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Growth performance of *Litopenaeus vannamei* in the presence and absence of probiotics with Bioflocs of different carbon sources

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Abstract

In the present investigation an attempt was made to evaluate the effects of Probiotics and using of different carbon sources for biofloc production on water quality, microbial population, production rates. The carbon sources selected in the present investigation are Sugarcane molasses, Rice flour, Wheat flour, for the production of bioflocs in the culture operation for *Litopenaeus vannamei*. The results obtained in the present study by using different carbon sources for the biofloc production with probiotics results in the better water quality due to a reduction in the levels of the various forms of nitrogenous wastes with in the system when this carbon sources are applied to the culture system. Due to the addition of carbon sources, to the culture system, Ammonia, Nitrate and Nitrite contents were found to be fluctuated and differences in the fluctuations of the nitrogenous wastes following the addition of these selected carbon sources related to the type of flour was explored. The results obtained in the present study clearly demonstrates that, the identification of best carbon source that could help mitigate the occurrence of high levels of nitrogenous wastes in BFT system for culture operation. All the carbon sources selected in the present study, were seems to be highly efficient in inducing highest and best growth potentials in *L. vannamei*.

Keywords: *L. vannamei* molasses, rice flour, wheat flour biofloc, probiotic

1. Introduction

Aquaculture is considered as one of the major food production sectors practiced worldwide due to its increased availability of proteins for human consumption. Aquaculture production from 3.9% of total production by weight in 1970 to 27.3% in 2000 and is growing more rapidly than all other animal food producing sectors [1]. Shrimp and prawn farming is one of the most important practices in all over the world. In recent times several major and innovative developments in shrimp culture operation including the introduction of probiotics of several types and bioflocs of different Carbon sources to improve the several and growth rates, simultaneously enhancing immune capacity and contributes to significant increase in the production rates. Both Probiotics and Bioflocs are known to improve shrimp health through controlling of infectious pathogens, are known to act as natural immune enhancers which provoke the disease resistance system in shrimp [2, 3, 4]. Maintenance of Ecological sustainability of shrimp farming activity is a major challenge, because of the inputs especially the feed and its ingredients were known to be accumulating in the culture environment and finally contributing towards the deterioration of water quality, results in the imposing of stress finally lead to disease outbreaks [5, 6]. In recent years, the introduction of usage of certain substances including Probiotics. Immuno-stimulants and adopting of newer technologies like biofloc technology paved way for the establishment of sustainability to a certain extent and reduced the rate of disease outbreaks to a large extent. Biofloc Technology is based on microbial manipulation in the aquaculture system in the form of Carbon: Nitrogen ratio feed has been shown promising results in aquaculture of candidate species [7, 8]. The biofloc technology was generated by supplementation of external carbon sources in the feed and contributes towards the increased productivity [9]. Different Carbon sources stimulate the growth of the indigenous microbiota in various ways and would greatly influence the water quality, insitu feed production and utilization of the flocs by the cultured organisms. Various products can provide a low-cost external carbon source for application in biofloc technology in

The culture of shrimp, but need extensive research before implementation on a larger scale Hence, this study evaluated the use of Molasses, Rice flour, Wheat Flour, as carbon sources for the production of bioflocs in terms of its effects on water quality, microbial population and yield during culture of shrimp. The effects of these selected products were compared against the performance of other substances, and can be used as an alternative source of carbon for biofloc production in shrimp culture.

2. Materials and Methods

The present set of experiments were conducted at shrimp culture units located in Ramayapatnam (Latitude 15^0 02^1 55^{11} N; Longitude 80^0 02^1 50^{11} E) Prakasam District of Andhra Pradesh, India. The Experiments were randomly designed which includes four feeding trail groups.

Group I: Control group without addition of Probiotics & Bioflocs
Group II: Control group with Probiotics added
Group III: Different type of bioflocs added
Group IV: Both Bioflocs and Probiotics added.

