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Technical Article

Biofloc production in activated sludge system treating shrimp farming effluent

Produção de bioflocos em sistema de lodos ativados tratando efluente de carcinicultura

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ABSTRACT

The release of wastewater and the shrimp feed cost are the main challenges faced by the shrimp farming industry. An alternative solution to both problems is biofloc production in a unit external to the farm, in an activated sludge system for effluent treatment. The treatment system's influent was composed of the shrimp farm wastewater supplemented with urea and sugarcane molasses. The results show that the average removal of chemical oxygen demand was 71% and the average biofloc production in the reactor was approximately 1.5g.L^{-1}. Adding molasses to the influent contributed to the increase in the quantity and diversity of existing microorganisms that are beneficial to cultured shrimp. The mass balance of nitrogen compounds confirmed that nitrification occurred in the system. Therefore, the use of the activated sludge system is a viable and environmentally suitable alternative to produce bioflocs and shrimp farming effluent treatment.

Keywords: Microbial flocs, nutritional supplements, *Litopenaeus vannamei*, wastewater treatment, biological systems.

RESUMO

A geração de efluentes e o custo com a alimentação do camarão são os principais desafios enfrentados pela carcinicultura. Uma solução conjunta para ambos os problemas é a produção de bioflocos em um sistema de lodo ativado para tratamento de efluentes. Neste trabalho, o afluente ao sistema estudado era composto pelas águas residuais do cultivo de camarão suplementadas com ureia e melaço de cana-de-açúcar. Os resultados mostraram que a remoção média de demanda química de oxigênio foi de 71% e a produção média de bioflocos no reator foi de aproximadamente 1,5 g.L^{-1}. A adição de melaço ao afluente contribuiu para o aumento da quantidade e da diversidade de microrganismos benéficos para a produção de camarão. Houve remoção de amônia e nitrificação confirmada pelo balanço de massa. Portanto, a utilização do sistema de lodos ativados é uma alternativa viável e ambientalmente adequada para produzir bioflocos e tratar efluentes de cultivo de camarão.

Palavras-chave: flocos microbianos; suplemento nutricional para camarão; *Litopenaeus vannamei*; tratamento de águas residuais; processo biológico.

INTRODUCTION

Shrimp is one of the most important commodities in the global seafood market (LEUNG & ENGLE, 2008). According to the FAO report (2015), 2×10^6 tons of shrimp were produced in 2015 in culture farms, located mainly in China, Thailand, Indonesia, India, Vietnam, Brazil, Ecuador, and Bangladesh (WWF, 2016).

However, this economic activity depends on the availability of a large amount of good-quality water, which has an impact on the estuarine aquatic environments. Environmental degradation occurs as a result of the intensive use of mangrove areas that are converted into farming areas, thereby causing the extinction of ecosystems that are essential for aquatic life (EMERENCIANO; GAXIOLA; CUZON, 2013).

In conventional shrimp farming, the quality of the culture environment is ensured by the continuous exchange of water in the farming ponds during the shrimp-fattening period (HERBECK *et al.*, 2013). Effluent water generated in the process of harvesting contains high levels of nutrients, suspended solids, organic matter, salts, microorganisms, and other chemical substances that, when discharged into adjacent water bodies without prior treatment, cause eutrophication, oxygen depletion, and sedimentation (CAO *et al.*, 2007; BARRAZA-GUARDADO *et al.*, 2013). According to Barraza-Guardado *et al.* (2013), there is still

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a lack of knowledge as to how effluents from these activities affect the ecosystems of coastal areas.

In addition, one of the main factors affecting the economic viability of shrimp farming is feed cost. The use of feeds based on fishmeal and fish oil accounts for up to 50% of the total production cost (AUDELO-NARANJO; VOLTOLINA; BELTRAN, 2012; EMERENCIANO *et al.*, 2012; SILVA-NETO *et al.*, 2012). The feed is generally provided at 5 to 10% of the estimated shrimp mass per day (FAO, 2016) and, according to Krummenauer *et al.* (2014), the feed-conversion rate for *Litopenaeus vannamei* is 1.5.

An alternative solution to both problems (environmental impact of harvest drainage water and feed costs) is the use of recirculating systems that reuse water, nutrients, and organic matter from wastewaters. Biofloc technology (BFT) is a technique for rational and sustainable development of aquaculture (FURTADO; POERSCH; WASIELESKY JR., 2014), wherein organic matter and nutrients present in farming pond water are converted into bioflocs that can partially or totally replace commercial feeds (KUHN *et al.*, 2009). This process involves the multiplication and control of heterotrophic microbial biofloc inside the shrimp-farming pond by introducing an additional source of carbon (AVNIMELECH, 1999; CRAB *et al.*, 2009; BAKAR *et al.*, 2015).

