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Biofilm developed on plant substrates enhances growth and survival of post larvae of *Macrobrachium rosenbergii*

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Abstract

A five-week laboratory experiment was conducted to evaluate the efficacy of plant-based substrates for biofilm production and their effect on water quality as well as growth and survival of freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) post-larvae. The experiment consisted of three treatments with a control, each with three replicates following a completely randomized design. The substrates evaluated were dried sugarcane bagasse (T1), paddy straw (T2) and *Eichhornia* (T3). Tanks without substrates acted as control. Seven days after the introduction of substrates at the rate of 300 g /tank, post-larvae were stocked at 40 nos/m^2. The mean initial length and weight of the post-larvae was 10.3 mm and 0.008 g, respectively. The post-larvae were fed with commercial pellet feed (30% protein) at 20% of initial biomass. Results revealed significantly higher ($p<0.05$) levels of total ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen in the control tanks (0.07 ±.004 mg L^{-1}; 0.03 ±.01 mg L^{-1}; 1.40 ±.05 mg L^{-1}, respectively) compared to treatment tanks. Addition of natural substrates enhanced survival and growth of post-larvae with highest growth recorded in paddy straw (0.15 ±0.05 g) followed by sugarcane bagasse (0.14±0.04 g) and *Eichhornia* (0.10±0.01 g) treatment. The enhancement of growth of PL in natural substrate corresponds to the heterotrophic bacteria that are using the substrate, probably as a food resource and as a probiotic. Further, there was no water exchange during the experimental period, which indicated that the presence of a biofilm reduces the necessity of water exchange and saves water during the post-larval rearing of *M. rosenbergii* at this density.

Keywords: Biofilm; growth; *Macrobrachium rosenbergii*; natural substrate; water quality

Introduction

Macrobrachium rosenbergii (De Man, 1879) is one of the high value aquaculture species in Asia. Its hardy nature, tolerance to wide range of temperature, high nutritional value and omnivorous feeding habit have made this species an excellent candidate for aquaculture (Chen & Chen, 2003). Large quantities of water and land are required for raising the freshwater prawn through expansion of pond area. The present challenge is to enhance the freshwater prawn production by increasing pond productivity per unit land area and water in a sustainable manner. A suitable way to maintain high water quality and to enhance sustainable production of freshwater prawn is to use plant substrates with a high surface area to volume ratio, pre-colonized by biofilm that absorbs excess nutrients from the water.

The aquaculture sector is growing more rapidly than any other animal food producing sector in the world. One of the main reasons that restricts further expansion of aquaculture is the pollution caused by discharge of wastewater from the culture system into the external water bodies. With the adoption of intensive and super-intensive methods of culture practices, the accumulation of ammonia, is of primary concern, affecting the food ingestion, growth and survival rates of culture animal (Tomasso, 1994; Wasielesky et al., 1994; Ostrensky & Wasielesky, 1995; Cavalli et al., 1996; Matias et al., 2002). Large amounts of water must be exchanged daily from the culture system, in order to keep these dissolved nutrients at low levels, which increases the cost of

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production. Moreover, most of the Asian aquaculture production comes from the small and marginal farms. Development of viable, low cost technologies for these small-scale farmers and their application to farming practices is needed. Production of high value finfish and shellfish species without the exchange of water has been a topic of interest in recent years.

Biofilm- a microbial consortium, is responsible for many biogeochemical cycles in aquatic ecosystems, especially nitrogen cycle (Decho, 1990; Meyer-Reil, 1994). Recently, zero water exchange management systems have been developed based on heterotrophic bacteria and promoted for the intensive production of finfish and shellfishes. Heterotrophic bacteria that proliferate through the addition of organic carbonaceous substrate, not only maintain good water quality in the system, but also provides an inexpensive feed source for the prawn and a higher efficiency of nutrient conversion of feed (Crab et al., 2007).

