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Water Quality and Nutrient Dynamics of Biofloc with Different C/N Ratios in Inland Saline Water

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ABSTRACT

A study evaluating water quality and nutrient dynamics in inland saline water was carried out using biofloc technology with different C/N ratios employed to raise *Litopenaeus vannamei* juveniles. The study was carried out for 60 days in FRP with no water exchange. Salinity, temperature, Dissolved oxygen, pH, nutrients, Biofloc Volume and Total Suspended Solids were monitored. All biofloc units indicated reduction in alkalinity at 40th day except the treatment with highest (25:1) C/N ratio, pH and alkalinity was not significant different among various treatments. Dissolved Oxygen was found to be significantly decreasing with increasing C/N ratios. The least Dissolved Oxygen was recorded at higher C/N ratios of 20:1 and 25:1 at the end of experiment. The Total Ammonical Nitrogen (TAN) and NO_2–Nin C/N ratios 15:1 and 20:1 were found to have a significantly decreasing in trend after 20th and 18th day respectively. The NO_3–N, PO_4^{3-}–P, BVF and TSS in biofloc were shown an increasing trend in all the experiments. The present study elucidates the suitability of optimum C/N ratios in biofloc for maintaining the water quality to raise *L. vannamei* in inland ground saline water. The finding could help in reducing the environmental concern saline waste water discharge from the shrimp pond to the land.

Keywords: Biofloc technology, C/N ratios, *L. vannamei*, Inland ground saline water, Water quality and Nutrient dynamics

Aquaculture has developed significantly since the last 20 years and is considered as one of the most important food production sector, for growing population. The number of secondary industries also developed with aquaculture and increasing food supply, creating new employment opportunities and raising nutritional security of the country.

In aquaculture shrimp farming considered to be a growing industry because of its huge export potential and high profitability. Shrimp farming mostly confined to the coastal regions in india and there are few farms which is away from coastal areas. Inland shrimp farming in ground saline water is a rising business in many countries of the world. Increasing inland salinity due to human activity has major economic, social and environmental consequences, threatening the viability of numerous rural communities. However, inland saline water could potentially be used for aquaculture purposes, providing source of income from an otherwise unutilized resource. Salinization of land and ground water has been affected a huge area of more than 80 million hectares worldwide (Ghassemin *et al.,* 1995) due to both natural and anthropogenic causes (Bennetts *et al.,* 2006) resulting in high water tables, reduced productivity, loss of fertility, and alienation of valuable cultivable land (Smith and Barlow, 1999) thus posing serious threat to agriculture. In India, the salt affected areas have spread into an area of 8. 62 mha (Lakra *et al.*, 2014). These water reserves could be potentially used for the production of commercial fish species, rendering a much prospective area in the saline aquaculture. In recent years the aquaculture industry has come under scrutiny for contributing to environmental

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degradation. Interest in closed aquaculture systems for the production of shrimp and fish is increasing, mostly due to some key environmental and marketing advantages that such systems have over extensive systems. When water is reused, the risk of discharging pollution is reduced. This is a benefit for protecting natural resources. Furthermore, environmental regulations and discharge fees are inhibitive in most regions. Another advantage is that introduction of contaminants and pathogen from the environment to cultured animals is unlikely, especially when biosecurity measures such as source water disinfection are employed. Using closed systems limits the chance of animal escapement, helping to prevent exotic species and disease introductions to the natural environment. Because of reduced water use, high biomass, and relatively high heat containment, marine shrimp can be cultured at inland locations. One of the major advantages of culturing shrimp in biofloc systems is that multiple external filtration systems are not required, thereby potentially reducing start up and operational expenses. There is no need for external biological filtration because the microbial processes which detoxify nitrogen compounds are found within the water column; sterilization devices such as ozonation systems would only hinder these processes. The manipulation of C/N ratios in the biofloc is a practical and inexpensive method for reducing the accumulation of nitrogenous wastes into pond water (Avnimelech, 1999). Nutrient dynamics in the system may have a great impact on cultured shrimp because of the presence of toxic nitrogen elements like ammonia and nitrite. Nutrient dynamics have been assessed in intensive (Jackson e*t al.* 2003) semi-intensive (Paez-Osuna *et al.,* 1997).

Similarly, commercial feed contains high protein levels (Ballester*et al.,* 2010) and high phosphorus (Kibria*et al.,* 1996; Montoya *et al.,* 2000) to fulfill the essential amino acid and energy requirements of cultured shrimps. Therefore, dissolved nitrogen, especially ammonia and phosphate are largely produced in intensive shrimp cultures due to excretion and decomposition of uneaten feed. Ammonia, the most toxic nitrogen form to shrimp, is mobilized by different pathways such as nitrification, assimilation by photoautotrophic and heterotrophic microorganisms or is lost to the atmosphere due to the volatilization of gaseous ammonia. Unlike ammonia, phosphate is not lost to the atmosphere and is usually removed from the water by phytoplankton. Thus, understanding nutrient cycling processes in biofloc technology compartments is important for developing nutrient removal strategies.

