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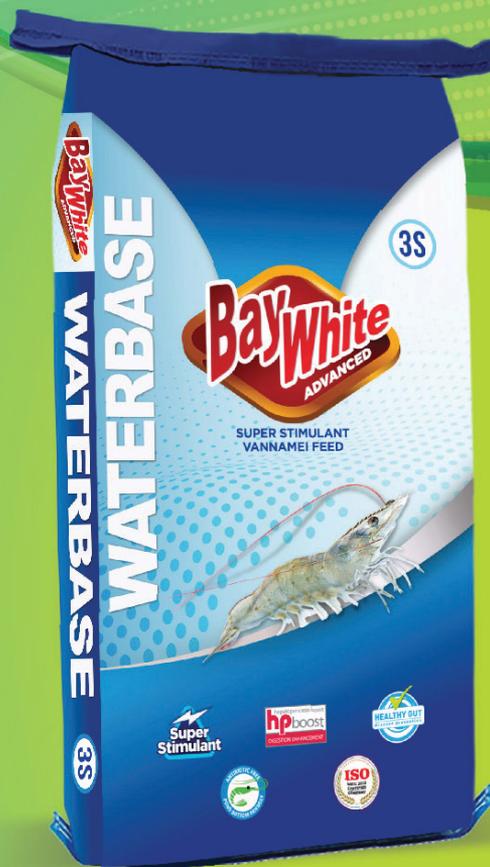
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BIO-FLOC TECHNOLOGY

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Introduction:

Globally, the aquaculture sector is the fastest-growing food-producing sector, as the demand for low-cost animal protein is increasing due to the shortage of protein food supply in many countries to feed the ever-growing population, besides its eliminates hunger and malnutrition.

Presently, the total world fishery production is about 178.5MT, of which aquaculture production is 82.1MT, which contributes about 50% of the total Production (Sofia 2020). This enormous increase in aquaculture production is due to the introduction of high-density intensive culture practices in fish/shrimp farming in the limited culture area. Apart from these benefits, intensive culture systems are also associated with some environmental and economic problems because, it requires more than 50% of the total production cost is driven to feed only. However, only 20-30% of feed is utilised by the culture species; the remaining 70-80% will be accumulated as organic waste (uneaten feed and excretory products) in pond water, leading to water quality deterioration (Avnimelech 1999), affect culture species in terms of growth, survival and ultimately causes disease outbreaks.

Moreover, aquaculture also has some limitations like less availability of land, water, feed ingredients and bio security measures. To overcome these above problems application of an environmental friendly culture system known as “Bio-floc Technology” is more advantageous, because it is a green culture system in which nutrients are reused and recycled continuously with minimum or zero water exchange. It is mainly based on the growth of microorganisms (bio-floc), which helps to improve the water quality by the uptake of nitrogen and to produce a microbial protein; in addition, FCR can be reduced, which ultimately reduces the feed cost.

Bioflocs are the heterogeneous macro aggregations of algae, diatoms, protozoa, microbial grazers, filamentous and floc forming bacteria with uneaten feed, faeces, and sludge, which were grouped into floc biomass by a biological adhesive such as poly-hydroxy alkanooates (PHA) released by the microorganisms within the water column. Bio-flocs are very light, highly porous, delicate and irregular in shape and vary from 50-200 microns (reach up to 1000microns) in size (Chu and Lee2004, Avnimelech 2009), Contains rich nutrients such as proteins, lipids, carbohydrates and ash (Crab et al., 2010a) and directly forms as feed to culture species in the Pond. By this process, 7-13% of nitrogen retention can be increased (Hari B et al. & Schneider O et al.). Protein utilisation is significantly higher in the bio-floc system (Protein conversion ratio-2), when compare to

