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Effect of different biofloc system on water quality, biofloc composition and growth performance in *Litopenaeus vannamei* (Boone, 1931)

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Abstract

The experiment was conducted with three biofloc treatments and one control in triplicate in 500 L capacity indoor tanks. Biofloc tanks, filled with 350 L of water, were fed with sugarcane molasses (BFT_S), tapioca flour (BFT_T), wheat flour (BFT_W) and clean water as control without biofloc and allowed to stand for 30 days. The postlarvae of *Litopenaeus vannamei* (Boone, 1931) with an Average body weight of 0.15 ± 0.02 g were stocked at the rate of 130 PL m^{-2} and cultured for a period of 60 days fed with pelleted feed at the rate of 1.5% of biomass. The total suspended solids (TSS) level was maintained at around 500 mg L^{-1} in BFT tanks. The addition of carbohydrate significantly reduced the total ammonia-N (TAN), nitrite-N and nitrate-N in water and it significantly increased the total heterotrophic bacteria (THB) population in the biofloc treatments. There was a significant difference in the final average body weight (8.49 ± 0.09 g) in the wheat flour treatment (BFT_W) than those treatment and control group of the shrimp. Survival of the shrimps was not affected by the treatments and ranged between 82.02% and 90.3%. The proximate and chemical composition of biofloc and proximate composition of the shrimp was significantly different between the biofloc treatments and control. Tintinids, ciliates, copepods, cyanobacteria and nematodes were identified in all the biofloc treatments, nematodes being the most dominant group of organisms in the biofloc. It could be concluded that the use of wheat flour (BFT_W) effectively enhanced the biofloc production and contributed towards better water quality which resulted in higher production of shrimp.

Keywords: biofloc, *Litopenaeus vannamei*, total heterotrophic bacteria, water quality, carbohydrate

Introduction

Crustaceans have high demand and economic value both in domestic and international markets. According to the Food and Agriculture Organization (2003, 2008), shrimp is the most important and profitable commodity among all seafood trades. Shrimp culture has a significant exploration among farmers, contributing to world's aquaculture production, but concerns about the environmental impacts due to this activity have also increased (Tovar, Moreno, Manuel-Vez & Garcia-Vargas 2000; Jory, Cabrera, Dugger, Fegan, Lee, Lawrence, Jackson, Mcintosh & Castaneda 2001). Aquaculture systems mainly depend on the exploitation of autotrophic and heterotrophic microbial food webs. Heterotrophic food web consists of decomposition of organic matter by microorganisms, leading to the formation of assimilable detritus and inorganic nutrients. The detritus and associated microbes are directly consumed by the cultured animals or by other small animals on which the cultured species feed (Moriarty 1997). The heterotrophic food web consistently appears as a major contributor to the total production of the target animals (Schroeder 1987). The shrimps assimilate only 15–30% of the

nitrogen added in the feed in a pond environment, the remaining quantity is lost to the system as ammonia and organic-N in the form of faeces and feed residue. The organic-N in faeces and uneaten feed undergoes decomposition resulting in ammonia production. Therefore, a high protein level in shrimp feed contribute to high concentration of ammonia in the water column which is detrimental to the cultured animals, and needs to be minimized. To rectify the above mentioned constraints, the biofloc technology (BFT) systems were developed to minimize effluent discharge, protect the surrounding water bodies and improve farm bio-security (Burford, Thompson, McIntosh, Bauman & Pearson 2003; Avnimelech 2007).

Farming of *L. vannamei* is generally conducted extensively in grow-out ponds, and has been developed in indoor high-intensive farming system to meet the growing world demand (Lin, Shan, Liu & Huang 2001; Zhou 2001). With rapid expansion and intensification, however, there is also a growing concern about the ecological sustainability of shrimp farming (Naylor, Goldburg, Primavera, Kautsk, Beveridge, Clay, Folke, Lubchencoi, Mooney & Troell 2000). The cultured shrimps retain only 20–30% of feed nutrient; therefore, 70–80% of high dietary protein is excreted and accumulated in water, which leads to deterioration of water quality (Avnimelech & Ritvo 2003). Moreover, deteriorated water quality has resulted in disease outbreaks and heavy financial losses (Samocha, Lawrence, Collins, Castille, Bray, Davies, Lee & Wood 2004). Such environmental issues have created a large demand for productive, efficient and sustainable shrimp farming systems that have low impact on the environment and are more likely to be disease free (Horowitz & Horowitz 2001). Hari, Kurup, Varghese, Schrama and Verdegem (2004, 2006) reported addition of tapioca flour into *Penaeus monodon* culture system can significantly reduce the TAN and NO_2-N concentrations. Samocha, Patnaik, Speed, Ali, Burger, Almeida, Ayub, Harisanto, Horowitz and Brock (2007) reported that the molasses addition does not result in a significant effect on *L. vannamei* culture system. Asaduzzaman, Wahab, Verdegem, Huque, Salam and Azim (2008) reported that the addition of tapioca flour into *Macrobrachium rosenbergii* culture system can result in a significant decrease in the TAN and NO_2-N concentrations. Although, the possibilities of utilizing tapioca as a carbohydrate source in animal feed formulations and induce biofloc for shrimp production has been established, the feed manufacturers and shrimp farmers have not incorporated cassava because of its high cost as compared with other cheap carbohydrate sources. Wheat is one of the cheap carbohydrate sources in India.