MATERIALS AND METHODS

The experiment was conducted for 60 days at aquaculture wet lab located at Central Institute of Fisheries Education, Rohtak centre, Haryana, India. The Inland ground saline water with 15ppt salinity used for the experiments were pumped out from the farm tube well and kept it for settled in large cemented tanks for few days. The ionic composition of the water prior to experiment initiation were analysed and fortified with Potassium Chloride (KCl) and Magnesium Chloride ($MgCl_2$) (Davis *et al.,* 2005). Potassium fortification was done using commercially available fertilizer Potassium Chloride (KCl) whose trade name is Muriate of potash (MOP) containing 50% K^+ while Magnesium fortification is done using commercially available fertilizer Magnesium Chloride ($MgCl_2$) whose Mg^{2+} content is 27%. The 18 FRP circular tanks (500L) with one control C (clear water) and five treatments (with different C/N ratios) T1 (10:1), T2 (15:1), T3 (20:1) and T4 (25:1) were filled with fortified 15 ppt inland ground saline waters upto 400 L each. The constant aeration provided to the experiment unit by air blower, through plastic tubing and air diffusers that maintained the biofloc present in the tanks in suspension and distributed throughout the water column. Flocs inoculums was developed by adding 20g of pond bottom soil was collected from CIFE centre Rohtak, India in well aerated water (1L) containing 10mg L^{-1} ammonium sulphate $(NH_4)_2SO_4$ and 400mg L^{-1} of carbon sources (Rice Bran) in (5L) glass tanks after 48 hours, the inoculums were distributed equally into the already prepared experimental tanks. The tanks were kept well aerated for 10 days to ensure optimum floc production. After optimum floc produced in experimental tanks the 60/M^2 SPF juveniles *Litopenaeus vannamei* (avg wt. 3.37±0.03 gm) were stocked. The SPF juveniles obtained from a commercial shrimp hatchery, Andhra Pradesh, India.

Input C/N ratios were calculated based on the carbon nitrogen contents of the feed and the carbon content of the Rice bran. The nitrogen content of feed estimated by the Kjeldahl method and accordingly each gram of feed, carbon source as Rice bran added into each treatment tank for maintain the C/N ratio. On average 75% of the feed nitrogen ends up in the water by ammonification of unutilised feed and excretion (Piedrahita *et al*., 2003). Since the final disposal of most (75%) of the added organic nitrogen is the culture medium, as inorganic nitrogen. The amount of carbohydrate addition needed to maintain C/N ratios was calculated by standard protocols of Schryver *et al.* (2008). All tanks were aerated and mixed continuously using air stones. Water temperature of the experimental tanks was maintained at around 26°C during the culture period. No water was exchanged during the experimental period and only freshwater was added to compensate for evaporation losses and maintain the salinity. The photoperiod was maintained on a 12:12 hour light-dark cycle.

Water samples were collected from the experimental tanks during morning hours between 8.00 and 10.00 am for the whole experimental period. Temperature, DO, salinity and pH were measured in the experimental unit every day. Other parameters such as total alkalinity, total hardness, Nitrate-Nitrogen (NO_3-N), Nitrite-Nitrogen (NO_2-N), Total Ammonia Nitrogen (TAN), Phosphate (PO_4^{-3}–P), Total suspended solids (TSS), Biofloc Volume (BFV), calcium and magnesium were carried out in the wet-lab following standard protocols at fixed interval. Dissolved Oxygen, Total Alkalinity and total hardness of the water was estimated titrimetrically following the standard methods (APHA, 2005). Calcium and Magnesium content of the water was estimated titrimetrically following the standard methods (APHA, 2005). Nitrate-Nitrogen (NO_3-N), Nitrite-Nitrogen (NO_2-N), Total Ammonia Nitrogen(TAN), Phosphate (PO_4^{-3}–P) of the water samples were estimated by using Spectroquant test kits (Merck, Germany) following the prescribed guidelines under Merck Spectroquant (NOVA 60). TSS measured by using portable TSS (Hach, India) and expressed as mg L^{-1}. Biofloc Volume (BFV), was measured using Imhoff cone (Borosil) in ml L^{-1}.

