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BIOFLOC TECHNOLOGY: AN INNOVATIVE APPROACH IN FISH NUTRITION AND HEALTHCARE

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ABSTRACT

A biofloc is a composite collection of living and dead particle organic matter, bacteria, and phytoplankton. Biofloc technology uses a zero water exchange system to improve water quality by adjusting the C/N ratio to transform harmful nitrogenous wastes into beneficial microbial proteins. It may serve as a comprehensive supply of nourishment for aquatic species, as well as some bioactive substances that will improve growth, survival, and defensive mechanisms. It also represents a novel method for managing animal health in aquaculture by enhancing innate immune systems. The floc biomass offers a complete supply of nutrition along with a number of bioactive substances that are beneficial for raising the welfare indices for all aquatic creatures. Natural probiotics and immunostimulants, such as organic acids, polyhydroxy acetate, and polyhydroxy butyrate, are produced by the beneficial microbial bacterial floc and its derivatives. The method helps keep water quality parameters at their ideal levels in a zero water exchange system, preventing eutrophication and effluent discharge into the environment. The method may also help ensure biosecurity because there is no water exchange other than sludge removal. The technology is socially and environmentally acceptable as well as economically viable.

Keywords: Biofloc technology, organic carbon, C/N ratio, and non-specific immunity

Introduction

Industries involved in food production, such as aquaculture, require both horizontal and vertical expansion to support the world's continually expanding population. It is impossible to overstate how quickly the aquaculture industry is expanding on a global basis. Increased aquaculture production results in massive amounts of surplus organic pollutants, which could potentially lead to both long-term environmental risks and immediate harmful impacts (Piedrahita 2003). The use of continual replacement of the pond water through the interchange of water has been the most popular strategy for addressing this issue (Gutierrez-Wing and Malone 2006). Even for small to medium-sized growth systems, the amount of water needed might be several hundred cubic meters per day. An alternative method for purifying the culture water of significant hazardous contaminants without endangering the environment is a recirculating aquaculture system (RAS) (Gutierrez-Wing and Malone 2006). Only 10% of the total water volume needs to be replaced daily (Twarowska *et al.* 1997), but RAS adoption is low among farmers, especially in poor nations, because of the high cost of operation and maintenance. As a result, there has been a long quest for a low-cost, environmentally friendly technology that is also sustainable.

Recent advances in biofloc technology (BFT) have drawn attention as a sustainable and environmentally friendly technique of aquaculture that regulates water quality and produces value-added microbial proteinaceous feed for aquatic species. There has been substantial research on the application of BFT systems in the aquaculture of marine shrimp (da Silva *et al*. 2013, Kumar *et al.* 2014). The technology promotes environmentally friendly and economically viable aquaculture (Avnimelech and Kochba 2009). Hence, this review has been designed to highlight the application of biofloc system on fish nutrition and aquatic animal healthcare especially biofloc microbial community, biofloc nutritional aspects and biofloc mediated fish immuno-physiological response.

Biofloc technology

Biofloc technology (BFT) is an innovative approach with the ability to solve problems of nitrogen-based toxic compounds. Using continuous aeration and the addition of carbohydrates to promote aerobic decomposition of the organic material, BFT maintains high levels of microbial bacterial floc in suspension (Avnimelech and Weber 1986). By including carbohydrates, heterotrophic bacterial growth is accelerated, and microbial proteins are produced via nitrogen uptake (Avnimelech 1999). The production of high-quality single-cell microbial protein can be increased while maintaining the C/N ratio in the aquaculture system through the external addition of carbon sources or an increased carbon level in the feed (Crab *et al.* 2012). Dense microorganisms grow in such environments, serving as a bioreactor that regulates water quality (Avnimelech et al. 1989) as well as a source of protein for fish and shrimp. With less water exchange and less feed input than conventional aquaculture methods, BFT offers a more affordable and environmentally friendly alternative, making it a low-cost technology for the development of a sustainable aquaculture industry in the future (Avnimelech and Kochba 2009). The intensive farming techniques with limited water exchange offer a sustainable alternative for farming intensification and biosecurity when compared to the productivity of other eco-friendly farming practices (Figure 1).

