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Research Article

Culture strategy for production of Indian white prawn, *Fenneropenaeus indicus* in semi-arid conditions using biofloc technology

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Abstract

An experiment was conducted to test efficiency of biofloc technique for production of Indian white prawn, *Fenneropenaeus indicus* in HDPE liner ponds (300 m^2) for 90 days. There were control (water exchange to maintain transparency at 40-50 cm) and treatment (zero water exchange) ponds and both were triplicated. Soya hull and molasses were added to treatment ponds as carbon sources to induce biofloc formation. Post larvae (PL_{20}) were stocked at the rate of $50/m^2$ and fed with a standard fishmeal based supplementary pellet feed. Physico-chemical parameters of water, microbial and plankton population, immune response, physical quality and shrimp growth were monitored during the period. High growth and survival was observed in treatment ponds compared to control. Heterotrophic bacteria, phytoplankton population and total haemocyte count (THC) were found to be enhanced in treatment ponds. A strong linear relationship (R^2=0.8758) was found between growth rate and biofloc content. Shrimp raised through biofloc culture strategy showed better colour and quality compared to control. Biofloc technology is an ideal culture method for biosecure production of white shrimp in semi arid lands.

Key words: *Fenneropenaeus indicus*, Biofloc technology, Water quality, Plankton, Immune response, Growth

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Introduction

Biofloc systems were developed primarily to improve environmental control over production and for enhanced biosecurity. Biofloc systems need limited water exchange and prevent introduction of disease to a farm from incoming water (Ogello *et al*., 2014). It uses a counter-intuitive approach which allows or encourages solids and associated microbial community to accumulate in water. Biofloc technology was developed by Avnimelech (2000, 2005 and 2012) and initially implemented commercially in Belize by Belize Aquaculture (McIntosh, 2000). It also has been applied with success in shrimp farming in Indonesia, Malaysia and Australia (Nyan Taw, 2010). Bioflocs are aggregates (flocs) of algae, bacteria, protozoans, and other kinds of particulate organic matter such as faeces and uneaten feed (Baloi *et al*., 2013). Each floc is held together in a loose matrix of mucus that is secreted by bacteria, bound by filamentous microorganisms, or held by electrostatic attraction (Supono *et al*., 2014). The biofloc community also includes animals that are grazers of flocs, such as some zooplankton and nematodes (Nyan Taw, 2014). Large bioflocs can be seen with naked eye, but most are microscopic (Avnimelech, 2012). Flocs in a typical green water biofloc system are rather large, around 50 to 200 microns, and will settle easily in calm water (Hargreaves, 2006).

Other than less water exchange, advantage with biofloc system is capacity to recycle waste nutrients through microbial protein into fish or shrimp. About 20 to 30% of nitrogen in feed is assimilated by fish, implying that 70 to 80 percent of nitrogen (85% according to Crab *et al*. (2012) is released to culture environment. In biofloc systems some of this nitrogen is incorporated into bacterial cells that are a main component of biofloc. Biofloc are nutritious with dry-weight protein content of 30 to 45%, fat content of 1 to 5% and good sources of vitamins and minerals, especially phosphorus (De Schryver *et al*., 2008). Bioflocs may also have probiotic effects (Rivera *et al*., 2014). Research with shrimp suggests that for every unit of growth derived from feed, an additional 0.25 to 0.50 units of growth are derived from microbial protein in biofloc systems (Crab *et al*., 2012). This benefit is reflected in improved feed conversion. Considering improved biosecurity, high production capacity, low FCR and cost of production per kg, a study was conducted to evaluate the effect of biofloc culture strategy for production of Indian white prawn, *F.indicus* an ideal candidate species for coastal aquaculture practice.

