



# The prospects of biofloc technology (BFT) for sustainable aquaculture development

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## ABSTRACT

As capture fishery sector continues to decline, aquaculture industry must be accelerated to bridge the fish supply gap especially in the developing countries. However, in most developing countries, aquaculture sector is characterized by low productivity due to inefficient technologies, hence the need for innovative aqua-technologies that can stimulate back yard fish production, for enhanced livelihood security among smallholder farmers. In aquaculture, biofloc technology (BFT) is considered as an innovative culture system with great potential for fish production. The BFT uses the principle of nutrient cycling through complex bio-pathways to produce natural food for fish. The working machines are the bacterial flocs that convert pond bio-wastes into edible nutrients for the cultured animals. This reduces feed cost by about 30% and ensures higher profitability. BFT is useful for mass production of live food resources, which are indispensable for successful larviculture in hatcheries. Bioflocs enhance gonad formation and ovary development in fish broodstock, thus improving reproduction of fish. Bioflocs are also natural biosecurity agents that reduce the use of antibiotics, which have various ecological consequences in aquaculture environment. Some species of bacteria are useful in the process of atmospheric CO<sub>2</sub> sequestration thus mitigating the effects of green-house-gasses (GHG). This article has articulated step-wise processes of establishing BFT and demonstrated its potential to achieving 'triple win' objectives of; a) increasing fish production, b) enhancing resilience of fish production systems, and c) efficient use of energy, water, land, and reduction of GHG emissions.

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## Introduction

Food and nutrition insecurity is a visible indicator of poverty in the developing countries [31]. In the developing countries, high prevalence of poverty has been linked to limited application of technology innovation management practices (TIMPs), and effects of changing climate in most agricultural production value chains [13,31]. In the recent past, the capture

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fishery sector supplied enough fish for human consumption, and promoted general socioeconomic stability in most developing countries [63,35,11]. Today, the capture fishery sector cannot supply sufficient fish for the growing human population, hence the need for sustainable aquaculture production technologies to bridge the fish supply gap [31]. In the developing countries, aquaculture has been recognized as a quicker means of enhancing livelihood security and economic growth through its value chain linkages [44,11,47]. However, the aquaculture sector is still characterized by low productivity due to lack of quality inputs and poor culture technologies. The low fish production has contributed to a lower fish consumption per capita of < 5 kg / person / year in most developing countries [60] compared to 20 kg / person/ year in developed countries [31]. This article advocates for creating an aquaculture production paradigm and shifting it towards innovative, highly productive, cost-effective and eco-friendly farming systems. The innovative aqua-systems should promote backyard fish production and stimulate livelihood security activities e.g. good health and wealth creation.

Biofloc technology (BFT) is a climate-smart technology that works on the basis of mass production of in situ microorganisms. The microorganisms are credited for (i) maintaining good water quality ([30], ii) increasing culture feasibility by reducing feed conversion ratio (FCR) and feed costs ([3], iii) biosecurity [21]; and (iv) sequestration of greenhouse gasses (GHG) [41]. These four biological functions of microorganisms in BFT units are factors of high fish production, profitability and environmental protection. One visible strength of BFT is that the initial investment cost is less than most conventional fish production systems, because only sunlight, a carbon source and sometimes aeration are needed. In continental Asia, top shrimp-industry players have shifted to BFT. As Mr. D James Lim, who is the CEO of Lim Shrimp Organization, the largest shrimp farming company in Asia, explains: “Without biofloc technology, our company wouldn’t be able to achieve its ambitious growth rates without compromising environmental integrity and animal-welfare principles. This system is a win-win situation for all stakeholders.”

Functionally, the BFT rely on heterotrophic process where uneaten feeds, feces and excess nutrients are converted into edible bioflocs, also called single cell proteins (SCP). The SCP are loosely bound by bacterial mucous to form visible floating clumps, which are nutritious food materials for cultured fish or shrimps. In an efficient BFT system, the cost of fish feed is reduced by 30% as each pellet is basically eaten twice (i.e. as fresh pellet, and, as SCP), thus leading to high aquaculture productivity and profitability [5]. The bioflocs not only contain essential nutrition but has probiotic effect that ensures biosecurity in the BFT systems [14,21,37]. The bioflocs consume ammonia to make own proteins, thus maintains good water quality in the culture systems [30]. The BFT requires minimal water exchange in aquaculture systems to maintain the flocs [30] and allows high stocking densities and increased fish productivity. The above attributes make BFT economically attractive to aquapreneurs [3]. BFT can be used for live food production in hatcheries to ensure supply of quality fish seeds throughout the year, for aquaculture [50,36]. Bioflocs are also efficient sinks of atmospheric carbon thus facilitates adaptation and mitigation of effects of GHG [34].