Maintenance of carbon-nitrogen (c/n) ratio

Maintaining the C/N ratio is essential for preventing the accumulation of organic nitrogen and promoting the development of microbial communities in water (Asaduzzaman *et al*. 2008) which may serve as a direct source of food for the cultivated organisms (Avnimelech 1999). When the organic matter's C/N ratio exceeds 10, inorganic nitrogen is immobilized (Lancelot and Billen 1985). An autotrophic system could become heterotrophic as a result of a change in the C/N ratio (Browdy and Bratvold 2001). In limited-discharge systems, it is possible to change the C/N ratio by adding a carbon source (direct or indirect C sources) to the culture medium, which will significantly increase the growth of beneficial microorganisms and their ability to fix harmful nitrogen compounds (Hari *et al*. 2006, Avnimelech and Kochba

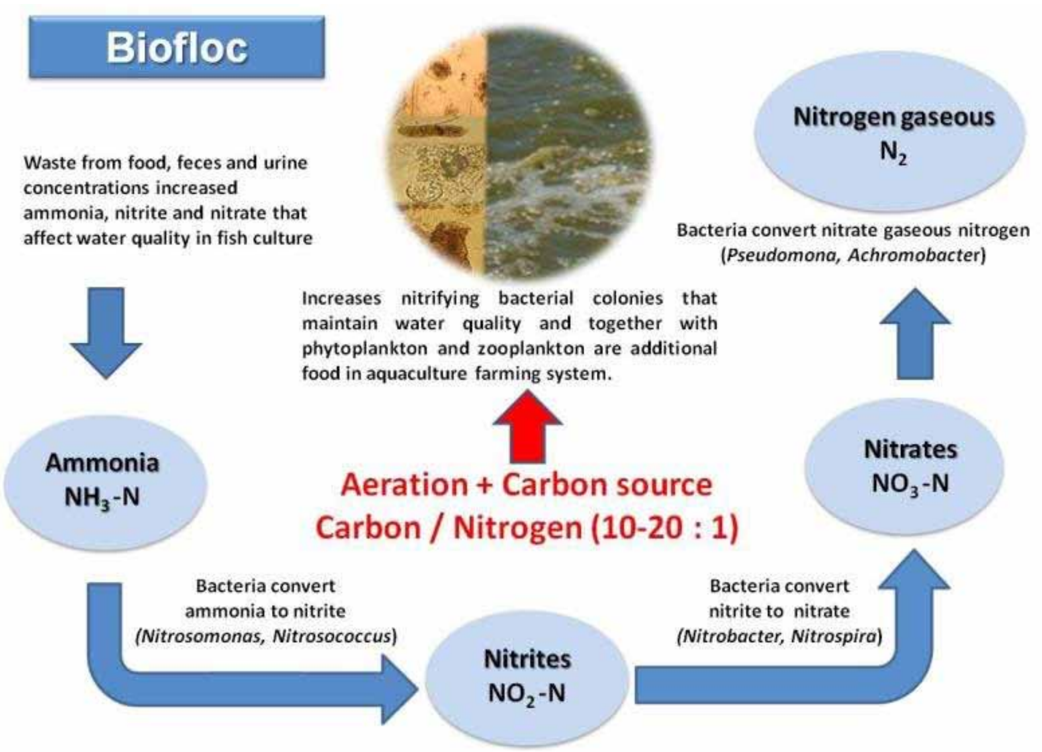

Figure 1. Mode of action of biofloc system (Gustiano *et al*. 2021).

2009, Crab *et al.* 2010). The bacterial process, known as nitrification, converts the harmful forms of nitrogen (ammonia and nitrite) into one that is toxic only at high concentrations (nitrate). Since the BFT is a zero water exchange system, nitrate tends to accumulate over time. As a result, the nitrate level in biofloc systems generally rises as the culture develops. According to Kuhn *et al.* (2009), carbon supplementation increased TAN removal rates to 26% per hour from 1% per hour in a control system. By using organic carbon sources and changing the C/N ratio in the feed, it is possible to reduce the hazardous nitrogenous chemicals produced by intensive, well-aerated systems (Avnimelech 1999, Browdy and Bratvold 2001). However, maintaining the high C/N ratio in the biofloc system will prevent the accumulation of hazardous inorganic components, including NH4+ and NO2, in the water due to the consumption of ammonium by the microbial community.

