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BIOLOGICAL REMOVAL OF NITROGENOUS WASTE COMPOUNDS IN THE BIOFLOC AQUACULTURE SYSTEM – A REVIEW

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Abstract

Aquaculture has experienced significant global expansion and is considered one of the fastest-growing sectors in food production. However, there exist additional challenges that restrict the capacity to achieve maximum efficiency in aquaculture systems, such as issues over water quality and shortages of appropriate live feeds. Intensive aquaculture systems involve the use of protein-rich prepared feed for feeding the cultured animals. This may give rise to the discharge of nitrogenous compounds into the water, which can pose a risk to the environment when present in excessive quantities beyond the acceptable levels. In recent years, an innovative method called biofloc technology (BFT) has become a practical solution to this issue. Undoubtedly, BFT offers a groundbreaking method for nutrient disposal that eradicates the requirement for excessive water use or equipment maintenance. Three primary types of microorganisms are crucial in alleviating the adverse impacts of nitrogen compounds in this technique. Photoautotrophs participate in the processes of removal and absorption, whereas chemoautotrophs promote nitrification and conversion. Heterotrophs contribute to the absorption process. Biofloc predominantly consists of heterotrophic bacteria, alongside algae, protozoa, rotifers, and nematodes. While there have been reviews carried out on multiple aspects of biofloc technology, there exists a lack of literature that tackles this particular field of research progress. This article discusses every aspect and techniques of biological management used for removing nitrogenous waste compounds in biofloc aquaculture systems.

Key words: aquaculture system, biofloc technology, nitrogen compounds, microorganism, heterotrophic bacteria

Aquaculture can have significant impacts on nutrient levels and coastal ecosystem (Khanjani et al., 2024 a). These impacts are often due to the discharge of effluents rich in nutrients and organic matter from the farms. The nutrients in these effluents can cause eutrophication in coastal waters, leading to increased algal growth and subsequent oxygen depletion, which can harm marine life (Yeganeh et al., 2020). Aquaculture practices are becoming more intensive as a result of the need to increase production (Khanjani et al., 2023 d).

Farm effluents rich in nutrients and organic matter often cause these impacts through their discharge. The nutrients in these effluents can cause eutrophication in coastal waters, leading to increased algal growth and subsequent oxygen depletion, which can harm marine life (Yeganeh et al., 2020). Aquaculture practices are becoming more intensive due to the need to increase production (Khanjani et al., 2023 d).

The rearing units receive significant nutrient additions in these situations. According to Gutierrez-Wing and Malone (2006), 85% of the phosphorus, 80–88% of the carbon, 52–95% of the nitrogen, and 60% of feed will end up in the rearing water as particulate matter, dissolved chemicals, or gases. Researchers are conducting significant research to find ways to reduce or eliminate nutrients, especially nitrogen compounds, from aquaculture systems. Understanding how to remove inorganic nitrogen compounds in aquaculture would help in the proper management of aquaculture wastewaters. Various biological treatment processes transform the undesirable nitrogen forms in wastewater into safer forms for discharge (Paul and Banerjee, 2022; Khanjani et al., 2022 d; Bai et al., 2023). Recent years have witnessed the use of new technologies for this purpose. A treatment method for aquaculture wastewater needs to take several factors into account, such as the type of polluting factor, the climate, the amount of available water, the amount of wastewater discharged, the amount of land available, the level and type of aquaculture and the cost-benefit ratio. The biofloc technology (BFT) offers these advantages,

but its success depends on its ability to remove, recycle or control harmful nitrogenous substances in the culture system (Souza et al., 2019; Abakari et al., 2021; Khanjani et al., 2023 a, 2024 b). Even relatively low levels of nitrogen can negatively impact growth performance in BFT systems (Bregnballe, 2010; Emerenciano et al., 2017; Timmons et al., 2002). There are several forms of nitrogen accumulation, including ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), total nitrogen (TN), and total ammonia nitrogen (TAN) (Ebeling et al., 2006).

In order to guarantee the success of the BFT system and the aquaculture industry, it is crucial to comprehend the dynamics and regulation of toxic nitrogenous compounds. Several studies have described the processes, dynamics, and methods for controlling nitrogen in BFT systems (Ebeling et al., 2006; Luo et al., 2017; Silva et al., 2013; Souza et al., 2019). A comprehensive document containing this knowledge can serve as a useful resource for practitioners and researchers of the BFT system if it is synthesized and compiled. Aquaculture has dubbed the BFT system as the "blue revolution" due to its many advantages and simplicity. As a "blue revolution", BFT involves the cycling of nutrients and their reuse within the same system, which is designed as a zero-exchange or minimal-exchange system (water). BFT operating method must be demonstrated to be different from most other aquaculture systems. To guide the industry, it is essential to gather information on nitrogen dynamics in these systems, along with the elimination process. The aim of this review is to synthesize the current understanding of nitrogen transport and regulation in BFT-based systems. Additionally, it examines the importance of BFT, the removal of nitrogen compounds in BFT-based systems, and the advancement of sustainable aquaculture through the use of BFT systems.

Aquaculture and inorganic nitrogen compounds

Aquaculture ponds have multiple types of nitrogen, and the quantity of nitrogen generated increases with the fish density. Fish consume both formulated and natural feeds that contain proteins. It is common for formulated feeds to contain 30 to 40% protein. In aquatic animals, they use part of the protein as a source of energy (Hepher, 1985). About 25% of the nitrogen in the feed is consumed and recycled by the fish, while approximately 75% is released into the water (Avnimelech and Ritvo, 2003; Crab et al., 2012). For instance, a fish pond containing 500 grams of fish per square meter, with a daily feeding of 10 grams (2% of fish weight), has a protein content of 30%, equivalent to 3 grams of protein or 0.465 grams of nitrogen per day. Out of this amount, 75% or 350 milligrams of nitrogen per square meter is excreted. This excretion results in approximately 0.350 milligrams of nitrogen per liter per day per meter of pool depth. In ponds with 5 kilograms of fish per square meter, nitrogen production is ten times higher (Crab et al., 2012). In 2009, Boy and Tucker estimated that fish excrete 30 grams of ammonia-

nitrogen per kilogram of feed containing 25–40% protein. Timmons et al. (2002) calculated the total ammonia nitrogen (TAN) excreted as follows:

$$P_{TAN} = F \times PC \times 0.092$$

Where P_{TAN} is the amount of TAN produced (kg/day), F is the amount of feeding (kg/day), and PC is the protein concentration in the feed. On the other hand, several aquaculture species require nitrogen and nitrogenous compounds as essential water quality parameters. It should not be neglected that nitrogen is an essential element required by aquaculture organisms for various physiological processes as well as a constituent of tissues, fluids and molecules such as body proteins (Wei et al., 2016), nucleic acids, nitrogenous bases, pigments, adenosine phosphates, etc. (Ebeling, 2013; Sigee, 2005). Thus, it is important to evaluate nitrogen in aquaculture during water quality analysis, particularly in the form of ammonia and nitrite (Bai et al., 2023).