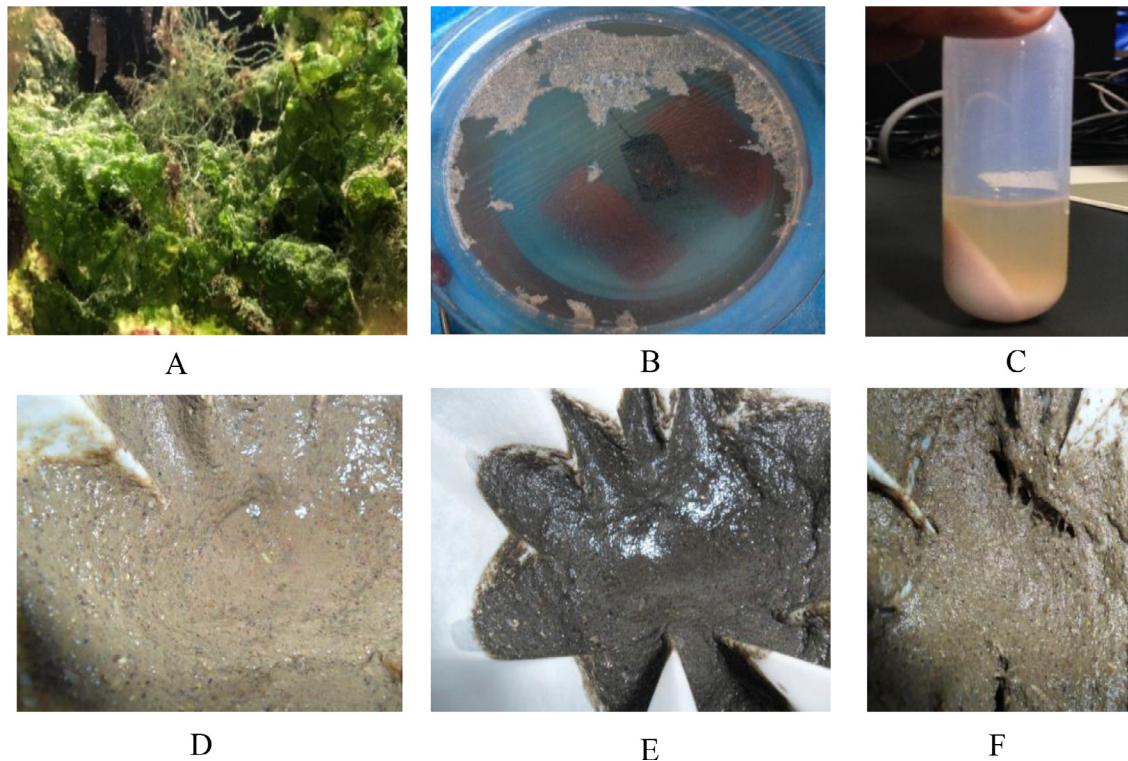
This article presents the prospects of BFT for sustainable aquaculture development. The authors have reviewed scholarly evidence to demonstrate the potential of BFT as a nutritional strategy to maximize the contribution of natural and supplemental feed in fish ponds to achieve sustainable aquaculture and livelihood security, especially in the developing countries. The authors have articulated step-wise processes for establishing various forms of BFT units (depending on financial capital). The authors have also demonstrated the potential of the BFT as a technology innovation and management practice (TIMP) to achieving ‘triple win’ objectives of; a) increasing fish production, b) enhancing resilience of fish production systems, and c) efficient use of energy, water, land, and reduction of GHG emissions.

## Bioflocs and flocculation process

Bioflocs are heterogeneous macro-aggregates of planktonic materials in the water column, which constitute a consortium of floc forming bacteria, diatoms, filamentous microalgae, micro- and macro-invertebrates, protozoa, fecal matter and uneaten feed (Fig. 1). The bioflocs form the basis of the food chain in aquatic ecosystems by converting to SCP. Therefore bioflocs are responsible for the initial nutrient cycling process in aquatic ecosystems [5,4].

### Bio-flocculation mechanisms

Bioflocs normally colonize new systems soon after accumulation of organic wastes. Microbial cells form matrix flocs through a complex flocculation processes controlled by physical, chemical and biological processes as described in De Schryver et al., [20]. In this process, the main constituents of the floc matrix are the extracellular polymeric structures that form microbial capsules, which bind the biofloc components [20,59]. The flocs are typically made up out of polysaccharides, protein, humic compounds, nucleic acids and lipids, and are mainly produced as slime or capsule layers under nitrogen limitation [9]. Under favorable conditions, biofloc aggregates vary in size from the microscopic to > 1 mm, which is similar to the size of most commercial fish pellets for juvenile fish. Densities of the microbial biomass average slightly above 1.0 g wet weight ml<sup>-1</sup> floc aggregate that make the bioflocs slow sinking particles (1–3 m h<sup>-1</sup>) [56]. The flocculation and sinking ability of the bioflocs is an adaptation mechanism to escape adverse ecological impacts e.g. of light and grazing pressure by organisms in higher-trophic chain [20,59]. In the process of sinking the bioflocs attach to available substrates and create mats that attract other aquatic microorganisms. The culture fish are able to graze upon the mat of microorganisms.



**Fig. 1.** Photos of various forms of bioflocs, A: amalgamation of bioflocs, detritus and algae; B: floating bioflocs in plastic tanks (white foams); C: high density bioflocs after centrifugation; D: aerobic flocs, E: anaerobic flocs and F: anoxic flocs (Courtesy of [50], and [40]).

### *The concept of BFT*

The BFT operates on the principle of nutrient recycling by maintaining a higher carbon / nitrogen (C/N) ratio e.g. above 15 to stimulate mass growth of heterotrophic bacteria [6]. Higher C/N ratio is maintained when more carbon source e.g. molasses, cassava, hay, sugarcane, starch, wheat bran, cellulose etc., is sprayed on the surface of pond water with continuous aeration. Under favorable BFT conditions, up to 0.5 g of heterotrophic bacterial biomass  $g^{-1}$  substrate of carbon can be produced [26]. With the information that 1 g of carbon produces 0.5 g of bacteria, farmers are able to estimate quantities of floc in the culture systems. The biofloc process stimulates natural growth of macro-aggregates of organisms that enhance self-nitrification in the culture water.

