Peer-Reviewed/ISSN: 2663-1040 AJ Life Sci. 2023, 6 (1): 8-39 Review Article

Abasyn Journal of Life Sciences

Open Access

DOI: 10.34091/AJLS.6.1.2

Biofloc Technology: Sustainable Technology for Fish Production

Ahmad Manan Mustafa Chatha[1], Saima Naz[2], Urooj Nazir3*

[1]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, PK63100, Bahawalpur, Punjab, Pakistan Email: manan.chatha@iub.edu.pk, ORC-ID: 0000-0002-8284-5896

PK63100, Bahawalpur, Punjab, PAKISTAN, manan.chatha@iub.edu.pk, ORC-ID: 0000-0002-8284-5896

[,3,2]Department of Zoology, Government Sadiq College Women University, Bahawalpur, Punjab, Pakistan, PK63100, Email:saima.naz@gscwu.edu.pk, ORC-ID: 0000-0002-7748-0490,

Abstract

Based on in-situ microorganism generation, biofloc technology is an aquaculture method that is favourable to the environment. The term "biofloc" means the lump of living and dead organic materials, phytoplankton and bacteria that feed on other bacteria that are suspended in water or other bodies of water. Using waste nutrients as fish food is a method of alternative fish farming known as biofloc technology. This analysis focuses on the prerequisites for a biofloc fish farming system, biofloc development, the microbial community, and the significance of microbial interaction in biofloc technology, where phytoplankton and bacterial populations play a more major role in preserving water quality. Between bacteria and algae, there is a complicated relationship. Due to bacteria, Algae can be decomposed into organic nutrients. Along with its ability to distribute bioactive chemicals, biofloc's nutritional value influences the entire aquatic food supply. The control and harmful consequences of nitrogenous chemicals have been covered in this review (such as ammonia, nitrites and nitrates). To create microbial protein from harmful fish waste and other organic debris in the water, Biofloc, or more particularly, cultivated microbes, are added to the water. This lowers costs while also maintaining water quality. Biofloc advantages and disadvantages are also discussed in this review and what improvement can be made to biofloc technology.

Key words: Fish, Biofloc, bacteria, phytoplankton, Microorganisms

Article Info:

Received:
Received:
March 25, 2023
Received Revised:
May 25, 2023
Accepted:
June 11, 2023
Available online:
June 23, 2023

Corresponding author:
Saima.naz@gscwu.edu.pk

1. INTRODUCTION:

Aquaculture output is typically increasing, which is shown by high level of stocking densities and higher feed inputs. While this can raise output which also created several difficulties, including higher nitrogen loadings and a greater danger of diseases that put farmers at greater risk in addition to the possibility to contaminate or transmit viruses to untreated natural water sources [1]. Recirculating aquaculture systems (RAS) were developed as one method of addressing these issues [2]. There is a need of vertical and horizontal expansions in aquaculture to fulfil raising population needs of the world. It is impossible to overstate how quickly the aquaculture business is expanding on a global scale given the potential obstacles posed by the environment and the economy. Increased aquaculture production results in enormous amounts of surplus organic contaminants, which have the potential to have both immediate harmful impacts and long-term environmental hazards [3]. The biofloc technology (BFT) is a recently developed economical method of growing fish that allows nitrogenous wastes from fish and shellfish as well as leftover feed to be transformed into protein-rich feed called biofloc [4]. Traditional aquaculture production systems have a number of economic and environmental issues that the BFT system (biofloc technology system) has proven to be able to address.

Aquaculture production must expand and become more intensive as a result of the increasing demand for aquatic food due to the estimated 7 billion people who currently inhabit the planet. The main objective of expanding aquaculture must be to increase production of aquaculture products while utilizing water and land more economically [5]. Fishes cultivated through BFT system are suitable for eating because a good nutritional value is found in Biofloc. The dry weight protein ranges from 25 – 50%, fat ranges 0.5 – 15%. It is a good source of vitamins and minerals, particularly phosphorous. It has an effect similar to probiotics.

2. HISTORY:

In the early 1970s at Ifremer-COP, the Oceanic Center of the Pacific, a French Research Institute for Sea Exploration, where biofloc technology was first discovered [6]. Understanding the success factor for Biofloc fish farming in Pakistan is crucial. Pakistan is one of the less developed nations. Comparatively speaking, the nation has fewer resources for knowledge. As a result, individuals and SMEs strain and toil on their own to develop the relevant skills by regularly exercising and learning this expertise. Therefore, people in Pakistan are able to get the required knowledge from reliable sources, have already implemented Biofloc fish farming utilising Biofloc Technology, and have begun working on it. Ten separate scenarios involving individuals and SMEs that switched from conventional farming to Biofloc fish farming have been examined. ten out of ten people have stated that they are happy and successful with it. For cultivating a wide range of species, such as *Litopenaeus vannamei, Litopenaeus stylirostris, Penaeus monodon*, and *Fenneropenaeus merguiensis*, this type of systems with active microbial suspensions through continuous water circulation system was used [7]. The discoverers of this technique notably Hepher, Schroeder and Wohlfarth (Israel), created the idea of the "Heterotrophic feed chain," and Steve Surfing, who founded Solar Aquafarms in California, a shrimp and fish farm based on active bacterial culture [8]. Under these circumstances, organic wastes that build up in the reservoir decompose and an active microbial community nitrifies or absorbs ammonia—N. The typical external bio filter or high-water exchange devices are replaced by this set of procedures. In this situation, these ponds' bacterial mechanisms act as a mechanism for treating the water to maintain pond quality of the water, and the bacterial protein acts as a feeding addition. Even though Biofloc technology was developed and operates on fairly straightforward principles, farmers did not start to accept this fish culture technology until the 2000s. Avnimelech [5] noticed that one factor in this reluctance was that the considerable turbidity brought on by the biofloc appeared to challenge the idea that cleaner pond waters are preferable. The eventual acceptance of Biofloc technology was largely a result of factors such as growing water shortages and some wealthy countries putting stronger controls on the quantity of wastewater discharged [1]. Today, a lot of research institutions are starting their Biofloc technology efforts, most often in relation to important areas like cultivation maintenance, nutritious food, and Biofloc technology applicable to reproduction, microbiological ecology, bioengineering, and economics[7].

3. NECESSITY OF BIOFLOC FISH FARMING SYSTEM:

More than billion people worldwide rely on fish as one of the most affordable and nutrient-dense sources of animal protein, which is vitally important in food and nutritional security [9]. Essential amino acids, lipids, vitamins, and minerals are abundant in fish. However, the world's constantly expanding fish-eating population presented many difficulties for researchers and fish producers to meet their expectations. The far more important and restricting elements for currently used aquaculture systems are the accessibility of land and water, as well as the price of fish feed, which makes up sixty percent of the overall production cost. Different aquaculture methods with increased production therefore need less land, water, and money. According to research, approximately fifteen to twenty five percent of the protein in feed is preserved by fish or shrimp in conventional aquaculture, and the remainder is flushed out primarily as ammonium, however protein consumption in a biofloc system can be enhanced up to 45 percent [10]. According to Da Silva et al. (2013), studies have shown that this mechanism assimilates food nutrients more effectively.

4. BIOFLOC DEVELOPMENT:

Biological polymers are needed to create the biofloc because they hold the various parts together and create a matrix that encloses the cells. This matrix serves as a substrate, gives the bacteria easy access to nutrients, and shields them from their predators [11]. In order to achieve the formation of heterotrophic bacteria found in the biofloc, the carbon/nitrogen (C: N) relation in the water reservoir must be adjusted. This is done by providing a food with less protein and one carbohydrate, including molasses, in adequate levels to integrate one unit of nitrogen, 20 units of carbon are required. The microorganisms in the culture can use the nitrogen that is not consumed to create microbial protein rather than hazardous chemicals, which aids in the direction of hazardous inorganic nitrogen, leftover food, and the remaining phytoplankton production [12] .As soon as this rate is sufficient, bacteria that are growing inside the microsystem begin to use potentially harmful substances like organic carbon, ammoniac nitrogen, nitrates, nitrites, and phosphates as energy sources, oxidizing them so that algae, fungi, other bacteria, and filtering microbes can also use them [13]. The microorganisms in the culture can use the nitrogen that is not consumed to create microbial protein rather than hazardous chemicals, which aids in the direction of hazardous inorganic nitrogen, leftover food, and the remaining phytoplankton production [12]. In biofloc technology the role of microbial community to improve water quality and fish yield in fresh water indoor and outdoor pond Aquaculture **(Fig .1).**

In systems with little to no water exchange (such as intensive care systems), heterotrophic ammonia-assimilating and chemoautotrophic nitrifying bacteria are principally in charge of preserving water quality [14]. The color change from green to brown occurs as the culture progresses through the transition from a predominantly algae-dominated to a bacterial biofloc-dominated system [13]. Phytoplankton, bacteria, and aggregations of live and dead organic particles make up the developed microbial communities [15].

Proteobacterium, *Bacillus amyloliquefaciens* specie and actinobacterium are the three most prevalent bacterial species found in bio floc. Other smaller bacterial species exist as well, including Cytophaga and Roseobacter species [16]. Heterotrophic bacterial population uses organic nitrogenous waste as well as ammonia to generate unicellular microbial protein [17]. Bio floc, which have several benefits for the culture, form in the ponds when fish are raised in high densities without water exchange. It has been hypothesized that the diversified microbial community in biofloc dominated systems is thought to increase competition with potentially harmful bacteria, such as vibrios, and lesson issues with non-excludable pathogens. [18]. The type of culture system, light, and salinity are a few of the variables that affect how the bacteria in Biofloc are arranged [19].

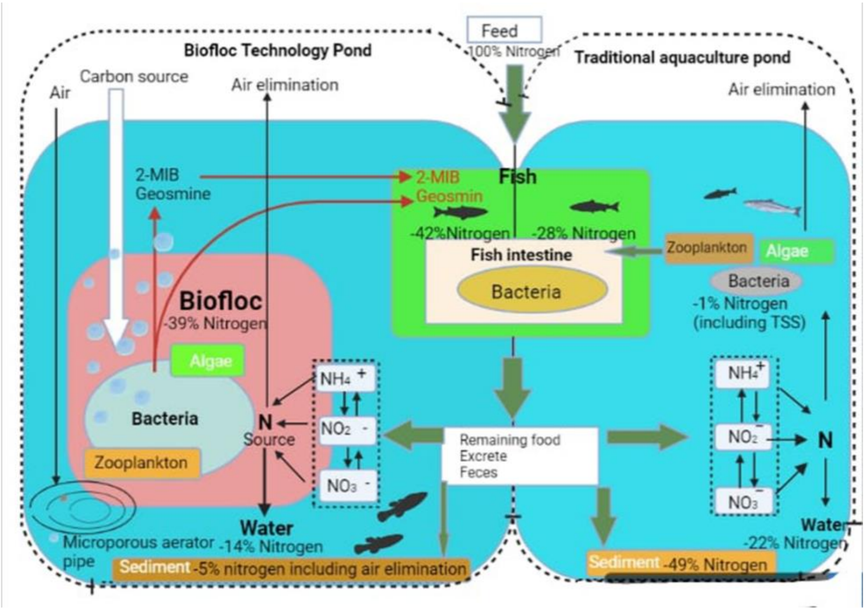

Figure 1 Biofloc Technology in fish pound.