Microbial community and bioremediation

It is interesting to note that both autotrophic and heterotrophic bacteria can grow in a biofloc system (Manan *et al.* 2017; Pacheco-Vega *et al.* 2018). *Acinetobacter, Sphingomonas, Pseudomonas, Rhodopseudomonas, Micrococcus, Nitrosomonas, Nitrospira, Nitrobacter, Cellulomonas,* and yeast are just a few of the heterotrophic beneficial microbial communities found in bioflocs. The water quality, growth efficiency, and health of cultured aquatic animals are all improved by these microbes in biofloc culture systems (Monroy-Dosta *et al.* 2013, Adel *et al.* 2017) (Figure 2). In biofloc systems, the accumulation of particulate and dissolved organic matter is a frequent occurrence. However, high concentrations of heterotrophic bacteria effectively reduce the amounts of organic nitrogen and carbon in the system. These heterotrophic bacteria are capable of acting as bioremediators because they produce a variety of metabolic enzymes that aid in the safe removal of toxins through either direct oxidation into less harmful compounds or conversion to safer ones. For instance, an experiment was conducted by Manan *et al.* (2017) to ascertain the function of aggregating biofloc in the bioremediation process, which includes the breakdown and decomposition of organic waste. The findings demonstrated that the bottom organic matter of shrimp (*L. vannamei*) culture biofloc tanks was devoured by heterotrophic bacteria from the *Aeromonas* (*Aeromonas salmonicida* and *Aeromonas hydrophila*) and *Pseudomonas* family (*Pseudomonas aeruginosa*). Additionally, by converting these bottom wastes through chemical processes, they assist in the creation of high protein flocs that are used by cultivated shrimp (Manan *et al.* 2017).