Therefore, the study aims to develop microbial biofloc for culture of *L. vannamei* by using carbohydrate materials (sugarcane molasses, tapioca flour and wheat flour) as a carbon source to boost the production by improving the conversion of nutrients into harvestable products while maintaining good water quality. The objectives of the study were: (1) to assess the effect of biofloc on the water quality, (2) to assess the effect of biofloc on growth of *L. vannamei* and (3) to investigate the effects of carbon source/C:N ratio of feed on proximate composition of biofloc.

Materials and methods

Experimental design

The experiment was conducted at Wet Laboratory of the Central Institute of Fisheries Education (CIFE), Versova, Mumbai, India. Uniform-size Fiberglass Reinforced Plastic (FRP) circular tanks of 500 L capacity with 0.98 m diameter, filled with 350 L of water were used for the experiment. Three treatments with a control in triplicates were set up using completely randomized design (CRD). Three treatments were biofloc tank fertilized with sugarcane molasses (BFT_S), tapioca flour (BFT_T) and wheat flour (BFT_W). All the treatments and control were fed with commercially available feed [Charoen Pokph (CP) and (India) Pvt. Ltd, Chennai, India] having 34.5% crude protein twice a day. The aeration (7 mg L^{-1}) was provided in all the experimental tanks from a centralized aeration unit. The aeration pipe in each tank was provided with an air stone and a regulator to control the air pressure in all the tanks.

The seawater was pumped from the Aksa Beach (Mumbai, India) during high tide time. The collected seawater was stored in the 5000 L reservoir tank. The seawater was allowed to settle down for a week and was diluted with tap water to achieve a salinity of 25 g L^{-1}. The 350 L of diluted seawater (25 g L^{-1}) was filled in each of the experimental tanks. The biofloc was produced using (25 g L^{-1}) in 500 L capacity tanks before stocking of the postlarvae. Sugarcane molasses, tapioca flour and wheat flour which contain 69–76 % nitrogen-free extracts

was used as carbohydrate source. The sugarcane molasses were prepared by fermenting boiled sugarcane juice with yeast (*Saccharomyces cerevisiae*) for 2 days. After 2 days, it was used as an input for biofloc production. Twenty gram of carbohydrate was added per gram of TAN released. The amount of TAN released was estimated assuming that added carbohydrate contains 50% carbon and that 50% of the dietary protein input was converted to ammonia. In consequence, 0.53 kg each carbon was applied for each kg of the 34.5% dietary protein feed administrated. The photoperiod was maintained at 12-h dark and 12-h light for the whole experimental period.

Stocking of shrimp seed and tank management

The specific pathogen free (SPF) *L. vannamei* (PL9) seeds were procured from Madha Hatchery, Chennai, India. The postlarvae were acclimatized in 500 L tanks for 2 weeks before being stocked in the experimental tanks. The juveniles (0.15 ± 0.02 g) were stocked when bioflocs measured as total suspended solids (TSS) and floc volume was higher than 100 mg L^{-1} and between 5 and 50 mL respectively. The shrimps were stocked in 30 day old biofloc treatments and control tanks at the rate of 130 PL m^{-2}. Tanks were aerated with an air pump to maintain dissolved oxygen content at saturation level. CP manufactured pelleted feeds (1.8–3.0 mm) were used for feeding throughout the experiment. Biochemical composition of the experimental diet and the carbon sources are given in Table 1. Feeding rates were based on observation of feeding behaviour of shrimp in the biofloc treatments during first few days and fixed at 1.5% of the total stocked biomass daily, and adjusted fortnightly after weighing shrimp sample. The same amount of feed was fed in all the treatment and control tanks. Daily feed rations were split into two equal quantities and fed at 08:00 and 17:00 hours in all the tanks. The shrimps were cultured for 60 days. When the pH of water dropped below 7.0, $NaHCO_3$ was added to raise the pH to 7.5. Addition of freshwater to compensate evaporation loss and removal of floc was carried out on a weekly basis.

Table 1 Biochemical composition of the experimental feed and carbon sources

		Carbon source		
Constituent (%)	Feed	Sugarcane molasses	Tapioca flour	Wheat flour
Crude Protein	34.50	6.30	11.46	14.72
Ether Extract	13.27	4.01	3.19	3.25
Crude Fibre	4.27	3.75	6.16	5.15
Total Ash	10.80	16.20	5.10	2.42
Nitrogen-free Extract	37.16	69.74	74.04	74.46
Moisture	5.35	22.67	8.26	6.64
Carbon Nitrogen ratio	7.68	17.43	10.11	9.40

Values are in dry weight basis.

Water quality parameters

Water samples were collected at fortnightly intervals from the experimental tanks during morning hours between 8:00 and 9:00 hours for a period of 90 days. Temperature (H-9283; Shenzhen Vicimeter Technology Co. Ltd., Shenzhen, Guangdong, China), pH (Eutech Instruments, klang, Selangor D.E., Malaysia) and electrical conductivity (EC) (Eutech Instruments) were measured in the experimental unit itself. Dissolved oxygen and biochemical oxygen demand (BOD_5) measured by following APHA (2005) guidelines for sample collection and preservation. Total alkalinity and total hardness of water were measured volumetrically (APHA 2005). Water samples were collected from each tank and filtered under vacuum pressure through pre-dried and pre-weighed GF/C filter paper. The filtered water was used for nutrient analysis and the filter paper for the estimation of total suspended solid (APHA 2005). Total ammonia-nitrogen (TAN), nitrite (NO_2-N) and nitrate (NO_3-N) were analysed spectrophotometrically following APHA (2005). The weight difference of the dried sample before and after ignition was taken for calculation of volatile suspended solids (VSS). Floc volume (FV) was measured by using the Imhoff Cone (Merck Specialties, Mumbai, India). Floc-morphostructure was observed using biological microscope and photographed with camera (JVC Color Video Camera, TK-C1481BEG, Victor Company, Yokohama, Japan) attached to microscope (H600L; Hund GMBH, Wetzlar, Germany). Chlorophyll *a* was measured following trichromatic method (APHA 2005) using spectrophotometer (Thermospectronic, UV 1, Cambridge, UK).

