Chapter – 1

General Introduction
1. Background of the study

Aquaculture, the farming of aquatic animals and plants has turned out to be an important industry worldwide. Aquaculture production systems used across the world differ widely depending on the species being cultured, the geographical location and socioeconomic context. Extensive, semi-intensive and intensive methods can be employed to produce shrimp and fish. Extensive methods have proved economically viable in the brackishwater area (Beveridge, 1987). Environmental issues have always been a point of debate in shrimp farm development. While the harvest from capture fisheries around the world has stagnated, aquaculture is viewed as a sound option to increase fish production and to play a vital role in providing food and nutritional security. However, the shrimp farming sector has been strongly opposed by environmental groups on many occasions not only in India but in many other countries around the globe. Legal interventions have been sought to curtail shrimp culture to preserve the coastal environment and the ecology. Though the polarization of opinion on the adverse impact of aquaculture in the nineties was very strong, there are signs of more tolerance lately to accommodate diverse views and opinions to allow development of shrimp farming in an environment friendly and sustainable manner.

In India, commercial shrimp farming started gaining roots during the mid-eighties only and during this period, this actually
attained peak in most of neighboring Asian countries especially China and Taiwan. Shrimp culture begun booming in early 1990's in India. However, the industry collapsed in 1995 - 96 due to disease outbreaks. Since then, shrimp aquaculture industry is facing severe criticism for the adoption of unsustainable culture practices, such as the discharge of pond water with high nutrients. Nitrogen plays a key role in aquaculture systems due to its dual functioning as a nutrient and toxicant. The excessive accumulation of toxic inorganic nitrogen in culture systems deteriorates the pond system by reducing the growth rate and survival rate of cultured organisms. Most of the coastal states in India are new to commercial scale shrimp farming. The lack of awareness on good farming practices and appropriate extension services have in fact led to a host of problems.

2. Sustainable development of shrimp farming – some issues for consideration

It is generally accepted that the days of maximum production oriented unsustainable shrimp farming practices are gone. Present day production has to take note of not only the markets but a host of technical issues as well as the concerns of the environment. The subject matter of sustainable shrimp farming is broad from farm level management practices to integration of shrimp farming into coastal area management, shrimp health management and policy,
socioeconomic and legal issues. The Aquaculture Authority permits
stocking of post larvae up to 6 nos m\(^{-2}\) for farms within the CRZ and
up to 10 nos m\(^{-2}\) outside the CRZ (Aquaculture Authority News,
2006). It is gratified to see that high percentage of farms are
embracing low stocking densities in the country and enjoying a high
success rate in doing so. The low stocking densities are working in
terms of economics as well. Adoption of low stocking densities will be
one of the key elements of sustainability in the years to come and
needs to be promoted among the shrimp farmers.

The sustainability of the shrimp farming in Kerala is facing
severe risks and crisis like degradation of estuarine ecosystem due to
indiscriminate discharge of shrimp farm effluents loaded with high
inorganic nitrogen, crop loss due to poor environmental conditions
and recurrence of diseases due to the stress and strain the farmed
shrimps are prone to. The shrimp farming sector has received
criticism for excessive use of formulated feed containing high protein
shrimp feed, of which around 50% is getting accumulated at the pond
bottom as unconsumed (Avnimelech, 1999; Hari et al., 2004; Hari et
al., 2006). The waste materials accumulated with the feces from the
cultured stock, dead organism and organic fertilizers undergo
decomposition. Thus, shrimps are exposed to toxic ammonia-N and
nitrite-N, which are responsible for stress and strain in cultured
shrimps. After oxygen, ammonia-N is the second most limiting factor

Carbon / nitrogen ratio optimization and periphyton development
in the culture system for shrimp stocking density (Raveh and Avnimelech, 1979; Blackburn et al., 1988; Piedrahita, 1988; Krom and Neori, 1989; van Dam, 1990; Colt and Oriwicz, 1991; Hargreaves, 1998; Monroya et al., 1999). Furthermore, the discharge of inorganic nitrogen rich pond effluents increases pollution in the main water sources. Maintenance of good water quality with minimum water exchange and retention of nitrogenous nutrient input into harvestable products are thus emerging as most important requirements for sustainable shrimp farming.

3. Development of shrimp aquaculture in India

India, by virtue of having 8118 km long coastline, 2.02 million sq. km of Exclusive Economic Zone (EEZ) and extensive geographical stretch with varied terrain and climate, supports a wide diversity of inland and coastal wetland habitats. There are 3.9 million ha of estuaries and 3.5 million ha of brackishwater areas in the country. Out of this, 1.2 million ha of coastal area have been identified as suitable for brackishwater aquaculture and by the adoption of sustainable practices, it can yield optimum quantities of shrimp and other commercially valuable finfish and shellfish species.

