MICROBIAL ROLE IN BIOFLOC SYSTEM

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The primary theme of biofloc technology is to convert nitrogenous waste of aquaculture system in to a microbial protein, a valuable nutrient for shrimp and fishes. The biofloc is generated by manipulation of C: N ratio. It serves multitude of function like provision of surplus microbial protein as feed, control toxic ammonia nitrogen and improve the immune system of aquatic animals. The present chapter deals with the various aspects of microbes and the role it plays in biofloc system.

1. Bacterial growth characteristics

Bacteria need simple nitrogen like ammonium ion and carbon source such as sugar, starch, cellulose etc. to run its cellular machinery. This makes it amenable for utilization of toxic ammonia nitrogen from intensive shrimp aquaculture system. Another characteristic feature of bacterial growth is its faster growth rate, as it almost doubles its number within 30 minutes. The bacterial efficiency of nutrient conversion is as high as 50%. Therefore, in nutshell bacteria with its fast multiplication rate are highly efficient in converting toxic product of the aquatic system into the useful, highly nutritious, much demanded microbial protein.

1.1. Carbon nitrogen ratio in bacterial growth

The adjustment of C:N ratio in the feed is the single most crucial factors for microbial growth. This has been derived by considering;

1. The carbon nitrogen ratio of microbial biomass is around 4.
2. Microbial conversion efficiency range between 40-60% with an average of 50%
3. Carbon content in the feed is roughly 50%

Taking these facts together the carbon nitrogen ratio of 10 has been found optimum for bacterial growth.

1.2 Bacterial nutritional composition

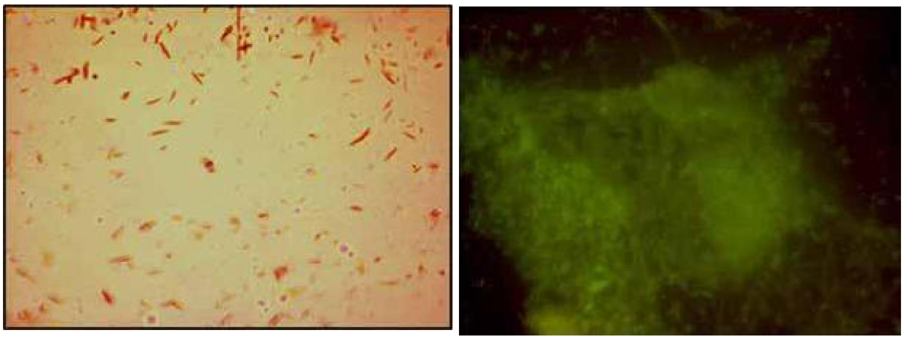
The nutritional composition of bacteria has been presented in Table 1. The table indicates that bacteria have almost 60% protein.

1.3 Aeration is crucial in biofloc formation

Intensive aeration is required in biofloc system to meet enhanced oxygen demand for microbial growth and shrimp. It also assists to keep biofloc in suspension. Our study indicated that a drop of oxygen above 1 ppm/h happens in biofloc system compared to control.

Table 1. Nutritional composition of bacteria

Dry matter	25%
On dry matter basis	
Carbon	48.9%
Hydrogen,	5.2%
Oxygen	24.8%
Nitrogen	9.46% (=61% crude protein)
Ash	9.2%



Bacteria in isolation **Bacteria infloc**

Fig 1. Microbial constituent in biofloc

2. Autotrophic verses heterotrophic microbial system

Three predominant pathways for ammonia-nitrogen assimilation function in aquaculture systems. This includes photoautotrophic, chemo-autotrophic and heterotrophic system. The photoautotrophic system is mediated by algae and diatoms and mostly works at nitrate level, the last and the least toxic metabolite of nitrogen cycle. However, the other two systems (chemo-autotrophic and heterotrophic) system is mediated by bacteria and start functioning from ammonia level, the most important toxic metabolite in shrimp culture. The chemo-autotrophic microbial system is managed by aerobic *Nitrosomonas* and *Nitrobacter* and the end product is nitrate. In contrast heterotrophic microbial system not only reduces ammonia level but also convert it into single cell microbial protein called biofloc.

