



Underwater structures for periphyton in bioflocs tanks for Nile tilapia submitted to feed restriction

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ABSTRACT. The objective of the present work was to assess the possible benefits of the installation of underwater structures for periphyton in bioflocs tanks for Nile tilapia, *Oreochromis niloticus*, submitted to feed restriction (artificial diet). The experimental treatments evaluated were: (1) BFT: bioflocs technology for aquaculture with no feed restriction; (2) BFT-30: the same as (1) but with feed restriction at 30%; (3) BP: bioflocs technology for aquaculture, that is, integration between bioflocs and periphyton in the same rearing unit, with no feed restriction; (4) BP-30: the same as (3) but with feed restriction at 30%. The underwater structures for periphyton in the BP and BP-30 tanks were not able to reduce the concentrations of ammonia, nitrite, nitrate and reactive phosphorus in the water to same respective level found in the BFT and BFT-30 tanks. The underwater structures for periphyton in the BP and BP-30 tanks have not significantly affected fish growth performance. No benefits were obtained by the installation of underwater structures for periphyton in bioflocs tanks submitted to feed restriction on water quality, water microbiology and tilapia growth performance.

Keywords: aquaculture, bioflocs, environmental sustainability.

Estruturas submersas para perifíton em tanques com bioflocos no cultivo de juvenis da tilápia do Nilo submetidos à restrição alimentar

RESUMO. O objetivo do presente trabalho foi avaliar os possíveis benefícios da instalação de estruturas submersas para perifíton em tanques BFT (bioflocos) de juvenis de tilápia do Nilo, *Oreochromis niloticus*, submetidos à restrição alimentar (ração comercial). Os tratamentos experimentais em avaliação foram os seguintes: (1) BFT: tecnologia bioflocos para aquicultura sem restrição alimentar; (2) BFT-30: o mesmo que em (1), exceto pela restrição alimentar de 30%; (3) BP: tecnologia bioflocos para aquicultura, isto é, integração entre bioflocos e perifíton em uma mesma unidade de cultivo, sem restrição alimentar; (4) BP-30: o mesmo que em (3), mas com restrição alimentar de 30%. As estruturas submersas para perifíton nos tanques BP e BP-30 não foram capazes de reduzir as concentrações de amônia, nitrito, nitrato e fósforo reativo da água, além dos respectivos níveis observados nos tanques BFT e BFT-30. As estruturas submersas para perifíton nos tanques BP e BP-30 não afetaram de modo significativo o desempenho animal. A instalação de estruturas submersas para perifíton, em tanques com bioflocos submetidos à restrição alimentar, não trouxe benefícios para qualidade da água, microbiologia da água e desempenho zootécnico da tilápia.

Palavras-chave: aquicultura, bioflocos, sustentabilidade ambiental.

Introduction

In general, fish and shrimp are not able to efficiently use the nutrients present in the artificial diets supplied. Only a minor fraction of the nitrogen and phosphorus contained in the artificial diets is retrieved in the harvested biomass, the majority is lost to the rearing environment (Bauer, Prentice-Hernandez, Tesser, Wasielesky Jr., & Poerch, 2012). One alternative to reduce the waste of nutrients is the bioflocs technology for aquaculture (BFT; Kuhn, Lawrence, Crockett, & Taylor, 2016). BFT

adjusts the C: N ratio of water aiming to produce a biosecure environment. Other benefits are reduced water use and effluent discharges, and lower artificial feed allowances (Wei, Liao, & Wang, 2016). Another alternative is the setting of underwater substrates for periphyton growth, which is a complex community formed by diverse organisms, mainly algae and cyanobacteria. Periphyton can act as a biological filter and as an important source of nutrients for the cultured animals (Liu, Wang, Liu, Tang, & Wu, 2016). A third management able to

increase the feeding efficiency in aquaculture is the restriction of the daily feed allowances to some level below the animal's *ad libitum* intake (Koch, Rawles, Webster, Cummins, & Kobayahi, 2016).

