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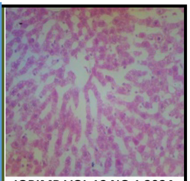

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Histoarchitecture of albino rat's liver

# Biofloc Technology as a Sustainable Alternative for Managing Aquaculture Wastewater

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**ABSTRACT**

The rapid expansion of the aquaculture industry has resulted in a significant increase in wastes generated from fish farming systems, including undigested feed, recycled nitrogen, and other resources. Consequently, the rise in fish production has slowed, as aqua-preneurs are unable to achieve maximum output and profitability. Bio-floc technology (BFT) is a cutting-edge system with enormous potential for application in fish farming. BFT works on the premise of converting the solid waste generated from spilt or undigested feed by the fish or from feces in the form of solid and dissolved waste mainly carbon, nitrogen ammonium, and phosphorus to probiotic or nutritious protein for fish consumption. Using the technology, bacteria may convert bio-waste into edible nutrients for farm animals. Adoption of BFT can offer biosecurity measures that can limit the use of antibiotics and chemicals that have been outlawed by WHO/EU owing to rising environmental issues associated with their application. This study examines and presents BFT as a sustainable alternative for managing aquaculture wastewater and in-house provision of nutrients for cultured animals. The coverage of this study includes an overview of Aquaculture wastewater treatment, aquaculture wastewater as media for Biofloc formation, pathogens, probiotics, and potential for biofloc formation in aquaculture systems and BFT as a sustainable means of nutrition in aquaculture. The cost-effectiveness and potential of BFT in treating aquaculture wastewater are also critically discussed. The present study highlights the importance of harnessing BFT for cost-effective aquaculture production and alternative means of managing aquaculture wastewater.

## INTRODUCTION

The continuously increasing demand for fish and growth of the aquaculture industry arising from the continuous increase in global human population has placed aquaculture systems as one of the major contributors to environmental pollution, predominantly in aquatic milieus. The high demand for seafood by the rapidly increasing human population is matched with corresponding rapid growth in aquaculture industry. This has led to increased environmental pollution from waste generated from aquaculture systems along coastal area and even in residential areas with artificial aquaculture set up [1]. As a result, fish farming accounts for over a third of all fish produced for human consumption, with aquaculture contributing 114.5 million tons of fish consumed globally in 2018. Consequently, as aquaculture has grown, so has the number of fish and feed produced per unit of culture area, resulting in increased waste output from the culture-producing system [2]. Thus, the aquaculture system tends to generate a lot of wastewaters containing total nitrogen, phosphorus, and suspended solids [3].

Naturally, Aquaculture, like any other food industry, requires more inputs for higher production. In such systems, waste is generated as by-products or as unutilized inputs [4]. Solid and dissolved waste, mostly carbon, nitrogen, and phosphorus, are the two types of waste created. The solid waste comes from spilled or undigested feed and excrement, whereas the dissolved waste comes from fish excreting metabolites (through the gills or urine) [3]. Aggregation of such pollutants degrades water quality and can lead to increased diseases in the fish as well as the consumers [5]. Thus, the reuse of such water may not be advised.

Aquaculture wastewater has been implicated as a major source of eutrophication in many lakes and rivers [5]. Hence, the need to recycle and treat wastewater and improve the technologies involved in the recycling of the waste need to be renewed and made cost-effective. Various methods have been proposed to treat wastewater from different aquaculture systems such as ecological ditches, biofilms, biofilters, hydrophytes, microalgae bioreactors and bioflocculant [5, 6, 7]. The multiple means employed in circulated aquaculture wastewater include plants, substrate, fauna and microbes at different stages of purification. This has led to the synchronism of physical, chemical, and biological processes, which removes surplus nutrients [8].

In natural or constructed aquatic systems, flocculation is defined as a process in which bigger complex aggregates of inorganic and organic material develop. Bioflocculants have gained biotechnological and scientific recognition due to their biodegradability and absence of secondary pollutants from their biodegraded intermediates in the 21st century [9]. Bioflocculants are natural organic macromolecular substances produced by microorganisms capable of flocculating suspended solids, colloidal solids and cells [10]. This same bio flocculant production potential of microorganisms has recently been harnessed for aquaculture wastewater treatment in biofloc technology.

The strong demand per person that has coincided with the world's population growth has sparked the need to enhance aquaculture production. Meanwhile, the industry's environmental impact and the restricted availability of natural resources significantly challenge the growth of a sustainable aquaculture sector [11]. Owing to these constraints, systems that, despite their high productivity and profitability, use less space, energy, water, and eventually capital while having a smaller environmental impact should be the focus of developing the sustainable aquaculture industry [12]. The biofloc technology have concurrent advantage of recycling the aquaculture wastewater and converting the unutilized feeds and other wastes in the culture system to rich proteins and nutritious substances for the fish and can thus reduce the cost of feed incurred by the fish farmers. The present study presents Biofloc technology as a sustainable alternative for remediating aquaculture wastewater.

