2.1 Sustainable aquaculture

Approximately 16% of animal protein consumed by the world’s population is originated from fish, and over one billion people worldwide depends on fish as their main source of animal protein (FAO, 2000). Aquaculture offers one way to supplement the production of wild capture fisheries and it will continue to increase in importance as demand increase in future (White et al., 2004). The ever growing demand for seafood leads to the intensification of aquaculture through high stocking density and intensification of the artificial feeds, leading to the aquaculture sector as most cost-effective as well as waste promoting industry. Like other form of intensive food production, industrial-scale fish farming generates significant environmental costs (White et al., 2004). Aquaculture development should be in a sustainable manner. Sustainable development is defined as the management and conservation of the natural resource-base and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development conserve land, water, plant and
animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable and socially acceptable (FAO, 1991).

### 2.2 Environmental problems of aquaculture

Aquaculture production has increased at an average annual rate of 8.9% since 1970, as compared with an annual growth rate of 1.2% and 2.8% for capture fisheries and terrestrial farmed meat production over the same frame. Yet, to supply demands, aquaculture production must grow by five fold in the next five decades. This development has to overcome three major constraints: a) Produce more fish without significantly increasing the use of basic natural resource of water and land, b) Develop sustainable systems that will not damage the environment, c) Develop systems providing a reasonable cost/benefit ratio, to support the economic and social sustainability of aquaculture (Avnimelech, 2009).

Alagarswami (1995) identified adverse impacts of aquaculture on social and physical environments and emphasized the need to adopt eco-friendly technologies. Intensive aquaculture systems are used to efficiently produce dense biomasses of fish or shrimp, intensive aquaculture industry faces two major problems. The first is the water quality deterioration caused by the high concentrations of metabolites and the second is the low feed utilization in cases when high water exchange, within or outside the pond system, is practiced (Avnimelech, 2007). Artificial formulated feed is the main investment in aquaculture i.e.; feed is the largest single cost item, as it constitutes 40-60% of operational cost in prawn production (Mitra et al., 2005).

The major portion (>80%) of artificial feed is lost in the aquaculture system as uneaten feed and faeces (Daniels and Boyd, 1989; Siddiqui and Al-Harbi, 1999; Rahman, 2006). Artificial feed, which is lost in the system,
has a great effect on water quality through decomposition (Horner et al., 1987; Poxton and Allouse, 1987; Cowey and Walton, 1989; Poxton and Lloyd, 1989; Wilson, 1994; Moreira et al., 2008). Higher dietary protein deteriorates the water and soil qualities in shrimp grow-out ponds (Boyd, 1989). To great extent water quality determines the success or failure of aquaculture operation. Physical and chemical characteristics such as suspended solids, temperature, dissolved gases, pH, mineral content and the potential danger of toxic metals must be considered in the selection of a suitable water source (Boyd and Tucker, 2009).