Highlight Points

- ▶ In Biofloc technology about 7 to 13% of nitrogen retention can be enhanced compared to conventional farming. In this article, we have updated the importance of Importance of C/N ratio in BFT (Bio-Floc Technology), Reutilization of excreted ammonia as a food source for culture species, which enhances species immunity and growth performance as well as disease resistance.
- ▶ Most Preferable species in BFT are *L.vennamei* and Tilapia, as they can withstand poor water quality, filter feeding habit makes them to culture in biofloc, which can utilize the nitrogen resources efficiently.
- ▶ Continuous oxygen supply should be maintained in BFT, as it is essential for floc formation with addition to that it helps in respiration for both cultural organisms and microbial species.
- ▶ Use of IOT(Internet of things)- based smart water quality management system in which sensors are used to analysis the water quality fluctuation in BFT tank.

conventional farming (PCR-4), and it shows more than 46% of PCR in the bio floc system (Chamberlain et al., 2001a)

Proximate composition of biofloc:

The nutritional values in the bio-floc mainly depend on what type of carbon source is used.

Table:1, Proximate composition of Biofloc

Component	Percentage
Protein	24.3 - 53.85%
Lipid	0.5-5.4%
Fibre	0.7-16.65%
Carbohydrate	21.1 - 81.1%

Ash	6.3- 31.9%
Nitrogen free extract	18 - 29.24%
Energy	12 - 19KJ/g

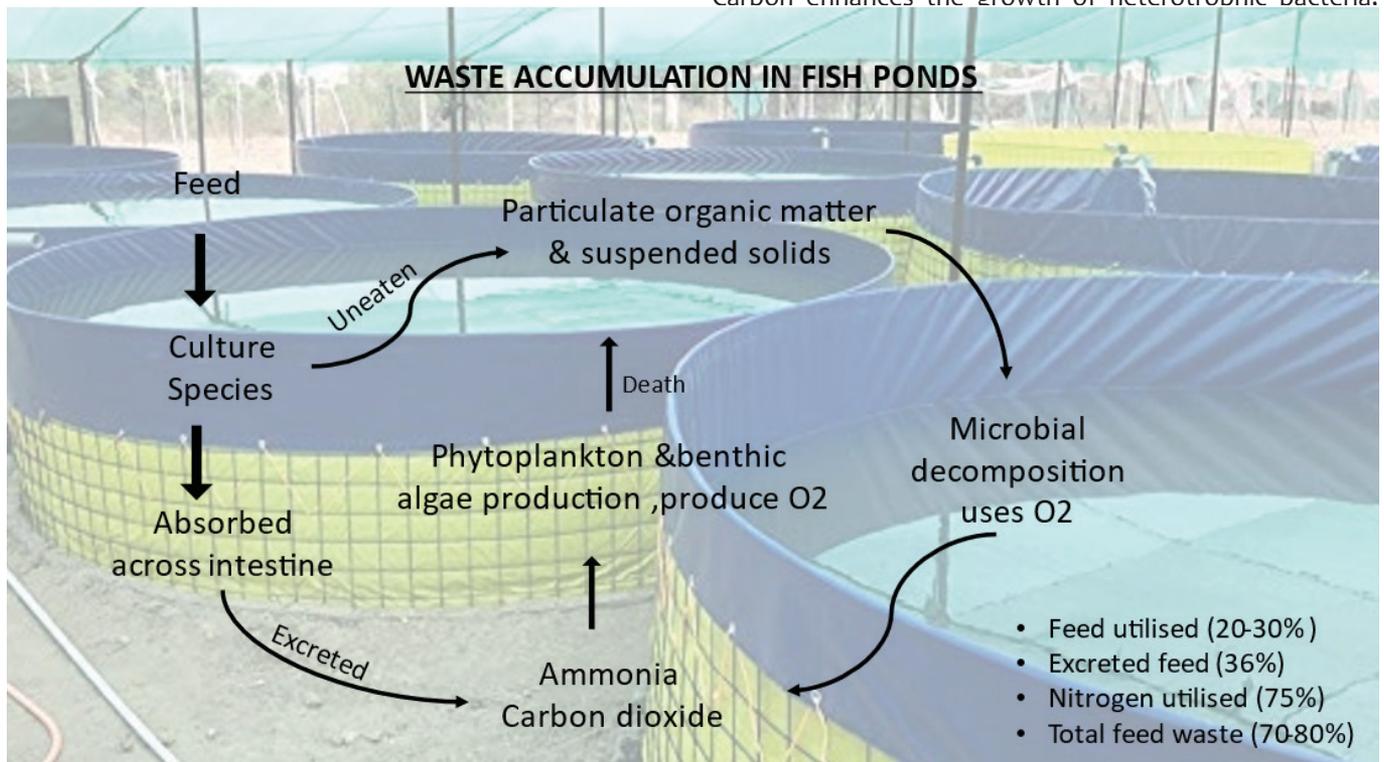
Principle of bio-floc Technology:

“Nitrogenous wastes present in the uneaten feed and animal excreta are converted into a consumable ‘bacterial floc’, with the addition of carbon sources at high oxygen levels with limited (less than 10%) or zero exchange of water “(Schneider et al. 2005, Avnimelech 1999; Crab et al. 2009)

due to continuous aeration, and carbon supply, usually highest densities of biofloc are observed in this system only. In this system, algae won't grow sufficiently or not at all grow and this biofloc system is solely based on bacteria, hence it is called as “True/brown biofloc system”. This system is alone recommended for shrimp/ fish culture.

Carbon - Nitrogen Ratio:

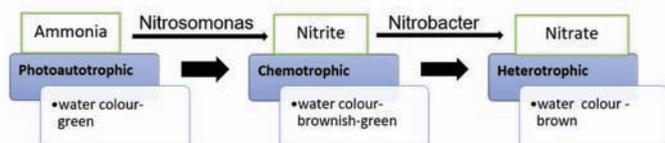
C/N ratio plays a vital role in the formation of bacterial biomass from the organic waste accumulated in the water. Carbon enhances the growth of heterotrophic bacteria.



Formation of biofloc:

Continuous aeration in the pond water promotes the bio-floc development by oxidising the ammonia in three stages that can be identified based on water colour as following

- 1. Photo-autotrophic stage:** This is the very initial stage of the biofloc system, the water appears in green colour due to the growth of filamentous microalgae such as chlorella, spirogyra, algae, anabaena and Oscillatoria are mostly dominated.
- 2. Chemotrophic stage:** It is the later nitrifying stage, in this system nitrifying bacteria such as Nitrosomonas, Nitrobacter and pseudomonas are mostly dominated as they convert the toxic ammonia to less toxic nitrate and water appears in the form of greenish brown in colour.
- 3. Heterotrophic stage:** Water appears in brown this indicates the domination of Heterotrophic Bacteria



and these bacteria take the nitrogen and produce a single-celled microbial protein (Avnimelech, 1999). Therefore, the optimal C/N ratio required by the heterotrophic bacteria is 12-15g:1g (Lechevallier et al., 1991; Henze et al., 1996; Avnimelech, 1999), but only 10g:1g is present in the fish feed, but the heterotrophic bacteria require C/N ratio higher than 10(Lancelot & Billen, 1985). Therefore, a well-balanced C/N ratio should be maintained by adding external carbon sources such as rice bran, jaggery, glycerol, molasses etc. However, the choice of using a carbon source depends on its price, availability, ease of application, and efficacy of the source.

Table:-2 Carbon percentage in different Carbon Sources

Carbon source	Percentage of carbon
Molasses	28%
Rice bran	46.52%
Sugar	42.11%

Jaggery	28.8%
Rice flour	40%
Glycerol	39.19%
Maize flour	46%
Acetate	40%
Wheat flour	40%
Dextrose	40.89%

Amount of carbon to be added :

By calculating the total fish / shrimp biomass and the total ammonia nitrogen produced in the pond / tank in a day the amount of carbon to be added can be calculated.