Total heterotrophic bacteria (THB) were enumerated on R2A agar medium{Enzymatic Digest of

Casein (0.25 g), Protease Peptone (0.25 g), Acid Hydrolysate of Casein (0.5 g), Yeast Extract (0.5 g), Dextrose (0.5 g), Soluble Starch (0.5 g), Dipotassium Phosphate (0.3 g), Magnesium Sulphate Heptahydrate (0.05 g), Sodium Pyruvate (0.3 g), Agar (15 g) and Final pH: 7.2 ± 0.2 at 25°C from all the treatment tanks following spread plate method. The inoculated plates were incubated at 37°C for 48 h and the THB is expressed as colony forming unit per millilitre (CFU mL^{-1}).

Proximate composition of biofloc and shrimps

Biofloc samples were collected for biochemical analysis at the end of the experiment from each tank using 100-μm mesh and were dried in an oven at 60°C. The dried samples were ground and processed for biochemical analysis following AOAC (2005). For moisture contents the known quantity of samples were dried in an oven at 105°C until constant weight obtained. The difference in the weight before and after drying of sample was taken and expressed in percentage. For ash contents, a known quantity of dry sample was burnt in a muffle furnace at 550°C for 4 h and the ash was cooled and weighed. The crude protein content was determined by the Kjeldahl method (KEL Plus – Classic DX VA, Pelican Equipments, Chennai, Tamil Nadu, India), Crude lipid by automatic fat extraction system (SOCS PLUS-SCS 08 AS, Pelican Equipments, Chennai, Tamil Nadu, India), crude fibre by automatic fibre analysis system (FIBROTRON-FRB 8, Tulin Equipments, Chennai, Tamil Nadu, India) and Nitrogen-free extract was estimated by the difference (Tacon 1990). The gross energy content of biofloc sample was determined by using bomb calorimeter (Parr Calorimetric Thermometer-6772, Parr Instruments, Moline, IL, USA) and C:N ratios were determined using CHNS analyser (ElementarAnalysysteme GmbH, Hanau, Germany). Similarly, the biochemical composition of all the experimental shrimps was estimated after harvesting.

Growth parameters of shrimp

The sampling was performed at fortnightly interval to assess the body weight of the shrimps. Shrimps were starved overnight before taking the weight. Twenty per cent of the test animals were randomly weighed for all the treatments. The weight was measured using an electronic balance. Shrimps were harvested at the end of the experiment after draining the tanks. Survival rate, average body weight (ABW), specific growth rate (SGR), protein efficiency ratio (PER) and total weight gain were calculated according to the formulae given below:

$$\text{Total weight gain (wet weight, g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{ABW (g)} = \text{total weight (g)}/\text{total Number of animals}$$

$$\text{SGR} = [(\ln \text{final weight} - \ln \text{initial weight}) \times 100]/\text{no. of days of experiment}$$

$$\text{PER} = \text{net weight gain (wet weight) (g)}/\text{protein consumed (g)}$$

Biochemical composition of biofloc

The biofloc samples were digested using supra pure concentrated acids (Merck Specialities Pvt. Ltd., Mumbai, Maharashtra, India), in a microwave-based digestion system (Multiwave 3000; Anton Parr, Suite, Houston, TX, USA). Three replicates of 0.250 g of biofloc samples were taken in the microwave digestion vessels, to which 5 mL of conc. HNO_3, 2 mL of conc. HCl and 1 mL of conc. HF were added. The vessels were capped and heated in the microwave unit at 1200 W to a temperature of 190°C for 30 min at a pressure of 25 bars. The digested samples were diluted to 50 mL and were used for the analysis of total phosphorus following ascorbic acid method APHA (2005). The digested biofloc samples were used for the analysis of sodium and potassium using flame photometer (Elico CL 378, Hyderabad, India) and analysed five elements (Ca, Mg, Zn, Cu and Fe) by atomic absorption spectrophotometer (AAnalyst 800; Perkin Elmer, Waltham, MA, USA) using flame atomization.

Taxonomic composition of biofloc

The plankton samples were collected in triplicate and concentrated to 50 mL by filtering the water using bolting silk cloth (no. 25) from the treatment tanks. The collected plankton samples were preserved in 5% formalin for further analyses

(Pennak 1978). The counting of plankton was carried out using a Sedgwick-Rafter counting cell following APHA (2005). The averages of three samples were taken into consideration and the results are given in terms of cells per litre. The photographs of all the major planktons were taken using a camera (JVC Color Video Camera, TK-C1481BEG) attached to Hund microscope (H600L; HundWetzlar). The morphometric features of the organisms were measured using the Bio-wizard software. Organisms were identified using keys and monographs given by Graham, Graham and Wilcox (2008).

Statistical analysis

All statistical analyses were performed using SAS v9.3 for Windows (Cary, NC, USA). Water quality parameters were compared by two-way repeated measures ANOVA with treatment as main factor and sampling date as repeated measures factor. One-way analysis of variance (ANOVA) was performed to examine difference of growth parameters, biochemical composition of shrimp and nutritional quality of biofloc among the treatments and control. The analyses were run at 5% significance level.