Materials and methods

Experimental set up and study management

The study was conducted in HDPE liner ponds (300m^2) for a period of 90 days at University Fish Farm at Obhur, Jeddah. Culture ponds were limed and sundried for one week prior to water culture. In order to develop algal bloom

(40-50cm Sechi disc transparency), water culture was done by the modified methods of Boyd (1990). On day 1, culture ponds were filled with seawater (30%) and manured by applying Urea (400 g), Molasses (1.5 liter) and Diammonium phosphate (200g) and the dose repeated on 4th and 8th day of culture. Pond water level increased to 60 and 100% before applying second and third dose. Two unit of aspirator aerator (1 hp) (Force-7, Acquaeco, Italy) were installed at 40 cm below water level with 35 cm angle downward in each pond. Ponds which were maintained transparency at 40-50 cm by water exchange at10cm/day were considered as control. Whereas the ponds in which zero water exchange was done (topping up was done to maintain the loss of water due to evaporation) were designated as treatment and both of them were triplicated. To boost up heterotrophic bacterial growth in treatment ponds, soya hull and molasses (2 kg) were applied as carbon sources once in three days. On 12th day of water culture, healthy and uniform size juvenile (1.84±0.2g: 5.7±1.8cm) produced at Farm Hatchery were stocked at the rate of 50 pieces /m^2 in each pond (hapa survival >95%). A standard fish meal based pellet feed having 35% protein (NAQUA, Jeddah) was supplemented to shrimp based on a standard feed table at 7:00am, 1:00 and 6:00pm daily.

Water quality control, heterotrophic bacteria, plankton and immune test

Water quality parameters such as temperature, dissolved oxygen, pH, salinity (YSI Incorporated, Yellow Springs, Ohio, USA) and biofloc were recorded daily. Ammonia (unionized), nitrates (NO_3), nitrites (NO_2), orthophosphates (PO_4) and alkalinity (as $CaCO_3$) were recorded (JBL Test kit, GmbH & Co., Germany) weekly. The floc volume (mL/liter) was determined by using Imhoff cones, which recorded the suspended organic solids for 20 minutes of settling (Taw, 2014). Heterotrophic bacterial population (Total plate count for colony forming units (CFU/mL) and plankton community (phyto and zooplankton) in the pond water were examined every month (APHA, 1995; Smith and Johnson, 1996). Immune response of shrimp was tested a week prior to harvest. Shrimp from control and treatment ponds were brought to laboratory and subjected to cold challenge test at 20°C for 24 hours. After the cold challenge, haemolymph sample was drawn from the heart of shrimp and subjected to haemocyte count using a compound microscope (Krupesha *et al*., 2009).

Sampling, Growth analysis and physical quality test

Shrimp sampling (200pcs/sampling) was done biweekly to assess the growth in weight and the feed quantity was re adjusted after every sampling. Upon harvest, all shrimp from control and treatment ponds were collected, survival and biomass were recorded. A panel of experts evaluated physical quality of shrimp such as colour, loose shell, soft shell and taste. Specific growth rate was calculated as Log_e W2-Log_eW1/T2-T1 (where W2 is the weight of shrimp at time T2 and W1 is the weight of shrimp at time T1). One-way analysis of variance (ANOVA) was employed to find out the statistical difference between growth, water quality parameters, biofloc content, bacterial population and haemocyte count of control and treatment. Linear regression analysis was done to find out relationship between biofloc content and growth of shrimp.

Results

Growth performance

Growth performance of control and treatment shrimp recorded during the culture period is shown in Figure 1 and production details are presented in Table 1. Shrimp grown in treatment ponds showed better growth in weight when compared to control and the observed difference was found to be non significant (p>0.01). Survival, average weekly growth, specific growth rate and biomass were high in treatment ponds; whereas, feed conversion rate was found to be low in treatment ponds. Significant relationship (R^2=0.8758) was observed between biofloc concentration and average body weight (g) in treatment ponds (Fig. 2).