RESULTS AND DISCUSSION

Inland saline aquaculture is known as the new environment and ecosystem for the culturing of shrimps and became a commercial venture in saline affected areas. In recent years, new management practices have been studied for the production of shrimps. These practices accentuate reduced water exchange and focus on the optimization of culture conditions and improvements in biosecurity. Biofloc technology improving water quality by addition of extra carbon consequential encouraged nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than nitrification (Hargreaves, 2006). The purpose of the present study was to evaluate the effects of biofloc with different C/N ratios manipulated by rice bran as a carbon source, on water quality and nutrient dynamics in inland saline water. Temperature is one of the important water quality parameter. In the present study, the temperature of the water recorded during 60 days culture period was in the range of 25.0^{o}C to 29.0^{o}C. This is almost with the conformity of recommended temperature range 23-30^{o}C for *L.vannamei* farming by (Wyban *et al.*, 1995; Van Wyk *et al.,* 1999 and Ravuru *et al.,* 2013). Salinity is one of the most basic parameters of the culture environment for marine shrimps. *L. vannamei* is known to inhabit brackish water of 1 to 2 ppt as well as hypersaline water of 40 ppt and higher (Menz and Blake, 1980; Stem *et al.,* 1990) and it is one of the most euryhalinepenaeid species, with juveniles and adults tolerating salinity from freshwater to more than 50 ppt salinity (Pan *et al.,* 2007). In the present study, salinity was maintained at 15 ppt throughout the experimental period by replenishing the evaporated water with fresh water. The experimental inland saline water of 15 ppt contained 841.25 mg L^{-1} Magnesium and 435 mg L^{-1} Calcium at 15 ppt with total hardness 4013 mg L^{-1}. The ratio of Mg^{++} /Ca^{++} was 1.93:1 in the raw inland saline water which is not enough for shrimp farming especially the white shrimp. Hence, Magnesium ion fortification was done using Magnesium Chloride ($MgCl_2$) to maintain Mg^{++} /Ca^{++} ratio of around 2-2.5:1. Potassium (K^+) ion concentration was maintained at par of natural sea water of corresponding salinity by fortifying with commercial fertilizer Muriate of Potash. Thus, the present study recorded mineral contents above the general recommended level for shrimp aquaculture (Wurts and Durburow, 1992; Van Wyk and Scarpa, 1999; Ebeling *et al.,* 2006; Hargreaves, 2013) which might have contributed to the better physiological conditions such as metabolism, moulting and hence, growth and survival in the present study was similar to earlier reports (Araneda *et al.,* 2008). The alkalinity was not significantly varying in control during the experimental period. By 30th day, all treatment groups showed an increasing trend in alkalinity. All biofloc units indicated reduction in alkalinity at 40th day except the treatment with highest (25:1) C/N ratio, significantly higher alkalinity was recorded in the treatments with C/N ratios 20:1 and 25:1at 60th day of experimental period.

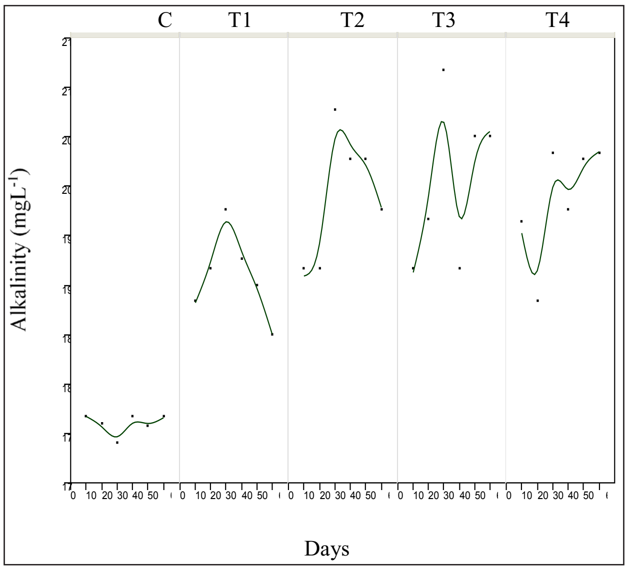

Fig. 1: Alkalinity (Mean±SE) in different treatment groups during the experimental period of 60 days

Dissolved Oxygen was found to be significantly decreasing with increasing C/N ratios. The least Dissolved Oxygen was recorded at higher C/N ratios of 20:1 and 25:1 at the end of experiment as 3.9±0.3 mg L^{-1} and 3.6±0.3 mg L^{-1} respectively.

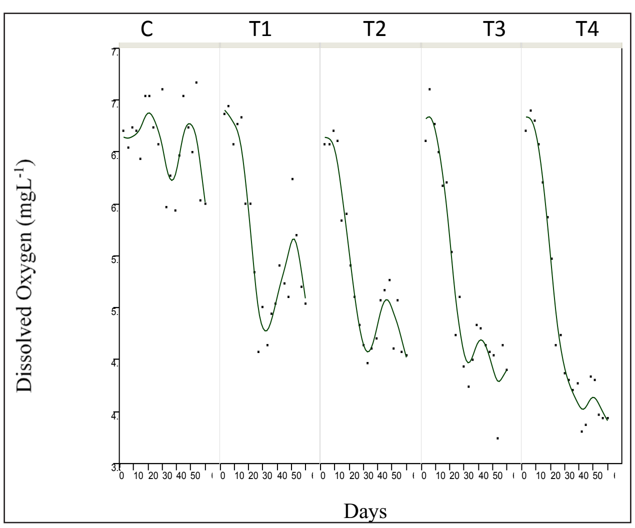

Fig. 2: Dissolved Oxygen Dynamics (Mean±SE) in different treatment groups during the experimental period of 60 days

The pH was not significantly varying among the treatments and control group. pH at the initial phase range between 7.6-8.0 and gradually fluctuated between 7.6 and 8.6 towards the end of the experiment period in all treatment groups and control.