2.1. Probiotic Feed Preparation

Probiotic supplemented Feeds were prepared as described by Naresh [10]. Probiotic bacterial species *Bacillus licheniformis* and *Lactobacillus rhamnorus* were obtained and maintained in the nutrient broth and were harvested by centrifuging at 10,000 rpm for 10 minutes subsequently washed with phosphate buffer, finally re-suspended in phosphate buffer saline (pH 7.4). These re-suspended bacteria were mixed uniformly to the feed pellets by spraying method. The Probiotic blended feed prepared was dried at 40^0C and packed in air tightpolythene covers and stored in Refrigerator for further use. The Probiotic blended feed with *L. rhamnorus* and *B. licheniformis* (@ 10 billion cfu/kg) feed were prepared once in seven days.

Penaeid shrimp *Litopenaeus vannamei* (2.52 ± 0.12 g) of uniform size were collected from local aquaculture farms and were acclimatized in field station in large cement tanks (2 × 1.5 × 0.75 Mts). The cement tanks were provided with soil collected from aquaculture ponds in order to provide earthen pond environment. The cement tanks were filled with water drawn from storage tank. The hydro biological parameters including Salinity (10±1 ppt), Temperature (27±1^0C), pH (7.7±0.1) were maintained constantly throughout the experimentation. All the experimental tanks wereintermittently aerated with the help of Air compressors. Feeding was done with a commercial feed obtained from local market and the shrimp were fed *ad libitum* twice a day both in the morning and evening at 6.00 AM and 6.00 PM @ 30% of the total biomass. The experimental trails were conducted for a period of 80 days.

2.2. Preparation of Biofloc

Sugarcane Molasses, Rice flour, Wheat flour were selected as sources for carbon and were incubated for 2 days in warm water at 40^0C and the same was added to use culture medium in the ratio of 1:3 with water. To stimulate Nitrogen loading in an aquaculture system. NH_4Cl, KH_2PO_4 and Na_2HPO_4 were added to each tank [11]. The ratio between substance for carbon source and the feed to reach designed Carbon: Nitrogen (C: N) ratio calculated based on assuming 50% nitrogen from feed eaten by the shrimp excreting in to water environment [12, 13].

On the above basis the formula of the ratio in weight between, the carbon source and feed can be given as follows:

$$\frac{\Delta CH}{\Delta F} = \frac{CN \times \%P(F) \times \%N(P) - \%CF}{\%C_{CH}}$$

Where
ΔCH: Weight of Carbon Source
ΔF: Weight of the Feed
CN: C: N ratio need to be required
% P(F): Protein content in Feed
% N(P): Nitrogen content in Protein (15.5%)
% CF: Carbon content in the Feed (50%)
% C_{CH}: Carbon content in the Carbon Source

Carbon content was determined by adopting the method of Walkley and Black [14]. Total Ammonia Nitrogen (TAN) concentration and other water quality parameters were measured with the procedures according to APHA [15]. Growth parameters including, average body weights, average body growth rates, specific growth rates, feed conversion ratio, protein efficiency ratio, feed efficiency ratio and productivity rates were monitored and tabulated. All the above parameters were calculated by adopting the following Formulae

$$\text{Survival rate (\%)} = \frac{\text{Total number of live shrimp}}{\text{Total number of shrimp stocked}} \text{ X 100}$$

Weight Gain (g)
Weight of the shrimp (g) - Weight of the shrimp (g)
At the end of the Expt. at the start of the Expt.

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total amount of feed consumed (Kgs)}}{\text{Total biomass of shrimp (Kgs)}}$$

$$\text{Average daily growth rates (ADGR)} = \frac{\text{Weight of the shrimp (g) At the end of the Expt} - \text{Weight of the shrimp (g) at the start of the Expt.}}{\text{Total number of days of Experiment}}$$

Bioflocvolume (FV)
V_{Floc} / $V_{collection}$
Where
V_{Floc} : Biofloc Volume (ml)
$V_{collection}$: Collected Sample Volume (ml)

The data obtained was analyzed statistically through Microsoft excel. The difference in variants between treatments was determined according to One-Way ANOVA using SPSS.

