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# Dynamics of nitrogenous compounds and their control in biofloc technology (BFT) systems: A review

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ABSTRACT

Controlling toxic nitrogenous substances in biofloc technology (BFT) systems is critical for the success of this novel technology. To effectively control nitrogen accumulation in BFT systems, it is important to first understand the dynamics and the removal pathways of this element and its related compounds from aquaculture water. This review focuses on synthesizing the information of nitrogen dynamics in BFT systems to provide researchers and practitioners with a guide to the fate of nitrogen and its control methods. This paper discusses the different types of nitrogenous compounds in BFT water, the transformation processes of ammonia to nitrites and nitrates, the relationship between the two forms of ammonia ($NH_3$ and $NH_4^+$) in water and the equilibrium between them. This paper also discusses nitrification as a major nitrogen removal pathway and the factors that influence the nitrification process. Notably, the control of nitrogen in BFT systems by manipulating the carbon to nitrogen ratio (C/N) using external carbohydrates is described in this paper. This paper suggests that further studies should focus on investigating the various factors that influence nitrogen dynamics in BFT systems and the means of controlling contaminants other than nitrogen.

## 1. Introduction

The biofloc technology (BFT) system has emerged as an outstanding technology capable of solving some of the environmental and economic challenges faced by traditional aquaculture production systems. This novel aquaculture technology has been described as an exceptionally ecofriendly technology due to the principle upon which it operates, which is the reliance on the activities of microorganisms (Emerenciano, Martinez-Cordova, Martinez-Porches, & Miranda-Baeza, 2017). These microorganisms function in three ways: 1) they control water quality through the immobilization of nitrogen, resulting in microbial protein; 2) microbial protein consequently serves as a source of nutrition for cultured species and 3) the microorganisms suppress the growth of pathogens through competition (Avnimelech, 2009, p. 182; Emerenciano et al., 2017).

More specifically, the BFT system, given its several advantages and simplicity, has been suitably adjudged as the novel "blue revolution" in the field of aquaculture. As a "blue revolution", BFT is based on the cycling/recycling of nutrients and their reuse in the same system, which is designed as a zero-exchange or minimal exchange (water) system (Emerenciano et al., 2017). The technology is noted for its positive role in maintaining water quality, enhancing fish reproduction, providing an alternative source of nutrition, and promoting the overall welfare and growth of fish in the culture units (Azim & Little, 2008; Ekasari et al., 2016; Luo et al., 2014). Given these advantages of the biofloc system, it is generally understood that the success of this technology rests upon its ability to remove, recycle or control harmful nitrogenous substances in the culture system (Souza, Cardozo, Wasielesky, & Abreu, 2019). Nitrogen (particularly nitrite and $NH_3$) accumulation in the culture units is a critical issue in the BFT system since even relatively low levels (0.02 mg/l for TAN and 2 mg/l for nitrites) (Bregnballe, 2010) could be harmful to growth performance (Emerenciano et al., 2017; Timmons, Ebeling, Wheaton, Summerfelt, & Vinci, 2002, p. 769). The accumulation typically occurs in several forms, including the following: ammonia ($NH_3$), ammonium ($NH_4^+$), nitrites ($NO_2^-$), nitrates ($NO_3^-$), total nitrogen (TN), and total ammonia nitrogen (TAN) (Ebeling, Timmons, & Bisogni, 2006). It is worth noting that of all these forms of nitrogen in aquaculture, those that are of critical concern and

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warrant immediate control are $NH_3$ and nitrite ($NO_2^-$), although nitrates ($NO_3^-$) may also be considered toxic when they accumulate above 100 mg/l in the system (FAO & EUROFISH, 2015). Therefore, the nitrification process must be carefully controlled to successfully operate a biofloc technology system (Souza et al., 2019). Nitrification is the process of transforming the harmful forms of nitrogen ($NH_3$ and nitrites) to less toxic forms (nitrates), which minimizes the impact on aquaculture species (Ebeling et al., 2006; Hargreaves, 2006). This process is typically carried out by autotrophic nitrifying bacteria including ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) and is subsequently completed by nitrite-oxidizing bacteria (NOB) (Souza et al., 2019). Understanding the dynamics and control of toxic nitrogenous compounds within the biofloc technology system is of key importance to the success of the biofloc technology system and the aquaculture industry.