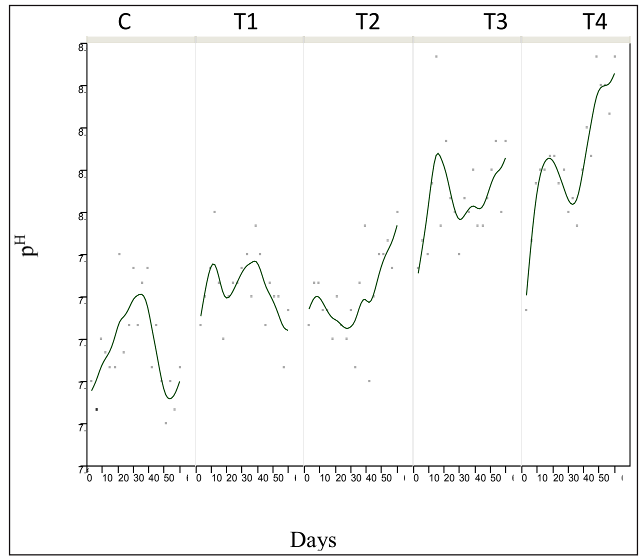

Fig. 3: p^H Dynamics in different treatment groups during the experimental period of 60 days

pH and total alkalinity of the water are closely related and the biofloc units require ample alkalinity reserves to maintain optimum pH. Total alkalinity of the system in the present study fluctuates from 175 to 210 mg L^{-1} which may be due to increasing biomass and hence, the increasing respiratory rate and the consequent carbon dioxide productions. In the present study, higher pH and alkalinity were recorded in treatments with higher C/N ratio of 20:1 and 25:1 as compared to lower C/N ratios and control. These results comply with the earlier reports of Silva *et al*. (2017), at C/N ratio of 20:1 which showed significantly higher alkalinity than those with C/N ratio of 10:1. In the present study significant difference was not found in pH and alkalinity among various treatments. However, earlier studies have indicated that alkalinity and pH may reduce due to consumption of inorganic carbon by autotrophic and heterotrophic bacteria that form the microbial biomass (Ebeling *et al.,* 2006; Perez-fuentes *et al.,* 2016 and Martins *et al.,* 2017).

The recorded dissolved oxygen values in the present study were fluctuated between 3.6 and 7.3 mg L^{-1} in all the treatments. In the present study, with the increase in C/N ratios, reduction of dissolved oxygen values were recorded in the biofloc treatments. The lower dissolved oxygen levels were recorded in treatments with higher C/N ratios 20:1 and 25:1 after 6 weeks of experiment as compared to control and treatments with lower C/N ratios. This observation confirms with the findings of Michaud *et al.* (2006), who observed that in higher C/N ratios, the heterotrophic bacteria compete for available oxygen and space in the biofilters. Several earlier studies (Hari *et al.,* 2004, Schveitzer *et al.,* 2013; Ray *et al.,* 2014) observed that biofloc with high C/N ratios increases the oxygen demand due to the rapid assimilation of TAN by heterotrophic bacteria, this characteristic of high C/N ratios can affect the species sensitive to different levels of dissolved oxygen. In the present study the low oxygen levels may be attributed to the low survival at high C/N ratios. The Total Ammonical Nitrogen (TAN) was found to be significantly lower in control due to periodic water exchange but in the biofloc units with C/N ratios 15:1 and 20:1 was found to have a significantly decreasing in trend. The maximum TAN was recorded in all the biofloc units on 20th days of experiment after that it was found to be in decreasing trend in all the biofloc units.

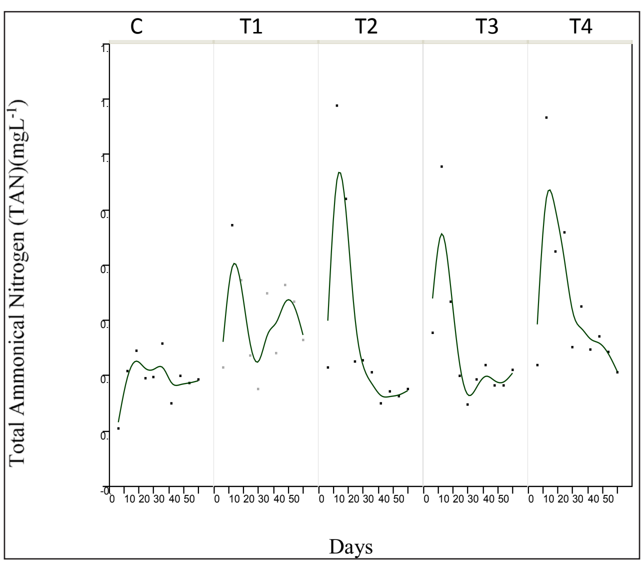

Fig. 4: Total Ammonical Nitrogen (TAN) Dynamics in different treatment groups during the experimental period of 60 days

The Nitrite Nitrogen (NO_2-N) was found to be significantly increasing up to 20th day of experiment in all the biofloc units but after that Nitrite Nitrogen (NO_2-N) showed a decreasing trend in all the biofloc units. The bioflocunits with C/N ratios 15:1 and 20:1 was found to have significantly lower Nitrite Nitrogen (NO_2-N) after 40th day of experiment.