Autotrophic and heterotrophic food production in the culture system can be enhanced through fertilization, thereby reducing the cost from the exogenous addition of artificial feed. But autotrophic food production in the system is light dependent, whereas microbial heterotrophic food production is independent of light. Therefore, promotion of heterotrophic food production in the pond even during night can be carried out using biofilm technology.

Earlier studies reported that both survival and growth of freshwater prawn even at higher stocking densities were significantly higher due to provision of substrates when compared to traditional production system without substrates (Cohen et al., 1983; Hem & Avit, 1994; Tidwell et al., 1998; Tidwell et al., 2000; Uddin et al., 2007; Asaduzzaman et al., 2008). Therefore, it is possible to increase production of prawns from a pond by increasing the amount of surface area available within the pond by addition of substrates. D'Abramo et al. (2006) reported that addition of substrates changes the culture system it from a two-dimensional area to a three-dimensional area.

Several studies have documented the benefits of adding different types of substrates like polyvinylchloride (PVC) frame with plastic mesh and suspended seine (Tidwell et al., 1998), plastic mesh and strips of oyster netting (Tidwell et al., 1999), bamboo poles, PVC pipes and sugarcane bagasse bundles (Keshavanath et al., 2001), hollow PVC pipe, high density polyethylene (HDPE) and black nylon netting (Mamun et al., 2010) to different finfish and shellfish culture units. Although considerable reports are available on the use of different artificial substrates, there are scant reports about the use of natural substrate (locally available and cheap) during the culture of freshwater prawn, *M. rosenbergii*. Considering the above, the present study was conducted with the following objectives: i) to evaluate the efficacy of different plant-based substrates, such as sugarcane bagasse, paddy straw and *Eichhornia* for biofilm production; and ii) to study the effect of biofilm on the water quality as well as growth and survival of freshwater prawn, *M. rosenbergii* post-larvae.

Materials and Methods

The present study was conducted in the wet laboratory of Central Institute of Freshwater Aquaculture (CIFA), Bhubaneshwar. The experimental design consisted of four experimental groups, each with three replicates following completely randomised design (CRD). Twelve tanks of 250 L capacity were filled with fresh water from the pond using a pump up to 45 ± 2 cm (height of the tank is 60 cm). All tanks were provided with uniform aeration using an air blower. Natural photoperiod of 12 h light and 12 h dark was maintained throughout the experimental period. Post-larvae of *M. rosenbergii* were collected from the prawn hatchery of CIFA. The mean initial length and weight of the post larvae recorded was 10.3 mm and 0.008 g, respectively.

Natural plant substrates, such as sugarcane bagasse (T1), paddy straw (T2) and *Eichhornia* (T3) were dried for a period of four days and are used for biofilm production. In case of *Eichhornia*, roots were removed and the remaining portion was well dried before introducing into the tanks. About 300 g of each substrate was divided into 6 bundles and was introduced into each tank as per Mridula et al. (2003). An area of 1 cm^2 was marked on six similar positions on all the substrates using a black marker pen for scraping the surface for determining total heterotrophic bacteria. The substrates were suspended in the water column 10 cm above the tank without touching bottom, using ropes tied on the walls of the circular tanks. The substrates remained submerged in the water column throughout the

study. Seven days after the introduction of substrates, post larvae of *M. rosenbergii* were hand-counted and stocked @ 40 PL/ m^2. The PL were fed with commercial pellet feed (Godrej Macro Gold, 30% protein, 5% lipid) at @ 20% (Mamun et al*.,* 2010) of initial biomass. The pellet feed for a day was divided into two equal portions and delivered at 0700 h and 1700 h daily. A regular record of supplied feed was kept for determining the feed conversion ratio (FCR).