Several studies (Ebeling et al., 2006; Luo, Zhang, Cai, Tan, & Liu, 2017; Silva, Wasielesky, & Abreu, 2013; Souza et al., 2019) have been conducted that describe the processes, dynamics and methods of nitrogen control in the biofloc system. It is equally important, however, to synthesize and compile this knowledge into a comprehensive document that can serve as a useful source of information on nitrogen dynamics for the practitioners and researchers of the BFT system. It is important to know whether the method of operation of the BFT system is demonstrably different from most other aquaculture systems. Thus, it is necessary to pull together information on nitrogen dynamics in these systems to guide the industry. This review focuses on assembling the current knowledge on nitrogen dynamics and the control of nitrogen in the biofloc technology system. This study also outlines important concepts for reducing the toxicity of harmful nitrogenous compounds such as nitrites that have not typically been discussed in previous review papers, particularly in regards to the biofloc technology aquaculture systems. This review will enable aquaculture scientists to identify areas of research that promote an understanding of the complexities of nitrogen species in BFT systems and the manipulation strategies for successful management.

## 2. Nitrogen and nitrogenous compounds in BFT systems

Nitrogen and its derivatives (nitrogenous compounds) are essential water quality parameters in the culture of several aquaculture species. Thus, it is important to evaluate nitrogen during water quality analysis in aquaculture, particularly in the forms of ammonia and nitrite. BFT, as a new aquaculture production system, has been described as a panacea to the problems of nitrogen toxicities that to date have confounded the aquaculture industry. In this system, toxic nitrogenous compounds are converted into useful products in the form of microbial protein, which becomes an additional source of nutrition for the aquaculture organism through the manipulation or adjustment of the carbon to nitrogen ratio (C/N) (Avnimelech, 1999; Hargreaves, 2013). This provision of an additional source of nutrition in BFT systems helps to reduce the overall production costs, particularly in regards to the reduction in feed costs, which significantly affect the economics of aquaculture (McIntosh, 1999; Rhode, 2014).

Although toxic nitrogenous compounds may be of significant concern in aquaculture, it is equally important to understand that nitrogen is an essential element required by aquaculture organisms for various physiological processes and as constituent of tissues, fluids and molecules including body proteins (Wei, Liao, & Wang, 2016), nucleic acids, nitrogenous bases (nucleotide bases), pigments, adenosine phosphates, etc. (Ebeling, 2013; Sigee, 2005).

According to Ebeling (2013), in the aquaculture system, nitrogen and its related compounds originate from sources such as leftover feed, fish feces, urea, the remains of dead animals and nitrogen from the atmosphere. Thus, nitrogen may exist in both organic and inorganic forms in the BFT system. All these forms can accumulate in the culture unit and be transformed into toxic compounds, which become a serious problem due to their detrimental effects on the cultured species. Significantly, nitrogen in the form of nitrates, nitrites and ammonia are largely soluble in aquaculture water. It is also important to understand that ammonia may occur in the aquaculture water (biofloc system) in two forms: the un-ionized form (ammonia, $NH_3$) and the ionized form (ammonium, $NH_4^+$) (Ebeling et al., 2006; Lekang, 2007). These forms of ammonia nitrogen exist in equilibrium. Controlling or reducing one form subsequently reduces the other, and depending on the temperature, salinity and pH, one form will dominate (Lekang, 2007). In the management, monitoring and water analysis processes of BFT systems, these two forms of ammonia are summed and referred to as total ammonia nitrogen (TAN) (Bregnballe, 2010; Lekang, 2007; Ebeling et al., 2006).

The terms typically associated with the various forms of nitrogen in aquaculture water quality analysis include nitrite-nitrogen ($NO_2^-$-N), ammonium-nitrogen ($NH_4^+$-N), nitrate-nitrogen ($NO_3^-$-N), and ammonia-nitrogen ($NH_3$–N), which makes the estimation of total ammonia nitrogen relatively simple (Ebeling et al., 2006). Given the above information, it is clear that nitrogen is a major concern for the biofloc technology system, and thus efforts should be directed at controlling its level and accumulation in the culture units.