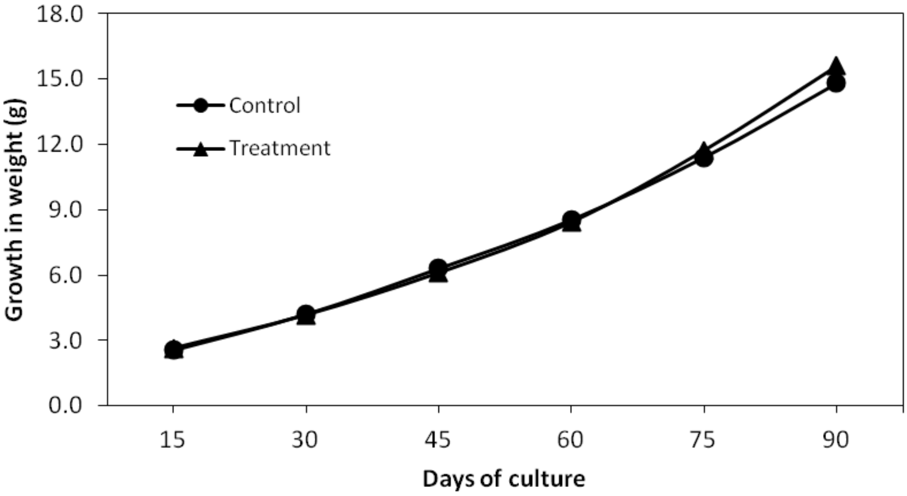

Figure 1: Growth of *F.indicus* during culture period.

Water quality parameters and biofloc content

Water quality parameters recorded during the culture period were found to be conducive for shrimp growth (Table 2). Significant difference was not observed in water temperature, dissolved oxygen, pH, salinity, nitrite, phosphate and ammonia between control and treatment ponds whereas, alkalinity and nitrate contents showed significant difference between control and treatment ponds.

Table 1: Growth perfromance, survival and biomass of shrimp

Parameters	Control	Treatment
	Mean ± SD	Mean ± SD
Initial weight (g)	1.84+0.2	1.84±0.2
Final weight (g)^{NS}	14.8±2.3^{a}	15.6±3.3^{a}
Net weight gain (g)	13.0±1.2	13.8±1.9
Average weekly growth (g)	1.0±0.1	1.2±0.1
Specific Growth Rate (%)	1.03±0.01	1.06±0.02
Survival (%)	77±8	83±12
Feed Conversion Rate	2.6±0.10	2.3±0.20
Biomass/pond (kg)	171±12	194±23
Biomass (kg)/ha	5700±121^{a}	6466±309^{b}

NS-$p>0.01$; (n =100); ** $p<0.01$
a, b. Means with the same superscript do not differ from each other.

Biofloc and Plankton community

Biofloc concentration during the culture period is depicted in Figure 3. An increase in biofloc concentration was noticed in treatment ponds compared to control and it attained a high concentration during the last week of culture. Plankton community in the ponds is presented in Figure 4. Phytoplankton community was found to be decreased in both control and treatment ponds in increasing culture days. However, it was high in treatment ponds during every sampling.

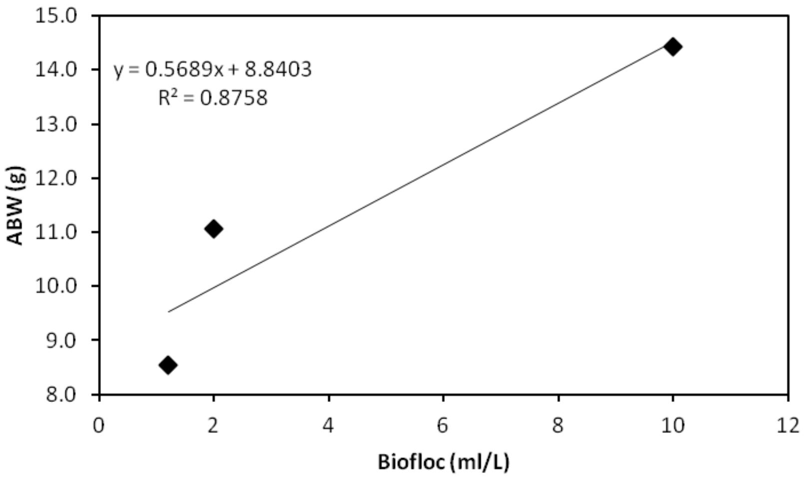

Figure 2: Linear relationship between Biofloc and ABW in treatment pond.

