Aquacultural Engineering 54 (2013) 93–103

Contents lists available at SciVerse ScienceDirect

Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online

Use of artificial substrates in the culture of *Litopenaeus vannamei* (Biofloc System) at different stocking densities: Effects on microbial activity, water quality and production rates

Rodrigo Schveitzer[a,*], Rafael Arantes[a], Manecas Francisco Baloi[a], Patrícia Fóes S. Costódio[b], Luis Vinatea Arana[a], Walter Quadros Seiffert[a], Edemar Roberto Andreatta[a]

[a] *Marine Shrimp Laboratory, Department of Aquaculture, Federal University of Santa Catarina (UFSC), Florianópolis, SC, 88061-600, Brazil*
[b] *Laboratory of Chemical Oceanography, Center of Technology, Earth and Sea Sciences, University of Vale do Itajaí (UNIVALI), Itajaí, SC, 88302-202, Brazil*

ARTICLE INFO

Article history:
Received 9 August 2012
Accepted 18 December 2012

Keywords:
Shrimp
Bioflocs
Artificial substrates
Periphyton
Stocking density

ABSTRACT

Although the use of artificial substrates can favor shrimp culture, some studies indicate that their presence in growth tanks does not improve water quality or the performance of the animals. One objective of this study was to evaluate whether the presence of artificial substrates modifies the microbial activity and the water quality of the culture of *Litopenaeus vannamei* with bioflocs. The substrate effects on the shrimp performance and the relationship between these effects and the stocking density/biomass of shrimp were also evaluated. The experiment consisted of four treatments: D238: 238 shrimp m^{-3}; D238 + S: 238 shrimp m^{-3} + substrates; D473: 473 shrimp m^{-3}; D473 + S: 473 shrimp m^{-3} + substrates. Twelve experimental units of 850 L were stocked with juvenile *L. vannamei* (2.6 g) that were grown for 34 days. The substrates did not appear to affect water quality since the concentrations of orthophosphate, ammonia and nitrite were not significantly different in tanks with or without substrates. The periphyton biomass was low and the biological activity on the substrates was not significant, indicating that the water quality variables were mainly controlled by the microbial community associated with the suspended bioflocs. The shrimp grown in the presence of the substrate exhibited greater weight gain (D238 + S = 1.40 ± 0.05 and D473 + S = 1.20 ± 0.04 g $week^{-1}$) than those grown without substrates (D238 = 0.73 ± 0.04 and D473 = 0.44 ± 0.13 g $week^{-1}$). The final biomass was 314% greater in the tanks with substrates. The shrimp survival was significantly higher in the tanks with substrates (93.9 ± 2.4%) than in the tanks without substrates (42.5 ± 35.9%). The results indicate that the substrates served to increase the surface area of the tank and to reduce the relative stocking density, which appears to reduce the stress levels of shrimp, indicated by higher shrimp performance. In tanks with higher biomass, where the negative effects of intensification were most severe, the presence of the substrates had a positive effect on the production indices.

1. Introduction

The use of artificial substrates is a management strategy employed in the growth of aquatic organisms to improve efficiency in culture tanks. Polyethylene and polypropylene screens, bamboo, plastic bottles and commercial products (Aquamats^{TM}) have been used as substrates in growth tanks (Azim et al., 2004; Bratvold and Browdy, 2001; Huchette et al., 2000; Richard et al., 2009; Zhang, 2011). In experiments with post-larvae and juvenile marine shrimp, the use of substrates is frequently associated with improved performance by the shrimp (Arnold et al., 2006, 2009; Audelo-Naranjo et al., 2010; Ballester et al., 2007; Bratvold and Browdy, 2001; Lezama-Cervantes and Paniagua-Michel, 2010; Moss and Moss, 2004; Otoshi et al., 2006a; Thompson et al., 2002; Viau et al., 2012; Zhang, 2011). In intensive cultures, substrates have been used in an effort to mitigate the negative effects of increasing the stocking density (Arnold et al., 2006). Abdussamad and Thampy (1994) suggest that the substrates provide an additional surface for the shrimp, which reduces their competition for space and negative behavioral interactions, such as cannibalism.

