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Original Research Article

Interplay of Substrate Variation and Biofilm Formation in Augmenting Carp Production

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Keywords

Substrates; Bacterial biofilm; Carp; Water Quality; TPC; Growth

A B S T R A C T

Field trial of six month was conducted to assess the efficacy of substrate based biofilm vis-à-vis fertilization on growth performance of carps. The trial had five treatments, control (without substrate), T_0; bamboo stick - side shoot (400 no. /0.01 ha), T_1; paddy straw (22 kg/0.01ha), T_2; sugarcane bagasse (24 kg/ 0.01 ha), T_3; wheat straw (22 kg/0.01ha), T_4. The ponds were stocked with rohu, (*Labeo rohita*); catla, (*Catla catla*); mrigal, (*Cirrhinus mrigala*) and common carp, (*Cyprinus carpio*) fingerlings at 10000 ha^{-1} in a ratio of 3:3:2:2. The substrate addition affected water quality; it decreased total ammonia and nitrite. Dissolved oxygen was low following manuring and introduction of the substrate, but improved subsequently. Total plate count of bacteria in water was significantly higher ($p<0.05$) in substrate based treatments than that in control with a highest value achieved in the treatment with sugarcane bagasse. The growth of fish was significantly higher in substrate-based treatments than that in control. This trial establishes the importance of substrate for formation of microbial biofilm and confirming that sugarcane bagasse can produce more bacteria in the water body. In such endeavour fish meritoriously utilized the biofilm grown on substrate therefore reducing the need of artificial feed which cost high for the carp culture.

Introduction

In India, aquaculture production comes largely from carps, contributing to nearly 85% of the freshwater fish production (Keshavanath, 2014), is based upon inputs of supplementary feed and fertilizer and hence profitability on the cost of these inputs. Increasing cost of inputs has posed threat to these practices and encouraged aquaculturists to search for alternatives. Moreover, in aquaculture practices, only about 15 to 30% of the nutrient input in feed-driven pond systems is converted into harvestable yields (Gross *et al.*, 2000). Dempster *et al.*, (1993) observed that many herbivorous fish species grazing periphytone more efficiently than filtering phytoplankton. There is thus growing interest in the potential of artificial substrates for biofilm production in ponds to reduce cost of production and increase

nutrient utilization for profitable aquaculture (Beveridge *et al.,* 1998). Proximate composition of biofilm have shown higher nutritive value under ungrazed situation having 41.4% protein and 7.9% fat whereas, under grazed situation they have 23-26% protein and 2.7% fat content (Verdegem *et al.,* 2001).

Microbial biofilms are meritoriously exploited by many fish species which thrive low in the food chain (Van Dam *et al.,* 2002), therefore substrate-based fish culture could be viable option to make semi-intensive aquaculture systems in terms of more nutrients efficient. By providing organic matter and suitable substrates, heterotrophic food production can be increased several folds, which in turn would support fish production. Substrates provide the site for epiphytic microbial production, consequently eaten by fish-food organisms and fish. Fish harvest microorganisms directly in significant quantities, either from microbial biofilm on detritus or from naturally occurring flocks in water column (Schroeder, 1978). The feasibility of using biofilm based culture systems has been explored and found to enhance primary production, food availability and fish production as compared to the traditional culture practices (Welcomme 1972; Hem and Avit, 1994; Konan-Brou and Guiral, 1994). Some recent trials suggests that artificial substrates are being used in the freshwater ecosystem for the development of microbial biofilm for enhancing fish and shellfish production (Ramesh *et al.,* 1999; Azim *et al.*, 2002a, b; Keshavanath *et al.*, 2002; Azim *et al.,* 2004; Mridula *et al.*, 2003, 2005; Rajesh *et al.,* 2008; Viau *et al.*, 2012; Gangadhar and Keshavanath, 2012). Provision of substrate would therefore, be useful for the growth of microbial biofilm. Apart from forming food for fish, biofilm improves water quality by lowering ammonia concentration (Langis *et al.*, 1988, Ramesh *et al.*, 1999), this implies the usefulness of substrates in improving water quality in culture systems, by lowering ammonia concentration.