In outdoor BFT systems, photosynthetic pathway that produces algae normally precedes the bio-floccing process. The algae provide substrate to which the bioflocs attach, and are usually referred to as green bioflocs. Under indoor conditions, bioflocs are mainly bacteria, and are referred to as brown bioflocs. With addition of adequate carbon source bacterial floc stimulates a secondary production line that involves degradation of organic wastes by bacteria to produce more billions of bacterial cells (heterotrophic cycle) under optimum aeration condition [17]. During this process, autotrophic and heterotrophic bacteria proliferate and attract billions of other cells including diatoms, fungi, algae, protozoans and various types of plankton [5,12]. The traditional aquaculture ponds lack injection of carbon source, aeration mechanisms and thus harbors fewer and less diverse bacterial communities, as opposed to BFT. Small quantities of bacteria cannot form substantial flocs in the culture system. The sediment of traditional ponds accumulates higher quantities (49%) of nitrogenous wastes while the BFT pond sediments have less (5%) nitrogenous wastes (Fig. 2).

### **Establishment of BFT systems**

#### *Tank and pond set-up*

Low-cost BFT tanks can be fabricated using wire mesh framework with plastic lining to hold water (Fig. 3A) or purely concrete walls (Fig. 3B). For tanks (preferably 13 m diameter, 1.5 m height), a leveled space of 200–250 sq feet is required. In construction, place a 3 - 4 mm iron mesh on a two-liner brick framework laid on the floor. A slope of 20 - 22° from the border area is created towards the center of the tank where a central drainage pipe is mounted to facilitate drainage of excess sludge. Aeration pipe lines are then introduced in the tank leading to several openings for aeration stones. The tank is filled with water and shadowed using an appropriate material, if necessary.