The salmon and shrimp aquaculture have proven to be destructive to the natural environment and population of aquatic animals (Gowen and Bradbury, 1987; Folke et al., 1994; Kautsky et al., 1997; Naylor et al., 2000; Milewski, 2001). Crustacean aquaculture is fraught with environmental problems that arise from: (i) the consumption of resources such as land, water, seed and feed; (ii) their transformation into products valued by society; and (iii) the subsequent release into the environment of wastes (Kautsky et al., 2000; Ronnback, 2001). The direct impacts include release of eutrophicating substances and toxic chemicals, the transfer of diseases and parasites to wild stock, and the introduction of exotic and genetic material into the environment. The environmental impact can also be indirect through the loss of habitat and niche space, and changes in food webs. Whereas traditional and extensive shrimp aquaculture uses natural production in the ponds or in the incoming waters, semi-intensive and intensive production systems are heavily dependent on formulated feeds based on fishmeal and fish oils. These latter systems use more than two times more protein, in the form of fishmeal, to feed the farmed shrimps than is ultimately harvested (Tacon, 1996). Most aquaculture systems are so-
called throughput systems (Daly and Cobb, 1989). This means that resources, collected over large areas, are introduced and used in the aquaculture production site, and released back into the environment in concentrated forms as nutrients and pollutants, causing various environmental problems (Folke and Kautsky, 1992). Uneaten food, faecal and urinary wastes may lead to eutrophication and oxygen depletion, the magnitude of which is dependent on the type and size of operation as well as the nature of the site, especially size, topography, and water retention time (Kautsky et al., 2000). In semi-intensive and intensive farms, artificial feeds provide most of the nitrogen (N), phosphorus (P) and organic matter inputs to the pond system. Only 17% (by dry weight) of the total amount of feeds applied to the pond is converted into shrimp biomass (Primavera, 1993). The rest is leached or otherwise not consumed, egested as faeces, eliminated as metabolites, etc. Effluent water during regular flushing and at harvest can account for 45% of nitrogen and 22% of organic matter output in intensive ponds (Briggs and Funge-Smith, 1994). Consequently, pond sediment is the major sink of N, P and organic matter, and accumulates in intensive shrimp ponds at the rate of almost 200 t (dry weight) per ha and production cycle (Briggs and Funge-Smith, 1994). During pond preparation between cropping the top sediment is removed and usually placed on pond dikes, from where it continuously leaks nutrients to the environment. Several methods have been proposed to ameliorate the impact of shrimp pond effluents on the water quality of the recipient: improved pond design (Dierberg and Kiattisimkul, 1996); construction of waste-water oxidation-sedimentation ponds, reduction of water exchange rates (Hopkins et al., 1995); reduction of nitrogen and phosphorus input from feed (Jory, 1995); removal of pond sludge; a combination of semi-closed farming systems with settling ponds and biological treatment ponds using polycultures (Dierberg and Kiattisimkul,
and the use of mangroves as biofilters for pond discharge prior to the release of effluent to estuarine waters (Robertson and Phillips, 1995). Furthermore, the use of fertilisers should be restricted to organic products.

In response to a public interest petition, the Supreme Court of India in 1996 directed the concerned authorities to abolish aqua-farms in the coastal regulation zone and to constitute an “Authority” to regulate aquaculture (Krishnan and Birthal, 2002). The Aquaculture Authority of India (AAI) has been constituted and guidelines on sustainable aquaculture development for regulating coastal aquaculture. By The Coastal Aquaculture Authority Act, 2005 enacted by the Central Government on 23 June 2005 the AAI was restructured to the Coastal Aquaculture Authority for regulating the activities connected with coastal aquaculture in coastal areas and matters connected therewith or incidental thereto. For making the aquaculture practices sustainable in the country, the Coastal Aquaculture Authority is giving directions, guide line and best management practices. According to Coastal Aquaculture Authority, activities such as construction of aquafarms in mangrove areas, conversion of agricultural field to aquaculture, use of ground water for aquaculture, collection and stocking of wild seeds, use of banned chemicals and drugs, releasing farm effluent into the natural aquatic environment, etc. are prohibited. CAA recommends maintenance of a buffer zone between farm and village facility, proper pre-stocking procedure, use of healthy and quality seeds from approved hatcheries, monitoring of soil and water quality at regular intervals, practicing suitable and recommended stocking density, raising seaweeds, mangrove saplings and bivalves in waste stabilization ponds and outflow canals.
2.3 Concept of biofloc technology and its application in aquaculture systems as a tool for waste management

Knoesche and Tscheu (1974) already adopted the idea of intensive heterotrophic bacteria growth in aquaculture systems and could retain 7% feed N and 6% feed P (estimated from 1% P feed, KarpiCo Supreme-7Ex, Coppens International, The Netherlands). They used an activated sludge process to treat water in a recirculation system, and proposed to mix produced sludge with grains for later re-use as fish feed for carps. The principles of growing fish or shrimp in limited water exchange intensive ponds were developed simultaneously for shrimp in the Waddel Mariculture Centre in the USA and for fish, mostly tilapia, in Israel (Avnimelech et al., 1989, 1994; Hopkins et al., 1993; Chamberlain and Hopkins, 1994) and practiced in the USA (Serfling, 2000), in the beginning of the 1990's. The idea of addition of carbohydrate for the immobilization of ammonia excreted by the fishes was suggested by Avnimelech and Lacher (1979), Boyd (1985), and Muthuwani and Lin (1996). But idea about the general water quality of the pond is essential before any modification or manipulation in aquaculture systems (Boyd and Tucker, 2009).