## Composition of aquaculture wastewater

### Nitrogen

Despite the fact that fish consume a high fraction of digestible protein as a source of energy and create huge amounts of nitrogenous metabolites, various species of fish utilize proteins in different ways [13]. Most fishes produce ammonia ($NH_3$) as a byproduct of protein metabolism, but certain species may also excrete urea as a large amount of nitrogenous waste. Creatine, creatinine, trimethylamine (TMA), trimethylamine oxide (TMAO), and uric acid are some of the other nitrogen (N) waste products explored in fish study [13]. Fish produce $NH_3$ mostly due to their protein consumption and metabolic control, both species-specific and regulated by waterborne $NH_3$ levels.

The majority of fish are fed protein-rich diets, and ammonia is the cheapest way to remove nitrogen created by deamination of amino acids from a metabolic standpoint [13]. According to these scientists, the variety of N content in meals explains the variability in the quantity of N excreted. Total ammonia nitrogen (TAN) excretion is higher in freshwater species than in marine ones. Ammonia is usually poisonous to fish and can stunt their development. Furthermore, a high occurrence of glutamic and aspartic acids has been discovered in the quantity of free amino acids present in fish tissue, which play an important role in the processes that localize and transfer extremely hazardous substances like ammonia that are created during amino acid deamination.

The quantity of ammonia and urea excreted in fish species might be connected to their nutrition, according to Lazzari and Baldisserotto [13]. This is crucial for highly intensive operations in fish culturing since the metabolism of protein in part affects how effective a specific nutritional program is. The type of fish species and the development phase determine the amount of protein fish consume. A significant association exists between the protein content available in the diet and the amount of ammonia generated. In general terms, about 16 percent of nitrogen is contained in proteins (14). Ammonia ($NH_3$) excretion is often greater in fish meals with protein sources that are low in amino acids [13].

*Phosphorous*

Phosphorus (P) is a significant mineral in nucleic acid and cell membranes, as well as the principal constituent of skeletal tissue structural components and a direct participant in energy processes [14]. Phosphorus supplemented diet is very important because of the low content of phosphorus available in water as fish absorbs them. Absorption of inorganic P supplemented into fish diet occurs in the gut as well as in the pyloric caeca in rainbow trout at 10 and 90 % respectively. If the food provides adequate P levels, the pyloric caeca intake accounts for roughly 92 percent of total inorganic P uptake with no inhibition of absorption.

On digested samples, total Phosphorus (TP) is tested to reveal the total quantity of P present, which includes both dissolved and particulate materials. Total phosphorus concentrations in aquaculture source waters vary widely, just as they do with TN; aquaculture activities typically elevate the concentration substantially over the average incoming level. The levels of TP in aquaculture raceways and pond effluent are hundreds to thousands of times lower than raw manures and RAS sludge. In general, the amount of phosphorus in aquaculture pond or raceway effluent is equivalent to, or less than, the amount of phosphorus in certain storm waters or runoff scenarios [15].

*Solid waste*

Solid waste originates from spilled or even undigested food materials excreted by the fish while dissolved waste materials result from excreted metabolic materials or products through the fish gill or urine [2]. Solid wastes are further grouped into suspendable solid and settleable solid (SS), the suspendable solid are filterable solid material suspended in water column whereas SS are solid material that settles from the water column, total suspended solid (TSS) is a measure of weight of suspended solid matters which can be filtered while settleable solids (SS), measures the volume or weight of material that will settle from the water column in an hour (15). TSS and SS is a representation of potentially recoverable material from effluent through prolonged settling or filtration treatments. The nitrogen, phosphorus and solid content of some aquaculture wastewaters reported are listed in **Table 1**.

**Table 1**. Nitrogen, phosphorus and solid content of aquaculture wastewater per ton of some species.

| Fish species | Nitrogen | Phosphorus | Solid waste | Reference |
|---|---|---|---|---|
| Rainbow trout (*Oncorhynchus mykiss*) | 57 | 13 | 30 | [16] |
| Barramundi (*Lates calcarifer*) | 101.7 | 15.4 | 302.3 | [17] |
| Mixed culture fish | 64.62 | 32.5 | - | [18] |
| Nile tilapia (*Oreochromis niloticus*, L.) | 44.95 | 14.26 | 1040.63 | [19] |
| Tilapia hybrid | 77.4 | 84 | 18.8 | [20] |
| Yellow catfish | 2.99 | 0.23 | - | [21] |
| Mixed fish culture | 1.18 | 0.07 | - | [22] |

### General overview of aquaculture wastewater treatment

Through the centuries, a lot of research has shown various means of treating aquaculture wastewater. These includes ecological ditches, microalgae bioreactors and bioflocculant hydrophytes [3, 7] biofilters, Aquaponics [23] for different aquaculture systems. The "ecological ditch" is an operative means of alleviating non-point-source effluence from agricultural source. Because Aquaculture wastewater is composed of nitrogen and phosphorus that can be influenced by a few factors and thus a relative variance of the natural drainage ditch, setting up an eco-ditch for aquaculture wastewater treatment involves ecological alteration, which will include plants, microbes, soil and appropriate substrates.

Bio-filters with suitable media can be used for treating aquaculture effluents that can subsequently recirculated through recirculating aquaculture systems. The microorganism attached to the filters (bacteria) tends to consume suspended organic solids to aid their metabolism. Milko *et al.* [24] have indicated bio-filters packed with media filter containing cross-link structures and have high bed porosity, accompanied by a bio-filter packaged with a surface roughness in the media yields optimal results. Other filtering methods include mechanical filters, gravity filters and pressure filters.