The over exploitation of shrimp from natural sources and the ever increasing demand for shrimp and shrimp products in the world food market has resulted in a wide gap between the demand and
supply shrimp. This has necessitated the need for exploring new avenues for increasing shrimp production. The state-wise potential area and status of shrimp culture development in India is given in Table 1.1. The estimated brackishwater area suitable for undertaking shrimp cultivation in India is around 11.91 lakhs ha spread over 10 states and union territories viz. West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Pondicherry, Kerala, Karnataka, Goa, Maharashtra and Gujarat. Of this, only around 1.2 lakhs ha are now under shrimp farming and hence lot of scope exists for entrepreneurs to venture into this field. The marine products export from India has been rising over the years and the current export is worth about US $ 1478 million (FAO, 2005). The major markets for Indian shrimp are Japan, Western Europe and USA. Frozen shrimp is the largest export item in terms of value contributing 64% of the total export earnings followed by frozen cephalopod (15%), frozen fish (11%), dried fish (2%), etc.

Today India stands amongst the major shrimp producing countries in the world with a growth rate of about 300.0 % over the last decade.

In India, shrimp farming has been traditionally practiced in the coastal states of West Bengal and Kerala. The traditional trap and hold farming system was characterized with low production levels of mixed species of finfishes and shell fishes. The importance of introducing scientific farming techniques will increase production and profitability from the traditional system.
Like any other agriculture / animal husbandry practice, shrimp culture has also been affected by health and disease problems. Initially, some bacterial diseases in localized shrimp farms with low mortality rates were noticed. However, viral diseases such as *Monodon baculo virus* and *white spot virus disease* syndrome were reported from shrimp farms in 1995 followed by a slump in Indian shrimp farming. Heavy stocking densities and poor farm management practices were attributed as major reasons for such disease outbreaks in the country.

4. Role of carbohydrate addition in the shrimp culture system

The tiger prawn, *Penaeus monodon* (Fabricius) is the most extensively cultured crustacean in South-East Asian countries. This species is known to possess high growth rate and adapt to various culture systems. *Penaeus monodon* is considered as a candidate species for brackishwater culture. Protein is an essential nutrient for this culture organism and the major source of ammonium-N in culture farms is typically protein rich feed. Aquatic animals excrete ammonium-N, which may accumulate in the pond. Protein is an expensive feed component and high dependency on artificial feeding increases feed cost considerably. The expensive protein fraction should, therefore, be at optimal level. Furthermore, the protein carbon / nitrogen ratio optimization and periphyton development.
sparing effect of non-protein nutrients such as carbohydrate may be effectively utilized for reducing the feed cost. In highly aerated ponds, ammonium-N is oxidized by bacteria to nitrite-N and nitrate-N. Unlike carbon dioxide, which is released to the air by diffusion or forced aeration, there is no effective mechanism to remove the nitrogenous metabolites out of the pond. Thus, intensification of aquaculture system is inherently associated with enrichment of the water with ammonium-N and other inorganic nitrogenous species. The management of such systems depends on developing methods to remove these compounds from culture pond.

Removal of excessive nitrogen from culture pond is commonly carried-out by frequent exchange and replacement of pond water. However this practice is constrained by the following reasons:

1. Environmental regulations prohibit the release of nutrient rich water into environment;
2. The danger of introducing pathogens into the external water;
3. The rich expense incurred in pumping huge volume of water.

Another approach is based on means to encourage and enhance nitrification of ammonium and nitrites to the relatively inert nitrate species. This is often done by employing biofilters, essentially immobile surfaces serving as substrate to the nitrifying bacteria. A
high surface area with immobilized nitrifying biomass enables a high nitrifying capacity in a controlled environment. One problem associated with biofiltration is the high cost involved and the need to treat and digest a large mass of feed residues.

An additional strategy that is getting more attention presently is the removal of ammonium from water through its assimilation into microbial proteins by addition of carbonaceous materials to the system. If properly adjusted, added carbohydrates can potentially eliminate the problem of inorganic nitrogen accumulation. A further important aspect of this process is the potential utilization of microbial proteins as a source of feed protein for fish or shrimp.

