



Latin American Journal of Aquatic  
Research

E-ISSN: 0718-560X

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Pontificia Universidad Católica de  
Valparaíso  
Chile

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Latin American Journal of Aquatic Research, vol. 43, núm. 3, julio, 2015, pp. 526-533

Pontificia Universidad Católica de Valparaíso  
Valparaíso, Chile

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**Research Article**

**Dextrose as carbon source in the culture of *Litopenaeus vannamei* (Boone, 1931) in a zero exchange system**

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**ABSTRACT.** This work compared the use of dextrose and molasses as carbon sources for biofloc development, water quality maintenance, microorganism composition and growth performance of *Litopenaeus vannamei* juveniles in biofloc technology (BFT). Two treatments, dextrose and molasses, were tested with four replicates each. Carbon was added to achieve a C:N-AT (N-(NH<sub>3</sub>+NH<sub>4</sub><sup>+</sup>)) ratio of 6:1. Physical and chemical water quality variables were monitored daily, and shrimp growth was estimated through periodic biometry. After 30 days, survival, final biomass, and feeding conversion rate (FCR) were determined. Dissolved organic carbon, chlorophyll-*a*, floc volume, total ammonia, nitrite, nitrate and phosphate concentrations, and microorganisms (qualified by groups), were measured every three days. Water quality variables remained within acceptable levels throughout the experimental period, except for nitrite, which reached higher levels than recommended for this species. The use of dextrose resulted in higher water transparency, which influenced the remaining centric diatoms. A superior shrimp performance was observed at this treatment, presumably because of variations on the microbial community. Therefore, it is concluded that the addition of dextrose results in a superior growth performance of *L. vannamei* when cultured in BFT systems.

**Keywords:** *Litopenaeus vannamei*, bioflocs, nitrogen, microorganisms, microalgae, aquaculture.

**Dextrosa como fuente de carbono en el cultivo de *Litopenaeus vannamei* (Boone, 1931) en un sistema sin recambio de agua**

**RESUMEN.** Se comparó el uso de dextrosa y melaza como fuentes de carbono en el desarrollo del biofloc, mantención de la calidad del agua, composición microbiana y crecimiento de juveniles de camarón blanco *Litopenaeus vannamei* en sistemas de cría con tecnología de biofloc (BFT). Se probaron dos tratamientos, dextrosa y melaza, con cuatro réplicas cada uno. Se agregó carbono para mantener la relación carbono: N-AT (N-(NH<sub>3</sub>+NH<sub>4</sub><sup>+</sup>)) en 6:1. Las variables físicas y químicas de calidad del agua se controlaron diariamente y el crecimiento de los camarones se estimó mediante biometría en forma periódica. Después de 30 días se determinó la sobrevivencia, biomasa final y tasa de conversión de alimento. Cada tres días se determinaron las concentraciones de carbono orgánico disuelto, clorofila-*a*, amonio total, nitrito, nitrato y fosfato, y microorganismos (de los grupos clasificados), en el agua. Las variables de calidad de agua se mantuvieron dentro de niveles aceptables durante el periodo experimental, excepto la concentración de nitrito que alcanzó niveles superiores a los recomendados para esta especie. El uso de dextrosa mostró una mayor transparencia del agua, lo que influyó en la mayor permanencia de diatomeas céntricas. En este mismo tratamiento los camarones tuvieron un mejor crecimiento debido probablemente a variaciones en la comunidad microbiana. Por esta razón, se concluye que la adición de dextrosa proporciona un mejor crecimiento del camarón blanco *L. vannamei* cultivado en sistema de bioflocos.

**Palabras clave:** *Litopenaeus vannamei*, biofloc, nitrógeno, microorganismos, microalgas, acuicultura.

**INTRODUCTION**

Aquaculture involves a number of risk factors, which include negative impacts to sediments, salinization of

water bodies, the introduction of exotic species, chemical and organic pollution, and the spread of diseases (Boyd, 2003). In superintensive aquaculture systems, the accumulation of potentially hazardous nitro-

gen products demands constant water quality monitoring. Biofloc technology (BFT) attempts to decrease or prevent the emission of effluents from aquaculture farms. Applying a source of organic carbon to the system to raise the carbon-nitrogen (C:N) proportion induces the removal of inorganic nitrogen from the culture environment. This process is driven by heterotrophic microorganisms that promote the transformation of these toxic products into microbial proteins. This process works by rapidly decreasing dissolved nitrogen concentrations in the water, transforming nitrogen into bacterial biomass (Avnimelech, 1999).

