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Aquaculture/ Original Article

Periphyton use on microbial dynamics, water quality, and Nile tilapia growth in rearing tanks

Abstract – The objective of this work was to evaluate the effect of the use of an artificial substrate for periphytic biofilm growth on the microbiological composition of the biofilm, water quality, and zootechnical performance of Nile tilapia (*Oreochromis niloticus*) in rearing tanks. The experiment consisted of two treatments: presence and absence of artificial substrate for the growth of periphyton, with five replicates. Water quality parameters were evaluated throughout the entire experimental period. Every two weeks, samples of the periphyton were collected for the analysis of its microbiological composition and biometric measurements of the fish were performed. The number of heterotrophic bacteria in the biofilm increased during the experimental period, but that of *Aeromonas* decreased with the use of the artificial substrate. The total ammonia nitrogen in the tanks with periphyton reduced in 30% compared with that of the control. Tilapia reared with the biofilm showed a final weight 2.4 times greater than that of those subjected to the control, as well as an increase of 59.19% in their productivity. The use of an artificial substrate for periphytic biofilm in Nile tilapia rearing tanks favors the maintenance of the quality of the culture water, the protection of the fish against pathogens, and the improvement of the zootechnical performance of the fish.

Index terms: *Oreochromis niloticus*, biofilm, microorganisms.

Uso de perifíton na dinâmica microbiológica, na qualidade da água e no crescimento de tilápia-do-nilo em tanques de cultivo

Resumo – O objetivo deste trabalho foi avaliar o efeito do uso de substrato artificial para o crescimento de biofilme perifítico sobre a composição microbiológica do biofilme, a qualidade da água e o desempenho zootécnico de tilápia-do-nilo (*Oreochromis niloticus*), em tanques de cultivo. O experimento consistiu de dois tratamentos: presença e ausência de substrato artificial para o crescimento de perifíton, com cinco repetições. Os parâmetros de qualidade de água foram avaliados durante todo o período experimental. A cada duas semanas, foram coletadas amostras do perifíton para análise da sua composição microbiológica e realizadas medidas biométricas dos peixes. A quantidade de bactérias heterotróficas no biofilme aumentou durante o período experimental, mas a de *Aeromonas* reduziu com o uso do substrato artificial. O nitrogênio amoniacal total dos tanques com perifíton reduziu 30% em relação ao do controle. As tilápias criadas com o biofilme apresentaram peso final 2,4 vezes maior que às submetidas ao controle, além de aumento de 59,19% na sua produtividade. O uso de substrato artificial para a formação de biofilme perifítico em cultivo de tilápia em tanques favorece a manutenção da qualidade da água de cultivo, a proteção dos peixes contra patógenos e a melhora do desempenho zootécnico dos peixes.

Termos para indexação: *Oreochromis niloticus*, biofilme, microrganismos.

Introduction

Aquaculture continues to grow at a faster pace than other food-producing sectors and has been counterbalancing the deficit in fishing supplies (FAO, 2018). However, to attain high productivity, it is necessary to develop an increasingly elaborate and complete feeding for fish. Artificial feed is used almost exclusively, which increases production costs and generates more polluting effluents due to its high content of phosphorus and nitrogen (Cyrino et al., 2010; Bentzon-Tilia et al., 2016). Another problem associated to intensive fish farming is the development of diseases, attributed to a high population density, a consequent increase of organic matter, and a decrease of dissolved oxygen levels (Hirsch et al., 2006; Cyrino et al., 2010).

New technologies have been used to improve feed, water quality, and effluents in fish farms. One of them is using microbial communities developed with the nutrients available in these farms as a natural food source. These communities can grow aggregated on substrates submerged in the culture environment and are called periphyton or periphytic biofilm (Silva et al., 2016). Periphyton is defined as a group of microorganisms formed by algae, bacteria, fungi, aquatic invertebrates, and protozoa, growing on any submerged substrate and debris (Azim et al., 2003; Pompêo & Moschini-Carlos, 2003; Martínez-Córdova et al., 2015; Kumar V. et al., 2019).