This, however, depends upon the ability of the animal to harvest such bacteria and to digest and utilize the microbial protein. One obvious constraint is the minimal size of particles that can be taken up by the fish and shrimp. Taghon (1982) reported that benthic invertebrates were able to take up microscopic glass bead when they were coated with proteins. This demonstrates that the chemical nature of particles may favor their harvesting by cultured organisms. The fact that relatively large microbial cell clusters are formed due to flocculation, alone or in combination with clay or feed particles (Harris and Mitchell, 1973; Avnimelech et al., 1982, 1984) and the resultant microbial protein additionally favors the growth of shrimp and fish.
Controlling inorganic nitrogen by manipulating carbon/nitrogen ratio is a potential method for aquaculture systems. This approach offers a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in culture ponds. Such a strategy can be practiced as an emergency response, i.e., addition of a carbonaceous substrate in case of increased ammonium concentration. It is possible to add cheap sources of carbohydrates such as cassava meal and flour. However, additional pond aeration may be required to compensate the additional oxygen consumption. The conventional control measures include intensive exchange of pond water. Furthermore, it is not always practical to stop feeding to slow down TAN (Total ammonia nitrogen) build up. The proposed method enables to keep a high biomass and to bring-out a corrective means in case of failure of conventional control measures.

A more advanced approach is to adjust protein level in feed so as to avoid the build up of inorganic nitrogen in pond water. This approach was tested and proven successfully in intensive ponds that are continually mixed and aerated. The intensive culture of fish in these ponds is based on a system that is similar to biotechnological reactors (Avnimelech, 1998). The addition of carbohydrates was done as a part of recycling and increased utilization of protein through the utilization of microbial proteins. Production and utilization of microbial proteins (SCP, single-cell protein) have been studied.
extensively during the last few decades (Tannenbaum and Wang, 1975). The major problem involved in economically sound utilization of SCP culture is harvesting, dehydration and packing of the material. In contrast, for in situ microbial protein culturing in the pond, all these expensive processing stages are not needed since harvesting is done by fish and shrimp, as part of the system.

Applicability of the same approach in earthen stagnant ponds is not trivial and has to be further studied in conventional fish and shrimp ponds. The addition of carbohydrates to feed may result in an accelerated sedimentation of organic matter to the pond bottom, where microbial biomass is not utilized by fish or shrimp and may increase the organic load in the pond.

The adjustments of the carbon / nitrogen ratio in feed as a means to control the pond water quality and sediment quality are studied. The objectives of this study are to evaluate the basic reactions and mechanisms affecting this process; to demonstrate its potential; to develop the quantitative means needed to adjust the carbon / nitrogen ratio and to control inorganic nitrogen accumulation in the farming system.

5. Periphyton based aquaculture

The term 'periphyton' refers to the microfloral community living attached to the surfaces of submerged objects in water (Wetzel, 1983).
This definition, however, does not include fungal, bacterial, protozoan and other attached animal components, which are included in the German word ‘Aufwuchs’. Depending on the substrate types, periphyton communities are again subdivided as ‘Epilithon’ grown on rock, ‘Epipelon’ on mud or silt, ‘Epipsammon’ on sand and ‘Epiphyton’ on submerged macrophyte substrates. In microbiology, periphyton is often referred to as ‘Biofilms’ (Nielsen et al., 1997; Shankar et al., 1998; Shankar and Mohan, 2001). In aquaculture, the term periphyton has been used in a broader sense. Periphyton is defined as the entire complex of sessile aquatic biota attached to the substratum and includes associated detritus and microorganisms. Thus, the periphyton community comprises bacteria, fungi, protozoa, phytoplankton, zooplankton, benthic organisms and a range of other invertebrates and their larvae. Any material providing surface area, including coral reef, branches of different trees, higher aquatic plants, bamboo, PVC pipes etc., can be used for periphyton production.

The idea of periphyton based aquaculture is originally derived from the traditional method, (bush trap testing) locally called ‘padal fishing’ a unique fishing method used in the Ashtamudi estuary, Vambanad is of Kerala (South India). Locally available tree branches such as mango, mangroves and bamboo poles are placed in shallow open waters. These branches are known as padals which act as shrimp and fish aggregating devices. A large number of post larvae of
shrimp and fingerlings find shelter beneath the paddles, foraging the peri and epiphyton developed from the submerged twigs and other structures used to construct them (Thomas et al., 2004), such as 'acadjas' of Cote Ivory Coast, West Africa (Welcomme, 1972) and the 'samarahs' of Cambodia (Shankar et al., 1998). Dense masses of tree branches or bamboo are established in lakes, lagoons or rivers the fish and shrimp are attracted by the provision of shelter from predators, suitable breeding habitats and the availability of natural food. These unique tools used in capture fisheries have recently been considered as models for novel periphyton based aquaculture systems.