Various sources of nitrogen exist in aquaculture systems, including nitrogen from leftover feed, fish feces, urea, dead animals, and atmospheric sources. Therefore, aquaculture systems can potentially contain both organic and inorganic nitrogen. Cultured species can be adversely affected by all these forms, which can accumulate in the culture environment and become toxic compounds in excessive levels. Nitrogen in the form of nitrates, nitrites, and ammonia is highly soluble in aquaculture water. Ammonia can occur in aquaculture water, specifically in biofloc systems, in two forms: unionized ammonia (NH_3) and ionized ammonium (NH_4^+) (Ebeling et al., 2006; Lekang, 2007). Ammonia nitrogen exists in equilibrium of these two forms that are collectively referred to as total ammonia nitrogen (TAN). Controlling or reducing one form results in a reduction of the other, and depending on the temperature, salinity, and pH, one form will be dominant. Species differences, age and physiological conditions affect fish tolerance to TAN. As an example, adult fish are more ammonia-tolerant than juveniles and fingerlings (Bregnballe, 2010; Lekang, 2007; Ebeling et al., 2006; Dauda et al., 2019). Its high concentration in the water can affect growth, molting (in crustaceans), oxygen consumption, and even kill fish or shrimp. Thus, it has been suggested that water ammonia levels in aquaculture systems should be less than 0.025 mg/L.

Nitrite is also toxic to cultured aquatic organisms. As a natural component of the nitrogen cycle, it is an intermediate product of the conversion of ammonia to nitrate by bacteria. However, nitrite is a very unstable compound that is easily oxidized into nitrate when oxygen is present or reduced to ammonia when oxygen is absent. Since nitrite is converted to nitrate by certain bacteria, environmental conditions that affect bacterial growth and metabolism can affect the rate of conversion and nitrite levels. Commercial fish and shellfish species, as well as ornamental fishes, can be adversely affected by high nitrite concentrations (Boyd, 1992; Alcaraz and Espina,

1995; Dvorak, 2004; Svobodova et al., 2005; Boyd and Tucker, 2009). Increasing of water nitrite concentration also has a negative effect on the growth and survival of fish and shrimp (Mallasen et al., 2006) and also, reduces resistance to diseases. When nitrite is absorbed by fish, it reacts with hemoglobin to form methemoglobin, which impairs oxygen-carrying capacity. This leads to hypoxia and cyanosis which cause stress and mortality (Colt and Armstrong, 1981; Colt, 2006). This effect is most noticeable in the gills but other tissues such as the liver, brain and muscle also tend to accumulate nitrite (Hu et al., 2012). When fish are exposed to nitrite for a long period of time, they can become anemic. In the nitrogen cycle, nitrate plays an important role. Nitrate is the final product of nitrification and is relatively less toxic to fish. In fish culture, 50 mg/L is generally accepted as a safe limit for nitrate nitrogen (Gutierrez-Wing and Malone, 2006). According to Yusoff et al. (2011), the acceptable level of nitrate for seawater culture is less than 20 mg/L. Moreover, NO_3^- may also be considered toxic when they accumulate above 100 mg/L in the system (Bregnballe, 2010).

Removal of ammonia from aquaculture systems

Biofiltration systems with bacteria have been widely used in closed culture systems, such as recirculated aquaculture systems (RAS), to remove ammonia from the water by nitrification process (Valenti and Daniels, 2000). While these methods can be effective, they can sometimes be costly and labor-intensive. Although, in some special circumstances, certain methods, such as reducing or stopping feeding, agitating the pool water with fresh water, reducing stocking density, aerating the pool, and lowering the pH level, may help us to reduce ammonia in RAS. However, these are only useful in case of emergency and can potentially harm the farmed aquatic animals and delay production (Thompson et al., 2002). As a result, the use of this system can be relatively expensive. Furthermore, when the process is disrupted, nitrite levels may increase in the water (Jensen, 2003). To address the issues raised above, a novel approach known as BFT was developed.

Biofloc technology (BFT)

Microbial-based cultivation method operates by introducing organic carbon sources into water or augmenting the carbon content in the feed to enhance the carbon-to-nitrogen ratio. This system promotes nutrient cycling and is therefore considered more eco-friendly (Yu et al., 2023; Li et al., 2023).

This technology not only minimizes water consumption but also recycles nutrients and organic materials. Furthermore, it reduces the entry of pathogenic agents into the breeding system and improves biological security on the farm (Padeniya et al., 2022; Khanjani et al., 2023 b).

The biofloc system contains nitrogen in various forms, including molecular nitrogen, ammonia, ammonium, nitrite, nitrate, and organic nitrogen. Among these, inorganic nitrogen (specifically ammonia and nitrite) presents the most significant threat. A bacterial community is established and maintained by the system. During the bacterial growth process, clumps of bacteria are formed, which are referred to as flocs. Flocs serve as a food source for finfish and shellfish and are also important for nutrient recycling (Serfling, 2006). The production of microbial biomass removes nitrogenous substances from the water. This material is primarily developed by bacteria, algae, fungi, and detritus (Burford et al., 2004; Holl et al., 2006; Serfling, 2006). Bacterial growth's nitrogen uptake reduces the ammonium concentration more quickly than nitrification, as stated by Hargreaves (2006). The BFT system includes three types of organisms: photoautotrophic, chemoautotrophic, and heterotrophic, which aid in the removal of nitrogenous waste compounds (Khanjani et al., 2022 a).

In a BFT system, the thriving of heterotrophic bacteria plays a significant role in absorbing inorganic nitrogen (Pimentel et al., 2023). The rate of nitrogen uptake by heterotrophic bacteria is superior to that of denitrifying bacteria, leading to a growth rate and production of microbial biomass per unit substrate 10 times greater than the latter. Consequently, the immobilization of ammonia by heterotrophic bacteria typically occurs swiftly in BFT over a period of hours or days with suitable C/N ratios (Ferreira et al., 2021). BFT largely relies on organic carbon sources for maintaining heterotrophic bacteria. The primary driver of floc formation is the growth of heterotrophic bacteria dependent on supplied carbon (Luo et al., 2023). Therefore, additional carbon sources are introduced to the BFT tank, providing an appropriate C/N ratio.

Photoautotrophic organisms

Phytoplankton and algae in this category are more efficient in the presence of light and have a high nutritional value, primarily due to their content of carotenoids and unsaturated fatty acids. Their growth and survival in aquatic environments may be influenced by fluctuations in environmental parameters, including oxygen (O_2), carbon dioxide (CO_2), pH, and ammonia.

Nitrogen can be recovered from wastewater through the use of microalgae and cyanobacteria. During the treatment of nutrient-rich wastewater, these microorganisms produce oxygen. Subsequently, bacteria use this generated oxygen to break down organic matter in wastewater into simpler inorganic molecules (Sood et al., 2015; Delgadillo-Mirquez et al., 2016).