The periphyton that grows on the substrates also helps to control water quality (Arnold et al., 2009; Asaduzzaman et al., 2009; Azim et al., 2004; Bratvold and Browdy, 2001; Lezama-Cervantes and Paniagua-Michel, 2010; Thompson et al., 2002; Viau et al., 2012) and serves as a natural food source for the cultured species

* Corresponding author at: Laboratório de Camarões Marinhos, Servidão dos Coroas s/n (fundos), Barra da Lagoa, 88061-600, Florianópolis, SC, Brazil.
Tel.: +55 48 37214118.
E-mail addresses: rschveitzer@hotmail.com, rodrigo@lcm.ufsc.br (R. Schveitzer).

0144-8609/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aquaeng.2012.12.003

(Abreu et al., 2007; Arnold et al., 2006; Ballester et al., 2007; Burford et al., 2004; Thompson et al., 2002). The periphyton, or biofilm, is characterized as a complex community of aquatic organisms adhered to submerged substrates, including associated non-attached organisms and detritus (van Dam et al., 2002). This assembly contains bacteria, fungi, protozoans, phyto- and zooplankton, benthic organisms and detritus (Azim and Asaeda, 2005). Although the use of substrates is considered beneficial for the shrimp culture, some studies have indicated that their presence in growth tanks did not affect the performance of the cultured animals (Kumlu et al., 2001; Samocha et al., 1993; Sandifer et al., 1987) or the water quality (Audelo-Naranjo et al., 2010; Samocha et al., 1993). Considering the diversity of experimental conditions in which the substrates have been tested, it is possible that such factors as species, culture system, stocking density/biomass, amount of substrate, age and the origin of the larvae affect the mechanisms of action of the substrates and their effects on the organisms.

Although the effectiveness of artificial substrates has not been tested in the culture of *L. vannamei* with bioflocs, their use is cited in the literature (Browdy and Moss, 2005; Krummenauer et al., 2011). Biofloc system involves the production of dense microbial communities that are manipulated to control the ammonia released mainly from the cultivated organisms (Avnimelech, 2012). Ammonia can be absorbed by microalgaes or heterotrophic bacteria or can be transformed by nitrifying bacteria (Ebeling et al., 2006), and the growth of these organisms occurs mainly in the form of microbial flocs (Schryver et al., 2008). One of the arguments employed for the use of artificial substrates is the creation of a habitat for nitrifying bacteria (Otoshi et al., 2006a). Otoshi et al. (2006b) observed, however, that 31% of the bacteria attached to the bioflocs in intensive cultures of *L. vannamei* were nitrifying. Therefore, even though these bacteria and other periphytic microorganisms are important in the control of toxic compounds, such as ammonia and nitrite, in culture systems based on bioflocs, the cycling of nutrients is performed by the microorganisms present in the water column. In fact, both the bioflocs in the water column, as well as the periphyton present on the substrates, function to accelerate the biological removal of organic and inorganic wastes (Crab et al., 2007), i.e., they both fulfill similar functions in controlling water quality. Therefore, although the presence of the periphyton is important in certain production systems, it is possible that in biofloc system, its presence is not relevant for water quality control.