There are two basic food sources for all organisms in extensive and semi-intensive ponds: primary productivity from algae and protein rich supplementary feed. Algae produce organic matter by using solar energy and carbon dioxide through photosynthesis which can be further utilized, indirectly through secondary trophic levels (zooplankton, benthos, and invertebrates etc.) and directly, through grazing by fish and shrimp. Heterotrophic microorganisms are essential components of the food web in these two food source as thus decompose organic matter and release nutrients which can again be utilized by algae or consumed by the cultured organism (Colman and Edwards, 1987; Moriarty, 1997). However, a common assumption particularly in aquaculture is that the phytoplankton community is
most important in terms of energy fixation and fuelling of the food web. Research has shown that macrophytes and periphyton are significant dominant contributor to primary production (Moss, 1998).

6. Objectives, hypotheses and outline of the thesis

Controlling the inorganic nitrogen by manipulating carbon / nitrogen ratio is a method gaining importance in aquaculture systems. Nitrogen control is induced by feeding bacteria with carbohydrates and through the subsequent uptake of nitrogen from the water for the synthesis of microbial proteins. The relationship between addition of carbohydrates, reduction of ammonium and the production of microbial protein depends on the microbial conversion coefficient. The carbon / nitrogen ratio in the microbial biomass is related to the carbon contents of the added material. The addition of carbonaceous substrate was found to reduce inorganic nitrogen in shrimp culture ponds and the resultant microbial proteins are taken up by shrimps. Thus, part of the feed protein is replaced and feeding costs are reduced in culture systems.

The use of various locally available substrates for periphyton based aquaculture practices increases production and profitability (NFEP, 1997; Ramesh et al., 1999; Wahab et al., 1999a; Azim et al., 2001; Keshavanath et al., 2001a; Azim et al., 2002). However, these
techniques for extensive shrimp farming have not so far been evaluated. Moreover, an evaluation of artificial substrates together with carbohydrate source based farming system in reducing inorganic nitrogen production in culture systems has not yet been carried-out. Furthermore, variations in water and soil quality, periphyton production and shrimp production of the whole system have also not been determined so-far.

This thesis starts with a general introduction (present chapter), a brief review of the most relevant literature, results of various experiments and concludes with a summary (Chapter – 9). The chapters are organised conforming to the objectives of the present study. The major objectives of this thesis are, to improve the sustainability of shrimp farming by carbohydrate addition and periphyton substrate based shrimp production and to improve the nutrient utilisation in aquaculture systems.

The specific objectives of the present study can be outlined as:

1. To optimize the protein percentage in shrimp feeds by the control of carbon / nitrogen ratio.

2. To evaluate the effect of various mode of carbohydrate application and diet having various protein levels for the production and sustainability of *Penaeus monodon*. 
3. To monitor the carbohydrate addition shrimp production relationship and culture sustainability for increasing the total revenue of the harvested shrimp and reducing the feed cost.

4. To examine the stocking density and carbohydrate addition relationship in the yield and sustainability of *Penaeus monodon*.

5. To assess the efficiency of various types of carbohydrates in the control of inorganic nitrogen and increasing production of *Penaeus monodon*.

6. To monitor the substrate based periphyton effect on water and soil quality parameters and to evaluate the quantitative production of additional excellent natural food source for the culture organism.

7. To optimize the fertilization rates in periphyton production in the absence of shrimp grazing pressure.

8. To assess the combined effects of periphyton and addition of carbohydrate in the production and sustainability of *Penaeus monodon*.

9. To reduce the water based inorganic nitrogen discharge in to environment this making shrimp farming more ecologically and environmentally sustainable.
Table 1.1
The state-wise potential area and status of shrimp culture development in India during 2005

<table>
<thead>
<tr>
<th>Sr.</th>
<th>State</th>
<th>Estimated brackish water area (ha.)</th>
<th>Area under cultivation (ha.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>West Bengal</td>
<td>405,000</td>
<td>34,660</td>
</tr>
<tr>
<td>2</td>
<td>Orissa</td>
<td>31,600</td>
<td>11,000</td>
</tr>
<tr>
<td>3</td>
<td>Andhra Pradesh</td>
<td>150,000</td>
<td>50,000</td>
</tr>
<tr>
<td>4</td>
<td>Tamil Nadu</td>
<td>56,000</td>
<td>2,879</td>
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<tr>
<td>5</td>
<td>Pondicherry</td>
<td>800</td>
<td>37</td>
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<tr>
<td>6</td>
<td>Kerala</td>
<td>65,000</td>
<td>14,657</td>
</tr>
<tr>
<td>7</td>
<td>Karnataka</td>
<td>8,000</td>
<td>3,500</td>
</tr>
<tr>
<td>8</td>
<td>Goa</td>
<td>18,500</td>
<td>650</td>
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<tr>
<td>9</td>
<td>Maharashtra</td>
<td>80,000</td>
<td>716</td>
</tr>
<tr>
<td>10</td>
<td>Gujarat</td>
<td>376,000</td>
<td>884</td>
</tr>
</tbody>
</table>

\[ \text{Total:} 1,190,000 \text{ ha.} \quad 118,983 \text{ ha.} \]