Good water quality is the key factor for the success of aquaculture and that ensures the survival, production and growth rate of the cultured animals (Boyd, 1990; Burford, 1997). Biofloc technology, BFT, (called also active suspension ponds, heterotrophic ponds, green soup and other terms) was first developed to solve water quality problems. Water quality management is based upon developing and controlling dense heterotrophic bacteria within the culture component (Avnimelech, 2007). The addition of fertilizers or carbon sources directly to the pond water is a way to augment the natural productivity (Crab et al., 2007; Uddin et al., 2007). The removal of toxic nitrogenous
compounds, especially ammonium, from water through its assimilation into microbial protein by the proper addition of carbonaceous materials to the culture system is the basic principle of biofloc technology. The success of this technology mainly depend on the selection of species, because the cultured animal should have the ability to harvest the bacterial floccules developed in the system, and should have the ability to digest and utilise the microbial protein. The bacterial floc produced as the result of bioflocculation serve as an important source of feed protein. Experimental trials showed that microbial flocs of different sizes can be taken up by fish or shrimp and serve as a feed source (Avnimelech et al., 1989; Beveridge et al., 1989; Rahmathulla and Beveridge, 1993; Tacon et al., 2002; Burford et al., 2004) which will help to reduce the cost of production by reducing the protein content of the artificial feed and improving the overall economics (Mc Intosh, 1999; Moss, 2002).

Avnimelech (2007) schematically represented and explained the process behind biofloc production and harvest by the fishes as

\[
\frac{D[BF]}{dt} = BF_{production} - (BF_{harvesting} + BF_{degradation})
\]

Where \( \frac{D[BF]}{dt} \) is the bio-floc concentration change with time, as affected by production, harvesting by fish and biodegradation. The process shown in this equation depends on a verity of factors:

1) Production of biofloc depends on the supply of organic substrates to the microbial community, both external sources (feed supply, algal activity) or by the excretion of un-utilized feed components by fish. In addition bioflocs production most probably depends on the quality of the added substrates, its C/N ratio, bio-availability and other factors.
2) Uptake of the bio-flocs by fish depends most probably on the fish species and feeding traits, fish size, floc size and floc density. It is possible that bioflocs harvesting depends also on the presence and rate of formulated feed added to the pond. In addition, feed eaten by the fish may be utilized and accumulate in the fish, or excreted and serve as a substrate for the production of more bioflocs.

3) Biodegradation of the floc depends on the microbial community associated with the bioflocs, be it bacteria, protozoans or others.

4) Finally, all of these processes may be affected by environmental and operational conditions such as temperature, water salinity, water exchange rate (affecting floc mean residence time), mixing intensity and many other.

Biofloc aquaculture is a sustainable solution for the development of aquaculture and this technology is fully based on the concept of carbon nitrogen (C/N) ratio. The control of inorganic nitrogen accumulation in the pond is based upon carbon metabolism and nitrogen immobilization into microbial cells (Avnimelech et al, 1989; Avnimelech, 1999; Crab et al., 2007). Bacterial cells are composed of proteins. carbon nitrogen ratio of most microbial cell is about 4-5 (Rittmann and Mc Carty, 2001). When bacteria fed with organic substrates that contains mostly carbon and little or no nitrogen (sugar, starch, molasses, cassava meal, etc.), they have to take up nitrogen from the water in order to produce the protein needed for the cell growth and multiplication. C/N ratio in an aquaculture system can be increased by the addition of cheap carbohydrate source or reduction of protein content in the feed (Avnimelech, 1999; Hargreaves, 2006).
Avnimelech and Mokady (1988) and Avnimelech et al. (1992) suggested managing the heterotrophic food web by the development of active suspension ponds, intensive ponds with zero or limited water exchange, accumulating high amounts of organic substrates.

In summary, inorganic nitrogen accumulation is controlled through the addition of carbonaceous substrates, raising the C/N ratio and leading to the immobilization of nitrogen through the production of microbial proteins (Avnimelech et al., 1989, 1992, 1994; Crab et al., 2007).

Fish harvest the bacteria, as bacterial flocs and utilize this protein source (Avnimelech et al., 1989; Milstein et al., 2001; Mc Intosh, 2000a). The amount of carbohydrate needed for reducing the ammonium was worked by Avnimelech (1999). The equation proved its success both in indoor and farm level experiments by various researchers (Hari et al., 2004, 2006; Varghese, 2007; Saritha, 2009).

