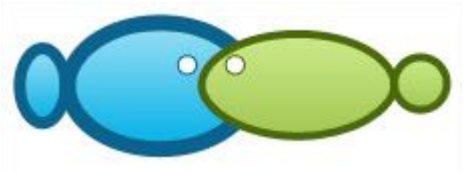

Dynamics and use of nitrogen in Biofloc Technology - BFT

Yira K. Jiménez-Ojeda, Luis F. Collazos-Lasso, José A. Arias-Castellanos

Instituto de Acuicultura de los Llanos IALL, Facultad de Ciencias Agropecuarias y Recursos Naturales, Universidad de los Llanos, Villavicencio, Meta, Colombia.
Corresponding author: Y. K. Jimenez-Ojeda, yirajimenez@hotmail.com

Abstract. Nitrogen compounds (ammonia and nitrite in particular) are important constraints in aquaculture because they are toxic metabolites for fish and other culture organisms. In water, bacterial microorganisms are responsible for the degradation of such forms of nitrogen, being that different groups of them use that compounds as a source of nitrogen for the construction of their biomass. The growth for aquatic products demand has generated the need to increase production, trying to minimize environmental damage, which has begun to be achieved with the recent development of new systems such as biofloc technology (BFT), which is based on the recycling of nutrients by bacteria that remove and transform into bacterial protein of high nutritional value in production containers, nitrogen compounds and other metabolites maintaining water quality conditions. The benefits from the protection of water and aquatic ecosystems with the use of this technology require the knowledge and correct use of it, given that without it the impact could be negative. This article reviews in detail the dynamics of nitrogen compounds in BFT.
Key Words: ammonia, nitrifying bacteria, nitrate, nitrite, nutrient recycling.

Introduction. In recent years the global aquaculture panorama has presented amazing changes; the most recent reports reveal that since 2014 the supply of fish for human consumption contributed by the aquaculture sector exceeds the catch, the difference being more and more noticeable, mainly due to the interest that has arisen from knowing about the breeding and cultivation of species for human consumption (FAO 2016). The increase in fish farming, in particular, has led to an increase in the deterioration of water and aquatic ecosystems, especially those that receive discharges from industrial waste (uneaten food and feces, the most common pollutants) (Avnimelech 2015), which has generated great alarm given that the discharge of organic and inorganic compounds in high concentrations and permanently, alter the biological and chemical balance of aquatic systems favoring nitrification and eutrophication, increasing sedimentation, harming benthic communities and deteriorating drastically the quality of water in the natural environment (Martínez-Córdova et al 2010).

One of the most abundant nutrients in effluents is ammonia, the most common protein metabolism product of aquatic organisms, soluble and very toxic even in low concentrations (1 mg L^{-1}) (Emerenciano et al 2017). This compound is converted into nitrite in the presence of oxygen, and later into nitrate by the nitrifying bacteria responsible for much of the dynamics of the nitrogen cycle, which allow other microorganisms to form less harmful molecules (Stein & Klotz 2016). In the natural environment, the cycle of nitrogenous compounds is slow due to the limited resources by which microorganisms must compete, in addition to nitrogen resources, oxygen, light, and other inorganic compounds (Sigee 2005).

In the course of the 21st century, knowledge and new technologies implementation for aquaculture production have been increased, several of them seek to minimize the environmental impact of the dumping of nitrogenous waste, one of them is biofloc technology (BFT), which is based on promoting development of aerobic microorganism communities in culture containers that use the different molecules to

grow and multiply, which results in an intricate trophic web capable of recycling nitrogen, maintaining water quality (Hargreaves 2013; Emerenciano et al 2017), and keep up comfort conditions for the main crop species, which results in faster development than in a normal production system, without competing for resources with the microorganisms that make up the biofloc (Ebeling et al 2006).