A novel approach is the integration between bioflocs and periphyton in the same rearing unit. Schweitzer et al. (2013) have observed better shrimp performance in BFT tanks with substrates for periphyton. However, in a previous work carried out in our laboratory, the underwater structures for periphyton in BFT tanks were not efficient in removing nitrogen compounds from the water or as a feeding supplement to Nile tilapia, *Oreochromis niloticus*, juveniles (Cavalcante, Lima, Rebouças, & Sá, 2016). In that study, however, regular feed rates were employed and, therefore, a great amount of artificial food was provided to the animals. We hypothesized that the periphyton gains importance as a biological filter and nutritional source in restricted-fed culture tanks. In this sense, the objective was to assess the possible benefits of the installation of underwater structures for periphyton in bioflocs tanks for rearing Nile tilapia submitted to feed restriction on water quality, water microbiology and growth performance.

Material and methods

One-thousand masculinized Nile tilapia juveniles were obtained from a nearby fish farm and transported to the laboratory by road. The study was carried out in twenty 250-L polyethylene outdoor tanks over 10 weeks. Fish (0.99 ± 0.04 g) were stocked in the culture tanks at nine fish per tank (36 fish m^{-3}). The C/N ratio of water in all tanks was adjusted to 15/1 by daily applications of dry molasses to the water, which were based on the feeding management, i.e., percentage of crude protein in the diet and dietary allowances (Schryver & Verstraete, 2009). All tanks were continuously aerated through air stones, silicone hoses and PVC pipes connected to one 2.5 hp air blower.

The experimental controls and treatments were the following: (1) BFT: bioflocs technology for aquaculture with no feed restriction (control 1); (2) BFT-30: the same as (1) but with feed restriction at 30% (control 2); (3) BP: biophyton technology for aquaculture, that is, integration between bioflocs and periphyton in the same rearing unit, with no feed restriction (treatment 1); (4) BP-30: the same as (3) but with feed restriction at 30% (treatment 2).

Two transversely interconnected 0.40 x 0.65 m (height x width) polyethylene boards were vertically set out in the water column of the BP and BP-30 tanks as underwater substrates for periphyton

development (Figure 1). Those boards had a useful area of 0.90 m^2 , which corresponded to 135% of the total surface water area. The polyethylene boards were thoroughly rubbed with a rough sponge before installation in the tanks. Fish were fed on appropriate commercial diets with crude protein content ranging from 43.4 – 49.4% at 0800, 1100, 1400 and 1700. The feeding rates were similar to those used by Oliveira-Segundo, Lima, Akao, and Sá (2013). No water exchange was performed over the entire study, only the replenishment to compensate for evaporation.

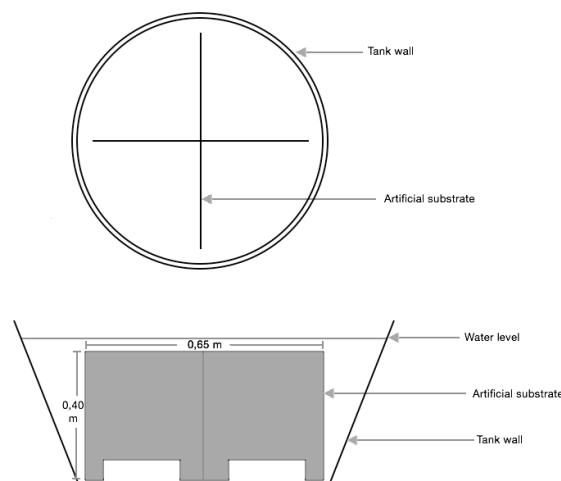


Figure 1. Layout of the Biophyton tank (BP and BP-30).