Sampling of water and substrates were done once in a week for analysis of physical, chemical and biological parameters. Biofilm colonized on the submerged natural substrates were quantified by scraping a known surface area (1 cm^2) already marked on the substrate. During each sampling**,** different marked positions of 1 cm^2 area is scrapped and the scraping is done at the similar positions on the substrates. The bacterial aggregates were then dispensed in suitable diluents by mechanical shaking, before dilution and plating by conventional plate count method (Anwar et al*.* 1992). Bacteriological study was done to evaluate the variation in heterotrophic bacterial populations in water and on the different natural substrates used in the experiment. Similarly, water samples were collected aseptically from the tanks under different treatments in uricol bottles and heterotrophic bacterial populations were enumerated by spread plate method using nutrient agar.

All the PLs stocked in all the treatment groups were sampled individually at the day of stocking (day 1), middle (day 15) and at the end of the experiment (day 35). The weight of PL during each sampling was measured using a analytical balance with precision of 0.01 g. Total length of *M. rosenbergii* PL was measured from the tip of the rostrum to the tip of the telson by using a centimetre scale. Both length and weight measurements were taken up to the nearest millimetre and up to the nearest gram, respectively. During sampling, the PLs were handled very carefully as the species is very susceptible to handling stress. Growth parameters**,** such as feed conversion ratio and survival percentage were estimated at the end of the experiment and were calculated as follows:

Survival (%) = (number of post larvae harvested/ number of post larvae stocked) × 100, Feed conversion ratio (FCR) = total dry feed fed (g)/total live weight gain (g)

No water exchange was carried out during the experiment. Water quality parameters like temperature, pH, dissolved oxygen, total alkalinity, ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen and phosphorus were measured as per standard methods (APHA 1998). The water quality parameters for each treatment were taken during morning hours (0700 h) at weekly intervals. The water samples were collected at the start of a week. Water temperature was measured using mercury-filled Celsius thermometer and dissolved oxygen were estimated by Winkler's modified method (APHA, 1998). The water pH was measured using digital pH meter (LABINDIA Ltd.) for all the experimental tanks.

Total alkalinity was estimated by titrimetric method (APHA, 1998) by titrating against standard H_2SO_4 and phenolphthalein and methyl orange as indicators. Ammonia, nitrite, nitrate and phosphate concentrations were estimated spectrophotometrically at 635 nm, 543 nm, 543 nm and 690 nm wavelength, respectively, (APHA, 1998) and compared with standard graph. The concentration was expressed as mgL^{-1}.

The comparison between all the treatments and control was done by one -way ANOVA at 5% probability level. Comparison between treatment means was evaluated by Duncan's Multiple Range Test (Duncan, 1955). A significance level of $p<0.05$ was used. All the statistical analyses were done using the SPSS 16 program.

Results and Discussion

Water quality parameters**,** such as temperature, pH, dissolved oxygen and alkalinity, measured weekly during the experimental period are shown in Table 1. The average water temperature recorded during the experimental period ranged from 26.7°C to 30.6°C (Table 1), suitable for the rearing of *M. rosenbergii* post-larvae. In the present study the pH values were recorded within the range of 6.61 to 8.24 (Table 1), slightly alkaline in most of the tanks, indicating favourable conditions for biological production. Dissolved oxygen levels in the tanks were also recorded within the suitable range for culture (4.3- to 7.1 mg L^{-1}**)** (Table 1).

Significantly higher total ammonia-nitrogen (Fig.1a), nitrite-nitrogen (Fig.1b) and nitrate-nitrogen (Fig.1c) were recorded in the control tanks compared to treatment tanks towards the last weeks (IV and V^{th}) of the experiment (Table 2). During the second and

third week of the experiment, the total ammonia-nitrogen levels of substrate-based treatments were significantly higher than the control but reduced significantly during the subsequent weeks (Fig.1a). The lowest value of ammonia-nitrogen was observed in bagasse (0.006±.003) followed by straw (0.02 ±.002), Eichhornia (0.02 ±.001) and the highest (0.07 ±.004) in control group (Fig.1a and Table 2).