During the tertiary treatment stage of wastewater treatment, microalgae and bacteria play a crucial role in removing inorganic contaminants, ensuring the safe discharge of treated water. Through a process known as dissimilatory nitrate reduction, these microorganisms assimilate nitrate and ammonia, converting them into biomass rather than releasing them as gaseous nitrogen into the atmosphere. Cyanobacteria and microalgae are more efficient than complex plants in wastewater treatment

due to their lack of structural carbon. This efficiency allows them to produce more biomass, with a carbon to nitrogen (C:N) ratio ranging from 18 to 120. Microalgae and cyanobacteria perform optimally when various parameters are controlled and monitored (Taziki et al., 2016; Gonçalves et al., 2017; Xin et al., 2011). As a result of this process, microalgal biomass can be used to produce fertilizer, bioenergy, animal feed, and pharmaceutical products.

During photosynthetic processes, microalgae convert dissolved inorganic nutrients, such as NH_3-NH_4, NO_2, NO_3, PO_4, CO_2 into "particulate nutrient packs" (Neori et al., 2004; Das and Dash, 2022). In a study conducted by Martínez-Cordova et al. (2009), they utilized a series of interconnected bioremediation ponds containing bivalves and benthic microalgae (specifically *Navicula* sp.) to culture penaeid shrimp and treat wastewater. They observed that the presence of microalgae significantly reduced levels of total nitrogen, ammonia, nitrite, and nitrate. Furthermore, the effluents containing microalgae were successfully used to culture another group of shrimp, leading to positive outcomes. According to Vymazal (1988), *Navicula* sp. can remove 80% and 70% of ammonium and orthophosphate, respectively, from polluted streams.

Control of inorganic nitrogen generated by algae in pond culture, especially in extensive aquaculture systems, is a common practice. Algae growing in these ponds have a C:N ratio of 5, which is sufficient to control nitrogen generated in ponds that contain between 0.5 and 1.2 kg of fish/m² (Avnimelech, 2015). However, in pools with a higher density, this mechanism is not very effective. Algal activity is also unstable, which limits algae control. Total alkalinity (TAN) in pools increases at night due to algae carbon absorption, which is dependent on light. On cloudy days, when solar radiation is limited, algal nitrogen control becomes ineffective, resulting in fish stress or the need to stop feeding (Brune, 2003). Therefore, Biofloc is not very efficient at removing nitrogen waste compounds in this way.

Chemoautotrophic organisms

Chemoautotrophic bacteria undergo three stages of transformation during their biological process. First, *Nitrosomonas* and *Nitrosococcus* bacteria are activated by ammonia, which is produced by leftover feed and shrimp excrement. The waste nitrogen compounds are oxidized to nitrites. *Nitrobacter* and *Nitrospira* bacteria convert nitrite into nitrate in the second stage. In the third stage, nitrate is converted into nitrogen gas through the process of denitrification by *Achromobacter* and *Pseudomonas* bacteria, which then leaves the system (Khanjani et al., 2022 a). In this system, aerobic nitrifying bacteria consume organic carbon sources for their growth under aerobic conditions, and produce molecular nitrogen (N_2) from nitrite and nitrate in the cultivation water through the process of denitrification. This process is the reason for the drastic reduction of nitrite in rearing tanks to which

starch has been added (Liu et al., 2014). Therefore, this technology is suggested as an efficient and environmentally friendly system for rearing aquatic animals that can tolerate suspended solids, high density, filter feeder feeding and moderate oxygen levels (3 to 6 mg/L) (Khanjani et al., 2023 c; Emericiano et al., 2013).

Nitrification

Two steps are required for nitrification: (1) oxidation of NH_4^+ to NO_2^- and (2) conversion of NO_2^- to NO_3^-. The first step is carried out by ammonium oxidizing bacteria (AOB, e.g., *Nitrosomonas*, *Nitrosococcus*), catalyzed by ammonia monooxygenase (AMO) (Sedlacek et al., 2016; Daims et al., 2016; Wang et al., 2018), along with hydroxylamine oxidoreductase (HAO) (Beman et al., 2010), which produces NH_2OH as an intermediate product. With the help of molecular oxygen, nitrite-oxidizing bacteria (NOB, e.g., *Nitrobacter*, *Nitrospira*) participate in the second phase of nitrification. Nitrite oxidoreductases (NXR) and nitrite-oxidizing systems catalyze this step. The NXR enzyme is an oxidation enzyme found in *Nitrobacter* (Rahimi et al., 2020).

The Calvin cycle provides nearly all nitrifiers with their carbon, and their only source of energy is the oxidation of ammonia (Philips et al., 2002). Research has shown that 80% of this produced energy is consumed by CO_2 fixation, and each fixed carbon atom oxidizes 35 molecules of NH_3 or 100 molecules of NO_2^- (Wood, 1986). It can take between four and eight weeks for nitrifiers to colonize water, depending on external factors such as water temperature, alkalinity, salinity, and other stresses (Emparanza et al., 2009; Malone et al., 2006).

Several factors influence the rate of nitrification, including pH, dissolved oxygen (DO), temperature (Timmons et al., 2002), ammonia-nitrogen concentration, C/N ratio, and alkalinity (Ebeling et al., 2006). Salinity is also a critical factor that affects nitrification in BFT systems (Bovendeur, 1989; de Alvarenga et al., 2018). It has been suggested that saline water or moderate salinity could reduce the toxicity of nitrite accumulation caused by nitrification in BFT systems (Luo et al., 2014). Particularly, the pH of the biofloc water is critical to the nitrification process because it affects the activity of the nitrifying bacteria. Odegaard (1992) observed that the rate of nitrification decreased by 90% when the pH was decreased from neutral (7) to 6. A pH range of approximately 7–8 is recommended to facilitate nitrification (Mook et al., 2012).

Nitrification in the BFT system is clearly affected by DO concentration. As nitrifying bacteria are obligate aerobics, oxygen enhances their activity, and increases their nitrification rate. Therefore, maintaining a sufficiently high oxygen concentration facilitates the activity of these bacteria (Lekang, 2007). *Nitrosomonas* activity declines at low oxygen levels, and *Nitrobacter* activity also declines when oxygen levels are below 2 mg/L (Huag and McCarty, 1971). Nitrification is strongly influenced by temperature. For instance, temperature greatly influences

the growth and activity of nitrifying bacteria. Bacteria may adapt to lower temperatures (Lekang, 2007; Timmons et al., 2002). Additionally, the initial concentration of ammonia in the system influences the growth of bacteria and the nitrification process. For instance, very low levels of ammonia can affect the growth of nitrifying bacteria, and similarly, high ammonia levels can also severely affect this growth.

Microorganisms in nitrification

Two phylogenetically unrelated groups, AOB and NOB, work cooperatively in the process of aerobic nitrification. AOB converts ammonia into nitrite, which serves as a substrate for NOB. Although AOB has lower energy yields during nitrite oxidation to nitrate, it is protected from the toxicity of accumulated nitrite by NOB, which inhibits its buildup (Stein and Arp, 1998).