Kurup (2009) summarised the advantages of the application of BFT to shrimp culture systems in India as:

- It is the best means for the control of toxic inorganic nitrogen in water and for accumulating production of microbial protein by adjusting C/N ratio
- It can convert uneaten nitrogen for being utilized to produce microbial protein rather than generating toxic component
- Microbial protein, the end product which is suspended in the system as microbial flocs can be utilized as feed by shrimps
- The level of protein utilization is doubled in microbial reuse system
The dense heterotrophic microbial biomass decreases the outbreak of microbial diseases and finally

The technology enables high yield in environmentally and economically sustainable system

Minimal-exchange, intensive aquaculture systems offer an environmentally attractive means of shrimp and fish production, allowing for high density culture and little or no water exchange (Ray et al., 2010b). The basic principle of the activated suspension technique and the C/N ratio controlled systems were recently referred as biofloc technology (BFT) is the retention of waste and its conversion to biofloc as a natural food within the culture system (Azim and Little, 2008). One of the features of natural aquatic environment is the ability to recycle the nutrients. Microbial floc generally consists of floc formers, filamentous bacterial particles, protozoans, zooplankton, colloids, organic polymers, cations and dead cells surrounded by a gelatinous matrix, which contains extra-cellular polymeric substances that encapsulate the microbial cells and play a major role for binding the floc components together (Jorand et al., 1995; Avnimelech, 2007). The grouping of bacteria within the floc mainly depends upon the zeta potential and Van der Waals forces (Sobeck and Higgins, 2002). Typical bacterial flocs are irregular by shape have a broad distribution of particle size, are fine compressible, highly porous and permeable to fluids (Chu and Lee, 2004). Accumulation of toxic inorganic nitrogen species (NH₄, NO₂) is prevented in bio-flocs system by maintaining a high C/N ratio and inducing the uptake of ammonium by the microbial community (Avnimelech et al., 1994; Mc Intosh, 2000b). Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in labscale sequencing batch reactor was studied by Schryver and Verstraete (2009).
Sesuk et al. (2009) demonstrated inorganic nitrogen control in a novel zero-water exchanged aquaculture system integrated with airlift-submerged fibrous nitrifying biofilters. The microorganisms not only remove excess nutrients, but have been implicated in nutritional provision for animals, including shrimp and tilapia, that can result in improved growth rate, feed conversion ratio (FCR) and weight gain (Moss and Pruder, 1995; Burford et al., 2004; Wasielsky et al., 2006 Azim and Little, 2008). This process was quantitatively formulated (Avnimelech, 1999), verified and practiced by farmers world-wide (Browdy et al., 2001; Panjaitan, 2004).

There are several factors that influencing floc formation and floc structure (Schryver et al., 2008). Ritvo et al. (2003) studied the salinity and pH effect on the colloidal properties of suspended particles in super intensive aquaculture systems. The mixing intensity imposed by a chosen aeration device at a certain power input will determine the steady-state floc size, this is in equilibrium between the rate of aggregation and the rate of breakage, and floc size distribution (Spicer and Pratsinis, 1996; Chaignon et al., 2002). Dissolved oxygen is another factor that influences the bioflocs. Previous studies revealed that biofloc with a higher floc volume index (FVI) are produced at lower dissolved oxygen levels. The organic carbon source of choice will to a large degree determine the composition of floc produced (Mikkelsen et al., 1996; Hollender et al., 2002; Oehmen et al., 2004). Organic loading rate, temperature and pH are the other factors that affect the biofloc formation (Schryver et al., 2008). pH level has a significant role in the floc formation both qualitatively and quantitatively in the culture of P. monodon, pH of 7.5 is found to be optimum for better biofloculation in shrimp aquaculture (Devi, 2009; Kurup and Devi, 2010).
Schneider et al. (2006) used molasses as carbon source for heterotrophic bacteria production on solid fish waste. Crab et al. (2010a) monitored effect of different carbon sources on the nutritional value of biofloc, in the culture system of *M. rosenbergii* post-larvae. In that experiment flocs were grown on acetate, glycerol and glucose as sources. Varghese (2007) evaluated performance of various locally available carbohydrate sources for biofloculation the culture system of *P. Monodon*; tapioca powder was the biofloculating agent selected by Hari et al. (2004, 2006), Varghese (2007), and Kurup and Varghese (2007a, 2007b).

