Bio-floc based farming Technology for shrimp aquaculture in India

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Focal Points at a Glance: Explaining what bio-floc is, the authors tell us about its importance and the higher levels of production per ha specially of vannamei shrimp that is achievable by its usage. As the main aspect of their effort, they provide the various aspects of this technology for the benefit of the readership, those consisting of the designing of the effort taking into account the engineering aspects, stocking densities, environmental requirements, and feeding aspects.

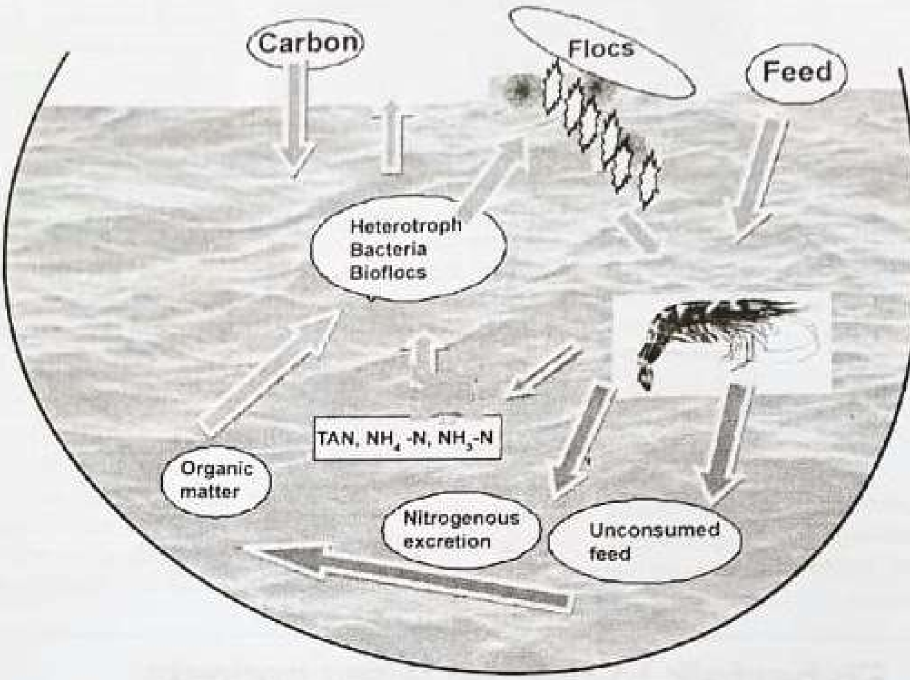

Fig1 : Schematic diagram showing the concept of biofloc in a grow-out system

What is bio-floc technology?

Intensification in aquaculture operations proceeded from clear water system with proper feed management to zero water exchange because of many advantages including the biosecurity. As part of the concept of delivering high productivity with sustainability, many concepts like bio-floc and periphyton based farming system are being evolved. Bio-floc technology (BFT) applied to aquaculture is based on the concept of the retention of waste within the culture system and its conversion to bio-floc as a natural food. Bio-floc is the conglomeration of heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans and detritus which acts synergistically to maintain the water quality in aquaculture units, for the reduction of the water exchange and for reutilisation of the feed and reducing the production cost. In the recent past, even those engaged in intensive farming system employ this technology using some kind of biomats and supplementing with carbon addition to manipulate C:N ratio. Bio-floc is rich in essential nutrients that can support and enhance growth of shrimp along with bioremediation of the nitrogenous metabolites. As the fish/shrimp ponds are rich in microbial community, the inorganic nitrogen added through the feed can be assimilated by the floc micro-organisms and convert the floc in to microbial protein through an adjustment of C:N ratio. There is a need for constant aeration and agitation of the water column and addition of carbon sources as organic matter substrate to allow aerobic decomposition and to maintain high levels of microbial floc in suspension in fertiliser-fed and/or fertilised ponds.