AOBs possess multilayered cell walls and flagella as their locomotory organs. Researchers have reported five genera of AOBs within two different subclasses of proteobacteria (Purkhold et al., 2000; Koops and Pommerening-Röser, 2001). In addition to *Nitrosomonas* (e.g., *Nitrosococcus mobilis*), *Nitrosospira*, *Nitrosovibrio*, and *Nitrosolobus*, this subclass of AOB also includes *Nitrosospira*. In contrast, literature reports the detection of *Nitrosococcus* clusters belonging to the γ-subclass (Junier et al., 2010; Fiencke et al., 2005). Although more than 25 species of AOB have been reported, *Nitrosomonas* and *Nitrobacter* remain the most widely studied (Mobarry et al., 1996; Stopforth et al., 2007). AMO and HAO are the key enzymes of AOB that convert ammonia to nitrite. AMO is a membrane-bound copper enzyme, while HAO is located in the periplasm (Ge et al., 2015). There are more NOBs in Proteobacteria than AOBs (Fiencke et al., 2005).

The literature reports eight species of NOB and four phylogenetically diverse groups (Lücker et al., 2010). *Nitrococcus* and *Nitrobacter* belong to the α- and γ-subclasses of Proteobacteria, respectively. A few years ago, *Nitrobacter* was considered to be the most important bacterium. In recent studies, *Nitrospira* has been reported to be the most prevalent NOB in wastewater treatment plants (WWTPs), drinking water, and soil systems (Stopforth et al., 2007; Wagner and Loy, 2002). In *Nitrobacter*, *Nitrococcus*, and *Nitrospira*, NXR is the key enzyme that mediates the conversion of nitrite to nitrate via NOB.

Denitrification

As a result of denitrification, nitrate is converted into nitrogen gas. Generally, heterotrophic denitrifiers perform this step after nitrification, although a small number of autotrophic nitrifiers can also perform it (Paul and Hall, 2021; Paul and Banerjee, 2022). Numerous factors are crucial in this process, specifically the maintenance of anoxic conditions, provision of a carbon source, and the subsequent treatment of the treated wastewater. Denitrifiers require an external organic carbon source, since external carbon acts as an electron donor. Glucose, methanol, ethanol, succinate, and acetate are some of the external sources of carbon (Razak et al., 2012; Miao and Liu, 2018).

Microorganisms in denitrification

The process involves both autotrophic and heterotrophic bacteria, although autotrophic bacteria are more widely applicable than heterotrophic bacteria. Furthermore, autotrophic bacteria exhibit slow growth and an inefficient ability to assimilate biomass (Khanjani et al., 2022 a). Autotrophic denitrifiers work by oxidizing inorganic matter and transporting discharged electrons to nitrate as a terminal acceptor. There are two types of autotrophic denitrifiers: hydrogen-based (*Micrococcus denitrificans* and *Paracoccus denitrificans*) and sulfur-based (*Thiobacillus denitrificans* and *T. thioparus*). In wastewater treatment processes, *Thiobacillus* sp. is the most commonly observed autotrophic denitrifier (Miao and Liu, 2018). *Pseudomonas* and *Bacillus* are the most widely observed heterotrophic denitrifiers (Zakhama-Sraieb et al., 2016). Denitrifiers utilize nitrate as a final electron acceptor, resulting in minimal dissolved oxygen consumption in wastewater and the ability to capture carbon from complex organic compounds. Moreover, these denitrifiers offer the advantage of requiring smaller reactor volumes for efficient bioconversion, thus reducing costs. The genera *Thauera*, *Paracoccus*, *Comamonas*, *Denitratisoma*, and the family Comamonadaceae are the most frequently encountered denitrifiers in wastewater treatment systems (Cowan et al., 2005).

Heterotrophic bacteria

Heterotrophic bacteria, such as Cyanobacteria (*Microcoleus chthonoplastes*, *Spirulina* sp., *Oscillatoria* sp., *Schizothrix* sp., *Calothrix* sp., *Phormidium* sp., etc.), have the ability to utilize ammonia nitrogen to produce harmless substances, including microbial biomass (Ebeling et al., 2006; Paniagua-Michel and Garcia, 2003). In addition to converting ammonia into biomass, heterotrophic bacteria can also break down organic waste like unconsumed feed, feces, and deceased organisms (Martínez-Cordova et al., 2017). One crucial method for controlling the accumulation of toxic nitrogenous compounds, specifically NH_3-N or TAN, in BFT-based systems is the manipulation of the C:N ratio to favor the activity of heterotrophic bacterial communities.

Based on several studies, it has been demonstrated that ammonia levels can be reduced in water exchange-free systems by manipulating the C:N ratio. This manipulation allows heterotrophic bacteria to absorb inorganic nitrogen and produce microbial biomass (Ebeling et al., 2006; Gao et al., 2012). Khanjani and Sharifinia (2021) observed a decrease in ammonia and nitrite levels in a zero-exchange system, which can be attributed to the presence of heterotrophic bacteria in biofloc rearing tanks. In

BFT systems, ammonia is rapidly assimilated (Khanjani and Alizadeh, 2024). Banerjee and Paul (2021) reported that a water body with an initial ammonia concentration of 10 mg/L disappeared within approximately 2 hours when glucose was added as a carbon source. Consequently, nitrite and nitrate concentrations in this BFT system would also remain low. However, in the control system without water exchange, the average ammonia concentration reached 6.35 mg/L, resulting in a high nitrite level of 11.78 mg/L (Huang, 2019). According to Cardona et al. (2016), *Litopenaeus stylirostris* survival rates were 27.2% higher in the BFT system compared to the conventional system with extensive water exchange. This also contributed to the low levels of ammonia and nitrite in the water.

Heterotrophic bacteria play a crucial role in reducing ammonia levels by utilizing external carbohydrates to combine carbon and nitrogen as part of their normal growth activity. This process is facilitated by maintaining an appropriate C:N ratio in BFT systems (Hargreaves, 2013). Generally, a C:N ratio between 12 and 15 is preferred by heterotrophic bacteria (Hargreaves, 2013; Rhode, 2014). However, Ebeling et al. (2006) found that a C:N ratio of 20:1 is more favorable for heterotrophic bacteria and promotes the immobilization of nitrogenous compounds. Other researchers have suggested that during the early stages of the BFT system, maintaining a C:N ratio range of 12–20:1 is essential to achieve optimal stimulation and stabilization of the heterotrophic bacterial community (Avnimelech, 2015).

Various external carbohydrates are commonly used to increase the C:N ratio in BFT systems, including dextrose, glycerin, sugar, sucrose (Rhode, 2014), starch, and molasses (Khanjani et al., 2021 a). Additionally, microorganisms can produce poly-beta-hydroxybutyrate (Zhang et al., 2016), and stable carbon sources like polycaprolactone or polypropylene can be utilized to maintain an appropriate C:N ratio in BFT systems (Luo et al., 2017). However, simple sugars or external carbohydrates are more effective in stimulating the uptake of nitrogen and converting it into microbial protein by heterotrophic bacteria. To achieve a balanced ratio of protein to carbohydrates, higher levels of carbohydrates are required when using high protein feed (Rhode, 2014). If a feed contains 30%–35% crude protein, the resulting C:N ratio would be 9–10, which is lower than the recommended ratio. To enhance the uptake of ammonia by the heterotrophic bacterial community, it is necessary to increase the C:N ratio or reduce the protein content of the feed (Abu Bakar et al., 2015; Hargreaves, 2013).