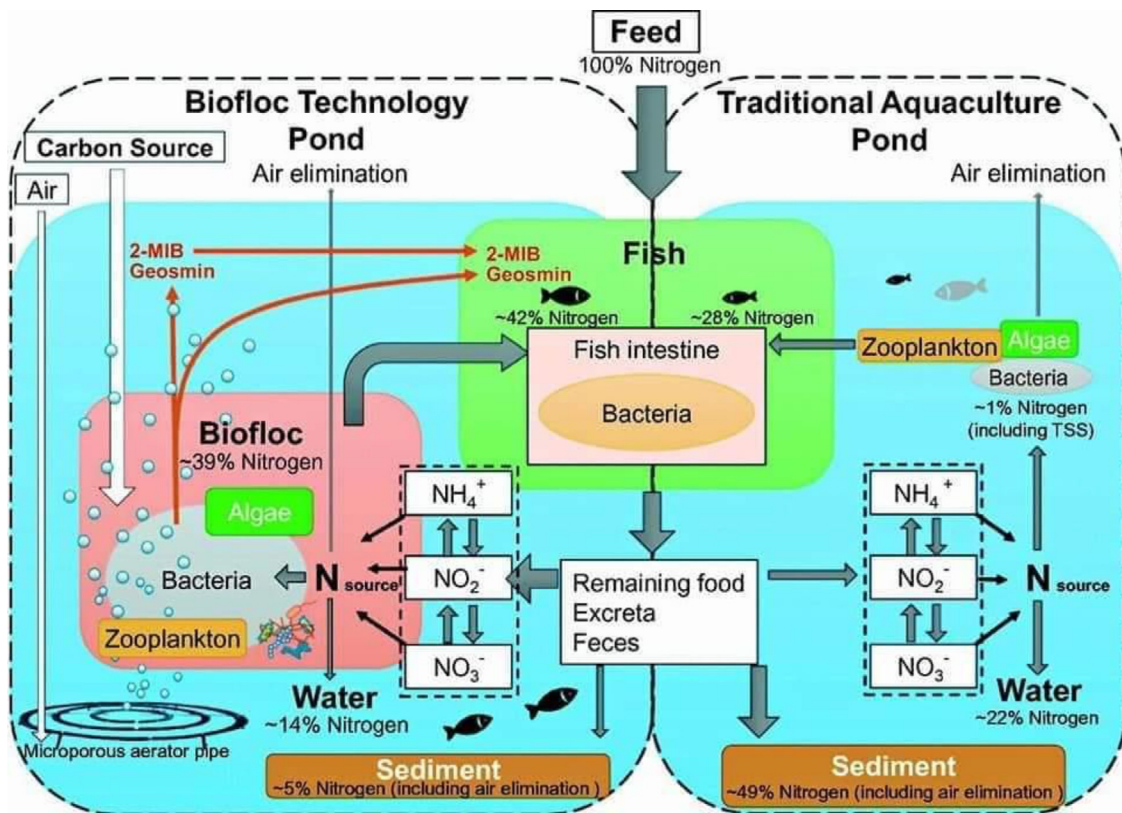


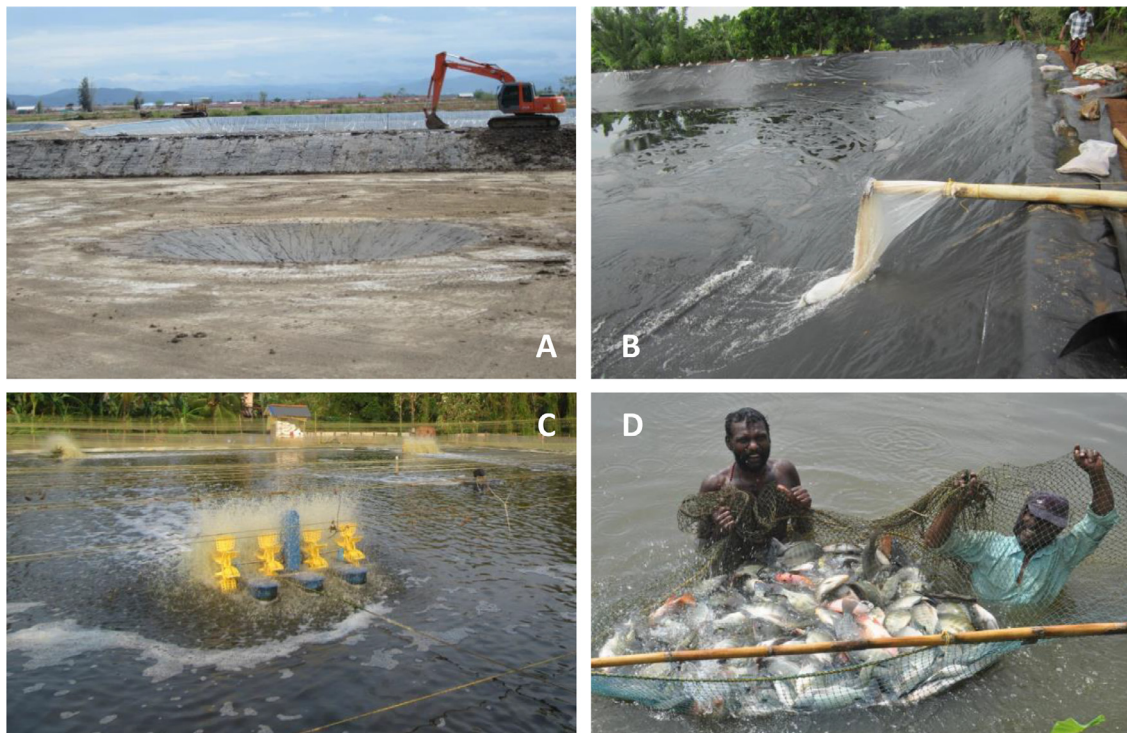
Fig. 2. Layout of biofloc technology pond and traditional aquaculture showing detailed biological processes (Accessed from <https://www.google.com/search?q=biofloc±system&client> on 20th February 2020).



Fig. 3. BFT tank construction process using wire mesh (A) and concrete material (B). Picture courtesy of Mr. Debtanu Barman (CEO Aqua-Doctor Solutions Co. Ltd, India).

*Pond set up*

Technically, traditional earthen ponds without liner material can be converted into biofloc systems to improve productivity. This can be achieved under the following condition: 1) the pond orientation should favor flow of wind so that the wind can facilitate effective water mixing. 2) At the inlet point, a 2 m<sup>3</sup> structure should be constructed preferably using bamboo trees, and regularly filled with biodegradable environmental wastes to generate bacteria, worms and plankton, which are direct food materials for fish. 3) The farmer needs to maintain higher carbon-nitrogen ratio by adding carbon source to promote proliferation of bacteria. Higher C/N ratio can also be achieved by feeding using feeds with lower crude protein (i.e. 18%).



**Fig. 4.** Texel HDPE Dame Liner lined Biofloc aquaculture unit at Goa, India with technical support of Texel Industries Limited. Layout and construction of high intensity BFT ponds (A), placement of HDPE lining and water filling (B), aeration mechanisms using paddlewheel (C) and harvesting using seine net (D) (Courtesy of Textel-Africa industries Ltd., Kenya).

Studies have shown that soil-bound microbes are important for the biological function of the BFT system [5]. However, there is need to inoculate probiotic bacterial community (especially the *Bacillus* spp) into the pond system and maintain an optimal dissolve oxygen level of  $5 - 8 \text{ mg l}^{-1}$  throughout in the pond. In the tropical countries, indoor BFT systems are only recommended in areas with very high rainfall throughout the year. Heavy rainfall can alter pond alkalinity and pH in outdoor systems. The optimal pH for BFT pond is  $7.5 - 8$  [61]. The magnitude of change of pH due to rains depend on the soil type in which acidic rains will lead to more alkaline condition in hard water area, and acidic condition in soft water area [61]. Nonetheless, outdoor BFT are preferred due to less cost of installation, and also, the fish benefit from photosynthetic algae (green bacteria), which are additional sources of nutrition to the cultured fish.

Highly intensive BFT ponds usually measuring  $2500 \text{ m}^2$  ( $50 \text{ m} \times 50 \text{ m} \times 1.5 \text{ m}$  depth) with 6 inch PVC central drainage pipe and HDPE lining require special expertise and high capital to establish. The pond is built above the ground level, with a top bund width of 1 m and bottom 5 m. Depth at side is 1.5 m and at center 2 m, that is sloping towards the center. In temperate areas where temperatures fall below  $20 \text{ }^\circ\text{C}$ , the pond should be under a poly house to maintain the required temperature of  $28 - 30 \text{ }^\circ\text{C}$ . For each pond of  $2500 \text{ m}^2$ , a toilet pond of  $300 \text{ m}^2$  is required for handling wastes and to reuse the waste water for irrigating other agriculture crops. The blue-cycling of nutrients and energy favors integration of BFT fish ponds to other agricultural crop production systems (Fig. 4).

#### Aeration

Intensive turbulent mixing is necessary for biofloc systems to maintain high dissolved oxygen levels and prevent settling of solids, respectively. The turbulence prevents anoxic dead ends that are lethal for the cultured organisms. Therefore, BFT systems need a properly planned layout of aerators (Fig. 4C), which should be regularly moved to avoid settlement of solid particles in areas with little or no current. Biofloc systems may require up to  $6 \text{ mg of oxygen l}^{-1} \text{ h}^{-1}$ , which translates to about 30 horsepower of aerators per hectare of pond [33]. Higher oxygen concentration should be maintained for 2 reasons: 1) the cultured fish and other planktonic organisms require oxygen for metabolism and, 2) the bacteria population requires oxygen to degrade wastes and continue proliferating. BFT ponds with low aeration mechanisms tend to accumulate organic wastes at the bottom and facilitates anoxic conditions. The anoxic zones (also called dead zones) are known to accumulate high ammonia. The accumulated ammonia could be harmful to the fish especially during water mixis. Farmers should be careful to avoid anoxic conditions or dead zones in the BFT systems.