Therefore, present study was designed to study the influence of different types of biodegradable substratum on microbial biofilm formation as well as carp production under pond conditions.

Materials and Methods

Pond facilities and experimental fishes

Pond trial was carried out in 10 earthen nursery ponds measuring 10 x 10 x 1 m (length, width and depth) at the Instructional Fish Farm of College of Fisheries, Dholi, Muaffarpur, Bihar. Fishes used for trial purpose were fingerlings of Indian Major Carp, *Catla catla* (catla), *Labeo rohita* (rohu), *Cirrhinus mrigala* (mrigal or naini) and Exotic Carp, *Cyprinus carpio* (common carp). All fish species were supplied from the Fish Seed Production Unit of College of Fisheries, Dholi. Prior to stocking fishes were given dip treatment of 0.1 g l^{-1} potassium permanganate in order to avoid any infection.

Experimental Design and Substrates

The experiment consists of five treatments which include, control (without substrate), T_0; bamboo stick - side shoot (400 no. /0.01 ha), T_1; paddy straw (22 kg/0.01ha), T_2; sugarcane bagasse (24 kg/ 0.01 ha), T_3; wheat straw (22 kg/0.01ha), T_4. These material were used as substrate material in replication of two. Before placing the substrates, all ponds were drained, dried and limed with quick lime @ 200 Kg ha^{-1}. After 4 days, water was filled to the ponds from a deep bore-well of fish farm and water

column of 0.8 m was maintained throughout the experimental period; the evaporation loss was compensated through regular pumping of water. Afterwards, bamboo sticks (approx 1.5 cm in diameter & 2 m in length) were fixed vertically to the bottom in inclined position. Paddy straw, sugarcane bagasse and wheat straw were sun dried and tied in bundles using nylon rope; these were introduced into the ponds randomly at the rate of 4.4 kg and 4.8 Kg each bundle respectively, by suspending the bundles at regular distances from bamboo poles kept across the ponds.

The substrates remained submerged in the water column throughout the study. Along with a substrate, each ponds were incorporated with 12.5 kg cattle dung (@ 1250 Kg ha^{-1}), 310 g urea (@ 31 Kg ha^{-1}) and 160 g triple super phosphate (@ 16 Kg ha^{-1}). Subsequent fertilization was done at fortnightly intervals with cattle dung @ 1000 kg ha^{-1}. The substrates and manure used were procured locally.

After 10 days of suspending substrates, fingerlings (n=100) of rohu (41g), catla (66g), mrigal (35g) and common carp (35g) were stocked at a 3:3:2:2 ratio respectively in each pond. The experiment was conducted for 6months.

Physico-chemical parameters

Water was analyzed once in every three days for different parameters. Temperature, pH and Dissolved oxygen were recorded on the site at 07:30 h using a Systronics Water Analyzer (Model 371). The transparency was determined by secchi disc in ponds and recording the reading on its complete disappearance at 09:30 h. Total alklanity, total ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen, phosphate-phosphorus, biological oxygen demand (BOD) and chemical oxygen demand (COD) was measured in the laboratory according to Welch (1948) and APHA (2005).

Biological parameters

Qualitative and quantitative analysis of planktons

Sampling for plankton analysis was carried out at 15 days interval. A known volume of water (40 l) was filtered through plankton net (mesh size 60 µ) and the freshly preserved plankton samples (in 6% formalin) were then used for quantitative and qualitative analyses. Qualitative and quantitative estimation of plankton were carried out using a Sedgewick Rafter Cell and identified using standard literature with the help of Olympus Microscope (BX 41).