3. Results and Discussion

The water quality parameters were monitored in all the Experimental groups i.e. Control, Control with Probiotics added, in another set three Carbon sources like Sugarcane Molasses, Rice flour, Wheat flour were selected for biofloc production but without addition of Probiotics, in another s*et al*ong with carbon sources, Probiotics were also added. The water quality parameters monitored throughout the experimental feeding trails were presented in Table.1. The water quality parameters like Salinity, temperature and pH were found to be almost constant without significant change throughout the experimental period of 80 days. The Salinity was maintained at 10 ppt. The temperature of water column also found to be around 26-28^{0}C. The Dissolved Oxygen (DO) content of the water samples ranges between 6.2-7.1. pH of the medium also falls in the range of 7.4-7.8. The DO reduction rate, Total suspended solids, Volatile suspended solids, and Alkalinity were found tobe significantly increased in all the experimental groups. Total Ammonia Nitrogen (TAN), Nitrite-N, Nitrate-N and Phosphate contents were found tobe significantly decreased in all the experimental trails. The electrical conductivity values were found to be almost static without any change. Biological Oxygen Demand (BOD), Biofloc Volume, Chlorophyll-a and Total Heterotrophic Bacteria (THB) were found to be significantly increased in all the experimental groups. The performance details of penaeid shrimp *Litopenaeus vannamei* were monitored for a period of 80 days and presented in Table.2. The percent survival rates were found to be in the range between 92-95%. At the start of the experiment specific pathogen free shrimp of average body weight of 2.52 ± 0.12 g were selected and stocked in all the Experimental tanks. For each Experimental group 1000 Nos were stocked and experimental trails were maintained. The final weights obtained were found to be in the range between 13.21 to 20.28 g and showed a significant in the all experimental trails. The FCR values obtained in the present study was significantly low and shows that all the feeds are ideal for growth of shrimp and was found to be around 2. The Daily growth rates (DGR) and Productivity values were found to be significantly elevated in all the experimental trails. In the present study the results obtained were found to be significantly elevated in the probiotics treated group compared to control group. In one set of experimentation only Sugarcane Molasses, Rice Flour, Wheat Flour were used for biofloc production but probiotics were not added, but in another s*et al*ong with carbon sources the probiotics were also added. The trends obtained were more prominent in the group added with probiotics compared to non addition of probiotics. In the present study, the biofloc development was observed through Total Suspended Solids (TSS) and floc volume (FV), and both were found to be significantly elevated in both biofloc added groups either with probiotic or without probiotic compared to control groups.

In many of the tropical countries, shrimp farming is a prominent and important activity in the coastal zones and also it may affect the environmental quality and interest of other uses by polluting coastal waters. The understanding of the ecological processes occurring in water bodies and in shrimp culture ponds can help us to understand and solve some of the disease issues faced by aquafarmers. Majority of farmers are depending on the site selection, water quality and good husbandry practices for *L. vannamei* culture, there by making the culture more successful and sustainable. The physico-chemical factors of the culture pond and their individual or synergistic effects play an important role on shrimp production and pond ecology. The maintenance of good water quality is essential for optimal growth and maximum survival of shrimp. The level of physical, chemical and biological parameters were known to control the quality of pond water[16]. In the present investigation, an attempt was made to monitor the effect of Probiotics and Bioflocs addition, both individually and also in combination in the culture operation of pacific shrimp *L. vannamei* to asses the water quality parameters and performance details in terms of productivity.