In India, with the introduction of *Litopenaeus vannamei* and its permitted stocking density of 60 pc/ sq m^{-2}, a production of 10-12 tonnes per ha is easily achievable. This production level itself is capital intensive, requiring at least Rs.12-15 lakhs of investment/ ha. At this

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stocking density, is it possible to have the emergence of microbial floc production system in the commercial culture of *L. vannamei*? A higher production of 16-18 tonnes of *Penaeus monodon* and even higher level of *L. vannamei* has been achieved in India using the substrate- based technique where carbon addition was practised along with very high aeration rate. On the other hand, low input and periphyton-based grow-out trial of *P. monodon* at CIBA have been demonstrated to give a better yield and returns consistently at a stocking density between 6-10 pc/ sq m (Anand et al., 2012) . There was substantial gain in the production as well as FCR by following these techniques where yeast-based extracts and other carbohydrate sources or periphyton were used. In another attempt, in extensive system, the application of carbohydrate addition at as low as 6 pc/ sq m stocking, even without aeration has shown merits with a production level of 65g/ sq m (Hari *et al.*, 2004). These authors showed a reduction in the TAN and other nitrogenous metabolites with carbohydrate addition and in turn a surge in the heterotrophic population.

Principle: The bio-floc principle combines the removal of nutrients from the water with the production of microbial biomass, which can *in situ* be used by the cultured species as additional food source. Under optimum C:N ratio, inorganic nitrogen is immobilised into bacterial cell, while organic substrates are metabolised. Some aquaculture species like shrimp, tilapia can use the microbial flocs and utilise the microbial proteins, doubling the feed protein efficiency. In these ponds the effect of C:N ratio can be predicted and the amount of carbohydrate demand can be estimated (Fig II). A C:N ratios 10-12:1 is most advantageous for optimising bio-floc production while minimising ammonia regeneration. The optimum C:N ratio in an aquaculture system can be maintained by adding different locally available cheap carbon sources and/or reduction of protein content in feed. In a typical brackishwater pond, 20–25% of fed protein is retained in the fish/shrimp and rest is wasted as ammonia and other metabolites, organic N in faeces and feed residue. In fact, increased C:N ratio through carbon addition enhances conversion of toxic inorganic nitrogen species to microbial biomass available as food for culture animals.

Bio-floc constituents and characterisation

In the floc, 70-80% Organic matter comprises heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans and detritus, though the composition changes rapidly and frequently through the cycle, 40-50% of the pond bacteria is in flocs and some are free living. Besides the bio-floc volume and the physico-chemical parameters, the level of protein, poly-ã-hydroxybutyrate and fatty acids can be used to characterise microbial flocs. The bio-floc bacteria include genera such as *Pseudomonas*, *Achromobacter*, *Flavoracterium*, *Alcaligenes*, *Arthrobacter*, *Citromonas* and *Zooglea* which are known as floc forming bacteria.

Bio-floc based Technology (BFT)

Designing and engineering consideration: In the pond system, the well prepared lining of pond can be used for effective utilisation and preventing erosion of dykes as very high aeration is required for bio-floc based system. However, earthy ponds also have their merits as far as interaction with soil phase and effective nutrient release is concerned. There is a need for sludge removal facility to serve as an effective control measure of the organic loading generated in the system. Bio-floc technology can be conveniently applied in recirculatory aquaculture system or raceways system either with *in situ* inclusion or *ex situ* floc production through activated sludge system and putting the harvested bio-floc in the production system. These systems can be adequately agitated and aerated to keep the microbial floc in suspension.

Species suitable and stocking density: BFT is suitable for species like *L. vannamei* which are effective at utilising natural productivity/flocs. Other species like *P. monodon*, *P. stylirostris* and *Macrobrachium* species. may not be so suitable for the purpose, at high density. Generally, higher stocking density is suitable for adoption of this technique as enough organic input is available and also the shrimps can help in stirring the pond bottom. Stocking to achieve 10-30 mt of shrimp / ha or 1-400 mt/ha for fish can be supported by following this technology. Again, a stocking of 100 pl/sq m under bio-floc was found to reduce protein requirement substantially, although still expecting the same growth pattern and better survival to a considerable extent. In contrast, there are reports where bio-floc system can be operated with as minimum as 6 pc/ sq m, aiming a higher growth.

