of over 10. In addition, employing a system for mixing and aerating the water, which increases energy costs, is required to sustain an active BFT in suspension and to meet the oxygen demand of elevated water respiration [64]. Thus, the BFT system requires a greater consumption of electrical energy than the conventional systems [85]. Therefore, the aerator model chosen should be as efficient as possible in both oxygen transfer and power consumption [86].

The suspended solid content in BFT is typically greater than 500 mg/L, and excessive solid concentrations can clog the gills of fish or shrimp, which affects their growth and welfare [87]. In addition, if total suspended solids concentration exceed the mixing capacity of the system, solid particles settle downward and can accumulate in anaerobic soil layers or pockets of ponds. Anaerobic spots in pond bottoms can lead to the production of toxic compounds and severely hamper fish growth [85].

Knowledge gaps about BFT engineering, feeding systems and bioenergetics, cost factors, and the economics also remain to be the biggest challenge relative to conventional aquaculture systems [84]. Despite the fact that the availability of suited species and cheap carbon sources to implement the system seems a good prospect for BFT application in Ethiopia, the knowledge gap and uncertainty of electric power make the future of the technology long-standing from its implementation in the country.

6. Conclusion and recommendations

Biofloc can be a novel strategy for disease management in contrast to conventional approaches such as antibiotic, antifungal, probiotic, and prebiotic application [88]. The natural probiotic effect in BFT could act internally and/or externally against *Vibrio sp.* and ectoparasites, respectively [9, 89]. This effect is stimulated by big assemblages of microorganisms, mainly bacteria, that are considered the first trophic levels in the system. Bacteria and their produced metabolites could act similar to organic acids and might be effective biocontrol agents by maintaining host's microbial stability in the gut [90].

It can be concluded that biofloc technology holds a firm position as a potential environmentally friend and sustainable production system in freshwater aquaculture. Results from various research consulted for this review indicated that BFT application in the three major freshwater aquaculture species (tilapia, catfish, and carp) improves culture water quality hence decreasing the wastewater discharge to the environment and allowing efficient water usage. Moreover, the technology proved to be efficient in producing an extra feed for the cultured species in the form of single-cell protein, providing an opportunity to substitute up to 20% of the conventional feed by biofloc, thus implying the potential of the technology in reducing the production cost of the cultured organisms. In addition, bioflocs alone or in combination with probiotic bacteria showed promising results in enhancing the immunity of cultured freshwater species providing an alternative to chemical therapeutics that have a detrimental impact on the environment. On the other hand, the organic carbon sources used to enhance flocs formation in BFT are relatively cheaper and readily available, which makes the technology economically viable. Although various carbon sources are being used in freshwater aquaculture, molasses was found to be the most frequently applied organic carbon source in this review while C:N ratios of 10, 15, and 20 are the most frequently reported ones so far. Despite all these potentials, BFT also has its own limitations related to the higher amount of total suspended solids and energy consumption for aeration and mixing. In addition, the technology requires relatively better know-how to successfully operate the system hindering its application at the farmer's level. Therefore, it is recommended that research activities under laboratory conditions should be up-scaled on to bigger size culture setups to better understand the economic feasibility of the technology, especially in catfish and carp aquaculture. Optimizations of the levels of total suspended solids which are safe for each species based on the morphology and feeding behavior of the species needs to be dealt well. Research activities should also focus on finding alternatives to the higher power consumption such as solar systems to mitigate the challenge related to power usage. Lastly, capacity building and promotion of the technology needs to be conducted especially in developing countries where the technology is still a potential rather than a real practice.

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