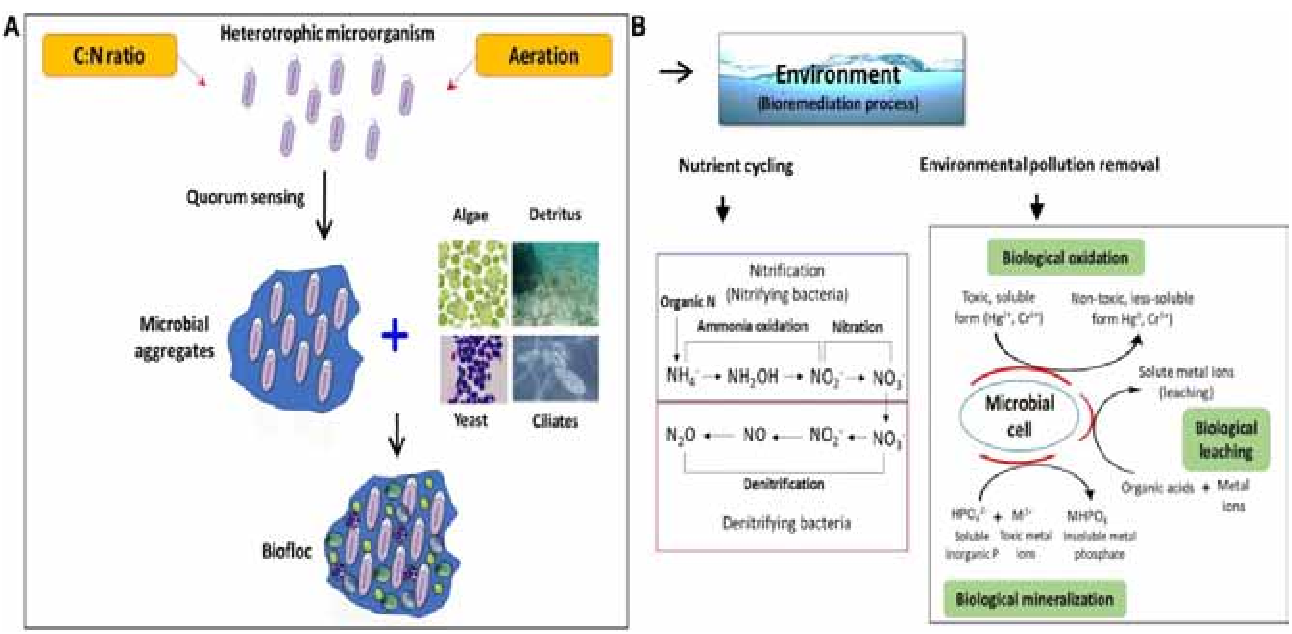

Figure 2. Schematic overview on the possible role of the biofloc microbiome (Kumar *et al.* 2021). (A) Development of a biofloc system; (B) Potential role of the biofloc system in the bioremediation process.

Nutritional composition of bioflocs

In terms of nutrition, the floc biomass may offer a comprehensive source of food as well as a variety of bioactive substances (Akiyama *et al.* 1992). The nutritional value of bioflocs depends on a number of elements, including the animal's food preferences, its capacity to consume and digest microbial protein, and the density of the flocs in the water (Hargreaves 2006). Shrimps, tilapia, and carps cultured in BFT system are fed on the single-cell protein produced by the heterotrophic bacterial population as a result of the uptake of inorganic N. (Burford *et al*. 2004, Mahanand *et al.* 2013).

Proteins, vitamins, and minerals are abundant in bioflocs (Tacon *et al.* 2002). It has 19 kJ/g of energy (on a dry matter basis), 38% protein, 3% fat, 6% fiber, and 12% ash (Azim and Little 2008). According to the analysis of amino acid profile of the organism, histidine and taurine are the two most prevalent amino acids in bioflocs (Ju *et al*. 2008). Bioflocs have a higher essential amino acid index (0.92-0.93). It provides a full source of cellular based nutrients that speeds up the rate of ingestion, nutritional absorption, and assimilation (Tacon *et al.* 2002). In *Farfantepenaeus duorarum and L. vannamei,* brood stock diets enriched with biofloc supplementation enhance reproductive performance in terms of fecundity, spawning, and egg biochemical composition (Emerenciano *et al.* 2014). The microbial community in biofocs boosts up shrimp and tilapia growth rates, feed conversion ratios, and weight gain (Burford *et al*. 2004).

Although bioflocs meet nutritional requirements, their nutritive qualities and their capacity to keep the water in the BFT system clean depend on the carbon source used in the flocs. Different carbon sources are used to alter the C/N ratio, stimulate particular bacteria, protozoa, and algae, as well as to affect the microbial composition and community structure of the bioflocs (Crab *et al.* 2009). Use of a biofloc system supplemented with dextrose or molasses allowed for the maintenance of *L. vannamei* production rates and water quality without the use of water exchange (Antonio *et al.* 2015). The bioflocs are rich in natural protein and lipid and thus serve as natural in situ food for culture organisms (Avnimelech 2007). In addition, they act as a bio-control to the system by treating the feeding waste and lowering ammonium concentrations (Crab *et al.* 2007, Hargreaves 2013), thereby maintaining the water quality.

Immuno-physiological response by bioflocs

Due to the environment and the fish's poikilothermic nature, the immune system of fish functions as an intersection between innate and adaptive immune responses (Tort *et al.* 2003). The inflammatory processes and phagocytosis that are part of the immune responses to injury or pathogenic invasion are aided by non-specific immune cells like neutrophils, macrophages, and non-specific cytotoxic cells (Corbel 1975). High molecular weight glycoproteins are released from fish skin mucus when the amount of bacteria in the nearby water increases. Once the fish are exposed to the pathogens, a variety of humoral factors are released by the fish, including cytokines, anti-proteases, peroxidases, lysozymes, etc. Lysozyme is one of them that is frequently used as a sign of an immune response. Lysozyme has a high potential for bactericidal or bacteriolytic activity against pathogenic gram-positive and gram-negative bacteria in addition to acting as an anti-inflammatory and antiviral agent (Saurabh and Sahoo 2008).