Plankton density was estimated using the formula: N = (P x C x 100) / L. Where, N=The number of plankton units per liter of original pond water; P= The number of planktons counted in 10 random fields of Sedgewick Rafter Cell; C= The volume of final; concentrated sample (ml); L= The volume (in liter) of the filtered pond water sample

Microbial enumeration

Total plate count (TPC) of bacteria in water and on substrate was estimated fortnightly on nutrient agar at room temperature by the spread plate method. Briefly, sample of substrate was collected and washed in physiological saline (0.85%) to remove free cells. A total of 1 g (wet weight) of substrate was then transferred to physiological saline, and mixed thoroughly by vortex for 4 min to dislodge biofilm cells. Appropriate serial dilutions of the homogenate were made in physiological saline and TPC estimated as number per gram of substrate.

Fish growth and Yield

Random sampling was done at every fifteen days interval to assess the growth and health of the stocked fishes in ponds. Fish weights were recorded on a top-loading electronic balance. Around thirty sampled fishes of four different species viz. Catla, Rohu, Mirgal and Common carp were sampled from each pond using drag net. Weight of the sample fishes was recorded. After weighing the sampled fishes were quarantined using 0.1 g l^{-1} potassium permanganate solution ($KMnO_4$) for disease prevention. However, during the sampling, efforts were made to minimize the stress due to handling. At the end of the culture period (6 month) all the fishes were harvested by draining the ponds.

Statistical Analysis

Statistical analysis of the data included the oneway analysis of variance (ANOVA), followed by Duncan's new multiple range test (DMRT) using the SPSS version 12.0 (statistical graphics corp. US). Significant mean difference were separated at 5%, whereas appropriate and values are expressed as mean ± SE. For convenience all the ANOVA tables were suppressed.

Results and Discussion

Fish Growth

The growth performance of cultured carp among different treatments is given in Table-1 and 2. Among treatments, the growth performance of carps were significantly higher ($p<0.05$) in T_3, followed by T_2 and T_4 treatments. SGR followed the growth trend, being the highest in T_3 treatment. The growth of carps was observed non-significant among treatment T_2 and T_4. The overall survival of carps was non-significant among control and treatment. Significant difference in fish yield was observed among treatments ($p<0.05$). The yield of carp was highest in T_3 compared to other treatments ($p<0.05$), while the yield of T2 and T4was non-significant but were significantly higher than control. The increase in production (%) due to addition of different substrate was 12.21, 28.68, 55.06 and 33.37% in T_1, T_2, T_3 andT_4 treatments, respectively compared to control.

Bacterial Biomass

The fortnightly observation during present study, the total plate count (TPC) of bacteria in water (no. ml^{-1}) showed a gradual increase in treatment ponds than control. In the ponds having sugarcane bagasse as substrate (T_3), bacterial biomass reached a peak on day 30. The TPC then declined steadily to reach minimum on day 60. Once again, bacterial biomass increased gradually after day 75, reaching a peak on day 90 and so on. This increase was due to re-addition of equal quantity of substrate on day between 61-65 and 121-125. The mean values of TPC in water was highest in T_3 ($1.65 \pm 0.17 \times 10^4.ml^{-1}$) followed by T_4 ($1.20 \pm 0.09 \times 10^4.ml^{-1}$), T_1 ($1.14 \pm 0.07 \times 10^4.ml^{-1}$), T_2 ($0.96 \pm 0.09 \times 10^4.ml^{-1}$) and T_0 ($0.46 \pm 0.01 \times 10^4.ml^{-1}$). The average values of bacterial biomass recorded in water were significantly higher ($p>0.05$) in T_3 compared to other treatment. No significant difference was observed in average values of TPC of water among treatments T_1 and T_4 (Fig.1 (a)).