### 2.1. *Effects of toxic nitrogenous compounds on fish and shrimp in biofloc technology systems*

In aquaculture and specifically in biofloc technology systems, it becomes necessary to control ammonia and other toxic nitrogenous compounds in the culture water because of the negative effects on the cultured species (Barbieri, 2010; Romano & Zeng, 2013; Wasielesky, Poerscha, Martinsa, & Miranda-Filho, 2017). This section briefly presents information on some of the effects of toxic nitrogenous compounds on the aquaculture organism. In most cases, gill irritation is the most likely effect on culture species, particularly for shrimp. This irritation generates stress in aquaculture species, and thus negatively affects growth performance, although the intensity of the effect may depend on the developmental stage (Souza et al., 2019). Ammonia toxicity can result from both the ionized and the non-ionized forms ($NH_4^+$ and $NH_3$) (Wright & Wood, 2012), but the non-ionized form ($NH_3$) tends to be more toxic, although both exist in equilibrium in water (Jiménez-Ojeda, Luis, Collazos-Lasso, & Arias-Castellanos, 2018). The accumulation of ammonia in the culture water may also cause histopathological changes such as the development of gill lesions and may affect the oxygen transport function of hemoglobin as a result of the compromised metabolic activity of the fish (Alabaster & Lloyd, 1982). Ammonia toxicity in the culture unit can be diagnosed by symptoms such as restlessness, gasping for air, darkened eyes and convulsions (Karasu et al., 2005).

The toxicity of nitrite to aquaculture species in the biofloc technology system is a critical consideration during the startup of a biofloc system. This is because nitrite accumulation may result in higher mortality, and thus typically the system is monitored till the nitrification process is established before introducing the culture species into the water (Hargreaves, 2006). Among the toxic nitrogenous compounds in aquaculture, nitrite toxicity is the primary cause of hypoxia because it affects oxygen transport by combining with hemocyanin to form metahemocyanin. This latter compound is unable to transport oxygen to the tissues, resulting in high mortality (Wasielesky, 2017). Another likely effect of nitrite accumulation in BFT systems is the inhibition of certain enzymes, including the metallo-enzyme carbonic anhydrase, which functions in ion transport across tissues and organs in fish and shrimp (Innocenti, Zimmerman, Ferry, Scozzafava, & Supuran, 2004). Nitrite may also affect the process of $Na^+$ absorption in the gills of fish and shrimp by affecting the production of the hormone T4, resulting in water retention by the kidney and additionally affecting the excretion of ammonia with lethal effects (Baldisserotto, 2013). Nitrite toxicity is often minimal in the presence of chlorine ions since they inhibit the diffusion of nitrite across the gills of aquaculture animals (Baldisserotto, 2013).

Nitrate, however, is less toxic to fish and shrimp except at high concentrations (> 100 mg/L) (Lekang, 2007) and in the event of synergistic effects resulting from the combined action of nitrates and other nitrogenous substances (Wasielesky et al., 2017). Given that biofloc systems are considered to be zero-exchange systems, the toxicity of nitrate becomes particularly important because it can accumulate to levels that become lethal (Luo, Avnimelech, Pan, & Tan, 2013). However, instances of nitrate toxicity are few. Some studies have reported that in tilapia culture, nitrates becomes a problem only at levels between 600 and 700 mg/l, and even those levels only affect the feed intake of the fish (Rakocy, Bailey, Martin, & Shultz, 2000).

As a result, attention is typically directed to the control of ammonia and nitrite in BFT systems with little focus on nitrate accumulation. Thus, to understand the dynamics of nitrogen and toxic nitrogenous compounds in BFT systems and allow their effective control or removal, their effects on the aquaculture animals should be known to determine which of the nitrogenous compounds deserves immediate attention based on their toxicity.

## 3. Nitrogen cycle/recycling and control in the BFT system

In BFT systems, nitrogenous compounds resulting from leftover feed and excretion products (tilapia or shrimp), are recycled by microorganisms including algae, autotrophic bacteria and heterotrophic bacteria. However, it is important to note that these categories of microorganisms differ in the manner in which they recycle, immobilize and transform the different nitrogenous compounds within the biofloc system (Martinez-Cordova et al., 2015). Additionally, it is worth knowing that the various forms of nitrogen in the biofloc system, including ammonia-nitrogen ($NH_3$–N), nitrite nitrogen ($NO_2^-$-N), nitrate-nitrogen ($NO_3^-$-N), total ammonia nitrogen (TAN) and total Kjeldahl nitrogen (TKN) can be utilized in specific ways by the microorganisms depending on their metabolic requirements (Shan & Obbard, 2001). For example, under aerobic conditions, the nitrifying bacteria (autotrophic bacteria) responsible for nitrification are able to transform harmful ammonia into less harmful nitrates. The nitrification process is carried out by two categories of autotrophic bacteria, the chemolithoautotrophic bacteria and the ammonia-oxidizing archaea (AOA), both of which oxidize ammonia to nitrite (Souza et al., 2019) via hydroxylamine (Martinez-Cordova et al., 2015). The specific bacteria responsible for this oxidizing step include bacteria belonging to the following taxa: *Nitrosovibrio, Nitrosolobus, Nitrosomonas, Nitrosococcus* and *Nitrospira* (Ray, 2012).