Environmental requirements for BFT: BFT works under zero water exchange too,which also makes the system more

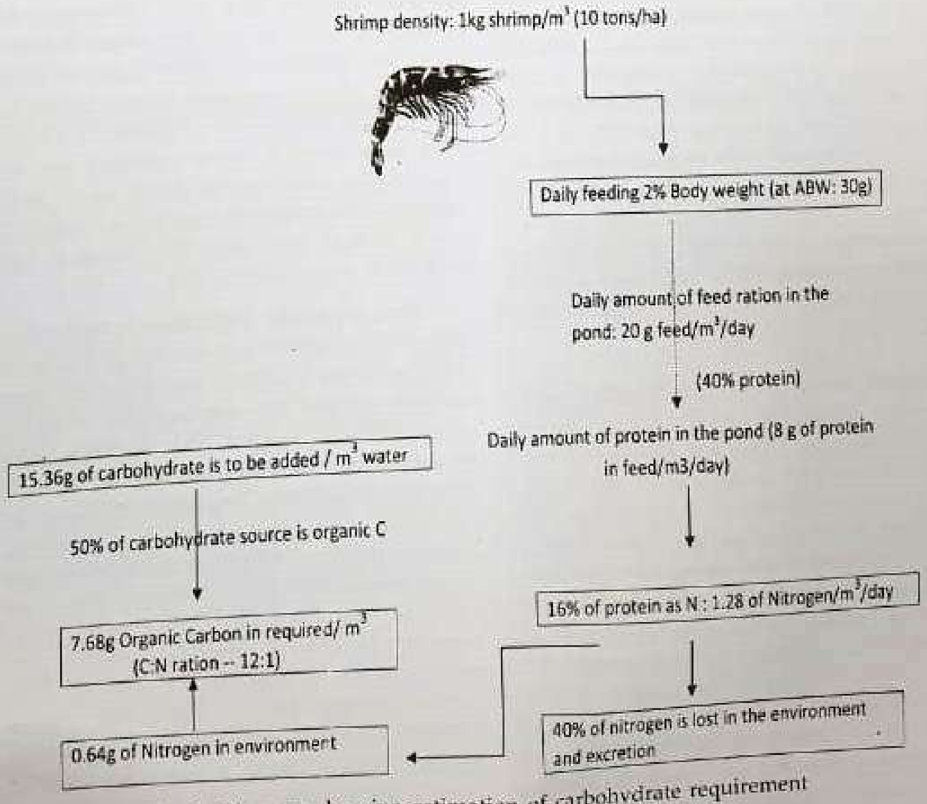

Fig 2: Diagram showing estimation of carbohydrate requirement

bio-secure. In floc system, ammonia is consumed by bacteria and nitrite increases, with tolerance upto a higher level. There is a need to correct the declining alkalinity which must be maintained at >75-150ppm. Another important requirement in the bio-floc system is to meet high oxygen demand. There will be also need to resuspend the aerator of aspirator type at the sludge pile. Typical aeration requirements are 1 HP/ 350-400 kg shrimp biomass. The bio-floc sytem requires high aeration/mixing with aerators, and use of blowers not only to provide DO, and remove CO_2 but also to keep bio-floc in suspension and prevent production of nitrogenous metabolites, sulphides and organic acids.

Feed Protein level **and** *feeding rates:* Based on observations of feeding behaviour of fish/shrimp in the BFT treatments during the first several days and rationed at 3-1.5% of the total standing biomass daily, feeding rates are to be adjusted depending on the day to day consumption and sampling. Daily feed rations are to be split into two to five times to be given at adjusted interval. Carbohydrate source is to be also added at an appropriate rate applied to BFT tanks to maintain an optimum C:N ratio of bacteria (Avnimelech, 1999).

Feed cost can substantially be reduced by reducing the protein level in feed. Even in the case of *L. vannamei*, the growth and survival is reported to be equal or better at 20-30% protein (7% lipid) than 40 % in commercial closed pond systems due to enhanced nutrition on flocs and reduced N-wastes. The guiding standard is that around 95% of N added to pond is from feed/fertilisers and more than 50% of N in feed is released into the environment. One should lower protein content to achieve good C:N ratio and reduce production of N-waste. At <25% feed protein, heterotrophic removal of TAN starts to dominate over autotrophic activity.