Table 2: Water quality parameters during the culture period

Parameters	Control	Treatment
	Mean ±SD	Mean ± SD
Temperature (^{o}C)^{NS}	27.22±2.72	27.30±2.76
Dissolved Oxygen (mg L^{-1})^{NS}	5.90±0.51	6.03±0.47
pH^{NS}	8.03±0.10	8.22±0.11
Salinity (g L^{-1})^{NS}	39.20±1.05	41.20±0.52
Alkalinity ((mg L^{-1})*	136.60±7.3	144.00±8.0
Nitrate (mg L^{-1})*	1.68±0.66	1.48±0.40
Nitrite (mg L^{-1})^{NS}	0.02±0.01	0.03±0.01
Orthophosphate (mg L^{-1})*	0.66±0.11	1.50±0.12
Unionized Ammonia (mg L^{-1})^{NS}	0.08±0.01	0.13±0.07

NS- $p>0.01$; *$p<0.05$

Species like *Tetraselmis*, *Peridinium*, *Trichodesmium*, *Ntzchia*, *Malassiosira*, *Cylotella*, *Ceratium*, *Amphora*, *Navicula*, *Psuedonizchium* and Blue green algae were the dominant groups in the community. Zooplankton community was found to be high in treatment and low in control ponds. Ciliates, protozoans, rotifers, crustacean larvae and copepods were observed in the group. Treatment ponds had low levels of micro flora, organic substances and other organisms compared to control.

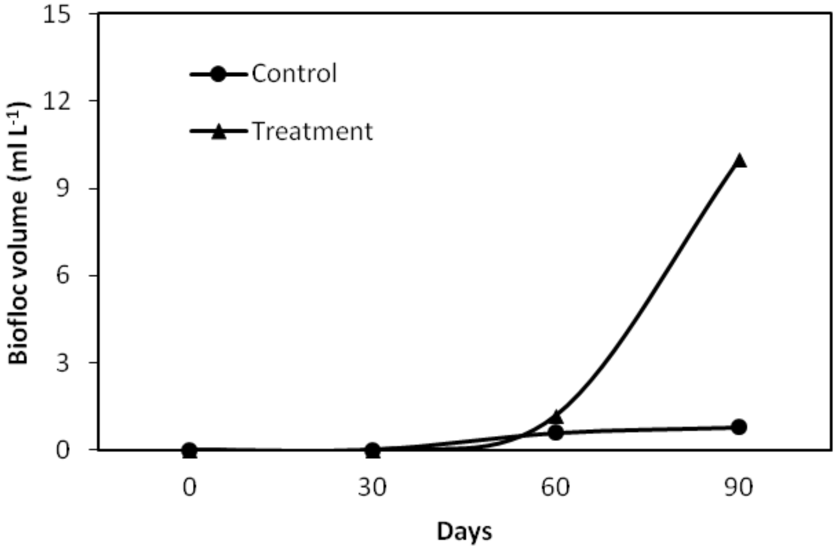

Figure 3: Biofloc (mL/L) in control and treatment ponds.