The induced formation of periphytic biofilm on the surfaces introduced in a culture environment is already being used to increase productivity and supply food for the cultured organisms. Periphytic biofilm cuts back the excess of nutrients, as nitrogen compounds and assimilates, converting them into microbial protein, which provides a source of organic carbon for the system, maintaining water quality (Kolek et al., 2019) and generating natural food for the cultured organisms (Khatoon et al., 2007; Pandey et al., 2014), reducing the feed conversion ratio (FCR) of the culture system (Kumar et al., 2019).

The microbial composition and diversity of periphytic biofilm play an important role in its resistance to external environmental stresses and in maintaining its functions, including pollutant removal (Wu et al., 2011; Tang et al., 2018). However, the nutritional quality of the periphyton as feed for cultivated animals represents a setback for the use of this kind of biomass in culture tanks, particularly due to the variability and instability of the microbiota installed on the substrates. The composition of such microbial communities is, consequently, variable and influenced by biotic and abiotic factors in water, as well as by the substrate used as a base for the fixation and growth of the microorganisms (Silva, 2018). In addition, spontaneously formed periphyton might contain harmful microorganisms and even ones that are pathogenic or toxic to the cultured organisms (Canada et al., 2020).

Therefore, the application of this technology as an effective strategy for nutritional complementation in cultures depends on the selection and maintenance of desirable components in the formed microbial community. In order to manipulate these microorganisms, it is necessary to understand these biological systems, identifying the microorganisms involved in the spontaneous formation of periphyton and understanding the role of the various microbial constituents in the development of the periphytic structure, besides their ecological interrelationships.

The objective of this work was to evaluate the effect of the use of an artificial substrate for periphytic biofilm growth on the microbiological composition of the biofilm, water quality, and zootechnical performance of Nile tilapia (*Oreochromis niloticus*) in rearing tanks.

Materials and Methods

A 60-day study was carried out, in September 2016, at the fish farming station of the Fisheries Engineering Department of Universidade Federal do Ceará, located in Fortaleza, in the state of Ceará, Brazil. The experimental system consisted of ten polyethylene 250 L tanks, assigned to the following treatments: juvenile Nile tilapia farmed in the presence (periphytic biolfim, PB) and absence (green water, GW) of artificial substrate for periphytic biofilm growth. The experiment was conducted in a completely randomized design with two treatments and five replicates. Two polyvinyl chloride (PVC) plates (0.40 m height × 0.65 m width) were used as an artificial substrate. The plates had a useful area of 0.90 m^2 and were vertically set out in the water column, corresponding to 135% of the total surface water area. The culture was a static outdoor system, in the presence of sunlight (natural photoperiod), without water renewal and filtration, only replacement to compensate for evaporation. The tanks of both treatments were naturally fertilized by the food wastes and fish feces generated by the system. Alkalinity correction products were not used.

Nile tilapia juveniles with an initial body weight of about 1.0 g were conditioned in the 250 L tanks at a density of 24 fishes per cubic meter. The fish were fed four times a day at 8:00 a.m., 11:00 a.m., 2:00 p.m., and 4:00 p.m. Two forms of commercial feed (Guabi Nutrição e Saúde Animal S.A., Sales Oliveira, SP, Brazil) were used: in powder, with 46.18% crude protein; and in 2.0–3.0 mm pellets, with 32.37% crude protein. The feeding rate started at 10% and was reduced according to the weight gain of the fish.

The following water physicochemical properties were measured weekly, totaling eight samples: pH, using the mPA-210 meter (MS Tecnopon Equipamentos, Piracicaba, SP, Brazil); temperature, by a portable thermometer; dissolved oxygen, with the 55 dissolved oxygen instrument (YSI Inc./Xylem Inc., Yellow Springs, OH, USA); total alkalinity, through titration with H_2SO_4 solution; total hardness, by titration with EDTA solution; total ammonia nitrogen (TAN), with the indophenol method; nitrite, by the reaction of sulphanilamide with N-1-naphthyl ethylenediamine dihydrochloride; nitrate, using the cadmium column reduction method; and reactive phosphorus, by the molybdenum blue method.