In addition to factors connected to nutrition and water quality, the low shrimp performance in intensive cultures has been attributed to negative social interactions between the animals, which can have an inhibitory effect (Araneda et al., 2008; Moss and Moss, 2004; Otoshi et al., 2007) or stimulate cannibalism (Abdussamad and Thampy, 1994). Some researchers suggest that the substrates mitigate the negative effects of intensification as they provide more space for the shrimp and reduce the stress that can detract from their performance (Arnold et al., 2006; Moss and Moss, 2004; Otoshi et al., 2006a; Zhang, 2011). Although the benefits of substrates in shrimp cultures are described in the literature, productivity of approximately 2 kg m^{-3} has been reported in published experiments and is lower than the biomasses achieved in biofloc cultures. One of the benefits of using artificial substrates is the increase in surface area of the tanks and the reduction of the relative stocking density. However, considering the maximum limit of substrate that can be placed in the tanks, the increase in the stocked biomass of shrimp can compromise the efficiency of the substrates because the available area for the animals will be smaller. Recently, Zhang (2011) observed that even in higher biomass cultures, the substrates improved the performance of the shrimp in tanks with high water turnover. Considering that the variability of the results in experiments testing the substrates in shrimp culture may also be related to the production system, it is important to evaluate the substrate efficiency in high stocking density/biomass biofloc systems.

Studies have demonstrated that artificial substrates can improve the production indices and water quality in shrimp cultures. In superintensive biofloc system, however, suspended microorganisms in the water are responsible for cycling nutrients, and the high stocking biomass can compromise the efficiency of the substrates. Studies evaluating the role of the substrates in biofloc cultures have not been reported, and studies with substrates and high shrimp biomass are limited. Considering the conflicting results concerning the use of substrates, their potential benefits for shrimp cultures and the costs involved in their use, the evaluation of this management tool in different production systems is important. The objective of this study was to evaluate whether the presence of artificial substrates modifies the microbial activity and water quality in biofloc systems. The effects of the substrates on the shrimp performance in biofloc system were also evaluated along with the effects of the stocking density/biomass of the shrimp.

2. Materials and methods

2.1. Biological material

The experiment was performed in the Marine Shrimp Laboratory of the Federal University at Santa Catarina, southern Brazil. Specific pathogen-free post-larvae (PLs 5) of *L. vannamei* were obtained from a commercial laboratory (Aquatec Ltda, Canguaretama, RN, Brazil). PLs were raised in a 50 m^2 circular tank until they reached an average weight of 0.02 g, at a stocking density of 1200 Ls m^{-2} (1500 PLs m^{-3}) in salinity of ~35. The water for the culture was prepared with the addition of the microalgae *Nanochloropsis oculata*. No water was exchanged during this time, and molasses was added to the tank when the total ammonia nitrogen (TAN) increased beyond 1 mg L^{-1}. Molasses addition was calculated based on Avnimelech (1999) assuming that 20 g of carbohydrate is needed to convert 1 g of TAN. The protein content of the feed ranged from 45% (INVE Aquaculture, Salt Lake City, UT, USA) to 40% (Guabi Nutrição Animal, Campinas, SP, Brazil) of crude protein.

Later, the shrimp were transferred into another 50 m^2 round tank (matrix tank) equipped with solids settling chamber, heating and aerating system. The shrimp were grown to 2.5 g at a stocking density of 214 shrimp m^{-2} (268 shrimp m^{-3}); the tank was operated as a biofloc system without water exchange. The water was prepared to receive the shrimp by adding 14 m^3 of water from the culture of the post-larvae and 26 m^3 of prechlorinated salt water. Shrimp were fed with commercial feeds (Guabi Nutrição Animal, Campinas, SP, Brazil) with crude protein level between 40% (Guabi Potimar 40) and 35% (Guabi Potimar 35). Calcium hydroxide was used to maintain the alkalinity above 120 mg L^{-1}, ammonia levels (TAN) were kept at up to 1 mg L^{-1} using molasses according to Avnimelech (1999), dissolved oxygen was maintained above 5.5 mg L^{-1}, total suspended solids (TSS) were maintained at levels of up to 400 mg L^{-1} and the water salinity was approximately 32. The average temperature of the water was 29 °C.

2.2. Experimental design, experimental units and system management

The experiment evaluated shrimp culture in the presence or absence of artificial substrates with different stocking densities (independent variables). The effects of these variables on the microbial activity, the water quality parameters and shrimp performance were evaluated. The biological activity on the substrate