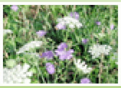

Photo Gallery

Microbial soup-Eco based approach for shrimp culture and management

A. Panigrahi*, M. Sundaram,P. Ravichandran and C. Gopal
CIBA (Indian Council of Agricultural Research),
Chennai- 600 028.
Email: apanigrahi2k@gmail.com;panigrahi@ciba.res.in

Abstract

The concept of 'microbial soup' eventually led to development of "biofloc" technology in changing the facet of intensive aquaculture with scope to attain high productivity through a sustainable approach. By developing dense heterotrophic bacterial community, the system becomes bacterial dominated rather than algae dominated and takes care of the waste generated in the aquaculture system through *in-situ* bioremediation. Biofloc technology approach promises a healthy rearing system, which is increasingly identified as one possible solution for disease problems. Protein is utilized two times once as feed for the shrimp and second time as microbial floc when the heterotrophic microbes converts the nitrogenous waste into protein.

Central Institute of Brackishwater Aquaculture has successfully demonstrated the biofloc and periphyton based nursery and grow out technology for Pacific white shrimp *Litopenaeus vannamei*. This study deals with the evolution of the concept, principles and progress in research including dynamics of biofloc production through addition of different carbohydrate sources and inoculums *vis–a-vis* protein levels for better yield. Advantage in providing better growth performance, nutrition, the "natural probiotic" effect in BFT and protective response against pathogens with biofloc based rearing is elaborated.

Keywords

Microbial soup, Biofloc, Periphyton, Protein, BFT and, Eco-based approach, bacteria

Introduction

The concept of delivering high production with sustainability is gaining momentum in aquaculture practices and many technologies like Biofloc and Periphyton based farming system are being evolved nowadays. Biofloc technology (BFT) is a promising technology which promotes the retention of waste and its conversion to biofloc as a natural food within the aquaculture system. Biofloc is the conglomeration of microorganisms (such as heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus). This is a relatively new biotechnological means to support high density, to maintain the water quality, reduce water exchange, maintaining biosecurity, reutilize the feed and reduce the production cost. In Asia, the shrimp-farming industry has been heavily affected by early mortality syndrome or, more descriptively, acute hepatopancreatic necrosis syndrome (AHPNS). Bio-floc technology approach promises a healthy rearing system and identified as one possible solution for these disease problems. Periphyton is a complex mixture of algae, cyanobacteria, heterotrophic microbes, and detritus attached to submerged surfaces in aquatic ecosystems. BFT in combination with Periphyton increases the natural production and in turn productivity.

Genesis and evolution

Going back to the genesis of this concept, Steve Surfling in 1976, put forward the 'microbial soup' concept that eventually led to development of "biofloc" based aquafarming. By developing dense heterotrophic bacterial community, this system becomes bacterial dominated. Accumulation of these bacteria, called soup or flocs, engulf up the nitrogenous wastes ten to hundred times more efficiently than algae, irrespective of the weather, and turn them into high-protein feed. The cell wall of the microbial constituent of this microbial soup (biofloc), such as bacterial lipopolysaccharide, peptidoglycan and ß-1, 3-glucans, stimulate nonspecific immune activity of fish/shrimp (Panigrahi *et al*., 2007).

BFT was first developed in early 1970s at Ifremer-COP (French Research Institute for Exploitation of the Sea, Oceanic Center of Pacific) with different penaeid species including *Penaeus monodon, Fenneropenaeus merguiensis, Litopenaeus vannamei*. Later, Prof. Yoram from Israel, contributed immensely for the growth of this promising technology. Biofloc technology has recently gained attention as a sustainable method to maintain water quality with the added value of producing proteinaceous feed *in-situ* (Crab *et al*., 2012). In addition to organic nitrogenous waste, ammonium will be converted into bacterial biomass if C:N ratio is balanced at a ratio of 10-15:1 (Schneider *et al*., 2005). The growth rate and microbial biomass yield per unit substrate of heterotrophs are higher than that of nitrifying bacteria, thus making many fold increase in heterotrophic bacteria (Hargreaves, 2006).

C: N Ratio and biofloc manipulation

The biofloc principle combines the removal of nutrients from the water with the production of microbial biomass, which can be used by the culture species, *in-situ* as additional food source. Application of immobile substrate and manipulation of carbon nitrogen ratio have drawn attention for their role in enhancement of aquaculture production through improved nutrients utilization and control of toxic nitrogen metabolites. Manipulation of C:N ratio of the feed affects the quantity and quality of periphyton production over the substrate. The optimum C:N ratio in an aquaculture system can be maintained (C:N ratio 12-15:1 is optimal for biofloc production) by adding different locally available cheap carbon sources and/or reducing protein percentage in feed. Under optimum C:N ratio, inorganic nitrogen is immobilized into bacterial cell, while organic substrates are metabolized. This technique was initially developed in Israel and subsequently spread to many other countries due to its several advantages. However, this is still in an initial stage and lot of research is necessary for its modification, standardization and implementation.