After completion of the first step of nitrification by ammonia-oxidizing bacteria (AOB), the second and final step is carried out by the nitrite-oxidizing bacteria (NOB). This group of bacteria are responsible for oxidizing nitrite to nitrates, which is the form of nitrogen that is generally harmless to the fish except at high levels (> 100 mg/L) (Chavez-Crooker & Obreque-Contreras, 2010). Relevant studies have established that the autotrophic bacteria responsible for oxidizing ammonia utilize carbon dioxide as a source of carbon and are classified as Beta- and Gammaproteobacteria (Koops & Pommerening- Röser, 2001). The nitrite-oxidizing bacteria are grouped under the Alpha- and Gammaproteobacteria and are classified under the phylum Nitrospirae (Martinez-Cardovo et al., 2015). Other bacterial taxa may include *Nitrospina, Nitrococcus*, *Nitrobacter* (Lekang, 2007; Ray, 2012), and gram-negative aerobic bacteria that convert nitrites into nitrates (Sigee, 2005). (The nitrification process is discussed in the next section).

In outdoor biofloc systems, the role of algae in the removal or uptake of nitrogenous compounds cannot be understated. Algal uptake of nitrogen is classified as one of the key pathways of nitrogen removal (Hargreaves, 2013; Jiménez-Ojeda et al., 2018). Depending on the situation, algal uptake may constitute the dominant removal pathway for ammonia nitrogen, although this removal method may be short-lived due to the possibility of an algal population crash (Ebeling et al., 2006; Hargreaves, 2006). This crash eventually results in the release of the immobilized nitrogen back into the water.

Notably, the work of Sigee (2005) has comprehensively described another pathway of nitrogen removal and control from aquaculture water that involves the reduction of nitrates into gaseous molecular nitrogen ($N_2$), a process known as denitrification. This process is carried out by heterotrophic bacteria rather than autotrophic bacteria. As in the case of nitrification, this group of bacteria relies on the reduction of nitrates to nitrogen gas as a mechanism of obtaining energy for their normal activities and therefore requires a carbon source to accomplish this under anaerobic conditions (no or minimal oxygen conditions) (Sigee, 2005). According to Jiménez-Ojeda et al. (2018), the direct removal of nitrates from the aquaculture water could be accomplished by algal species in the system. The role of the above pathways of uptake or removal of nitrogenous compounds from the water in biofloc systems cannot be ignored due to the significance of those processes; however, removal by heterotrophic bacteria eventually becomes the dominant mode of nitrogen control in biofloc systems after the nitrification process is fully established.

The heterotrophic removal of ammonia from biofloc systems has been the major focus of most previous studies, probably due to the benefits derived from their activities. These benefits include their roles in the immobilization of nitrogen compounds and conversion into protein, which eventually becomes a secondary feed source for the culture organism. These benefits also include their key function of outcompeting other microorganisms in the system, particularly pathogenic bacteria (Hargreaves, 2013; De Schryver, Crab, Defoirdt, Boon, & Verstraete, 2008; Avnimelech, 2009, p. 182; 1999). The microbial flocs, also called bioflocs, serve as a good source of nutrition for the cultured species in BFT systems (Avnimelech, 2015). This conversion of nitrogen into protein by the heterotrophic bacteria eventually controls the levels of toxic nitrogenous compounds in the system. It must be remembered that the success of the heterotrophic bacteria in the BFT system in converting toxic nitrogen compounds into bacterial biomass is largely dependent on the carbon/nitrogen ratio (C/N), which is regarded as a control parameter (Emerenciano et al., 2017; Avnimelech, 1999). It is typically recommended that the C/N ratio be maintained within the range of 15–20:1 to achieve optimal activity of heterotrophic bacteria (Avnimelech, 2009, p. 182).

Another ammonia removal method that is not typically mentioned is the dissimilatory reduction of nitrite to ammonia and its eventual conversion to nitrogen gas. In this process, ammonia is directly broken down by some bacteria during the nitrification process. The above discussion of ammonia removal and control pathways are summarized in aquaculture and in environmental chemistry into the following processes: mineralization, nitrification, denitrification and nitrogen fixation as described by Stein and Klotz (2016).