Results

Water quality parameters

The biofloc development was observed in terms of FV and TSS. The FV and TSS gradually increased during the experiment. Bioflocs were observed as brown in colour, and were composed of suspended organic particles in the form of flocculated aggregates, which were colonized by a number of heterotrophic bacteria, microalgae and protozoa. All the water quality parameters in the three experimental tanks were found within suitable ranges for *L. vannamei* culture throughout the experimental period. There were no significant differences ($P > 0.05$) among the treatments in terms of water temperature, pH, alkalinity, electrical conductivity and total hardness (Table 2). All other parameters showed significant variation ($P < 0.05$) among the treatments. The treatment, BFT_S (5.99 mg L^{-1}) had significantly lower DO than that of control and other treatments. The TAN, nitrite-N and nitrate-N concentrations in control were significantly higher than those of biofloc treatments except TAN in BFT_S. The treatments BFT_T and BFT_W showed significantly higher TSS, VSS, BOD, chlorophyll and plankton density

Table 2 Water quality parameters of different experimental groups

		Treatment		
Parameter	**Control**	**BFT_S**	**BFT_T**	**BFT_W**
Temperature (°C)	23.29 ± 0.24	23.12 ± 0.20	23.08 ± 0.20	23.16 ± 0.23
Alkalinity (mg $CaCO_3$ L^{-1})	138.89 ± 8.11	153.52 ± 10.91	150.61 ± 9.39	138.68 ± 9.43
pH (no unit)	7.8 ± 0.0	7.8 ± 0.0	7.6 ± 0.0	7.7 ± 0.0
Hardness (mg $CaCO_3$ L^{-1})	4635.85 ± 84.16	4261.36 ± 200.10	4456.11^{a} ± 152.01	4453.97 ± 109.59
Dissolved oxygen (mg L^{-1})	6.57 ± 0.43^{ab}	5.99 ± 0.26^{b}	6.70 ± 0.36^{ab}	7.39 ± 0.44^{a}
Total ammonia-nitrogen (mg L^{-1})	0.78 ± 0.10^{a}	0.57 ± 0.10^{ab}	0.40 ± 0.08^{bc}	0.24 ± 0.05^{c}
Nitrite-N (mg L^{-1})	1.89 ± 0.14^{a}	0.93 ± 0.09^{b}	0.75^{bc} ± 0.11	0.61^{c} ± 0.05
Nitrate-N (mg L^{-1})	3.21 ± 0.14^{a}	2.42 ± 0.12^{bc}	2.56 ± 0.12^{b}	2.09 ± 0.12^{c}
Total suspended solids (mg L^{-1})	158.5 ± 29.66^{b}	285.08 ± 36.73^{b}	493.50 ± 69.31^{a}	484.94 ± 65.46^{a}
Volatile suspended solids (mg L^{-1})	282.00 ± 42.54^{b}	427.83 ± 51.93^{ab}	494.89 ± 56.15^{a}	526.22 ± 59.21^{a}
Electrical conductivity (mS cm^{-1})	33.39 ± 2.09^{a}	31.60 ± 2.47^{a}	46.73 ± 21.23^{a}	37.64 ± 1.82^{a}
Chlorophyll *a* (μg $L^{-1)}$	283.44 ± 39.41^{b}	439.14 ± 49.13^{a}	516.77 ± 64.30^{a}	553.67 ± 59.99^{a}
Biological oxygen demand (mg L^{-1})	15.98 ± 2.42^{c}	64.34 ± 15.16^{b}	77.54 ± 17.58^{ab}	108.44 ± 16.25^{a}
Floc volume (mg L^{-1})	15.50 ± 1.75^{c}	32.17 ± 2.88^{b}	33.64 ± 3.25^{b}	43.06 ± 3.60^{a}
Total beterotrophic bacteria $\times 10^6$ (CFU mL^{-1})	19.40 ± 139.57^{b}	93.50 ± 107.23^{ab}	131.88 ± 107.69^{a}	148.16 ± 118.00^{a}
Plankton $\times 10^6$ (cells L^{-1})	1.45 ± 0.08^{b}	1.93 ± 0.23^{ab}	2.23 ± 0.18^{a}	2.38 ± 0.21^{a}

BFT_S, sugarcane molasses; BFT_T, tapioca flour; BFT_W, wheat flour.

The values are means (±SE, $n = 18$) of three replications in six sampling date for the treatment and control. Mean values in the same row with different superscript differ significantly ($P < 0.05$).

than the control. The parameters, VSS, chlorophyll, THB and plankton density in BFT_S was on par with those of BFT_T and BFT_W. Among the treatments, BFT_W and BFT_T, floc volume in BFT_W was significantly higher than that of BFT_T.

Growth parameters

There was a significant difference ($P < 0.05$) in the ABW, growth rate, PER and SGR among the treatments and control. There were no significant differences in the survival rate. The ABW, growth rate, PER and SGR of shrimps in BFT_W treatment was significantly higher than those of other biofloc treatments and control. The total weight gain was also significantly ($P < 0.05$) higher in BFT_W than that of other treatments and control (Table 3).