However, biofloc production in the farming pond itself creates problems related to increase in the concentration of total solids, which can lead to oxygen depletion, obstruction of shrimp gills, and mortality due to asphyxiation (HARGREAVES, 2013; SCHVEITZER *et al.*, 2013b). The alternative is to produce bioflocs in a unit external to the farm, in a system known as "activated sludge". This system is usually used for the biological treatment of domestic and industrial wastewater, but its design can be used to recycle shrimp harvest drainage water and promote biofloc production.

The activated sludge system comprises an aeration tank and a settling tank. Part of the organic matter is oxidized to CO_2 by aerobic microorganisms in the aeration tank, while the remaining part is converted into new cells that form flocs (bioflocs). The mixed liquor containing a high concentration of flocculated microorganisms is then decanted in the settling tank; part of the solid material returns to the aeration tank and the rest is discarded or, in the case of a biofloc production system, used as a feed component. In a conventional activated sludge system, the concentration of total suspended solids in the form of bioflocs in the reactor can reach a value of 5 $g.L^{-1}$ (VON SPERLING, 2007).

The concentration of organic matter in the wastewater from shrimp harvest ranges between 68 and 132 mg COD/L (ANH *et al.*, 2010). However, this is a suboptimal concentration if the aim is to produce bioflocs for shrimp feed. The solution is to induce the production of biofloc similar to that in a BFT system, by feeding an activated sludge system with water from shrimp farming and supplementing it with an external source of carbon while the C:N ratio is adjusted.

Based on the above, the aim of the present study was to maximize the microbial biofloc production in an activated sludge system, and consequently, treat shrimp-farming effluents.

MATERIAL AND METHODS

The study was conducted at the Center for Coastal Environmental Studies (Centro de Estudos Ambientais Costeiros — CEAC) and the Laboratory of Effluent Treatment and Water Quality of the Institute of Marine Sciences of the Federal University of Ceará (LABOMAR/UFC). The CEAC had 200 shrimp-culture tanks, of which six were 3,000 L nurseries, 94 were 1,000 L fattening ponds, and 100 were 500 L fattening ponds. Each fattening pond received approximately 2 kg of feed during a culture cycle of ten weeks.

The wastewater used in the experiments came from the *Litopenaeus vannamei* shrimp (Boone 1931) farms from the CEAC. The main physical and chemical characteristics were the following ($mg.L^{-1}$): BOD_5^{20} (331), COD (713), NO_3^- (2.7), NO_2^- (6.4), PO_4^{3-} (2.0), total suspended solids (TSS) (150), volatile suspended solids (VSS) (117), non-volatile suspended solids (NVSS) (33). The influent to the biofloc production system was composed by this wastewater enriched with urea and sugarcane molasses to adjust the C:N ratio to 20:1, as recommended by Schveitzer *et al.* (2013b).

Biofloc production from shrimp farming effluent was performed in a pilot-scale activated sludge system (ASS) built in fiberglass and sized according to the recommendations of van Haandel and van der Lubbe (2007).

The ASS comprised a cylindrical aeration tank (0.49 m in diameter, 0.60 m in height, and 115 L of usable volume), followed by a secondary cylindrical-conical decanter (diameter, 0.38 m; usable height of the cylinder, 0.30 m; cone height, 0.40 m; usable volume, 49 L). The system was continuously aerated with an air compressor to maintain the concentration of oxygen at approximately 2 $g.L^{-1}$, and the mixing was performed mechanically.

The inoculum was produced from 6,000 L of wastewater from nursery tanks for the reproduction of Pacific white shrimp, *Litopenaeus vannamei* (Boone 1931). One hour after the wastewater was decanted, the sediments were transferred to a 1,000 L tank, to which molasses (1 $g.L^{-1}$) (SAMOCHA *et al.*, 2007; SCHVEITZER *et al.*, 2013a), a nutrient solution (1 $mL.L^{-1}$) comprising ($mg.L^{-1}$): NH_4Cl (25), K_2HPO_4 (25), $MgSO_4 \cdot 7H_2O$ (8.6), $CaCl_2$ (5.3), $FeCl_2$ (0,3), H_3BO_3(0.08), $ZnCl_2$(0.02), $CuCl_2 \cdot 2H_2O$ (0.01), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.2), and $CoCl_2 \cdot 6H_2O$ (0.3) (KIM & PAGILLA, 2003; DIEZ *et al.*, 2005), were added on a daily basis. The tank was continuously aerated for 25 days. After this period, the inoculum was deemed as formed and the supernatant was discarded. Subsequently, a volume of 80 L of inoculum with 105 mg $TSS.L^{-1}$ and 68 mg $VSS.L^{-1}$ was added to the aeration tank of the activated sludge system.