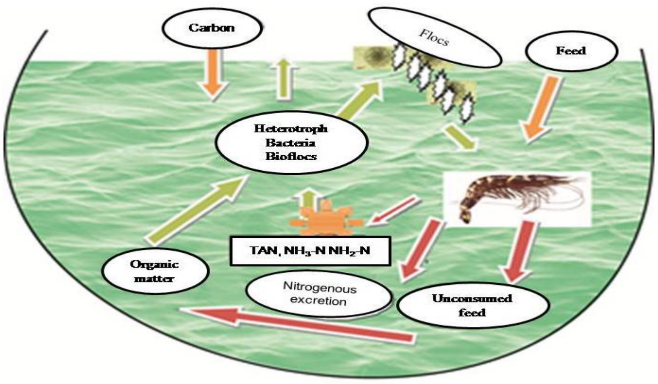

Fig 1: Schematic diagram showing the concept of biofloc in a grow – out system

Biofloc research in India

Central Institute of Brackishwater Aquaculture (CIBA), Chennai has initiated research for standardizing nursery and grow-out culture methods for *L. vannamei* on periphyton and biofloc based technology. A series of experiments on pilot scale was conducted at CIBA to standardise the bio-floc system and optimise the carbohydrate, C:N ratio and inoculums, nutritional characterisationof different types of biofloc and protein requirement for maximum gain of their inclusion in feeds. There was substantial gain in the production as well as FCR in tiger shrimp *P. monodon* farming by following these eco based techniques (Anand *et al.*, 2013). In an outdoor growth trial, carbon nitrogen ratio (C:N ratio 10 and 20) was manipulated with shrimp feed (containing 32% crude protein and rice flour as carbohydrate source) resulted in 42% increase in final body weight. Ray *et al.* (2010) described this as a complex interaction between organic matter, physical substrate and large range of microorganisms. Bacteria and other microorganisms act as very efficient “biochemical systems” degrader and metabolize organic residues (Avnimelech,1999).

Biofloc constituents and characterization

The constituents of biofloc were characterised and found to be comprised of 70-80% of microorganisms such as phytoplankton, free and attached bacteria, aggregates of particulate organic matter and grazers, such as rotifers, ciliates and flagellates protozoa and copepods. Besides the biofloc volume and the physicochemical parameters, the level of protein, poly-ß-hydroxybutyrate and fatty acids can be used to characterize microbial flocs.

CHO source and reduction in TAN and Nitrite

Carbohydrate (CHO) sources like molasses, wheat, tapioca or some other inexpensive carbon sources were added to make carbon/nitrogen ratio of 12:1, or higher, to promote heterotrophic bacteria (HB) as these bacteria use organic carbon as energy source and uptake of nitrogen to grow through the production of microbial proteins (Avnimelech, 1999). Simple sugars like sucrose and molasses found to induce the growth of the floc faster, however, it require frequent additions. In contrast, complex starches like corn, cassava, tapioca, wheat and cellulose most stable but slow to react, can also act as bacterial substrates and contain suites of enzymes which facilitate digestion once ingested by shrimp. The impact of the carbon source type on bio?oc characteristics and shrimp production economics is being investigated.

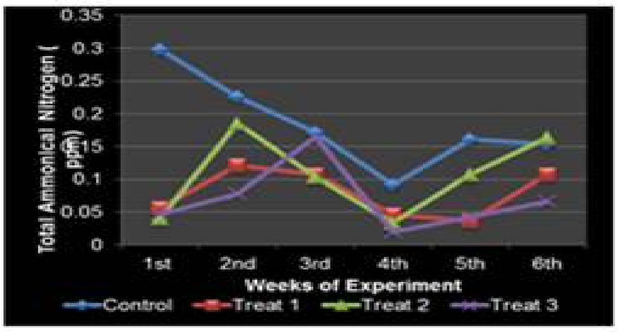

Fig 2: Reduced level of TAN in Biofloc treatments

Biofloc technology for improving production efficiency in aquaculture

CIBA have successfully demonstrated the biofloc and periphyton based nursery technology for shrimp culture in tank system, where a very high survival of 98-99% was achieved compared to the conventional system (91-92%). Interestingly, it is found to reduce the risk of early mortality¸ due to any disease, at nursery phase, and ensuring better survival during grow-out phase. Recently in another demonstration trial, a production up to 4 to 4.5 kg/ cu m (40-45 tonnes/ha) was achieved at the institute facility using different protein levels in feed through biofloc based farming system. The results also revealed a very high growth rate in the biofloc system compared to that of the conventional system and a very high standing crop could be maintained without any risk.