Initial studies were conducted to optimize the addition of carbohydrate source in aquaculture systems for the process of biofloculation and standardize the quantity of carbohydrate source required for biofloculation. Mechanism and principle behind this technology was also interesting field of research (Avnimelech, 1999, 2009). Eminent Israeli scientist Yoram Avnimelech is the pioneer in this field. Species selection is one of the major factors that decide the success of the BFT aquaculture (Avnimelech, 2007; Azim and Little, 2008). BFT is proved to be beneficial for both shrimp and finfish culture (Milstein et al., 2001; Burford et al., 2003; 2004; Serfling, 2006; Wasielsky et al., 2006). Tilapia was the first studied species for the biofloc ponds, the nibbling habit and feeding behaviour were the reasons for the selection of this fish. Later, marine shrimps were also identified as the suitable species for BFT aquaculture (Burford et al., 2003, 2004; Wasielsky et al., 2006; Hari et al., 2004, 2006; Varghese, 2007; Kurup and Varghese, 2007a, 2007b; Arnold et al., 2009; Ballester et al., 2009; Kuhn et al., 2009).

Studies by Avnimelech (2007) revealed that microbial flocs developing in BFT ponds are effective potential food source for tilapia; according to his observation, fish growing in the BFT pond did not rush on
to the added feed pellets, since the pond contained flocs as a potential feed, feed that is available 24 hours per day. Avnimelech et al. (1994) estimated that feed utilization is higher in BFT systems, while tilapia in such ponds is fed a ration 20% less than conventional one. Varghese (2007) studied various aspects of BFT in the culture of P. monodon. Optimization of protein in the feed by the application of C/N ratio, effect of various modes of carbohydrate application, on farm application of BFT, optimization of stocking density in BFT tanks, performance of carbohydrate sources, and combined application of BFT with periphyton based aquaculture were the focal theme of the study. The author summarises the study as, C/N ratio optimization by the addition of suitable carbohydrate sources is a potential method for controlling the inorganic nitrogen species in aquaculture systems.

Kurup and Varghese (2007a), and Varghese (2007) explained the suitability of various carbohydrate sources for controlling C/N ratio in shrimp culture system. They also studied the combined effect of BFT and periphyton based aquaculture. Biofloc can act as a feed for different cultured species such as Nile tilapia (Oreochromis niloticus) and whiteleg shrimp, L. vannamei (Azim and Little, 2008; Crab et al., 2009; Kuhn et al., 2009). Burford et al. (2004) suggested that “floculated particles” rich in bacteria and phytoplankton could contribute substantially to the nutrition of L. vannamei in intensive shrimp ponds. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for L. vannamei was studied by Wasielsky et al. (2006).

Several researchers followed the uptake of microbial protein through the use of 15N enriched microbial biomass (Preston et al., 1996; Epp et al., 2002; Burford et al., 2004; Avnimelech, 2007). Avnimelech and Kochba (2009) conducted a detailed study on the uptake and utilization of microbial
protein by tilapia, using N15 tagging to get data on the dynamics of the biofloc system and to critically evaluate the available experimental procedures. Authors also estimated the residence time of bioflocs. It was estimated that the residence time of bioflocs was about 8 hours, i.e. bioflocs were regenerated three times a day.

Ray et al. (2010b) characterized microbial communities in minimal-exchange, intensive aquaculture systems and studied the effects of suspended solids management. Characterisation of microbial community structure in shrimp biofloc cultures using biomarkers and analysis of floc amino acid profile was done by Ju et al. (2008). Ray et al. (2010 a) also studied the mechanism behind the suspended solids removal to improve production of *L. vannamei* and part of the same study the team has evaluated a plant based feed in minimal-exchange, super intensive culture systems. According to their study, shrimp biomass production (kg/m$^{-3}$) was increased 41% when biofloc concentration was managed through the use of external settling chambers. They also showed a 60% reduction in nitrate-nitrogen concentration and a 61% reduction in phosphate concentration when biofloc concentration was managed.