Proximate and biochemical composition of biofloc

The parameters, crude protein, ether extract, crude fibre and GE content of biofloc in BFT_W were significantly higher than those of control. The moisture, ash and total carbon contents in control were significantly lower than those of BFT_W. The C:N ratio of the treatments BFT_T and BFT_W was on par and significantly higher than that of BFT_S and control (Table 4). There was a significant difference in the biochemical composition among the biofloc treatments and control. There was a significant difference in the nutrient composition among biofloc treatments and control. The concentration of Fe and Ca was significantly higher in BFT_T than that of other treatments, whereas, the concentration of Mg, Na and K was significantly higher in BFT_W than that of others (Table 5).

Biochemical composition of shrimp reared in biofloc-based system

There was a significant difference ($P < 0.05$) in the crude protein in the treatment BFT_S among the treatments. The treatment, BFT_W had significantly higher ether extract than that of BFT_S and control

Table 3 Growth parameters of shrimp in different biofloc treatments and the control (means ± SE)

		Treatment		
Parameters	**Control**	**BFT_S**	**BFT_T**	**BFT_W**
Average body weight (g)	5.97 ± 0.03^{d}	6.64 ± 0.13^{c}	7.25 ± 0.09^{b}	8.49 ± 0.09^{a}
Growth rate (g day^{-1})	0.06 ± 0.00^{d}	0.07 ± 0.001^{c}	0.08 ± 0.002^{b}	0.09 ± 0.001^{a}
Specific growth rate	4.10 ± 0.03^{d}	4.25 ± 0.04^{c}	4.43 ± 0.02^{b}	4.57 ± 0.03^{a}
Protein efficiency ratio	1.58 ± 0.01^{d}	2.16 ± 0.03^{c}	2.34 ± 0.03^{b}	2.59 ± 0.08^{a}
Survival (%)	86.90 ± 3.8^{a}	82.2 ± 3.4^{a}	85.6 ± 3.5^{a}	90.3 ± 0.96^{a}
Total weight gain (g)	623.00 ± 12.99^{c}	656.08 ± 27.14^{c}	744.63 ± 37.64^{b}	919.41 ± 14.76^{a}

BFT_S, sugarcane molasses; BFT_T, tapioca flour; BFT_W, wheat flour.
Mean values in the same row with different superscript differ significantly ($P < 0.05$).

Table 4 Proximate composition of bioflocs (mean ± SE) produced in shrimp feeding experiment with of bioflocs

		Treatment		
Parameter (%)	**Control**	**BFT_S**	**BFT_T**	**BFT_W**
Moisture	74.34 ± 5.43^{a}	63.51 ± 2.14^{ab}	63.74 ± 2.51^{ab}	56.51 ± 3.12^{b}
Ash	29.03 ± 0.93^{a}	22.53 ± 0.22^{c}	14.88 ± 0.46^{d}	25.04 ± 0.39^{b}
Crude protein	25.29 ± 0.75^{c}	45.98 ± 0.59^{b}	52.03 ± 1.57^{a}	53.65 ± 0.7^{a}
Ether extract	0.48 ± 0.09^{b}	0.57 ± 0.07^{b}	0.70 ± 0.03^{ab}	0.92 ± 0.003^{a}
Crude fibre	11.88 ± 1.10^{c}	12.92 ± 0.8^{bc}	15.25 ± 0.06^{ab}	16.65 ± 0.02^{a}
Carbon	45.20 ± 1.77^{a}	30.92 ± 0.45^{b}	32.39 ± 1.93^{b}	20.39 ± 0.45^{c}
Carbon nitrogen ratio	12.35 ± 0.06^{c}	7.2 ± 0.08^{b}	6.52 ± 0.16^{a}	6.011 ± 0.14^{a}
Gross energy*	19.42 ± 1.02^{c}	22.45 ± 0.56^{c}	25.4 ± 1.25^{b}	27.25 ± 0.86^{a}

BFT_S, sugarcane molasses; BFT_T, tapioca flour; BFT_W, wheat flour.
Mean values in the same row with different superscript differ significantly ($P < 0.05$).
*Values expressed in kJ g^{-1}.

Table 5 Biochemical composition (dry weight basis) of biofloc in different treatments and control (means ± SE, $n = 9$)

Parameter (mg kg^{-1})	Control	Treatment		
		BFT_S	BFT_T	BFT_W
Iron	4918.33 ± 69.17^{b}	4417.33 ± 45.04^{c}	11200 ± 179.50^{a}	4479 ± 204.86^{bc}
Zinc	161.5 ± 0.43^{b}	146.2 ± 0.40^{c}	200.77 ± 0.26^{a}	200.63 ± 0.58^{a}
Magnesium	3537 ± 7.77^{d}	7315 ± 61.582^{b}	6302.33 ± 10.14^{c}	12300 ± 124.23^{a}
Potassium	5773.87 ± 60.84^{c}	6162.7 ± 75.51^{b}	6057.21 ± 66.93^{b}	7982.96 ± 58.83^{a}
Phosphorous*	14 ± 2.00^{b}	23.8 ± 1.20^{ab}	27 ± 3.0^{a}	31.5 ± 3.50^{a}
Calcium*	18.9 ± 1.04^{c}	23.9 ± 3.78^{b}	37.1 ± 3.66^{a}	22.7 ± 2.070^{b}
Sodium*	15.45 ± 2.73^{b}	16.52 ± 1.91^{a}	15.71 ± 1.81^{b}	16.81 ± 1.82^{a}

BFT_S, sugarcane molasses; BFT_T, tapioca flour; BFT_W, wheat flour.
Mean values in the same row with different superscript differ significantly ($P < 0.05$).
*Values expressed in mg g^{-1}.