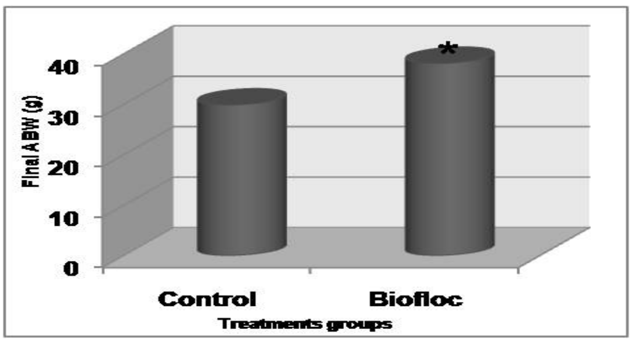

Fig 3: Gain in average body weight in the bio-floc group of shrimp during a grow-out experiment.

In our study, it was found that the protein content in pelletized feed can be reduced substantially without compromising growth and survival. It was observed that shrimp fed with minimum crude protein under biofloc conditions performed better, especially at higher density,compared to those fed with 40% protein under regular autotrophic intensive culture. This would help to reduce feed cost. It is important to reduce the protein content to achieve good C:N ratio and reduction of N-waste formation. At lower protein content, heterotrophic removal of total ammonium nitrogen (TAN) begins to dominate over autotrophic and the growth and survival was found to be better than higher protein feed in commercial closed pond systems due to enhanced nutrition on flocs and reduced N-wastes (Ballester *et al*., 2010; Nunes *et al*., 2010).

Bioflocs as a biocontrol measure, Immunomodulation and protective response

The "natural probiotic" effect in bio-floc could act internally and/or externally against, *Vibrio* sp. and ectoparasites. The regular addition of carbon in the water is known to select for polyhydroxy alkanoates (PHA) accumulating bacteria which produce several biodegradable polymer storage products, like poly-ß-hydroxy butyrate (PHB), having antibacterial or biocontrol properties which provide immunity to the host (Sinha *et al*., 2008). The number of total bacteria in biofloc group was significantly higher (10^6 to 10^8 cfu mL^{-1}) than in the conventional system. The cellwall components of these beneficial microbes have potential immunomodulatory properties. More than 50% of the bacteria in these systems were free living and the reminder was associated with detritus in the form of flocculated matter (Burford *et al*., 2004). Our studies suggest that microbes associated with bioflocs may enhance expression of certain immune-related genes. Studies in the similar line also revealed an upregulation of immune genes like prophenoloxidase, prophenoloxidase activation enzyme and serine proteinase1 genes with exposure to biofloc thus implying immunomodulation in the shrimp. Probiotic interventions of probionts *L. rhamnossus* and *B. subtilis* were found to be advantageous in terms of better growth and survival rate (Panigrahi and Azad, 2007). Again, the expression of certain immune related genes were significantly up-regulated explaining the possible immunomodulations and in turn better protectionin to fish (Panigrahi *et al*., 2007; 2011) which opens the scope of probiotics driven biofloc interventions.

Microbial biomass application as feed for aquaculture species

The macro aggregates (biofloc), in aquatic medium, is proteinlipid rich natural source available "*in-situ*" throughout the day (Avnimelech, 2007). It is possible to replace 1/3 of a conventional diet by low-protein biofloc meal without interfering survival and performance of the shrimp. Harvested bioflocs have been biochemically characterised as premium quality feed ingredients. This can be used instead of fishmeal in shrimp diets (Crab *et al*., 2007) and is also cost effective (Kuhn *et al*, 2009). This could also be incorporated into broodstock diet. Growth enhancement has been attributed to both bacterial and algal nutritional components, which is up to 30% of conventional feeding ratio and can be lowered due to biofloc consumption in shrimp. Burford *et al*., (2004) reported that more than 29% of daily food consumed by *L. vannamei* could be of biofloc. Shrimps could also be grown on a diet without any of the marine protein sources in a biofloc based rearing system without compromising growth.

Fig 4: (A-F)Biofloc technology commercial-scale at CIBA –harvest festival (A, B), harvested shrimp (C), pilot scale tanks (D), biofloc measuring cones (E) and commercial scale Hitide Sea farm (F).