After the addition of the inoculum, the aeration tank and the secondary decanter were filled with water from the Pacoti river estuary that had been disinfected with chlorine but was free of residual chlorine.

The BOD_5^{20}/COD ratio of the wastewater from the shrimp farming system was 0.46, which indicated that the ASS can be used for biofloc production (PI *et al.*, 2009). However, the concentration of biodegradable organic matter was low (BOD=331 mg $O_2.L^{-1}$), which would result in a very low yield of biofloc. Therefore, an external source of carbon (sugarcane molasses) was added. During the first 30 days, the reactor was fed three times per week with wastewater from the shrimp ponds, along with 100 g of molasses and 100 ml of a solution containing urea (110 $mg.L^{-1}$), monodicalcium phosphate (40 $mg.L^{-1}$), and a solution of nutrients (1 $mL.L^{-1}$). The C:N:P ratio was adjusted to 100:5:1 (MARROT *et al.*, 2006).

After the initial period of 30 days, the system was continuously fed for 90 days until the concentration of total suspended solids (TSS) in the aeration tank was approximately 5.0 $g.L^{-1}$, which, according to van Haandel and van der Lubbe (2007), is the minimum value to initiate the removal of excess sludge. At this stage, 500 g of molasses, 107 g of urea, 43 g of monodicalcium phosphate, and 500 mL of the aforementioned nutrient solution were directly added to the reactor on a daily basis.

The system was monitored weekly, at every exchange of shrimp culture water, to determine the physical and chemical parameters (COD, SS, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_3$, TKN, and PO_4^{3-}) of the system's influent and effluent. Dissolved oxygen (DO), pH, and salinity were measured daily using the methods described by Eaton *et al.* (2005).

The ASS was operated with an influent flow rate (Q_{INf}) of 1.08 $L.h^{-1}$, a recirculation flow rate (Q_{re}) of 72 $L.h^{-1}$, a hydraulic retention time (HRT) of 10.8 h, and a cell residence time (CRT) of 7.15 days. Biofloc consisted of the flocs formed by microorganisms present in the mixed liquor in the reaction tank of the activated sludge system. Therefore, biofloc concentration was measured by determining the VSS ($mg.L^{-1}$).

The nitrogen mass balance in the system was calculated based on Equation 1, as described by Barker and Dold (1995) and Lee *et al.* (2008), who assumed that the mass of nitrogen entering the system is equal to the sum of nitrogen mass in the effluent, nitrogen mass in the sludge, and the fraction of nitrogen mass after denitrification.

$$Q_{if} \times ([TKN]_{inf}\,[NO^{3-}]_{inf} + [NO^{2-}]_{inf}) = \{Q_{inf} \times ([TKN]_{eff} + [NO^{3-}]_{eff} + [NO^{2-}]_{eff})\} + N_x + N_{slud} + N_{den} \quad (1)$$

In which:

Q_{inf} = average flow rate ($L.d^{-1}$);

$[TKN]$ = concentration of total Kjeldahl nitrogen ($mg.L^{-1}$);

$[NO^{3-}]$ = concentration of nitrate ($mg.L^{-1}$);

$[NO^{2-}]_{inf}$ = concentration of nitrite ($mg.L^{-1}$);

inf and eff = concentrations of influent and effluent, respectively;

N_X = mass of nitrogen in the sludge inside the reactor ($mg.d^{-1}$);

N_{slud} = mass of nitrogen removed from the sludge ($mg.d^{-1}$);

N_{den} = mass of nitrogen removed via denitrification ($mg.d^{-1}$) (considered null because denitrification did not occur in the system).

The masses of nitrogen entering and exiting the ASS were determined by the sum of the masses of oxidized forms of nitrogen (NO_3^- and NO_2^-) and the mass of TKN present in the influent and in the effluent, respectively. The nitrogen mass in the sludge produced inside the reactor (calculated according to Equation 2), nitrogen mass in the excess sludge (calculated according to Equation 3), and the fraction of nitrogen mass after denitrification (considered null because only nitrification occurs in the aerobic process).

$$N_x = \frac{V_r}{SRT}[VSS]_r \times f_n \quad (2)$$

$$N_{SS} = Q_{inf} \times [VSS]_{SS} \times f_n \quad (3)$$

In which:

V_r = volume of the reactor (L);

SRT = sludge retention time (sludge age) (d);

$[VSS]_r$ = concentration of volatile suspended solids in the aeration tank ($mg.L^{-1}$);

f_n = fraction of nitrogen in the sludge (0.1 mg N.mg VSS^{-1});

N_{SS} = mass of nitrogen in the excess sludge ($mg.d^{-1}$);

$[VSS]_{SS}$ = concentration of volatile suspended solids in the excess sludge ($mg.L^{-1}$).