### Floc preparation and pre-seeding

Flocs can be prepared in a 20 l bucket containing 300 g of molasses, 100 g of probiotic bacteria and 10 kg of sea salt (non-iodine). This mixture is aerated for 1 - 2 days before release into the BFT unit to create bioflocs (Aqua-Doctor Solutions Co. Ltd, India). The common bacteria used in BFT include *Lactobacillus* sp., *Bacillus* sp., *Enterococcus* sp., yeast and *Saccharomyces cerevisiae* [10]. The bacteria are commercially available. To accelerate flocculation, stabilization and quick production of probiotic and prebiotic microbes, it is recommended to pre-seed the culture water by adding some commercial (i.e. yoghurt) or homemade recipes. Farmers in some parts of Asia have perfected the use of locally available wheat pollard and Red Cap 48 as a homemade recipes. The receipt and flocs mixtures are placed in a drum and fermented for 2 days before use. The floc quantity should be confirmed before releasing into the culture system. Quantification of flocs can be done using a one-liter cylinder, in which settled flocs should be at least  $50 \text{ ml l}^{-1}$ .

Also, carbonized pond technology (CPT) can be used to enhance C/N ratio in ponds and promote productivity. In CPT, carbonized rice hull is mixed with goat or chicken manure and moisturized up to 60%. Lactic Acid Bacteria (LAB), which is a product of fermented rice wash and fresh cow or goat milk is added to the mixture and fermented for 2 weeks in an airtight container. After 2 weeks, the mixture is either broadcasted into the pond or put in into a sack and submerged into water as slow release fertilizer. The carbon provides energy to LABs, which decomposes all the excess feeds and inorganic matter (wastes) in the pond bottom and convert the wastes into natural feeds for the cultured animals. CPT has potential to improve pond productivity and profitability in both BFT and traditional fish ponds. However, more studies are recommended to establish the efficacy of broadcasted or submerged CPT in traditional and BFT ponds.

### Balancing carbon and nitrogen

Carbon sources can be used or better still, the protein content in the feeds can be reduced to achieve the optimum C/N ratio. However, it is recommended to investigate cost-effective and efficiency of carbon sources for use in BFT units. Maintaining C/N balance is the key to controlling nitrogen toxicity in BFT units. However, controlling C/N ratio is normally a challenging task in successful implementation of BFT. Sufficient availability of carbon at the beginning of farming cycle is a sure method of preventing ammonia peaks. The carbon enables heterotrophic bacteria to multiply and consume ammonia present in the water, thus maintaining low ammonia concentration. It is advisable to use carbon sources and feed mixtures with a C/N ratio above 10 [6,25]. Since most fish feeds have a C/N ratio of 9 or 10, additional inputs are needed to raise this ratio to between 12 and 15 [16]. Calculation of optimum C/N ratio for specific BFT is summarized in the following example.

- i. Assuming the quantity of fish meal diet used = 0.015 kg, crude protein (CP) of the diet = 15%, and Carbohydrate = 14%. Assuming carbon source used is wheat, whose CP = 10%, and carbohydrate = 76%.
- ii. Total Ammonium Nitrogen (TAN) produced: = Diet used (kg)  $\times$  CP  $\times$  0.144<sup>a</sup>) = 0.015 kg  $\times$  0.15  $\times$  0.144 = **3.24  $\times 10^{-4}$  kg**
- iii. Carbon requirement = [TAN  $\times$  15.17<sup>b</sup>] - [Diet used (kg)  $\times$  % carbon in feed] = [0.000324 kg  $\times$  15.17] - [0.015  $\times$  0.14] = **2.81  $\times 10^{-3}$  kg**
- iv. Carbon constant = Carbon in wheat - [CP of wheat  $\times$  0.16<sup>c</sup>  $\times$  15.17] = **0.52**
- v. Carbohydrate needed = carbon requirement / carbon constant = **5.44  $\times 10^{-3}$  kg**
- vi. C/N ratio = carbohydrate needed / TAN = **16:1**

**Note:** <sup>a</sup>Ebeling constant [25]; <sup>b</sup>15.17 = the constant representing the required carbohydrate needed to eliminate 1 unit of nitrogen [25]; <sup>c</sup>0.16 = the constant in the ammonia generation equation assumes that protein is 16% nitrogen [25].

### Species selection and stocking densities in BFT

BFT culture species should be wholly or partially filter feeders to exploit biofloc and detritus particles. Both shrimp and tilapia are excellent candidates because of their ability to gobble up bioflocs, thereby improving the feeding efficiency and feed conversion ratio (FCR). It is recommended to stock tilapia seeds at  $12.5 \text{ g l}^{-1}$  in tank-based BFT. The optimal carrying capacity of a 10,000 l BFT tank is estimated at  $0.4 \text{ kg l}^{-1}$  (Aqua-doctor solutions, India). The fish should be fed with feeds of high buoyancy and crude protein content of between 20 - 18%, if the BFT is working efficiently. Due to high productivity, farmers are sometimes tempted to use higher stocking densities. However, the carrying capacity of BFT should not be exceeded as this may compromise both the health of the cultured animals and productivity of the units.