Water quality of the tanks was monitored by determination of the following variables: (1) pH (pH meter mPA210 - MS Tecnopon), (2) temperature and specific conductance at 0800 and 1500 (conductivity meter CD-4303 - Lutron), (3) dissolved oxygen (0800; dissolved oxygen meter YSI 55), (4) total ammonia nitrogen (TAN; indophenol method), (5) nitrite (sulfanilamide method), (6) nitrate (Cd reduction technique), (7) reactive phosphorus (molybdenum blue method), (8) total alkalinity (titration with H_2SO_4 standard solution), (9) total hardness (titration with EDTA standard solution), (10) organic matter (consumed KMnO_4) and (11) settleable solids (sedimentation in Imhoff cones). After the determinations of N-NO_2^- and N-NO_3^- , the results were multiplied by 3.28 and 4.43, respectively, to determine the concentrations of nitrite and nitrate. After filling each Imhoff cone with 1 L of culture water, there was a 15-min period of interval before the volumes of settleable matter were recorded. The water quality variables were monitored daily (1, 2), weekly (3, 4) and fortnightly (5-12). All water

quality determinations were carried out according to Clesceri, Greenberg, and Eaton (1998).

The periphyton biomass grown on the underwater structures in BP and BP-30 were sampled at the 20th, 40th and 60th days after the onset of the experiment. For that, areas of 10 x 10 cm² were scraped in each structure. The wet periphyton biomass was then put on to dry in an oven at 60°C for 12 hours.

Microbiological analyses of the culture water were performed in the beginning and on the 40th experimental day (soon after the middle of the period), comprising the number of *Aeromonas* sp. (culture on Agar GSP with ampicillin) and *Bacillus* sp. (culture on Agar nutrient). There were five dilutions for each culture medium. The methodology used for the bacteria count was the 'Pour plate' (Rall, Bombo, Lopes, Carvalho, & Silva, 2003). The bacteria colonies were counted after 48 hours of incubation at 37°C. The results were expressed in colony-forming units per milliliter (CFU mL⁻¹).

Growth performance variables analyzed were the followings: survival (%), fish final body weight (g), specific growth rate (% day⁻¹; SGR = [Ln (final weight) - Ln (initial weight)]/days of culture x 100), fish yield (g m⁻³ day⁻¹), feed conversion ratio (FCR = feed consumed/body weight gain), and protein efficiency ratio (PER = weight gain/protein consumed).

The results were tested by one-way ANOVA. When a significant difference was detected between the treatments (p < 0.05), the means were compared two by two with the Tukey's test for equal-variance variables or Games-Howell's test for unequal-variance variables. Assumptions of normal distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test) were checked before

analyses. The SPSS v.15.0 and Windows Excel 2010 software were used for the statistical analyses.

Results and discussion

Water quality

The temperature, specific conductance, and the concentrations of dissolved oxygen and reactive phosphorus in water were not affected neither by the installation of underwater structures for periphyton nor by the feed restriction imposed (p > 0.05; Table 1). Therefore, the rate of phosphorus removal from the water has not increased in any of the periphyton-bioflocs tanks (BP and BP-30), as initially expected. The same was also observed by Haque et al. (2015) with *Macrobrachium rosenbergii* and *O. niloticus*. These results suggest that is worthless to install underwater structures in BFT tanks aiming to reduce concentrations of phosphorus in the water. Regardless of the treatment, the tanks under feed restriction presented higher pH and total alkalinity. Less organic matter means lower concentrations of free CO₂ in water and, consequently, higher bicarbonate levels (Caldini, Cavalcante, Rocha-Filho, & Sá, 2015). On the other hand, the underwater structures for periphyton have not significantly affected the pH, total alkalinity and total hardness of water (Table 1).

There was a decrease in the concentrations of TAN, nitrite, nitrate and organic matter in the tanks under feed restriction (BFT-30 and BP-30). The same was also reported in the study of Haque et al. (2015), where the installation of underwater structures for periphyton in bioflocs tanks was not able to reduce the concentrations of TAN, nitrite, nitrate and organic matter in the water (Table 1).