Microbiological analyses of the effluent and biofloc were performed based on the quantity of cultivable aerobic heterotrophic bacteria (CHB), quantity and species of *Vibrio* bacteria, estimates of the number and species of nitrifying and ammonifying bacteria, and the quantity of fungi.

The samples were serially diluted and inoculated in culture media specific for each microbial group. The cultures were isolated from the culture media for subsequent identification.

Quantification of CHB was performed according to the method described by Downes and Ito (2001), using the standard plate count procedure in plate count agar (PCA) medium diluted with seawater and salinity adjusted to 20. To quantify *Vibrio* bacteria, 100-µL aliquots of the serially diluted samples were spread onto the surface of thiosulfate-citrate-bile-sucrose (TCBS) agar medium plates using the spread plate technique (KAYSNER & DEPAOLA JR., 2004). The identification of the isolates followed the procedure proposed by Noguerola and Blanch (2008).

The estimation of the most probable number (MPN) of communities of ammonifying and nitrifying bacteria was performed using

the multiple-tube technique (EATON *et al.*, 2005) with media whose single source of nitrogen was arginine (for ammonifying bacteria) and ammonia and nitrate (for nitrifying bacteria). The isolates were identified using the method established by Brenner, Krieg and Stanley (2005).

The quantification of culturable fungi was performed according to the method described by Downes and Ito (2001). The standard plate count method with potato dextrose agar (pour plate technique) was used.

RESULTS AND DISCUSSION

According to Fróes *et al.* (2012) and Schveitzer *et al.* (2013a), the addition of sugarcane molasses as an external source of carbon to the ASS favors the transition from an autotrophic to a heterotrophic medium and the control of nitrogen compounds in the system, which is in agreement to the findings of this work. Avnimelech (1999) reported that bacteria require 20 g of carbon to metabolize one gram of nitrogen. Krummenauer *et al.* (2014) state that the addition of carbohydrates to culture water is essential to accelerate biofloc development. Therefore, sugarcane molasses is one of the most commonly used sources of carbohydrates to fertilize farming water (FRÓES *et al.*, 2012; FERREIRA *et al.*, 2015; SABRY-NETO, SANTAELLA & NUNES, 2015; XU; MORRIS; SAMOCHA, 2015).

The average concentration of orthophosphate was 2.04±2.50 $mg.L^{-1}$ and that of ammoniacal nitrogen was lower than the detection limit of the method (5.0 mg $N\text{-}NH_3.L^{-1}$). Therefore, there was a need to supplement the system with a source of nitrogen and phosphorous to maintain the C:N:P ratio at 100:5:1 for guaranteeing biofloc growth.

After the shrimp farming wastewater was supplemented with molasses and nutrients at a ratio of 100C:5N:1P, as suggested in the literature (RATSAK; MAARSEN; KOOIJMAN, 1996; KARAMALIDIS *et al.*, 2010; WIJEKOON; VISVANATHAN; ABEYNAYAKA, 2011; SHAHI *et al.*, 2016), the value of COD increased to 1,170±430 mg $O_2.L^{-1}$, and the concentration of TSS increased to 323±128 $mg.L^{-1}$, thus, reaching adequate levels for the growth of a heterotrophic community (AVNIMELECH, 1999). This increase in the concentration of TSS positively affected the system because, as reported by Fróes *et al.* (2012) and Schveitzer *et al.* (2013b), the removal of ammonia is hindered when the concentration of TSS is lower than 200 $mg.L^{-1}$ due to slow nitrification by autotrophic bacteria.

Biofloc technology has been used in aquaculture not only to control the concentration of nitrogen compounds and farming water quality, but also as a form of protein supplementation in feeds for farmed species, possibly leading to a reduction in feed costs (EMERENCIANO *et al.*, 2012). According to Ekasari *et al.* (2014b), the ingestion of biomass in the form of biofloc increases protein and lipid assimilation by shrimp, which enhances its immune response.