The colonization of bacterial biomass in all the substrates increased steadily over time and highest bacterial count (cfu × 10^7 cm^{-1}) was observed higher in paddy straw (3.43 ±0.61) followed by sugarcane bagasse (2.62 ±0.61) and *Eichhornia* (1.98±0.61) (Table 3).

Addition of natural substrates enhanced survival and growth of *M. rosenbergii* post larvae in substrate

Table 1: Physicochemical quality parameters of water in different experimental groups

Water quality parameters	Temperature (ºC)	pH	D.O (mg l^{-1})	Alkalinity (mg l^{-1})
Control	26.8-30.5	6.99-8.04	4.9–7.1	92-108
	28.63^{a}±1.2	7.66^{a}±0.3	6.00^{c}±1.0	100.4^{a}±2.5
T1	26.7-30.4	6.91-8.27	4.3-6.8	104-120
	28.55^{a}±1.5	7.84^{ab}±0.4	5.6^{ab}±1.2	115.1^{b}±4.0
T2	26.9-30.6	7.01-8.24	4.3-6.9	100-180
	28.64^{a}±2.0	7.98^{b}±0.5	5.5^{a} ±1.5	139.7^{c}±3.5
T3	26.7-30.1	6.61-8.32	4.7-7.1	124-134
	28.56^{a} ±1.8	7.93^{b}±0.5	5.7^{b}±2.0	125.3^{b}±3.0

In each experimental group results the first row indicates the range of parameters and second row indicates mean ± SD. In each column mean ± SD followed by different letters were found to differ at 0.05 probability level by Duncan's multiple range test. n = 3, where n is the number of observations

Table 2. Ammonia, Nitrite and Nitrate content (mgL^{-1}) of water (mean ± SE) in different treatment groups

	Experimental period (week)	C	SB	PS	E
Ammonia	I	0.003^{a}±.002	0.005^{a}±.003	0.003^{a}±.001	0.007^{a}±.004
	II	0.06^{a}±.011	0.14^{c}±.01	0.12^{bc}±.035	0.08^{ab}±.01
	III	0.07^{ab}±.006	0.10^{b}±.03	0.06^{a}±.009	0.11^{b}±.001
	IV	0.07^{b}±.006	0.05^{a}±0.005	0.04^{a}±.01	0.05^{a}±0.003
	V	0.07^{c}±.004	0.006^{a}±.003	0.02^{b}±.002	0.02^{b}±.001
Nitrite	I	0.009^{a}±.001	0.01^{ab}±.002	0.02^{b}±.001	0.018^{b}±.002
	II	0.018^{a}±.015	0.024^{b}±.015	0.029^{c}±.015	0.024^{b}±.01
	III	0.022^{a}±.008	0.037^{b}±.008	0.048^{c}±.008	0.031^{a}±.008
	IV	0.035^{d}±.007	0.022^{a}±0.007	0.033^{c}±.007	0.030^{b}±0.007
	V	0.034^{d}±.013	0.009^{a}±.01	0.012^{b}±.01	0.016^{c}±.01
Nitrate	I	1.21^{c}±.05	0.23^{a}±.02	0.18^{a}±.02	0.46^{b}±.03
	II	1.14^{c}±.05	0.34^{b}±.02	0.19^{a}±.02	0.23^{a}±.03
	III	1.22^{c}±.05	0.26^{a}±.02	0.17^{a}±.02	0.55^{b}±.03
	IV	1.22^{c}±.05	0.29^{b}±.021	0.22^{a}±.02	0.57^{b}±.03
	V	1.40^{c}±.05	0.50^{b}±.02	0.38^{a}±.02	0.61^{b}±.03

The mean ± S.E in each row followed by different letters were found to be differ at 0.05 probability level by Duncan's Multiple Range Test (David B. Duncan, 1955). C-control, SB- sugarcane bagasse, PS- paddy straw, E- Eichhornia