Biometry measurements of the fish were performed every 15 days to establish a growth curve and adjust the feed. The following variables of zootechnical performance were obtained: survival (%), final weight (g), final length (cm), specific growth rate (%), productivity (g m^{-3} per day), FCR, protein efficiency rate (%), and evenness index (%).

The first periphyton sample was collected right after the visual observation of biofilm formation on the artificial substrates, which happened ten days after the beginning of the culture, and sampling continued every two weeks. The periphyton samples were scraped off a 10 cm^2 area on the substrate, stored in an amber glass bottle, and transported in an isothermal box to the Fisheries and Environmental Microbiology laboratory of Universidade Federal do Ceará for the microbiological composition analysis.

The quantification of culturable heterotrophic bacteria (CHB) and of the *Aeromonas* genus was conducted through the plate counting method using the pour plate technique (Huguet & Ribas, 1991; Apha, 2000). Fungi were quantified using the spread plate technique. For an estimation of the nitrogen-fixing, nitrifying, and denitrifying bacterial populations, the most probable number (MPN) technique was used, besides the multiple tubes method (Marin et al., 2012) with modifications.

The results of water quality and zootechnical performance were statistically analyzed using the analysis of variance for completely randomized designs. Whenever statistically significant differences ($p<0.05$) were observed between treatments, averages were compared two by two, using Tukey's test. Statistical analyses were performed with the BioStat, version 5.0 (AnalystSoft, Walnut, CA, USA), and Excel 2010 (Microsoft Corporation, Redmond, WA, USA) software.

Results and Discussion

Water temperature, pH, alkalinity, and orthophosphate were similar between the treatments with and without substrate for periphytic biofilm growth (Table 1). The obtained values are within the acceptable range for tilapia growth (El-Sherif & El-Feky, 2009a, 2009b). Aquaculture environments with high alkalinity maintain the pH stable throughout the day, without oscillations (Mercante et al., 2011), preventing the occurrence of stress in the cultured organisms. In addition, the photoautotrophic microorganisms present in the periphytic biofilm consume CO_2 during the photosynthetic process and induce an increase in the underlying water pH (Wu et al., 2018).

The concentration of dissolved oxygen showed a stable pattern in the tanks used for both treatments, always being above 4.0 mg L^{-1} (Table 1). However, in the last week, oxygen concentration in the water of the PB treatment reached a higher value of 10.9 mg L^{-1}. This high concentration of oxygen is due to the phytoplankton present in the water column and fixed to the substrate, helping to incorporate the gas into the water during the day.

Total hardness concentration, however, differed between treatments (Table 1). The water in the GW tanks showed a higher hardness of 185.4 mg L^{-1} eq. $CaCO_3$. However, the values obtained for both treatments were

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above the reference levels for aquaculture, which are between 60 and 150 mg L^{-1} $CaCO_3$ (Sá, 2012).

The average values for TAN and nitrate were 4.005 and 1.358 mg L^{-1} in the GW treatment, higher than those of 1.20 and 0.003 mg L^{-1} in the PB tanks, even though the concentration of nitrite did not vary significantly in the culture water, regardless of the used treatment (Table 1). Viau et al. (2013) also observed a significant decrease in ammonia and nitrite concentrations in the culture of *Farfantepenaeus brasiliensis* (Latreille, 1817) shrimp when the periphytic biofilm treatment was used, in addition to artificial feed. The presence of the periphytic biofilm in the culture water helps in the removal of nitrogen compounds due to the presence of nitrifying and heterotrophic bacteria fixed to the substrates and of the algae community (Thompson et al., 2002; Ebeling et al., 2006; Anand et al., 2013b).