In the present investigation, all the water quality parameters recorded were found to be with in the suitable range for *L. vannamei* survival and growth for a period of 80 days. In the present study, Temperature, pH and Salinity contents were found tobe almost static and no significant changes were noticed throughout the experimental period. The maintenance of DO levels are dependent on several factors including, quantity of Phytoplankton, Zooplankton, Microbial population interms of production and utilization of oxygen by adopting several processes. Due to addition of bioflos, which stimulates the growth of heterotrophic bacteria which inturn require oxygen for their growth. Due to Photosynthesis the Oxygen loading will occur into the system. So, the DO levels maintained in the present study seams to be more ideal for *L. vannamei* culture operation. Ammonia and its management during culture operation also plays a vital role in the maintenance of water quality. By maintaining adequate quantities of DO levels and addition of sufficient quantities of Carbohydrate sources, induces good amounts of bioflocs, thereby significantly reducing the higher concentrations of TAN, Nitrite-N and Nitrate-N in the water column of culture tanks. Moreover, due to TAN level reduction to the maximum possible, helps to maintain better water quality under biofloc added culture system. Another important event i.e. very low levels of Nitrite-N were recorded in the present study also suggests that successful oxidation of ammonia to nitrate-N [17]. The Floc Volume and total Suspended Solids (TSS) were found tobe significantly increased consequent of adding both Probiotics and Bioflocs in to the culture feeding trails. The colour obtained in the water column was appeared tobe brown, which indicates the presence of flocculated aggregates, with heterotrophic bacteria colonies, microalgae, Protozoans etc. The main aim of adding Probiotics and Bioflocs to stimulate growth of Heterotrophic bacteria which will be functioned to immobilize inorganic ammonia in the water. FV and TSS are the true indicators for the formation of bioflocs in the culture operation [18, 19]. The Biofloc Volume (BV), BOD, DO reduction rates were shown a considerable increase by the application of both Probiotics and Bioflocs. Due to increase in Phytoplankton, Zooplankton, THB contents in the present study (unpublished data), contributes for the demand of Oxygen from the water column, which also reflects the demand for Oxygen in terms of DO reduction rate from the water DO levels.The Chlorophyl-a content also showed a significant increase during probiotic and biofloc added feeding trails, gains a strong support from the increased production of Phytoplankton during culture operation. By maintaining adequate balance in the C: N ratios in the culture operation due to the addition of carbon sources, facilitate the driving force for the development of THB populations in the present study. Moreover, relatively lower values of Nitrate contents in the present study in the biofloc fed groups probably due to the conversion of ammonia to Nitrate by HB, which will be subsequently utilized by microalgae for their growth. This clearly demonstrates that the nitrification process took place in the culture medium in the presence of bioflocs. Due to the addition of Probiotics and external carbon sources as biofloc induces, the improvement of shrimp performance, through the production of supplemental food in the form of Phytoplankton, Zooplankton and HB, made available in the culture operation. The Presence of higher bacterial population in the biofloc produced system as a result of the addition carbon sources should have resulted in the increased volume of the flocs. Higher biofloc production triggered the increased levels of TSS and also subsequently rise in respiration, which led to higher BOD levels. It is interesting to find that whether a correlation exists between bacterial population and the levels of nitrogenous wastes in the rearing water as this relationship could provide insight on the possible role of the bacteria in the dynamics of nitrogenous wastes in the culturing water.

Growth Performance parameters like Average Body Weights (ABW), Daily Growth Rates (DGR), Feed Conversion Ratio (FCR), and productivity rates were recorded for *L. vannamei* under Probiotic and Biofloc added feeding trails and are found tobe significantly increased during feeding trails. Due to the addition of probiotics introduced into the culture environment, they tend to compete and control the pathogenic bacteria as well as will promote growth of candidate sps and also known to improve water quality and condition of pond bottom, substantially stimulating the immune system of the shrimp. Similar kind of observations i.e. due to the addition of carbon sources in to the culture system lead to the higher protein utilization and substantially higher productivity rates reported in shrimp including *L. vannamei* [20, 21]. So both probiotics and bioflocs addition into the culture environment facilitates the increase in the growth potentials, which reflected through higher productivity rates in the present study. The present study may be concluded that both probiotics and bioflocs facilitates the maintenance of good water quality and is ideal for maximum growth potentials; probiotics are helpful in making the culture environment clean and hygienic for promotion of higher growth rates, whereas the bioflocs, due to addition of Carbohydrate sources facilitates for production of supplemental food materials, there by Promotes growth. Several authors also reported that addition and maintenance of adequate quantities of C: N ratios by way of carbohydrate inputs into the culture environment will pave way for induction of growth potentials by using different carbohydrates sources like Molasses, Sucrose, Wheat bran, Maize, Starch, Dextrose, Rice bran in the culture operation produces higher rates of yield.

The results obtained in the present study by using different carbon sources for the biofloc production results in the better water quality due to a reduction in the levels of the various forms of nitrogenous wastes with in the system when this carbon sources are applied to the culture system. Due to the addition of carbon sources, to the culture system, Ammonia, nitrate and nitrite contents were found to be significantly decreased. How these differences in the fluctuations of the nitrogenous wastes following the addition of these selected carbon sources related to the type of flour was envisaged. The results obtained in the present study clearly demonstrates that, the identification of best carbon source that could help mitigate the occurrence of high levels of nitrogenous wastes in BFT system for culture operation. All the sources of carbon sources added for the production of bioflocs seemed tobe beneficial inlowering nitrogenous wastes during culture operation.