Although any carbohydrate may be used as a carbon source, sugar cane molasses is more commonly used. This product effectively controls nitrogen in the form of total ammonia ( $\text{N}-(\text{NH}_3+\text{NH}_4^+)$ ) (Samocha *et al.*, 2007). Further studies are required to investigate the impacts of different carbon sources on the microbial community and biofloc formation in super intensive aquaculture environments with zero water exchange. There is also a need to determine the effects of other carbon sources on the performance of shrimp and the maintenance of water quality. Dextrose is a simple carbohydrate industrially obtained from starch (Lehninger, 1995), and is an alternative source of carbon to be used in superintensive shrimp culture systems. The use of this sugar is supposed to provide better light penetration compared to molasses, which in turn supports a more permanent phytoplankton community. Baloi *et al.* (2012) tested the performance of *L. vannamei* in biofloc system with different light intensities and found that higher light penetration may favor the permanence of microalgae in the environment, and thus it improves the performance of shrimp due to the supply of fatty acids, amino acids, and vitamins.

The goal of this study was to evaluate the use of dextrose as a carbon source for the water quality parameters, microbial community, determine its effect on the development of shrimp, and observe its effect on reduction of ammonia concentration. Each of these parameters is compared to those of molasses, the carbon source most often used for this type of culture systems.

## MATERIALS AND METHODS

Postlarvae of *L. vannamei* were kept in a nursery system for 30 days. Shrimp ( $1.44 \pm 0.33$  g) were stocked in eight 163 L PVC tanks and divided into two treatments (dextrose and molasses) each with four replicates. Experimental units were individually aerated and maintained within a static system with no water

recirculation and under a natural light regime. The microalgae *Thalassiosira weissflogii* was added at a density of  $6 (\pm 1) \times 10^4$  cells  $\text{mL}^{-1}$ .

A commercial (38% crude protein) diet was supplied in feeding trays. The amount given was 7.5% of the total shrimp biomass (Jory, 2001). A stocking density of 300 shrimp  $\text{m}^{-2}$  was used. On days 15 and 30, 30 shrimp from each tank were weighed to determine growth at each treatment. Survival was determined by counting the remaining shrimp in each tank at the end of rearing period. The apparent feeding conversion rate (FCR) was calculated according to the formula:  $\text{FCR} = \text{RF}/(\text{B}_f - \text{B}_i)$ , where FCR: apparent feeding conversion rate, RF: quantity of diet supplied,  $\text{B}_f$ : final biomass,  $\text{B}_i$ : initial biomass.

Every morning dissolved oxygen concentrations (Handylab OXI/SET, Schott), temperature (mercury thermometer), pH (digital pH-meter DMpH-1, Digimed) and water transparency (Secchi disk) were monitored. Salinity was measured every two days using an optical refractometer (Atago). Alkalinity ( $\text{mg L}^{-1}$  of  $\text{CaCO}_3$ ) was measured every three days (Strickland & Parsons, 1972). Water samples were analyzed every two days for total ammonia ( $\text{N}-\text{AT}(\text{N}-\text{NH}_3+\text{NH}_4^+)$ ) (UNESCO, 1983), and every three days for nitrite ( $\text{N}-\text{NO}_2^-$ ) and phosphate ( $\text{P}-\text{PO}_4^{3-}$ ) (Strickland & Parsons, 1972). Nitrate ( $\text{N}-\text{NO}_3^-$ ) levels were measured weekly (Strickland & Parsons, 1972). Before nutrients analysis, samples were filtered through GF50-A fiberglass filters (Schleicher & Schuell,  $-47 \pm 0.5$  mm  $\varnothing$ ) using a vacuum pump (Diapump®). The determinations of the carbon and nitrogen contents of the diet, dextrose, molasses, and wheat bran were done using a CHN Analyzer (PerkinElmer® Series PE 2400).