Table 1. Water quality in tanks after 10 weeks (mean ± S.D.¹; n = 5).

Variable ²	Treatment ³				ANOVA P
	BFT	BFT-30	BP	BP-30	
Temp	28.0 ± 1.3	27.8 ± 0.8	28.0 ± 0.8	27.8 ± 1.0	NS ⁴
pH	7.77 ± 0.16 b ⁴	8.20 ± 0.19 a	7.64 ± 0.21 b	8.17 ± 0.21 a	0.025
SC	738 ± 268	737 ± 130	740 ± 129	730 ± 127	NS
DO ₂	7.78 ± 0.56	7.73 ± 0.54	7.77 ± 0.54	7.57 ± 0.52	NS
TA	87.6 ± 26 b	138.9 ± 32 a	90.4 ± 25 b	131.3 ± 24 a	< 0.001
TH	229.3 ± 39 a	170.7 ± 41 b	227.4 ± 40 a	171.6 ± 38 b	< 0.001
TAN	0.64 ± 0.13 ab	0.41 ± 0.08 c	0.79 ± 0.14 a	0.52 ± 0.12 bc	0.001
NO ₂ ⁻	0.32 ± 0.11 a	0.22 ± 0.07 b	0.36 ± 0.06 a	0.21 ± 0.05 b	< 0.001
NO ₃ ⁻	2.45 ± 0.23 a	1.33 ± 0.22 b	2.29 ± 0.20 a	1.42 ± 0.58 b	< 0.001
P-react	0.39 ± 0.12	0.38 ± 0.19	0.32 ± 0.19	0.34 ± 0.18	NS
Org Mat	246.7 ± 19 a	197.2 ± 12 b	246.1 ± 13 a	195.6 ± 12 b	< 0.001
SS	142.9 ± 12 ab	123.6 ± 19 ab	146.2 ± 12 a	120.4 ± 19 b	0.044

¹These results were obtained in the last monitoring (10th week); ²Temp: temperature (Celsius), SC: specific conductance (μS cm⁻¹), DO₂: dissolved oxygen (mg L⁻¹), TA: total alkalinity (mg L⁻¹ CaCO₃), TH: total hardness (mg L⁻¹ CaCO₃), TAN: total ammonia nitrogen (mg L⁻¹), NO₂⁻: nitrite (mg L⁻¹), NO₃⁻: nitrate (mg L⁻¹), P-react: reactive phosphorus (mg L⁻¹), Org Mat: organic matter (mg L⁻¹) and SS: settable solids (mg L⁻¹); ³BFT: Bioflocs technology for aquaculture with no feed restriction. BFT-30: Bioflocs technology for aquaculture with feed restriction at 30% in relation to BFT. BP: Biophyton technology (bioflocs + periphyton) with no feed restriction. BP-30: Biophyton technology with feed restriction at 30% in relation to BP; ⁴For the same variable, means with distinct letters are significantly different by Tukey's test (p < 0.05); ⁵Non-significant (p = 0.807, 0.983, 0.230 and 0.113 for temperature, SC, DO₂ and P-react, respectively).

Again, it seems meaningless to install underwater structures for periphyton in bioflocs culture tanks aiming to improve their water quality. Moreover, it is suggested that feed restriction is a better management practice than the installation of underwater structures to obtain a cleaner culture water.

Water microbiology

No significant differences were detected between BP and BP-30 for the periphyton biomass formed onto the surface of underwater structures ($p > 0.05$). On average, the dry periphyton biomass was 0.24 ± 0.07 and 0.22 ± 0.06 mg cm⁻² for BP and BP-30, respectively ($n = 5$).