5. ROLE OF MICROBIAL INTERACTION IN BIOFLOC TECHNOLOGY

BFT is also known as suspended growth systems [15], autotrophic-heterotrophic systems without exchange [20], activated sludge or suspended bacteria-based systems [21], single cell protein production system [13], and microbial floc system [22]. However, BFT is attempting to protect water quality by utilizing bacteria in fish farming systems [23]. The main goal of this system is to eliminate nitrogenous waste while promoting the growth of bacteria by increasing carbon/nitrogen ratio, mostly by adding an organic carbon source. Microorganisms associated with biofloc have three major roles: maintaining water quality, providing nutrients to cultivated aquatic species, and create probiotic properties.

6. ALGAE-BACTERIA INTERACTIONS IN BFT

Complex interactions are present between the various types of microorganisms (complementary or competitive) in BFT, and algae and bacteria in the system play a variety of stimulatory and inhibitory roles [24]. In the water the productivity of bacteria is increased, for instance, might be caused by higher primary productivity. This will enable heterotrophs to use the organic carbon by converting simple sugars into complex polysaccharides that algae may release. Algae are also short-lived organisms, and when they perish, more organic carbon becomes available for faster heterotrophic reproduction [15]. Minerals, vitamins, and other bioactive compounds that can promote the growth of phytoplankton and are present in organic debris are decomposed by bacteria. Bacteria and microalgae may be inhibited by antagonistic growth compounds such as anatoxin, microcystin, and hemagglutinin [24]. There may be some influence on nitrogen removal from the major microbial groups. This is because nitrifying bacteria only change the hazardous nitrogen metabolites into less toxic nitrate-N, while phytoplankton and heterotrophic bacteria are able to remove nitrogen from the system [25]. It is realistic to expect that a mature biofloc will serve a complementary role in removing nutrients from the culture medium after it has achieved equilibrium among the diverse microbial community. However, less hazardous nitrate may still be present in bacteria or filamentous bacteria in nitrification-dominated settings [4]. This happens as a result of filamentous bacteria that accumulate nitrate-N and release it when dissolved oxygen levels are low [26]. **Table 1** compares systems controlled by bacteria and alga.

Table 1: compares systems controlled by bacteria and algae

Character	Algal system	Bacterial system
Source of energy	**Solar energy**	**Mostly organic matter**
Occurrence	Low organic matter concentration ponds. Density of algae rises with nutrients readily available up to restriction of light	Dominant in pond having high concentration of organic matter, usually limited in ponds with little to no water exchange.
Sensitivity to environment-related factors	Light is necessary (activity is declined on overcast days).	Do not require light. Therefor able to adopt in variety of conditions.
Oxygen impact	Day time production of oxygen and night time consumption of it.	Only oxygen consumption is present.
Suitable activities	Primary production includes oxygen and organic matter. Uptake of ammonia	Decay of organic matter. Nitrification. Protein production by microbes.
Regulation of inorganic nitrogen	Primary production drives uptake of nitrogen. 0.7g $NH+/m^2$/day is the maximum capacity	The carbon nitrogen ratio in organic matter affects nitrogen uptake. Practically capacity is appeared to be unlimited.
Possible capacity	Daily primary production is typically not more than 4 g O_2/m^2	Limited because of constant rate of degradation and concentration of substrate.

Source: [69] Aquaculture Society

7. MICROBE AGGREGATION IN THE FLOC AND VARIABLES INFLUENCING FLOC FORMATION:

When a chemical coagulant is added to water, it facilitates the joining of the particles, resulting in larger aggregates that are simpler to separate. This process is known as flocculation. In addition to being frequently employed in water treatment facilities, the technique can also be used when processing samples for monitoring applications. A complex process involving numerous physical, chemical, and biological events is called microbial flocculation. Bacteria create extracellular polymeric substances (EPS), which are made up of polysaccharides, proteins, humic materials, and nucleic acids. A matrix is made of lipids and acids. Several other factors could also be behind this flocculation, such as: B. Dehydration through proton translocation [27], cation bridging and steric interactions. Variations in size and quality improve accessibility in an organism's life cycle. The main factors that affect floc formation are temperature, organic carbon source, mixing intensity, dissolved oxygen concentration, hydrogen ion concentration, and organic load rate (Floc volume index, floc size, communal representatives, nutritional aspects, etc.). All other dependent parameters are given much less weight than mixing intensities. For flocculation, extremely turbulent mixing that is kept in suspension is required. The unsuspended, settling microbial floc that create an anaerobic zone in the water produce toxic metabolites such as hydrogen sulphide, methane, ammonia, and other gases [28].

8. NUTRITIONAL VALUE OF BIOFLOC

A complete aquatic food source, biofloc deliver bioactive compounds and has a nutritional dynamic [29]. Because of this, it's crucial to examine the factors affecting the nutritional value of biofloc, including their capacity to consume and digest microbial protein and their density in water [15]. Red tilapia *Oreochromis niloticus,* mussel *Perna viridis,* and shrimp *Litopenaeus vannamei* all perform better in floc with particle sizes of > 100 and 48 µm because of their higher nutritional value and nitrogen recovery [30]. They also found that the highest concentrations of protein and fat were found in particles larger than 100 µm, whereas the most important amino acids were found in floc less than 48 µm in size.

Aquatic animals create a complex food chain that they use in the presence of biofloc and artificial diet, which is natural food supplemented with a designed diet. This is linked to improving aquatic species' growth performance in the biofloc system. Khanjani, Sajjadi [31] looked into how BFT affected feed conversion ratio (FCR) and discovered that clean water and BFT treatments had FCR values of 1.52 and 1.2-1.29, respectively. He also Compared to clear water treatment (1.52 for FCR and 66.81% for feed efficiency), the presence of biofloc can cause FCR to decrease (1.20-1.29), and feed efficiency to increase (78.61-84.26%). **Table 2** also lists the nutritional benefits of biofloc, from several studies.

Table 2: Different study record of Nutritional value of biofloc.

Crude protein (%)	Carbohydrate (%)	Lipid (%)	Crude fibre (%)	Ash (%)	Reference
13.50	_	0.35	7.43	61.40	70
35.00	–	5.00	6.00	5.00	71
35.20	_	2.40	_	15.30	72
26.91	_	2.05	_	32.04	31
31.70	_	3.06	_	28.23	73
30.04	24.50	0.60	_	_	74
20.50	20.00	0.50	-	-	74
21.60	16.00	0.50			74
13.97	16.99	_	0.33	48.46	75
66.03	_	3.00	0.87	6.46	76
67.98	_	3.67	1.08	6.48	76
35.78	31.73	16.32	_	10.17	77
29-34	_	6.5-90	7.00	7.80-10.80	78
28.70	_	2.30	_	43.00	79
32.74	_	_	6.62	22.34	80
38.00	----	3.00	6.00	12.00	81

21.30-32.10	-------	1.60-2.80	-----	43.40-61.40	[82]
33.40-37.60	17.10-25.50	0.40-0.50	-----	-----	[83]

9. NITROGENOUS COMPOUNDS IN BIOFLOC:

The presence of nitrogen and its derivatives, commonly known as nitrogenous compounds, play a significant role in maintaining water quality in aquaculture, particularly when following the biofloc technique. Therefore, assessing the water quality in aquaculture requires careful consideration of nitrogen, especially ammonia and nitrite. One innovative aquaculture production method, known as biofloc technology (BFT), has been introduced to address the longstanding problem of nitrogen toxicity in the industry. By regulating the carbon to nitrogen ratio (C/N), BFT facilitates the conversion of hazardous nitrogenous compounds into microbial protein, which can serve as an additional source of nutrition for the aquaculture species.[28].

Aquaculture species rely on nitrogen for various physiological functions, including the composition of tissues, fluids, and compounds such as proteins, nucleic acids, nitrogenous bases, pigments, and adenosine phosphates. The most common soluble forms of nitrogen found in aquaculture water are ammonia, nitrates, and nitrites. In biofloc systems, it's important to recognize that ammonia can exist in two different forms: unionized (ammonia, NH3) and ionized (ammonium, NH4+). To monitor water quality in aquaculture, total ammonia nitrogen can be estimated by examining the levels of nitrite-nitrogen (NO2 - N), ammonium-nitrogen (NH4+- N), nitrate-nitrogen (NO3 - N), and ammonia nitrogen (NH3- N), as these terms are widely used to describe the various forms of nitrogen present in the water. [14].

10. TOXIC EFFECT OF NITROGEN COMPOUNDS:

10.1. Ammonia

The primary waste product produced by aquatic organisms is non-ionized ammonia (NH_3) [32]. According to a gradient from a higher blood ammonia concentration to a lower blood ammonia concentration, normal ammonia excretion happens through the gills through diffusion as NH_3. The liver typically has the highest concentrations of ammonia, followed by skeletal muscle, kidneys, and the liver. The ionized form of ammonia is not hazardous to most aquatic organisms; however, the ammoniac form is extremely toxic. Total Ammonia Nitrogen (TAN) pertains to the two nitrogen forms that are linked to excretion in aquatic species. The rate of each molecule (NH3 or NH4+) depends on the pH of the medium in which it is present, whether it is in the blood or water. Consequently, an alkaline pH would lead to a lower proportion of NH4+ and a higher concentration of NH3, while an acidic pH would result in the highest concentration of NH3.[33].

Indications of intoxication in aquaculture species include the darkening of the eyes and body, frequent jerky movements, convulsions, spiral movements, and difficulty breathing. Systems that involve high-density culture with limited or no water exchange and a protein-rich diet can rapidly elevate TAN concentration and reduce the diffusion of ammonia through the gills due to the decreased gradients between the water and blood. [33].When facilitated diffusion is unable to remove ammonia, an excretion mechanism is activated that involves the use of ATPases in the exchange of sodium ions (Na+) and ionised ammonia (NH4+), which takes energy. [34].This process maintains the Total Ammonia Nitrogen balance both inside and outside of the fish, thereby reducing the toxicity of NH3. **Table 3** illustrates the lethal concentration of ammonia for various aquaculture species in different physicochemical water conditions.

10.2 Nitrite and nitrate

The pH and chlorine concentration (Cl^-) have impact on nitrite toxicity [35]. Acidic pH levels (about 5.5) are less tolerant of low quantities of nitrite than are higher pH values [36]. This may be because nitrous acid (HNO_2) is formed under these circumstances [37].

The following effects are associated with nitrite toxicity [38].

i) Competition with the entry of Cl- to the branchial exchange cells and the activation of the potassium ion (K+) exit within skeletal muscle cells and erythrocytes, leading to a loss of intracellular and extracellular potassium levels,

ii) Impairment of Na+ absorption in the gill exchange cells,

iii) Reduction in oxygen levels,

iv) Conversion of haemoglobin to methaemoglobin, which is incapable of carrying oxygen through the blood,

v) Increase in heart rate,

vi)Thyroxine (also known as T4) is a crucial component of your endocrine system. It controls a number of things, including your body temperature, mood, and metabolism. Inhibition of T4 hormone production, causing the kidney to retain water, and

vii) Changes in the excretion levels of urea and ammonia. Table 4 presents the lethal concentration of nitrite for various aquaculture species under specific physicochemical water conditions.

Nitrite causes a variety of physiological changes in aquatic organisms, including:

According to studies, an organism's susceptibility to these substances can depend on its species [38] size or developmental stage, temperature [39] salt, and pH [33]. The toxic concentration of ammonia and nitrite varies greatly, as seen in **Tables 3 and 4**. The toxicity of nitrite can be influenced by the proportion of calcium ions to chloride ions in water as well as other ionic components of water [36].