Ray and Lotz (2014) studied the effect of different types of carbohydrates as sources of carbon supplementation on bacterial biofloc production in a shrimp farm in heterotrophic systems. They used glucose, molasses, and glycerol at a C:N ratio of 22:1, which was higher than the C:N ratio (20:1) used in the present study. They observed that adding molasses resulted in a higher production of total and volatile solids in the system compared with other sources of carbon.

In this research, after the wastewater was fertilized, the concentration of TKN increased to 30.49±17.75 $mg.L^{-1}$, and that of orthophosphate increased to 8.11±2.41 $mg.L^{-1}$.

The operating parameters in the ASS were the following: DO=5.5±0.8 mg $O_2.L^{-1}$, pH=7.3±0.5, temperature=29.8±1.0ºC, and salinity=31.4±4.2. All parameters were within the ranges recommended for treatment of ASS effluents. Figure 1 shows the average values and confidence intervals (α=0.05) of total and soluble COD of the ASS influent and effluent. The average removal efficiency of total and soluble COD was 71.3 and 71.6%, respectively, which indicated good conversion of organic matter into bioflocs or CO_2.

The activated sludge system promoted a reduction of 25% in influent VSS. In other words, the formed sludge exhibited good sedimentation, which resulted in a decrease in the concentration of solids in the treated effluent. Moreover, the average biofloc production in the reactor was high (1,461 $mg.L^{-1}$ or 676%), which proved that the activated sludge system is a feasible option for biofloc production as a feed supplement in shrimp farming (KUHN *et al.*, 2009; FERREIRA *et al.*, 2015).

Figure 2 shows the average concentrations and confidence intervals (α=0.05) of inorganic and total Kjeldahl nitrogen in the influent and effluent of the reaction tank during activated sludge system operation. There was a reduction in ammonia starting from the second week of operation, and complete removal was achieved during some periods; this result was expected because nitrification of ammonia to nitrate occurs in aerobic systems, which results in nitrate accumulation. Because nitrate is the form of nitrogen that is best assimilated by microorganisms, the generation of nitrate in the activated sludge system contributed to the increase in VSS in the reaction tank.

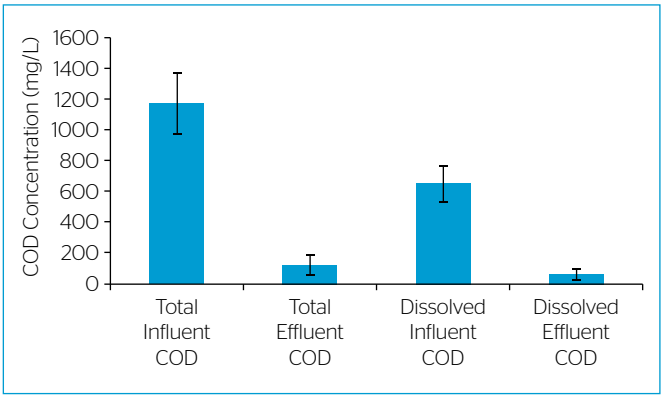

Figure 1 – Average COD concentrations (total and soluble). Error bars indicate the confidence interval (α=0.05).

There was an accentuated reduction (73%) in TKN in the ASS effluent, which confirms the hypothesis that ammonia and organic nitrogen are assimilated to produce biomass. The majority of the nitrogen compounds present in the influent were probably incorporated into bioflocs in the form of organic nitrogen. The comparison between ammonia and TKN concentrations (Figure 2) showed that organic nitrogen was the major component of TKN, because the concentration of ammonia in the system's effluent was only 3.92 mg.L^{-1}. This is because increasing the C:N ratio in heterotrophic systems favours the growth of heterotrophic microbiota.

Table 1 shows the mass balance results of the nitrogen compounds in the ASS. There was a decrease in the concentrations of TKN and NO_2^- in the system's effluent, whereas NO_3^- concentration increased. There was a slight difference between the sums of the different forms of nitrogen due to experimental errors during the determination of concentrations as well as errors inherent to experiments with living organisms.

The main routes of transformation of nitrogen compounds in activated sludge system include nitrification, denitrification, ammonification, and volatilization (BABU *et al.*, 2011; ZHAO *et al.*, 2013). Table 1 shows an increase in the concentration of NO_3^- as a consequence of the nitrification process that occurred in the system. The increase in NO_3^- concentration in the system's effluent indicated that ammonia was oxidized to nitrogen oxides. NO_2^- was probably rapidly converted to NO_3^- because it is an unstable compound, which justifies its low concentration in the effluent (0.46±0.5 mg N-NO_2^-.L^{-1}). This was an aerobic system, therefore there was no removal of nitrogen, only conversion of ammonia by heterotrophic bacteria (SCHVEITZER *et al.*, 2013b) to forms that are less toxic to microorganisms, such as NO_2^- and NO_3^-; thus, all the nitrogen that entered the ASS was detected in the output.