According to Emerenciano et al. (2017), the manipulation of the C:N ratio occurs in two phases. During the initial formation phase, a C:N ratio of 12–20 is utilized, while in the maintenance phase, the ratio is adjusted to 6:1 based on the recorded TAN levels. By supplementing the biofloc system with carbohydrates, it is possible to effectively control toxic nitrogenous compounds (Jimenez-Ojeda et al., 2018). Furthermore, the heterotrophic uptake of nitrogenous waste from the biofloc system is considered more stable and reliable compared to removal by nitrification or algae (Hargreaves, 2013). However, the continuous supply of external carbohydrates can lead to the accumulation of solids in BFT-based systems, which can deplete oxygen levels and negatively impact the growth and development of the organisms (Hargreaves, 2013). Zhang et al. (2016) suggest that poly-beta-hydroxybutyrate and polycaprolactone (Luo et al., 2017) can address the issue of high solids accumulation and yield similar results to simple carbohydrates. It is important to note that while the biofloc system can be manipulated to favor the activity of the heterotrophic bacterial community, the nitrifying (chemoautotrophic) bacterial community also plays a significant role in controlling nitrogenous waste within the system (Emerenciano et al., 2017).

Heterotrophic bacteria play a crucial role in optimizing biofloc formation and nutrient absorption. As a result, they effectively remove TAN and nitrite from the system (Khanjani et al., 2021). In a study by de Alvarenga et al. (2018) on a BFT system, the impact of moderate salinity on the growth of tilapia fingerlings was assessed. Additionally, the researchers explored the potential of moderate salinity in reducing mortality of tilapia fingerlings during nitrite peaks in the biofloc system. The findings from this research indicate that controlling the toxicity of nitrogenous compounds, specifically nitrites (NO_2), was achievable.

Nootong et al. (2011) observed that heterotrophic bacteria are able to control or remove nitrogen from the biofloc system even before nitrification is fully established. Effective nitrogenous waste control was observed after 6–7 weeks, when nitrification was fully established. This indicates that the heterotrophic bacterial community plays a role in controlling nitrogenous waste through both nitrification and assimilation. Therefore, it can be concluded that toxic nitrogenous compounds in BFT systems are controlled by both nitrifying bacteria and heterotrophic bacteria through immobilization into bacterial biomass. Therefore, nitrification processes and heterotrophic bacteria play an important role in understanding nitrogen dynamics and its control in BFT systems.

Adding carbohydrates

Carbon sources are crucial for the growth and success of biofloc systems.

They supply the energy required for microbial growth, aid in the breakdown of organic waste, and improve the nutritional value of the bioflocs. Aquaculturists can improve the performance and sustainability of their biofloc systems by carefully selecting and taking advantage of different types of carbon sources. An effective solution for addressing waste material toxicity is the gradual addition of carbonaceous materials

in smaller quantities, as opposed to adding them all at once to the pond (Crab et al., 2010). Introducing carbohydrates to systems used for intensive shrimp culture, without water exchange, has been found to significantly enhance water quality, bacterial activities, and zooplankton growth, resulting in improved growth performance (Khanjani et al., 2022 c; Guo et al., 2012). When a carbon source is introduced into the culture environment, it is rapidly metabolized by the biofloc community present in the environment. It is important to select carbon sources based on criteria such as carbohydrate digestibility, protein content, and cost per unit. To maintain a high C:N ratio, complex carbohydrates are often preferred as they typically contain protein (Avnimelech, 2015; Khanjani et al., 2023 c). Khanjani et al. (2016) found that improving the quality of the crop can be achieved by adding carbonaceous organic sources (molasses, starch, and wheat flour) and maintaining the C:N ratio at an optimal level (above 10) in the cultivation system without water exchange. As a result, water was preserved and toxic ammonia compounds were prevented from increasing. Table 1 presents the values of different nitrogen compounds in the biofloc system under various test conditions (type of carbon source used, carbon to nitrogen ratio, salinity, and stocking density).

Numerous materials have been identified as potential carbon sources for BFT systems. These include acetate and glycerol (Crab et al., 2010), dextrose (Suita et al., 2015), cassava meal (Sena Fugimura et al., 2015), cellulose (Deng et al., 2018), corn flour (Wang et al., 2016; Khanjani et al., 2021 a), glucose (Crab et al., 2010), molasses (Khanjani et al., 2021 c; Khanjani and Sharifinia, 2022), tapioca (Ekasari et al., 2014), wheat flour (Khanjani et al., 2016; Panigrahi et al., 2019 a), rice flour (Kumar et al., 2017), wheat bran (Zhao et al., 2016), rice bran (Serra et al., 2015), starch (Bakhshi et al., 2018; Khanjani et al., 2021 b), poly-β-hydroxybutyrate (Zhang et al., 2016), brewery residues (Sena Fugimura et al., 2015), and sugar (Bakhshi et al., 2018). These diverse carbon sources offer a range of options for optimizing the performance and efficiency of BFT systems.

The addition of carbonated materials to rearing ponds has been found to effectively prevent the accumulation of inorganic nitrogen (Crab et al., 2012). To estimate the amount of carbon utilized, it is possible to consider the feeding practices. For instance, following a daily feeding of 2% of fish weight with a food ration consisting of 25% protein (Craig and Helfrich, 2002), approximately 20 grams of food (5 grams of protein) would be provided per kilogram of fish. Since about 16% of protein is nitrogen (Craig and Helfrich, 2002), this would result in the addition of 0.8 grams of nitrogen to the fish each day. Additionally, it has been found that 75% of the nitrogen in food is released into the water (Piedrahita, 2003), leading to the release of 0.6 grams of nitrogen into the water per kilogram of fish every day. According to Crab et al. (2009), microorganisms require a carbon to nitrogen ratio of at least 10, while the formation of biofloc requires

6 grams of net carbon per kilogram of fish per day. Considering that most organic compounds are composed of 50% carbon, it is necessary to provide 12 grams of carbonaceous organic compounds for the daily formation of biofloc.

Manipulation of carbon to nitrogen ratios

Maintaining the right carbon-to-nitrogen (C/N) ratio is vital for the success of a biofloc system. This ratio influences the composition and activity of the microbial community, as well as the overall quality of the water. A balanced C/N ratio facilitates the efficient removal of nitrogenous compounds and guarantees optimal microbial growth. The ideal C/N ratio varies depending on the specific needs of the targeted aquatic organisms and the system conditions (Li et al., 2023).