Neither the installation of underwater structures for periphyton nor the feed restriction have affected the *Bacillus* count in the water ($p > 0.05$; Figure 2). On average, the number of *Bacillus* sp. in rearing waters increased up from 60 ± 15 CFU mL⁻¹, at the beginning, to 1155 ± 259 CFU mL⁻¹, in the end of the study. Anand et al. (2014), studying *Penaeus monodon*, have obtained similar results. In that work, the *Bacillus* counts increased 2000+ times over a 60 day culture period. Therefore, the installation of underwater structures for periphyton in the culture tanks was not capable to increase the *Bacillus* counts. The massive presence of *Bacillus* sp. in healthy and productivity culture tanks qualify that genus of bacteria as a worthy source of probiotics for aquaculture (Nayak, 2010).

There was a reduction in the number of *Aeromonas* sp. in water up from 765 ± 93 CFU mL⁻¹, at the beginning, to 160 ± 30 CFU mL⁻¹, in the end of the study. The differences between the treatments for the number of *Aeromonas* in water were not significant ($p > 0.05$; Figure 2). Probably, the reduction in the number of *Aeromonas* was due to the competition with other bacterial groups, such as *Bacillus*. Crab, Chielens, Wille, Bossier, and Verstraete (2010) have also observed a reduction in the number of harmful bacteria in water, such as *Vibrio* and *Aeromonas*, and an increase in the number of beneficial bacteria, such as *Bacillus*, when they stimulated the development of bioflocs in water. Those results suggest that the bioflocs technology can promote a probiotic effect in aquaculture tanks, preventing the outbreaks of harmful bacteria in the culture.

Fish growth performance

Fish survival was not significantly affected by the treatments, averaging $91.6 \pm 6.4\%$. The feed restriction used in the present work has significantly impaired the fish growth performance, with lower

results of final body weight, specific growth rate and fish yield (Table 2). Therefore, the partial withdrawal of the artificial food was not appropriately compensated for the natural food available in the tanks (bioflocs and periphyton). These results indicate that the level of feeding restriction applied herein, i.e., -30%, was excessive or that the bioflocs concentrations in water were not high enough as expected. Possibly, better growth performance results would have been achieved if the adopted feed restriction level had been moderate (10-15%) instead of high. Yet Burford et al. (2004) were able to reduce the feeding allowances of *Penaeus esculentus* post-larvae reared in bioflocs tanks up to 30% without any growth impairment. Therefore, it is suggested that the maximum level of feeding restriction adopted by the fish producer should be directly proportional to the level of bioflocs in the rearing water.