Table 1: Water quality parameters during *L vannamei* culture operation under different feeding trails

			Type of Biofloc without Probiotics			**Type of Biofloc with Probiotics**		
Parameter	C	C+P	M	RF	WF	M	RF	WF
Salinity (ppt)	10.23	10.34	10.43	10.31	10.24	10.25	10.13	10.39
	± 0.88	± 0.85	± 0.79	± 0.84	± 0.84	± 0.82	± 0.78	± 0.74
Temperature (^{o}C)	27	28	28	28	27	28	28	26
	± 1	± 1	± 1	± 1	± 1	± 1	± 1	± 1
pH	7.83	7.74	7.78	7.75	7.73	7.75	7.49	7.58
	± 0.24	± 0.22	± 0.21	± 0.22	± 0.23	± 0.24	± 0.25	± 0.27
Dissolved Oxygen (DO) (mg/lit)	6.24	6.35	6.49	6.78	7.03	7.12	7.13	6.84
	± 0.21	±0.22	±0.23	± 0.31	± 0.28	± 0.24	± 0.22	± 0.22
DO Reduction Rate (mg/lit/hr)	0.44	0.76^{a}	1.32^{a}	1.21^{a}	1.11^{a}	1.44^{a}	1.27^{a}	1.19^{a}
	± 0.05	± 0.05	± 0.11	± 0.14	± 0.12	± 0.12	± 0.11	± 0.12
	PDC	+73	+200	+175	+152	+227	+189	+170
Total Suspended Solids (TSS) (mg/lit)	263.45	374.34^{a}	578.43^{a}	549.74^{a}	539.14^{a}	604.31^{a}	564.18^{a}	543.14^{a}
	± 35.14	± 29.73	± 37.74	± 32.38	± 31.72	± 38.39	± 32.72	± 30.76
	PDC	+42	+120	+109	+105	+130	+115	+106
Volatile Suspended Solids (VSS) (mg/lit)	312.42	504.18^{a}	695.19^{a}	650.18^{a}	639.74^{a}	714.18^{a}	614.18^{a}	618.74^{a}
	± 35.76	± 45.12	± 35.78	± 38.94	± 34.18	± 38.25	± 38.74	± 39.79
	PDC	+61	+123	+108	+105	+129	+97	+98
Alkalinity (mg Ca CO_3/lit)	163.35	243.43^{a}	528.44^{a}	490.11^{a}	473.15^{a}	513.42^{a}	492.18^{a}	450.73^{a}
	± 28.74	± 29.94	± 28.39	± 27.13	± 24.42	± 27.34	± 27.74	± 24.79
	PDC	+49	+224	+201	+190	+215	+202	+176
Total Ammonia N TAN (mg/lit)	0.83	0.78^{c}	0.54^{a}	0.68^{a}	0.72^{b}	0.47^{a}	0.61^{a}	0.63^{a}
	± 0.08	± 0.07	± 0.07	± 0.04	± 0.05	± 0.05	± 0.04	± 0.04
	PDC	-6	-35	-18	-13	-43	-27	-24
Nitrite-N (mg/lit)	1.37	1.11^{a}	0.58^{a}	0.73^{a}	0.81^{a}	0.52^{a}	0.68^{a}	0.72^{a}
	± 0.12	± 0.12	± 0.07	± 0.05	± 0.06	± 0.05	± 0.05	± 0.06
	PDC	-19	-58	-47	-41	-62	-50	-47
Nitrate N (mg/lit)	4.22	3.24^{a}	2.12^{a}	3.14^{a}	3.04^{a}	1.87^{a}	2.74^{a}	2.92^{a}
	± 0.14	± 0.11	± 0.11	± 0.11	± 0.11	± 0.11	± 0.11	± 0.11
	PDC	-23	-50	-26	-28	-56	-35	-31
Phosphate P (mg/lit)	5.34	4.12^{a}	27.4^{a}	3.17^{a}	3.44^{a}	2.62^{a}	2.85^{a}	3.07^{a}
	± 0.13	± 0.11	± 0.11	± 0.12	± 0.13	± 0.11	± 0.11	± 0.12
	PDC	-23	-49	-41	-30	-51	-47	-43
Electrical Conductivity (mg/cm)	40.18	38.72^{c}	41.31^{c}	40.18^{c}	41.34^{c}	42.71^{c}	41.82^{c}	40.75^{c}
	± 2.75	± 2.14	± 2.72	± 2.19	± 2.28	± 2.44	± 2.04	± 2.18