Source: Aquaculture Authority News, September 2006
Review of Literature
Review of Literature

As demand for fish, crustaceans and other aquatic organisms is increasing and capture fisheries reaches its maximum level of exploitation, food production through aquaculture attracts global attention. For the successful rearing of tiger prawn, development of a balanced artificial ration is essential (Banerjea, 1967). The optimum amounts of dietary protein and energy play key roles in the growth of shrimp in culture ponds (Tiemeier et al., 1965; Hastings, 1967). Studies in shrimp have indicated that those diets which are low in both protein and total energy resulted in reduced weight gain (Lee and Putnam, 1973; Garling and Wilson, 1976). High dietary protein essential for faster tissue growth and maintenance, is an expensive component of formulated diets and protein may be catabolised to meet the energy requirements of somatic growth (Capuzzo and Lameasten, 1979; Sedgwick, 1979). Higher dietary protein feed deteriorates the water and soil quality in shrimp grow-out ponds (Boyd, 1989). Furthermore, Avnimelech and Lacher (1979) and Boyd (1985) reported that total fed feeds only 50% of the feed is utilized by the cultured organism. The remaining 50% dietary protein feed is assumed to constitute feed waste, which is a major source of ammonium-N (Gaudy and Gaudy, 1980; Heaper, 1988). Colt and Armstrong (1981) reported that accumulation of toxic inorganic nitrogen species such as $\text{NH}_4^+$ and $\text{NO}_2^-$ in water is one of the major
problems affecting the sustainability of shrimp farming. Ammonia-N is a highly toxic compound because it can easily cross most biological membranes and cause pH alterations which may reduce survival rates and impair various physiological mechanisms (Schmidt-Nielson, 1983; Campbell, 1991). The release of dissolved nutrients by a shrimp farm leads to an increase in their concentration in the receiving water body. This increase has been termed as 'hypernutrification' (Gowen and Bradbury, 1987). Increasing levels of total nitrogen, total inorganic nitrogen and total phosphorus in shrimp pond water poses serious environmental problem (Boyd, 1990).

The most widely documented process associated with commercial culture is the high accumulation of organic matter due to the deposition of solid waste from undigested feed (Brown et al., 1987; Gowen et al., 1991). The aquaculture industry is focusing on the development and refinement of water recycling technologies due to concerns related to the potential negative impacts of production on the environment (Klontz, 1979; Rosenthal, 1994). Typically, mechanical filtration removes particulate matter, while biological filtration removes dissolved wastes, including ammonia (Brune and Gunther, 1981; Kaiser and Wheaton, 1983; Losordo, 1991). However, it is not economically viable in a high surface area due to the higher cost. At the same time, good water quality is essential for ensuring survival and adequate growth rate (Boyd, 1990; Burford, 1997).
Aquatic animals excrete ammonium, which may accumulate in the pond bottom. A different approach is to estimate the amount of carbohydrate needed to be added in order to immobilize the ammonium excreted by the fish or shrimp in the culture system (Avnimelech and Lacher, 1979; Boyd, 1985; Muthuwani and Lin, 1996). The manipulation of shrimp culture system for improved water quality and shrimp production requires a definite understanding of various physical, chemical and biological processes (Boyd, 1986). Reduction of dissolved inorganic nitrogen can be established in intensive, well aerated and circulated fish or shrimp ponds by the application of organic carbon sources (Avnimelech et al., 1989). The organic carbon rich substrates such as glucose, cassava and sorghum meal were used to control carbon / nitrogen ratio (Avnimelech et al., 1994). The control of inorganic nitrogen accumulation in pond is based upon carbon metabolism and nitrogen immobilizing microbial process. Bacteria and other microorganisms use carbohydrates (sugar, starch and cellulose) as a food, to generate energy and to produce proteins and new cells (Avnimelech, 1999). The resulting heterotrophic bacterial production (single cell protein) may be utilized as a food source of fish and shrimp (Beveridge et al., 1989; Rahmathulla and Beveridge, 1993; Burford et al., 2004a) and thus lowering the demand for supplemental feed protein (Avnimelech, 1999). Schroeder (1978) reported that carp can filter out particles
larger than 20 – 50 μm. According to Odum (1968) *Mugil cephalus* take up particles small as 10 μm. Interestingly Taghon (1982), found that benthic invertebrates were able to take up microscopic glass bead when they were coated with proteins.