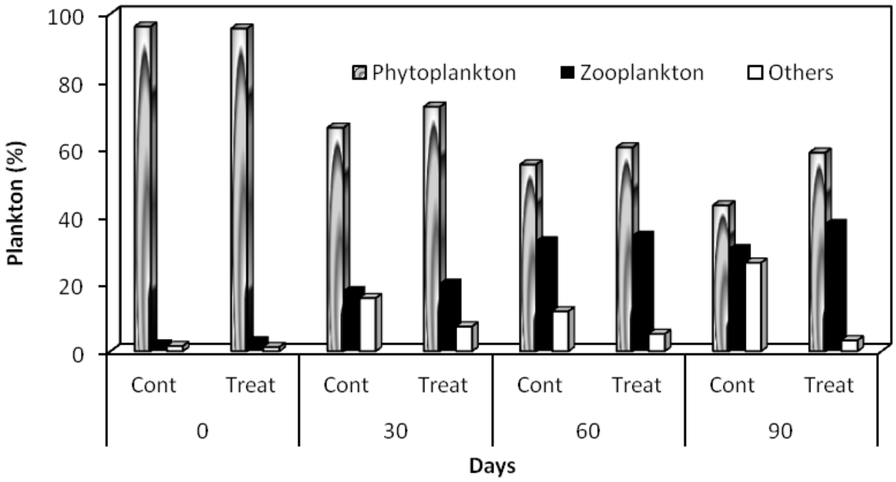

Figure 4: Plankton community in control and treatment ponds.

Heterotrophic bacteria and THC count

Total heterotrophic bacterial population in pond water during the culture period is presented in Table 3. Significant difference ($p<0.01$) in Total plate count (TPC) for colony forming units (CFU) was found between control treatment ponds. Total Yellow *Vibrio* (TYV) colonies were found to be very low in treatment ponds when compared to control. Total Green *Vibrio* (TGV) colonies were found to be decreased in treatment ponds. It is noted that harmful *Vibrio* colonies were found to be considerably reduced in treatment pond water compared to control ponds. Details on total haemocyte count/mL of haemolymph are shown in Figure 5. Shrimp grown in Treatment ponds showed high haemocyte count compared to control and it did not change even after cold challenge test done at 20^{o}C for 24 hours.

Table 3: Heterotrophic bacterial population in control and treatment ponds

Days	Ponds	TPC (CFU/mL)	TYV (CFU/mL)	TGV (CFU/mL)
30*	Control	10000±232^a	10000±432^a	130±0.001^a
	Treatment	8500±134^b	8200±267^b	13±2^b
60*	Control	12333±366^a	3833±384^a	170±21^a
	Treatment	10833±376^b	1333±98^b	313±13^b
90*	Control	63000±298^a	11093±399^a	690±28^b
	Treatment	47333±243^b	1066±201^a	23±9^a

TPC-Total plate count; TYV- Total Yello Vibrio; TGV- Total Green Vibrio
CFU- Colony forming unit
* p<0.01; n =3; ANOVA showing significant difference between control and treatment in each sampling.
a, b. Means with the same superscript do not differ from each other.

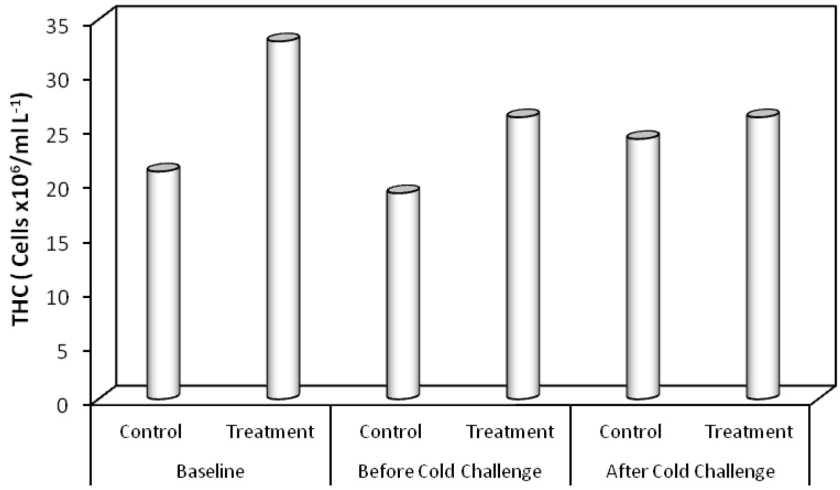

Figure 5: Total haemocyte count (THC) before and after cold challenge test.