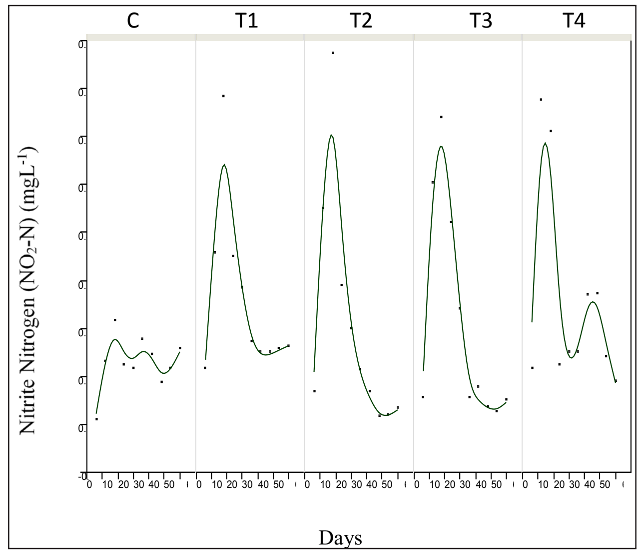

Fig. 5: Nitrite Nitrogen (NO_2-N) Dynamics in different treatment groups during the experimental period of 60 days

The Nitrate Nitrogen (NO_3-N) was significantly varying in all the biofloc units during the experimental period. An increasing trend with significantly higher Nitrate Nitrogen (NO_3-N) was found in the biofloc unit with 20:1 C/N ratios.

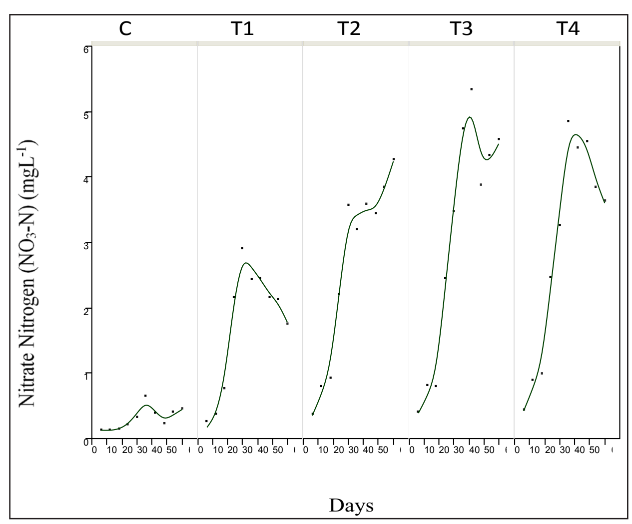

Fig. 6: Nitrate Nitrogen (NO_3-N) Dynamics in different treatment groups during the experimental period of 60 days

Similarly, the Phosphate (PO_4^{-3} – P) was also significantly varying in all the biofloc units during the experimental period. The significantly higher Phosphate (PO_4^{-3} – P) was found in the biofloc units on 40th day in 20:1 and 25:1 C/N ratios.

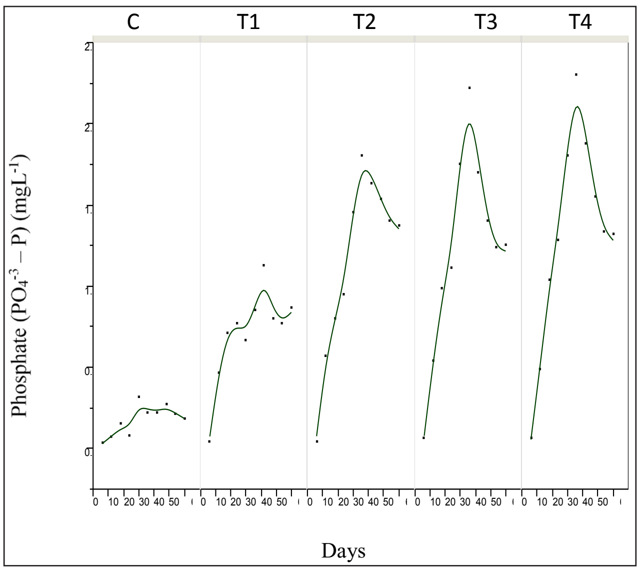

Fig. 7: Phosphate (PO_4^{-3} – P) Dynamics in different treatment groups during the experimental period of 60 days