Pond production systems in Southern Asian countries are becoming increasingly reliant on external resources (feed, fertilizers) to supplement or stimulate autochthonous food production in fish pond. Hickling (1962) and Heaper (1988) have demonstrated that fish production in fertilized ponds does not increase in direct proportion to increased fertilizer addition and that above a certain level, increasing fertilizer rates does not further increase fish yield. In most feed driven pond production systems, only about 15 – 30% of nutrient input is converted into harvestable products, the remainder being lost to the sediment, effluent water and the atmosphere (Acosta-Nassar et al., 1994; Gross et al., 2000). In nutrient rich environment, the substrate acted merely as a platform for periphyton (Moss, 1998). On the other hand, periphyton biomass in open water habitats strongly depended on substrates type (Blinn et al., 1980; Hansson, 1992; Vymazal and Richardson, 1995). The amount of periphyton biomass per surface area was found to be highly variable and was influenced by water depth (Konan-Brou and Guiral, 1994; Light and Beardall, 1998; Keshavanath et al., 2001a), nutrient availability (Elwood et al., 1981; Fairchild et al., 1985) grazing pressure (Hatcher and Larkum, 1983;
Hansson et al., 1987; Hay, 1991; Huchette et al., 2000) and seasonality, including environmental parameters (Hatcher and Larkum, 1983; Carpenter, 1986; Bothwell, 1988; Arfi et al., 1997; Ledger and Hildrew et al., 1998).

The term 'periphyton' is applied to the complex of sessile biota attached to submerged substrata such as stones and sticks and includes not only algae and invertebrates but also associated detritus and microorganisms. The assemblage of attached organisms on submerged surfaces, including associated non-attached fauna are referred to as periphyton (van Dam et al., 2002). The feasibility of periphyton based systems has been explored in brackishwater fish ponds in West Africa (Welcomme, 1972; Hem and Avit, 1994; Konanbrou and Guiral, 1994). Periphyton is a complex matrix of algae and heterotrophic microbes attached to submerged surfaces in streams and other shallow waters. It serves as an important food source for invertebrates and some fish (Apesteguia and Marta, 1979; Newman and McIntosh, 1989; Cattaneo et al., 1993). Periphyton based aquaculture systems offer the possibility of increasing both primary production and food availability for culture organism (Legenedre et al., 1989; Hem and Avit, 1994; Guiral et al., 1995; Wahab et al., 1999a). Greater abundance of net phytoplankton in ponds probably relates to higher nutrient input in those ponds (Boyd, 1989). Biochemical oxygen demand and ammonia-nitrogen were most closely related to
phytoplankton abundance and community variation in the grow-out ponds. Ammonia-nitrogen is an important algal nutrient (Boyd, 1989) and BOD is a measure of the organic material in ponds which is closely related to phytoplankton communities (Tookwinas and Songsangjinda, 1999). In shrimp ponds, ammonia concentration was not significantly affected by pond depth, although nitrite and nitrate were inversely related to pond depth (Carpenter et al., 1986). Presumably, reducing water depth in a pond with a high phytoplankton density will reduce light limitation of phytoplankton growth and thereby enhance nutrient uptake (Piedrahita, 1991). A periphyton mat consists of a solid matrix embedded with bacteria, algae, protozoa, fungi, zooplankton and small invertebrates (Kalpan et al., 1987; Bender and Phillips, 2004; Garcia-Meza et al., 2005). The selection of suitable species (Wahab et al., 1999a; Azim et al., 2001), selection of locally available substrates and the optimization of fertilizer dose are the major steps in periphyton based aquaculture. It is evident that periphytic algae need to be grazed constantly and kept at low biomass to maintain their high productivity (Hatcher, 1983; Hay, 1991; Huchette et al., 2000). Trials have demonstrated that fish production from ponds supplied with additional substrates for periphyton production is higher than that from substrate free controls (Legendre et al., 1989; Konan et al., 1991; Hem and Avit, 1994; Guiral et al., 1995; NFEP, 1997; Wahab et al., 1999a; Azim et al., 2001).
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Laboratory based grazing trails also indicated that algal feeding species, such as Tilapias, can ingest more plant based food per unit time when presented as periphyton than as plankton (Dempster et al., 1993).