Recently, scientists have proposed that the immunostimulatory properties of bioflocs may enhance the immunity and antioxidant status of shrimp and fish, resulting in a broad-based resistance to numerous infections (Ahmad *et al.* 2016). It is believed that the heterotrophic microbial biomass has a regulating effect on pathogenic bacteria (Michaud *et al.* 2006). Floc carotenoids have been demonstrated to perform numerous bioactive physiological functions in animal tissue, including boosting the animal immune system (Ju *et al.* 2008). A novel approach to managing bacterial infection in aquaculture involves disrupting the quorum-sensing, bacterial cell-to-cell communication system. It's interesting to note that a similar phenomenon was seen in bioflocs grown on glycerol to combat *Vibrio harveyi* in *Artemia franciscana* culture (Crab *et al*. 2010); it was also hypothesized that probiotic bacteria are present in large numbers in microbial flocs (Bairagi *et al.* 2002).

A variety of live and synthetic substances derived from bacteria and bacterial products, as well as extracts from plants and animals, comprise the majority of the immunostimulants that are currently on the market (Wang *et al.* 2008). Biofloc technology may contain some immunostimulatory substances that are advantageous for the wellbeing of cultured organisms because it deals with the bacterial environment. Shrimp's innate immunity and antioxidant status have been improved by using microorganisms and their cell components as probiotics or immunostimulants, increasing their disease resistance (Smith *et al.* 2003, Vazquez *et al.* 2009). Although bioflocs have been shown to be the richest source of microbial and bioactive substances, little research has been carried out on how they affect the physiological health of cultured shrimp, especially in terms of their impact on the immune and antioxidant defense systems. According to Jang *et al.* (2011), long-term biofloc rearing of shrimp significantly increased the expression of a prophenoloxidase activating enzyme 1 (lvPPAE1) in hemocytes of *L. vannamei*. Recent research by Becerra-Dorame *et al.* (2014) found that *L. vannamei* raised in biofloc-based systems displayed improved physiological performance as evidenced by a number of hemolymph parameters, including superoxide dismutase activity. The host's immune system is most likely modulated by some active microorganisms that continuously enter a shrimp body while the shrimp is ingesting biofloc (Johnson *et al.* 2008). These microorganisms may be either living organisms or microbial components (Jang *et al*. 2011). Therefore, more research must be done to understand the precise nature of the humoral innate or cellular immune response as well as to establish the protective biofloc life of bacteria in order to improve the welfare of fish in aquaculture. The last section of this article provides a comprehensive view of bioflocs that can be strategically employed to combat diseases in aquaculture (Figure 3).

Biofloc technology in future aquaculture

BFT has been effectively implemented to aquaculture, particularly in shrimp farming due to its financial and environmental advantages over traditional culture systems.