Pond preparation for biofloc based farming: There are different approaches to prepare the pond for bio-floc based shrimp farming depending on the design and compatibility of the system, the species to be cultured, intensity of farming and the buffering time for bio-floc generation. Further, the system can operate with complete bio-floc or semi bio-floc mode with or without integrating substrates for periphyton growth. Ponds are prepared following the Standard Operating Procedure (SOP) like drying, ploughing refilling the ponds with aged (minimum for 4-5 days) and disinfected water properly sieved through 100 i screen and adhering to all biosecurity protocols. Autotrophic bloom is developed by fertilising with nitrogen and phosphorous (like 10-30kg Urea + 2-6 kg TSP/ha) fertilisers and/or in combination with any other biofertilisers. The pH is maintained by liming with dolomite or hydrated lime. In the beginning, nitrogen level is built up by using feed or any organic substance. Subsequently, CHO source like molasses is applied depending on the feed quantity and the TAN level so that a high C:N ratio (15:1) can be maintained to stimulate the flocs. Typically ponds start autotrophically - algal dominated and after few days water turns brown and foamy as flocs develop and system goes mostly heterotrophic without much algae. Ammonia levels rise , peak and then fall, following the rise of nitrite which stabilises after sometime and is controlled by adding more C. Transition from algae to bacteria stimulated by adding molasses every 1-3 days interval for 2-3 weeks. Once heterotrophic population establishes, the molasses or other carbohydrate addition can be regulated to keep high C:N ratio based on the TAN level and the feed quantity. There can be modification in the process if the purpose is to generate bio-floc in a shorter duration. If the nursery reared juveniles are stocked, feed quantity is high enough to quick start the bio-flocs with addition of good amount of carbohydrates. To hasten the process bio-floc inoculum from other ponds or in its preserved form can also be applied. However, care should be taken before using any commercial inoculum for this purpose. Heavy aeration is required for keeping the floc under suspension. Pond liner allows easier maintenance of floc in suspension and avoids dead spots and stops accumulation of inorganic material from pond banks/walls caused by excessive water circulation.

Carbohydrate addition: CHOs are added to promote heterotrophic bacteria (HB) as these bacteria use organic C as energy source and for uptake of N to grow. Simple sugars like sucrose and molasses induce to grow the floc faster, however requiring frequent additions. In contrast, complex starches i.e. corn, cassava, tapioca, wheat and cellulose are most stable but slow to react. They can also act as bacterial substrates and contain suites of enzymes useful for digestion once ingested by shrimp. The lower the feed protein level, the less of CHO is required. In another study using tilapia farm effluent, it was determined that 1 kg of microbial floc could be produced per 1.49 kg of sucrose. In minimal-exchanging intensive systems, excess nutrients are assimilated and mineralised by a dense microbial community in the water column, thus alleviating potential toxicity.

Management and Control mechanism: Floc management is basically done keeping in mind the needs of the bacteria and not the shrimp, as at times total bacterial biomass is 2-5 times than that of the shrimp. Floc volumes typically measure 2-4 ml/l is first 1-2 months, then 6-20 ml/l later. Total soluble solids should be managed to be less than 300 ppm (3 mt/ha) to reduce aeration requirements. With C addition, TAN can be limited at 0.5-1 ppm. pH should be controlled by addition of lime /dolomite/bicarbonate and better alkalinity should be maintained.

Controlling the unregulated growth of bio-floc in the pond system is required to avoid a number of critical problems like declining oxygen level and increase in sludge. So, proper handling of C addition and feeding quantity and schedule as per the standing biomass is required for having a control over bio-floc generation.

Other Characteristics of Bio-floc: One important property of bio-floc is their bio-control effect. Among the important micro-organism groups, algae utilise toxic total ammonia nitrogen (TAN), as well as less dangerous nitrate-nitrogen and phosphate compounds to construct cellular structures such as proteins and sugars. Again, certain groups like diatoms are nutritious and can benefit shrimp production by contributing qualities such as essential amino acids and highly unsaturated fatty acids. Many pathogens in aquaculture have been found to control virulence factor expression by quorum sensing. It is hypothesised that, the "natural probiotic" effect in bio-floc could act internally and/or externally against, i.e., to *Vibrio* sp. and other disease agents like ectoparasites. Bio-floc can be comprised of beneficial microflora having probiotic properties through probiotic driven biofloc system. Different probiotic strains have been shown to be capable of modulating the immune system of aquatic animals in several ways (Panigrahi et al, 2007). Antagonistic properties of bio-flocs can be explored to understand their mechanism to control the infection.