The TPC of bacteria on substrates (no. g^{-1}) showed increase in number of bacteria on all treatment groups. It can be clearly observed that in treatment T_1, TPC was more or less consistent. In treatment T_3, increase in TPC was observed till day 30, followed by a

steady decrease till day 60. The TPC once again increased reaching a peak on day 90, followed by steady decline till day 120. This increase in bacterial biomass was due to re-addition of substrate during day 61-65 and day 121-125. The TPC of treatment T_2 and T_4, followed the trend of rise and decline of TPC as in T_3, but the bacterial number on substrate was considerably low in T_2 as compared to T_3. The mean values of TPC on substrate was highest in T_3 (163.08 ± 9.85 x $10^4.g^{-1}$) followed by T_1 (110.75 ± 1.71 x $10^4.g^{-1}$), and T_4 (108.33 ± 2.53 x $10^4.g^{-1}$). The least mean value of TPC was observed in T_2 (78.09 ± 4.42 x $10^4.g^{-1}$). Significantly higher ($p>0.05$) bacterial biomass was recorded on substrate were in treatment T_3 compared to other treatment. Significant difference was not observed in mean values of TPC of water among treatments T_1 and T_4 (Fig. 1 (b).

Plankton abundance

The plankton abundance among different experimental groups is given in Table-3. The average values of phytoplankton recorded in water were significantly higher ($p>0.05$) in T_0 compared to other treatment however zooplankton were higher in other treatments compare to control. No significant difference was observed in average values of phytoplankton and zooplankton among treatments T_1 and T_4. The average phytoplankton and zooplankton (specimen per litre) observed were 93 and 83 in T_0, 118 and 101 in T_1, 103 and 92 in T_2, 131 and 112 in T_3 and 113 and 105 in T_4 treatments, respectively.

A total of 25 phytoplankton species, belonging to four classes, were recorded for the during the study period. Among phytoplanktons, class Bacilliariophyceae (Diatoms) were represented by 7 species *viz*. *Bacillaria*, *Coscinodiscus*, *Cyclotella*, *Diatoma*, *Navicula*, *Nitzachia* and *Synedra*. The class Chlorophyceae was represented by 12 species *viz*. *Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Cladophora*, *Microspora*, *Oedogonium*, *Pediastrum*, *Pandorina*, *Scenedesmus*, *Spirogyra*, *Tetradeon* and *Ulothrix*. The class cyanophyceae (Blue-Green algae) was represented by 4 species *viz*. *Apanocapsa*, *Microcystis*, *Nostoc* and *Oscillatoria*. The class Euglenophycease represented by 2 species *viz. Euglena* and *Phacus*.

A total of 18 zooplankton species, belonging to three classes, were recorded during the study period. During the present investigation class Rotifera was dominated among all the zooplanktonic groups in all the seasons. However, the diversity of zooplankton varied from season to season. Among zooplanktons, class Rotifera was represented by 10 species *viz*. *Asplanchna*, *Brachionus*, *Filinia*, *Keratella*, *Lecane*, *Lepadella*, *Notholca*, *Polyarthra*, *Rotaria* and *Trichocerca.* The class Cladocera was represented by 4 species *viz*. *Ceriodaphnia*, *Daphnia*, *Macrothrix* and *Moina*. The class Copepoda was also represented by 4 species *viz*. *Cyclops*, *Diaptomus*, *Heliodiaptomus* and *Nauplius.*

Traditionally, fish farmers only fertilize their ponds or apply a combination of fertilizers and feed. Therefore study was aimed to compare the newly derived microbial biofilm technology with the traditional management techniques. In the present study, utilization of four locally available substrates viz. bamboo stick (side shoot), paddy straw, sugarcane bagasse and wheat straw to support enhanced microbial biofilm formation and subsequently fish growth. Despite some variability in substrate types and density, there is no doubt that fish production was consistently higher in the substrate based systems than in the