The addition of organic carbon for the formation of microbial flocs was divided into two phases. During the three initial days, organic carbon was added in the form of dextrose or molasses and wheat bran in a C:N ratio of 20:1 (Avnimelech, 1999). This ratio was established by calculating the amount of nitrogen from the diet added to the system, and then determining the amount of carbon needed to achieve such ratio. Wheat bran was employed as a substrate for biofloc formation, and made up 5% of the total supplied organic fertilizers. From day 4 onwards, dextrose and molasses were added when total ammonia concentrations exceeded  $1 \text{ mg L}^{-1}$ , keeping C:N-AT at a ratio of 6:1, where 6 g of carbon are necessary to convert 1 g of nitrogen in the form of ammonia into microbial protein (Ebeling *et al.*, 2006).

The concentration of chlorophyll-*a* (Welschmeyer, 1994) was determined every three days from 50 mL samples of water filtered through GF 50-A fiberglass filters (Whatman GF/F). The extraction of chlorophyll

was carried out by dipping filters in 10 mL of 90% acetone kept in dark flasks at  $-12^{\circ}\text{C}$ . After 24 h, chlorophyll-*a* was measured using a Turner TD700 fluorometer. Dissolved organic carbon was measured in water samples three times a week using the same methodology used for chlorophyll-*a* and then analyzed using the “NPOC Analysis” (TOC-V CPH) method.

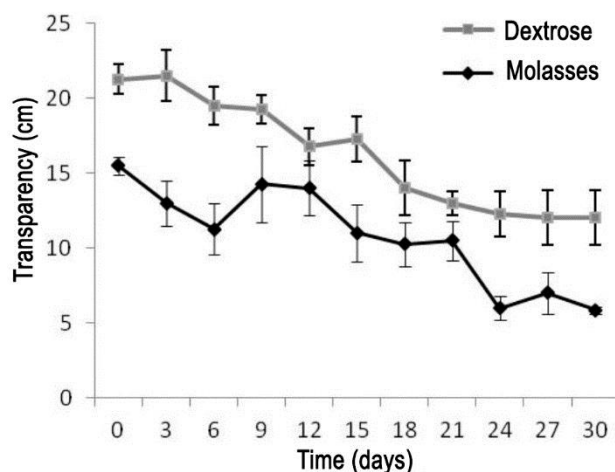
Samples of shrimp and microbial flocs were collected on the last experimental day for the measurement of the contents of crude protein, fiber, ether extract, ash, and dry matter (AOAC, 1984). The total volume of flocs ( $\text{mL L}^{-1}$ ) was measured using Imhoff cones (Avnimelech, 2007). To quantify and characterize the microbial community in the water and microbial flocs, water samples were collected from each tank, fixed in 4% formalin, and stored in amber flasks. To determine bacterial abundance, the fixed samples were filtered through polycarbonate membrane filters (Nucleopore,  $0.2\ \mu\text{m}$  of pore and 2.5 mm of diameter) and dyed with Acridine orange at the concentration of  $1\ \mu\text{g mL}^{-1}$  (Hobbie *et al.*, 1977).

Images of bioflocs were captured at 1000x magnification using a camera attached to an epifluorescence microscope (Zeiss Axioplan). A set of 487709 (BP3450-490, FT510, LP520) light filters were also used. Bacteria were counted in 30 randomly chosen fields, analyzed using the software “Image tool” version 3.00. A Zeiss Axiovert inverted microscope was used at 400x magnification to count ciliates, flagellates, and microalgae. Aliquots containing 0.5 mL of water were placed in a sedimentation chamber and 50 random fields were counted (Utermohl, 1958). Microorganisms were separated into groups based on size.