Some authors have noted the advantages of adding substrates to fishing farms, in order to stimulate the growth of nitrifying bacteria (Thompson et al., 2002; Furtado et al., 2015). Since the substrates added to the tanks are positioned in the water column where the dissolved oxygen is more readily available, the nitrification process is accelerated (Milstein et al., 2009; Asaduzzaman et al., 2008).

The concentration of TAN during tilapia culture presented two peaks in the PB treatment, on the second and seventh weeks (Figure 1). Despite this, overall, a stable pattern was observed for this treatment, without a great oscillation. The peak in the seventh week, of 1.35 mg L^{-1}, can be explained by the lower MPN of nitrifying bacteria (Table 2). In the last week, a decrease in the concentration of TAN was observed, coinciding with the increase in the MPN of nitrifying bacteria.

In the GW treatment, there was a steady increase in TAN from the fourth week onwards, reaching the maximum values of 3.95 and 4.05 mg L^{-1}, respectively, in the two last weeks (Figure 1). The presence of a substrate in the culture system resulted in a lower concentration of TAN and in its higher stability, as there was no great fluctuation. The TAN values started low in the GW tanks, at 0.79 mg L^{-1}, increasing as time passed, but increased in the water when there was a higher entry of organic matter due to feed.

The nitrite concentration in the PB treatment reached the highest value in the first week, followed by a steep decrease (Figure 1), which coincided with the decrease in TAN concentration, showing the action of nitrifying bacteria. However, in the GW system, the nitrite concentration did not vary, being stable from the third week until the end of the culture period. The high nitrite values in the tanks with periphyton are indicative of the presence and continuous activity of the biofilm nitrifying groups.

The nitrate concentration was lower in the water of the PB system than in that of GW (Figure 1). This result confirms that the nitrification process was completely carried out, with the transformation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB) and of nitrite to nitrate by nitrite-oxiding bacteria (NOB) (Martins et al., 2019).

Table 1. Variables indicating the quality of the water of the culture of Nile tilapia (*Oreochromis niloticus*) juveniles, subjected to the green water (GW) and periphytic biofilm (PB) treatments, after eight weeks (average ± standard deviation; n = 5).

Variable	Treatment[(1)]		p-value
	GW	PB	
Temperature (ºC)	27.4±0.1	27.7±0.3	0.09
pH	8.1±0.07	8.2±0.2	0.65
Total alkalinity (mg L^{-1} eq. $CaCO_3$)	159.1±4.7	165.2±9.5	0.185
Total hardness (mg L^{-1} eq. $CaCO_3$)	185.4±5.5a[1]	171.2±9.5b	<0.01
Dissolved oxygen (mg L^{-1})	9.86±0.4b	10.9±0.7a	<0.01
Total ammonia nitrogen (mg L^{-1})	4.005±0.084a	1.20±0.35b	<0.01
Nitrite (mg L^{-1})	0.0002±0.0001	0.0002±0.00001	0.892
Nitrate (mg L^{-1})	1.358±0.18a	0.003±0.0002b	<0.01
Orthophosphate (mg L^{-1})	0.694±0.0001	0.689±0.005	0.749

[(1)]Means followed by equal letters in the line do not differ by Tukey's test, at 5% probability.

The fish survival rate was not affected by the used treatments (Table 3), which did not differ significantly from each another, showing a rate above 90%. A similar result was reported by Asaduzzaman et al. (2009b), while Uddin et al. (2009) found a lower survival rate, below 76%, for tilapia culture with the addition of substrate plus artificial feed.

At the end of the experimental period, the tilapia cultured in the PB treatment showed a final weight 2.4 times greater than that of the juveniles cultured without periphyton (Table 3). The positive effect of periphyton on final body weight shows that the adherence of particulate organic matter (such as fixed bacteria, microalgae, and protozoa) to the substrate contributed to the growth of the confined organisms (Arnold et al., 2006), working as a supplementary source of natural food (Uddin et al., 2009).