## 4. Nitrification in BFT systems

In BFT systems, nitrification is a very important process due to its role in converting harmful ammonia to nitrate. Nitrification is a process that requires serious consideration during the startup and operation of a BFT system. Monitoring the nitrification process is necessary due to the effects of the intermediate product of nitrification (nitrite) on the culture organism. As indicated previously, nitrification involves two steps performed by two different groups of autotrophic bacteria, the ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (NOB) (Ebeling et al., 2006; Lekang, 2007). The first step of the nitrification process describes the transformation of ammonia to nitrite by bacteria belonging to the genera *Nitrosovibrio, Nitrosolobus, Nitrosomonas, Nitrosospira,* and *Nitrosococcus* (Bregnballe, 2010; Ebeling, 2013; Ray, 2012). The second step involves the oxidation of nitrite to nitrates, which is carried out by autotrophic bacteria belonging the genera *Nitrobacter, Nitrospira, Nitrospina* and *Nitrococcus* (Ray, 2012).

These two processes involved in the nitrification process are illustrated below:

It important to note that these two groups of nitrifying bacteria are obligate autotrophs (Sigee, 2005), which utilize carbon dioxide as their energy source (Lekang, 2007). In BFT systems, there is intense competition between the autotrophic and heterotrophic bacterial communities during the startup stage. Therefore, it is always important to promote the growth of the heterotrophic bacteria by supplying adequate external carbon sources until the nitrification process is fully established (Hargreaves, 2006). Although the heterotrophic bacterial community fast-growing, it should be noted that they are challenged by grazing from protozoa (Hahn & Hofle, 1999; Silva et al., 2013).

Due to the importance of the nitrification process in the biofloc system, most studies on these systems typically report trends in the forms of nitrogen to help document the establishment of the nitrification process. Total ammonia nitrogen, nitrite and nitrate levels are typically studied throughout the experiment and are used as indicators to study the microbial community and the efficiency in converting toxic nitrogen compounds to the less toxic forms. Ebeling (2013) has presented general trends for the nitrification process and this could be used as a framework during the startup of the biofloc system. It should be understood that as part of the nitrification process, nitrite peaks typically occur over a period of time and eventually decline and are accompanied by a rise in nitrate levels (Souza et al., 2019). Several other studies have demonstrated these trends, although the number of days for the establishment of the peak might vary.

It is important to note that the nitrification process results in a decline in pH, and thus the necessary steps must always be taken to balance the pH to enhance the activity of the nitrifying bacteria (Ebeling et al., 2006).

## 5. Factors influencing the nitrification process in BFT systems

The rate of nitrification is influenced by a number of factors including pH, dissolved oxygen (DO), temperature (Koops & Pommerening- Röser, 2001; Timmons et al., 2002), ammonia-nitrogen concentration, C/N ratio, and alkalinity (Ebeling et al., 2006). Studies have also confirmed salinity as another critical factor that affects the nitrification process in BFT systems (Bovendeur, 1989; De Alvarenga et al., 2018). It has been proposed that the toxicity of nitrite accumulation as a result of nitrification in BFT systems could be reduced by employing saline water (Luo et al., 2014) or moderate levels of salinity (De Alvarenga et al., 2018).

Specifically, the pH of the biofloc water is critical to the nitrification process because it affects the activities of the nitrifying bacteria. At a lower pH, the rate of nitrification decreases; Odegaard (1992) observed that the rate of nitrification declined by 90% when there was a decrease in pH from neutral (7) to 6. It is recommended that the appropriate pH range is approximately 8–9, which favors the nitrification process (Henze & Harremoës, 1990). The availability of free hydrogen ions that are associated with lower pH values depends on the buffering capacity of the water (Lekang, 2007). Therefore, liming agents such as calcium carbonates may be used to increase the pH when nitrification has caused a decline in pH, as is expected in biofloc systems as nitrification progresses (Alves et al., 2017; Azim & Little, 2008).

The concentration of dissolved oxygen (DO) is clearly a factor that affects nitrification in the BFT system. Because nitrifying bacteria are obligate aerobes, oxygen enhances their activity and thus increases the rate of nitrification. Therefore, it is important to keep the oxygen concentration sufficiently high to facilitate the activity of these bacteria (Lekang, 2007). It has been shown that low levels of oxygen ($< 4$ mg/L) cause a decline in the activity of *Nitrosomonas*, and *Nitrobacter* is affected at oxygen levels below 2 mg/L (Huag & McCarty, 1971).

The effect of temperature on nitrification cannot be overstated. For example, the growth and therefore the activity of nitrifying bacteria is greatly influenced by temperature. The recommended optimal temperature is approximately 30 °C, although bacteria may adjust to lower temperature environments (Lekang, 2007; Timmons et al., 2002).