In natural settings, the typical range of C/N ratio is 5-10:1, where the substrate is rich in organic carbon and poor in nitrogen, which encourages the bacteria to grow and multiply at a moderate rate (Goldman et al., 1987). In Table 2, the different C/N ratios used in various biofloc studies are presented. In intensive and super-intensive cultivation systems like BFT, a high feed rate with a high protein percentage (30–50%) can produce significant amounts of ammonium and nitrite. These can be assimilated by microorganisms under high C/N ratios, hence the need to add carbohydrates. The relationships in BFT are typically reported as approximately 10:1, which favors photoautotrophic microorganisms; approximately 15:1, which favors chemoautotrophs; and to 20:1, which favors heterotrophic microorganisms (Ebeling et al., 2006; Perez-Fuentes et al., 2016). Research has shown that a C/N ratio of 12-15:1 is generally favored by the heterotrophic bacterial community (Hargreaves, 2013; Rhode, 2014). However, Ebeling et al. (2006) have suggested that a carbon to nitrogen ratio of 20:1 is more beneficial for the heterotrophic bacterial community and promotes strong microbial immobilization of nitrogenous compounds. It has been suggested elsewhere that the carbon to nitrogen ratio should be maintained within the range of 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 used to elevate the carbon to nitrogen ratio to 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 et al., 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).

Table 1. Amounts of nitrogen compounds under different test conditions in the biofloc system

Cultivated species	IW (g)	SD	RP (days)	CSU	C/N	Temp. (°C)	Salinity ppt	pH	TAN mg/L	NO_2 mg/L	NO_3 mg/L	Highlight	Reference
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Oreochromis niloticus	30	3 kg/m³	50	Tapioca starch	16:1	29		7.5	0.23	0.12	43.7	The addition of organic carbon may be conducted until the establishment of complete nitrification or used as a strategy to quickly reduce TAN and nitrite concentrations.	Nootong et al., 2011
Fenneropenaeus merguiensis	4.5	1 shrimp/L	30	Molasses	15:1	29.39–29.45	29.04–29.16	8.07–8.13	0.35–0.64	1.98–2.92	2.37–4.26	The higher TAN in the control (and the decrease in TAN and NO_2 as biofloc addition increased) was due to the availability of organic carbon (from molasses and biofloc addition) allowing heterotrophic metabolism of ammonia.	Khanjani and Sharifinia, 2022 b
H. fossilis	0.5–0.7		70	Molasses	12:1 15:1 18:1 21:1	27.15–29.86		7.23–7.78	0.29–0.82			The manipulation of the C/N ratio significantly impacts the development of the biofloc system and water quality. A C/N ratio of 15:1 was found to be adequate for bioflocs based on stinging catfish curlicue.	Saha et al., 2022
O. niloticus	1.68	300 fingerlings/m³	63	Molasses, sugar, and cassava starch	10:1 20:1				1.6–2.0	0.5–1.1	6.8–13.6	Starting a BFT system can cause constant variations in water quality parameters, which can hinder the performance of tilapia fingerlings. However, molasses and sugar can be used as sources of organic carbon in the C:N ratio of 10:1 and 20:1 for rearing Nile tilapia with BFT.	Silva et al., 2017

Table 1 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gibelion catla	1.6 mg	3, 4, and 5 spawn/L	20	Molasses and wheat bran	10:1 15:1 20:1	26.76–26.81		7.4–7.7	0.22–0.26	0.022–0.030	12.27–14.45	Increasing the C/N ratio to 20:1 can improve the nursery rearing of *G. catla* in the biofloc system at a stocking density of 3 spawn/L.	Solanki et al., 2023
L. vannamei	0.8	270 shrimp/m^3	63	Glucose	8:1 16:1 24:1	28.8 28.7 28.7	5‰ 5‰ 5‰	6.9 7.6 7.7	7.1 2.0 1.3	14.0 4.9 1.9	77.0 14.7 4.6	The water parameters in the treatments with a C:N ratio higher than 16:1 (CN16 and CN24) were all within the acceptable range for shrimp culture. However, in the treatment with a C:N ratio of 8:1 (CN8), the pH and biofloc volume were lower than the recommended levels, and the TAN and nitrite levels were higher than their safe levels.	Huang et al., 2022
C. carpio	11.75		56	Sugar	10:1 15:1 20:1	24.72 24.31 24.23	0.45 0.45 0.46	7.87 7.69 7.65	1.13 0.81 0.95	8.22 9.16 17.13	12.20 16.10 1.00	The TAN concentration was significantly higher in treatments with a C:N ratio of 10:1. Manipulating the C:N ratio had an anti-stress effect, and the culture of common carp in the biofloc system with the lowest daily water exchange could be recommended.	Qoljaei et al., 2023
C. carpio	15.2	5 g/L	120	Maize flour Wheat flour Barley flour	15:1				0.057 0.088 0.074		0.395 0.367 0.121	The highest ammonia and urea excretion rates were recorded in the biofloc treatment containing barley flour. Therefore, it was concluded that the use of barley flour as a carbon source in the biofloc system can be used for the rearing of common carp.	Ghaleshiri et al., 2022

Table 1 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
O. niloticus	24.2	2.3 g/L	62	Molasses	14:1	24.19		7.72	0.25	0.29	142	The lowest ammonium and nitrite concentrations were recorded in the BFT20 and control groups. Dynamics of water quality parameters in BFT systems are influenced by the C:N ratio used, through the control of the balance between autotrophic and heterotrophic communities, which are responsible for the removal of nitrogenous substances.	Dilmi et al., 2022
					17:1	24.12		7.82	0.18	0.18	77		
					20:1	23.97		7.85	0.14	0.10	44		
C. carpio	17.10	125 fish/m³	40	Molasses	20:1	24.95		7.9	0.27	1.38	126.8	The water quality parameters including TAN, NO_2–N, and NO_3–N concentrations were considerably higher in the group with a stocking density of 500 fish/m³ than the other groups. Finally, stocking juvenile common carp at a density of 125 up to 250 fish/m³ in BFT caused optimal floc density with improved growth and water quality.	Nazarpour and Mohammadiazarm, 2023
		250 fish/m³				25.11		7.86	0.36	1.46	129.8		
		375 fish/m³				25.1		7.83	0.39	1.49	133.2		
		500 fish/m³				24.9		7.57	0.63	1.80	145.0		
L. vannamei		100 shrimp/ m³	112	Molasses	5:1	28.9	30.1	8.15	0.86	0.39	0.22	The TAN levels were reduced gradually when the addition of carbon sources increased. Specifically, the carbon addition to a C:N ratio of 20:1 (CN20) significantly reduced the TAN concentration. Similarly, NO_2–N and NO_3–N were reduced in the water of all the CN treatments, whereas in the control group, the values were significantly higher.	Panigrahi et al., 2019 b
					10:1	28.8	30.15	8.15	0.6	0.24	0.21		
					15:1	28.45	30.32	8.07	0.55	0.21	0.11		
					20:1	28.54	30.77	7.87	0.46	0.14	0.10		