substrate-free (control) system. Growth of cultured carps were significantly higher in sugarcane bagasse, followed by paddy straw and wheat straw treatments. SGR followed the growth trend, being the highest in sugarcane bagasse treatment. Between the treatments paddy straw and wheat straw, non-significant growth was observed. Bagasse effortlessly biodegradable and has more surface area compare to other treatments moreover higher fiber content favor higher growth of fish through higher bacterial colonization. Therefore in such situation bamboo as treatment has lesser growth than other treatment our finding is accordance with Hem and Avit, 1994. Analysis of variance of the data showed overall significant effect on percentage body weight gain in the treatment and control group. However, there was no significant difference in survival of fish between the treatments. Better growth and survival of carps in tanks with substrate supplemented with manure compared with manure or substrate alone has been reported by other authors (Ramesh *et al.*, 1999; Umesh *et al.*, 1999; Joice *et al.*, 2002; Mridula *et al.*, 2003, Gangadhar and Keshavanath, 2012). Incorporation of biofilm to the intensive culture of *Litopenaeus vannamei* (Moss and Moss, 2004; Zhang *et al.*, 2010), and *Penaeus monodon* (Stuart *et al.*, 2006), improved both weight gain and survival. Biofilm also showed to contribute with several additional nutrients to post-larvae of *Penaeus esculentus*, reared at high densities (Burford *et al.*, 2004). This kind of benefits was also found in other species of high economic value, such as the prawn *Macrobrachium rosenbergii* (Tidwell *et al.*, 1998) and the shrimp *Farfantepenaeus paulensis* (Ballester *et al.*, 2007).

The maximal net fish yield reached 28.04 kg per $100m^2$ after 180 rearing days in the treatment sugarcane bagasse and were significantly higher than other treatment. Treatment having bamboo stick (T1) gave lowest yield among other substrate based treatments. The calculated extrapolated gross yield of carps in T3 treatment was 3149.0 kg ha^{-1} 6 $months^{-1}$, whereas in the other treatments 2030.70, 2278.70, 2613.20, 2708.40 kg ha^{-1} 6 $months^{-1}$ in T0, T1, T2 and T4, respectively.

Our finding strengthen by others funding such as the gross yield of rohu in sugarcane bagasse substrate tanks ranged from 2325.6 kg ha^{-1} 6 $months^{-1}$ to a tune of 3161.6 kg ha^{-1} 6 $months^{-1}$ was reported in a recent study (Gangadhar and Keshavanath, 2012). Ramesh *et al.*, (1999) reported rohu and common carp production of 1235 kg ha^{-1} in 133 days, using sugarcane bagasse as substrate. Keshavanath *et al.*, (2001) reported a production of 1310 kg ha^{-1} 6 $months^{-1}$ in a polyculture study with catla, rohu and common carp, where sugarcane bagasse was suspended vertically in water column. While the results of the current experiment clearly confirmed previous results on the effects of periphyton, the mechanisms of enhanced fish production in the substrate-based system are still not fully elucidated. The present experiment demonstrated 55.06% higher net carp yield using sugarcane bagasse as substrate. The increase in gross production of rohu in monoculture due to bagasse substrate was recorded as 68, 129, 123 and 119% in 1.5, 2.0, 2.5 and 3.0 t bagasse ha^{-1} treatments, respectively (Gangadhar and Keshavanath, 2012). The paddy straw and wheat straw as substrate resulted in 28.68% and 33.37% increase in net yield, respectively. The bamboo stick resulted in mere 12.21% increase in net yield compare to control. Saikia and Das (2010) reported 25% increase in the annual yield of fishes in ponds with bamboo substrates than bamboo substrate free ponds.