Fish biomass = Total number of fish X average body weight (ABW)

1kg of Fish, upon feeding with 25% protein @ 2-3% body weight

Feed to be added is 20g -30g and contains 5g - 6g of protein

↓ (1kg feed protein contains 160g of nitrogen)

Nitrogen in the added feed is about 0.8g - 0.96g

↓ 75% of feed-N remains unutilized (uneaten+ excreted)

About 0.6 – 0.72g of Nitrogen is produced per day

↓ (Heterotrophic bacteria require a C/N ratio of more than 10)

More than 6g -7.2g of carbon to be added per 1kg fish per day

Bio-floc tank setup:

If the tank diameter is 16m, the area of tank is calculated by $A = \pi \times r^2$ (where, $\pi = 3.14$)

Radius = diameter/2 i.e 16/2=8m

Then, area = $3.14 \times (8)^2 = 3.14 \times 64 = 200.96m^2$ (Ideal depth of the tank should be 1.5m)

Then the volume of the tank will be $200.96 \times 1.5m = 301.44m^3$ (1m³ can hold 1000litres of water)

Then the tank water holding capacity is $301.44m^3 \times 1000L = 3,01,440$ litres



Table-3- Required equipment for bio-floc tank setup:

Equipment	Purpose
Iron frame /fish pond	holds the tarpaulin tank
Tarpaulin tank	used for water storage to culture
Aeration pump	used for aeration; to maintain DO
Air tube Air tube connectors Air stones	distributes the air and helps in lifting water near the substrate to the top of the tank
Test kits	to monitor the various parameters of water like NH ₃ , pH, TDS, NO ₂ , NO ₃ , temperature etc,
Imhoff cones	The volume of Bio floc mass is measured
PVC pipes and fittings	For inlet and outlet; to maintain the structure of the tank

Preparation of bio-floc inoculum:

Wash the tank/pond cleanly and dry it properly.



Now fill the water up to 35-50% of the tank capacity and start aeration



Start aeration and keep for 24hrs; (Total dissolved solids (TDS) initially 800 – 1000)



Add 1kg of salt/10,000lit. of water to maintain the total dissolved solids (TDS) range between 1400 to 1600. (Do not use iodised salt, use only raw salt).



Check pH should be between 7.5 to 8.5 (add CaCO₃ to maintain)



Now add urea @1g/1000litres or Triple Super Phosphate @ 0.14gms/1000litres of water.



Then add carbon source 7gms/1000litres, if using jaggery add 2gms/10litres



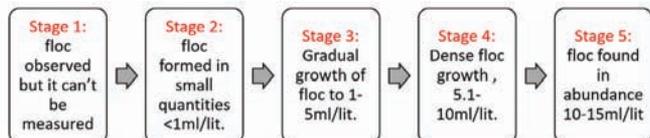
After one day add probiotic powder @ 20g/1000lit. of water



Continuous aeration is required



Within 7 to 10days, the floc will be formed



*From 1gm of ammonia, about 15.85gm of algae biomass; 0.2gm of nitrifying bacteria; 0.87gm of heterotrophic bacteriacan be produced

*Daily add 600gm of carbon for every 1kg of feed (maintenance phase@ 0.6:1) added until the floc reaches10-15ml/Lto maintain a C/N ratio of 10:1

*Ideal density of floc is 10-15ml/L for shrimp and 25 - 35ml/L for fish

Preferable species for culture in Bio-floc Technology:

Fish / shrimp species that can tolerate high stocking density, high total suspended solids concentrations, intermediate levels of DO, TAN and filter-feeding / omnivorous habit (Taw N,2010) are best suitable

- 1. Non-air-breathing fishes** such as *Labeo rohita*, Common Carp, Grass Carp, Silver carps, Tilapia (*Oreochromis aureus*, *O. niloticus*, *O. mossambicus*)
- 2. Air-breathing fishes** include Magur (*Claries batrachus*), Murrels, Anabas, Channel catfish, and *Clarias gariepinus*.
- 3. Shell fishes** such as *Litopenaeus vannamei* (Indian white leg shrimp) and *Penaeus monodon*(Tiger shrimp)

Table - 4, Management practises in BFT of shrimp and tilapia culture

Requisite	Shrimp	Tilapia
Stocking density	250 - 500 PL/m ²	150-200 fingerlings/m ²
Feeding rate	3-6% body weight	2-3%
Survival	85-90%	80%
Growth rate	1.3 – 2g/week	6-12g/week
Culture period	80days	6months
Final weight	22g	600-800g
Production	5-9kg/m ³	20-30kg/m ³