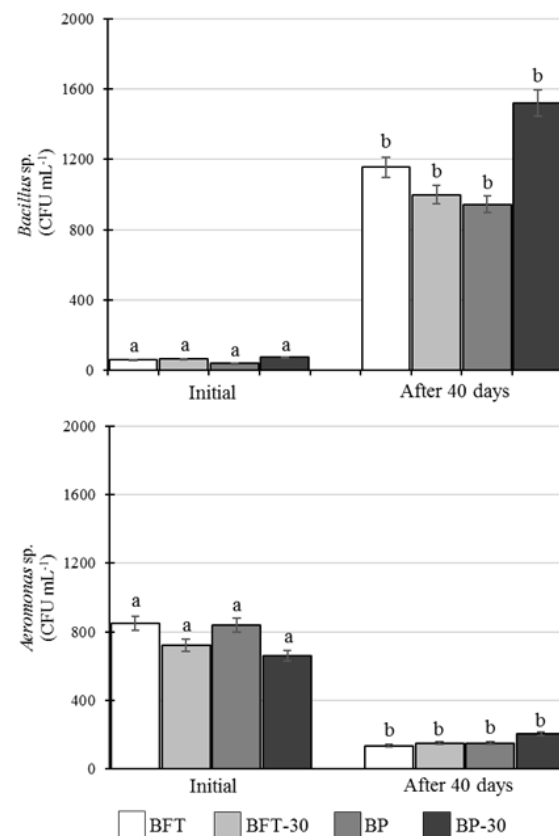


Figure 2. Initial and 40th day number of *Bacillus* and *Aeromonas* in tanks for rearing Nile tilapia after 10 weeks (mean \pm S.D.; $n = 5$). For the same time (initial and final), the bacterial counts of the four treatments were not significantly different from each other by Tukey's test (ANOVA, $p > 0.05$). BFT: Bioflocs technology for aquaculture with no feed restriction. BFT-30: Bioflocs technology for aquaculture with feed restriction at 30% in relation to BFT. BP: Biophyton technology (bioflocs + periphyton) with no feed restriction. BP-30: Biophyton technology with feed restriction at 30% in relation to BP.

Table 2. Growth performance of Nile tilapia juveniles (initial body weight = 0.99 ± 0.04 g) submitted to different rearing systems for 10 weeks (mean \pm S.D.; n = 5).

Variable ¹	Treatment ²				ANOVA P
	BFT	BFT-30	BP	BP-30	
Surv	93.3 \pm 6.1	91.1 \pm 9.3	93.3 \pm 6.1	88.9 \pm 13.6	NS ³
FBW	36.6 \pm 3.9 a ⁴	24.6 \pm 2.4 b	37.7 \pm 1.4 a	28.1 \pm 2.7 b	< 0.001
SGR	5.57 \pm 0.20 a	4.95 \pm 0.18 b	5.57 \pm 0.08 a	5.12 \pm 0.09 b	< 0.001
FY	19.1 \pm 1.4 a	12.3 \pm 1.0 b	19.7 \pm 1.4 a	13.7 \pm 1.9 b	< 0.001
FCR	0.95 \pm 0.08 a	0.86 \pm 0.11 b	0.91 \pm 0.08 ab	0.78 \pm 0.05 b	0.026
PER	2.54 \pm 0.20 b	2.83 \pm 0.35 ab	2.63 \pm 0.22 ab	3.08 \pm 0.19 a	0.018

¹Surv: survival (%), FBW: final body weight (g), SGR: specific growth rate (% day⁻¹) = (ln final body weight - ln initial body weight)/days of culture x 100, FY: fish yield (g m⁻²day⁻¹), FCR: feed conversion ratio = food intake/body weight increase, PER: protein efficiency ratio = body weight gain/protein intake; ²BFT: Bioflocs technology for aquaculture with no feed restriction. BFT-30: Bioflocs technology for aquaculture with feed restriction at 30% in relation to BFT. BP: Biophyton technology (bioflocs + periphyton) with no feed restriction. BP-30: Biophyton technology with feed restriction at 30% in relation to BP; ³Non-significant (p = 0.852); ⁴For the same variable, means with distinct letters are significantly different from each other by Tukey's test (survival, FBW, SGR, FY and PER) or Games-Howell's test (FCR; p < 0.05).

The installation of underwater structures for periphyton in BFT tanks has not significantly affected any of the growth performance variables, including FCR and PER (e.g. compare BFT vs. BP and BFT-30 vs. BP-30; Table 2). Arnold, Coman, Jackson, and Groves (2009) have also observed the same lack of response from tiger shrimp, *P. monodon*. Nevertheless, Schweitzer et al. (2013) found a positive effect of underwater structures for periphyton on *L. vannamei* growth. Therefore, the benefits of the installation of underwater structures in BFT tanks will depend on the definite technical parameters applied to the culture system, such as the quantity and the quality of the periphyton developed on the structures.

Conclusion

No benefits were achieved by the installation of underwater structures for periphyton in bioflocs tanks under feed restriction on water quality, water microbiology and tilapia growth performance.

Acknowledgements

We would like to thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasília, DF, Brazil), Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasília, DF, Brazil) and Funcap (Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico, Fortaleza, CE, Brazil) for providing scholarship and financial support.

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Received on August 17, 2016.

Accepted on November 8, 2016.

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