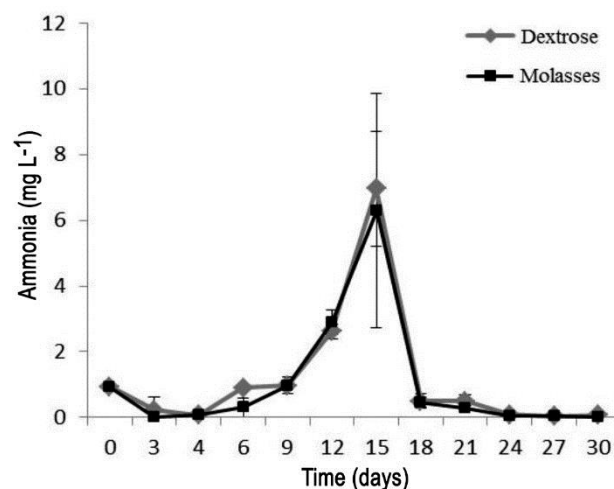
Significant differences ( $P \leq 0.05$ ) of survival, growth, and final biomass were determined using the *t*-test. Prior to the analysis of survival, data were arcsine-square root transformed. A two-way analysis of variance (ANOVA,  $\alpha = 0.05$ ) (time  $\times$  treatment) was used to detect differences of water quality parameters between treatments. All tests were conducted after the confirmation of homogeneity of variances (Lévene's test) and normality distribution of data (Kolmogorov-Smirnov's test).

## RESULTS

Values of water transparency were significantly different between the treatments throughout the experimental period. Decreases in water transparency were observed in the molasses treatment after day 3, whereas for the dextrose treatment, a significant decline was seen only after day 18 (Fig. 1). Except for alkali-



**Figure 1.** Mean ( $\pm$  SD) water transparency (cm) during the culture of *Litopenaeus vannamei* in a biofloc system provided either dextrose or molasses.



**Figure 2.** Mean ( $\pm$  SD) total ammonia concentration ( $\text{mg L}^{-1}$ ) during the culture of *Litopenaeus vannamei* in a biofloc system provided either dextrose or molasses.

nity, nitrite and transparency, there were no significant differences between treatments.

Significant differences in alkalinity were found between treatments after day 12, with the molasses application possessing higher mean alkalinity. Alkalinity in the dextrose treatment decreased on day 12, approximately seven days earlier than in the molasses treatment.

Concentrations of ammonia were not significantly different between treatments throughout the entire study. There was a significant increase in concentration around 15<sup>th</sup> day for both treatments, followed by a decrease on 18<sup>th</sup> day, (Fig. 2). Although significant differences in nitrate were not found between the

treatments, the dextrose treatment exhibited a higher concentration ( $34.81 \pm 22.41 \text{ mg L}^{-1}$ ) on the last experimental day than the molasses treatment ( $14.62 \pm 11.86 \text{ mg L}^{-1}$ ).

The means for physical and chemical water parameters obtained from both treatments are shown in Table 1.

The concentrations of dissolved organic carbon (Fig. 3) were significantly different between treatments on days 9 and 15, and from day 21 onwards, there was no difference between the treatments, although dissolved carbon values were lower in the dextrose treatment. Both treatments showed a significant increase in biofloc volume on the third experimental day. By the end of the experiment, the biofloc concentrations of both treatments were very similar (Fig. 4).

The amount of crude proteins and ashes of biofloc were similar within each treatment. However, the dextrose treatment had higher levels of ethereal extract (1.6%) and 1.7% for molasses and 11.6% crude fiber for dextrose and 9.8% molasses. As concentrations of crude protein was obtained 31.40% and 31.8% for dextrose and molasses respectively, and the ash content of 47.6% and 47.8% for dextrose and molasses respectively.

The dextrose treatment produced shrimp with a larger final weight, which was statistically different from the molasses treatment. The dextrose treatment also resulted in a significantly higher final biomass value than the molasses treatment. The feeding conversion rates (FCR) were significantly lower for the dextrose treatment than for the molasses treatment (Table 2).

Although both treatments have similar microbial colonization, there was a clear distinction in quantification of the microbial community between treatments over time. On day 21, significant differences in density were observed for all groups, but the centric diatoms. However, until day 5 this group had a higher density in the dextrose treatment. In the molasses treatment, there was a positive relationship between coccoid bacteria adhered to the bioflocs and production time, while in the dextrose treatment this relationship occurred with increasing density of filamentous bacteria. The means for microorganisms of both treatments are presented in Table 3.