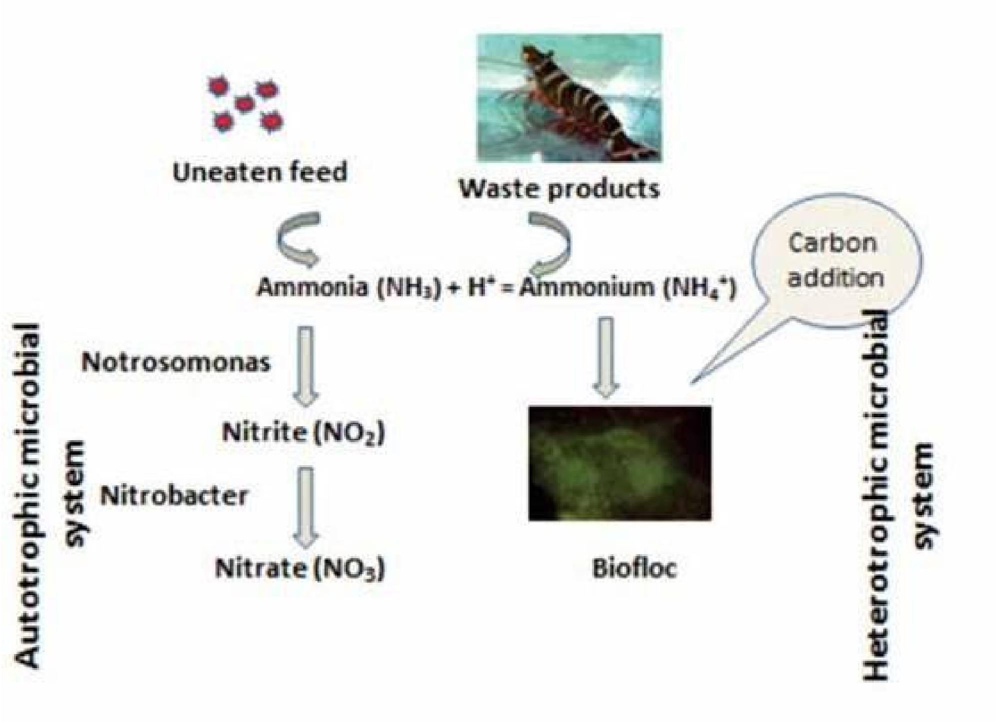


Fig 2. Autotrophic and Heterotrophic microbial system in nitrogen cycle

Table 2. Difference between chemoautotrophic and heterotrophic system

Feature	Chemoautotrophic (Nitrifying bacteria)	Heterotrophic
Multiplication speed	Very slow	Very fast
Generation time of bacteria	Many hours to days	30 min
Oxygen demand	Must, as bacteria are obligate aerobe. Without oxygen these bacteria will die.	Required to keep biofloc in suspension but not indispens-able for bacterial growth.
Nitrite accumulation	Chances is high, if aeration is low	

Table 3. Difference between photoautotrophic and heterotrophic system

PROPERTY	BACTERIA CONTROL	ALGAE CONTROL
Energy source	Mostly organic matter	Solar radiation
Occurrence	Dominance in ponds with high supply and concentration of organic substrate, normally limited to intensive ponds with zero or low water exchanges	Ponds with low organic matter concentration. Algae density increases with the availability of nutrients up to limitation of light
Sensitivity toward environmental variables	Does not need light. Adapts to a variety of conditions and stable	Light is essential (activity lowered in cloudy days) Crashes are common and less stable
Effect on Oxygen	Oxygen is consumed and demand is high	Oxygen is produced during the day, consumed at night
Relevant activities	Degradation of organic matter. Production of microbial protein through uptake of inorganic nitrogen	Primary production produces organic matter and oxygen uptake of dissolved nitrate and phosphate
Inorganic nitrogen control	Uptake of nitrogen affected by the C/N ratio of organic matter. Practically unlimited capacity	Uptake driven by primary production
Potential capacity	Limited by substrate concentration and rate of application	Normally, daily primary production <4gO /m^2

3. Microbial consortium of biofloc

The last few years have witnessed extensive research on the microbial composition of biofloc. The recent study suggests that biofloc is mostly dominated by Gram negative bacteria. Recently researchers reported that most of the screened bacteria belong to Proteobacteria phylum followed by Bacteroides and Cyanobacteia. The member of phylum proteobacteria is widely dispersed in the marine environment and plays an important role in the process of nutrient cycling and the mineralization of organic compounds. It was also found that among Proteobacteria, *Vibrio* group is the most predominant one. Our study indicated that biofloc system had increased level of *Bacillus* and *Lactobacillus* bacterium which have probiotics properties.

4. Microbial role in bioflocsystem

4.1. Bioremediation of toxic ammonia: The biofloc system maintains adequate water quality especially toxic nitrogen metabolites. At higher C: N ratio, bacteria immobilize toxic ammonia into microbial protein within fewhours as compared to slow conventional nitrification process which takes a month to get established.

4.2. Biocontrol agent: Numerous studies have reported that shrimps are healthiest and grow best in aquaculture systems that have high levels of algae, bacteria and other natural microbiota. Probiotics are viable microbial cells and have beneficial effect on health of shrimp by stimulation of immune system and microbial equilibrium in intestine, and by inhibition of pathogenic microbes. Microbes store poly-β-hydroxy butyrate (PHB) as a stored product of carbon and energy. Its synthesis is stimulated in the condition of limited nitrogen supply and with excess carbon supplementation. Condition available in biofloc system thus enhances its production. The PHB particles offer preventive and curative protection in *Artemia* nauplii against luminescent pathogenic *Vibrio campbelli*. This indicates that biofloc can serves as novel strategy for disease management on long term basis.

4.3. Healthy supplementary food: The protein content of bacteria is almost 60%. Therefore, its consumption becomes an alternate source of protein for aquatic animals like shrimp.

4.4 Probiotics and immunostimulant: Biofloc is a microbial consortium, which has large number of bacteria, which could play a powerful role in digestive enzyme secretion and as immunostimulant. Our study indicated that biofloc system improves the load of *Bacillus* and *Lactobacillus* bacterium which is expected to play role in probiotics and immunostimulant effect. Our experimental trial on biofloc work carried at ICAR-CIBA and Kakdwip Research Centre of CIBA, WestBengal revealed that biofloc formation starts 24 hours after addition of carbon source when bacterial count reaches 10^6-10^7 cfu. Biofloc was mainly composed of bacterial aggregates, zooplankton and phytoplankton. The increase in C:N ratio reduced the total ammonia nitrogen level. The CN20 was most effective while CN5 was least effective in reducing ammonia level. This corresponded to increased biofloc volume and the highest floc volume was observed in CN20 while the least was observed at CN5. Integration of substrate with biofloc system have profound effect on growth performance and immunity improvement in juvenile and sub adult stages of penaeid shrimps.

Reference

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