Table 1 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Litopenaeus setiferus	0.1	150 shrimp/m³	90	Molasses		27.49 / 28.02	5 / 35	8.50 / 8.03	0.11 / 0.29	1.37 / 1.47	87.34 / 92.34	For the BFT treatment (with salinity 5 ppt), NH_4 high values were observed in 4 weeks, and NO_2 in 6 weeks respectively. This tendency was similar for the BFT treatment (with salinity 35 ppt) only for NO_2.	Valenzuela-Jiménez et al., 2022
Mugil cephalus	117.36	7.2 kg·m⁻³	87	Chancaca	15:1	15.48		7.23	2.07	0.91	17.34	In BFT, especially autotrophic nitrifying and heterotrophic bacteria, consume alkalinity, reducing pH, increasing TSS, and transforming ammonium into nitrate, due to the nitrification process.	Garcés and Lara, 2023
O. niloticus	48	2 kg fish/m³	70	Rice bran / Wheat milling by-product	15:1	27.03 / 27.0	1.5 / 1.5	8 / 7.92	1.61 / 1.62	1.37 / 1.55	12.58 / 12.33	TAN, NO_2 and NO_3 and biofloc volume were significantly affected by the different carbon sources.	Mansour and Esteban, 2017
Clarias gariepinus	9	500 fish/m³	90	Cassava / Rice bran	15:1	25.6 / 24.2			0.11 / 0.17	0.23 / 0.29	23.03 / 25.2	Addition of carbohydrate lessens the need for dietary protein concentration and also decreases the TAN level in the system	Popoola et al., 2021
F. merguiensis	10 mg	1000 shrimp/m³ / 2000 shrimp/m³ / 3000 shrimp/m³ / 4000 shrimp/m³	32	Molasses	15:1	28.37 / 28.41 / 28.36 / 28.35	30.17 / 30.11 / 30.08 / 30.17	8.15 / 8.14 / 8.06 / 7.97	0.24 / 0.33 / 0.46 / 0.59	2.15 / 2.55 / 2.9 / 3.34	3.16 / 4.08 / 5.05 / 5.47	Stocking density affects the water quality. The most suitable water quality, growth performance and survival rate of banana shrimp were observed at the lowest density of 1000 shrimps/m³ with limited water exchange	Khanjani et al., 2022 b
L. vannamei	74.46 mg	1 shrimp/L	32	Molasses	15:1	30.49 / 30.51 / 30.54	8 / 21 / 32	8.12 / 8.18 / 8.22	0.46 / 0.35 / 0.23	1.60 / 2.46 / 3.78	2.12 / 2.62 / 3.7	Salinity was confirmed as a factor affecting water quality and growth	Khanjani et al., 2020 a

Table 1 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Cyprinus carpio	14.17	1.06 kg /m³	90	Molasses	11:1 15:1 19:1 23:1	27.71 27.70 27.67 27.65		8.04 8.00 8.00 8.00	0.62 0.42 0.36 0.36	0.12 0.11 0.09 0.08	48.76 45.83 42.91 39.92	The biofloc system with C/N 19:1 improved the water quality and growth performance of common carp	Minabi et al., 2020
Huso huso	168.2	10.09 g/L	56			18.47	0.22	7.60	0.217	0.004	0.076	In the biofloc system, water quality improved compared to the control group.	Aghabarari et al., 2021
L. vannamei	2.5	1 shrimp/L	35	Molasses	10:1 14:1 18:1 22:1	30.43 30.47 30.42 30.41	33.76 33.68 33.67 33.74	8.09 8.06 7.92 7.83	0.52 0.32 0.3 0.28	6.44 5.01 4.59 4.61	5.48 7.64 8.32 8.78	Growth performance and water quality were suitable for rearing in biofloc system with C/N ratio of 14 compared to other treatments.	Khanjani and Sharifinia, 2022 a
O. niloticus	1.7	1 fish/L	30	Molasses Starch Corn flour Barley flour	15:1	26.08 26.25 26.31 26.12	8.9 8.91 8.89 8.87	7.35 7.45 7.42 7.28	5.08 7.15 7.37 4.91	0.4 0.42 0.48 0.4	19.36 15.34 15.84 20.22	Simple carbon sources such as molasses and starch have a higher effect on reducing and absorbing total ammonia nitrogen than complex carbon sources such as corn and barley flour.	Khanjani et al., 2021 a
O. niloticus	14	100 fish/m³	70	Glucose Molasses Starch Bran Cellulose	10:1	28.72 28.7 28.74 28.75 28.77		8.25 8.31 8.39 8.30 8.32	1.21 1.12 1.22 1.15 0.95	0.24 0.23 0.33 0.34 0.18	1.18 1.22 1.10 1.53 1.15	The slow degradation of complex carbon source (wheat bran and cellulose) delayed the improvement of water quality at the beginning of the experiment with less fluctuation values of NH_4^+ and NO_3	El-Husseiny et al., 2018
L. vannamei	0.01	16 shrimp/L	90	Sugarcane bagasse Rice bran	16:1	28.08 28.24	32.14 32.07	7.54 7.65	0.089 0.091	0.160 0.154	0.288 0.291	Promoted biofloc was used to effectively regulate inorganic chemicals, such as TAN, NO_2, and NO_3	Mansour et al., 2022
L. vannamei	2.56	1 shrimp/L	35	Molasses	15:1	30.3 30.24	10 32	8.00 8.05	0.42 0.31	4.39 4.73	6.61 7.38	The water quality of the rearing tanks is improved in the presence of biofloc, along with the addition of carbonaceous molasses	Khanjani et al., 2020 b

Abbreviations: Initial weight = IW, Rearing period = RP, Stocking densities = SD, Carbon source used = CSU, Carbon to nitrogen ratio = C/N, Temperature = Temp.
Total ammonium nitrogen = TAN, Nitrite = NO_2, Nitrate = NO_3.
The general highlight of the table: Various studies show that parameters such as the used carbon source, carbon to nitrogen ratio, salinity and stocking density affect the amount of nitrogen compounds in the biofloc system.

Table 2. Carbon sources and different carbon to nitrogen ratios in biofloc system