### Biofloc monitoring and maintenance

The minimum quantity of floc required in a BFT system depends on the intensity and size of the culture system. Biofloc concentration of  $15 \text{ ml l}^{-1}$  (as settleable solids) should be maintained by adding grain pellets (18% crude protein - CP) and 300 g molasses, to maintain a C/N ratio greater than 15 [33]. Under favorable environmental conditions, the bioflocs numbers increase fivefold  $\text{ml}^{-1}$  within 2 - 3 weeks to reach over 10 billion bacteria cells  $\text{cm}^{-3}$  with over 2000 bacterial species [6]. However, whenever floc levels exceeded  $500 \text{ mg l}^{-1}$  sludge should be reduced by removing 10 - 15% of bottom water through the drainage system [7]. The sludge can also be removed when ammonia level is higher than  $1 \text{ mg l}^{-1}$  or



when there is bad smell with lots of algal forms. Higher ammonia level in the BFT system can be reduced through addition of carbon source (molasses), which facilitates proliferation of bacteria to consume excess ammonia. Even though literature is not particular on the estimated biofloc peak time, perhaps due to other extraneous factors, it is probable that biofloc peak time is synchronized with the time at which maximum flocculation occurs. Biofloc sampling should be done every 15 - 20 days to monitor biofloc types (i.e. green algae and brown bacteria) and densities.

It is easier to monitor growth of biofloc using Imhoff cones developed by Avnimelech [6]. The Imhoff cone is used to collect water samples at a depth of about 15 cm to 25 cm, preferably in the late morning and left to settle for about 20 min. The floc solids stick to the sides of the cone and are easily counted. Too much flocs can suffocate the cultured fish through clogging of gills. During biofloc monitoring, water quality parameters especially dissolved oxygen, pH and ammonia levels should also be checked. The water quality are also indicators of the health of the BFT.

### Applications of BFT in aquaculture

#### a) Hatchery, larviculture and live food production

The increasing aquaculture activities in tropical countries has created huge demand for fish seeds and feeds. Successful larviculture (fish seed production) require sufficient live food resources such as rotifers, copepods, cladocerans and *Artemia* nauplii as starter food ('baby' fish food) in hatcheries [48]. The preference of live food for fry and fingerling culture is attributed to the small size (for ease of ingestion by fish larvae), high digestibility, palatability and nutritional completeness [51]. The conventional method of live food production involves the use of high density microalgal pastes, whose culture protocol is expensive, fragile and stressful [50]. Therefore, new protocols have been developed [49] for mass production of live food resources using BFT. High densities ( $> 1200$  individuals  $\text{ml}^{-1}$ ) of single strains of rotifers i.e. *Brachionus rotundiformis* [50] and *Proales similis* [36] were produced using fish wastes diet (FWD) in BFT units. In another study, high densities of mixed zooplankton communities i.e. rotifers, copepods and cladocerans were obtained using BFT under outdoor conditions [48]. Therefore, BFT appears to be a major leap toward making pre-planning of fish seedling production in aquaculture facilities feasible throughout the year.

Besides promoting faster population density of live food resources, the bioflocs can be used as nutritional supplements due to the higher nutritional factors. Since studies have established the presence of essential PUFAs in biofloc paste [50], the paste can be used as an enrichment emulsion to live food resources (rotifers, copepods, cladocerans and *Artemia*) and larval fish, thus reduce or eliminate the use of expensive commercial enrichment emulsions. Currently, live food resources (algae, rotifers, *Artemia*) are first supplemented with expensive commercial emulsions in Asian and European hatcheries. The biofloc emulsion could be better than other homemade emulsions (of fish oil and yolk sac), which have short shelf life that limits their application in aquaculture. The biofloc PUFAs are more protected against oxidation, and provide a variety of other natural nutrients that meet the species-specific nutritional requirements of the cultured fishes [32].

#### a) Proteins, lipids and amino acids for grow-out and broodstock

The potential effect of BFT in breeding of fish and shellfish is largely unknown. Studies have shown the superior effects of biofloc nutrition for first stages of broodstock's gonads formation and ovary development, thus enhances spawning performance [29]. The constant supply of biofloc nutrients promotes better sexual tissue formation and reproduction activities in brooders [28]. Indeed, BFT has been successfully applied in nursery phase for different shrimp species such as *Litopenaeus vannamei* [54], *Penaes monodon* [2] and *Farfantepenaeus* sp [28]. Bioflocs caused a 50% increase in weight and 80% in final biomass in *F. paulensis* early postlarval stage compared to conventional clear-water system [28]. In addition, the survival rate of 56 - 100% was achieved for *L. vannamei* [54]. For grow-out tilapia, *Oreochromis niloticus*. Better growth rate of fish has been reported in BFT tanks using 24% CP than fish fed with 35% CP in non-BFT [7]. The higher growth rate could be linked to the nutritious bioactive compounds in BFT [58].

The production of finfish species using BFT has been generally successful especially in the Asian continent (see reviews in [46]). Many authors consider BFT as a more sustainable and environmentally friendly aquaculture system both in laboratory studies and commercial scale for various aquaculture species including tilapia species [5,7,18], *Cyprinus carpio* [45] and catfish, *Clarias gariepinus* [67]. Nonetheless, the tilapines are the most farmed fin fish species using the BFT, due to the filter feeding and grazing strategies on suspended and attached bioflocs and periphytons [1]. Indeed, studies have recorded a growth rate of 0.27 to 0.29 / day [18] and yield ranging from 150 to 300 tons / ha of tilapia [5] or higher in all males [65].