The result of mass balance showed that the main process for transformation of nitrogen compounds was nitrification, because a strictly aerobic system would not be capable of removing nitrogen by converting it to N_2, unlike combined systems that operate under aerobic/anoxic conditions (FOCO; LOPES; NOUR, 2015). However, a large proportion of the nitrogen compounds (2,350 mg N.d^{-1}) was incorporated in the bioflocs, which was a desired result as the latter would be studied as a source of protein for nutritional supplementation of shrimp.

Although the activated sludge system was operated in order to reach the concentration of 5.0 g SST.L^{-1}, this concentration obtained was not reached and the maximum concentration obtained was 4.6 g SST.L^{-1} (average=2.8±0.9 g.L^{-1}). Within the total solids, the concentration of VSS was 1.9±0.7 g.L^{-1} (70%), corresponding to the organic fraction of bioflocs. The concentrations of TSS and VSS are shown in Figure 3.

Microbial biofloc has been studied as a nutrition source for shrimp and can be used to supplement feeds and recycle nitrogen into microbial protein, thus, favoring the control of ammonia in the system (SCHVEITZER *et al.*, 2013a).

The addition of molasses to the wastewater entering the activated sludge system was performed to promote the removal of nitrogen compounds from the water, as they were incorporated into biomass by heterotrophic bacteria. This procedure increased the level of suspended solids in the tank. Ammonia uptake takes place faster in heterotrophic bacteria than in autotrophic bacteria. This is because of

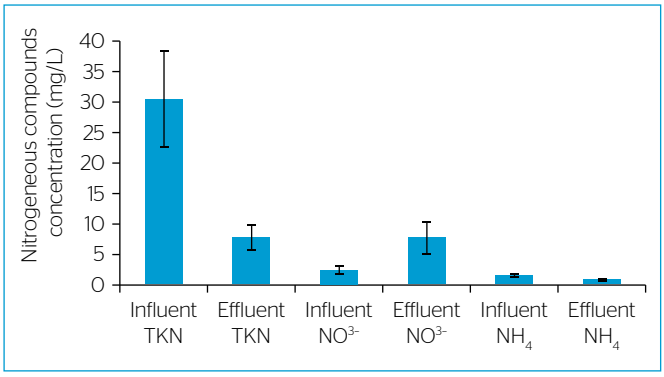

Figure 2 – Variation in the levels of nitrogen compounds in the activated sludge system. Error bars indicate the confidence interval (α = 0.05).

Table 1 – Mass balance of the nitrogen compounds in the activated sludge system.

Parameter	Unit	Number of samples	Influent	Effluent
[a]Q	L.d^{-1}	18	260	260
[TKN]	mg N.L^{-1}	18	30.49±17.75	7.91±4.89
[NO_3^-]	mg N.L^{-1}	17	2.55±2.42	7.88±6.24
[NO_2^-]	mg N.L^{-1}	16	0.766±1.15	0.46±0.5
[b]N_{ss}	mg N.d^{-1}	-	-	0.11008
[c]N_{Slud}	mg N.d^{-1}	-	-	2,350.10
Total	mg N.d^{-1}		8,790.09	9,069.71

[a]Flow rate; [b]N_{ss} is the nitrogen mass that was removed along with the treated effluent; [c]N_{Slud} is the nitrogen mass removed with the sludge; values after ± refer to the Standard Deviation.

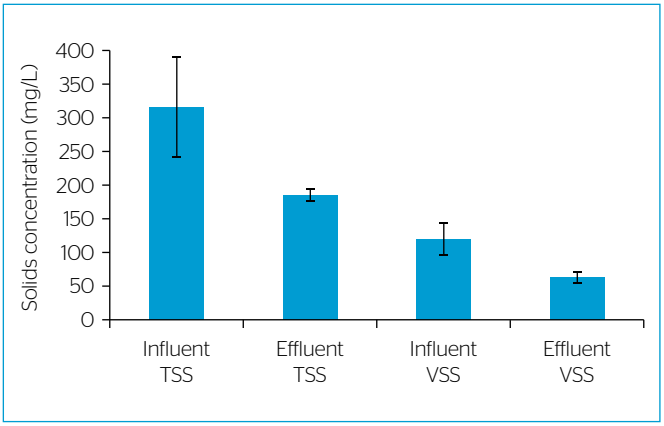

Figure 3 – Mean concentration of TSS and VSS in the reaction tank. Error bars indicate the confidence interval (α=0.05).

the higher growth rate and production of microbial biomass per unit substrate of heterotrophic bacteria (0.5 g of carbon biomass/g of used C), which is approximately ten times greater than that of autotrophic bacteria (HARGREAVES, 2006; CRAB *et al.*, 2012).