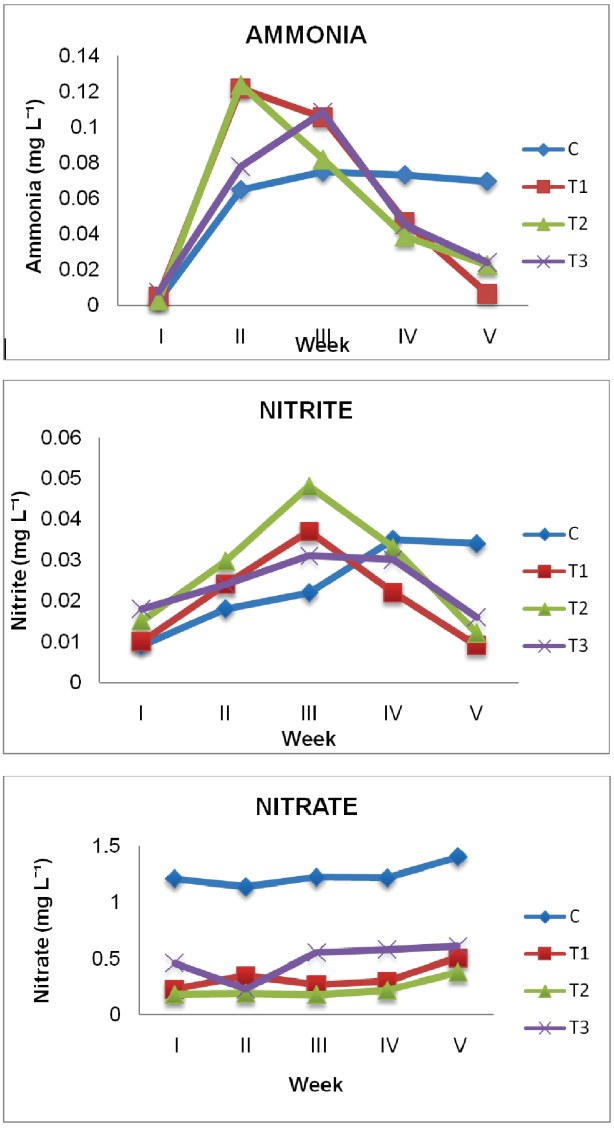

Fig. 1 (A) Ammonia content (mgL^{-1}), (B) Nitrite content (mgL^{-1}), (C) Nitrate content (mgL^{-1}) of water in different treatment groups

based treatments with highest growth recorded in paddy straw (0.15 g) followed by sugarcane bagasse (0.14 g) and *Eichhornia* (0.10 g) treatment (Fig. 2). There were significant ($p<0.05$) effects of natural substrates on final mean length and weight of PLs (Table 4). However, no significant differences ($p>0.05$) existed between the final mean lengths and weights of PLs in straw and bagasse treatments but these value were significantly higher than those in *Eichhornia* treatment and control. The survival percentage of all the treatment groups were also significantly higher than that of the control group (Table 4). Feed conversion ratio (FCR) in all the

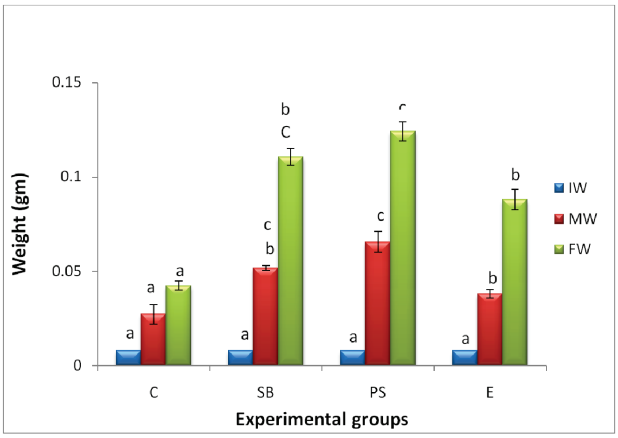

Fig. 2. Mean body weight (g) of *Macrobrachium rosenbergii* post larvae from different treatments during the experimental period

treatment tanks were significantly lower than that of the control group. The mean FCR decreased from 1.45 in control tanks without substrate to 0.42 in tanks with natural substrate.