Although the BFT system has been commercially driven since the beginning of the 1990s, it is in the last decade that begins to appreciate its potential in intensive production systems of consume species such as tilapia *Oreochromis niloticus* (Avnimelech 2015; Luo et al 2017), *Oreochromis mossambicus* and *Oreochromis andersonii* (Day et al 2016), channel catfish *Ictalurus punctatus* (Green & McEntire 2017), white shrimp *Litopenaeus vannamei* (Khanjani et al 2017), pink shrimp *Farfantepenaeus duoradum* (Emerenciano et al 2014), red shrimp *Farfantepenaeus pauliensis* (Emerenciano et al 2011), tiger shrimp *Penaeus monodon* (Kumar et al 2017), giant freshwater shrimp *Macrobrachium rosenbergii* (Crab et al 2010); regional species such as pacu *Piaractus brachypomus* (Poleo et al 2011), Bocachico *Prochilodus magdalenae* (Pertúz-Buelvas et al 2016) and exotic species as African cichlid *Pseudotropheus saulosi* (Harini et al 2016) and golden carp *Carassius auratus* (Castro et al 2016).

Due to the need to carry out new studies where biofloc technology is implemented for the production of different species, with a variety of conditions, this review aims to gather information that allows clarity of some of the phenomena involved in this work.

Nitrogen dynamics. Nitrogen is an essential component of all living organisms, aquatic animals get it along with the proteins in the diet (Wei et al 2016), which are incorporated as amino acids, nucleic acids and other biomolecules, constituting about 5% of the dry weight (Sigee 2005). In aquatic ecosystems it is found in a wide range of organic (urea and amino acids mainly) and inorganic forms, including the gaseous compounds (N_2), as anion (NO_3^-, NO_2^-) and as a cation (NH_4^+) (Baldisserotto 2013). The soluble state of nitrogenous molecules is of great importance for aquatic organisms because it depends on them being assimilated and incorporated into their biomass, however some heterotrophic bacteria, protozoa and algae have the capacity to take nitrogen in an undissolved state (Sigee 2005).

The microorganisms are responsible for the transformation and dynamics of nitrogen in the aquatic environment. The sequence of transformations is known as the nitrogen cycle (Figure 1), with four recognized processes: nitrogen fixation, mineralization, nitrification and denitrification, which are regulated by the greater or lesser discharge of nitrogen-rich compounds in ecosystems, most coming from agricultural activities that mainly influence the nitrification, denitrification and accumulation of ammonium, nitrite and nitrous oxide that end up in excess contaminating the water and affecting the organisms that live in it (Hayatsu et al 2008; Milhazes-Cunha & Otero 2017). The study of the microorganisms involved in the nitrogen cycle and especially in the processes of nitrification and denitrification has shown other metabolic pathways involved in the transformation of nitrogen compounds.

According to Stein & Klotz (2016), the main routes of the nitrogen cycle include: ammonification, nitrification, denitrification, anammox, mineralization and assimilation.

Ammonification involves the formation of ammonia in the form of NH_4^+ or NH_3 and can occur in two ways, the first is the fixation of nitrogen gas in organic nitrogen, made only by bacteria and archaebacteria in the absence of oxygen, and the second is the reduction of nitrate or nitrite to ammonium by anaerobic assimilatory and disimilatory ways, which are carried out by some bacteria and fungi. Nitrification is mainly carried out by chemolithotropic microorganisms and involves the oxidation of ammonia in nitrite and later in nitrate in the presence of oxygen, nitritation and nitratation being denominated to these processes respectively. The anammox pathway is a reaction carried out by some microorganisms in the ocean in places with very little oxygen and in which the anaerobic oxidation of nitrite and ammonium is carried out to form gaseous nitrogen; the bacteria with anammoxosome of the Order Planctomycetales, are the ones in charge mainly, what turns them into first option for the treatment of residual waters since they allow to remove ammonium and nitrite without producing nitrous oxide. Denitrification is

described as the process of anaerobic respiration from nitrate (NO_3^-) or nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) to nitrogenous gas (N_2), is performed by denitrifying microorganisms. This process involves fungi, protists, bacteria and archaebacteria, which with different "incomplete" metabolic reactions, release nitrogenous gases into the atmosphere in different pathways. Finally, mineralization and assimilation are the last steps of the cycle, in which i) the transformation of organic matter into inorganic or ammonification occurs and ii) the regulation of ammonia generation and its use.