Post harvest status and shrimp quality

Pond bottom status after harvest showed that an accumulation of sludge at102±12kg at the centre of treatment ponds; whereas in control ponds, it was 29.4±5.8kg. Physical quality test of shrimp reveals that treatment shrimp had better colour than that of the control. The percentage of hard shell, loose shell and soft shell was 89.1, 5.5 and 5.4 respectively in treatment shrimps and 78.5, 8.5 and 13 was in control. No significant difference in taste observed between control and treatment shrimp.

Discussion

Biofloc technology is a technique of enhancing water quality in aquaculture through balancing carbon and nitrogen in the system (Crab *et al*., 2012, Ray, 2014, Taw, 2014). Results of the present study show that biofloc strategy influences shrimp growth and production. Even though high growth was observed in biofloc ponds, the difference on growth between control and treatment was found to be non significant. This indicates that zero water exchange could bring growth and production as equal as traditional semi extensive culture and is an effective method to control the frequent discharge of pond water to the environment. The positive linear relationship found between biofloc content and growth rate in treatment ponds shows that biofloc formation influences to create a favorable environment for shrimp growth. Similar enhanced growth coupled with biofloc were observed in pacific white shrimp, *Litopenaeus vannamei* grown in zero water exchange system (Avnimelech, 2012; Manecas *et al*., 2013; Rivera *et al*., 2014; Ray, 2014; Taw, 2014). Therefore, it is suggested that biofloc culture strategy can be considered as is an ideal method to improve growth and production of Indian white shrimp, *F.indicus.*

All the water quality parameters remained within the ranges reported as conducive for the culture of white shrimp (Wickins, 1976; Van Wyk and Scarpa, 1999). The high salinity recorded in biofloc ponds may be the result of evaporation of water due to zero water exchange (Emerenciano, 2012). Studies evaluating water quality in zero-exchange systems report low concentrations of nitrate (Ray *et al*., 2010a,b; Vinatea *et al*., 2010). Low concentrations of nitrate observed during culture period suggest oxidation of ammonia (Cohen *et al*., 2005). According to Avnimelech and Ritvo (2003), only about 25% of the feed nutrients are converted into harvestable products hence contributing to high nitrogen residues in pond water, especially total ammonia nitrogen (TAN), which is the sum of both ammonia and ammonium which will adversely affect shrimp growth. This may be a reason for accumulation of ammonia in treatment ponds. Orthophosphate level in pond water regulates proliferation of phytoplankton and diatoms (Chaignon *et al*., 2002). The increased phosphate level found in treatment pond may lead the phytoplankton growth and pursued the formation of biofloc in treatment ponds.

Biofloc is explained as a medium rich in organic matter made of particulate biomass, friendly bacteria and phytoplankton (Rivera *et al*., 2014). From a nutritional point of view, it helps shrimp to gain weight owing to an abundance of native protein sources from protozoa, filamentous bacteria, nematodes, ciliates, flagellates, and rotifers (Decamp *et al*., 2002; Ray *et al*., 2010a,b). This is true in the case of present study, which shows that treatment pond has high phyto and zooplankton community compared to control. Presence of these organism in biofloc may served as source of natural food and this probably a reason for the high survival recorded in biofloc ponds as stated by Burford *et al*. (2003).