Cultivated species	Carbon source	C/N ratio	Reference
L. vannamei	Rice brand, molasses	20:1	Gomes Vilani et al., 2016
Labeo rohita	Tapioca, wheat, corn, sugar bagasse	15:1	Ahmad et al., 2016
O. niloticus	Poly-ß-hydroxybutyric acid, glucose	15 –20:1	Luo et al., 2017
Penaeus monodon	Rice flour, molasses	10:1	Kumar et al., 2017
Pseudotropheus saulosi	Sugar	15:1	Harini et al., 2016
O. niloticus	Molasses	15:1	Cavalcante et al., 2016
L. vannamei	White sugar	15:1	Ferreira et al., 2020
Clarias gariepinus	Glycerol	10:1, 15:1, and 20:1	Dauda et al., 2018
L. vannamei	Corn starch and molasses	12:1	Tinh et al., 2021 a
L. vannamei	Tapioca powder	12:1	Tinh et al., 2021 b
O. niloticus	PHB	>15:1	Wu et al., 2021
L. vannamei	Mixture of sucrose and basal diet	6:1, 10:1, and 15:1	Guo et al., 2020
O. niloticus	Molasses	5:1, 10:1, 15:1, and 20:1	Bhattacharyyaa and Ghosh, 2023
Heteropneustes fossilis			
Penaeus vannamei	Molasses	8:1, 12:1, and 16:1	Xu et al., 2022
H. fossilis	Sugarcane molasses	12:1, 15:1, 18:1, and 21:1	Saha et al., 2022
Cyprinus carpio	Wheat meal	10:1, 15:1, and 20:1	Azimi et al., 2022
O. niloticus	Molasses, sugar, and cassava starch	10:1, 1 and 20:1	Silva et al., 2017
Ctenopharyngodon idella	Powdered banana peels	10:1, 15:1, and 20:1	Tayyab et al., 2023
L. vannamei	Molasses	5:1, 10:1, 15:1, and 20:1	Panigrahi et al., 2018
L. vannamei	Glucose	8:1, 16:1, and 24:1	Huang et al., 2022
Clarias gariepinus	Fine bran	10:1	Aziz and Oktaviana, 2022
Gibelion catla	Molasses and wheat bran	10:1, 15:1, and 20:1	Solanki et al., 2023
L. vannamei	Molasses	12:1, 15:1, and 18:1	Xu et al., 2016
L. vannamei		7.4:1, 12:1, and 16:1	Tinh et al., 2023
P. monodon	Tapioca		Hari et al., 2006
Macrobrachium rosenbergii	Tapioca	10:1, 15:1, and 20:1	Asaduzzaman et al., 2008
O. niloticus	Molasses	10:1, 12:1, 15:1, 17:1, and 21:1	Perez-Fuentes et al., 2016
C. carpio	Sugar	0, 10:1, 15:1, and 20:1	Qoljaei et al., 2023
L. vannamei	Molasses	15:1	Vázquez-Euan et al., 2022
O. niloticus	Molasses	14:1, 17:1, and 20:1	Dilmi et al., 2022
P. vannamei	Molasses	8:1, 12:1, and 16:1	Xu et al., 2022
C. carpio	Molasses	20:1	Nazarpour and Mohammadiazarm, 2023
L. vannamei	Molasses	5:1, 10:1, 15:1, and 20:1	Panigrahi et al., 2019 b

Abbreviation: PHB = poly-beta-hydroxybutyrate acid.

In BFT culture systems with a C:N ratio of 20:1, the addition of organic carbon in the presence of high TAN levels can increase the demand for oxygen due to the rapid absorption of TAN by heterotrophic bacteria. This aspect can impact the crop, especially in species sensitive to different levels of dissolved oxygen (Schveitzer et al., 2013; Ray and Lotz 2014). Another significant aspect of the 20:1 ratio is the rapid generation of solids due to the accelerated growth of microorganisms, which can increase the floc volume and must be controlled to avoid high oxygen consumption, the generation of anoxic zones in the tank for excess solids, and the impact on the crop species (Hargreaves, 2013). In *O. niloticus* at different C:N ratios, Perez-Fuentes et al (2016) reported a 200% increase in solids at the 20:1 ratio compared to the 10:1 control, and a significant decrease in dissolved oxygen concentration from 3.2 to 1–1.5 mg/L, when molasses was added at a concentration higher than 0.12 g/L. To maintain stable levels of TAN and dissolved oxygen, it is essential to ensure adequate aeration and carry out the addition of carbohydrates based on the feed provided, which allows maintaining the stable C:N ratio and the development of the biofloc (Luo et al., 2017). Manipulating the C/N ratio is an effective method when running a BFT system. Numerous studies have evaluated the C/N ratio to enhance the overall performance of the BFT system.

Specific C/N ratio ranges have been recommended for different culturing animals. Azhar et al. (2020) found that an increase in C/N ratio from 10 to 15 had positive effects on the feed utilization efficiency and water quality in the tilapia–redclaw crayfish co-culture BFT system. Azimi et al. (2022) stated that increasing the C/N ratio from 10 to 20 enhanced the innate immunity of carp (*Cyprinus carpio*) reared in BFT. Similarly, positive effects of the increase in C/N ratio from 10 to 20 were also reported on immunological parameters and stress indicators of *C. carpio* (Haghparast et al., 2020). Chakrapani et al. (2021) stated that the BFT water quality and growth performance of *L. vannamei* were better at C/N ratios of 10 and 15 compared to 20. It seems plausible that an increased C/N ratio leads to a high possibility of carbon loss from BFT systems. Nevertheless, the aspect of carbon loss is rarely mentioned when selecting C/N ratios in BFT. One study investigated the influence of C/N (0, 10, 15, and 20) on the input/output ratio and found that the input/output ratio increased with increasing C/N ratios (Dauda et al., 2018). Liu et al. (2021) concluded that as the real-time nitrate was controlled at extremely low levels in BFT, less carbon could be applied to convert the heterotrophic denitrifying BFT biofilters into nitrifying ones, through which carbohydrates were saved. Also, Ferreira et al. (2020) stated that the supply of carbohydrates to BFT tanks is necessary only in emergency situations, when occasional ammonia spikes need to be controlled.

Thus, from the standpoint of system sustainability, reducing carbon supply to BFT may be a viable option without negatively impacting overall system performance, with the exception of emergency conditions, in which carbon emissions into the environment can be regulated to some extent. In a study conducted by Xu et al. (2022), it was confirmed that manipulating the input C/N ratio could influence the bacterial community of both water biofloc and shrimp gut in the environment of a commercial BFT system with intensive production of *P. vannamei*. During the trial, the differences in nitrogen dynamics and culture management measures of the tank systems probably resulted from the responses of the bacterial community in water bioflocs to different C/N ratios.

Aeration

In BFT, heterotrophic bacteria play a crucial role in ammonia assimilation. To enhance their activity, aeration is essential. Heterotrophic microorganisms require a significant amount of oxygen due to the oxygen consumption by blooming bacteria and the oxidative fermentation of organic materials secreted by bacteria (Khanjani et al., 2022 a). To maintain a high level of dissolved oxygen in the water, a robust air blower is typically necessary. The recommended dissolved oxygen level is at least 5 mg/L (Van Wyk, 1999). In certain cases, pure oxygen can also be utilized. Research has demonstrated that biofloc systems equipped with air blowers, like root blowers (Huang, 2019), can consistently sustain dissolved oxy-

gen levels. To ensure a high level of dissolved oxygen, it is advisable to introduce air into the water at a rate of 1–5% of its volume per minute. For instance, in a pond with a water volume of 1000 m³, a root blower should flow at a rate of 10–50 m³ per minute. This guarantees an adequate oxygen supply for the heterotrophic bacteria and other organisms in the system.

Conclusion

The aquaculture industry has been confronted with environmental pollution resulting from recent advancements. Consequently, it is crucial to prioritize environmentally friendly breeding systems and management practices. Sustainable development necessitates the consideration of not only biological sciences, but also social, economic, and environmental factors. An encouraging solution to this issue is the adoption of BFT, which eliminates the need for extensive water exchanges or even enables zero exchange. In a BFT system, ammonia can be rapidly removed within a few hours. Three primary groups of microorganisms, namely photoautotrophs, chemoautotrophs, and heterotrophs, play a pivotal role in controlling nitrogen compounds in the biofloc system. Different microorganisms specialize in bioremediation through various mechanisms. However, there is currently considerable knowledge gap about nitrogen dynamics in BFT systems. This is critical because factors affecting the dominance of heterotrophic or autotrophic bacteria might affect nitrogen dynamics. Finally, researchers should focus on ways for reducing the toxicity of nitrogenous compounds that accumulate in BFT systems.

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