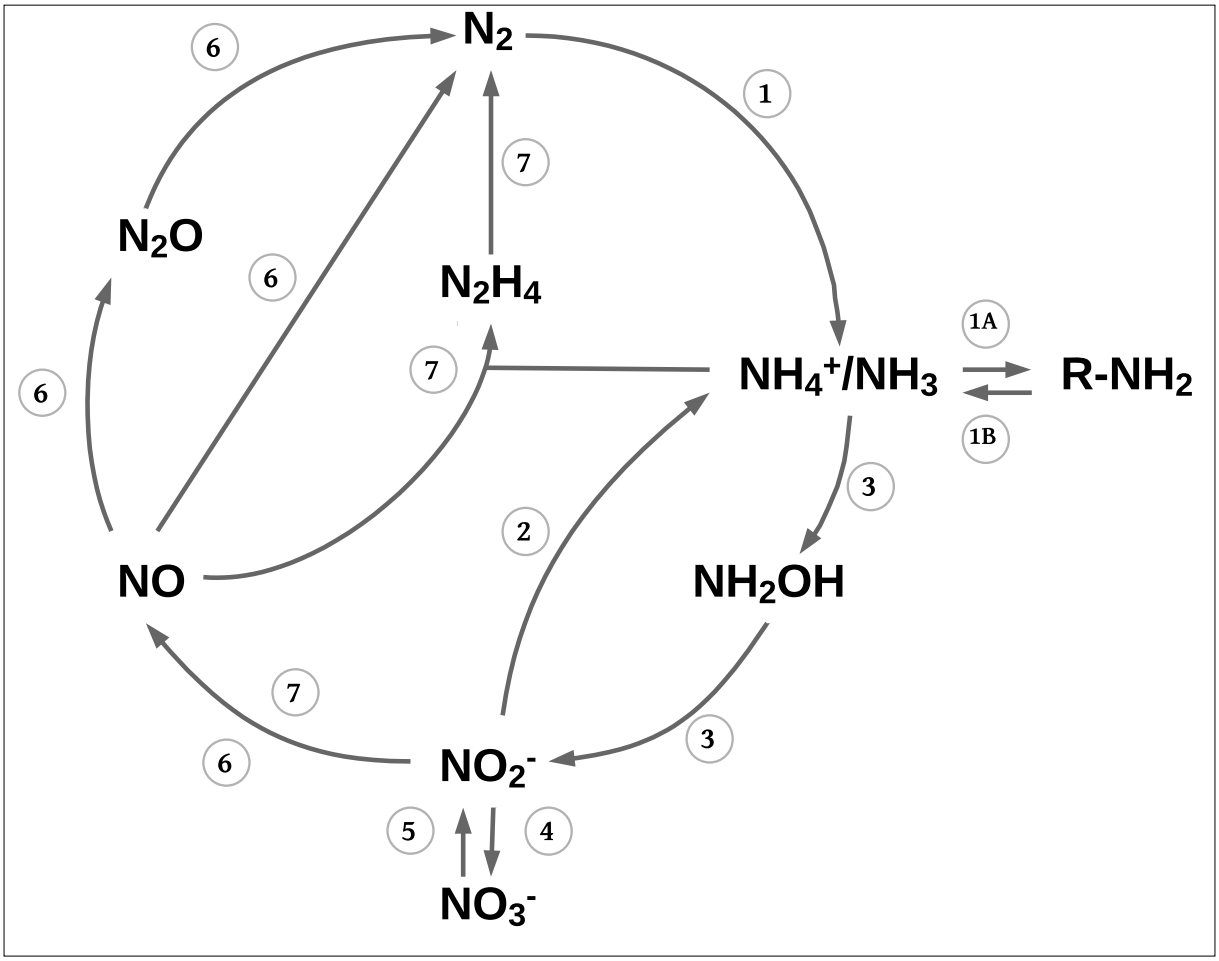

Figure 1. Major pathways in nitrogen cycle. 1 nitrogen fixation; 1A assimilation; 1B mineralization; 2 dissimilatory nitrite reduction to ammonium; 3 nitritation; 4 nitratation; 5 reduction nitrate to nitrite; 6 denitrification; 7 anammox pathways (adapted from Stein & Klotz (2016)).

The nitrogen cycle is directly related to production systems, so knowledge of the processes involved in the dynamics of nitrogen can improve production from aquaculture and minimize environmental impact (Stein & Klotz 2016), preventing water pollution and global warming (Camargo & Alonso 2006). Aquaculture production with the BFT system reduces the environmental impact generated by open and conventional aquaculture production systems, decreasing the discharges of nutrients and pollutants that alter the dynamics of ecosystems and the nitrogen cycle itself (Avnimelech 2015), although the processes of ammonification, nitrification, assimilation and mineralization can be carried out constantly in this cultivation system, the pathways of nitrogen removal and the compounds that predominate in the water will depend on the C:N ratio and the microorganisms that such proportion favors.

Little is known about the denitrification pathways involved in the nitrogen cycle, which certainly have an influence on the dynamics of nitrogen compounds in BFT. Ray & Lotz (2014) report the possible existence of partial denitrification processes in the sedimentation chambers comparing different carbon sources in heterotrophic and

chemoautotrophic systems. These authors argue that the increase in alkalinity in some heterotrophic systems may be due to the presence of processes of partial denitrification in the solids maintained in the chambers, which are then returned and that may favor the formation of nitrous oxide or the formation of nitrite but not nitrate formation. The ability to control denitrification processes with simple and easy-to-implement technologies will safely reduce the concentration of nitrogenous compounds in effluents from BTF systems in the future, thus optimizing technology while minimizing the negative impacts they cause.