Biofloc materials are nutritionally superior to provide essential food items for aquaculture [38]. On a dry-weight basis, biofloc contains 12 - 50% protein, 0.5 - 41% lipids 14 - 59% carbohydrates and 3 - 61% ash [7]. BFT is also credited for improvement of the nutritional value of the live food individuals. Ogello et al., [50] detected up to 0.35 and 0.39  $\text{mg g}^{-1}$  of DHA and EPA, respectively in the rotifers cultured under BFT conditions. The DHA and EPA are lipids that are known to facilitate survival and faster growth of fish larvae. Bioflocs in marine waters are rich in the amino acids e.g. valine, lysine, leucine, phenylalanine, and threonine, but can be deficient in arginine, methionine, and cysteine, and vitamin C [14]. The variations in these figures could be caused by differences in composition between new and old floc aggregates [27]. It is important to maintain old biofloc community because of efficient heterotrophic processes [66]. Total ammonium nitrogen (TAN) concentrations can be effectively controlled by either heterotrophic assimilation or autotrophic nitrification that helps maintain acceptable TAN ranges even at high stocking densities [66]. Mixed type of biofloc i.e. green and brown bioflocs

is more beneficial in high density zero-exchange culture systems than systems dominated by heterotrophic bacteria only. Mixed biofloc systems reduce production costs by reducing organic carbon input and oxygen use [66].

Tilapia may consume biofloc accounting for up to 50% of the regular feed ration (assuming daily feeding of 2% body weight) [14]. Assuming that only 25% of feed is consumed by fish and 75% of feed is not consumed, this amount, together with fish bio-wastes, can be recycled into bioflocs, thus reducing cost of feed by 30% as a feed is literally 'eaten twice' i.e. as feed and as biofloc [3]. In traditional farming systems, only about 25% of the protein content of feeds are actually utilized by farmed species. By converting ammonium into microbial proteins that can be consumed by filter feeders, biofloc systems are able to double this figure, thus reducing production cost significantly. In case of complete conversion into bioflocs and that the fish will consume 25% of the bioflocs, the protein uptake through the bioflocs will be around 0.056 kg protein per kg fish multiplied by amount of conventional feed added [19]. The direct and indirect uptake of commercial feed, leads to a total uptake of the feed by the fish by a factor of 1.75, which is higher than in tilapia cultured using non-BFT units [19]. However, it should be noted that biofloc alone is not sufficient to guarantee the level of growth and survival required by high-density fish/shrimp culture. Thus, supplemental formulated feed is still required to satisfy the nutritional requirements of the fish/shrimp cultured in BFT. Bioflocs are highly proteaceous. Therefore, additional carbohydrates are needed to maintain optimal protein-carbohydrate ratio, which is an important parameter for growth of somatic cells in fish.

#### a) **Biofloc meal**

Bioflocs can be used as substitute to fish meal (FM), which is an expensive and scarce protein source in fish feed industry. The biofloc paste can be harvested and processed as an ingredient for formulating compounded feed in local hatcheries. This could provide a positive leap towards low-cost fish feed formulation in cottage industries in the developing countries. Initiatives geared towards developing nutrition strategies such as bioflocs and periphyton that maximizes the contribution of natural and supplemental feeds in culture facilities would help to expand aquaculture production. In this respect, it is important to have a predominance of easily digestible bacteria containing energy rich compounds promoting BFT in aquaculture sector. However, the idea of biofloc meal still require in-depth scientific study.

#### a) **Factors influencing quality and quantity of bioflocs in BFT**

Studies have shown that biofloc nutrition is a function of C/N ratio, dietary protein level, available light intensity, and environmental culture conditions [14,27]. The C/N ratio above 15 coupled with minimal water exchange produces high quality and quantity of bioflocs that promotes survival, growth, and immune activity of shrimps [52]. The types of carbon source also influences the quality of biofloc. For example, glycerol-based bioflocs has higher protein, vitamin C and n-6 fatty acid content than glucose-based bioflocs [15]. On the other hand, other studies have reported highest protein content in the Glucose-based bioflocs than starch- and glycogen- based bioflocs [64]. The essential and nonessential amino acid contents were similar in glucose- and glycogen-based bioflocs, but were higher in starch-based bioflocs [64]. Lower dietary protein level provides room for more starch and promotes biofloc growth under zero-water exchange systems [42]. Light intensity is known to promote development of green biofloc through photosynthetic process. The green bioflocs contain additional nutrition that benefit cultured fish. Manipulation of BFT environmental conditions is necessary to maintain stability of bioflocs. For example, highly acidic conditions would reduce the population of bioflocs.

#### *Bioflocs and water quality in aquaculture*

The major water quality problems in intensive aquaculture systems is the accumulation of toxic nitrogenous compounds. BFT was first developed to remove ammonia-nitrogen in aquaculture systems by utilizing natural processes. This process include photoautotrophic removal by algae, autotrophic bacterial conversion of ammonia-nitrogen to nitrate-nitrogen, and heterotrophic bacterial conversion of ammonia-nitrogen directly to microbial biomass [25]. In BFT, the processes are accelerated by developing and maintaining dense heterotrophic microbial flocs (Azim et al. 2003) though addition of carbohydrates [17,18].

The attached microbial community (periphyton) control water quality in aquatic system through entrapping organic detritus, photosynthetic removal of nutrients and, autotrophic processes [62]. The autotrophic processes of periphytons are capable of assimilating  $0.2 \text{ g N m}^{-2} \text{ day}^{-1}$  [8].

#### *Bioflocs for biosecurity*

Today, closed aquaculture systems are safer for biosecurity reasons. The closed systems have environmental and marketing advantages over conventional extensive and semi intensive systems [53]. The fact that water is re-used reduces chances for introduction of external pathogens into the system. Bacterial flocs are normally controlled by cell-to-cell communication through signal molecules (i.e. N-acyl-homoserine lactones or AHL's in Gram-negative bacteria and peptides in Gram positive bacteria) in a process called quorum sensing [43]. Quorum sensing occurs when a certain cell density is reached [39] and regulates the expression of genes encoding for the production of lytic enzymes and toxins in biofilms [21]. Reduction of the toxic biofilms require disruption of cell-to-cell communication in flocs through inactivation of the signaling molecules [21]. Some bacterial communities control virulence factor expression by quorum sensing through natural disruption of cell-cell



communications [21], thus protect cultured animals from pathogenic bacterial infections [16]. BFT appears to offer alternative natural immunoprophylactic agents for biosecurity than artificial antibiotics, which attract ecological consequences [21,22].