The average water temperature recorded during the experimental period ranged from 26.7°C to 30.6°C, suitable for the rearing of *M. rosenbergii* post-larvae. Indulkar et al. (1994) reported that 25°C to 27°C was suitable temperature for the growth of freshwater prawn post-larvae and Hossain & Islam (2006) reported that about 27°C-29°C is suitable for post-larval rearing of *M. rosenbergii* in a recirculatory system, which were similar to those in the present study.

Boyd & Zimmermann (2000) reported that a pH range between 7.0- 8.5 is recommended as ideal for nursery rearing phase of prawn post-larvae. In the present study the pH values were recorded within the range of 6.61 to 8.24, slightly alkaline in most of the tanks, indicating favorable conditions for biological production (Mridula et al., 2003). Dissolved oxygen levels in the tanks were also recorded within the suitable range for culture (4.3- to 7.1 $mg.L^{-1}$).

Strauss et al. (1991) reported that NH_3-N concentration higher than 1 and 2 mgL^{-1} at pH values of 9 and 8.5, respectively, cause harmful effects to prawn juveniles. Ammonia concentrations in this experiment ranged between 0.003 and 0.14 mg l^{-1}, which might be considered safe for freshwater prawn post-larvae culture. The ammonia levels throughout all treatments, including the control, are quite low and would not be expected to be toxic or even retard

Table 3: Bacterial biomass production in different substrates over the experimental period (cfu × 10^7 ml^{-1}) (Mean ± SE)

Week	T1 (Sugarcane bagasse)	T2 (Paddy straw)	T3 (Eichhornia)
I	0.212^{b}±0.033	0.249^{c}±.033	0.134^{a}±0.033
II	0.573^{a}±0.122	0.960^{c}±.122	0.616^{b}±0.122
III	1.890^{b}±0.301	2.040^{c}±0.301	1.073^{a}±0.301
IV	2.043^{b}±0.412	2.990^{c}±0.412	1.502^{a}±0.412
V	2.620^{b}±0.610	3.432^{c}±0.610	1.980^{a}±0.610

The means in each row followed by different letters were found to be differ at 0.05 probability level by Duncan's Multiple Range Test (David B. Duncan 1955).

Table 4: Growth parameters of *M.rosenbergii* post larvae in different experimental groups

Parameters	Control	Bagasse	Straw	Eichhornia
Initial mean length (cm)	1.0^{a}±0.01	1.0^{a}±0.01	1.0^{a}±0.01	1.0^{a}±0.01
Final mean length (cm)	1.66^{a}±0.3	2.44^{c}±0.8	2.44^{c}±1.0	2.22^{b}±0.5
Initial mean weight (g)	0.008^{a}±0.01	0.008^{a}±0.02	0.008^{a}±0.01	0.008^{a}±0.02
Final mean weight (g)	0.04^{a}±0.02	0.14^{c}±0.04	0.15^{c}±0.05	0.10^{b}±0.01
Average daily growth (g/d)	0.0011^{a}	0.004^{c}	0.0042^{c}	0.0025^{b}
Survival (%)[1]	81.1^{a}±2.43	86.1^{ab}±3.09	92.2^{b}±1.47	88.3^{ab}±2.54
FCR[2]	1.45^{b}±0.10	0.45^{a}±0.02	0.42^{a}±0.02	0.60±0.05

The mean ± S.E in each row followed by different letters were found to be differ at 0.05 probability level by Duncan's Multiple Range Test (David B. Duncan 1955).
1 Survival (%) = number of post larvae harvested/ number of post larvae stocked × 100
2 Feed conversion ratio (FCR) = total dry feed fed (g)/total live weight gain (g)

growth of PL at the present stocking density of 40/ m^2.