Toxic effect of nitrogen compounds

Ammonia. Aquatic organisms excrete mainly non-ionized ammonia (NH_3) (Remen et al 2008). Under normal physiological conditions, ammonia occurs in greater proportion in the liver, followed by skeletal muscle, kidneys and gills, being transported by the blood in ionized form as NH_4^+ and excreted through the gills by diffusion as NH_3 following a gradient of greater concentration in the blood to one of lower concentration in the water (Baldisserotto 2013). In water, for most animals that inhabit it, the form of ionized ammonia is not toxic, while the ammoniacal form is very toxic. The two states of nitrogen related to the excretion of aquatic organisms are known as Total Ammonia Nitrogen (TAN). The rate of each molecule (NH_3 or NH_4^+) is depending on pH of the medium in which it is found (blood or water), so an alkaline pH the NH_4^+ will be in a lower proportion and the NH_3 will be more abundant and acid pH the first will be the most abundant (Baldisserotto 2013):

$$NH_3 + H^+ \leftrightarrows NH_4^+$$

The excess of ammonia decreases the metabolism of fish causing histopathological lesions in the gills, blood and other tissues, reduction in the oxygen transport capacity by hemoglobin, depletion of energy as a result of hyperactivity and hyperventilation, and finally increase of water absorption and death of the individual (Alabaster & Lloyd 1982). Karasu Benli & Koksal (2005) reported other signs that can help identify intoxication such as: darkening of the eyes and body, frequency of movement, convulsions, spiral movement and effort to take air.