In intensive culture farms with a biofloc system, the concentration of total suspended solids in the farm should be kept approximately at 500 mg.L^{-1}, which is the concentration deemed ideal for the formation of bacterial biofloc (SAMOCHA *et al.*, 2007; EKASARI *et al.*, 2014a). However, if the biofloc production system is set up in a unit external to and downstream from the farm, as in the activated sludge system used in the present study, it is possible to operate with TSS concentrations up to ten times higher than 500 mg.L^{-1}. This facilitates the formation of denser bioflocs and eliminates the mortality of farmed species caused by the obstruction of gills and loss of osmotic and ionic regulation. Schveitzer *et al.* (2013a) worked with concentrations of up to 1.0 g SST.L^{-1} in farms with a biofloc system and reported a decrease in the survival rate of farmed species, due to respiratory disorders caused by the obstruction of gills by particulate materials and decreased osmoregulation. However, this occurs only when the biofloc is produced inside the farm, which was not the case in the present study.

Table 2 shows the results of the quantification of microbial populations. As expected, their quantity was higher in the sludge than in the water. The numbers of CHB were higher than that of autotrophic (ammonifying and nitrifying) bacteria in both samples. This value increased in the samples of sludge, which indicated that the addition of sugarcane molasses to the reactor influent favoured the growth of the heterotrophic community. According to Ebeling, Timmons and Bisogni (2006), the maximum growth rate of heterotrophic bacteria is significantly higher than that of nitrifying bacteria, and they can multiply up to five times faster depending on the environmental conditions. *Vibrio* bacteria and fungi also had a higher abundance in the sludge than in the water.

The dominant species in the sludge sample was V. calviensis, a bacterium native of marine and estuarine environments, which was first identified in shrimp farms in the state of Ceará (Brazil) by Vieira *et al.* (2010). To date, there are no reports of this *Vibrio* species being pathogenic to aquatic animals. The bacterial community present in a super-intensive shrimp culture system that uses biofloc technology serves as food for the farmed animals, in addition to inhibiting the proliferation of pathogenic agents via competitive exclusion for space. However, pathogenic and opportunistic bacteria such as *Vibrio* spp. might be found (CRAB *et al.*, 2012).

Four dominant genera of CHBs were identified in the sludge and the highest percentage of isolates belonged to the Bacillus genus (Figure 4). Microorganisms belonging to these genera are used to improve the animals' health and water quality in the farms, with the added benefit of controlling the populations of *Vibrio* species that are pathogenic to cultured shrimp (FERREIRA *et al.*, 2015).

The diversity of microorganisms in the aquaculture environment has a beneficial effect on the health of cultured animals. The cell wall of microorganisms such as bacteria and fungi is composed of lipopolysaccharides, peptidoglycans, and 3-glycans that activate the non-specific immune system in teleosts and crustaceans, increasing their resistance against viral and bacterial infections (KIM *et al.*, 2014).

In a biofloc system, these bacteria utilize compounds that are potentially toxic to shrimp cultures (such as organic carbon, ammoniacal nitrogen, nitrates, nitrites, and phosphates) as sources of energy by oxidizing them and making them biologically available to algae, fungi, and other bacteria. The proliferation of microorganisms increases the biofloc biomass, which is used by cultured animals as a source of protein (CASTRO-NIETO *et al.*, 2012).

In addition, the microbial community in aquatic systems plays a key role in nutrient cycling that involves the processes of nitrogen fixation, ammonification, nitrification, and denitrification. Species belonging

Table 2 – Quantification of microbial groups in the samples of water and sludge of effluents from shrimp farming.