The initial concentration of ammonia in the system is also a factor influencing the growth of bacteria and the nitrification process. High ammonia levels could significantly affect the growth of nitrifying bacteria as do very low levels of ammonia (Lekang, 2007). In some systems such as recirculating systems and waste water treatment, the optimal value for ammonia is set above 3 mg/L to attain maximum growth of nitrifying bacteria (Odegaard, 1992).

Manipulation of the carbon to nitrogen ratio affects nitrification. At higher C/N ratios, the activity of heterotrophic bacteria is favored over that of nitrifying autotrophic bacteria, which results in the heterotrophic bacteria outcompeting the autotrophic bacteria due to a higher growth rate and a reduced doubling time (Ebeling et al., 2006; Hargreaves, 2006). Lekang (2007) observed a decrease in the rate of nitrification by 60%–70% when the C/N ratio in the form of chemical oxygen demand (COD)/N was increased.

It has been reported that the presence of free chloride ions in saline water may reduce the activity of nitrifying bacteria, resulting in slower growth, and studies have indicated that nitrification in freshwater occurs more rapidly than in saline water (Nijhof & Bovendeur, 1990). A recent study by De Alvarenga et al. (2018) has illustrated this phenomenon in which the nitrification in freshwater biofloc systems was more rapid than in saline biofloc systems.

Other factors that might affect the nitrification process due to their effect on the nitrifying bacteria include toxic substances such as metal ions and organic substances such as formaldehyde (Nijhof & Bovendeur, 1990). It should be noted that the factors discussed above interact with one another (Lekang, 2007).

## 6. Control of toxic nitrogenous compounds in BFT systems by manipulation of carbon to nitrogen ratios (C/N)

One critical method to control the accumulation of toxic nitrogenous compounds in biofloc technology systems, particularly ammonia-nitrogen ($NH_3$–N) or total ammonia nitrogen (TAN), is the manipulation of the C/N ratio to achieve a ratio that favors the activity of the heterotrophic bacterial community. Avnimelech (1999) has specifically reported that to stimulate the removal of toxic nitrogenous compounds ($NH_4^+$ and $NO_2^-$) by heterotrophic bacteria through the assimilation into microbial biomass, it is necessary to supply an external carbon source. By providing external carbohydrates, the heterotrophic bacterial community is able to combine carbon and nitrogen in a specific ratio as part of their normal growth activity, resulting in a reduction in ammonia levels. Hence, the carbon to nitrogen ratio becomes a control method for inorganic nitrogenous compounds in BFT systems (Avnimelech, 1999; Hargreaves, 2013). Studies have demonstrated that a carbon to nitrogen ratio of 12–15 : 1 is generally preferred by the heterotrophic bacterial community (Hargreaves, 2013; Rhode, 2014). However, Ebeling et al. (2006) have indicated that a carbon to nitrogen ratio of 20 : 1 is more favorable for the heterotrophic bacterial community and stimulates strong microbial immobilization of nitrogenous compounds.

It has been proposed elsewhere that the range for the carbon to nitrogen ratio should be maintained at 12–20 : 1 during the early stages of the biofloc system to achieve optimal stimulation and stabilization of the heterotrophic bacterial community (Avnimelech, 2015). The external carbohydrates most often employed to raise the carbon to nitrogen ratio to the optimal levels include dextrose, glycerin, sugar, sucrose (Rhode, 2014), starch and cellulose (Avnimelech, 1999). Notably, other researchers have reported that poly-beta-hydroxybutyrate obtained from microorganisms (Zhang, Luo, Tan, Liu, & Hou, 2016) and polycaprolactone could also be used as stable carbon sources capable of maintaining the acceptable C/N ratio in BFT systems (Luo et al., 2017).

However, it is important to remember that simple sugars or external carbohydrates better stimulate the uptake and conversion of nitrogen to microbial protein by heterotrophic bacteria and that a high protein content in the feed would require higher levels of carbohydrates to balance the ratio (Rhode, 2014). For example, a feed with 30%–35% crude protein will have a carbon to nitrogen ratio of 9–10 : 1, which is lower than the recommended C/N range. Therefore, necessary steps must be taken to raise the carbon to nitrogen ratio or reduce the protein content of the feed to favor the uptake of ammonia by the heterotrophic bacterial community (Abu Bakar et al., 2015; Hargreaves, 2013).