Miller and Falace (2000) suggested two mechanisms for increasing fish production in artificial reef based system: (1) the additional shelter provided by the substrate allows more of the resource to flow into cultured organism biomass and (2) the new primary production and attached benthic secondary production fostered by the artificial substrate support a new food web, part of which will end up in cultured species biomass. Maximum periphyton biomass level is reported to be coinciding with the euphotic zone (Konan-Brou and Guiral, 1994; Keshavanath et al., 2001c). Wahab et al. (1999a) reported 53 genera of periphyton collected from scrap bamboo in fish ponds among which 12 genera were observed in verity. Huchette et al. (2000) identified 32 species of diatom as periphyton along with other micro and macro organisms from both animal and plant kingdoms growing on artificial substrates in Tilapia cages. The periphyton quantity varied substantially with substrate type, fertilization level, environment conditions and taxonomic composition (Paine and Vadas, 1969; Heaper, 1988; Makarevich et al., 1993; Napolitano et al., 1996; Ledger and Hildrew, 1998; Huchettu et al., 2000; Keshavanath et al., 2001a). The increased fertilization rate
amplified pond productivity. However, it results in drastic production of inorganic nitrogen in the culture system (Bormann et al., 1968; Vitsousek et al., 1979; Schimel and Firestone, 1989; Dail et al., 2001). Konan-Brou and Guiral (1994) and Keshavanath et al. (2001a) reported that maximum periphytic biomass levels coincided with photosynthetic compensation and the depth of culture pond. The biodegradable substrates viz. sugarcane bagasse, paddy straw, dried water hyacinth (*Eichornia crassipes*), kanchi, PVC pipes and bamboo poles were used in the culture system for the periphyton production but the highest periphyton growth occurred on bamboo poles (Ramesh et al., 1999; Umesh et al., 1999; Azim, et al., 2001; Keshavanath et al., 2001a; Azim et al., 2002; Joice et al., 2002; Mridula et al., 2003). Microbial communities containing algae, blue green algae, bacteria, protists, zooplankton and fungi embedded in an extracellular polysaccharide matrix develop on submerged surfaces. Within these communities, autotrophic or heterotrophic biomass dominates, depending on light, dissolved oxygen and nutrient availability (Hepher et al., 1989).

Worldwide aquaculture has been increasing rapidly in the last decade, approximately at an average rate of more than 10% per year (Muir, 1995; Tacon, 1997; Pedini and Shehadeh, 1997; World Bank, 1998; FAO, 2001), mainly due to the combined effects of increasing world population (Caddy and Griffiths, 1995), and the increasing
demand for aquaculture products in developed countries (Tacon, 1997; Lem and Shehadeh, 1997). Modified extensive production of juvenile shrimp is gaining increased attention worldwide as a potential means to improve aquaculture production via application as a transitional nursery system (i.e. between the hatchery and grow-out ponds). Stocking juvenile shrimp into grow-out ponds, as opposed to post larvae, is thought to improve production mainly by; increasing early survival rates in ponds, because juveniles are likely to be hardier and therefore more able to adapt to pond conditions (Samocha et al., 2002), and also to reduced grow-out duration (Samocha et al., 1993; Peterson and Griffith, 1999). Reduced growth and survival at higher densities are attributed to a number of factors like, a decrease in the availability of space and natural food sources (Maguire and Leedow, 1983; Peterson and Griffith, 1999); an increase in adverse shrimp behavior such as cannibalism (Abdussamad and Thampy, 1994); the degradation of water quality (Nga et al., 2005); and accumulation of undesirable sediment (Arnold et al., 2005, 2006). It is believed that added surface area created by the substrates enhance the colonization of epiphytic biota, which in turn provides a natural food supplement for the shrimp (Moss and Moss, 2004; Burford et al., 2004b). Shrimp was also cultured both with and without the addition of artificial substrates, on the surface of artificial substrates colonized with epiphytic biota, at each density to ascertain if *Penaeus monodon*
benefit from the addition of substrates during intensive production (Moss and Moss, 2004).