Table.1 Growth performance (mean ±SE) of cultured carp in different treatments

Species	Treatment	Initial Mean Weight (g)	Final Mean Weight (g)	Weight Gain (%)	Per Day Weight Gain (g)	Specific Growth Rate
Rohu (*L. rohita*)	T_0	41 ± 2.93	250^{d} ± 12.6	509.75^{d} ± 31.9	1.16^{d} ± 0.06	1.00^{d} ± 0.05
	T_1	41 ± 2.81	305^{c} ± 15.3	643.90^{c} ± 44.5	1.46^{c} ± 0.12	1.11^{c} ± 0.11
	T_2	41 ± 2.91	340^{b} ± 11.2	729.27^{b} ± 37.4	1.66^{b} ± 0.09	1.17^{b} ± 0.10
	T_3	41 ± 3.07	410^{a} ± 17.8	900.00^{a} ± 51.5	2.05^{a} ± 0.13	1.28^{a} ± 0.12
	T_4	41 ± 3.11	355^{b} ± 12.4	765.85^{b} ± 47.3	1.74^{b} ± 0.11	1.19^{b} ± 0.10
Catla (*C. catla*)	T_0	66 ± 4.35	345^{d} ± 19.2	422.72^{d} ± 31.2	1.55^{d} ± 0.11	0.92^{d} ± 0.05
	T_1	66 ± 4.09	392^{c} ± 21.5	493.94^{c} ± 39.7	1.81^{c} ± 0.17	0.99^{c} ± 0.08
	T_2	66 ± 4.42	452^{b} ± 17.1	584.85^{b} ± 33.5	2.14^{b} ± 0.09	1.07^{b} ± 0.04
	T_3	66 ± 5.02	507^{a} ± 15.3	668.18^{a} ± 41.7	2.45^{a} ± 0.21	1.13^{a} ± 0.05
	T_4	66 ± 4.73	440^{b} ± 19.7	566.66^{b} ± 39.1	2.07^{b} ± 0.13	1.05^{b} ± 0.08
Mrigal or Naini (*C. mrigala*)	T_0	35 ± 2.31	233^{d} ± 17.1	565.71^{d} ± 33.5	1.10^{d} ± 0.06	1.05^{d} ± 0.06
	T_1	35 ± 2.77	252^{c} ± 15.6	620.00^{c} ± 58.3	1.20^{c} ± 0.12	1.09^{c} ± 0.08
	T_2	35 ± 3.05	295^{b} ± 11.4	742.85^{b} ± 36.4	1.44^{b} ± 0.09	1.18^{b} ± 0.07
	T_3	35 ± 2.55	332^{a} ± 16.3	848.57^{a} ± 48.7	1.65^{a} ± 0.13	1.24^{a} ± 0.09
	T_4	35 ± 2.73	301^{b} ± 13.2	760.00^{b} ± 37.1	1.47^{b} ± 0.11	1.19^{b} ± 0.07
Common carp (*C. carpio*)	T_0	35 ± 3.02	247^{d} ± 31.3	605.71^{d} ± 48.1	1.18^{d} ± 0.13	1.08^{d} ± 0.09
	T_1	35 ± 2.31	271^{c} ± 23.7	674.28^{c} ± 62.8	1.31^{c} ± 0.14	1.14^{c} ± 0.11
	T_2	35 ± 2.83	327^{b} ± 31.6	834.28^{b} ± 68.0	1.62^{b} ± 0.13	1.24^{b} ± 0.09
	T_3	35 ± 3.11	394^{a} ± 32.9	1025.71^{a} ± 82.5	1.99^{a} ± 0.17	1.35^{a} ± 0.14
	T_4	35 ± 2.57	335^{b} ± 34.3	857.14^{b} ± 73.4	1.66^{b} ± 0.13	1.25^{b} ± 0.12

Mean values with different superscript in the same column among treatments are significantly different ($p<0.05$)

Table.2 Combined weight gain (%), survival, yield and increase in production (%) of cultured carp in different treatment groups

Treatments	Combined weight gain (%)	Survival (%)	Net Yield (Kg/ 100m^2)	Gross Yield (Kg/ 100m^2)	Extrapolated Gross Yield (Kg ha^{-1})	Increase in production (%)
T_0	525.98^d ± 42.3	92.50	16.90^d	20.31^d	2030.70^d	--
T_1	608.03^c ± 57.9	91.25	19.45^c	22.79^c	2278.70^c	12.21
T_2	722.81^b ± 56.1	90.00	22.80^b	26.13^b	2613.20^b	28.68
T_3	860.62^a ± 63.8	93.75	28.04^a	31.49^a	3149.00^a	55.06
T_4	737.42^b ± 49.8	92.50	23.67^b	27.08^b	2708.40^b	33.37

Mean values with different superscript in the same column among treatments are significantly different ($p<0.05$).