Future Research

Immunomodulatory properties of the bioflocs for the host, molecular characterization of biofloc constituents, poly-ß-hydroxybutyrate like constituents which influences the protective response, the potential antagonistic properties, and efficacy of bioflocs to influence viral and bacterial load are the other areas which need further investigation. Mechanism to control the unregulated growth of bio-floc in the existing pond systems and evaluating biofloc and/or periphyton based system for advantage also needs research attention. Further fine-tuning and implementation of this technology will also attract more focused research and development. Researchers are challenged to refine this technique to farmers friendly to implement in their future aquaculture systems. Many research issues can be fashioned to optimise microbial floc production, maintaining the water quality, reducing the water exchange, risk of disease and reutilizing the nitrogenous metabolites and understanding its working mechanism with altering factors such as C:N ratio, temperature, dynamics of TAN and other metabolites.

Conclusion

Biofloc technology will enable aquaculture practices towards an environmental friendly approach. This technology as revealed through our studies brings an obvious advantage of minimizing water requirement, recycling *in-situ* nutrients and organic matter and in turn improving farm biosecurity by exclusion of pathogens, augmentation of natural food and improvement of FCR, zero/minimal water exchange system and *in situ* bioremediation to maintain water quality, protein requirements in feed could be brought down in these systems, providing stress-free environment, use of chemicals and other medicines are not required and negligible environmental impact. This 'microbial soup' based technology BFT have potential to revolutionise the aquaculture sector.

References

Anand, P.S.S, Sujeet Kumar, Panigrahi, A., Ghoshal, T.K., SyamaDayal, J., Biswas, G., Sundaray, J.K., De, D., Ananda Raja, R., Deo, A.D., Pillai, S.M. and Ravichandran, P. (2013). Effects of C: N ratio and substrate integration on periphyton biomass, microbial dynamics and growth of Penaeus monodon juveniles. Aquacul.Intl, **21**:511–524.

Avnimelech, Y. (1999). Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture, **176**, 227–235.

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture, **264**:140–147.

Ballester, E.L.C., Abreu, P.C., Cavalli, R.O., Emerenciano, M., Abreu, L. and Wasielesky, W. (2010). Effect of practical diets with different protein levels on the performance of F. paulensis juveniles nursed in a zero exchange suspended microbial flocs intensive system. Aquacul. Nutri. **16**:163-172.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H. and Pearson, D.C. (2004). The contribution of flocculated material to shrimp (Litopenaeus vannamei) nutrition in a high intensity, zero-exchange system. Aquaculture, **232**:525–537.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P. and Verstraete, W. (2007). Nitrogen removal in aquaculture towards sustainable nitrogen in aquaculture systems. Aquaculture, **257**: 346–358.

Crab, R., Defoirdt, T., Bossier, P. and Verstraete, W. (2012). Biofloc technology in aquaculture: Beneficial effects and future challenges. Aquaculture, **356–357**: 351–356.

Hargreaves, J.A. (2006). Photosynthetic suspended-growth systems in aquaculture. Aquacul. Engg., **34**: 344–363.

Kuhn, DD., Boardman, G.D., Lawrence, A.L., Marsh, L. and Flick, G.J. (2009). Microbial flocs generated in bioreactors is a superior replacement ingredient for fishmeal or soybean meal in shrimp feed. Aquaculture, **296**:51–57.

Nunes, A.J.P., Castro, L.F. and Sabry-Neto, H. (2010). Microbial flocs spare protein in white shrimp diets. Global Advocate, **10**:28-30.

Panigrahi, A. and Azad, I.S. (2007). Microbial intervention for better fish health in aquaculture: the Indian scenario. Fish Physiol. Biochem., **33**:429-440.

Panigrahi, A., Kiron, V., Satoh, S., Hirono, I., Kobayashi, T., Sugita, H., Puangkaew, J. and Aoki, T. (2007). Immune modulation and expression of cytokine genes in rainbow trout Oncorhynchusmykiss upon probiotic feeding. Develop. Comp. Immunol., **31**: 372-382.

Panigrahi, A., Kiron, V. and Suichi, S. (2011). Real time quantification of the immune gene expression in rainbow trout fed different forms of probiotic bacteria Lactobacillus rhamnosus. Aquacul. Res., **42**: 906-917.

Ray, A.J., Seaborn, G., Leffler, J.W,, Wilde, S.B., Lawson, A. and Browdy, C.L. (2010). Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. Aquaculture, **310**:130–138.

Schneider, O., Sereti, V., Eding, E.H. and Verreth, J.A.J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. Aquacul. Engg., **32**: 379–401.

Sinha, A.K., Baruah, K. and Bossier, P. (2008). Horizon Scanning: the potential use of biofloc as an anti-infective strategy in aquaculture – an overview. Aquacul. Health Intl., **13**:8-10.

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