Michaud et al. (2006) studied the effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Addison et al. (2010) studied the affect of biofloc replaced fish meal and soybean meal in semi-purified shrimp diets. Ju et al. (2008) studied the enhanced growth effect on shrimp (*L. vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. The results suggest that inclusion of the floc material in shrimp diets could enhance the shrimp growth. They observed enhanced growth when compared with that of the control (P<0.05); the diet preference and pellet
stability study were also positive. Shrimp preferred the experimental diets over commercial diet and the feed stability was same as that of commercial diet. Addison et al. (2010) reported increased growth of *L. Vannamei* by replacing fishmeal and soybean with biofloc. Microbial flocs produced in suspended growth bioreactors could offer the shrimp industry a novel alternative feed. Study by Kuhn et al. (2009) showed that bioflocs harvested from the sequencing batch reactors (SBRs) and membrane biological reactors proved to be a suitable and often superior ingredient, to soybean protein and fishmeal in lab-scale feeding trials with *L. vannamei*. High quality control diets were compared against experimental diets in 35-day feeding trials. Result was that experimental diets were varied greatly and notable independent variables included complete replacement of soybean protein, two-thirds replacement of fishmeal, and no fish oil. Biofloc inclusion always increased growth rates and ranged from a low average increase of 4% to a high average of 67% over the control diets; the latter percent increase was significant at P < 0.01. It seems that biofloc technology represents a promising option for sustainability of the aquaculture industry. Ballester et al. (2010) compared the performance of *Farfantepenaeus paulensis* reared with partial diet with different protein levels under zero exchange microbial floc intensive system. Forty five days experimental trial concluded that shrimps fed with a protein percentage of 35 which are maintained in biofloc ponds showed maximum growth parameters. It was significantly greater when compared to the feed with 25, 30, 40 and 45% of crude protein. Kuhn et al. (2010) compared the possibilities of incorporating two types of microbial flocs in the feed of *L. vannamei* derived from biological treatment of fish effluent. Logan (2010) presented the possibilities of commercializing the biofloc feeds in recent World Aquaculture Society conference held at California, the
In addition to being a tool for water quality and feed management, biofloc technology also has potential to protect the cultured organisms from infections with pathogenic bacteria, which are responsible for major economic losses in aquaculture (Crab et al., 2010b). Biofloc can be a novel strategy for disease management on a long-term basis, in contrast to conventional approaches such as antibiotic, probiotic and prebiotic application (Sinha et al., 2008). Recent research questions focus on the possible extra added value of bioflocs, more specifically regarding pathogen control, because infectious diseases burden the aquaculture industry. It was observed that the regular addition of carbon to the culture is known to select for polyhydroxyalkanoate (PHA) accumulating bacteria (Salehizadeh and Von Loosdrecht, 2004) such as *Alcaligenes eutrophus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans* and others that synthesise PHA granules. Such granules are synthesised under conditions of nutrient stress, that is, when an essential nutrient like nitrogen is limited in the presence of an excess carbon source (Avnimelech, 1999). These PHAs are polymers of β-hydroxy short chain fatty acids and if degraded in the gut, they could have antibacterial activity similar to short chain fatty acids (SCFAs) or organic acids. The breakdown of PHA inside the gastrointestinal tract can be carried out via enzymatic and chemical hydrolysis (Yu et al., 2005). Apart from inhibiting the growth of pathogenic bacteria by lowering the pH of surrounding milieu, SCFA have also been shown to specifically down-regulate virulence factor expression and positively influence the gut health of animals (Teitelbaum and Walker, 2002). Moreover, these compounds are capable of exhibiting bacteriostatic
and/or bacteriocidal properties, depending on the physiological status of the host and the physiochemical characteristics of the external environment (Ricke, 2003). Defoirdt et al. (2006) reported increased survival of artemia nauplii when fed formic, acetic, propionic, butyric and valeric acid and challenged with a luminescence pathogenic Vibrio campbellii strain. In another study, the same authors (Defoirdt et al., 2007) reported that commercial polyhydroxy butyrate (PHB) particles or PHB accumulating bacteria offered a preventive and curative protection to artemia against luminescent vibriosis. Crab et al. (2010b) studied the use of bioflocs as new bio-control agents for aquaculture using a model system with gnotobiotic brine shrimp (Artemia franciscana). They found that the bioflocs can also be used by brine shrimp (A. franciscana) nauplii as a feed. The technique has also proved to be beneficial in the nursery rearing of P. monodon, wherein the use of carbon source and artificial substrates was found to positively influence growth and production of shrimp juveniles in addition to providing more favourable water quality conditions irrespective of elevated stocking densities (Arnold et al., 2009; Anjalee and Kurup, 2011).