Aquaponics refers to food production approach through combined traditional aquaculture techniques and recirculating aquaculture system. It involves cultivation of aquatic animals like snails, fish, crayfish or prawns along with hydroponics in the same tank. The plant is cultivated in water in a symbiotic relationship with the aquatic animals. In traditional culture systems, wastes from the cultured animals accrue in the water and increases water toxicity. To overcome this through aquaponic system, the water from the culture system is conveyed to hydroponic, the waste products in the water is then degraded by bacteria to release nitrate and ammonium that can be assimilated by the plant as nutrients. The detoxified water is then pump back to the aquaculture system [23].

The Sedimentation system tends to separate unused feed and feces. Fish feces and water density are closely similar, thus leading to low settling rate of solid particles. Suspended solids also travel through the tank due to turbulence created by fish swimming and water speed which leads to feces in constants suspension. Sedimentation can be achieved through simple sedimentation, channels, Centrifugal concentrators (Hydro clones or cones) as detailed in the work of Ozigbo *et al.* [25].

The work of Yeo *et al.,* [15] showed different aquaculture systems and the methods employed in treating wastewater. The author indicated pond culture being practice in North Central Region (NCR) of the United States, some major fish producing countries in Africa also practice pond culture, but such culture system use is declining in Africa as a result of adopting intensive systems of fish culture [25, 26, 27]. Pond culture system majorly depends on the internal natural processes for the purification of the water.

Flow-through system, also known as raceway system, involves the continuous flow of water to keep the necessary water quality standards needed for fish culture at high stocking density [28]. According to Anetekhai [28], in Nigeria the system is mostly employed in hatcheries production for Catfish fingerlings. The methods adopted for treatment of flow-through systems are employed by the Idaho Salmonid industry [15].

RAS is a closed, high-intensity system in which water is pumped into fish tanks via mechanical and biological water filtering devices before being recycled [25, 28]. RAS, according to Chen *et al*., [29], have an essential trait of recycling all or a large amount of their raising water several times. RAS often include various parts that collect as well as eliminate solid waste quickly, oxygenate water, and prevent harmful metabolite build-up. Chen *et al*., [29], Yeo *et al.,* [15], highlighted the adopted method for RAS waste treatment as microbe biofilter put in place to convert dissolved nitrogenous toxic metabolic waste to non-toxic states.

Recirculating Aquaculture Systems (RAS) are typically closed devices that include fish aquaria as well as filtration and water treatment systems. Tanks are used to house the fish, and the water is changed on a regular basis to ensure that they have the best possible growth circumstances, especially in terms of oxygen. Water is pumped into the tanks via biological and mechanical filtering processes before being returned. However, because it is difficult to assure that all waste materials are transformed or eliminated by the treatment process, not all water is completely exchanged. Depending on stocking and feeding rates, most culturing systems propose a daily water exchange rate of 20-66 percent. RAS takes up extremely little space, allowing growers to keep fish in large numbers and get substantial yields per unit area. Because these systems are so demanding, they need a high degree of stock, apparatus and water quality observation. They give a consistent and predictable environment for fish to develop in. RAS may be costly to acquire and run, thus it's typically only practical for high-value species farming [30].

Bioremediation is the process of removing hazardous contaminants using living organisms (actinomycetes, bacteria, fungus, cyanobacteria, and occasionally plants). The organisms might be found in nature or cultivated in a laboratory. Depending on the species grown and the farming technique used, the quality and amount of components in aquaculture effluent varies. Residual food and feces, metabolic by-products, residues of biocides and biostats, and fertilizer generated wastes have all been identified in aquaculture wastewater [31]. The use of microbes/enzymes to the ponds, termed as "bioremediation," is a recent application to enhance water quality in aquaculture. Bioremediators or bioremediating agents are macro and microorganisms or their products that are used as additives to enhance water quality. Bioremediation, which includes the employment of microorganisms in ponds to increase mineralization of organic matter and get rid of undesired waste products, is another growing attempt being undertaken to improve water quality in aquaculture [25, 28].

Efficient bioremediation requires the presence of bacteria capable of successfully removing carbonaceous pollutants from water. As a result, the microorganisms' capacity to grow quickly and have strong enzymatic activity will be very beneficial. *Bacillus subtilis, Bacillus licheniformes, Bacillus cereus, Bacillus coagulans*, and the species *Phenibacillus polymyxa* are all good examples of bacteria that may bioremediate organic waste. Generally, three interconnected pathways are involve in converting nitrogen in biofloc technology namely photoautotrophic assimilation by algae, chemoautotrophic nitrification of noxious ammonia to less noxious nitrate by bacteria and heterotrophic direct bacteria integration of ammonia-nitrogen to biomass [32].

Geng, *et al*., [33] worked on the use of mussels, microalgae and bacteria in the treatment of aquaculture wastewater. The research made use of mussels (*Hyriopsis cumingii*), microalgae (*Chlorella vulgaris*) and bacteria (*Bacillus subtilis* and *Bacillus licheniformis*), *Chlorella vulgaris* biomass absorbed the contaminants in wastewater, which Hyriopsis cumingii continuously filtered out. Meanwhile, *Bacillus subtilis* and *Bacillus licheniformis* increased the mussels' ability to digest their food.. The study indicated the use of mussels/microalgae/bacteria as the best sustainable and efficient characteristics of aquaculture wastewater bioremediation. The work of Muskan, *et al.,* [34] also show the positive use of microalgae as bioremediation of water contaminated by pesticides.