Table-5, Water quality parameters in Bio-floc technology

Parameter	Ideal range (shrimp)	Ideal range (Tilapia)	Recommendations
Temperature	28-30°C	26-34°C	High temperatures cause stress; low temperatures slower the growth
pH	7.8-8.0	7.5 -8.0	Fluctuations in pH effect the nitrification process
Dissolved oxygen (DO)	> 5mg/L (60%saturation)	>5mg/L	Essential for culture species and microbiota respiration
Salinity	4-35ppt	<5ppt	ranges between 0-50ppt depending on culture species
Alkalinity	>160mg/L	120 - 280mg/L	Helps in moderating pH fluctuations; helps in nitrogen assimilation and nitrification process

- *Among all the culturable species *Litopenaeus vannamei* and Tilapia are highly recommended to culture in bio-floc technology as they are filter feeders with omnivorous/detritivorous habit and can also withstand poor water quality conditions

Shrimp (vennamei) culture with bio-floc technology:

Litopenaeus vannamei is a most successful cultured shrimp species using bio-floc technology. In bio floc technology, vennamei are stocked at very high densities of about 200-500 PL/m²and the post-larvae of 10 to 11 days size are used. Very high aeration is required to meet the oxygen demand of the shrimps and bacterial biomass. Feeding rate is about 3-6% of the body weight the average growth is about 1.3 – 2g/week. They reach the final body weight up to 22g within the 3month period of culture. Survival of shrimp obtained in this system is about 85%to 90%.Production upto 5-9kg/m³ can be obtained in bio-floc system.

Tilapia culture with bio-floc technology:

Tilapia is an omnivorous fish and can be capable to feed upon suspended bio-floc mass detritus and commercial feed. It is the second important commercial fish after the carps. In BFT the stocked density of tilapia is about 150-200 fingerlings/m²and the survival rate is about 80%. Floating type of pelleted feed is used and fed at 2 – 3% of body weight. Tilapia utilises the 51% of the incorporated nitrogen and the FCR is reduced to 1.3.The average growth rate is about 6-12gm/week with a protein efficiency ratio (PER) of 2.62gm weight gain per 1gm protein consumed. Tilapia will reach up to 600 – 800g within 6months of culture period and the production obtained is about 20 – 30 kg/m³.

TAN	0.03mg/L	< 1mg/L	Toxicity depends on pH
Nitrite	0.3mg/L	0.3mg/L	Rises with increasing salinity
Nitrate	18-23mg/L	0.5-20mg/L	High levels increase stress
Settling solids	10-15ml/L	20-50ml/L	High amounts lead to anaerobic conditions; gill occlusions
Orthophosphate	0.5-10mg/L	0.5-20mg/L	Safe levels for culture species
Total suspended solids	400-500mg/L	<600mg/L	Excess amounts cause gill irritation; less amounts leads to poor production

Advancements in bio-floc Technology:

1. In bio-floc technology, an IoT (internet of things)-based smart water quality management system is developed in which sensors are used to analyse the water parameters such as pH, temperature, DO, TAN, suspended solids, salinity etc. They transmit the interpreted data to mobile phones, which helps farmers easily control water quality problems and reduces the labour cost for maintaining these parameters in water.
2. In Bio-floc technology, about 2000 different kinds of bacteria will be developed, of which few bacteria show mRNA expressions of the genes related to disease resistance that helps to enhance the immunity of the shrimp and helps in controlling WSSP and infectious myonecrosis virus disease in shrimp (Nyan Tawet *et al* 2015) and in *Labeo rohita* on consuming the bio-floc improved the resistance towards *Edwardsiella tarda*.
3. Biofloc helps reduce the typical off flavours in the channel catfish (*Ictalurus punctatus*). Geosmin and 2-methylisoborneol (MIB) produced by cyanobacteria give earthy or muddy flavours to the fish cultured in earthen ponds; these off flavours reduce the palatability of fish; this can be controlled in Bio floc technology because continuous aeration and no earthen bottom minimises the growth of off-flavour producing cyanobacteria (Kevin K. Schrader *et al.* 2013)
4. Bio-floc technology helps in controlling various diseases in shrimps, such as the pathogenic effect of *Vibrio harveyi* and *V. parahaemolyticus* in *Litopenaeus vannamei*, *Penaeus monodon* and in gnotobiotic brine shrimp, *Artemia franciscana* (Crab *et al.*, 2010) controlled by disrupting the pathogen's quorum sensing mechanism through which the pathogen communicates cell-to-cell, using signalling molecules (Spoering and Gilmore, 2006, Defoirdt *et al.*, 2008)
5. High stocking density in less space
6. Consuming the floc biomass enhances the immune system of culture species
7. Control the pathogenic effects of microbes by quorum sensing
8. Bio-floc forms as an alternative feed source and reduce the feeding cost
9. Efficient use of land and water resources
10. Low risk of pathogen entry into the culture
11. Improves the water quality by recycling the nutrients
12. Reduction of sensitivity to light fluctuations of water parameters
13. Minimum (<10%) or zero exchange of water
14. Minimize effluent discharge in the surrounding water bodies
15. Improves farm biosecurity