Table 3: Lethal concentration of ammonia nitrogen and un-ionized ammonia in some species used in aquaculture

Species	W/L	Ammonium Nitrogen /Un-ionized ammonia (mg/l)	Salinity in percentage	Temperature (%)	W C	Author
Ictalurus punctatus (Channel catfish)	3.5±0.0 g	1.29 [D]	Null	19.9	pH 7.6 Dissolved oxygen 5.4-13.60 mg/l Alkalinity 95-176 mg/l Hardness 118-209 mg/l	84
Pimephales promelas (Fathead minnow)	1.7±0.0b	2.55 [D]	Null	26.7	pH 8.1 Dissolved oxygen 5.4-13.60 mg/l Alkalinity 95-176 mg/l Hardness 118-209 mg/l	84
Litopenaeus. schmitti (Southern white shrimp)	1.9±0.6 g / 1.6±0.5 cm	41.72/1.66 [A] 54.52/1.90 [A] 55.32/1.97 [A] 32.67/1.57 [B] 38.70/1.60 [B] 47.77/1.44 [B] 24.68/0.98 [C] 26.76/0.99 [C] 41.76/1.25 [C] 19.32/0.79 [D] 25.65/0.76 [D] 38.98/1.22 [D]	8 26 33 7 24 33 4 20 30 6 24 31	19.6	pH 7.0-7.05 Dissolved Oxygen 7.80±1.50 mg/l	85
Piaractus mesopotamicus (Flat fish)	1.2±0.3 g / 1.6±0.4 cM	5.52/0.028 [A] 4.91/0.043 [A] 4.18/0.929 [A] 4.98/0.814 [B] 3.99/0.919 [B] 3.98/0.726 [B] 3.98/0.913 [C] 3.55/0.616 [C] 2.88/0.518 [C] 2.565/0.609 [D] 2.53/0.019 [D] 2.07/0.018 [D]	---	18 24 23 17 22 27 19 29 29 18 25 29	pH 7.0(6.88-7.45) Dissolved oxygen 7.8±0.70 mg/l Hardness 50 mg/l	39
Penaeus. monodon (Giant tiger prawan)	4.82±1.40g 91.4±9.1 mm	98.0/1.76 [A] 88.0/1.69 [B] 56.4/1.86 [D]	24	42.4	pH 6.77 Alkalinity 2.80 mg/l Hardness 3185 mg/l	86
Penaeus monodon (Giant tiger prawan)	0.27±0.06g 35.4±2.2 mm	97.96/2.98 [A] 63.09/1.93 [B] 49.47/1.55 [C] 47.58/1.89 [D]	22	28	pH 6.5 Dissolved Oxygen 7.8-6.7 mg/l Hardness 3300 mg/l	38
Oreochromis niloticus (Nile tilapia)	Larvae 0.056±0.008g Fingerlings 10.41±0.845 g	1.807-1.81 [B] 7.490-7.51 [B]	Null	23	pH 8.0 ± 0,2 Dissolved oxygen 7.2 0 mg/l Nitrogen Dioxide 0.001 mg/l	38

Litopenaeus vannamei (King prawn)	22.0±2.4 mm	63.72/3.05 [A] 66.68/3.03 [A] 67.75/2.98 [A] 40.98/2.90 [B] 49.03/2.66 [B] 54.04/2.88 [B] 32.75/2.59 [C] 43.77/2.01 [C] 45.03/5.82 [C] 24.79/1.80 [D] 35.80/1.87 [D] 39.94/2.00 [D]	25 15 37 17 26 39 14 27 38 17 24 39	25	pH 8.15 Dissolved oxygen 6.12±0.60 mg/l	87
Fidicinoides pauliensis (paulo shrimp)	Egg stage Naupliis Zoeae Myses Postlarvae Juvenile 5.45±0.4 g Adult 31.43±1.3 g	6.25[A] 122.3 [A] 45.80[B] 29.59[C] 23.93 [A] 14.93 [B] 11.94 [C] 9.99 [D] 75.07[A] 41.80[B] 32.85[C] 21.98 [D] 24.19 [A] 8.59/ [B] 5.65 [C] 5.49 [D] 1.87[A] 3.11[B] 0.25 [C] 38.92 [D] 1.67[A] 0.55 [B] 6.57[C] 2.49 [D]	28	25	pH 7.71-8.24	88
Fidicinoides carpio (European carp)	570±0.00g	123/45.5 [A]	Null	19-22	pH 7.1-7.5 Dissolved oxygen 7-8 mg/l	89

Table 4: Determine the Lethal concentration of nitrite in the warm water species of aquaculture.

Species	W/L	Lethal concentration of Nitrite (mg/l)	Mortality (%)	Salinity (%)	WC	Author

Penaeus monodon	5.07 ± 2.4 g / 91.6 ± 9.0 mm	220 ^{A} 198 ^{A} 177 ^{A}	53	25	Potential hydrogen 7.57 Temperature 24.50 C° Alkalinity 1.60 mg/l Hardness 3180 mg/l Calcium carbonate	86
Penaeus monodon	0.77 ± 0.16 g/38.4 ± 2.12 mm	219.85 ^{A} 185.83 ^{B} 88.94^{C} 54.96 ^{D}	52	24	Potential Hydrogen 7.9 Temperature 28 C° Dissolved Oxygen 5.9-6.6 mg/l Alkalinity 83 mg/l Calcium carbonate Hardness 3300 mg/l Calcium carbonate	38
Prochilodus.magdalenae	0.4-6.6 gram	4.2 ^{D} 133 ^{D} 223 ^{D}	25	Null	Potential Hydrogen 7.4-7.6 Temperature 27 C° Alkalinity 24-27.6 mg/l Calcium carbonate Hardness 21-26 mg/l Calcium carbonate Chloride 6-9 mg/l	90
Litopenaeus vannamei	4.34 ± 1.42 gram / 56.89 ± 9.66 millimeter	188.9 ^{A} 275.1 ^{A} 526.2 ^{A} 144.2 ^{B} 245.0 ^{B} 425.9 ^{B} 92.76 C 224.98 ^{C} 376.0 ^{C} 76.58 ^{D} 178.39 ^{D} 321.79 ^{D}	51	16 28 36 18 23 39 17 29 34 18 29 32	Potential Hydrogen 8.12 Temperature C° Dissolved Oxygen 6.8-0.2 mg/l	87
Piaractus brachypomus	39.9 ± 0.19 gram	5 ^{B} 7 ^{B} 18 ^{B} 35 ^{B}	26 48 14 58	0.45 ±0.906	Potential Hydrogen 5.27 ± 0.94 Temperature 28 C° Dissolved Oxygen 7.7 ± 0.2 mg/l	91
Brycon amazonicus	69.1 ± 13.9 gram	0.6 ^{D}	50	0.05 ± 0.006	Potential Hydrogen 5.7 ± 0.04 Temperature 24 C° Dissolved Oxygen 7.7 ± 0.2 mg/l	91

11. MANAGEMENT OF NITROGENOUS COMPOUNDS IN BFT:

In biofloc technology systems, microorganisms like algae, autotrophic bacteria, and heterotrophic bacteria reuse nitrogenous compounds arising from residual feed and metabolism products (tilapia or shrimp). The nitrifying bacteria (autotrophic bacteria) can nitrate (convert hazardous ammonia to less hazardous nitrates) in an aerobic environment. Two types of autotrophic bacteria, chemolithoautotrophic bacteria (an autotrophic microorganism that obtains energy by oxidizing inorganic compounds) and ammonia-oxidizing archaea (AOA), carry out the nitrification process by oxidizing ammonia to nitrite [40] via hydroxylamine. The second and final stage of nitrification is achieved by nitrite-oxidizing bacteria after ammonia oxidizing bacteria (AOB) have finished the initial step (NOB). This particular bacterial group is in charge of converting nitrite to nitrates, a form of nitrogen that is usually safe for fish to consume up to a concentration of hundred milligram /Liter [41]. In the field of aquaculture, DC. Sigee's research has introduced an alternative technique to remove and regulate nitrogen from water called denitrification, which converts nitrates to gaseous molecular nitrogen (N2). Unlike autotrophic bacteria, heterotrophic bacteria are responsible for this process, which immobilizes nitrogen compounds and converts them into protein that serves as a secondary feed source for the culture organism. Previous studies have primarily focused on heterotrophic ammonia removal from biofloc systems due to their advantageous operations and their ability to outperform harmful bacteria in the system. Another method of eliminating ammonia that is underutilized is the dissimilatory reduction of nitrite to ammonia and its conversion to nitrogen gas, a process that some bacteria directly degrade during nitrification. [42].

12. TYPES OF CARBON SOURCE IN BIOFLOC TECHNOLOGY:

Carbon is a particularly important nutrient for biofloc effectiveness because it is used as food sources for the bacteria that are important in biofloc (or zero-water-exchange) farming.

Categorization based on chemical composition:

12.1 Carbohydrates:

For the majority of heterotrophic bacteria, carbohydrates may be the most plentiful organic carbon source. Typical carbohydrates include glucose, sucrose, starches, molasses, and cellulose [43]. Due to their high cost, organic carbon sources such as dextrose and glucose ($C_6H_{12}O_6$) may be commercially impractical [44]. Sucrose ($C_{12}H_{22}O_{11}$), which is naturally present in the majority of plants and is widely available in markets, has proven effective at facilitating bacterial digestion of NH_4 –N [43]. In Biofloc technology systems, there are additional varieties of complex carbohydrates, including as starch, molasses, and cellulose. A naturally occurring, high-nutrient carbohydrate is starch ($C_6H_{10}O_5$).

12.2 Organic acid and alcohols:

The production of biodiesel results in the creation of glycerol (C3H8O3), which can serve as a substitute carbon source for industrial bioprocesses due to its high degree of reduction potential. Additionally, glycerol has been shown to facilitate the production of nutrient-rich biofloc [45]. Denitrifying bacterial cultures can directly use acetic acid through a straightforward bio-degradation pathway to produce acetyl-CoA [46] (acetyl coenzyme A). Sodium acetate (CH_3COONa) has been used as a readily degradable source of carbon for biofloc technology systems in several studies. [45]

12.3 Biodegradable polymers:

The release of dissolved organic carbon (DOC) by biologically degradable polymers (BDPs) is facilitated by microbial enzymes [46]. Outer organic sources of carbon such as polyhydroxyalkanoate (PHA), poly(3-hydroxybutyrate) (PHB), polycapro-lactone (PCL), and polybutylene succinate have been utilized to modify denitrifying conditions or processes [47]. Utilizing BDPs as organic carbon sources has the primary benefit of lowering the possibility of overdose or scarcity with minimal control. PHAs are polyesters made from distinct hydroxyalkanoates that are produced by different microorganisms [48].PHB is made up of tiny ($C_4H_6O_2$) monomer units that are synthesized by bacteria as an energy storage molecule, particularly in low-nutrient environments [49]. **Table 5** shows the effect of different carbon sources on cultured organism in biofloc and their impact on biofloc characteristic

12.4 Impacts of different carbon sources on the efficiency of the BFT system

It has been discovered that carbonaceous substrates in BFT systems have a variety of effects on BFT systems, including bacterial populations, water quality, culture organisms, and biofloc characteristics. These impacts might result from items that have degraded the effectiveness of maintaining the C/N ratio, or other unidentified variables. The mechanism causing these effects has not yet been the subject of any study. The impacts of carbon sources on the aforementioned components are covered in more detail in the following subsections.