Emerenciano et al. (2017) discussed that manipulation of the carbon to nitrogen ratio occurs in two phases: the early phase of formation involves a carbon to nitrogen ratio of 12–20 : 1, and the maintenance phase involves a carbon to nitrogen ratio of 6 : 1, depending on the TAN levels recorded. To effectively control the toxic nitrogenous compounds in the biofloc system by supplementation with carbohydrates, it is necessary to know that the carbon to nitrogen ratio and feed protein content have an inverse relationship (Avnimelech, 2015; Jiménez-Ojeda et al., 2018). It is also important to understand that the heterotrophic uptake of nitrogenous waste from the biofloc system is considered more stable and reliable compared with the removal of nitrogenous waste via the nitrification process or by algae (Hargreaves, 2013).

However, the continuous supply of external carbohydrates to biofloc systems eventually results in the accumulation of solids in the system. This negatively affects the normal growth and development of the culture species by depleting oxygen in the system and clogging the gills of the animals (Hargreaves, 2013). Zhang et al. (2016) proposed that the use of poly-beta-hydroxybutyrate and polycaprolactone (Luo et al., 2017) could solve this problem of high solids accumulation in the system and produce results to similar those achieved with the use of simple carbohydrates. Although the biofloc system can be manipulated to favor the activity of the heterotrophic bacterial community, it must remembered that the nitrifying (chemoautotrophic) bacterial community also plays a significant role in the control of nitrogenous waste within the biofloc system (Emerenciano et al., 2017).

To manage the carbon to nitrogen ratio (C/N), Emerenciano et al. (2017) derived a mathematical framework that could be adopted in biofloc systems depending on the TAN in the system. This framework is based on an external carbohydrate with a carbon content of approximately 50%. It is further assumed that the protein retention rate of the fish and shrimp is approximately 35% and 20%, respectively. These assumptions could be adjusted depending on the carbon content of the external carbon source and the aquaculture species under consideration.

## 7. Recent research on nitrogen dynamics and C/N manipulation in BFT systems

In recent years, a number of studies have attempted to understand the dynamics of nitrogen and the effects of the carbon to nitrogen ratio and how they impact the water quality and the biofloc formation process. For example, Nootong, Pavasant, and Powtongsook (2011) investigated the effects of external carbon additions and how they influenced the control of nitrogen in a biofloc system by maintaining a carbon to nitrogen ratio of 16:1. Specifically, they evaluated the effects of the carbon addition on the effectiveness of the heterotrophic bacterial assimilation of nitrogen and its effects on nitrification. Nootong et al. (2011) observed that heterotrophic bacteria control or removal of nitrogen from the biofloc system was profound and noticeable even before the nitrification process was fully established, and effective control of nitrogenous waste in the system was observed when nitrification was fully established after a period of 6–7 weeks. It was therefore reported that both nitrification and assimilation by the heterotrophic bacterial community were responsible for controlling the nitrogenous waste in the system. Given this finding, it can be concluded that in BFT systems, control of toxic nitrogenous compounds is accomplished through the activities of both the nitrifying bacteria and those of the heterotrophic bacterial communities (immobilization into bacterial biomass). Thus, to understand nitrogen dynamics and its control in BFT systems, it is necessary to understand the nitrification process and the role of heterotrophic bacteria.

In another study, Li et al. (2018) reported that different solid carbon sources (including Longan powder, polyhydroxybutyrate, hydroxyvalerate and polybutylene succinate) used in biofloc systems differed in their impact on the water quality and the bacterial community. The nitrite ($NO_2^-$) accumulation rates and levels in the systems differed significantly among groups with different solid carbon sources. As a result, nitrate ($NO_3^-$) accumulation rates were also different, although the level of total ammonia nitrogen (TAN) in all the three systems observed did not differ significantly. Additionally, the bacterial community in the water, including the microbiota in the gut of the fish, showed different results for the different types of solid carbon sources utilized in the system (Li et al., 2018). From these findings, it can be concluded that using different carbon sources in the biofloc system affects the nitrification process (as indicated by different nitrite and nitrate accumulation rates) and influences the bacterial community composition.