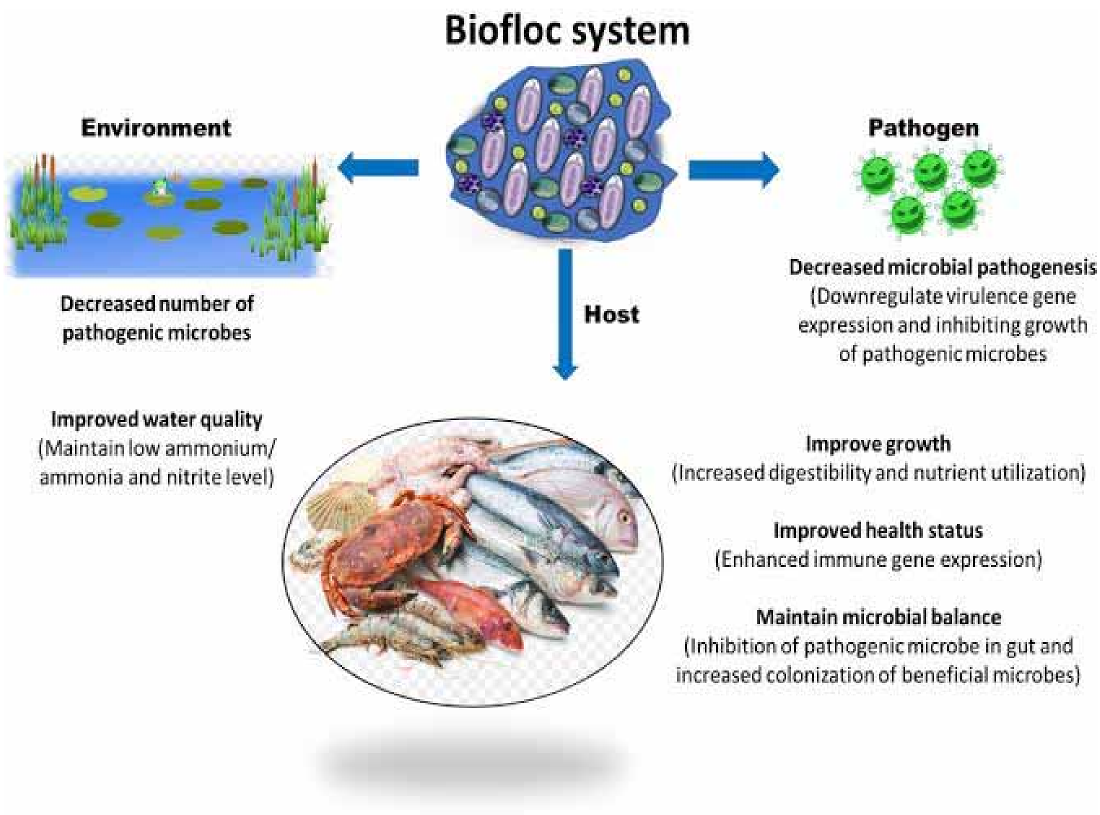

Figure 3. Potential role of biofloc system in host, pathogen, and environment in a culture facility (Kumar *et al*. 2021).

It has been widely used to reduce ammonia accumulation by converting it into heterotrophic bacterial biomass. The benefits of this new aquaculture technology are well known including low feed and water input, reduced risk of pathogen introduction and disease, increased biosecurity, increased growth and survival, and consequently higher crop yield (Krummenauer *et al*. 2011, Perez-Fuentes *et al*. 2013). It also lowered the feed conversion rate by utilizing the in situ natural feed. Additionally, it is reliable, simple to use, and affordable (Crab *et al*. 2012). Even when the system is experiencing high levels of organic matter and biochemical oxygen demand, it supports nitrogen removal. However, more study is needed to determine the best method for controlling BFT in culture ponds because it can also act as a low-cost and efficient immunostimulant for the organisms that are being grown in culture. It will nevertheless be crucial for the future BFT to comprehend the microbial mechanisms involved in flocculation, specifically quorum sensing and its effect on pathogenic microbes. But it will be essential for the future BFT to fully understand the microbial mechanisms, including such quorum sensing and controlling effect on pathogenic microbes that are involved in the flocculation process.

CONCLUSION

Water scarcity, rising protein demand, and conflict for land usage for the expansion of aquacultural practices has combined to create a significant issue on a worldwide scale. Intensive aquaculture is one of the main possibilities to meet the rising demand for animal protein. However, intensifying aquaculture methods will produce a lot of effluents that will harm the aquatic ecosystem. Additionally, intensification will cause socioeconomic conflicts, disease outbreaks in cultured organisms, environmental deterioration, and a significant reliance on fish meal, a rare resource. As a sustainable alternative to intensification, BFT has grown in popularity as a means of reducing the effects of the environmental, health, and economic issues connected with aquaculture. This system has a promising future because it can assist in achieving the high levels of output necessary to satisfy the demands of a growing human population. The introduction of biofloc technology can meet the needs for environmentally friendly and sustainable aquaculture development.

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