13. BACTERIAL COMMUNITY

To promote the growth and activity of heterotrophic bacteria that assimilate inorganic N into their biomass, we alter the carbon-nitrogen ratio. Water quality in maintained by BFT aquaculture[4]. This results in the formation of communities of microorganisms, such as algae, bacteria and protozoa, which are found in detritus and other organic particles [50]. Despite the fact that heterotrophic bacteria are stimulated to grow by carbon sources, these sources have different effects on bacterial communities [51]. For instance, they may have an impact on the quantity, diversity, and relative abundance of bacteria.

13.1 Bacterial counts

Microbial communities in biofloc aggregates are responsible in eliminating water exchange and producing microbial proteins that can be used as supplemented feed Because they change the metabolism of bacteria in BFT systems, carbonaceous substrates can change the quantity or population of bacteria [51]. Different bacterial types can affect bacterial populations as a carbon source, especially autotrophic bacteria and some heterotrophic bacteria gain energy from organic molecules [28]. Compare this mechanism to how organic carbon molecules affect chemosynthetic nitrifying bacteria, which acquire their energy from inorganic sources [14]. As a result, carbon-supplemented systems have larger bacterial biomass than carbon-deficient ones. The specific bacterial communities that prevail in BFT systems need to be identified through studies [52].

13.2 Relative abundance

Despite the fact that only heterotrophic biofloc systems are expected, chemo-autotrophic bacterial population activities have been seen [53]. Therefore, populations of these two types of bacteria can both find the best conditions for development in BFT systems. The carbon-nitrogen ratio in the culture water, the type of carbon source employed or the number of suspended particles could all be the contributing factor of this phenomenon. [52]. To promote the growth of heterotrophic bacteria in BFT system, glycerol and glucose has been used [50].In addition dextrose and molasses(highly soluble carbonaceous substrates), have been reported to significantly support heterotrophic bacterial populations in BFT [22]. In BFT systems, phytoplankton communities can be impacted by the carbon supply in addition to bacterial communities [54]. In BFT treatments employing treacle as an organic soluble carbon source, chlorophyll a was also seen to accumulate more, indicating an abundance of the microalgal population in the system. Because these phytoplankton are

Table 5: Organic Carbon sources, additional strategies, effects on culture organism, water quality and bio-floc characteristics

Ratio of Nitrogen and Carbon	Supplemental plans	Cultured Organisms	Impact on growth	Impact on quality of water	Impact on characteristics of biofloc	Reference
To start up,.4.33 kilogram of food put in every vessel. During the substantial period no other fertilizer was added	Depended on feed's input carbon concentration, additional carbon source was not added	*Litopenaeus vannamei (Whiteleg shrimp)*	With a mean value of 71+- 8% the rate of survival was same for all therapies. The different feed used do not had considerable differences in Food conversion ratio. While in groups with solids food conversion ratio was lower.	Despite no additional carbon being introduced. For shrimp water parameters did not build up to harmful standards. pH somewhat decreased, however NaHCO3 was used to restore it.	In this study, proximate composition of floc was not determined. However, growth performance of shrimp was better due to controlling floc level(Total suspended solid and VSS).	92
Wheat, gramme, rice, corn, milet, Maida, molasses and multigranular flour all have carbon nitrogen ratio greater than fifteen	According to the derivation of Avni Melech (1999), which is 0.465 feed, carbon sources were put in.	*Litopenaeus.vannamei (Whiteleg shrimp)*	Growth indices such as; Average Daily Gain For millet, molasses and multigranular treatment Food Conversion Ratio, Sustainable Growth Rate, Survival, and ADW were similar. In general, millet and multigranular therapies had low death rates. In these therapy groups, it was also shown that immunological markers like SOD, Mn Super oxide dismutase, and BGBP had increased levels.	The predominant oxidation products of Total ammonia nitrogen and nitrites were nitrates, and in the Carbon supplemental groups the quantities of Nitrogen subsequently decreased. It kept the quality of water indicators within the orbit that is ideal for growth of shrimp	There was high protein quality in molasses, millet and multigranule treatments flocs because limiting amino acids (arginine, methionine and lysine) were identified.	51
wheat flour, corn flour, sugar, twelve ratio one	In order to establish the proper Carbon nitrogen ratio for heterotrophic bacterial growth, one gram per hundred Litre of carbon source was given. In particular,2.7g of sugar, and 3.5g of corn flour,3.7g of wheat flour were included.	*Oreochrmis niloticus (Nile Tilapia)*	The sugar group had the largest fish weight among the carbon treatments. The final Food Conversion ratio was in the range of 1.28 to 1.51, and significant variations between the various carbon source treatments were found. Overall, the sugar and corn flour groups both showed same zoo technical qualities.	For total ammonia nitrogen (0.15, 0.30, 0.33mg/l, respectively) and levels of nitrates, there was no considerable difference between the wheat flour, corn flour, and sugar treatments.	Total suspended solid collected more when wheat flour was added with Carbon recorded the highest levels (226.6 mg/l). But neither the estimated conformation nor any additional floc features were mentioned.	93

Molasses, corn flour, wheat bran carbon nitrogen ratio greater than sixteen	Different combinations of sources of carbon had evaluated, but 60 % molasses + 20 % maize flour + 20 % wheat bran proved to be the greatest addition for shrimp growth.	*Litopenaeus.vannamei* (Whiteleg shrimp)	In the biofloc treatments. food conversion ratio and survival rate was same.In relation of the combination of various C source , the group that consisted of 60 percent molasses, 20 % maize flour, and 20 % wheat bran showed the highest growth efficiency.	Water quality was maintained within acceptable range by all carbon groups to grow shrimp with the irregularity in pH that because of carbon source inclusion decreased rarely to non- optimal levels.	There was effect of Carbon source on protein content of biofloc (23.95–32.32%) and lipid content of flocs (2.92–5.33%). From all carbon treatments activities of protease, amylase, cellulose and lipase were recorded in flocs.	94
Molasses, put in every 4th day, ten ratio one to twenty ratio one	On the basis of protein content of the feed carbon nitrogen ratio were determined. Tilll the end of the experiment carbon was put in with an interval of four day.	Youngs of *Oreochrmis niloticus* (Nile Tilapia)	The lifespan of young of tilapia was same though carbon nitrogen ratios were not similar in biofloc treatments. But greater because molasses had put in as extra carbon source. In some biofloc therapies employing molasses as the carbon source, growth responding measures like gain in weight, total biomass,gain in weight per day, and final weight were at their maximum.	The oxygen levels dropped (3.2-1-1.5 mg/l) in an early experiment where molasses was added to the pulse (>0.12 gram/l). As a result, the levels of total Nitrogen, ammonia, nitrates, and nitrites in the water were kept at a level suitable for tilapia. While, the inclusion of molasses caused the pH to drop more gradually.	Flock concentration was highest in the treatment with Nirogen Carbon ratio 1:20 employing which determined by molasses inclusion. Without molasses addition, it was 200%higer in magnitude than the control.	93
Sucrose is just employed during the initial phase or floc formulation phase	During the onset phase, fifteen grams of sugar were supplied if the ammonia concentration was 1.5 mg per liter or greater.	*Litopenaeus.vannamei* (Whiteleg shrimp)	In the biofloc technology treatment life span of shrimp was greatest (86.2 T 1.7%) than other groups. , In biofloc technology group Food conversion ratio (1.1 T 0.1) and specific growth rate(1.4 T 0.1%) were superior than non-biofloc technology treatment.	Turbidness was greater in BFT treatment (15.1 T 5.7 NTU). As compared to non biofloc technology Total ammonia nitrogen,nitrogen dioxide and nitrate concentrations were higher (1.5 T 0.8, 9.2 T 4.5 and 21.4 T 8.8mg L-1,accordingly)in the Biofoic technology group. This may be because sucrose was only applied during onset.	In Biofloc technology treatment higher N isotope level tell that the flocs act as additional protein source for the shrimp. Hence, there as great nutritive value of bioflocs.	95
No additional carbon	In the BFT group, in situ carbon flocs were used as the only carbon source; and there was no additional source of carbon.	*Litopenaeus vannamei* (Whiteleg shrimp)	Without any additional carbon source, the Rular advisory service group (1.8 T 0.1) outperformed the biofloc technology group (1.5 T 0.1) in terms of growth indices like total biomass, harvest weight, and Food conversion ratio. The ultimate average weight of	In Biofloc technology treatment minimum rate of ammonia was detected than rular advisory service However, pH decreased in biofloc technology than rular advisory service. In Biofloc technology group Turbidity, nitrite and nitrate were higher than rular advisory service.	Since C13 was higher in the biofloc technology than the Rular advisory service, flocs were used as the carbon source. According to isotope analyses, bioflocs gave shrimp roughly eighteen % and sixty % of their carbon and sixteen % of their nitrogen	92

			shrimp treated with Rular advisory service was greater than that of shrimp treated with Biofloc technology			
In the first four weeks, molasses utilised. When Total ammonia nitrogen exceeded 0.6 mg/l, 15:1 sugar cane molasses was utilised.	Molasses was added according to input nitrogen that is anytime total ammonia nitrogen surpassed 0.6mg/molasses included to regulate nitrogen. Anytime total ammonia nitrogen surpassed one mg per liter carbon source was added for the maintenance of carbon nitrogen ratio.	Youngs of *Litopenaeus vannamei* (Whiteleg shrimp)	The shrimp acquired a specific growth ratio of 6.9 % daily and total biomass was 2.17 T 0.05g with food conversion ratio of 0.87 T 0.08	Nitrogen compounds, phosphorus and phosphate continuously collected. In total inorganic Nitrogen, nitrate reached up to 80%.	Less food conversion ratio tells that the flocs were highly nutritive for shrimp	96
sugarcane molasses fifteen ratio one	Every time carbon source was put in total ammonia nitrogen surpass one mili gram L-1 to maintain carbon nitrogen ratio	*Lipopenaeus vannamei* (White shrimp)	All treatment the shrimp survival was greater than ninety-five %. Although, among treatment with distinct floc sizes end values for weight, biomass, and weight increase vary importantly with the exception of one fifty and three hundred micre meter floc size group	Throughout the trial, ammonia level fluctuated between 5 and 0mg/l. After day 13, nitrate fluctuated up to the study's conclusion. By the end of the research, nitrate levels had reached 15 mgL^{-1}.	Sizes of floc (such as 50,150, and 300 um) had little impact on the nitrification process.	40
Molasses 6gCVS1g total ammonia nitrogen, carbon and nitrogen 15:1	Every time carbon source was put in total ammonia nitrogen mass 0.8 mgL-1 or higher. Avnimelech served as the basis for the induction (1999).	*Lipopenaeus vannamei* (Whiteleg shrimp)	In biofloc technology groups the elevated biomass and yield rating was set down make use of Na2CO3 and the pH-correcting agents CA(OH)2. The final biomass run to 630.0 T 61.1 and 615.7 T 93 g, sequentially. In addition, for two groups the yield rates were 2.3 T 0.1 and 2.2 T 0.1 kg m-3, sequentially.	In the group with no carbonate administration total ammonia nitrogen levels differ commonly. Regardless of the alkalinity manipulation the NO2 content was maintained below 25.7 mg/l that was within shrimp acceptable levels.	All treatment had microbial floc mass beyond those necessary for shrimp production, which were subsequently brought under control eliminating some floc and total suspended solids.	97