A comparative study using mussels, mussels/microalgae, mussels/bacteria system, and the treatment ability of mussels/microalgae/bacteria indicated 94.67% of $NH_3$-N, 92.89% of TP and 77.78% of chemical oxygen demand (COD) decreased after 6 days of action [33].

**Aquaculture wastewater as a suitable media for biofloc formation**

Aquaculture or Aquafarming is a method of cultivating aquatic animals such as crustaceans, molluscs, finfish, aquatic plants; mainly algae, lotus, using brackish water, sea water, inland saline water and fresh water. The report according to the FAO (aquaculture production in 2018 was 114.5 million tons, comprising 26,000 tons of seashell and pearl decorations, 32.4 million tons of aquatic organisms, and 82.1 million tons of aquatic animals. China, Indonesia, India, Vietnam, Bangladesh, Philippine, South Korea, Egypt, Norway and Japan top the list of the leading aquaculture producing countries [35].

Aquaculture cannot be mentioned without stating the tremendous wastes generated in forms of wastewater, unused feed, waste substances generated by the animals themselves as well the impact of these wastes on the environment. Aquaculture is becoming a major source of food production in Asia, with China being the top exporter of aquaculture goods, with 2.5 million ha of freshwater aquaculture ponds producing 22.11 million tons in 2018 [36]. According to Halwart [37], Africa's contribution to global aquaculture production is small, despite considerable increases in large-scale investment in Egypt, Nigeria, Ghana, and Uganda. Between 1995 and 2018, Africa's production rose twentyfold, from 110,200 to 2,196,000 tons [37].

Aquaculture monocultures have changed over the previous several decades, it is nowadays more than fish rearing in ponds for easy harvesting to sophisticated techniques in fish farming where fish feed, hormones, as well as antibiotics, for which many researchers are looking for alternatives for antimicrobials [1] [38]. This global tremendous increase in aquaculture systems and fish farming has both known and unknown impact on the environment via the waste and wastewater generated. In order to achieve sustainability, better technology such as water recirculation systems and adequate treatment must be used to maximize this precious resource while minimizing the related negative environmental effect. In addition, it is critical to reduce the strain observed on coastlines while yet producing significant quantities of fish in inland aquaculture systems which is nearer to the final consumers [3].

The rapid expansion of pond aquaculture has resulted in an overabundance of effluent being released into natural aquatic bodies. Part of the primary issue of aquaculture production systems is their effluent; chemical-laden waters discharged by aquaculture operations are a concern all over the globe because they have the potential to become environmental toxins [39]. Aquaculture effluents are high in dissolved solids and suspension, including mostly nitrogen and phosphorus from fish excretion, feces, and uneaten food [39]. According to a study, 36% of fish feed is expelled as organic waste, while roughly 75% of nitrogen and phosphorus absorbed goes unused and ends up as a residue in the water. Approximately 85 percent of phosphorus, 80–88 percent of carbon, and 52–95 percent of nitrogen that enters a fish production system is lost in the environment, depending on the species [40]. According to research [39], the amount of dissolved nutrients released in a system is dependent on the species, food quality, and culture system management. Furthermore, it was recently shown that increasing feed intake with water exchange rates resulted in extremely changing macro-nutrient proportions during the aquatic production cycle [6].

Mahari et al. [3] indicated nitrogen and phosphorus as major pollutants caused by fish farming. Several fish use diffusion and ion exchange to release nitrogenous waste products through their gills, urine, and feces. Due to the toxicity of ammonia and nitrite, as well as the potential for nitrate hyper-trophication of the environment, festering and cycling of nitrogenous compounds is crucial in recirculating aquaculture [2, 3, 6]. Aquaculture effluent may therefore be divided into solid and dissolved waste, with the primary components being carbon, nitrogen, and phosphorus. Solid waste comes from spilled or undigested feed or excrement, according to Dauda *et al*. [2], whereas dissolved waste comes from metabolites produced by fish (through gill or urine).

The expelled organic waste, unused nitrogen, carbon and phosphorus are no doubt suitable media for natural microbial flora of the culture system. These microorganisms which are mostly heterotrophic in nature used the above stated components of the aquaculture wastewater for floc formation in a natural and inducible process called bioflocculation. Many studies have demonstrated the contribution of the extracellular polymeric substances (EPS) produced by the microbial community of the aquaculture system to aquaculture production and sustainability. Their contribution is in form of providing nutrient to the cultured species, waste elimination and provision of hygienic condition in closed aquaculture systems [41]. EPS occurs in variable amounts in sludge and are highly important in the remediation of pollutants from wastewater generally through bioflocculation, settling, and dewatering of activated sludges.

In the presence of flocculant, fine colloids dispersed in the wastewater get agglomerated to produce bigger particles call flocs that settles and clarifies the system. The flocculants exist in chemically synthesized types and natural organic flocculants otherwise called bioflocculant. The bioflocculants (composed of bacterial EPS) is emerging as a substitute to conventional flocculants due to their safety, biodegradability and no secondary pollutant production and is therefore applicable in aquaculture systems to provide even more advantages such as production of natural probiotics to the culture species.