## DISCUSSION

Bacterial metabolism of carbohydrates removes inorganic nitrogen and produces protein, forming bioflocs of different sizes. These microbial commu-

nities contain bacteria, protozoa and other microorganisms that can serve as potential prey for fish or shrimp (Samocha *et al.*, 2007). Therefore, the use of bioflocs as a natural food source may impact the survival and growth of shrimp. In diatom-rich BFT culture systems, microalgae may serve as a source of nutrients and increase shrimp growth (Abreu *et al.*, 2007). These authors reported that *Farfantepenaeus paulensis* preferentially feed on large centric diatoms.

All parameters of water quality (temperature, salinity, dissolved oxygen, and pH) remained at concentrations recommended for this species. High concentrations of ammonia can retard growth and, in extreme situations, may lead to death of shrimp (Ostrensky & Wasielesky, 1995; Lin & Chen, 2003; Li *et al.*, 2007). Nitrite is an intermediate product of the nitrification of ammonia and the denitrification of nitrate, and increases with time, possibly having a synergistic effect on ammonia. In addition, the accumulation of nitrogen can damage water quality, retard shrimp growth, increase oxygen consumption, and cause high mortality rates (Lin & Chen, 2003).

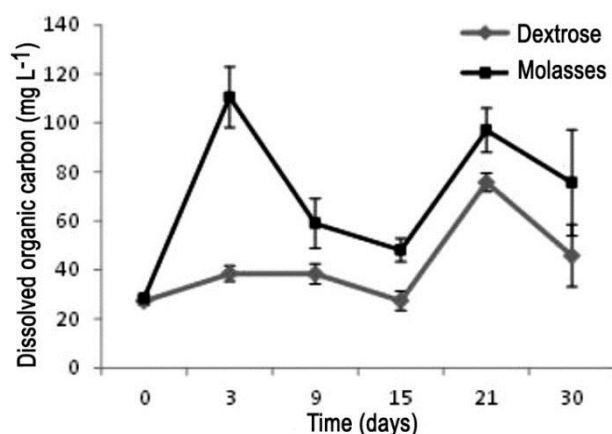
The maximum ammonia levels in this study, which varied between 6 and 7  $\text{mg L}^{-1}$  for both treatments on day 15, may be harmful to shrimp. Nitrite remained above recommended levels for the species in both treatments. According to Van Wyk & Scarpa (1999), the maximum tolerable level of nitrite for *L. vannamei* is 1  $\text{mg L}^{-1}$ . Although a higher nitrite concentration was observed in the molasses than in the dextrose treatment, this factor was not likely the sole cause of the low final weight and survival observed in this treatment.

High nitrification rates can affect the aquaculture system itself as the oxidation rate of ammonia may exceed that of nitrite, hence causing nitrite to accumulate in the environment. A similar process was observed in this study. Compared to other nitrogen compounds, nitrate is of least concern, because shrimp performance is affected only at concentrations above 60  $\text{mg L}^{-1}$  (Van Wyk & Scarpa, 1999). Thus, even the maximum levels observed here ( $\sim 40 \text{ mg L}^{-1}$ ) would not affect shrimp performance.

Bioflocs have a dual function as they absorb accumulated dissolved inorganic nutrients, helping to maintain water quality, and are a food source for reared organisms (Avnimelech, 1999, 2007; Hari *et al.*, 2004; Ballester *et al.*, 2010). Microbial flocs contain high levels of nutrients for the shrimp, such as proteins, fatty acids, and amino acids (Burford *et al.*, 2004). Reported levels of crude protein (about 31%) in microbial flocs by Wasielesky *et al.* (2006) and Emerenciano *et al.* (2011) are consistent to the ones found in this study (31.4% and 31.8% for dextrose and molasses, respectively). However, in this study, the lipid content

**Table 1.** Mean ( $\pm$  SD) physical and chemical variables of water quality from dextrose and molasses treatments in superintensive culture of *Litopenaeus vannamei* in biofloc systems. Different superscripts in the same row denote significant differences ( $P < 0.05$ ).