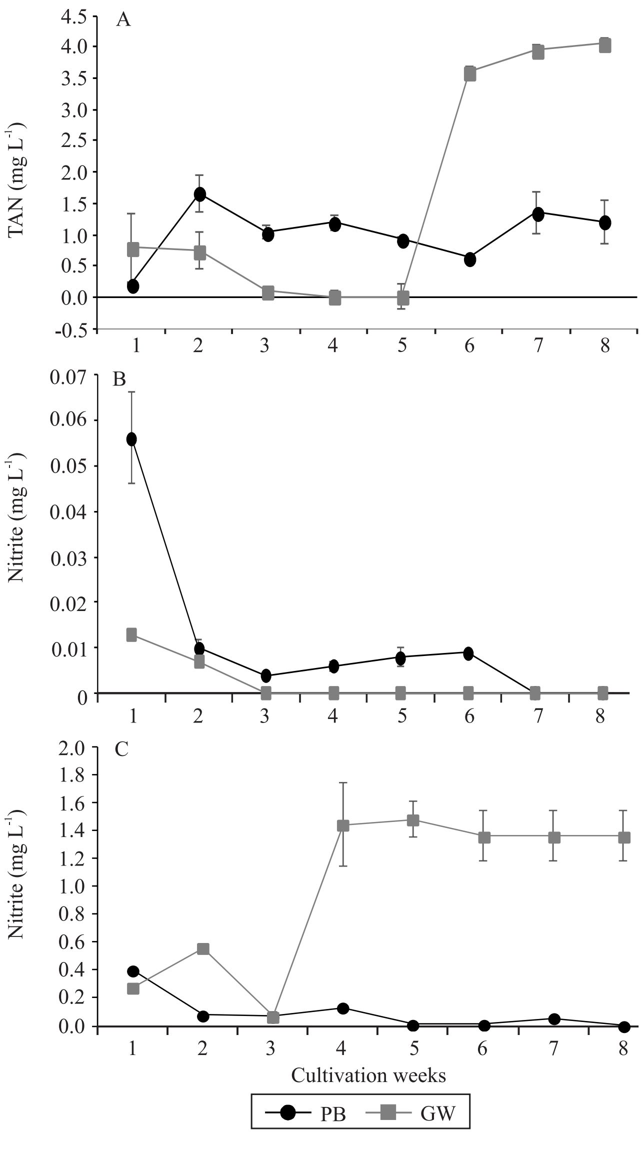

Figure 1. Concentration of total ammonia nitrogen (TAN) (A), nitrite (B), and nitrate (C) during eight weeks of the culture of juvenile Nile tilapia (*Oreochromis niloticus*) in the green water (GW) and periphytic biofilm (PB) treatments (average ± standard deviation; n = 5).

The values obtained for specific growth rate and protein efficiency rate were 11.39 and 39.16% higher, respectively, for the tilapia confined in the PB tanks (Table 3); however, FCR was 30.30% lower, differing between treatments. The addition of substrate to the tanks influences the performance and efficiency of nutrient conversion (Sakr et al., 2015), directly affecting fish development. As previously mentioned, the microorganisms present in the culture tanks convert the inorganic nitrogen in water and make it available to fish in the form of microbial protein (Kolek et al., 2019), increasing protein conversion from 20–25 to 45%. Some studies suggest that periphyton ingestion raises the activity of intestinal enzymes, providing a higher amount of protein to fish (Mridula et al., 2005; Anand et al., 2013a; Gangadhar et al., 2017).

In the productivity analysis – one of the most crucial variables to aquaculture –, the obtained numbers were significantly higher, over 59.19%, in the PB tanks (Table 3). Uddin et al. (2009) reported a productivity 59% higher when adding artificial substrate for periphyton growth in the culture. However, no significant difference was observed between treatments for the evenness index, which was above 70% in both of them (Table 2). This shows that the addition of PVC plates did not influence negatively the well-being of the tilapia; on the contrary, the plates possibly reduced the competition for food between the fish (Marques et al., 2003), contributing to an even growth.