	PDC	-3.61	+2.27	0	-2.29	+6.3	+4.08	+1.42
Biological Oxygen Demand (BOD) (mg/lit)	17.37 ± 1.39	38.48^a ± 2.45	89.75^a ± 4.28	72.74^a ± 3.78	66.33^a ± 3.79	94.78^a ± 4.25	76.74^a ± 3.82	70.44^a ± 4.72
	PDC	+122	+417	+319	+282	+446	+342	+306
Biofloc Volume (mg/lit)	12.34 ± 0.58	24.38^a ± 0.72	43.42^a ± 1.78	31.39^a ± 1.85	35.44^a ± 1.41	46.73^a ± 1.99	33.49^a ± 1.34	37.82^a ± 1.49
	PDC	+98	+252	+154	+187	+279	+171	+206
Chlorophyll A (mg/lit)	0.33 ± 0.06	0.42^a ± 0.08	0.82^a ± 0.09	0.61^a ± 0.07	0.72^a ± 0.08	0.88^a ± 0.09	0.63^a ± 0.07	0.75^a ± 0.07
	PDC	+27	+148	+85	+118	+167	+91	+127
Total Heterotrophic Bacteria (THB) (cfu/ml) X 10^6	20.13 ± 1.12	60.19^a ± 2.72	149.75^a ± 10.12	124.11^a ± 10.18	134.18^a ± 10.72	170.18^a ± 11.12	132.15^a ± 10.72	145.74^a ± 11.25
	PDC	+199	+644	+517	+567	+745	+556	+624

C: Control; C+P: Control + Probiotic; M: Molasses; RF: Rice Flour; WF: Wheat Flour
All values are Mean ± SD of six individual observations.
PDC: Percent Deviation over Control
All values are Statistically Significant at $^aP< 0.05$; $^bP< 0.01$; ^{c}NS (Not Significant)

Table 2: Performance of *L vannamei* under different feeding trails

			Type of Biofloc without Probiotics			**Type of Biofloc with Probiotics**		
Parameters	C	C+P	M	RF	WF	M	RF	WF
Shrimp stocked	1000	1000	1000	1000	1000	1000	1000	1000
Percent survival	92	94	95	93	94	95	94	94
Final weight (g)	13.21 ± 0.32	17.28^a ± 0.32	19.77^a ± 0.31	16.05^a ± 0.22	17.18^a ± 0.33	20.28^a ± 0.42	17.89^a ± 0.32	18.77^a ± 0.34
	PDC	+31	+50	+22	+30	+54	+35	+42
Weight gain (g)	10.69 ± 0.28	14.76^a ±0.32	17.25^a ±0.34	13.53^a ± 0.29	14.66^a ± 0.34	17.76^a ± 0.34	15.37^a ± 0.32	16.25^a ± 0.33
	PDC	+38	+61	+27	+37	+66	+44	+52
Feed Conversion Ratio	2.34	2.21^c	1.94^b	2.11^b	2.14^b	1.86^a	2.07^b	2.03^b
	PDC	-5.5	-17	-9.8	-8.5	-20.5	-11.54	-13.24
Daily Growth Rate	0.13	0.19^a	0.22^a	0.17^a	0.18^a	0.22^a	0.19^a	0.20^a
	PDC	+46	+70	+31	+38	+70	+46	+54
Productivity	12.15	16.24^a	18.78^a	14.93^a	16.15^a	19.27^a	16.82^a	17.64^a
	PDC	+34	+55	+23	+33	+59	+38	+45

C: Control; C+P: Control + Probiotic; M: Molasses; RF: Rice Flour; WF: Wheat Flour
All values are Mean ± SD of six individual observations.
PDC: Percent Deviation over Control
All values are Statistically Significant at $^aP< 0.05$; $^bP< 0.01$; ^{c}NS (Not Significant)
Shrimp of Initial weight of 2.52 ± 0.12 g stocked.