Teleost fishes excrete 70 to 95% of the nitrogen in the form of ammonia, while 15% of the total excreted nitrogen is urea and uric acid (Kaushik 2000), basically because producing these last molecules requires a high cost of energy (Baldisserotto 2013). Passive diffusion, also called transcellular diffusion or facilitated transport, is the way in which ammonia is excreted in fish, a way that allows NH_3 to circulate from a place of higher concentration to one of lower concentration mediated by Rhesus glycoproteins, which are channels for the NH_3 located in the apical and basolateral membrane of the exchange gill cells (Wright & Wood 2012). In intensive culture systems with little or no water exchange and feed with high protein level, the concentration of TAN in the water can easily increase and the diffusion of ammonia through the gills can decrease, since the gradients in the blood and in the water are reduced (Baldisserotto 2013). When the facilitated diffusion does not help the elimination of ammonia, an excretion system is activated in which ATPases intervene through an exchange between the sodium ion (Na^+) and the ionized ammonia (NH_4^+) with energy expenditure (Wood 2001), this mechanism maintains the TAN equilibrium inside and outside the fish, decreasing the toxicity of NH_3 (Ip et al 2004). Table 1 shows the lethal concentration of ammonia in different crop species under certain physicochemical water conditions.

In aquaculture, ammonia excretion can be affected by different factors, these can be: environmental, biological or induced by the diet provided. In intensive systems such as BFT, where the feeding rate and culture densities are high, ammonia levels can easily be increased, all these factors must be carefully reviewed to avoid affecting the crop species and to maximize the comfort conditions that allow it to develop quickly, generating minimal stress (Avnimelech 2015). Among the environmental factors that affect ammonium excretion are: salinity, which depends on the osmoregulatory capacity of the species (Regnault 1987; Chen & Chia 1995), temperature (Schmitt et al 1992; Wilson & Taylor 1992), and pH, which in species with a tendency to inhabit alkaline

environments will have a protective effect on unionized ammonia and on its excretion (Kaushik 2000). The increase in the levels of environmental ammonia lead to an increase in the concentrations of this molecule in the blood or hemolymph (Fromm & Gillette 1968), as well as in intensive production systems with minimum water exchange where the pH can vary considerably, shock measures must be taken to avoid the possible accumulation of un-ionized ammonia and fish poisoning (Avnimelech 2015).

The main biological reason that can influence the ammonium excretion is body weight. It has been shown that the rate of ammonia excretion tends to decrease when body weight increases (Clifford & Brick 1978; Valbuena-Villareal & Vasquez-Torres 2011).

Among the food factors are mainly the measure and quality of the protein that is supplied in the food, since there is a direct relationship between the intake of nitrogen (N) and the excretion of ammonia (Kaushik 2000). In the majority of teleost fishes the total nitrogen excretion increases with the increasing N intake (Chakraborty & Chakraborty 1998; Schmitt & Santos 1998; Martinez-Lopez 2002; Valbuena-Villareal & Vasquez-Torres 2011), however, it has been found that this response time may vary depending on the species and the protein requirements (Schmitt & Santos 1998). The diet quality plays a fundamental role that can improve the use of the protein.

Nitrite and nitrate. The toxic effect of nitrite depends to a large extent on the pH and concentration of chlorine (Cl^-) (Tomasso et al 1979). At acid pH (close to 5.5), low concentrations of nitrite are less tolerated than at higher pH (Jensen 2003), this may be due to the presence of nitrous acid (HNO_2) that is formed under such conditions (Colt et al 1981).

In trout, for example, the lethal concentration in 96 hours at pH 6.4 is 0.21 mgL^{-1}, while at pH 9.0 it is 1.6 mg L^{-1} of nitrite (Baldisserotto 2013). Regarding the concentration of chlorine ions in the water, it has been found that high concentrations of chlorine diminish the toxic effect of nitrite since they prevent the latter from entering the gills via Cl^-/HCO_3^- (Baldisserotto 2013). Fish with a high chlorine uptake rate such as trout and perch are more sensitive to nitrite than fish with low uptake rates such as carp and tench (Williams & Eddy 1986).