**Pathogens, probiotics and potential for biofloc formation in Aquaculture systems**

Despite the fact that aquaculture is the fastest expanding food industry, illnesses, particularly bacterial infections, tend to stifle its progress [42]. Antibiotics are often used to treat bacterial, viral, and other infections. More recently, there has been a significant rise in the utilization of veterinary pharmaceuticals for disease prevention and control. However, questions have always risen in the effectiveness of antimicrobials as well as antibiotics as a treatment option.

Antibiotics in such large quantities have put a lot of strain on bacteria that have adapted to this situation, mostly through horizontal and philandering resistance gene transfer [43]. This is passed on to the consumer, resulting in allergies and illnesses, as well as an imbalance in the intestinal mucosa due to removal of helpful bacteria from the gastrointestinal tract [44]. As a result of these, the EU and WHO forbid the use of antibiotics as growth promoters in aquaculture and animal husbandry, many researchers are searching for alternatives. Among the supplements that have been suggested are phytobiotics, antimicrobial peptides (AMP), inhibitors of bacterial quorum sensing (QS), feed enzymes, immunomodulatory agents, bacteriophages and associated lysins, biofilm and virulence, probiotics, prebiotics, and symbiotics [45].

Therefore, the application of extra measures as alternative medicine in fish culture, such as probiotics and prebiotics, has shown to be highly effective. It also seems to be an important step for aquaculture operations to increase growth and disease resistance, as well as strategic biological management [44]. Beneficial microorganisms modify the gut microbiota by food supplementation, which is a unique nutritional and immunological strategy. Many studies have shown that using prebiotics and probiotics in fish feed has a favorable impact on the expansion of various species of fish, including *Mozambique tilapia*, *Oreochromis mossambicus*, European Sea bass juveniles, *Dicentrarchus labrax* [46], and Rainbow trout*, Oncorhynchus mykiss* [45] [47].

The adoption of probiotics and prebiotics in animal husbandry is on the rise, with benefits such as increased growth rate, reduced mortality, and improved immune function. To limit the number of opportunistic infections while concurrently activating host immune responses, several disease management approaches have been explored; Gene expression, malabsorption, anti-oxidant enzyme activity, feed utilization, observed non-immune effects include gut morphology, gut microbiota alteration, intermediate stress response, improved nutrition, and decreased risk of certain cancers (blank, colon), as well as lactase production, symptoms of lactose intolerance, and digestive enzyme activity [44].

Probiotic comes from the Greek words "pro" and "bios," which mean "for life," as opposed to "antibiotic," which means "against life" [48]. Probiotics came into limelight when "The Prolongation of Life" by Metchnikof in 1907 noticed a prolong life in birds after consuming yogurt and postulated health benefits of Lactic Acid Bacteria (LAB). Other definitions of probiotics have arisen over time, including chemicals and organisms that contribute to intestinal microbial balance, additionally to live microbial feed supplements that improve the gut microbial equilibrium of the host. Probiotics in water or food may prevent harmful microorganisms while also providing growth nutrients.

Probiotics influence the host's normal microbiota's enhancement and stability, as well as pathogen colonization. They also have a tropic impact on intestinal epithelia, which affects the mucosal barrier and activates both specific and nonspecific immune system components [1]. They also contribute to improved nutrient utility and development by preventing intestinal instabilities and pre-digestion of antinutritional substances included in the components. Probiotics may also detoxify potentially hazardous substances in fish feeds by using hydrolytic enzymes like amylase and protease to denature the potentially indigestible components in the feed. Probiotics can also boost feed utilization, which means they can reduce the quantity of feed required for animal development, lowering production costs [1].

Bioflocs are sometimes refer to probiotics since they are both compose of living cells. However, in addition to living cells bioflocs contains no living cells, particles, cations and polymeric substances. Biofloc also contains substances like bromophenols, carotenoids, chlorophylls, poly-beta-hydroxybutyrate and phytosterols that have been demonstrated to have anti-bacterial activities [2]. For instance, poly-b-hydroxybutyrate synthesized by polyhydroxyalkanoates (PHA) accumulating bacteria in a biofloc (16–18% of the floc) is produced because of the physiological stress [49] and serve as a prebiotic and is used in BFT to obstruct pathogens. Crab *et al.* [50] reported that glycerol grown bioflocs inhibited pathogenic *Vibrio harveyi* in aquaculture of *Artemia franciscana.* Pathogenic *Aeromonas hydrophila* was also inhibited in an aquaculture of *Labeo rohita* [51]. *Litopenaeus vannamei* (white leg shrimp) cultured in a BFT system demonstrated increased total hemocyte count, phagocyte antioxidant activity.

There was an enhanced defiance of African catfish grown in glycerol based biofloc to *Aeromonas hydrophila* [2]. Administration of probiotics to tilapia increased non-specific enzymes which was noted by various parameters like, lysozymes actions, neutrophile movement and bactericidal effect resulting in elevation of fish resistance to red disease (caused by *Edwardsiella tarda*) [52], the work of Robertson *et al.,* [53] who administered isolated *Carnobacterium sp* from salmon bowel and administered to Atlantic salmon and rainbow trout, showed antagonistic effects on known pathogens (*Flavobacterium psychrophilum, Aeromonas hydropila, Photobacterium damselae, A. salmonicida and Vibrio species*). It has been demonstrated that using Vibrio alginolyticus as probiotics increases the survival and growth of white shrimp, it also increased hatchery production of Ecuadorian shrimp by 35% whereas use of antimicrobes decreased it by 94%. [54].