Demerits:

1. Cost-effectiveness
2. Maintaining a constant C/N ratio is problematic during culture
3. Monitoring floc volume, oxygen saturation, and ammonia levels requires highly skilled persons and a fully equipped laboratory at the farm only
4. Constant supply of electricity is needed because continuous aeration is necessary to maintain the bio-floc imposing higher energy costs.
5. High turbulence may also lead to floc breakage as they held together by a loose matrix

Conclusion:

Bio-floc technology doubles the farmer's income with efficient use of available resources. Unless the initial cost bio-floc system is more economical in production, in regular pond-based farming, 2000kg /6months is produced in a 1acre area, but the same crop yield can be obtained in 4small tanks of each 4m dia. and 1.2m depth in a floor area of 100-150m². Fast growth and improved disease resistance help the farmer overcome the financial loss due to disease outbreaks. In this system, adding supplementary

Merits:

1. It is a green approach to reducing the environmental impact
2. High productivity compared to conventional fish farming
3. Increased growth and survival rate of cultured species
4. Lower the feed conversion ratio (FCR) and reduce the feeding cost

feed is reduced to half as the culture species mainly feed upon the floc biomass in water; thus, the input feed cost is minimized. Unlike the conventional fish farming, frequent exchange of water is minimum or zero in this system; this reduces the labour cost, saves time, and prevents the entry of pathogens and also environmental degradation caused due to aquaculture effluents can be controlled by using the bio-floc culture system

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*More References can be provided on request.

IMPACT OF CORAL BLEACHING ON MARINE LIFE

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Abstract

Coral reefs are the most diverse ecosystems present around the world supporting rich biodiversity and livelihood of millions of people. But currently they are threatened by rising CO₂ levels through increase in sea surface temperature and ocean acidification. Changes in environmental conditions, such as water temperatures, pH values, and turbidity will directly affect the symbiotic relationship between corals and zooxanthellae and thus coral bleaching occurs. Bleached corals are expected to have reduced growth rates and decreased reproductive capacity which increases the susceptibility to diseases and higher mortality rates.

Awareness programme and collaborations with national and international institutes is required for the reduction in the climate change phenomenon and for the successful management and conservation of the coral reef ecosystem.

Introduction

Coral reefs are the most diverse marine ecosystems. They are living, colorful, multi-faceted underwater ecosystems, hosting a variety of organisms such as fish, invertebrates, (crabs, shrimp, sea stars), algae, benthos, and many more. They teem with life, with perhaps one-quarter of all ocean species depending on reefs for food and shelter. This is a remarkable statistic when you consider that reefs cover just a tiny fraction (less than one percent) of the earth's surface and less than two percent of the ocean bottom. Because they are so diverse, coral reefs are often called as rainforests of the sea. The value of coral reefs has been estimated at 30 billion U.S. dollars and perhaps as much as 172 billion U.S. dollars each year, providing food, protection of shorelines, jobs based on tourism, and even medicines.