In shrimp culture systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo and Rimon, 1982; Diab and Shilo, 1988). The use of low protein feed led to a significant reduction in the feed based inorganic nitrogen accumulation in the pond (Li and Lovell, 1992). The addition of carbohydrate enhances the total heterotrophic bacterial population in the pond, which in turn results in further reduction of inorganic nitrogen. Middelburg and Nieuwenhuize (2000a), Benner (2002) and Bronk (2002) found that the presence of microbial community uptake the different nitrogenous substrates. Heterotrophic bacteria nitrogen uptake focused on dissolved inorganic nitrogen (DIN), especially ammonium-N (NH₄⁺) and nitrate-N (NO₃⁻) as an important nitrogen source (Antia et al., 1991; Middelburg and Nieuwenhuize, 2000b; Bronk, 2002; Zehr and Ward, 2002; Berman and Bronk, 2003). Thus, the low toxic inorganic nitrogen levels in the pond (Wahab et al., 2003) and utilization of microbial cells as feed act as favorable factors for the augmented shrimp production (Avnimelech, 1999; Burford et al., 2003, 2004b). The utilization of microbial protein depends on the ability of the target animal to harvest the bacteria and its ability to digest and utilize the microbial protein (Avnimelech, 1999). Extensive conditions and carbohydrate addition to the water column also
resulted in a significant increase in the THB count, together with observed lower TAN concentrations in water and sediment (Bronk, 2002). Carbohydrate addition also caused a significant reduction in NO$_2^-$-N concentration in the water column, which can be attributed to low availability of TAN as substrate for nitrification (Avnimelech, 1999; Hari et al., 2004). Furthermore, lower TAN in the sediment positively influenced the food intake and health of the shrimps (Avnimelech and Ritvo, 2003). The addition of carbohydrate to intensively well-mixed production systems will reduce the TAN concentration through immobilization by bacterial biomass (Avnimelech and Mokady, 1988; Avnimelech et al., 1989; 1994; and Avnimelech, 1999). According to Avnimelech (1999) TAN concentrations were found low (2.0 mg l$^{-1}$) in carbohydrate added shrimp culture, when compared to the findings of Chen and Tu (1991) (6.5 mg l$^{-1}$) and Thakur and Lin (2003). Cotner et al. (2000) showed that water samples collected from Florida Bay having a TAN concentration of 7.4 – 17.1 μg l$^{-1}$ enhanced microbial growth with glucose addition. Water exchange in ponds is limited or even null, leading to the accumulation of organic residue and to the development of dense heterotrophic microbial population (McIntosh, 2000; Avnimelech, 2003). In intensive aquaculture systems, inorganic nitrogen, including toxic ammonia and nitrite accumulate in the water (McIntosh, 2000). This problem is prevented through the addition of
carbonaceous substrates leading assimilation of the soluble inorganic nitrogen and its incorporation into microbial protein (Chamberlain et al., 2001; Tacon et al., 2002). The microbial protein, aggregated in microbial flocs serves as a rich source of amino acids and growth factors to fish and shrimp, leading to a significant recycling of protein and higher utilization of feed (Avnimelech et al., 1994; Chamberlain et al., 2001; Tacon et al., 2002). Recent research showed that carbohydrate addition in extensive shrimp ponds improved the nitrogen retention efficiency and had a positive effect on production (Hari et al., 2004).

The shrimp growth recorded in carbohydrate added culture conditions was not limited by any of the water quality parameters as they fell in the favorable limits for *Penaeus monodon* production (Chen et al., 1990; Hariati et al., 1996). The comparable net shrimp yield and FCR showed the possibility of reducing the dietary protein level in favor of addition of carbohydrate to the water column without any significant reduction in shrimp production (Hari et al., 2004). The reduction in TAN and NO$_2^-$-N levels observed in carbohydrate added treatments could only be attributed to the increased THB population, which immobilized TAN for the synthesis of new bacterial cells (Hari et al., 2004). Burford et al. (2004b) suggested that 'flocculated particles' rich in bacteria and phytoplankton could contribute substantially to the nutrition of the *Litopenaeus vannamei* in intensive shrimp ponds.
Survival rates were similar in various experiments showed that water and sediment quality were favorable for *Penaeus monodon* cultivation (Hariati et al., 1996). Shrimp rely on natural foods even in fed ponds. Studies using stable isotope have shown that the natural biota can contribute to shrimp nutrition in less intensive systems (Parker and Anderson, 1989; Cam et al., 1991; Burford, 2000). Focken et al. (1998) found that 71% natural food in the stomachs of *Penaeus monodon* in semi-intensively managed fed pond. Four to ten percent of $^{15}$N-enriched natural biota were retained by shrimp within 48 hours (Burford, 2000). O'Keefe (1998) suggested a reduced dietary protein level (25 – 30%) for *Penaeus monodon* in extensive type of shrimp culture systems against 30 – 40% and 40 – 50% in semi-intensive and intensive type of culture, respectively. Depending on the culture system, 16 - 21% of the total amount of nitrogen available in the system was retained in shrimp biomass. These values concur with 14% retention in semi intensive *Penaeus vannamei* ponds (Teichet-Coddington et al., 2000), 18% retention in semi-intensive Thai shrimps ponds (Briggs and Funge-Smith, 1994) and 21 – 22% retention in intensive *Penaeus monodon* shrimps ponds (Jackson et al., 2003). However, in closed intensive *Penaeus monodon* rearing systems, a 23 - 31% N recovery was recorded (Thakur and Lin, 2003). The nitrogen budget in the carbohydrate added shrimp culture system revealed that 16 - 21% of the total nitrogen input was retained in the
shrimp, 0.22 - 0.49% in the water, 67 - 71% in the sediment, and 2.1 - 2.7% was lost through water exchange (Hari et al., 2006). Extensive shrimp farming with low water exchange pollutes less surrounding surface waters than all other shrimp farming systems (Hari et al., 2006).