De Alvarenga et al. (2018) evaluated the beneficial effects of moderate salinity on the growth performance of tilapia fingerlings in a biofloc-based system. They also sought to evaluate whether moderate salinity in a biofloc system was capable of reducing mortality in tilapia fingerlings during nitrite peaks. Their findings were remarkable, as they were able to control the toxicity of nitrogenous compounds such as nitrites ($NO_2^-$), which is a significant aspect of nitrogen dynamics. The effects of moderate levels of salinity on the activity of nitrifying bacteria has also been studied by Dincer and Kargi (2001) and Somville (1984). The results of these studies indicated that salinity influences the nitrification process by causing a decline in the population of nitrifying bacteria at higher salinity levels. However, lower levels of salinity enhanced the activity of bacteria (Altendorf et al., 2013; Wood, 2015). Therefore, as part of the nitrogen dynamics in BFT systems, salinity may be described as a control parameter for nitrite toxicity because it affects the bacterial community and reduces the nitrite levels as assumed by De Alvarenga et al. (2018).

As stated previously, nitrification is a critical process in the BFT system, and nitrite accumulation in the culture system over time is an indication of the progression nitrification as a result of the activity of nitrifying bacteria as observed in several studies (Burford & Longmore, 2001; Hari, Kurup, Varghese, Schrama, & Verdegem, 2006; Luo et al., 2014; Widanarni, Ekasari, & Maryam, 2012). The nitrification process is considered an important process because the overall removal of toxic nitrogenous substances from the system is largely depend upon it (De Schryver et al., 2008; Souza et al., 2019). Hence, recent studies have focused on identifying the factors influencing the process.

Recently, in an attempt to gain insight into nitrification and its drivers in BFT systems, Souza et al. (2019) investigated whether the biofloc size affects the rate and process of nitrification. The study concluded that biofloc size per say does not influence the nitrification process, although the perturbation of the biofloc through agitation (sifting) or rupture of the biofloc affects the autotrophic bacterial community (nitrifying bacteria). This subsequently reduces the rate of nitrification. These new insights could significantly impact the BFT industry by helping practitioners and researchers to adequately understand the variation in nitrification that occurs in BFT systems. Aeration causes turbulence in the water column in biofloc systems, but there is limited research on the effects of aeration on water quality parameters and nitrogen dynamics in biofloc systems. Lara, Krummenauer, Abreu, Poersch, and Wasielesky (2017) found that some aerators rupture bioflocs or particles in the system, and they concluded that the rupture of the flocs may negatively affect the rate of nitrification in BFT systems. This phenomenon could result in the buildup of ammonia in the culture unit, culminating in negative effects on the growth and survival of the culture species. From these findings, one could hypothesize that a reduction in the particle size of bioflocs affects the nitrification process. In contrast, Souza et al. (2019) have demonstrated that the size of the biofloc does not affect nitrification in the biofloc system. However, what affects nitrification is the breakage/rapture of flocs through turbulence (Rusten, Eikebrokk, Ulgenes, & Lygren, 2006) or disturbance due to its effects on the activity of the nitrifying bacterial community. This study stands in contrast to numerous studies that have suggested that the particle size or the size of the biofloc could affect the nitrification process (Carvalho, Meyer, Yuan, & Keller, 2006; Vlaeminck et al., 2010).

One widely acknowledged factor that significantly affects and typically slows nitrification in the biofloc system is the accumulation of organic matter (Wijeyekoon, Mino, Satoh, & Matsuo, 2004). The accumulation of organic matter in the system results in a decline in the activity of nitrifying bacteria, but enhances the activity of heterotrophic bacteria; the heterotrophic bacterial community thus outcompetes the nitrifying bacteria due to their more rapid growth rate and thereby affects the nitrification process (Ma et al., 2013).

## 8. Conclusions

To effectively control the toxic nitrogenous compounds in BFT systems, it is necessary to understand the dynamics of these compounds in the system. Nitrogen dynamics and control are critical aspects of BFT systems. Additionally, understanding the fate of nitrogen and its related compounds, including their control through the manipulation of the carbon to nitrogen ratio, are key to successful BFT systems. The effectiveness of BFT systems is largely dependent on their ability to remove nitrogen from the water through various pathways. However, there is an inadequate understanding of how to effectively manipulate the system to favor the nitrification process or the heterotrophic removal of ammonia since, in most cases, both processes occur simultaneously. Therefore, this paper proposes that further research should be conducted to identify the best method to manipulate the biofloc system to favor either the nitrifying bacteria or the heterotrophic bacteria. A significant knowledge gap that needs to be addressed by future studies relates to understanding the complexities of nitrogen dynamics in BFT systems, which is relevant because the nitrogen in BFT systems may be controlled and influenced by different factors that affect the dominance of heterotrophic or autotrophic bacteria. Finally, research should also focus on exploring strategies that could reduce the toxicities of the nitrogenous compounds that accumulate in BFT systems.

## Declaration of competing interest

The authors declare no conflict of interest.

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