Nutrient dynamics in the biofloc system may have major impact on cultured shrimp because of the presence of toxic nitrogen elements (ammonia and nitrite) or the presence of nitrate and phosphate directly responsible for productivity and food chain in aquatic ecosystem. Nitrogen is an essential component of all living organisms, aquatic animals get it along with the proteins in the diet (Wei *et al.,* 2016). The microorganisms are responsible for the transformation and dynamics of nitrogen in the aquatic environment. The sequence of transformations is known as the nitrogen cycle. The two states of nitrogen related to the excretion of aquatic organisms are known as Total Ammonia Nitrogen (TAN). In the biofloc units, the TAN was primarily from the decomposition of organic matter such as shrimp waste and dead microbes in the tanks. The TAN can be rapidly taken up and stored by the biofloc microbes by adding organic carbon to stimulate the growth of heterotrophic bacteria. In the present study, highest concentrations of TAN were observed on 12th day of experiment in all treatments and the highest concentrations of NO_2–N were observed on 18th day of experiment in all treatments after that with the acclimation of the system, the TAN and NO_2–N started to decrease. The NO_3–N was shown an increasing trend in all the experiments. In the earlier studies, (Guozhi *et al.,* 2014) stated that in the biofloc system, increasing the C/N ratios and sustaining a high dissolve oxygen in the culture water enabled the assimilatory activity of heterotrophic bacteria to convert ammonium into bacterial biomass. This process is more competent than nitrification, which maintains the water quality, oxygen and alkalinity levels. Moreover, the biofloc formed a heterogeneous microenvironment that led to more opportunities for nitrogen cycle in the system. NO_2–N and NO_3–N are produced through nitrification in the biofloc system which undergo incomplete denitrification to produce NO_2–N, and dissimilatory NO_3–N reduction to ammonia (NH^{3+}) might also occur under the experimental conditions (Wu *et al.,* 2012). However, the packaging of nitrogen in microbial cells is temporary because cells turn over rapidly and release nitrogen as NH^{3+} when they decompose. Therefore, the TAN was repeatedly cycled between the dissolved NH^{3+} and the floc solids, exhibiting an obvious fluctuation. NO_2–N is an intermediate during both nitrification and denitrification (Chuang *et al.,* 2007; Ruiz *et al.,* 2003). The accumulation of NO_2–N is common in intensive aquaculture (Wang *et al*., 2004) probably because of the free NH^{3+} inhibition during nitrification and denitrification (Shi *et al.,* 2011). Meanwhile, the accumulation of NO_3–N concentration,which occurred in all the treatments, indicates that nitrifying bacteria were also present in the biofloc (Ebeling *et al.,* 2006; Xu *et al.,* 2012). Samocha *et al.* (2007) also reported no significant effect on TAN dynamics in the grow-out phase when the shrimp were fed a low protein feed (30%) with molasses supplementation under limited water exchange. These results suggest that once a mature biofloc community is established in the culture water, TAN and NO_2–N concentrations can be effectively controlled by either heterotrophic assimilation or autotrophic nitrification. Therefore, the nitrogen cycle in biofloc is complex and dynamic.

The biofloc system is designed to use little or no water exchange, hampering the elimination of phosphorus through water renewal. Moreover, phosphorus cannot be lost atmospherically. In the present study, the PO_4^{3-}–P was shown an increasing trend in all the experiments with increase in C/N ratios. The results of PO_4^{3-}–P dynamics in the present study are in agreement with the findings of Wu *et al.* (2016) where increase in C/N ratio had significant impact on the concentration of PO_4^{3-}–P. The PO_4^{3-}–P concentration in the culture water significantly increased, showing a faster increase with the higher input of organic carbon. On one hand, more PO_4^{3-}–P was produced from uneaten feed over the time, suggesting saturation of microalgae in the water. This was more obvious in treatments with higher C/N ratio, in which the biofloc were dominated by more heterotrophic bacteria and less microalgae.

The Biofloc volume (BFV) was found to be significantly increasing in all the biofloc units during the experimental period, in which all the treatments were found to be significantly different from each other.

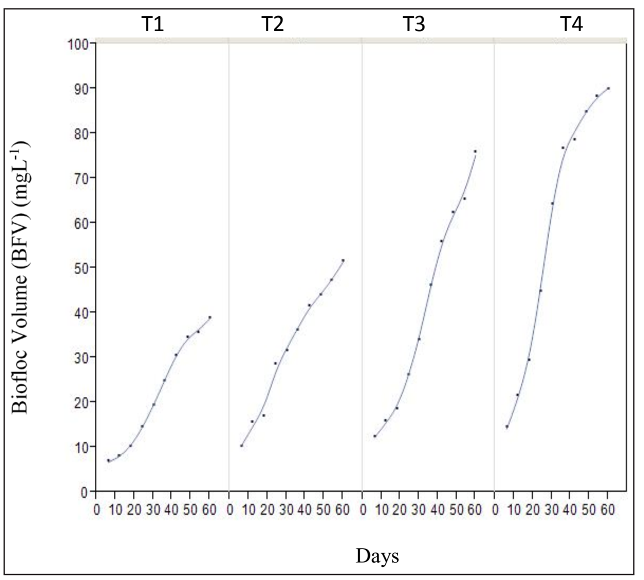

Fig. 8: Biofloc Volume (BFV) Dynamics in different treatment groups during the experimental period of 60 days

Similarly, Total suspended solids (TSS) was found to increase significantly in all the biofloc units during the experimental period in which all treatments were found to significantly differ from each other.