2.4 Application of biofloc technology in giant freshwater prawn aquaculture

Eventhough more than 25 species of freshwater prawns have been reported from Kerala, M. rosenbergii is the only species with aquaculture importance. There is a vast potential for the development of prawn culture and nearly 65,000 ha comprising ponds, tanks and check dams are found suitable for freshwater prawn farming in monoculture or polyculture with carps (Kumar and Velayudhan, 2006). Prawn farming has been considered as an important aquaculture activity in the state due to its domestic as well as export market and the possibility of integration or rotation with paddy
cultivation which in turn provides an additional income. The increase in the aquaculture production from the state of Kerala mentioned earlier may be due to the utilisation of many of these potential areas for culture purposes as denoted by the increase in the area brought under the culture. Raising of freshwater prawn production through expansion of pond area would demand large additional quality of water and land area, both are very scarce resources. The most practical way to raise freshwater prawn production is by increasing pond productivity per unit land area and water (Asaduzzaman et al., 2008). *M. rosenbergii* is omnivore in the feeding behaviour and is more or less similar to the feeding of the tiger shrimp, *Peanaeus monodon*. The benefits of BFT has been described extensively for shrimp culture (Buford et al., 2003, 2004; Hari et al., 2004, 2006; Wasielsky et al., 2006) and for finfish culture (Avnimelech, 1999; Avnimelech, 2007; Milstein et al., 2001; Serfling 2006). There are only few works on its applicability and advantages of the technology to *M. rosenbergii* grow outs (Asaduzzaman et al., 2008, 2009a, 2009b 2010a, 2010b) and in larviculture (Saritha, 2009; Saritha and Kurup, 2011).

Asaduzzaman et al. (2008, 2009a, 2009b, 2010a, 2010b) conducted a series of experiments on diversified application of C/N ratio control in giant freshwater prawn culture. In 2009, the research team studied the effects of addition of tilapia, *Oreochromis niloticus*, and substrates for periphyton developments on pond ecology and production in C/N-controlled *M. rosenbergii* farming systems. In the same year the team investigated effects of stocking density of *M. rosenbergii* and addition of different levels of *Oreochromis niloticus* on production in C/N controlled periphyton based system. Asaduzzaman et al. (2010a) explained the effects of C/N ratio and substrate addition on natural food communities in freshwater prawn
monoculture ponds. Asaduzzaman et al. (2010b) investigated the possibilities of combining C/N ratio control, fish-driven re-suspension and periphyton based aquaculture in freshwater prawn culture with varying stocking densities of tilapia and *Labeo rohita*. They also studied the effect of two carbohydrate sources for controlling the C/N ratio in the culture pond. In the same year, the team scanned various carbohydrate sources for maintaining a high C/N ratio and fish driven re-suspension on pond ecology and production in periphyton-based freshwater prawn culture systems.

The biofloc technology has been widely used in shrimp culture for reducing the accumulation of toxic metabolites like ammonia by its conversion to heterotrophic bacterial biomass, especially in static systems. The modified static green water system of seed production of *M. rosenbergii* faces the problem of elevated levels of metabolites like ammonia and nitrite. Saritha (2009), Kurup and Saritha (2010) and Saritha and Kurup, 2011) made an attempt to evaluate the effectiveness of biofloc technology in larval rearing of the giant freshwater prawn wherein the quantity of carbohydrate addition has been optimised to assimilate the toxic metabolites generated and consequently converting them into bacterial floccules.

Application of BFT making culture more sustainable by 54% increase of total revenue from the harvested shrimp, reduce water based nitrogen species discharge to the environment and also reducing the protein content of the feed substantially (Kurup, 2009). Enhanced pond productivity through stimulation of suspended and attached algal development and by using them to improved water quality, provide additional food, higher carrying capacity in combination with reduced nutrient discharge, improving environmental sustainability. This technology is simple and cheap, making it also socially and
economically sustainable, even for small scale or poor farmers (Hari et al., 2004, 2006; Varghese, 2007; Kurup, 2009).

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