Table 6 Proximate composition of *L. vannamei* cultured in different biofloc treatments and control (% means ± SE, $n = 3$) at the end of the experiment

Parameter (%)	Control	Treatment		
		BFT_S	BFT_T	BFT_W
Moisture	76.82 ± 0.78	76.20 ± 0.14	75.91 ± 2.38	78.53 ± 2.0
Ash	2.24 ± 0.15	2.26 ± 0.56	2.21 ± 0.07	2.19 ± 0.11
Crude protein	20.12^{c} ± 0.18	20.07^{c} ± 0.73	21.62^{b} ± 0.06	20.96^{a} ± 2.0
Ether extract	1.39^{c} ± 0.18	1.31^{c} ± 0.41	1.28^{b} ± 0.58	1.25^{a} ± 0.29
Crude fibre	1.08^{b} ± 0.31	1.06^{b} ± 0.3	0.98^{a} ± 0.12	1.01^{a} ± 0.14
Carbon	44.55^{a} ± 0.3	44.3^{a} ± 1.25	36.78^{b} ± 0.6	31.15^{c} ± 2.16

BFT_S, sugarcane molasses; BFT_T, tapioca flour; BFT_W, wheat flour.
Values are expressed on wet weight basis. Mean values in the same row with different superscript differ significantly ($P < 0.05$).

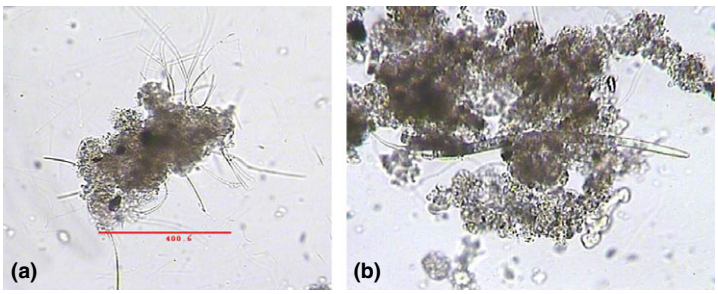

Figure 1 Morphology of floc under microscope (a) biofloc particle flocculation with filamentous algae and bacteria (b) biofloc flocculates with nematodes.

and was at par with BFT_T. The treatment BFT_W had significantly higher crude fibre than that of control and was at par with BFT_S and BFT_T control and was at par with BFT_T (Table 6).

Taxonomic composition of biofloc

In the bioflocs treatment group, floc was seen in anomalous flocculation with bacteria and zooplankton especially nematodes (Fig. 1). However, in the relative control group, there were a few of detritus and sloughs in the sediment of the Imhoff cone. The taxonomic compositions of different floc-associated planktonic organisms are given in Fig. 2. The group of organisms was identified as tintinids, ciliates, copepods, cyanobacteria and nematodes. Nematodes were the most dominant group in all the biofloc treatment tanks. The total number of organisms was significantly high in the BFT_W followed by BFT_T and control. These micro-organisms were found to be grazing on the flocs, when fresh samples were observed under micro-

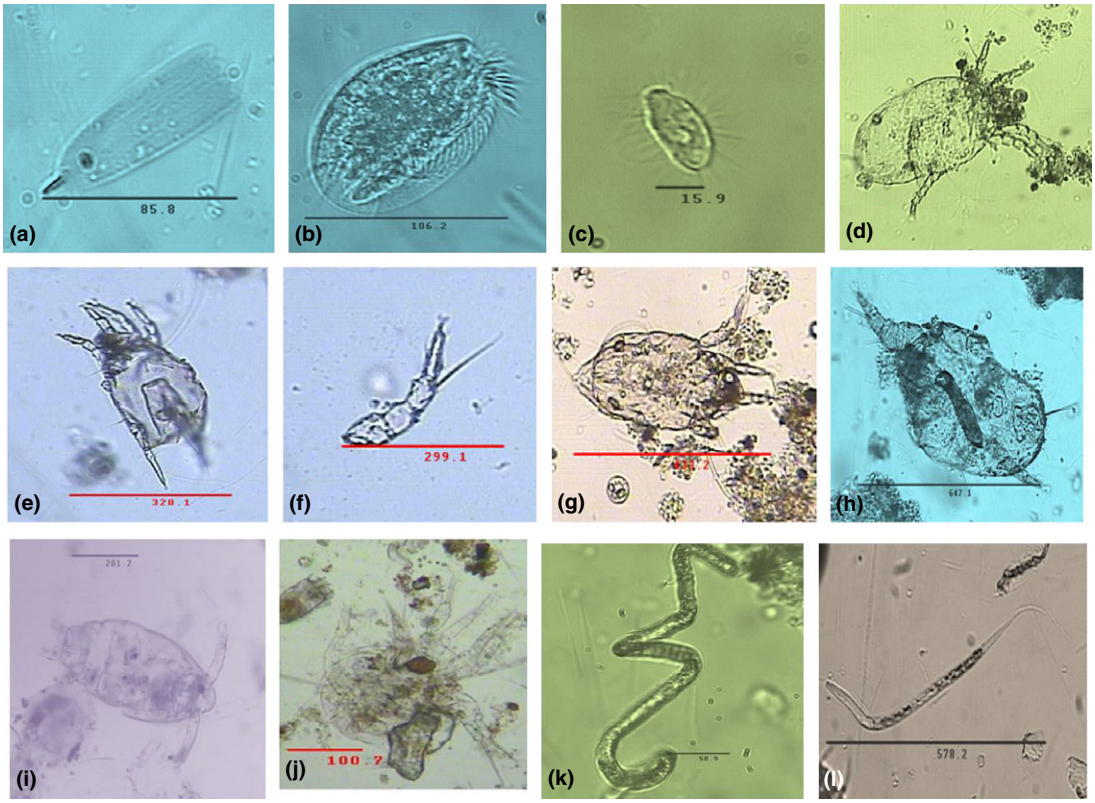

Figure 2 Common zooplankton and phytoplankton observed in the biofloc treatments (a) Tintinids, (b and c) Ciliates, (d–j) Copepods, (k) Spirulina, (l) Nematodes.

scope. In all the biofloc treatment tanks, mostly the zooplanktons were dominant and very limited number of phytoplankton could be visualized under the microscope. The only one phytoplankton (*Spirulina* sp.) was observed along with zooplankton.