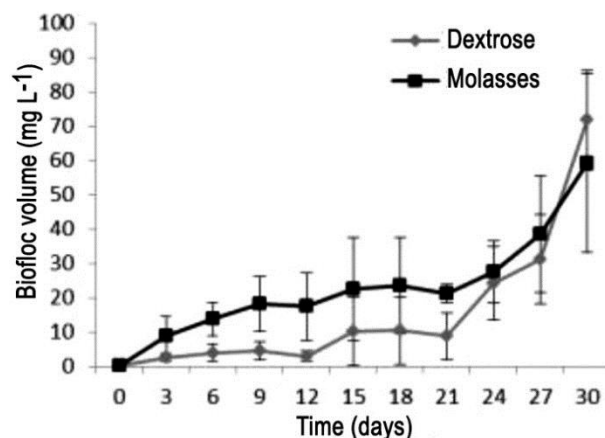
	Dextrose	Molasses
Temperature ( $^{\circ}\text{C}$ )	$26.23 \pm 3.55$	$26.52 \pm 3.56$
Dissolved oxygen ( $\text{mg L}^{-1}$ )	$7.08 \pm 0.8$	$7.07 \pm 0.78$
Salinity	$34.28 \pm 0.94$	$34.30 \pm 0.93$
pH	$7.94 \pm 0.33$	$7.95 \pm 0.32$
Alkalinity ( $\text{CaCO}_3 \text{ mg L}^{-1}$ )	$126.59 \pm 33.7^a$	$143.86 \pm 34.01^b$
Transparency (cm)	$16.59 \pm 3.59^a$	$10.56 \pm 3.19^b$
N-NH <sub>4</sub> <sup>+</sup> ( $\text{mg L}^{-1}$ )	$1.16 \pm 1.96$	$1.03 \pm 2.01$
N-NO <sub>2</sub> ( $\text{mg L}^{-1}$ )	$14.34 \pm 17.02^a$	$17.81 \pm 20.82^b$
N-NO <sub>3</sub> ( $\text{mg L}^{-1}$ )	$15.8 \pm 16.84$	$13.32 \pm 13.58$
PO <sub>4</sub> ( $\text{mg L}^{-1}$ )	$1.22 \pm 1.19$	$1.48 \pm 1.2$



**Figure 3.** Mean ( $\pm$  SD) concentration of dissolved organic carbon ( $\text{mg L}^{-1}$ ) during the super intensive culture of *Litopenaeus vannamei* in a biofloc system provided either dextrose or molasses.

of the bioflocs were considerably higher in the dextrose (1.6%) and molasses (1.4%) treatment in comparison to Wasielesky *et al.* (2006) and Emerenciano *et al.* (2011) (0.47% and 0.49% lipids, respectively). The rather high lipid concentrations observed here are likely caused by the presence of microalgae and ciliates. Microorganisms, especially diatoms, are important sources of lipids and essential fatty acids (Silva *et al.*, 2008).

In this study, a higher feed conversion was observed in the dextrose treatment, indicating that shrimp prefer to feed on this type of microbial community. Again, this was probably due to the presence of a larger number of nutritious diatoms in this treatment (Silva *et al.*, 2008). Results indicated that natural food is an important factor in the performance of *L. vannamei*, suggesting that the nutritional quality of the microbial community grown with organic dextrose is somehow higher. This treatment produced a higher final weight for the shrimp.



**Figure 4.** Mean ( $\pm$  SD) volume ( $\text{mg L}^{-1}$ ) of microbial bioflocs during the culture of *Litopenaeus vannamei* provided either dextrose or molasses.

**Table 2.** Mean ( $\pm$  SD) survival, final weight (g), final biomass (g) and apparent feed conversion rate (FCR) in the culture of *Litopenaeus vannamei* in a static bioflocs system. Different superscripts in the same row denote significant differences ( $P < 0.05$ ).

	Dextrose	Molasses
Survival (%)	$79.11 \pm 11.44$	$68.64 \pm 7.48$
Final weight (g)	$3.49 \pm 0.78^a$	$3.06 \pm 0.81^b$
Final biomass (g)	$298.63 \pm 24.32^a$	$231.58 \pm 15.54^b$
FCR	$1.67 \pm 0.17^a$	$2.21 \pm 0.14^b$