It has been reported that natural productivity in zero-exchange shrimp production systems provide supplemental food resources, reducing feed costs and improving shrimp growth rate (Otoshi *et al*., 2011, Manecas *et al*., 2013). Divakaran and Moss (2004) correlated higher shrimp growth with higher concentration of Chlorophyll a in phytoplankton. Becker (1994) and Olvera-Novoa *et al*. (1998) reported that all type of microalgae biomass are rich in polyunsaturated fatty acids and can be an important source of essential fatty acids for shrimp growth. The better colour of shrimp grown in biofloc pond may be due to the presence of high plankton (due to carotenoids) in the ponds. This assumption is in agreement the findings of Ju *et al*. (2009) who reported that microalgae in the microbial floc play a key role in improving shrimp growth rates and quality. Zooplankton consumes algae and bacteria and they can play an important role in transfer of nutrients from primary producers to secondary consumers (Moss *et al*., 2001). Zooplankton such as rotifers can contribute significantly to protein and energy requirements of shrimp (Focken *et al*., 1998). This may be another reason for the enhanced survival and quality of shrimp recorded in biofloc ponds.

Natural production of some substances by bacteria in biofloc has been reported to inhibit growth of co-habiting pathogenic species such as *Vibrio harveyi* (Hsieh *et al*., 2007; Iyapparaj *et al*., 2013). Results of the present study show that harmful *Vibrio* colonies were found to be decreased in biofloc water and this can be attributed to the inhibitory effect of substance in bioflocs as stated above. Bianchi (1979) observed that bacteria in biofloc fluctuate and can have some antibiotic activity. The low yellow and green *Vibrio* colonies noticed in biofloc water may be due to the antibiotic activity of bacteria present in bioflocs. It is suggested that biofloc can control the growth and proliferation of pathogenic bacteria and harmful *Vibrio* colonies in pond water and thereby creating a favorable environment for shrimp growth.

Shrimp health is influenced by a range of factors, one of the most important being environmental stress (Xu and Pan, 2013). Under culture conditions, wide range of stresses caused by various adverse environmental factors damage the host defense system resulting in an increased susceptibility to infections (Perazzolo *et al*., 2002; Vazquez *et al*, 2009). Recently, scientists have hypothesized possibilities of immunostimulatory features of the bioflocs leading to enhancement of the immunity to provide broad-based resistance towards many infections (Crab *et al*., 2012). According to Wang *et al*. (2008), existing immunostimulants are group of live and synthetic compounds including bacteria and bacterial products, complex carbohydrates, nutritional factors, animal extracts, cytokines, lectins and plant extracts. Therefore, bioflocs might also contain immunostimulatory compounds since biofloc technology deals with bacteria and bacterial products. Total haemocyte count (THC) in haemolymph is considered as index of shrimp immunological functions and a higher THC is responsible for high immune status (Rodriguez and Moullac, 2000; Krupesha *et al*., 2009). Results of the present study show that shrimp grown under stressed environment (treatment ponds had high ammonia, pH and salinity) had high THC. It is interesting to note that the treatment shrimp had a capacity to maintain the high THC even after a cold challenge was given at 20°C for 24 hours. This suggests that shrimp grown in biofloc environment may have strong immune capacity/response than those grown in clean water.

High accumulation of organic sludge was observed in all treatment ponds after harvest. This sludge formation was due to the deposition of dead algae and other organic compounds formed as a result of zero water exchange (Mikkelsen *et al*., 1996; Gonzalez-Felix *et al*., 2007). Studies show that this formed sludge would spoil pond bottom environment and act as medium for the proliferation and growth of harmful bacteria (Ju *et al*., 2008: Crab *et al*., 2012). Pathogenic bacteria act as agents to convert nitrogenous compounds into toxic ammonia and foul gases at pond bottom, elevating ammonia and pH level of water (Ebeling *et al*., 2006). This can be correlated as a factor for the enhanced ammonia, and pH observed in the biofloc ponds.

Overall performance of biofloc culture technique in the present study shows that it enhances shrimp growth similar to clean water culture. Heterotrophic bacterial population and phytoplankton community in biofloc ponds enhanced the colour and quality of shrimp. If better management methods are implemented to control ammonia and pH, biofloc technology would certainly be an ideal strategy for farmers for the biosecured production of shrimp and also to reduce environmental pollution due to coastal aquaculture.

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