The higher dietary protein levels and the absence of substrate to produce microbial biomass might have resulted in significantly higher total ammonia-nitrogen concentrations in control tanks. Lovell (1992) reported that ammonia concentration in the culture system increases with increasing dietary protein concentration as well as the feeding rate. The initial increase in total ammonia content in treatment tanks may be due to the introduction of nutrient rich plant substrates.

The lowest value of ammonia-nitrogen was observed in treatments and the highest in control group. The addition of nutrient rich plant substrates in treatment tanks lead to an increased bacterial count, which immobilized total ammonia-nitrogen for the synthesis of new microbial biomass; thus reducing the level of these nitrogenous compounds in substrate-based treatments (Avnimelech & Mokady, 1988; Avnimelech et al., 1989; Avnimelech, 1999 and Hari et al., 2004). Therefore it indicates that biofilm on natural substrate can be utilized in longer nursery duration or higher stocking density fresh-water PL culture to reduce the rate of ammonia and nitrite accumulation thereby reducing the water exchange.

Lower total ammonia concentrations in the treatment tanks also might be due to higher nitrification rates in substrate treatments. Langis et al. (1988); Joice (1999) and Ramesh et al. (1999) reported that the bacterial biofilm on the substrates reduced ammonia and nitrite levels through nitrification process. Even though substrates are absent in control tanks there is a possibility of nitrification due to the microbial biomass attached to the walls of the control tanks and phytoplankton present in pond water and this may resulted in higher NO_2-N and NO_3-N concentrations in control tanks.

The nitrogen uptake by biofilm might reduce the occurrence of pathogenic bacteria in the culture system, as they normally require high amount of nitrogenous compounds (Austin & Austin, 1999; Brock & Main, 1994; Thompson et al., 2002). It was reported earlier that in scampi culture systems, phytoplankton as well as bacteria plays an important role in the processing of nitrogenous wastes (Shilo & Rimon, 1982; Diab & Shilo, 1988).

The colonization of bacterial biomass in all the substrates increased steadily over time and highest bacterial count (cfu × 10^7 cmÉ^1) was observed higher in paddy straw followed by sugarcane bagasse and *Eichhornia* which is in agreement with Mridula et al. (2003). The higher bacterial population in natural substrates indicates the utilization of nutrient source of these substrates by heterotrophic bacteria. This is in agreement with the studies of Chang & Rittman, (1988) and Ramesh et al. (1999). Rough carbon surface of the substrates provide attachment sites as well as protection to the biofilm developed. Heterotrophic bacteria proliferated through the addition of organic carbonaceous substrate, maintained good water quality and also provided an inexpensive feed source for the PL in the culture system.

Higher bacterial colonization observed in paddy straw compared to sugarcane bagasse may also be due to the presence of low lignin complex in paddy straw (12%) compared to sugarcane bagasse (20-30%) (Shilta et al., 2016). Srinivasan (1987) showed that the microbial decomposition of plant substrate in water depends on contents of cellulose, lignin, C: N ratio hence restricts the digestibility of the natural substrates. Mridula et al. (2003) also reported higher bacterial colonization in paddy straw than sugarcane bagasse. Therefore, the difference in the morphology, chemical composition and surface area of natural substrates which, despite the same "amount", may determine the quantity of harvestable attached microbial biomass.