The length of the tilapia was 1.06 cm longer in the treatment with periphyton. Therefore, the presence of extra natural food in the tanks resulted in greater weight and length gains.

An expressive amount of colony forming units (CFU) – 425.0×10^3 per millimeter – was verified for cultivable heterotrophic bacteria (CHB) in the initial culture period. However, a decrease occurred in the second sampling at 30 days, followed by an increase of 250.0×10^3 and 800.0×10^3 CFU mL^{-1} in the third and last samplings, at 45 and 60 days, respectively. This increase in CHB in the culture throughout time is relevant to tilapia farming, as this bacterial group has a fast growth rate and is an important source of nutrients for omnivorous species (McGraw, 2002).

According to Asaduzzaman et al. (2009a), a high concentration of CHB – 3.06×10^7 CFU g^{-1} in their study – leads to an increase in organic matter decomposition, contributing to the liberation of inorganic nutrients, which stimulate bacterial development. The positive effects are observed in the quality of the culture water, as well as in the extra protein food source for the cultured organisms. In their work, Anand et al. (2013b) detected a gradual growth of CHB in the periphyton during the experimental period. Kumar V. et al. (2017) observed values equal to 3.6×10^5 CFU mL^{-1} CHB in the water column in the presence of periphyton, which they attributed to two main factors: type of organic matter present and grazing pressure.

The number of *Aeromonas* groups declined over time, with a value of 9.55×10^3 at the begging of the experiment and of <10.0 CFU mL^{-1} (estimate considering the highest concentration) in the last sampling period (Table 2). The decrease of this bacterial group in the biofilm is advantageous for the tilapia culture, as these bacteria are known for containing various potentially virulent species (Hu et al., 2012), which cause infection outbreaks. In the present study, the presence of periphytic biofilm in the culture environments reduced the occurrence of pathogens (Thompson et al., 2002). Contrarily, Silva et al. (2016) did not detect the species from the genus *Aeromonas* during the cultivation of tilapia in the presence of periphyton.

Fungi increased until the thirtieth culture day (second sampling), reaching 135.0×10^3 CFU mL^{-2}

Table 2. Quantification of the culturable heterotrophic bacteria (CHB), *Aeromonas* spp., and fungi, as well as of the nitrogen-fixing, nitrifying, and denitrifying bacteria, present in the periphyton samples from the culture of Nile tilapia (*Oreochromis niloticus*) juveniles[(1)].

Group	Unit[(1)]	Sample			
		First	Second	Third	Fourth
CHB	CFU mL^{-1}	425.0×10^3	88.0×10^3	250.0×10^3est	800.0×10^3
Aeromonas spp.	CFU mL^{-1}	9.55×10^3	60.0 est[(2)]	35.0 est[(2)]	<10 est[(2)]
Fungi	CFU mL^{-1}	420.0	135.0×10^3	2.5×10^3	1.295×10^3
N_2 fixing	MPN mL^{-1}	11.0	240.0	<1.8	<1.8
Nitrifying	MPN mL^{-1}	13.0×10^2	79.0	38.0	470.0
Denitrifying	MPN mL^{-1}	<1.8	<1.8	<1.8	<1.8

[(1)]CFU, colony forming unit; and MPN, most probable number. [(2)]est, estimate obtained considering the highest concentration.

Table 3. Zootechnical performance of Nile tilapia (*Oreochromis niloticus*) juveniles cultured in the presence and absence of periphytic biofilm (average ± standard deviation; n = 5)[(1)].