Table.3 Mean plankton abundance (specimen per litre) in different treatment groups during the experimental period

Treatments	Phytoplankton	Zooplankton
T_0	93^d ± 8.28	83^c ± 7.11
T_1	118^b ± 9.71	101^b ± 8.38
T_2	103^c ± 9.27	92^c ± 7.62
T_3	131^a ± 11.82	112^a ± 9.63
T_4	113^b ± 10.27	105^b ± 8.65

Mean values with different superscript letters in the same column are significantly different ($p<0.05$)

However, Azim (2001) recorded 77% higher gross production in monoculture of rohu and 180% higher production in polyculture of rohu with catla when bamboo was provided as substrate. *Labeo fimbriatus* showed 75% higher net production in bamboo-based culture tanks (Keshavanath *et al.*, 2002).

Current data confirm that addition of substrate in cultured pond resulted in higher carp production than the non-substrate pond. As regards the direct effect of substratum on biofilm development, our results show that, under field conditions, the nature of substrate have the potential to modify the pattern of biofilm development in terms of quantity through time. It seems that the increased fish production partly results from the additional food that the substrate provides (Miller and Falace, 2000). The growth of fish was highest in substrate-based treatments compared to that in control, and among the three substrates, sugarcane bagasse favoured better growth. Considering the cost of artificial feeds, the present approach of promoting fish food through bio-conversion of regionally available waste plant biomass is seen as a better strategy.

Water quality parameters were within the suitable range for carps, with no intense deviation between treatments. No much effect of different substrate on mean water quality parameters was observed in other studies such as bamboo (Azim *et al.*, 2002b; Keshavanath *et al.*, 2004, Rai *et al.*, 2008), paddy straw (Ramesh *et al.*, 1999, Rai *et al.*, 2008, 2012), sugarcane bagasse (Ramesh *et al.*, 1999; Keshavanath *et al.*, 2001; Dharmaraj *et al.*, 2002; Gangadhar and Keshavanath, 2012), hizol, jute stick (Azim, 2001), dried *Eichhornea* (Ramesh *et al.*, 1999) employing vertically hung or fixed substrate. Following manuring and introduction of the substrate in the culture

ponds, dissolved oxygen showed a significant decrease ($p<0.05$) in the treatment T2, T3 and T4 after the first week, being the corresponding values below the recommended limits for carp culture, but improved subsequently. Low dissolved oxygen is characteristic for water with predominantly heterotrophic food production which accounts for bulk of the oxygen consumption (Moriarty, 1997). A similar observation has been reported in substrate used tanks (Ramesh *et al.,* 1999; Umesh *et al.,* 1999; Dharmaraj et. al., 2002, Rai *et al.,* 2008). Besides, ammonium and nitrite concentrations were low during the whole culture-periods. A clear benefit of biofilm to both survival and growth of cultured species is related to water quality. Biofilm community consists of bacteria and microalgae are able to actively uptake ammonium and other nitrogenous waste, to synthesize protein that can be consumed by either fish or crustaceans, finally supporting in maintenance of water quality and nutrition of cultured animals (Viau *et al.,* 2012; Rajesh *et al.,* 2008; Mridula*et al.,* 2003; Azim *et al.,* 2001; Bratvold and Browdy, 2001; Ramesh *et al.,* 1999). Some of researcher suggested that nitrogenous uptake by biofilm micro-organisms can reduce the proliferation of pathogen bacteria in cultures (Alabi *et al.,* 1999; Avnimelech and Ritoo, 2001; Thompson *et al.,* 2002; Rajesh *et al.,* 2008).