Species	Water	Sludge
CHB (CFU.mL^{-1})	236 × 10^4	1,408 × 10^5
Vibrio spp. (CFU.mL^{-1})	<100	545 × 10
Fungi (CFU.mL^{-1})	<100	12.5 × 10
Ammonifying bacteria (MPN.mL^{-1})	1,600 × 10^2	1,600 × 10^2
Nitrifying bacteria (MPN.mL^{-1})	1,600 × 10^2	1,600 × 10^2

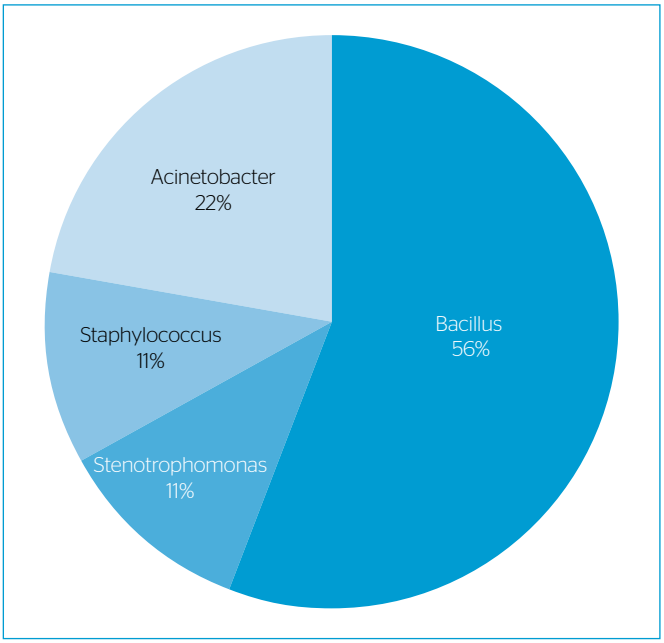

Figure 4 – Identification of bacterial isolates in samples from the activated sludge system reactor of the CEAC, Fortaleza (CE), Brazil.

to the Nitrosomonas and Nitrospira genera were identified among the culturable ammonifying bacteria. Among nitrifying bacteria, the Nitrobacter and Nitrococcus genera were the most frequently isolated ones in water and sludge samples. According to Srithep *et al.* (2015), there is a great diversity of autotrophic and heterotrophic microorganisms that mediate these processes, and they are strongly affected by environmental factors.

Sabry-Neto, Santaella and Nunes (2015) analyzed the nutritional content of biofloc formed in this system and reported that the percentage of ash (64.9%) was higher than that obtained in other studies (24.7 and 11.8% by Kuhn *et al.* in 2009, 27.39% by Ferreira *et al.* in 2015, and 53.95% by Fugimura *et al.* in 2014). The high ash content found in the biofloc probably originated from salts, since the water used to feed the reactor had high salinity (31.44±4.2).

Furthermore, Sabry-Neto, Santaella and Nunes (2015) obtained a value of 9.59% for crude protein, which is significantly lower than the values reported by Kuhn *et al.* (2009), Ferreira *et al.* (2015), and Fugimura *et al.* (2014) (40.5, 33.48, and 15.98%, respectively). The concentration of crude protein in commercial feeds is variable (25 to 45%) (CORREIA *et al.*, 2014; NG *et al.*, 2015; KHATOON *et al.*, 2016). According to Hu *et al.* (2008), L. vannamei requires a diet with 34% crude protein, which is a higher percentage than that obtained in the analyzed biofloc.

Although the levels of ash and crude protein in the bioflocs produced in the activated sludge system were different from those reported in other studies and incompatible with the requirements of L. vannamei, Sabry-Neto, Santaella and Nunes (2015) observed that shrimp gained body mass when provided with feeds containing 30% biofloc, which was produced in an activated sludge system. The production of crude protein in the system probably increases when the C:N ratio decreases.

Since the level of mineral matter might have been a result of the high salinity of culture water, it is recommended that the biofloc produced in an activated sludge system be thoroughly washed in water to remove the salts.

CONCLUSIONS

It was possible to produce large quantities of bioflocs in activated sludge system. The addition of molasses to the influent of the activated sludge system contributed to the increase in VSS (biomass), which aggregated in the form of flocs, thereby decreasing their concentration in the effluent.

The microbiological analysis of the biofloc demonstrated that the quantity and diversity of microorganisms were beneficial to the cultured shrimp, serving as feed and immune response inducers on one hand, and ensuring water quality on the other hand.

The mass balance of nitrogen compounds confirmed that nitrification occurred in the system, i.e., there was conversion of ammonia to less toxic nitrogen compounds, which was demonstrated by a 309% increase in the concentration of nitrate.

The conversion of organic matter to biofloc and CO_2 in the activated sludge system was 71%, and there was a 41% decrease in the concentration of ammoniacal nitrogen through conversion to nitrite and nitrate, which are forms of nitrogen assimilated by heterotrophic bacteria.

Therefore, the use of activated sludge system to produce biofloc, and consequently, treat wastewater from shrimp farming is a viable and environmentally sound option.

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