Journal of Environmental Management 215 (2018) 206–215

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

# Biofloc improves water, effluent quality and growth parameters of *Penaeus vannamei* in an intensive culture system

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ARTICLE INFO

*Article history:*
Received 18 November 2017
Received in revised form
1 February 2018
Accepted 4 March 2018

*Keywords:*
Biofloc
Effluent nitrogen
Water quality
*Penaeus vannamei*
Growth

ABSTRACT

Biofloc technology was evaluated with a view to analyse utilization of nitrogenous waste from the effluent and to improve water quality and growth parameters of *Penaeus vannamei* in intensive culture system. The experiment was carried out in two different treatment outdoor earthen ponds of 0.12 ha, one supplemented with carbon source (molasses, wheat and sugar) for biofloc formation and other was feed based control pond with a stocking density of 60 animals $m^{-2}$ in duplicate for 120 days. Water, sediment and *P. vannamei* were sampled at regular intervals from the both set of ponds for evaluating physico-chemical parameters, nitrogen content and growth parameters, respectively. A significant reduction in the concentration of total ammonia nitrogen (TAN) and nitrite ($NO_2$-N) were found in the biofloc pond than that of control pond. A significant low level of nitrogen was recorded in the effluents of biofloc pond in comparison to the control. In biofloc system, a significantly elevated heterotrophic bacterial count along with reduction in total *Vibrio* count was noticed. A significant improvement in the feed conversion efficiency (FCR) and growth parameters of *P. vannamei* was noticed in the biofloc pond. Growth of *P. vannamei* in the biofloc pond showed positive allometric pattern with an increased survival. The microbial biomass grown in biofloc consumes toxic inorganic nitrogen and converts it into useful protein, making it available for the cultured shrimp. This improved FCR and reduced the discharge of nitrogenous waste into adjacent environment, making intensive shrimp farming an eco-friendly enterprise.


## 1. Introduction

The world population is expected to grow by another 2 billion to reach 9.7 billion by 2050 (UNDESA, 2015). Food producing sectors have a huge challenge ahead, to meet the growing food demand with limited land availability. In this regard, aquaculture is likely to play a key role in maximizing the utilization of various aquatic resources to produce varieties of food organisms by incorporating intensive culture practices. However, these intensive culture practices are associated with rising concerns such as effluent discharge, excessive use of feed, pollutants, disease outbreaks and adverse environmental impact such as eutrophication (Páez-Osuna, 2001; Piedrahita, 2003). Further, high level of nitrogen released in effluents, possess major threats to the homeostasis of aquatic ecosystem (Nixon, 1995; Howarth et al., 2000). Among all the identified sources, aquaculture releases more nitrogen to the receiving water bodies. Around 70–80% of nitrogen, added as input in aquaculture system, remains unutilized and released to the adjacent environment (Funge-Smith and Briggs, 1998; Jackson et al., 2003; Sahu et al., 2013a, 2013b; Sun and Boyd, 2013). These unutilized nitrogen converts into toxic inorganic nitrogen (ammonia and nitrite) and deteriorates the in-situ water quality as well as the receiving water bodies (Páez-Osuna, 2001). Effluent treatment system (ETS) plays a key role in removing the waste nutrients released from the effluents of shrimp farm especially larger than 5 ha (CAA, 2005). However, high capital investment and operating cost, make this practice inconspicuous among the farming community. This specifies the need for developing a novel strategy to remove unutilized nutrients from the effluents without affecting economic performance of the shrimp culture.

Biofloc technology has been gaining importance and found to be

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https://doi.org/10.1016/j.jenvman.2018.03.015
0301-4797/

a lucrative alternative for treating in-situ culture water quality without affecting the shrimp production. Manipulation of C/N ratio in the culture water through the addition of carbon source via feed and other external source (molasses, sugar, wheat, etc.) stimulates microbial biomass (Avnimelech, 1999). In the optimum range of C/N ratio (15–20:1), heterotrophic microbes, present in the culture water, converts inorganic nitrogen metabolites into utilizable biofloc. This biofloc acts as an feed ingredient in shrimp diet (Kuhn et al., 2010; Anand et al., 2014), improves the water quality, growth performance and microbial performance in a zero water exchange intensive culture system of shrimp (Schveitzer et al., 2013; Rajkumar et al., 2015; Xu et al., 2016). A prerequisite study on nutrient dynamics of the system is necessary to undertake before evaluating biofloc system as waste removal technology. Nutrient budgeting has been studied in semi-intensive (Páez-Osuna et al., 1997), intensive (Jackson et al., 2003), substrate installed (Kumar et al., 2017) shrimp culture systems in earthen ponds and nitrogen dynamics of biofloc system was studied in a lab scale experiment in indoor tanks (Da Silva et al., 2013). Inadequacy in reports regarding the nitrogen budget of biofloc treated dynamic pond ecosystem has been witnessed. Hence, the present study aims to budget the nitrogen in biofloc pond, and evaluate the effect of biofloc on water quality, microbial load and growth performance of *P. vannamei* in an intensive culture system.