Within the BFT, alternating periods of excess carbon and limitations for the microorganisms in the water triggers the accumulation of poly  $\beta$ -hydroxybutyrate (PHB), which is a bacterial storage compound [24]. Under limited essential nutrients like nitrogen with the presence of excess carbon source, PHB is produced and degraded in the gut of culturing organism [23]. PHB have an antibacterial activity and acts as a preventive curator against vibrios [23]. The mechanism behind the probiotic effect of biofloc is the competition between microorganisms in the floc and pathogen for space and some essential nutrients, and block multiplication of pathogenic bacteria.

#### *Bioflocs sink atmospheric carbon*

Under outdoor BFT systems, the flocs contain phytoplankton consisting of Chlorophyta (green algae), Dynophyta (dinoflagellates), Chrysophyta (golden-brown algae), and Cyanophyta (cyanobacteria) [55]. The phytoplankton species utilize carbon through photosynthesis, hence act as carbon sinks [34]. The phytoplankton convert dissolved carbon dioxide (CO<sub>2</sub>) to oxygen (O<sub>2</sub>) and energy (glucose) efficiently, thus natural carbon attractors [41]. Study on the carbon sink by biofloc phytoplankton from species *Oocystis* sp. (Chlorophyta) and *Chroococcus* sp. (Cyanophyta) revealed that *Chroococcus* sp. sequestered higher amount of carbon dioxide (CO<sub>2</sub>) as compared to the amount of carbon can be sequestered by *Oocystis* sp [41].

#### *Cost-benefit analysis of BFT*

The application of BFT in aquaculture is an economically viable technology [57]. The viability is linked to reduced cost of feeding, increased growth rate and high survival rates [4]. In addition, with BFT, the cost of organic and / or inorganic fertilizers is eliminated [4]. As discussed earlier, fish feed always account for the highest production costs in aquaculture. However, biofloc systems consume less feed compared to earthen ponds. Due to faster growth rate and high survival in BFT, it would suffice that BFT consume higher quantity of feeds. Despite faster increase in biomass in the biofloc system, any rise in feed cost is quickly accounted for in the higher profit margins, which is still superior to conventional earthen pond systems [57]. In addition, BFT excludes the cost of organic and inorganic fertilizers but includes the cost of carbon source only, which is much cheaper. Studies have reported reduced culture period coupled with higher growth and survival in BFT, thus making it more profitable over the other systems [4]. Studies of Sontakke and Haridas [57] confirmed that the nursery rearing of milkfish using BFT provided more economic returns and ensured continuous supply of fingerlings for grow-out culture operation year-round.

#### *Challenges and limitations of BFT*

Despite the many advantages of BFT, there are limiting factors that impede effective production. Even though high bacteria population is encouraged, the multiplication of the heterotrophic bacteria may cause excessive turbidity in the system, which can cause clogging in shrimps and fish gills of fish species, especially those not adaptable to growing in turbid waters. BFT requires increased use of energy for mixing and aeration. Stable energy is one of the challenges in the developing countries due to frequent power blackout. The BFT requires starter period of about 2 weeks during which the microbes develop. The starter period may lengthen production cycles. There is need for supplementation of alkalinity to maintain conducive conditions for biofloc proliferation. High microbial population imbalance (i.e. very low bacteria) may increase chances of pollution from accumulation of nitrate compounds. Also, the sunlight-exposed systems may suffer from inconsistent and seasonal performance (i.e. production may have to be stopped during heavy rain seasons). Other challenges include the possibility of over performance of filamentous bioflocs, which may cause floc bulking, system instability and incomplete nitrogen removal.

#### *Conclusions and recommendations*

There is no doubt that bacteria is running the food production industry. This article has demonstrated the potential of BFT to achieve climate-smart aquaculture objectives of a) increasing fish productivity, b) enhancing resilience of fish production systems, and c) efficient use of energy, water, land, and reduction of green-house-gas (GHG) emissions. BFT offer promising aqua-preneur opportunities for both smallholder populations and big companies. BFT benefit the fish whose feeding behavior include filter feeding and scraping, thus selection of fish species with these characteristics is critical. The BFT also maintains good water quality in situ, manufactures food, and ensures biosecurity. However, the effects of physico-chemical parameters on flocculation processes in BFT needs depth investigation. The biosecurity aspect is critical for adoption of BFT in larval rearing (hatchery) and grow-out stages of fish and shrimps, to improve production and increase wealth.

There is need to promote the adoption of BFT through capacity building initiatives and pilot demonstrations. The shifting of microbial population and biofloc monitoring technique should be studied to provide a good understanding of the microscopic mechanisms that are involved in bio-flocculation. Succession patterns in BFT needs special scrutiny to unravel scientific myths surrounding probiotic organisms in the microbial community of the bioflocs. Indeed this could solve the

problems of antibiotic use in aquaculture sector. The biofloc paste as potential biofloc meal should be investigated as potential media for supplementing fish feeds towards reducing the need for expensive commercial emulsions. This could be potentially viable for reducing overreliance on fish meal, which is already facing overexploitation challenges. Aquaculture policies should be tailored to stimulate innovative aquaculture techniques for sustainable production and livelihood security of the fish farmers and the value chain players.

## Declaration of Competing Interest

None

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