The microbiological status of the water in ponds depends on a wide variety of factors influencing the environment, the most important being the organic matter content (Rheinheimer, 1980; Zmyslowska *et al.,* 2003). Variations in the abundance of heterotrophic bacteria in water samples (cfu ml^{-1}) in the treatments were the result of addition of substrate and showed a gradual increase with time in treatment ponds than control. Among treatments, TPC in the water was higher in sugarcane bagasse treatment compared with other substrate based treatment groups. A similar observation has been reported in substrate used tanks (Ramesh *et al.,* 1999; Dharmaraj et. al., 2002; Gangadhar and Keshavanath, 2012). It is interesting that control ponds had a lower total plate count with any unusual deviation. TPC on sugarcane bagasse substrate was 120 fold higher than those calculated for the water per unit. However, there was significant difference in TPC on substrate between sugarcane bagasse and other substrates used in this trial. Therefore, overall, it is total higher TPC on substrate and in water in the sugarcane bagasse than that in bamboo stick, paddy straw or wheat straw. The reason for higher bacterial biofilm in bagasse could be higher fiber content in sugar and higher surface area of bagasse (Ramesh *et al.,* 1999). This may be the reason for the enhanced growth and survival in these treatment groups.

Earlier studies have also demonstrated clearly that higher TPC on substrate in substrate based treatments, such as sugarcane bagasse (Ramesh *et al.,* 1999; Dharmaraj et. al., 2002; Gangadhar and Keshavanath, 2012), paddy straw (Ramesh *et al.,* 1999), bamboo stick (Azim *et al.,* 2001a; Saikia and Das, 2010). Nutrient enrichment of water is known to increase both the thickness and cellular density of bacterial biofilm. The higher bacterial density in water from bagasse based tanks is due to the organic matter contributed by manure as well as the nutrients present in bagasse (Dharmaraj et. al., 2002). Among substrates, sugarcane bagasse, paddy straw, wheat straw and bamboo stick, the surface area of the sugarcane bagasse is much higher favouring better bacterial colonization. The growth performance in these four substrates treatment appears to support this. The decrease in plate count

values in the present experiment clearly points out to effective grazing by cultured fishes.

Zooplankton production in substrate based treatments was significantly higher ($p<0.05$) than phytoplankton. On the other hand, in control, zooplankton production was lower than phytoplankton and however reverse was the case in phytoplankton production. The phytoplankton density was higher in control. The higher bacterial biomass in the enrich treatments would have suppressed phytoplankton population by competing for micronutrient moreover dark colour in enrich treatments would have hinder light penetration for phytoplankton growth (Ramesh *et al.,* 1999).The cumulative density of heterotrophic population in the substrate based treatments was significantly higher than control. The higher zooplankton production in treatments can be attributed to higher biofilm density on substrate, that could have serve food for zooplankton. Moreover, higher phytoplankton density in control was not reflected in production. Increased zooplankton density with enhanced biofilm has been demonstrated by Langis *et al.,* 1988; Umesh *et al.,* 1999; Rai *et al.,* 2008.

From the results of the present investigation, it could be concluded that under the field conditions of, a dose of sugarcane baggasse as substrate @ 2.4 t ha^{-1} could be considered better for the accumulation of periphytic and heterotropic population in water thereby the fish can effectively utilize biofilm which interns reduces the need for artificial feed. The use of other substrate however, could not be ignored as round the year availability of baggasse, paddy straw or wheat straw is limited due to mechanization and other reasons in Bihar. Biofilm is also involved in the maintenance of water quality by reducing ammonium and nitrite which is essential for better growth and survival of fish. Use of such agro-based substrates in ponds can really improve economic viability of aquaculture.

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