The decrease of microalgae density was probably caused by the lack of light, especially in the molasses treatment, and by shrimp grazing. The increased water transparency in the dextrose treatment was likely a property of the chemical characteristics of dextrose, which is a monosaccharide refined from starch (Lehninger, 1995). These properties allowed more light to reach microalgae in the dextrose treatment. Liquid molasses is a compound sugar (Lehninger, 1995) obtained from glucose residue, so it is less labile than dextrose. Increased water turbidity, and decreasing light penetration, may affect the growth of microalgae. Baloi *et al.* (2012) used molasses as carbon source and observed a reduced penetration of light after the second week trial. The fact that dextrose is a labile source of carbon also benefits the development of heterotrophic bacteria, because this kind of carbohydrate allows bacteria to be the first to use the inorganic nitrogen available in the medium (Ebeling *et al.*, 2006). Thus, in the dextrose treatment, a greater proliferation of microalgae occurred with a simultaneous reduction of chlorophyll-*a*. This observation suggests that phytoplankton were declining, likely through predation by heterotrophic plankton and shrimp. The availability of nutrients and light decreased after the second week.

**Table 3.** Mean ( $\pm$  SD) concentration of microorganisms ( $\text{ind L}^{-1} \times 10^3$ ) in the culture of *Liopenaeus vannamei* in a static biofloc system provided either dextrose or molasses as carbon source. Different superscripts, in the same row denote significant differences ( $P < 0.05$ ).

Days	Dextrose			Molasses		
	9	15	21	9	15	21
Free coccoid bacteria	20700 $\pm$ 1034.7 <sup>a</sup>	1840 $\pm$ 528.3 <sup>a</sup>	3540 $\pm$ 917.7 <sup>a</sup>	5700 $\pm$ 892.1 <sup>b</sup>	8990 $\pm$ 1956.1 <sup>b</sup>	25100 $\pm$ 2504.5 <sup>b</sup>
Small filamentous adhered bacteria	0 <sup>a</sup>	1810 $\pm$ 398 <sup>a</sup>	1490 $\pm$ 491.77 <sup>a</sup>	140.92.17 <sup>b</sup>	1080 $\pm$ 685.55 <sup>b</sup>	637 $\pm$ 241.31 <sup>b</sup>
Large filamentous adhered bacteria	1510 $\pm$ 428.64 <sup>a</sup>	0	0	400 $\pm$ 219.37 <sup>b</sup>	0	0
Flagellates	1.64 $\pm$ 0.39 <sup>a</sup>	2.53 $\pm$ 0.63 <sup>a</sup>	1.2 $\pm$ 0.39 <sup>a</sup>	4.17 $\pm$ 0.9 <sup>b</sup>	3.46 $\pm$ 0.72 <sup>b</sup>	1.74 $\pm$ 0.5 <sup>b</sup>
Small ciliates	0	0.46 $\pm$ 0.3	3.18 $\pm$ 0.78 <sup>a</sup>	0	1.05 $\pm$ 0.68	5.04 $\pm$ 0.96 <sup>b</sup>
Large ciliates	0.67 $\pm$ 0.23 <sup>a</sup>	0.5 $\pm$ 0.25 <sup>a</sup>	0.29 $\pm$ 0.25 <sup>a</sup>	4.5 $\pm$ 0.75 <sup>b</sup>	2.44 $\pm$ 0.54 <sup>b</sup>	1.86 $\pm$ 0.56 <sup>b</sup>
Centric diatoms	13.4 $\pm$ 1.65 <sup>a</sup>	7.25 $\pm$ 0.79 <sup>a</sup>	1.03 $\pm$ 0.37	5.1 $\pm$ 0.88 <sup>b</sup>	3.08 $\pm$ 0.14 <sup>b</sup>	1.4 $\pm$ 0.5

The addition of dextrose and molasses on days 9, 12 and 15 at a ratio of 6:1 (C:N-NH<sub>4</sub><sup>+</sup>) may have influenced the formation of bacterial biomass by removing ammonia from the system. This is seen in the increase in floc volume from the second week onwards, mainly in the dextrose treatment. Moreover, the reduction of ammonia on day 18 indicates that the formation of microbial flocs is caused in part by the immobilization of nitrogen compounds.

Even with the addition of organic fertilizers, a decline in the concentration of dissolved organic carbon was observed on days 9 and 15. These observations precede the immobilization of ammonia, and may be related to the action of heterotrophic organisms. Therefore, the immobilization of nitrogen may have occurred heterotrophically, through the formation of bacterial biomass, and chemo-autotrophically through the development of nitrifying bacteria.