4. References

1. Food and Agriculture Organization (FAO). The State of World Fisheries and Aquaculture opportunities and challenges 2016.
2. FAO/WHO. Evaluation of Health and Nutritional Properties of Probiotics in Food including powdered milk and Live Lactic Acid bacteria. FAO/WHO Report 2001. http:// www.fao.org/es/ESN/Probio/Probio.htm.
3. Irianto A, Austin B. Probiotics in aquaculture. J Fish. Dis 2002;25:633-642.
4. Krishnaprakash R, Sarvanan R, Murugesan P, Rajagopal R. Usefulness of Probiotics in the production of high quality shrimp *Penaeus monodon* seeds in hatcheries. World Journal of Zoology 2009;4(2):144-147.
5. Kautsky N, Ronnback P, Tendenglen M, Trocell M. Ecosystem perspectives on management of disease in shrimp pond farming. Aquaculture 2000;191:145-161.
6. Funge-smith SJ, Briggs MR. Nutrient budgets in intensive shrimp ponds: Implications for sustainability. Aquaculture 1998;164:117-133.
7. Avnimelech Y. Carbon/Nitrogen ratio as control element in Aquaculture system. Aquaculture 1999;176:227-235.
8. Crab R, Detoudt T, Bossier P, Verstraek W. Biofloc Technology in aquaculture: Beneficial effects and future challenges. Aquaculture 2012;356:351-356.
9. Kumar S, Anand PSS De D, Sundaray JK, Raja RA, Biswas G, Ponnaiah AG *et al*. Effects of carbohydrate supplementation on water quality, microbial dynamics and growth performance of giant tiger prawn *Penaeus monodon*. Aquaculture International 2014;22:901-912.
10. Naresh S, Suneetha Y, Srinivasulu Reddy M. Effect of Lactobacillus rhamnorus and Bacillus subtilis supplemented probiotic diets on the growth patterns and Antioxidant enzyme activities in *Penaeus monodon* and *Penaeus indicus*. Int. J Res. App. Nat. Sciences 2014;14:76-80.
11. Ekasari J, Crab R, Verstrate W. Primary nutritional content of bioflocs cultured with different organic carbon sources and salinity Hayati J Biosci 2010;17:125-130.
12. Avnimelech Y. Feeding with microbial flocs by Tilapia in minimal discharge bioflocs Technology ponds. Aquaculture 2007; 264:140-147.
13. Naik MK, Srinivasulu Reddy M. Effect of biofloc system on growth performance in shrimp *Litopenaeus vannamei* under different C:N ratios with sugarcane molasses. International Journal of Scientific & Engineering Research 2020;11(5):243-262.
14. Walkey A, Black IA. An examination of Degtrareff method for determining soil organic matter and a proposed modification of the chromic acid titration

method. Soil. Sci 1934;37:29-37.
15. APHA. Standard methods for the examination of water and waste water (21st Edition). American public Health Association. USA. 2005.
16. Boyd CE. Water quality for pond Aquaculture Research and Development Series No.43. International and center for Aquaculture and Aquatic Environment. Alabama Agricultural & Experiment Station. Auburn University. USA 1998.
17. Cohen JM, Samocha TM, fox JM, Gandy RL, Lawrence AL. characterisation of water quality factors during intensive raceway production of juvenile *Litopenaeus vannamei* using limited discharge and biosecure management tools. Aquacultural Engineering 2005;32:425-442.
18. Ray AJ, Lewis BL, Browdy CL, Leffler JW. Suspended solids removal to improve shrimp *Litopenaeus vannamei* production and an evaluation of a plant based feed in minimal exchange; Super-intensive culture systems. Aquaculture 2010;299:89-98.
19. Vinatea L, Galvez AO, Browdy CL, Stokes A, Venero J, Haveman J *et al*. Photosynthesis, water respiration and growth performance of *Litopenaeus vannamei* in a super intensive race way culture with zero water exchange: Interaction of water quality variables. Aquaculture. Eng 2010;42:17-24.
20. Gao-L, Shan HW, Zhang TW, Bao WY, Ma S. Effects of carbohydrate addition on *Litopenaeus vannamei* intensive culture in a zero water exchange system. Aquaculture 2012;342:89-96.
21. Naik MK, Srinivasulu Reddy M. Performance of shrimp *Litopenaeus vannamei* with the addition of probiotics and bioflocs: A field study. International Journal of Fisheries & Aquatic Studies 2020;8(3):286-291.