Molasses20 g C VS 1 g total ammonia nitrogen	Every time molasses was put in total ammonia nitrogen surpass one mili gram L-1 and based their deliberation Avnimelech (1999)	Children of *Litopenaeus vannamei* (White leg shrimp)	Shrimp survival rates were higher in lower floc levels than in higher levels. However, across all floc treatments, the average weight growth and end weight were comparable.	Then higher floc level groups (400-600 and 800-1000mg/l), lower floc level groups had higher PH (7.8).	The amount of biofloc has an impact on the protein amount of floc. Then higher floc level group (20.2% and 18.6%, consequently), lower floc mass had higher CP (28%).	98
Sucrose Carbon nitrogen 15:16 g C VS 1 g total ammonia nitrogen	According to Avnimelech (1999) and Ebelimg *et al*. (2006), daily additions of sucrose required six grammes of carbon to transform just one gramme of total ammonia nitrogen.	Children of *Litopenaeus vannamei* (White leg shrimp)	The pH 8.1 (14 51g) group had a higher final weight than the pH 7.6 (13.76g) group. PH 8.1 had a higher survival rate (87.5%) than pH 7.6(80.8%) than the control (71.7 %). Food conversion ratio was lower and did not change across the pH-adjusted (7.6 and 8.1) groups, but it did in the biofloc technology control group that did not get a pH adjustment. Shrimp phagocytic activity and tetra hydrocannabidinnol levels were higher in the pH 8.1 and 7.6 groups compared to the control group, that was considerably less.	The group with a high pH (8.1) had more alkalinity. The pH (8.1) group had a lower total ammonia nitrogen concentration (0.25mg/l). NO2 in the pH (8.1) group was considerably less than in the pH 7.6 group and the control group(9.08mg/l).	In contrast to the control, the pH 7.6 and pH 8.1 groups had increased Poly-β-hydroxybutyrate (PHB)concentration in the flocs. However, all groups' flocs had identical amounts of carbohydrates and carotenoids.	44
Rice bran carbon nitrogen 15:1 and wheat milling by-product	Two hours after feeding daily adding up of carbon inception	*Oreochrmis niloticus* (Nile talipia)	In biofloc treatment fish growth production was greater than clear water group. Especially, in the group where the carbon source was wheat grinding by products weight gain,feed conversion ratio etc were higher in biofloc group.Globulin, albumin, total humoral innate immunity and protein parameters (such as lysozyme) were non- specific immunological variables that were noticably elevated.	Dissolved oxygen, temperature and salinity did not affect by carbon source. Although, total ammonia nitrogen, nitrogen dioxide and nitrate were affected by different carbon source importantly. In biofloc treatment nitrogen level was greater than clear water group	Floc size was high in the group employing wheat milling by-product as C source.	99

Glucose Carbon and nitrogen ten, fifteen and twenty	Every third day Glucose (40% C) was put on the based on the amount of protein in food. 15.6g of glucose to 10g must be present in the meal.	*Oreochrmis niloticus* (Nile talipia)	In C/N 10 and 15 group the growth production of talipia was greater than carbon and nitrogen 20 group. In carbon and nitrogen 10 and 15. Trypsin and lipase, as well as immunological indicaters like lysozyme and alkaline phosphatase, were also measured greater than carbon and nitrogen 20	In carbon and nitrogen 20 group NO2 levels were lower than the carbon and carbon nitrogen 15 groups. In all groups total nitrogen ammonia vary generally.	The colours of flocs were affected by carbon and nitrogen ratio. The browner flocs intensity was correlate with increasing carbon and nitrogen ratio.	57
Tapioca starch 16:1	Every day adding up of tapioca starch according to input N from food	*Oreochrmis niloticus* (Nile talipia)	Average daily growth rate was greater compared to the control (1 day); the tapioca treatment was (1-4 day). In addition, the tapioca treatment (ninety-six %) survival rate was higher as compared to control.	In carbon group, total nitrogen oxides and ammonia nitrogen assembled at steady rates than the control. This suggested the formation of nitrification slow up by adding carbon	Increased floc concentration and carbon and the amount of nitrogen in the floc by addition of tapioca than the control treatment	23
Sodium acetate	According to Gao et al (2012).'s technique C inception was calculated as follows: 0.465* feed* food protein content.	*Oreochrmis niloticus* (Nile talipia)	In Biofloc Technology treatment fish weight/ individual was twenty-two % higher than recirculating aquaculture system treatment from Biofloc technology treatment the particular growth rate and total weight in fish were, sequentially, one twenty-eight % and one hundred twelve % greater than recirculating aquaculture treatment. Feed conversion ratio was eighteen % lower in biofloc technology than recirculating aquaculture system.	Biofloc Technology data elevated mean levels of total ammonia nitrogen (60 T 0.45mg/l) and NO-(119T2.0.1mgL-1) as compared to the recirculating aquaculture system. In biofloc technology NO growth was not notice	The biofloc technology flocs' impurified protein concentration was 30.90T9.04 %, which was appropriate for tilapia nutrition.	100
After feeding glucose was put in, polyhydroxy butyrate and precaudal length carbon Nitrogen of 16.84	After feeding 46.5 % of tarate glucose added, Poly hydroxy butyrate and precaudal length was supplemented with carbon by placing twenty gram of the particles in	*Oreochrmi niloticus (Nile tilapia)*	Fish from the biofloc system have greater all superoxide dismutase task than recirculatory aquaculture system ultimate biofuel had same in the Precaudal treatment and poly hydroxy	During steady condition NO_3 concentrations and total ammonia nitrogen in aquaculture were below three mg L−1and 0.8mgL−1, respectively. Ongoing nitrification, In all group's total ammonia nitrogen in aquaculture and	CP (crude protein) content of floc was same and varied from 31 to 39% for poly hydroxy butyrate and Precaudal length treatment. Further, Crude protein did not dissimilar between glucose procedure and poly hydroxy butyrate	45

	eight polyethylene containers, which were then hung in the tank		butyrate (37.93 kilogram m−3) and same between glucose ministration (44.1 kgm−3) and poly hydroxy butyrate	Nitrification and assimilation of NO3 was observed.		
There were two categories of carbon source additions; through the initial weeks (INI) only molasses was used every day sugarcane molasses supplements (CONT),12:1	Molasse was added daily to the system in the treatment	Juveniles of *Litopenaeus vannamei (whiteleg shrimp)*	The surviving estimate of krill in Initial weeks was greater (76.9 T 6.7%) than in the CONT group (57.0 T 8.6%). Also, Food conversion ratio was lower in the Initial weeks group (1.5 T 0.1) than CONT group (2.4T0.0). Final biomass in Initial weeks (1.8 T 0.3 kg m−3) was considerably greater and less in the CONT group (0.8 T 0.0 kg m−3)	Both treatments, with carbon nitrogen proportion of 6.41 T and 7.5 T 1.6, subsequently, had less carbon nitrogen proportion at quasi static. Additionally, all two classes had less biological oxygen demand: total suspended solid proportions. Compared to the CONTROL group sludge buildup was more steady in initial group. Values of Total ammonia nitrogen in aquaculture, NO—2, NO—3, temperature and oxygen were same in both classes	Total suspended solids accumulated greater among group CONT (i.e., 0.25-kilogram kilogram−one of given feed) than group initial weeks The Total suspended solids accumulation in initial weeks group was 0.16-kilogram kilogram−one of feed	101
Mature bioflocs used No supplemental carbon was used	Mature biofloc with already established niotrification condition and which was also in stage of mixotrophy or chemotrophy was used. Thus, no supplemental carbon was put in	*Litopenaeus vannamei (whiteleg shrimp)*	lesser weight gainis resulted when less protein feed (24.3) % wass added in biofloc treatment, final weight values and specific growth rate than biofloc groups fed with high protein content feed (i.e.,30.3%,32.9% and 36.7%)	Levels of pH (7.48–8.56), Dissolved oxygen (4.7 to 6.3mgL-1), T (27.9–30.2°C) and salinity (20–21%) were suitable for shrimp culture in all biofloc treatment. Same as, Total ammonia nitrogen in aquaculture, phosphate (3.36–4.91 mg L−1), NO2 (0.05–0.31mgL−1) and NO3 (7.4–79.9 mg L−1) were within the bearable restriction for krill	Biofloc volume and Total suspended solids was high and showed considerable difference on day 14 of the experiment resulting in beginning the elimination of some flocs.	102

glycerol, sucrose and molasses Carbon nitrogen 22:1	Daily every kind of carbon source was put in for two times between the feeding time to maintain C-N ratio of 22:1 in heterotrophic based tanks.	*Litopenaeus vannamei (whiteleg shrimp))*	In all groups, surviving estimate for krill same along carbon supplements (sucrose, molasses and glycerol) with values of53.2% 21.6% and49.2%, respectively, except that the values foe molasses and source groups differ considerably GR (growth rate) by week was 0.7-o.1g for CA0.3-0.2g for molasses treatment, 0.7 T 0.0 g for sucrose treatment and 0.6 g for glycerol treatment.	In the heterotrophic classes (H), as compared to chemoautotrophic based group(0.2mg/l), total ammonia Nitrogen was higher (0.3-1.1mg/l) .NO-level did not differ in all treatments (0.2-1.5mg/l). NO—3levels was considerably higher (9.08mg/l) in Chemo autotrophic group compared to Heterotrophic group (0-1.4mg/l). Basicity was greater in heterotrophic group(276-352mg/l) than the chemoautotrophic group(169mg/l).	Particular features of flocs were not reported. although, during latter stages of experiment, a noticeable increase in solids was recorded in the glycerol and sucrose procedures. Variability in settle able solids was relatively greater; although there were no considerably differences notices in groups.	103
Starch Wheat, feed in 20:1 (carbohydrates /Total ammonia nitrogen	On the basis of Avni Melech starch Wheat was put in according to sixty % of the feed added	Mixed sex *Oreochrmis niloticus (Nile tilapia)*	Fish welfare indicators such as blood haemoglobin concentration, fin condition, serum hydro cortisone, within a stone throw configurations, gill histology were unaffected by the treatments (thirty five % feed, twenty four % cater for and control category). In Biofloc technology reserviores, net fish manufacturing amounted to forty-five % more than in the control group	In the biofloc groups wide fluctuations in alkalinity was noticed (eight –two hundred fifty mg L−1). although, steadiness in the degree of all basicity (eighteen –twenty-seven mgL−1) in the command without carbon supplements was noticed. Total ammonia nitrogen in aquaculture, NO—2and NO3 wide variability in biofloc technology categories as compared the con	In all biofloc technology groups nutritional parameters of floc were same. In biofloc technology group Flocs constituted twenty-seven –twenty-eight % polyunsaturated fatty acids and twenty-eight –twenty-nine % mono unsaturated fatty acids.	81
Tapioca starch and Plant cellulose Carbon nitrogen scale. 13.0–14.7	Every day additing carbon inception 0.6 gram/g of totted cater for twice.	*Pelteabaggrus vachelli*	As compared to other treatments in tapioca starch Weight gain ratio showed considerably greater value (94.47 %). Specific growth rate was considerably greater in Tapioca starch (1.58 T 0.20 %) and short in the command category (1.19 T 0.20 %). in other ways Food conversion ratio exhibit same rate in	In carbon supplemented group Total ammonia nitrogen in aquaculture was generally lower (i.e., 2.4, 1.8 and2.2 mg/l), subsequentially, for Tapioca starch and plant cellulose VS that of control (3.6 mg Liter−one). NO—stayed below 1.5 milligram L−1till the ending. Dissolved oxygen carbon was considerably greater among Tapioca starch (sixteen	In carbon Supplemented groups and the control biofloc volume didn't differ. Total suspended solids were greater in biofloc technology groups (90.1 T 30.3 mg L−1) than the control (51.9 T 22.6 mg L−1). In plant cellulose treatments sludge volume index (SVI) was greater (PCwas51.7 andTS+PCwas53.2mgL−1) than the control group (34.6 mg)	60