Advantage

Advantages already established:

√ Since there is less/ no water

exchange, biosecurity of the system can be maintained;

- √ Heterotrophic bacteria can reduce toxic metabolites (NH_3, NO_2),
- √ Diurnal changes (pH, O_2, CO_2) in pond water is reduced, stabilising environment and reducing stress;
- √ Easier management and environmental friendly approach (reduced protein requirement, fish meal usage and water/nutrient discharge);
- √ Doubling the protein utilisation, as shrimp use proteins twice - eat feed and then consume flocs;
- √ Bio-floc -fed shrimp have less, but more of diverse gut flora that reduce pathogens, and
- √ Reduced costs (15-20% lower cost of production) including 30-50% cost savings in feed.

Additional advantages yet to be fully established:

- Enhancing digestion (with enzymes and growth promoters) ,
- Role in immune response by stimulating humoral and cellular immunity,
- Probiotic action - reducing pathogenic bacteria (*vibrios*),
- *Virus Removal:* potential of bacterial flocs to remove viruses from water requires further investigation.

Conclusion

Depending on the investment possibility, a farmer can go either for earthen pond or lined pond with substrate based periphyton or suspension-based bio-floc intervention to get better production and sustainability. Bio-floc technology with all the required control over microbial manipulation, heavy aeration and sludge removal facility can be followed at higher stocking density (30-60 pc/ sq m), whereas periphyton based farming can be adopted for extensive low input based farming systems, with low stocking and even with no provisions for aeration. Even in low input based farming systems addition of molasses or other yeast derivatives are being followed by farmers to have good bacterial population and stable plankton bloom. Microbial flocs were produced in sequencing batch reactors (SBRs) using fish farm effluent and sugar as a growth media. Further, for the recirculatory aquaculture system with super intensive stockings, microbial flocs produced in suspended growth bioreactors could offer the shrimp industry a novel alternative feed. The continuous crop of shrimp obtained in the farms where bio-floc technique is followed is an enough proof in its support, though more robust scientific studies are required to elucidate this concept. It is required to understand further the principle of this microbial intervention to reap maximum benefit from these potential techniques across the farming systems and intensifications.

Suggested readings

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Consultation Workshop on Self-Sufficient and Sustainable Aquaculture in North Eastern Region

One day consultation workshop on "Self-sufficient and sustainable aquaculture in North Eastern region" at Pragyan Bhavan, Agartala on 5th February 2014 was organized by the Central Institute of Freshwater Aquaculture, Bhubaneswar in collaboration with Department of Fisheries of Tripura Government. Hon'ble Chief Minister, Mr. Manik Sarkar inaugurated the workshop in the presence of Fisheries Minister ShriKhagendraJamatia and other dignitaries. In his inaugural address, he said that Tripura has demonstrated the successful development of the aquaculture in the region and stressed on increasing per capita production of fish to reduced dependence on fish from other states. He said the per capita consumption has increased up to 17 kg which was 8 kg earlier, which is due to increase in production. He expected that production of fish will increase further in future.Welcome address was delivered by Dr. P. Jaysankar, Director,CIFA. Dr. Dilip Kumar, Advisor (Fy.), BTC, Bihar also delivered a speech on the occasion.Representing CIFT Dr. M.M Prasad, PS& SIC and Ms. Jesmi Debbarma, Scientist has participated in the workshop.

The inaugural session was followed by opening of exhibition and visit of Chief Guest Mr. ManikSarkar to all the stalls that included stall of CIFT. Other dignitaries namely Directors of different Fisheries Departments of different North Eastern states, ICAR Institutes, Scientists, personnel from Sate Departments, aquaculturists and fish farmers from different North Eastern states also visited CIFT stall. Most of the Directors of Fisheries Departments from different north eastern state who visited CIFT stalls have evinced keen interest on the technologies developed at CIFT and were eager to adopt them.Mr.ManikSarkar,hon'ble Chief Minister of Tripura had a brief meeting with Miss. Jesmi Debbarma and Dr. M.M.Prasad and he appreciated works carried out by CIFT.In the technical session as a part of technological interventions experience and opportunities, Dr. Prasad made a presentation on " Roadmap for development of harvest and post harvest fisheries in north eastern states". After the completion of technical session plenary session was held. Meeting came to a conclusion with vote of thanks from the Director of CIFA.