**Biofloc technology (BFT) as a sustainable means of nutrition in aquaculture**

A primary approach to boosting feed nutrient utilization and aquaculture sustainability should be through.

(i) Improving feed quality and feeding technique to enable effective delivery and eventual utilization of the nutrients.

(ii) Modifying the culture system to repurpose the nutrient waste.

Numerous natural biogeochemical processes, primarily involving microorganisms with diverse roles in nutrient cycles, can eliminate nutrients from an aquatic system. [32,50]

Enhancing aquaculture productivity through the use of Biofloc Technology (BFT) can help accomplish sustainable development objectives. With less of an influence on the environment, this technology has increased production. In order to promote productive integrated systems and produce more food and feed from the same amount of land with fewer inputs, BFT may also be created and implemented in conjunction with other forms of food production.

The practice of maintaining high levels of microbiological bacterial floc in suspension via continuous aeration and carbohydrate addition to promote aerobic decomposition of organic waste is known as biofloc technology (BFT). Biofloc technology is based on the system's simple principle of flocculation (co-culture of heterotrophic bacteria and algae) [55]. Flocculation is a water treatment process in which particles aggregate into larger clusters or flocs, which are subsequently removed from the water [55,56]. The key force behind biofloc technology is the exhaustive growth of heterotrophic microbes that consume organic carbon.

The addition of carbohydrates aids heterotrophic bacterial growth, whereas nitrogen absorption aids microbial protein synthesis [56]. According to Xu *et al*., [57], when a biofloc community is fully matured, TAN and NO2-N concentrations can be beneficially managed through heterotrophic assimilation or autotrophic nitrification, which aids in maintaining their concentrations at appropriate levels for cultured organisms even at increasing stocking densities.

The biofloc system maintains a C/N ratio of 15–20 under intensive fish farming with regulated discharge, resulting in the development of sufficient microbial floc to digest dangerous nitrogenous species. A lot of work in biofloc technology regarding adjusting the C/N ratio has recently been published, and Biofloc Technology: A Practical Guidebook, aimed at farmers and researchers, is a big advancement in providing this technical knowledge [58].

Bioflocs are small, easily squeezable, very porous and penetrable to liquids. The living cells in sludge flocs constitute only about 2–20% of the entire organic portion, 60–70% total organic and 30–40% inorganic matter. The microbial biomass of the biofloc is about 1.0 g wet weight·$mL^{-1}$ and can thus falls slowly to the base of the tank in the clarifier. The aggregated microorganisms can then be recycled to the aeration tank containing fresh nutrients. At this stage, poor flocs containing filamentous organisms that could not settle at the tank's base are washed out from the system. In aquaculture setups, the ability of the flocs to settle confers some advantages on the flocs such as escaping the damaging effect of light, protection against other top-layer organisms that may graze on the flocs [59]

Avnimelech [60] indicated Biofloc as suspended particles and a variety of microorganisms, mostly bacteria and planktonic organisms which associates with extracellular polymeric substances to form flocs. Flocs visible to the eye are larger flocs but most are microscopic. Bioflocs' nutritional content changes depending on the ecological condition, carbon source given, total suspended solids, salinity, stocking density, light intensity, plankton and bacteria, and other factors. Protein, lipid, and ash content of dried bioflocs biomass can range from 12 to 50 percent, 0.5 to 15 percent, and 13 to 46 percent, respectively. It's high in a variety of vitamins and minerals, including phosphorus. Another important value of BFT in aquaculture in addition to potential saving on the cost of feed obtained from the floc, is the mitigation of cost of the wastewater treatment. The cost mitigation analysis found in the work of De Schryver, [59] showed a gain in the order of 10–20% in terms of feed costs·$kg^{-1}$ fish produced.

**Composition and nutritional value of biofloc**

Bioflocs are irregular in shape, can size up to1000 µm and are most often composed of heterogeneous microbial species (phytoplanktonics, cyanobacteria, flagellates, ciliates, Proteobacteria and Firmicutes) floc forming and free-living bacteria, colloids, polymeric substances, positively charged ions and no living cells [61,62]. Khanjani and Sharifinia [63] reported dominant bacterial groups in aquaculture biofloc, including Proteobacterium, *Bacillus* species, nitrifying bacteria and Actinobacterium.