			entirely procedures (2.02–3.11) encompasses the command category	milligram Liter−one) as compared to supplemental categories in order of Tapioca starch > Tapioca starch and plant cellulose > Plant cellulose > Control		
Molasses	Molasses was put in to the Biofloc technology reserviores at any moment Total ammonia nitrogen in aquaculture surpass 0.5 miligram Liter−one. Ebeling et al (2006) and Avnimelech(2009) states that molasses was put in formed on the computation of six gram carbon for every one gram total ammonia nitrogen	*Litopenaeus vannamei (Whiteleg shrimp)*	When compared to the regulated classes with zero % enhancement, the krill ultimate weight was greater in the biofloc cultivate (twenty-five %, fifty %, seventy % and hundred %: i.e., 8.01 to 8.42 gram) classes (7.37g) In all treatments surviving estimate didn't considerably dissimilar and vary from 90.93–99.06% than the the regulatd categories (1.52) Food conversion ratio was considerably less in the biofloc-supplemented categories (0.84 to 1.23)	Total ammonia nitrogen concentration was considerably lessin the biofloc- supplemented categories (vary from 0.0001–1.5 miligram Liter−one) than observed in regulated categories.NO—2concentration was considerablygreateramong Regulated categories (10.11 miligram Liter−one) than biofloc-supplemented classes (0.54–1.85 miligram Liter−one)	Total suspended solids concentrations were same in all treatments (three hundred eighty-three –six hundred sixty-three mg/l) except the hundred % biofloc enriched group which was considerably greater in Total suspended solids (714mgL−1). Levels of Settle able solids were considerably greater in the biofloc-enriched groups (12.51–16.20 mg L−1) than the control (8.48 mg L−1). Levels of settleable solids were considerably greater in biofloc enriched-groups (12.51-16.20mg/l) as compared to control group (8.48mg/l).	104

communities are numerous, the resulting biofloc may include larger concentrations of crude lipids and fatty acids, improving their nutritional value [55].

13.2 Water quality

By manipulating C/N, it is possible to maintain ideal water quality for target culture species when employing carbonaceous substrates in BFT systems. But maintaining a healthy C/N ratio is known to be important for a number of processes, such as the growth of nutrient-rich biofloc, a decrease in the concentration of total ammonium nitrogen (TAN), and perhaps even an improvement in water quality [51]. Variants in the C/N ratio N influences the competition between autotrophic and heterotrophic bacterial communities through supplied carbon substrates and thereby affects water quality[51]. In BFT systems, carbon substrates like glucose, glycerol, acetate, molasses, and starch are frequently employed to regulate the carbon-nitrogen [17].

13.3 Dissolved inorganic nitrogen:

TAN (total ammonia nitrogen) control at low concentrations or quantities that are less hazardous to the farmed organism is the main problem in aquaculture practise [56]. Because of this, TAN is kept at safe levels in biofloc aquaculture systems using carbonaceous substrates. TAN levels in BFT systems are impacted by carbon source effects and addition techniques. For instance, maintained total ammonia nitrogen at safe level (0.096-0.02) for the growth of shrimp by utilising molasses. Similar to this, [57] observed positive outcomes when sugarcane treacle was utilised to keep TAN levels appropriate for shrimp development. When comparing the efficiency of sugarcane and soybean molasses as a carbon source in BFT, identical TAN levels were found in another study [58].

13.4 Carbon source's impact on TSS or floc volume

Due to impact of C addition on the flocculation process in BFT aquaculture systems, TSS or higher floc levels has been seen in biofloc system[59]. It has been discovered that nutrient dynamics, processes, and routes in BFT systems are affected by flake volume (FV) or TSS (Luo et al. 2019). The carbon source does not significantly alter floc volume, as shown by Deng, Chen [60]; nevertheless, a significant effect was seen for TSS concentration (Table 5). Compared to the release rates of other types of carbon sources, some carbon sources may discharge carbon more slowly (BDP) (e.g., glucose, sucrose, glycerol). Consequently, depending on the type of carbon chosen, this phenomenon may alter either the TSS concentration or floc volume. Several reports have been written about this aspect of BFT aquaculture systems.

13.5 Effect on Cultured organisms

The important impact of various carbon sources on culture organism performance, including growth, welfare, immunological state, and health of aquaculture species, has been shown by BFT investigations [61].

14. APPLICATIONS OF BIOFLOC

In Pakistan, biofloc fish farming is one of the most lucrative and successful industries. In addition to meeting its own local need for various fish species for consumption, Pakistan is also able to fulfil orders from overseas.

- The technique used to improve water quality in aquaculture by balancing the carbon-nitrogen ratio in the system is called biofloc. Biofloc technology can improve aquaculture systems by achieving a balance between carbon and nitrogen through photosynthesis and nitrification, as indicated by the following citation. [4].
- The bacterial biofloc system heavily relies on bacteria, and actinobacteria are particularly significant as they promote biofloc formation and offer additional advantages by safeguarding fish against pathogens. [57].
- The Biofloc system is used as dietary stimulants. Common carp grow faster and have higher levels of digestive enzyme activity when biofloc are included in the diet at BFT 75%. Penaeus monodon also grow faster and have higher levels of digestive enzyme activity when biofloc is added to shrimp feed at 4% [62].

- Biofloc have recently been proposed as a potential novel disease treatment strategy with a natural probiotic impact, in contrast to conventional approaches like antibiotic, antifungal, and external application of probiotics and prebiotics [7].
- When a bacterial cell dies or lyses in a culture system, PHB is degraded by the activity of extracellular PHB depolymerase enzymes, which are widely found in microbes.
- In recirculating aquaculture systems, biofloc is a key source of food (RAS). RAS is a cutting-edge aquaculture technique that could be applied to enhance production strategies for fish farming in a biosecurity environment[29].
- Biofloc is a protein-rich feed that occurs naturally and is alive, produced by the conversion of leftover feed and excrement in an aquaculture system under the influence of micron solar radiation, as indicated by the source. [63].
- A combined BFT and RAS system would more efficiently convert excess feed for fast-growing farm animals into microbial biomass, which the animals could then consume as food, reducing water pollution. [64].
- Biofloc is a secure and integrated aquaculture system that allows for intensive aeration, higher stocking densities, and organic input with little to no water exchange. It also involves intensive mixing and provides an additional source of feed for some species suitable for these conditions. [28].
- The Implications of BFT for Disease and Health Management is the most significant application. Bacterial cell-to-cell communication, known as quorum sensing, has been hypothesised to be an efficient substitute for managing pathogenic microorganisms in aquaculture. A potential method for employing biofloc to prevent harmful organisms in BFT is quorum sensing. [65]
- The immune system of fish and shrimp is strengthened in the presence of biofloc and there is less incidence of diseases in biofloc systems [4].
- Biofloc production also plays an important role in a bioreactor that can treat wastewater from aquaculture facilities and convert dissolved nutrients into single-cell protein [66].

15. ADVANTAGES OF BIOFLOC:

The biofloc technology (BFT) has several advantages over disadvantages and other contemporary fish farming methods, making it an eco-friendly culture technique that does not require the draining of water after culture. One of its main benefits is its ability to reduce environmental impact, optimize the use of land and water, and maintain appropriate water quality with minimal water usage and exchange within the rearing ponds. Additionally, BFT produces protein-rich biofloc, which serves as an additional feed for aquatic organisms, further enhancing its effectiveness compared to other systems. Additionally, according to [67], Biofloc technology has several advantages over conventional aquaculture systems. It provides a clean and secure environment for aquatic organisms by decreasing wastewater pollution and reducing the possibility of disease transmission. Compared to conventional culture systems, BFT is more cost-effective and can improve the growth and survival rates of fish. It also reduces the need for protein-rich feed, resulting in more cost-effective production of cultured animals. Biofloc is an inexpensive and sufficient source of nutrition that can be used as a substitute for capturing and marketing cheap food fish and waste fish for fishmeal. In summary, BFT offers significant benefits, including improved water quality, increased biosecurity, lower production costs, and a sustainable alternative to traditional feed sources. [63].

16. DISADVANTAGES:

There are various flaws in the biofloc system, such as the immediate necessity of ventilation and fast water velocity, which leads to higher energy costs for aeration. Additionally, some drawbacks have been noted for Bluefin tuna, including a risk for pollution due to nitrate buildup, a shortened reaction time caused by dissolved oxygen uptake in the water, and a faster breathing rate. As well as the need for a beginning period and alkalinity additives. Additionally, for systems exposed to sunlight, the performance of biofloc technology may be erratic and depending on seasonal variations in the day time [63]. Finally, biofloc technology has relatively regular maintenance requirements, which can occasionally be prohibitively expensive [68].

17. FUTURE DIRECTIONS:

Major issues include a lack of water supplies, an increase in seafood consumption, and a lack of land for expanding aquaculture activities. One of the key solutions to meet the rising demand for animal protein is extensive aquaculture. The application of biofloc technology can meet the demands for sustainable development and the advancement of environmentally friendly aquaculture. Aquatic supplementing demands are met by biofloc, which can also serve as a suitable replacement for fish meal in the diet of aquatic animals. The biofloc production system offers advantages such as decreased feed intake, decreased water interchange, increased biosecurity, decreased disease risk, increased growth and survival, and thus increased productivity and output. Additionally, the usage of organic foods has grown in the same cultural region while industrial foods have declined, reducing the environmental effects of sewage. Additionally, little is known about the impact of environmental conditions, microbiological mechanisms engaged in the process, and water quality on the biofloc, which calls for further research. The use of biofloc technology to recover nitrogen from the culture system is thought to have a number of advantages in addition to a 10–20% estimated feed yield [4].

18. CONCLUSION

Aquaculture production is increasing every day along with the advantage of higher productivity; it has some problems with increased nitrogen content and more chance of disease. The Biofloc system became necessary due to the increasing demand for fish worldwide. The requirement of an ecological aquaculture system can be achieved with biofloc. The microbial community plays a major role in biofloc technology. Biofloc has essential nutritional value. Nitrites act on aquatic organisms and causes physiological damage such as ion imbalance, Na^+ absorption in gills changes cells, promotes haemoglobin oxidation to methaemoglobin, inhibits hormone synthesis. In BFT, nitrogenous compounds are recycled by microorganisms and their removal from aquaculture is done by denitrification. In addition, biofloc technology includes different types of carbon sources: carbohydrates, organic acids and alcohol, a biodegradable polymer. Different carbonaceous substrates have a large effect on BFT. It has an important role in maintaining water quality for target culture species.

References

1. Boyd CE. Guidelines for aquaculture effluent management at the farm-level. Aquaculture 2003; 226(1): 101-112.

2. Malone RF, Pfeiffer TJ. Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. Aquacultural Engineering 2006; 34(3): 389-402.