Discussion

Water quality parameters

The lowest level of dissolved oxygen observed in this study (4.07 mg L^{-1}) is sufficient for good survival and growth of *L. vannamei* (McGraw, Teichert-Coddington, Rouse & Boyd 2001). The sufficient DO level in all the treatments during the experiment period is attributed to continuous aeration in biofloc tanks. The water hardness ($CaCO_3$) and alkalinity levels were above the recommended levels of 4200 and 130 mg L^{-1} respectively (Boyd, Thunjai & Boonyaratpalin 2002), and might therefore, have provided the physiological conditions allowing shedding and proper formation of the exoskeleton, promoting growth and survival of organisms (McGraw & Scarpa 2003).

Concentrations of TAN and nitrite-N, recorded during the experiment, were at optimum levels as recommended for juveniles of Pacific white shrimp (Lin & Chen 2003; Samocha *et al.* 2004). The low concentrations of nitrite-N, observed during the culture period, suggest oxidation of ammonia to nitrate (Cohen, Samocha, Fox, Gandy & Lawrence 2005). Studies evaluating water quality in zero-exchange systems report low concentrations of ammonia and nitrite (Burford, Thompson, McIntosh, Bauman & Pearson 2004; Wasielesky, Atwood, Stokes & Browdy 2006; Ray, Lewis, Browdy & Leffler 2010; Vinatea, Galvez, Browdy, Stokes, Venero, Haveman, Lewis, Lawson, Shuler and Leffler (2010)), resulting from the removal of these compounds by microbial community (Ebeling, Timmons & Bisogni 2006). The assimilation of nitrogen is also evidenced from the significantly higher crude protein content observed in the biofloc of the treatments. Avnimelech (2007), with the help of a series of

experiments, proved that addition of carbohydrate reduces the need of dietary protein concentration and also decrease the TAN level in the system. In this experiment, the wheat and tapioca flour significantly reduced the TAN level when compared with control.

The presence of high concentration of NO_3-N in BFT treatments indicates the occurrence of nitrification processes in the culture systems. While NO_2-N concentration in BFT treatments seems to be relatively stable, the opposite was observed in the control which could be explained by the high rate of nitrification processes in these treatments. For the first 60 days of the experiment, NO_3-N accumulation was observed in all the biofloc treatments and control which were followed by a sharp decline on 75th day. This decrease probably relates to NO_3-N uptake by microbes in the treatments in particular when there was limited availability of ammonia-nitrogen in the water (Hargreaves 1998). As most of the ammonia in the culture system is taken up by heterotrophic bacteria, the availability of NO_3-N in BFT system thus allows the phytoplankton to grow (Middelburg & Nieuwenhuize 2000). Nitrogenous constituents were at safe levels for ammonia (Kuhn, Lawrence, Boardman, Patnaik, Lori Marsh & Flick 2010), nitrite (Lin & Chen 2003) and nitrate (Wickins 1976) in the biofloc treatments and control which are suitable for shrimp culture.

The TSS, observed in this study, was within the recommended level of <500 mg L^{-1} for penaeid shrimps (Samocha *et al.* 2007). Several authors have indicated that a similar trend of concentration of TSS and VSS which is beneficial to the shrimp and to the system stability (Schryver, Crab, Defoirdt, Boon & Verstraete 2008; Baloia, Arantes, Schveitzer, Magnotti & Vinatea 2013). The EC value was recorded in the range of 31.60–46.73 mS cm^{-1} which is within permissible range for shrimp aquaculture (Araneda, Perez & Gasca-Leyva 2008). The decrease in the concentrations of chlorophyll *a* during the study is probably associated with the use of carbon sources to control ammonia. The use of carbon sources in biofloc systems promotes succession and dominance of bacteria over microalgae (Gonzalez-Felix, Ponce-Palafox, Valenzuela-Quinonez, Arredondo-Figueroa & Garcia-Ulloa 2007; Ju, Forster, Conquest, Dominy, Kuo & Horgen 2008). Chlorophyll *a* concentrations was (553.67 μg L^{-1}) lower than those reported (Burford *et al.* 2004; Decamp, Conquest, Cody, Forster & Tacon 2007) for pacific white shrimp production biofloc systems. The BOD, potentially high in the biofloc tanks due to increased level of microbial density, led to decreased oxygen availability for shrimp (Azim & Little 2008).

Floc volume levels increased gradually during the experimental period and the fluctuation over the time was consistent. The floc development in the first 30 days was slow due to the clean surfaces of the tank. The development of adhesiveness of the biofloc would develop slowly in the initial period. The recorded level of floc volume (50 mL) was sufficient for the growth of shrimp (Avnimelech 2012). If the floc volume is higher than 50 mL will leads to the depletion of DO during morning time. To avoid that, in this study the floc volume was maintained at the required level by adding water. The total heterotrophic bacterial count in the BFT_W was higher than that reported (Burford *et al.* 2003). Burford *et al.* (2004) reported that the plankton density in the zero water exchange system was 10^4 cells mL^{-1}. The plankton density was very low in all the treatments due to the limited light and the presence of carbon source. The results show application of carbon source enhances the microbial growth rather than microalgae.