The carbon-to-Nitrogen ratio plays an important role in influencing the predominance of species or groups found in bioflocs. Nor *et al.* [61] isolated over 125 bacteria from a biofloc and identified 9 species as *Halomonas venusta*, *H. aquamarina*, *Vibrio parahaemolyticus*, *Bacillus infantis*, *B. safensis*, *B. cereus*, *Providencia vermicola*, *Nitratireductor aquimarinus* and *Pseudoalteromonas sp.* These isolates belong to the families of *Proteobacteria* and *Firmicutes*. Further, biofloc formation, intensity, structure and predominancy of microbial groups or species in the biofloc is influenced by temperature of the culture system, dissolved oxygen in the culture system, intensity of the light, shear rate, nature and source of the carbon in the aquaculture system as comprehensively reviewed in the work of De Schryver *et al.* [59]

Bioflocs are generally dynamic in terms of nutritional composition; they contain bioactive substances that can be used as a complete nutrient pool for aquacultures. The nutrient value of the bioflocs is affected by aquatic nourishment precedence, the ability of the aquatic animals to consume microbial proteins, the farmer's nutrition priority, and the cultured species' ability to ingest and digest microbial protein and biofloc density in water [63]. The higher contents of the flocs greater than 100 µm is attributable to the concentration of EPS that has been reported to be 80–95% of the bioflocs' organic matter. Similarly, the amino acid composition of the flocs also depends on their size; however it was reported that bioflocs generally contain valine, lysine, leucine, phenylalanine and threonine. Ju *et al*. [64], in their study on amino acid profiling of biofloc reported a good essential amino acid index of 0.92–0.93 with histidine and taurine as the predominant amino acids. However, cysteine arginine and lysine [55] were not found in the bioflocs. For detrimental inorganic nitrogen molecules to be immobilized into beneficial bacterial cells (single-cell protein), which can serve as a direct source of food for cultured species, the kind of carbon source and the carbon-nitrogen ratio (C/N) in the aquatic environment are crucial [56].

The important of the carbon source for single-cell protein synthesis, floc formation and resulting clarification of the aquaculture wastewater is evident in the studies compiled in Table 2 below. When different carbon sources (Pearl millet, Sorghum, Tapioca, Finger millet) were added to the culture wastewater at same conditions, survival and body indices (weight gain, specific growth rate (SGR), blioflioc volume and Feed efficiency ratio (FER) of *Pangasianodon hypophthalmus* fingerlings all changes [65] as tabulated in **Table 2**. Similarly, the nature of the carbon source also affects the treatment efficiency of aquaculture wastewater. This is also evident in the water quality parameters for different fish species and carbon sources outlined in **Table 2**. Thus, for effective fish farming using Biofloc technology, many carbon sources should be tested and optimized for any aquatic animal of choice. Growth rate and feed conversion ratio are generally essential parameters that contribute to the cost of aquaculture and are enhanced in biofloc systems in comparison to the traditional system [63]. Crab *et al.* [66]. In the presence of bioflocs, fish and shrimp's immune systems were strengthened, and disease incidence decreased in biofloc systems. Several studies had reported probiotic effects of bioflocs on *Streptococcus, Vibrio sp.* and ectoparasites, as well serving as effective bio-control agents. In contrast to traditional techniques, Biofloc might be a revolutionary disease management strategy [63].

**Prospects and challenges of biofloc technology in aquaculture system**

With a human population of around 7 billion people, there is a growing need for aquatic food, necessitating the need for aquaculture output to be intensified. One of the industries that produces food the fastest, aquaculture, offers many opportunities to reduce poverty, hunger, and malnutrition while fostering economic growth and guaranteeing improved resource management [71]. This technology's returns in aquatic farming include little feed and or no water exchange, reduced pathogens and ailments, biosecurity, and improved growth and survival rate [56]. It is a robust, easily operated, and economically viable technology.

Aquaculture intensification has increased by waste creation. Biofloc technology has recently gained popularity as one of the most efficient methods for treating aquaculture effluent. Biofloc technology (BFT) may be seen as a climate-smart technological invention that operates utilizing the principles of in situ large-scale microbial production [71]. These microbes have been held accountable for the following greenhouse gas (GHG) sequestration [71], improved culture feasibility by lowering the feed conversion ratio (FCR) and feed costs [58], biosafety, and ensuring high-quality water [49].

The characteristics of BFT bacteria have resulted in great profitability, fish output, and environmental conservation. Because BFT requires a carbon source, sunshine, and occasional aeration, the initial investment cost is lower than most conventional fish production systems. BFT is primarily based on a heterotrophic process that converts leftover feeds, extra nutrients, and wastes into bioflocs that can be consumed, it is also known as single-cell proteins (SCP). This consists primarily of uneaten feeds loosely connected by bacterial mucus, generating apparent floating clumps that are very nutritious feed material for farmed fish and shrimp. BFT decreases feed costs by 30 % because each pellet is consumed twice, resulting in increased aquaculture production as well as huge profit [60]. Bioflocs provide essential nutrients and a probiotic impact, which plays a key role in the BFT systems for biosecurity [50, 72].

Bioflocs maintain good water quality by consuming ammonia and creating their proteins; in the aquaculture system, there is limited water exchange, which keeps the flocs alive and allows for higher-density stocking and enhanced fish output [49]. Due to the characteristics highlighted by BFT above, which make it economically appealing to aquapreneurs, it also functions as live feed production in hatcheries, and bioflocs serve as efficient carbon sinks, allowing for the mitigation and adaptation of GHG impacts [58,73,74].