## 2. Materials and methods

### 2.1. Experimental design

The experiment was conducted at Hitide Sea Farms (11$^0$21′34.1″N, 79$^0$48′45.4″E), Mahendrapalli, Nagapattinam district, Tamilnadu, India for a period of 120 days during August to November, 2015. It was conducted in 4 uniform HDPE (High Density Polyethylene) lined ponds with an area of 0.12 ha (40 × 30 × 1.5 m), by following completely randomized design (CRD) in duplicates, with and without the addition of 3500 kg carbon source for the development of biofloc and considered as biofloc (treatment) and control pond, respectively. The ponds were prepared as per the guidelines 5.0 of Coastal Aquaculture Authority (CAA), India. The biofloc from previous crop was added as an inoculum and reared for 10 days, followed by the addition of urea (11.5 kg) and 3500 kg carbon source such as molasses, sugar and wheat in the ratio of 8:1:1, respectively to maintain the C/N ratio of 15:1 (Avnimelech and Kochba, 2009). Fourteen horse power aerators were placed at 4 corners and 3 m away from the pond dyke in order to maintain a clockwise circulation of water. Aspirators were placed at pond bottom to keep the floc in suspension. Floc volume was estimated by collecting 1 L water sample in the imhoff cone and left undisturbed for 15–20 min and settled floc was read and noted as ml $L^{-1}$.

Fifteen-days old post larvae (PL 15) of *P. vannamei* (0.015 ± 0.001 g) were procured from CAA approved commercial hatchery in Marakanam, Tamilnadu, India and were acclimatized to the pond water conditions prior to stocking. Healthy and uniform sized PL 15 of *P. vannamei* were selected and stocked at the rate of 60 number $m^{-2}$ in each pond. The shrimp larvae were fed with commercial sinking pelleted shrimp feed (Chareon Pokphand India Pvt. Ltd., Chennai; India) at the rate of 5–3% of shrimp biomass having crude protein 35% (Table 1) ad libitum four times a day. Weights of 10% of total number of shrimp were measured individually at weekly interval after 30$^{th}$ day of culture (DOC) with a view to estimate the shrimp biomass and to adjust the feeding rate accordingly.

Water samples were collected at 10 days interval from two locations in each pond and samples were pooled for analyzing the physico-chemical and microbiological parameters. Nitrogen contents of inputs such as fertilizers, water, stocked shrimp, feed and carbon source and outputs such as harvested shrimp, effluents (sediment removal + harvest drainage) of ponds were used for calculating the nitrogen budgeting. Nitrogen retention in the system and output was calculated by estimating the corresponding nitrogen content at the time of stocking and at the end of the experiment (120$^{th}$ DOC). Nitrogen utilized by the biofloc was calculated by collecting the water samples at 30 days interval after biofloc formation, filtered, dried and the mean nitrogen content was estimated. The shrimps were sampled and weighed at the time of stocking and at the end of experiment for analyzing the growth performance.

**Table 1**
Proximate composition of biofloc and feed used in experimental pond.

| Nutrients (%) | Proximate composition | |
|---|---|---|
| | Biofloc | Feed |
| Crude protein | 23.19 | 35 |
| Crude fat | 4 | 5.32 |
| Crude fiber | 3.56 | 4 |
| Moisture | 7.8 | 11.97 |
| Ash | 30.38 | 17.2 |
| [a]Nitrogen free extract (NFE) | 31.07 | 26.51 |
| [b]Gross Energy GE (Kcal 100g$^{-1}$) | 309.61 | 371.31 |
| [c]Organic Matter (OM %) | 69.62 | 82.8 |

[a] NFE = 100 - (CP + EE + CF + ash + moisture).
[b] GE = (CP x 5.6) + (EE x 9.44) + (CF x 4.1) + (NFE x 4.1) Kcal 100g-1 (N. R. C., 1993).
[c] OM = 100- Crude ash (%).

### 2.2. Water quality parameters

Water quality parameters, such as dissolved oxygen (portable DO meter -Eutech Instruments, Singapore), Salinity (Refractometer; Master Atago, (Master- S/MillM), Japan), transparency (Secchi disc, India) and pH (pH Scan-Eutech Instruments, Singapore) of the experimental ponds were measured between 7.00 and 8.00 h. For estimating nitrate–N ($NO_3$–N), nitrite–N ($NO_2$–N), and total ammonia nitrogen (TAN), water samples were filtered through a GF/C Whatman glass fiber filter and the filtrate was analyzed spectrophotometrically (UV/VIS Spectrophotometer, PerkinElmer Lambda 25, USA) following standard methods (APHA, 2005). Total alkalinity, calcium, magnesium, hardness, total dissolved solids, total suspended solids, total solids and chemical oxygen demand (COD) were analysed, following standard methods (APHA, 2005). Two separate amber coloured bottle were used for collecting samples for estimating chlorophyll-*a* and biological oxygen demand (BOD) content by following standard methods, (APHA, 2005).

### 2.3. Nitrogen budgeting

Nitrogen contents of all the inputs and outputs of experimental ponds were calculated, following the standard procedures (AOAC, 1995). Nitrogen entry through the feed was calculated as follows:

$$Nitrogen\ in\ feed = NF \times TF$$

Where, NF = Nitrogen content in 1 kg of feed, TF = Total amount of feed applied.

Likewise, the entry of nitrogen through fertilizers, input water and carbon source were analyzed.

Nitrogen released via effluents represents the sum of nitrogen released through the harvest drainage and nitrogen pumped out as sediment/sludge from the pond bottom.