Taxonomic composition of biofloc

Zooplankton was highly abundant throughout the study in all the biofloc treatment tanks. Nematodes, ciliates and copepods were present in low abundance at the beginning of the study and became less due to grazing by the shrimp. Nematodes and copepods were seen almost exclusively grazing on and within the particles and cyanobacteria were also principally located within biofloc. Shrimp might have consumed a portion of the zooplankton community. Previous authors have shown that these nutritious planktons are an important food item for shrimp (Moss, Divakaran & Kim 2001). In terms of potential nutrition, a decrease in the abundance of zooplankton might not be desirable for the shrimp. Phytoplankton abundance was limited in all the biofloc treatments due to the light limitation and carbon source application. The similar types of plankton community were observed by Ray *et al.* (2010).

Growth parameters

Growth rate and final individual shrimp weight were significantly higher in the treatment BFT_W

than those of others. Although no significant differences were observed on survival but there were significant differences in ABW, SGR and PER between the biofloc and control treatments, indicating that the appropriate quantity of carbohydrate addition was helpful in good growth and survival of *L. vannamei*. These might be due to the synergistic effects of the improved water quality, higher bacterial and zooplankton densities. Studies have indicated that carbohydrate addition can result in the production and accumulation of bioflocs (Avnimelech 2007; Emerenciano, Ballester, Cavalli & Wasielesky 2011; Gao, Shan, Zhang, Bao & Ma 2012), which could serve as an important food source for the zooplankton and thus could increase the growth of the shrimp. It has been demonstrated that zooplankton serve as a supplemental food source for *L. vannamei* (Chen & Chen 1992) and therefore could, increase the conversion efficiency of microbial protein into *L. vannamei* protein. Studies have also demonstrated that bioflocs serve as a good source of protein for shrimp and lower the demand for feed protein (Tacon, Cody, Conquest, Divakaran, Forster & Decamp 2002; Burford & Lorenzen 2004). Avnimelech, Verdegem, Kurup and Keshavanath (2008) also demonstrated that carbohydrate addition can lead to increase in protein utilization and supply of essential lipids and vitamins for the growth of shrimp.

Biochemical composition of shrimp

The bio chemical composition of shrimp depends on the proximate value of the biofloc. It seems that the higher crude protein was observed in all biofloc treatments ranges from 20% to 21.6% and low CP observed in control. The shrimps cultured in the biofloc system have more nutritional quality than the shrimps reared in the control system. It was confirmed that presence of high proximate composition in the biofloc leads to better growth of shrimp. The proximate composition of *L. vannamei* was similar to the observation made by Xu and Pan (2012).

Biochemical and nutrient composition of biofloc

The biochemical and nutrient composition of the biofloc, derived from biofloc tanks, fed with different carbon sources, were significantly different with regard to all the nutritional parameters between the treatments and control, indicating the importance of carbon source. In the study, the high protein content was observed in the biofloc produced in the treatment BFT_W and BFT_T followed by BFT_S (45.98%). Biofloc that contains more than 50% crude protein, 4% fibre, 7% ash and 22 kJ g^{-1} energy on dry matter basis can be considered as appropriate in fish nutrition especially for herbivorous/omnivorous fish and shrimp species (Webster & Lim 2002). It was confirmed that the biofloc produced in the study provided good nutrition to the shrimp. Proximate compositions of the bioflocs from the current experiment revealed that they contained appropriate level of crude protein and crude lipid for omnivorous *L. vannamei* (Cuzon, Lawrence, Gaxiola, Rosas & Guillaume 2004). The biofloc was able to provide sufficient nutrient content for growth of the shrimp. The nutrient composition (Fe, Zn, Ca, Mg, Na, K and total phosphorus), observed in the biofloc, were similar to the nutrient composition observed by Kuhn *et al.* (2010). The higher nutrient level of Ca and Fe was observed in the biofloc treatment BFT_T, whereas Mg, Na and K in BFT_W. The C/N ratio of the biofloc was inversely proportional to the protein content of the biofloc. If the protein content of the biofloc is high, the C/N ratio is low.

Conclusions

This study evaluated the biofloc effect of biofloc on growth of *L. vannamei*, water quality and biofloc composition. Among the biofloc system, BFT_W effectively reduced the total ammonia-nitrogen while maintaining good water quality for shrimp culture. The use of wheat flour (BFT_W) for the biofloc production could effectively enhance the biofloc production and contributed towards good water quality which resulted in higher production of shrimp. The wheat flour has easily digestible and available carbon for the growth of microorganism. Hence, the microorganisms easily assimilated and multiplied faster than other treatments. The higher nutritional value of the biofloc developed by wheat flour would have increased the growth of *L. vannamei* than other treatments. In generally, the biofloc system would increase growth rates of *L. vannamei* as the shrimps that feed on the biofloc would get the additional supplement nutrition from the assimilated nutritious planktons, bacteria and organic compounds. From

the results of this study, it is confirmed that biofloc provided sufficient nutrition leads to the growth of shrimp. This system can play a key role in developing a sustainable aquaculture via better water quality maintenance decrease in feed requirements and higher production to achieve more profit in shrimp farming.

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