**Table 2**. Effect of carbon source on the growth parameters of the culture species and water quality of the treated aquaculture wastewater.

| Fish species | Carbon sources | Weight gain (g) | SGR (%) | FCR | BFV $(mL·L^{-1})$ | Survival (%) | Water Quality parameters $(mg·L^{-1})$ | References |
|---|---|---|---|---|---|---|---|---|
| *Pangasianodon hypophthalmus* | Pearl millet | 21.92±0.21 | 1.28±0.01 | 1.37±0.02 | 25.50±3.42 | 100±0. 00 | - | |
| | Sorghum | 21.34±0.50 | 1.26±0.02 | 2.37±0.05 | 2.37±0.05 | 100±0. 00 | | (65) |
| | Tapioca | 20.67±0.20 | 1.23±0.01 | 2.44±0.03 | 19.61±0.28 | 100±0. 00 | | |
| | Finger millet | 24.34±0.50 | 1.37±0.02 | 2.12±0.03 | 25.50±3.42 | 100±0. 00 | | |
| *Heteropneustes fossilis* | sugarcane molasses | 42.97±1.32 | 1.98±0.04 | 0.85±0.01 | 26.0±0076 | 96.74±1.34 | D.O - 5.43±0.06<br>TDS - 245.17±8.16<br>TSS - 247.6±2.51<br>NH3-N - 0.002±0.0006<br>NO3-N - 2.00±0.01 | (67) |
| *Clarias gariepinus* | Fermenting rice bran | 213.56±10.32 | 2.85±0.08 | 0.82±0.03 | - | 96.00±2.31 | D.O - 7.89±0.07<br>TDS - 245.17±8.16<br>TSS - 578.06±52.43<br>NH3-N - 1.63±0.38<br>NO3-N - 0.47±0.12 | (68) |
| *Oreochromis niloticus* | Wheat mill waste | 100.02±3.92(%) | - | 1.72±0.08 | 29.92±4.10 | 100±0. 00 | D.O - 5.28±0.56<br>NH3-N – 1.62±0.06<br>NO3-N - 12.33±0.67 | (69) |
| | Rice bran | 88.23±1.81(%) | | 1.80±0.05 | 18.58±3.84 | 98.4±1.00 | D.O - 5.62±0.62<br>NH3-N – 1.61±0.05<br>NO3-N - 12.58±0.64 | |
| *Litopenaeus vannamei* | Corn | 22.99±1.66 (%) | 2.83±0.05 | 1.5±0.2 | 12.9±2.5 | 82.5±3.5 | D.O - 5.7±0.85<br>NO3-N - 0.089±0.07<br>TSS - 57.3±25.2<br>Turbidity (NTU) - 12.9±2.5 | (70) |
| | Molasses | 82.85±1.55 (%) | 3.19±0.01 | 0.91±0.1 | 23.9±3.8 | 99.0±1.1 | D.O - 4.2±0.48<br>NO3-N - 0.018±0.02<br>TSS - 305.6±21.5<br>Turbidity (NTU) - 23.9±3.8 | |
| | Wheat | 21.17±8.26 | 2.76±0.07 | 1.5±0.1 | 15.2±3.8 | 84.0±1.4b | D.O - 5.1±0.70<br>NO3-N - 0.122±0.04<br>TSS - 79.0±61.1 | |

In designing a biofloc technology for any fish species, there is a need to move from pilot trials to full-scale experimentations. Research into development and engineering of Biofloc technology that can work appropriately in the culture systems that contain multifaceted mixtures of dissolved and suspended contaminants that are not distributed evenly in the environment is needed. Research into elucidating microbial mechanisms and microbial genes involved in Biofloc formation is highly needed.

Standard methods and tools for fishpond or culture house designs, stock management, and fish reaping in BFT aquaculture are some of the requirements that are yet to be accorded the needed research attention; as such, Biofloc studies that put these requirements into consideration will further enhance the potentials of this technology. The viability of BFT with fermented intricate carbohydrates on the poor filter-feeding aquatic species needs exploration to widen the application of this technology to more species and enhance the aquaculture industry sustainably. The development of biofloc technology is still in its infancy. Much more research is required to improve the system's operating parameters with respect to nutrient recycling, MAMP production, and immunological effects. Furthermore, farmers will need to be informed of research findings because biofloc technology will require them to upgrade their skills [75]. The oversimplified concept of turning aquatic life excrement into feed may deter buyers from purchasing these goods. Additionally, mixing and aeration need more energy, and there's a chance that nitrate build-up would contaminate the surrounding area.

## CONCLUSION

As the demand for seafood climbs with the rising global population, the expansion of aquaculture has escalated environmental concerns, primarily due to the effluent discharge that contributes to aquatic pollution. Biofloc Technology (BFT) offers a robust response, serving as an innovative and sustainable approach to the challenges of aquaculture waste management. It ingeniously repurposes organic waste, including excess feed and fish metabolites, into bioflocs—nutrient-rich biomass that aquatic species can consume, thus slashing feed costs and closing the loop on waste. BFT curtails the environmental burden by recycling waste and enhances the nutritional intake of cultured species, contributing to healthier and potentially faster-growing stock. Moreover, this technology underpins water conservation efforts by reducing the need for water exchange, a critical advantage in regions where water is scarce. The efficiency of BFT in improving water quality transcends to disease prevention, lowering the reliance on antibiotics and chemicals, and fostering a more natural aquaculture environment. Looking forward, BFT is a pillar for sustainable aquaculture practices, aligning industry growth with environmental stewardship. It epitomizes the quest for innovative methods that deliver economic benefits while preserving ecological balance, ultimately supporting the goal of sustainable food security on a global scale.

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