3. Piedrahita RH. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 2003; 226(1): 35-44.

4. Crab R, Defoirdt T, Bossier P, Verstraete W. Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture 2012; 356-357: 351-356.

5. Avnimelech Y, *Biofloc technology: a practical guide book*. 2009: World Aquaculture Society.

6. Emerenciano M, Cuzon G, Goguenheim J, Gaxiola G, Aquacop. Floc contribution on spawning performance of blue shrimp Litopenaeus stylirostris. Aquaculture Research 2012; 44(1): 75-85.

7. Emerenciano M, Cuzon G, Paredes A, Gaxiola G. Evaluation of biofloc technology in pink shrimp Farfantepenaeus duorarum culture: growth performance, water quality, microorganisms' profile and proximate analysis of biofloc. Aquaculture International 2013; 21(6): 1381-1394.

8. Hepher B. Aquaculture intensification under land and water limitations. Geo Journal 1985; 10(3): 253-259.

9. Gordon A, Tupper M. Small-scale Fisheries and Oil and Gas Operations in Trinidad and Tobago-Scoping Study. 2011.

10. Burford MA, Thompson PJ, McIntosh RP, Bauman RH, Pearson DC. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 2003; 219(1): 393-411.

11. Da Silva, K.R., Wasielesky, W., and Abreu, P.C. (2013) Nitrogen and phosphorus dynamics in the biofloc production of the pacific white shrimp, Litopenaeus vannamei. J. World Aquac Soc 44: 30–41.

12. De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture 2008; 277: 125-137.

13. Avnimelech Y. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 1999; 176(3): 227-235.

14. Avnimelech Y. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 2007; 264(1): 140-147.

15. Ebeling JM, Timmons MB, Bisogni JJ. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture 2006; 257(1): 346-358.

16. Hargreaves JA. Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering 2006; 34(3): 344-363.

17. Zhao P, Huang J, Wang X-H, Song X-L, Yang C-H, Zhang X-G, Wang G-C. The application of bioflocs technology in high-intensive, zero exchange farming systems of Marsupenaeus japonicus. Aquaculture 2012; 354-355: 97-106.

18. Schneider O, Sereti V, Machiels MAM, Eding EH, Verreth JAJ. The potential of producing heterotrophic bacteria biomass on aquaculture waste. Water Research 2006; 40(14): 2684-2694.

19. Bratvold D, Lu J, Browdy CL. Disinfection, Microbial Community Establishment and Shrimp Production in a Prototype Biosecure Pond. Journal of the World Aquaculture Society 1999; 30(4): 422-432.

20. Anand PSS, Kumar S, Panigrahi A, Ghoshal TK, Syama Dayal J, Biswas G, Sundaray JK, De D, Ananda Raja R, Deo AD, Pillai SM, Ravichandran P. Effects of C:N ratio and substrate integration on periphyton biomass, microbial dynamics and growth of Penaeus monodon juveniles. Aquaculture International 2013; 21(2): 511-524.

21. Ballester ELC, Abreu PC, Cavalli RO, Emerenciano M, De Abreu L, Wasielesky Jr W. Effect of practical diets with different protein levels on the performance of Farfantepenaeus paulensis juveniles nursed in a zero-exchange suspended microbial flocs intensive system. Aquaculture Nutrition 2010; 16(2): 163-172.

22. Zaki MAA, Alabssawy AN, Nour AE-AM, El Basuini MF, Dawood MAO, Alkahtani S, Abdel-Daim MM. The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (Oreochromis niloticus) reared under biofloc conditions. Aquaculture Reports 2020; 16: 100282.

23. Wasielesky W, Atwood H, Stokes A, Browdy CL. Effect of natural production in a zero-exchange suspended microbial floc based super-intensive culture system for white shrimp Litopenaeus vannamei. Aquaculture 2006; 258(1): 396-403.

24. Bossier P, Ekasari J. Biofloc technology application in aquaculture to support sustainable development goals. Microbial Biotechnology 2017; 10(5): 1012-1016.

25. Fuentes JL, Garbayo I, Cuaresma M, Montero Z, González-del-Valle M, Vílchez C *Impact of Microalgae-Bacteria Interactions on the Production of Algal Biomass and Associated Compounds*. Marine Drugs, 2016; 14, DOI: 10.3390/md14050100.

26. Timmons MB, Ebeling JM, Wheaton FW, Summerfelt S, Vinci BJ, *Recirculating aquaculture systems*. 2002: Northeastern Regional Aquaculture Center, Ithaca (New York).

27. Su Y, Zhang Y, Zhou X, Jiang M. Effect of nitrate concentration on filamentous bulking under low level of dissolved oxygen in an airlift inner circular anoxic-aerobic incorporate reactor. Journal of Environmental Sciences 2013; 25(9): 1736-1744.

28. Tay J-H, Xu H-L, Teo K-C. Molecular mechanism of granulation. I: H+ translocation-dehydration theory. ournal of Environmental Engineering 2000; 126(5): 403-410.

29. Hargreaves J. *Biofloc production systems for aquaculture*. 2013.

30. Ahmad I, Babitha Rani AM, Verma AK, Maqsood M. Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition. Aquaculture International 2017; 25(3): 1215-1226.

31. Ekasari J, Angela D, Waluyo SH, Bachtiar T, Surawidjaja EH, Bossier P, De Schryver P. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture 2014; 426-427: 105-111.

32. Khanjani M, Sajjadi M, Alizadeh M, Sourinejad I. Effect of different feeding levels on water quality, growth performance and survival of western white shrimp (Litopenaeus vannamei Boone, 1931) post larvae with application of biofloc technology. Iranian Scientific Fisheries Journal 2015; 24(2): 13-28.

33. Remen M, Imsland AK, Stefansson SO, Jonassen TM, Foss A. Interactive effects of ammonia and oxygen on growth and physiological status of juvenile Atlantic cod (*Gadus morhua*). Aquaculture 2008; 274(2): 292-299.

34. Baldisserotto B, *Physiology of peixes applied to fish farming*. 2002.

35. Wood CM, *Influence of feeding, exercise, and temperature on nitrogen metabolism and excretion*, in *Fish Physiology*. 2001, Academic Press. p. 201-238.

36. Tomasso JR, Simco BA, Davis KB. Chloride Inhibition of Nitrite-Induced Methemoglobinemia in Channel Catfish (*Ictalurus punctatus*). Journal of the Fisheries Research Board of Canada 1979; 36(9): 1141-1144.

37. Jensen FB. Nitrite disrupts multiple physiological functions in aquatic animals. Comp Biochem Physiol A Mol Integr Physiol 2003; 135(1): 9-24.

38. Colt J, Ludwig R, Tchobanoglous G, Cech JJ. The effects of nitrite on the short-term growth and survival of channel catfish, *Ictalurus punctatus.* Aquaculture 1981; 24: 111-122.

39. Chen J-C, Lei S-C. Toxicity of Ammonia and Nitrite to Penueus monodon Juveniles. Journal of the World Aquaculture Society 1990; 21(4): 300-306.

40. Barbieri E, Bondioli ACV. Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus,* Holmberg, 1887) at different temperatures levels. Aquaculture Research 2015; 46(3): 565-571.

41. Souza J, Cardozo A, Wasielesky W, Abreu PC. Does the biofloc size matter to the nitrification process in Biofloc Technology (BFT) systems? Aquaculture 2019; 500: 443-450.

42. Chávez-Crooker P, Obreque-Contreras J. Bioremediation of aquaculture wastes. Current Opinion in Biotechnology 2010; 21(3): 313-317.

43. Stein LY, Klotz MG. The nitrogen cycle. Current Biology 2016; 26(3): R94-R98.

44. Dictionary M-W. Disponível em: https://www. merriamwebster. com/dictionary/profection. Acesso em 2020; 10.

45. Zhang K, Pan L, Chen W, Wang C. Effect of using sodium bicarbonate to adjust the pH to different levels on water quality, the growth and the immune response of shrimp Litopenaeus vannamei reared in zero-water exchange biofloc-based culture tanks. Aquaculture Research 2017; 48(3): 1194-1208.

46. Luo G-z, Avnimelech Y, Pan Y-f, Tan H-x. Inorganic nitrogen dynamics in sequencing batch reactors using biofloc technology to treat aquaculture sludge. Aquacultural Engineering 2013; 52: 73-79.

47. Song JH, Murphy RJ, Narayan R, Davies GBH. Biodegradable and compostable alternatives to conventional plastics. Philosophical Transactions of the Royal Society B: Biological Sciences 2009; 364(1526): 2127-2139.

48. Chu L, Wang J. Nitrogen removal using biodegradable polymers as carbon source and biofilm carriers in a moving bed biofilm reactor. Chemical Engineering Journal 2011; 170(1): 220-225.

49. Reddy CSK, Ghai R, Rashmi, Kalia VC. Polyhydroxyalkanoates: an overview. Bioresource Technology 2003; 87(2): 137-146.

50. Bhuwal AK, Singh G, Aggarwal NK, Goyal V, Yadav A. Isolation and Screening of Polyhydroxyalkanoates Producing Bacteria from Pulp, Paper, and Cardboard Industry Wastes. International Journal of Biomaterials 2013; 2013: 752821.

51. Wei Y-f, Wang A-l, Liao S-a. Effect of different carbon sources on microbial community structure and composition of ex-situ biofloc formation. Aquaculture 2020; 515: 734492.

52. Panigrahi A, Sundaram M, Saranya C, Swain S, Dash RR, Dayal JS. Carbohydrate sources deferentially influence growth performances, microbial dynamics and immunomodulation in Pacific white shrimp (Litopenaeus vannamei) under biofloc system. Fish & Shellfish Immunology 2019; 86: 1207-1216.

53. Luo G, Chen X, Tan J, Abakari G, Tan H. Effects of carbohydrate addition strategy and biofloc levels on the establishment of nitrification in biofloc technology aquaculture systems. Aquaculture 2020; 514: 734441.

54. Nootong K, Pavasant P, Powtongsook S. Effects of Organic Carbon Addition in Controlling Inorganic Nitrogen Concentrations in a Biofloc System. Journal of the World Aquaculture Society 2011; 42(3): 339-346.

55. Du X, Almeida D, Song D, Zhao Z, Luo L, Wang Ca, Li J, Wang L, Ji F, Xu Q. Effects of organic carbon addition on water quality and phytoplankton assemblages in biofloc technology ponds. Aquaculture 2018; 497: 155-163.

56. Fugimura MMS, dos Reis Flor H, de Melo EP, da Costa TV, Wasielesky W, Oshiro LMY. Brewery residues as a source of organic carbon in Litopenaeus schmitti white shrimp farms with BFT systems. Aquaculture International 2015; 23(2): 509-522.

57. Boyd CE, Tucker CS. Handbook for aquaculture water quality. 2014; 439.

58. Liu H, Li H, Wei H, Zhu X, Han D, Jin J, Yang Y, Xie S. Biofloc formation improves water quality and fish yield in a freshwater pond aquaculture system. Aquaculture 2019; 506: 256-269.

59. do Espírito Santo CM, Pinheiro IC, de Jesus GFA, Mouriño JLP, Vieira FdN, Seiffert WQ. Soybean molasses as an organic carbon source in the farming of Litopenaeus vannamei (Boone, 1931) in a biofloc system. Aquaculture Research 2017; 48(4): 1827-1835.

60. Zhang N, Luo G, Tan H, Liu W, Hou Z. Growth, digestive enzyme activity and welfare of tilapia (Oreochromis niloticus) reared in a biofloc-based system with poly-β-hydroxybutyric as a carbon source. Aquaculture 2016; 464: 710-717.