One week after initiation of trial, the color of the water in the tanks began to differentiate between the treatments. These results of BVF and TSS dynamics are in agreement with the findings of Xu *et al.* (2012) reported that with the increase in C/N ratio, there was a shift in the dominant microbial community from the photoautotrophic microalgae and chemoautotrophic bacteria responsible for increasing BVF and TSS in biofloc.

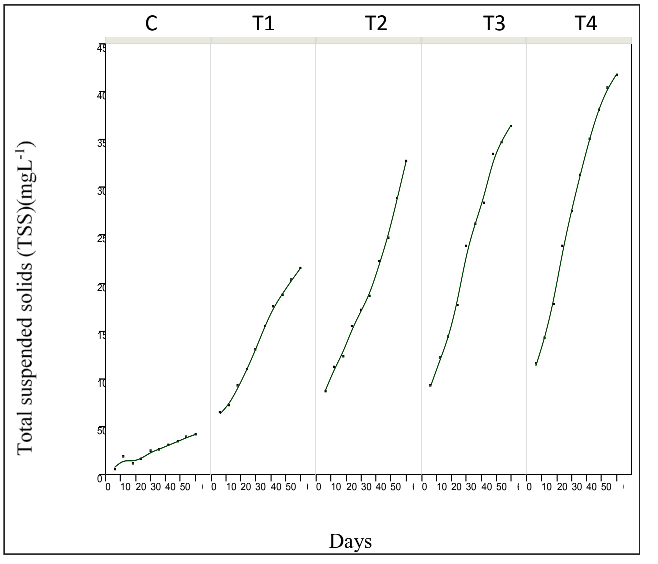

Fig. 9: Total suspended solids (TSS) Dynamics in different treatment groups during the experimental period of 60 days

Similarly, Ebeling *et al.* (2006) also reported that rapid increase in TSS level, from the second week onwards, was proportional to the level of carbon supplementation and also accompanied by higher production of heterotrophic microbial biomass. In other words, organic residues and excreted nitrogen are continually converted into microbial biomass consequently increasing in BFV and TSS levels rather than accumulating as toxic ammonia and nitrite in the system.

CONCLUSION

The present study demonstrated that development of biofloc with C/N ratios (15:1 and 20:1) in inland ground saline water effectively regulate the water quality deterioration with in a very short time. The nutrients level also increased with the high C/N ratios, but they always need more aeration for maintaining the optimum dissolve oxygen in culture water. An important potential conclusion of this work is to encourage shrimp farmers in inland saline areas to use low cost carbon source as rice bran with appropriate C/N ratios for development of biofloc to minimizing the water exchange in inland saline areas for shrimp farming.

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REFERENCES

APHA, 2005. Standard methods for the Examination of the Water and Wastewater, 22nd edition. American Public Health Association, Washington, D.C.

Avnimelech, Y. 1999. Carbon/Nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, **176**: 227–235.

Ballester, E.L.C., Abreu, P.C., Cavalli, R.O., Emerenciano, M., de Abreu, L. and Wasielesky, J.W. 2010. Effect of practical diets with different protein levels on the performance of *Farfantepenaeus paulensis* juveniles nursed in a zero exchange suspended microbial flocs intensive system. *Aqua. Nutri.,* **16**: 163–172.

Bennetts, D.A., Webb, J.A., Stone, D.J.M. and Hill, D.M. 2006. Understanding the salinisation process for groundwater in an area of south-eastern Australia, using hydrochemical and isotopic evidence. *J. Hydrol*., **323**(1): 178- 192.

Chuang, H.P., Ohashi, A., Imachi, H., Tandukar, M. and Harada, H. 2007. Effective partial nitrification to nitrite by down-flow hanging sponge reactor under limited oxygen condition. *Water Res.,* **41**: 295–302.

Davis, D.A., Saoud, I.P., Boyd, C.E. and Rouse, D.B. 2005. Effects of potassium, magnesium, and age on growth and survival of *Litopenaeus vannamei* post-larvae reared in inland low salinity well waters in west Alabama. *J. W. Aqua. Soc.*, **36**: 403-406.

Ebeling, J.M., Rishel, K.L., Welsh, C. and Timmons, M.B. 2006. Impact of the carbon/nitrogen ratio on water quality in zero-exchange shrimp production systems. In Proceedings of International Conference Recirculating Aquaculture. Virginia. Virginia: Virginia Tech University, pp. 361-369.

Ghassemi, F., Jakeman, A.J. and Nix, H.A. 1995. Salinisation of land and water resources: human causes, extent, management and case studies. CAB international.