There were significant ($p<0.05$) effects of natural substrates on final mean length and weight of post-larvae. However, no significant differences ($p>0.05$) existed between the final mean lengths and weights of post-larvae in straw and bagasse treatments but these value were significantly higher than those in *Eichhornia* treatment and control. The enhancement of growth of PL in natural substrate based treatment may be due to the heterotrophic bacteria in the substrate, acting probably as a food resource and as a probiotic. (Tidwell et al., 2000, 2002; van Dam et al., 2002; Miller & Falace, 2000; Milstein et al., 2003). Therefore it can be concluded that natural substrate in pond-based growth out systems could also allow the use of supplemental rather than completely balanced feeds. For example, the 30% crude protein feed used in this experiment could be reduced to 22-25% because of the contribution of the heterotrophic bacteria.

The growth of other organisms such as algae, fungi and protozoans were observed along with microbial biofilm formed on substrate (Khatoon et al. (2007). These organisms have a higher protein to energy ratio and due to their ability to synthesize long chain polyunsaturated fatty acids, they enrich the quality of microbial biofilm (Zhukova & Kharlamenko, 1999; Ballester et al., 2007). Freshwater prawn can graze on these food items more efficiently than filter feeding on planktonic food (Dempster et al.,1993).

Besides the nutritional contribution provided by the biofilm, the presence of natural substrates within the culture system provides refuge for moulting prawns as well as increased the surface area for prawn distribution by utilizing the three dimensional volume of the tank, rather than only the walls and bottom. Since post-larvae are easily subjected to predation, cannibalism and fluctuating environmental conditions (Fujimura & Okamoto, 1972), their survival at the end of the nursery phase can vary substantially when cultured at high densities. The use of natural substrates during the rearing phase of freshwater prawn could have reduced the negative effects of cannibalism and increased their survival. In the present study survival percentage of all the treatment groups were significantly higher than that of the control group. Earlier studies have indicated improved culture performance of the freshwater prawn, *M. rosenbergii* with the use of artificial substrates (Tidwell et al., 1998; 1999) but the result of the study conducted by Mamun et al. (2010) indicated that growth of *M. rosenbergii* PLs are improved in presence of artificial substrates but the artificial substrates did not improved their survival.

Feed conversion ratios in all the treatment tanks were significantly lower than that of the control group (Uddin et al., 2006). Hossain & Islam (2006); Mamun et al. (2010) reported FCR value range of 1.73-1.93 and 1.85- 1.88, respectively, when prawn

PLs were fed with formulated diets in a recirculatory system using artificial substrates. Gwak (2003); Tidwell et al. (2002) showed that artificial substrates had no statistically significant impact ($p<0.05$) on feed conversion ratios. But in the present study, mean FCR decreased from 1.45 in control tanks without substrate to 0.42 in tanks with natural substrate. Although prawn post-larvae were fed a high quality artificial diet throughout the experiment, they were observed to actively graze on the biofilm and, therefore, better results were obtained in the substrate- based tanks.

In the present study the nursery phase is limited to 30 days with 300 g substrate yielding a mean post larval weight of approximately 0.1 g in substrate treatments. Addition of more substrate per unit area is necessary for larger post-larvae stocking.

Easily biodegradable bagasse and paddy straw with higher production of heterotrophic bacterial population was associated with significantly higher growth, survival and lower FCRs of *M. rosenbergii* post larvae when compared to control tanks.

The results of present study revealed that biofilm developed on natural substrates (paddy straw and bagasse) enhanced the growth of *Macrobrachium rosenbergii* post-larvae probably by providing additional food source in the form of biofilm and by improving water quality of the culture system.The best substrate was found to be paddy straw followed by sugarcane bagasse. In freshwater prawn ponds, plant substrates can act as a shelter for growing post-larvae, thereby reducing the heterogeneous individual growth rate. By promoting bacterial biofilm on cheaply available plant substrates can be an important eco-friendly, low cost technology to boost the freshwater prawn production. Further research should focus on quantifying the amount of natural substrates if culture duration is increased. However, further studies are needed in order to evaluate the biofilm efficiency in large-scale freshwater prawn culture systems, and how the different trophic interactions among microorganisms may affect the production of freshwater prawn post-larvae.

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