Hari *et al.* (2004) obtained a better feed conversion rate with tapioca flour as a source of organic carbon at a C:N ratio of 20:1. This ratio seems to be effective for *L. vannamei*, cultured with microbial flocs, and has been applied in several other studies (Avnimelech, 1999; Hari *et al.*, 2004; Samocha *et al.*, 2007). In this study, as molasses were added, the fertilization used in phase 1 was found to be sufficient to stimulate the formation of flocs. However, the addition of dextrose increased the floc volume (from 2.93 to 10.13 mL L<sup>-1</sup>) from day 15 onwards. As the conversion efficiency of ammonia did not differ between treatments, this probably occurred due the contribution of microalgae to the removal of ammonia in the dextrose treatment.

Levels of dissolved organic carbon also contribute to the development of bacteria and cyanophytes (Esteves, 1998). Bacteria are responsible for most of the uptake of N and P, and have an advantage over phytoplankton because bacteria assimilate more nutrients due to their higher surface-to-volume ratio (Kirchman, 2000). The use of different carbohydrate sources led to different values of bacterial abundance in both treatments. On day 9, the number of coccoid bacteria in the dextrose treatment was much higher than in the molasses treatment, and there was an inversion in the evolution of abundance values throughout the experiment. The decrease in number of bacteria in the dextrose treatment may represent the consumption of these microorganisms by flagellates, which in turn were consumed by ciliates, a trophic interaction typical of the Microbial Loop concept (Azam *et al.*, 1983). The processes involved in the performance of the microbial community in this study seems to corroborate the claims of Zhukova & Kharlamenko (1999), who say that polyunsaturated fatty acids synthesized by ciliates and flagellates can be processed from the ingestion of bacteria, indicating the occurrence of the microbial loop.

A larger light penetration in BFT systems may improve shrimp performance, but may also result in the presence of potentially harmful organisms, such as filamentous bacteria (Ray *et al.*, 2009). Small ( $\leq 20 \mu\text{m}$ ) filamentous bacteria adhered to the bioflocs appeared on day 15 in the dextrose treatment, a likely result of the higher availability of phosphorus from day 9 onwards (Burford *et al.*, 2003). Moreover, the growth of cyanobacteria (filamentous bacteria) may be related to the addition of dissolved organic carbon in the system (Esteves, 1998) because the abundance of these microorganisms on the last day was preceded by reductions in the levels of organic carbon, suggesting an uptake for the development of these organisms. However, the low number of small ( $\leq 20 \mu\text{m}$ ) filamentous bacteria attached to the bioflocs found in the molasses treatment may have been caused by a N:P ratio harmful to cyanobacteria. This unfavorable ratio may have also promoted the dominance of coccoid bacteria, which may better assimilate nutrients because of their higher surface-to-volume ratio.

The increase in the density of small ciliates in both treatments by the end of the experiment was likely caused by a low predation by shrimp, as they were concentrated in consuming larger ciliates. Thus, there was a decrease in the abundance of large ciliates in both treatments. However, significant differences in the number of large ciliates between the treatments may be caused by a higher rate of consumption of these ciliates by shrimps, indicating a selection process.

## CONCLUSIONS

The differences in the structure microbial community of the biofloc affected overall biomass production. Although the use of molasses is currently well established in BFT systems, a superior growth performance of shrimp was observed when dextrose was used as carbon source.

Albeit more expensive than molasses, the addition of dextrose results in increased water transparency, which may contribute to a higher availability of microbes for shrimp to feed on. Longer-term experiments are needed to observe the stabilization of microbial flocs and the conversion of nitrite to nitrate. Moreover, applying techniques to characterize bacterial groups may help identifying important information about microbial dynamics in BFT culture systems.

## ACKNOWLEDGEMENTS

The authors are grateful for the financial support provided by the National Council for Scientific and

Technological Development (CNPq), Ministry of Fishery and Aquaculture (MPA) and Coordination for the Improvement of Higher Level Personnel (CAPES). Special thanks to Centro Oeste Rações S.A. (GUABI) for the experimental diets. W. Wasielesky Jr., P.C.V. Abreu and E.L.C. Ballester are research fellows of CNPq.

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Received: 4 October 2013; Accepted: 10 February 2015