Variable[(2)]	Treatment		p-value
	Green water (GW)	Periphytic biofilm (PB)	
Survival (%)	97.0±7.47	97.0±7.47	0.995
Initial body weight (g)	0.89±0.18	1.2±0.37	0.126
Final body weight (g)	15.74±2.09b	38.48±2.49a	<0.01
Final length (cm)	9.76±0.29b	10.37±0.32a	<0.05
SGR (% per day)[(2)]	4.9±0.36b	5.53±0.35a	<0.01
Productivity (g m^{-3} per day)	6.28±0.83b	15.39±0.99a	<0.01
FCR[(3)]	1.32±0.16b	0.80±0.05a	<0.01
PER[(4)]	1.74 0.19b	2.86±0.20a	<0.01
Evenness index (%)	75.04±10.27	71.22±7.04	0.767

[(1)]Means followed by equal letters, in the lines, do not differ by Tukey's test, at 5% probability. [(2)]SGR, specific growth rate = [(Ln final weight - Ln initial weight)/culture days] × 100. [(3)]FCR, feed conversion ratio = offered feed (g)/gain in body weight (g). [(4)]PER, protein efficiency rate = weight gain (g)/protein consumed (g).

(Table 2). After this period, their amount decreased until the end of the culture period, reaching 1.29×10^3 CFU mL^{-1}. According to Tant et al. (2015), aquatic fungi are important for processing organic matter and influencing the transformation and conversion of pieces of particulate organic matter into dissolved ones. Moreover, fungi, such as yeasts, can be a source of protein food, presenting probiotic and immunostimulatory effects for cultured aquatic organisms (Wijayanti et al., 2020).

Differently from CHB, there was a decrease in the concentration of nitrifying bacteria. Heterotrophic bacteria have a growth rate and biomass production ten times greater than those of nitrifying bacteria (Furtado et al., 2015). This generates an unfavorable competition, as CHB will be present in a larger concentration and, consequently, will consume a higher amount of nutrients and dissolved oxygen, while also demanding more space. Therefore, the addition of a substrate for the development of periphyton stimulates the growth of nitrifying bacteria by supplying a larger area and fixed organic matter. The importance of substrate addition in fish farms to stimulate bacterial growth has already been highlighted by Asaduzzaman et al. (2010) and Furtado et al. (2015).

Nitrogen-fixing bacteria presented higher values in the first and second samples. The activity of these bacteria contributed to the increase in the ammonia levels in the water of the culture tanks. The nitrifying bacterial group, responsible for the oxidation of ammonia to nitrite and later of nitrite to nitrate, showed a high value of 13.0×10^2 MPN mL^{-1} in the first sampling at 15 days, decreasing at the second and third samplings, but increasing again to 470 MPN mL^{-1} in the last sampling period at 60 days (Table 2). These results are an indicative of the presence of two main groups responsible for the nitrification process: autotrophic bacteria, known as AOB and NOB; and nitrifying heterotrophic bacteria. Santos et al. (2019) found a greater number of nitrifying bacteria, compared with heterotrophic bacteria, in artificial substrates (bioballs) in the culture of pacific white shrimp (*Litopenaeus vannamei* Boone, 1931).

The low presence of denitrifying bacteria (<1.8 MPN mL^{-1}) in the culture period might be a consequence of the efficiency of oxygenation in the tanks, since this bacterial group transforms nitrite or nitrate in molecular nitrogen in the absence of oxygen (Jiménez-Ojeda et al., 2018). According to Li et al. (2017), a mature periphytic biofilm supplies a microbial habitat that contains both aerobic and anaerobic conditions, providing the necessary environment for the biological removal of nitrogen by aerobic nitrification, anaerobic denitrification, and anaerobic ammonium oxidation. Despite the low quantification of denitrifiers in the tanks with periphyton, there was an efficient removal of nitrate in this system, considering the low concentrations of this substance in the PB treatment.

Conclusions

1. The use of an artificial substrate for periphytic biofilm in Nile tilapia (*Oreochromis niloticus*) rearing tanks favors the maintenance of the quality of the culture water, the protection of the fish against pathogens, and the improvement of the zootechnical performance of the fish.
2. The formation of a native periphytic biofilm on an artificial substrate surface in tilapia culture tanks presents a temporal succession of distinct microbial groups.
3. Groups of nitrifying bacteria that are components of the periphytic biolfim maintain water quality in the tilapia culture tanks.

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