Guozhi, L., Gao, Q., Chaohui, W., Wenchang, L., Dachuan, S., Li, L. and Hongxin, T. 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (*Oreochromis niloticus*) cultured in a recirculating aquaculture system and an indoor biofloc system. *Aquaculture,* **422**: 1–7.

Hargreaves, J.A. 2006. Photosynthetic suspended-growth systems in aquaculture. *Aqua. Eng.,* **34**: 344-363.

Hari, B., Kurup, B.M., Johny, T.V., Schrama, J.W. and Verdegem, M.C.J. 2004. Improved sustainability in extensive shrimp culture system: control of carbon nitrogen ratio through addition of carbohydrate to the pond. *Aquaculture*, **241**: 179 –194.

Johnson, C.N., Barnes, S., Ogle, J., Grimes, D.J., Chang, Y.J., Peacock, A.D. and Kline, L. 2008. Microbial community analysis of water, foregut, and hidgut during growth of Pacific white shrimp, *Litopenaeus vannamei*, in losed system aquaculture. *J. W. Aqua. Soc.,* **39**: 251-258.

Kibria, G., Nugegoda, D., Lam, P. and Fairclough, R. 1996. Aspects of phosphorus pollution from aquaculture. *NAGA, The ICLARM Quarterly,* **19**: 20–24.

Lakra, W.S., Reddy, A.K. and Harikrishna, V. 2014. Technology for commercial farming of Pacific white shrimp *Litopenaeus vannamei* in inland saline soils using ground saline water. CIFE Technical Bulletin-1, pp. 1-28.

Martins, G.B. 2017. The utilization of sodium bicarbonate, calcium carbonate or hydroxide in biofloc system: water quality, growth performance and oxidative stress of Nile tilapia (*Oreochromis niloticus*). *Aquaculture,* Amsterdam, **468**(1): 10-17.

Michaud, L. 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. *Aqua. Eng.*, **34**: 224-233.

Montoya, R.A., Lawrence, W.E. Grand, and Velasco, M. 2000. Simulation of phosphorus dynamics in an intensive shrimp culture system: effects of feed formulations and feeding strategies. *Eco. Mod.,* **129**: 131–142.

Piedrahita, R.H. 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture,* **226**: 35-44.

P´aez-Osuna, R. Guerrero-Galvan, A.C., Ruiz-Hernandez, A.C. and Espinozangulo, R. 1997. Fluxes and mass balances of nutrients in a semiintensive shrimp farm in North-Western Mexico. *Mar. Pollut. Bull.,* **32**: 290–297.

Pérez-fuentes, J.A. 2016. C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. *Aquaculture*, Amsterdam, **452**(1): 247-251.

Ray, A.J. and Lotz, J.M. 2014. Comparing a chemoautotrophic-based biofloc system and three heterotrophic-based systems receiving different carbohydrate sources. *Aqua. Eng.,* **63**: 54–61.

Ruiz, G., Jeison, D. and Chamy, R. 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Res.,* **37**: 1371–1377.

Samocha, T.M. Patnaik, S., Speed, M., Ali, A.M., Burger, J.M., Almeida, R.V., Ayub, Z., Harisanto, M., Horowitz, A. and

Brock, D.L. 2007. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei. Aquac. Eng.,* **36**: 184–191.

Schveitzer, R., Arantes, R., Costodio, P.F.S., Santo, C.M.D., Arana, L.V., Seiffert, W.Q. and Andreatta, E.R. 2013. Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. *Aqua. Eng.,* **56**: 59–70.

Shi, Y.J., Wang, X.H., Yu, H.B., Xie, H.J., Teng, S.X., Sun, X.F., Tian, B.H. and Wang, S.G. 2011. Aerobic granulation for nitrogen removal via nitrite in a sequencing batch reactor and the emission of nitrous oxide. *Bioresour. Technol.,* **102**: 2536–2541.

Silva, U.L., Falcon, D.R., Nogueira M. Da Cruz Pessôa, Souza Correia E.D. 2017. Carbon sources and C:N Ratios on Water Quality For Nile Tilapia farming In Biofloc System. *Rev. Caatinga, Mossoró*., **30**: 1017 – 1027.

Smith, B. and Barlow, C. 1999. Inland Saline Aquaculture: Proceedings of a Workshop Held in Perth, Western Australia, 6-7 August 1997 (No. 83). Australian Centre for International Agricultural Research. http://purl.umn.edu/135193.

Wang, W.N., Wang, A.L., Zhang, Y.J., Li, Z.H., Wang, J.X. and Sun, R.Y. 2004. Effects of nitrite on lethal and immune response of *Macrobrachium nipponense. Aquaculture,* **232**: 679–686.

Wei, Y.F., Liao, S.A. and Wang, A.L. 2016. The effect of different carbon sources on the nutritional composition, microbial community and structure of biofloc. *Aquaculture,* **465**: 88-93.

Wu, J.X., Timothy, C., Morris, T. and Samocha, M. 2016. Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. *Aquaculture,* **453**: 169-175.

Xu, W.J. and Pan, L.Q. 2012. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. *Aquaculture,* **357**: 147–152.