61. Deng M, Chen J, Gou J, Hou J, Li D, He X. The effect of different carbon sources on water quality, microbial community and structure of biofloc systems. Aquaculture 2018; 482: 103-110.

62. Dauda AB, Romano N, Ebrahimi M, Karim M, Natrah I, Kamarudin MS, Ekasari J. Different carbon sources affects biofloc volume, water quality and the survival and physiology of African catfish Clarias gariepinus fingerlings reared in an intensive biofloc technology system. Fisheries Science 2017; 83(6): 1037-1048.

63. Najdegerami EH, Bakhshi F, Lakani FB. Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. Fish Physiology and Biochemistry 2016; 42(2): 457-465.

64. Jamal MT, Broom M, Al-Mur BA, Al Harbi M, Ghandourah M, Al Otaibi A, Haque MF. Biofloc technology: Emerging microbial biotechnology for the improvement of aquaculture productivity. Polish journal of microbiology 2020; 69(4): 401-409.

65. Choo HX, Caipang CMA. Biofloc technology (BFT) and its application towards improved production in freshwater tilapia culture. Aquaculture, Aquarium, Conservation, Legislation 2015; 8(3): 362-366.

66. Defoirdt T, Boon N, Sorgeloos P, Verstraete W, Bossier P. Quorum sensing and quorum quenching in Vibrio harveyi: lessons learned from in vivo work. The ISME Journal 2008; 2(1): 19-26.

67. Kuhn DD, Lawrence AL, Boardman GD, Patnaik S, Marsh L, Flick GJ. Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei.* Aquaculture 2010; 303(1): 28-33.

68. Arias-Moscoso JL, Espinoza-Barrón LG, Miranda-Baeza A, Rivas-Vega ME, Nieves-Soto M. Effect of commercial probiotics addition in a biofloc shrimp farm during the nursery phase in zero water exchange. Aquaculture Reports 2018; 11: 47-52.

69. De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W. The basics of bio-flocs technology: The added value for aquaculture. Aquaculture 2008; 277(3): 125-137.

70. FAO FJO, Food c, Nations AOotU. The state of world fisheries and aquaculture. 2012.

71. Binalshikh-Abubkr T, Hanafiah MM, Das SK *Proximate Chemical Composition of Dried Shrimp and Tilapia Waste Bioflocs Produced by Two Drying Methods*. Journal of Marine Science and Engineering, 2021; 9, DOI: 10.3390/jmse9020193.

72. Becerril D, Del M, Monroy Dosta M, Emerenciano M, Castro G, Cienfuegos K, Lara-Andrade R. Nutritional importance for aquaculture and ecological function of microorganisms that make up Biofloc, a review. International Journal of Aquatic Science 2017; 8: 69-77.

73. Cang P, Zhang M, Qiao G, Sun Q, Xu D, Li Q, Yuan X, Liu W. Analysis of Growth, Nutrition and Economic Profitability of Gibel Carp (*Carassius auratus gibelio*♀× *C. carpio*♂) Cultured in Zero-water Exchange System. Pakistan Journal of Zoology 2019; 51(2): 619-630.

74. Khanjani MH, Alizadeh M, Sharifinia M. Rearing of the Pacific white shrimp, Litopenaeus vannamei in a biofloc system: The effects of different food sources and salinity levels. Aquaculture Nutrition 2020; 26(2): 328-337.

75. Sakkaravarthi DKS, *ENHANCEMENT OF LOW PROTEIN FEED FOR INTENSIVE SHRIMP CULTURE THROUGH BIOFLOC TECHNOLOGY*. 2019.

76. Uawisetwathana U, Situmorang ML, Arayamethakorn S, Haniswita, Suantika G, Panya A, Karoonuthaisiri N, Rungrassamee W *Supplementation of Ex-Situ Biofloc to Improve Growth Performance and Enhance Nutritional Values of the Pacific White Shrimp Rearing at Low Salinity Conditions*. Applied Sciences, 2021; 11, DOI: 10.3390/app11104598.

77. Barzamini M, Harsij M, Adineh H, Jafaryan H. Research Article: The effect of biofloc-supplemented diets on the Pacific white shrimp (*Litopenaeus vannamei*): Analysis of water quality, growth performance, and biochemical composition. Sustainable Aquaculture. Health. Management journal 2021; 7(2): 30-43.

78. Suloma A, Gomaa AH, Abo-Taleb MA, Mola HR, Khattab MS, Mabroke RS. Heterotrophic biofloc as a promising system to enhance nutrients waste recycling, dry diet acceptance and intestinal health status of European eel (*Anguilla anguilla*). Aquaculture, Aquarium, Conservation Legislation 2021; 14(2): 1021-1035.

79. Lunda R, Roy K, Dvorak P, Kouba A, Mraz J. Recycling biofloc waste as novel protein source for crayfish with special reference to crayfish nutritional standards and growth trajectory. Scientific Reports 2020; 10(1): 19607.

80. Lee C, Kim S, Lim S-J, Lee K-J. Supplemental effects of biofloc powder on growth performance, innate immunity, and disease resistance of Pacific white shrimp Litopenaeus vannamei. Fisheries and Aquatic Sciences 2017; 20(1): 15.

81. Prabu E, Rajagopalsamy C, Ahilan B, Jeevagan I, Renuhadevi M. Effect of dietary supplementation of biofloc meal with tryptophan on growth and survival of GIFT Tilapia. International Journal of Current Microbiology and Applied Sciences 2017; 6(8): 3426-3434.

82. Azim ME, Little DC. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (Oreochromis niloticus). Aquaculture 2008; 283(1): 29-35.

83. Xu W-J, Pan L-Q. Dietary protein level and C/N ratio manipulation in zero-exchange culture of Litopenaeus vannamei: Evaluation of inorganic nitrogen control, biofloc composition and shrimp performance. Aquaculture Research 2014; 45(11): 1842-1851.

84. Peiro-Alcantar C, Rivas-Vega ME, Martínez-Porchas M, Lizárraga-Armenta JA, Miranda-Baeza A, Martínez-Córdova LR. Effect of adding vegetable substrates on Penaeus vannamei pre-grown in biofloc system on shrimp performance, water quality and biofloc composition. Latin american journal of aquatic research 2019; 47(5): 784-790.

85. Arthur JW, West CW, Allen KN, Hedtke SF. Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bulletin of Environmental Contamination and Toxicology 1987; 38(2): 324-331.

86. Barbieri E. Acute toxicity of ammonia in white shrimp (Litopenaeus schmitti) (Burkenroad, 1936, Crustacea) at different salinity levels. Aquaculture 2010; 306(1): 329-333.

87. Chen J-C, Liu P-C, Lei S-C. Toxicities of ammonia and nitrite to Penaeus monodon adolescents. Aquaculture 1990; 89(2): 127-137.

88. Lin Y-C, Chen J-C. Acute toxicity of nitrite on Litopenaeus vannamei (Boone) juveniles at different salinity levels. Aquaculture 2003; 224(1): 193-201.

89. Ostrensky A, Wasielesky W. Acute toxicity of ammonia to various life stages of the São Paulo shrimp, Penaeus paulensis Pérez-Farfante, 1967. Aquaculture 1995; 132(3): 339-347.

90. Peyghan R, Takamy GA. Histopathological, serum enzyme, cholesterol and urea changes in experimental acute toxicity of ammonia in common carp Cyprinus carpio and use of natural zeolite for prevention. Aquaculture International 2002; 10(4): 317-325.

91. González J, Suárez R, Gil B, Torres GZ. Exposición aguda a tres concentraciones de nitrito en juveniles de bocachico (*Prochilodus magdalenae*). Revista de la Facultad de Medicina Veterinaria y de 2005; 52(1): 36-43.

92. Torres-Tabares A, Sierra-Fontecha F, Medina-Robles V, Cruz-Casallas P, Velasco-Santamaría Y. Acute toxicity of nitrite (N-NO2) in two fish species of the Colombian Orinoquia: Yamú (Brycon amazonicus) and white Cachama (*Piaractus brachypomus*). ornada de Acuicultura, Universidad de Los Llanos 2007; 13: 80-85.

93. Ray AJ, Dillon KS, Lotz JM. Water quality dynamics and shrimp (Litopenaeus vannamei) production in intensive, mesohaline culture systems with two levels of biofloc management. Aquacultural Engineering 2011; 45(3): 127-136.

94. García-Ríos L, Miranda-Baeza A, Coelho-Emerenciano MG, Huerta-Rábago JA, Osuna-Amarillas P. Biofloc technology (BFT) applied to tilapia fingerlings production using different carbon sources: Emphasis on commercial applications. Aquaculture 2019; 502: 26-31.

95. Wang C, Pan L, Zhang K, Xu W, Zhao D, Mei L. Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of Litopenaeus vannamei in zero-exchange culture tanks. Aquaculture Research 2016; 47(10): 3307-3318.

96. Tierney TW, Ray AJ. Comparing biofloc, clear-water, and hybrid nursery systems (Part I): Shrimp (Litopenaeus vannamei) production, water quality, and stable isotope dynamics. Aquacultural Engineering 2018; 82: 73-79.

97. da Silva KR, Wasielesky Jr W, Abreu PC. Nitrogen and Phosphorus Dynamics in the Biofloc Production of the Pacific White Shrimp, Litopenaeus vannamei. Journal of the World Aquaculture Society 2013; 44(1): 30-41.

98. Furtado PS, Poersch LH, Wasielesky W. Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture 2011; 321(1): 130-135.

99. Schveitzer R, Arantes R, Costódio PFS, do Espírito Santo CM, Arana LV, Seiffert WQ, Andreatta ER. Effect of different biofloc levels on microbial activity, water quality and performance of Litopenaeus vannamei in a tank system operated with no water exchange. Aquacultural Engineering 2013; 56: 59-70.

100. Mansour AT, Esteban MÁ. Effects of carbon sources and plant protein levels in a biofloc system on growth performance, and the immune and antioxidant status of Nile tilapia (*Oreochromis niloticus).* Fish & Shellfish Immunology 2017; 64: 202-209.

101. Luo G, Gao Q, Wang C, Liu W, Sun D, Li L, Tan H. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquaculture 2014; 422: 1-7.

102. Arantes R, Schveitzer R, Magnotti C, Lapa KR, Vinatea L. A comparison between water exchange and settling tank as a method for suspended solids management in intensive biofloc technology systems: effects on shrimp (*Litopenaeus vannamei*) performance, water quality and water use. Aquaculture Research 2017; 48(4): 1478-1490.

103. Jatobá A, Vieira FdN, Silva BCd, Soares M, Mouriño JLP, Seiffert WQ. Replacement of fishmeal for soy protein concentrate in diets for juvenile Litopenaeus vannamei in biofloc-based rearing system. Revista Brasileira de Zootecnia 2017; 46: 705-713.

104. Ray AJ, Lotz JM. Comparing a chemoautotrophic-based biofloc system and three heterotrophic-based systems receiving different carbohydrate sources. Aquacultural Engineering 2014; 63: 54-61.

105. Krummenauer D, Poersch L, Romano LA, Lara GR, Encarnação P, Wasielesky W. The Effect of Probiotics in a Litopenaeus vannamei Biofloc Culture System Infected with Vibrio parahaemolyticus. Journal of Applied Aquaculture 2014; 26(4): 370-379.