Nitrite generates multiple physiological effects in aquatic organisms, among them: i) ionic imbalance when competing with the entry of Cl^- to the branchial exchange cells and activation of the potassium ion (K^+) exit inside the skeletal muscle cells and erythrocytes, causing the intracellular and extracellular levels of potassium to be lost; ii) affects the absorption of Na^+ in the gill exchange cells; iii) promotes the oxidation of hemoglobin to methaemoglobin which is inefficient in the transport of oxygen in the blood; iv) increase of the heart rate; v) inhibits the synthesis of T4 hormone, triggering the retention of water by the kidney and vi) alters the excretion levels of ammonia and urea (Jensen 2003; Baldisserotto 2013). Table 2 shows the lethal concentration of nitrite in different crop species under certain physicochemical water conditions.

Table 1

Lethal concentration (CL50) of ammonia nitrogen (NH_4^+/NH_3) and un-ionized ammonia (NH_3) in some species used in aquaculture

Species	*Weight/length*	*$NH_4^+ + NH_3 / NH_3$ (mg L^{-1})*	*Sal (‰)*	*T (ºC)*	*Other water conditions*	*Author*
Ictalurus punctatus	3.5±0.0 g / ---	---/1.29 [D]	---	19.6	pH 7.8 DO 4.4-13.1 mg L^{-1} Alc 94-186 mg L^{-1} $CaCO_3$ Hrd 112-206 mg L^{-1} $CaCO_3$	Arthur et al (1987)
Pimephales promelas	1.7±0.0 g / ---	---/2.55 [D]	---	26.1	pH 8.1 DO 4.4-13.1 mg L^{-1} Alc 94-186 mg L^{-1} $CaCO_3$ Hrd 112-206 mg L^{-1} $CaCO_3$	Arthur et al (1987)
Litopenaeus schmitti	1.5±0.4 g / 1.4±0.5 cm	40.72/1.46 [A]	5	20±1	pH 8.0-8.05 DO 6.8±0.5 mg L^{-1}	Barbieri (2010)
		53.52/1.80 [A]	20			
		54.32/1.67 [A]	35			
		32.63/1.17 [B]	5			
		38.60/1.30 [B]	20			
		47.87/1.47 [B]	35			
		24.63/0.88 [C]	5			
		27.76/0.93 [C]	20			
		41.67/1.28 [C]	35			
		19.12/0.69 [D]	5			
		25.55/0.86 [D]	20			
		38.88/1.20 [D]	35			
Piaractus mesopotamicus	1.2±0.3 g / 1.6±0.4 cm	5.32/0.018 [A]	---	15	pH 6.98 (6.78-7.15) DO 6.8±0.5 mg L^{-1} Hrd 40 mg L^{-1} $CaCO_3$	Barbieri & Vigliar Bondioli (2015)
		4.81/0.023 [A]		20		
		4.16/0.029 [A]		25		
		4.19/0.014 [B]		15		
		3.97/0.019 [B]		20		
		3.79/0.026 [B]		25		
		3.79/0.013 [C]		15		
		3.25/0.016 [C]		20		
		2.58/0.018 [C]		25		
		2.85/0.009 [D]		15		
		2.50/0.012 [D]		20		
		1.97/0.014 [D]		25		

Penaeus monodon	4.87±1.4 g / 91.0±8.0 mm	97.9/1.76 [A] 88.0/1.59 [B] 53.4/0.96 [D]	20	24.5	pH 7.57 Alc 1.6 mg L^{-1} Hrd 3180 mg L^{-1} $CaCO_3$	Chen et al (1990)
Penaeus monodon	0.27±0.06 g / 35.4±2.2 mm	94.96/2.68 [A] 61.09/1.73 [B] 47.47/1.35 [C] 45.58/1.29 [D]	20	27	pH 7.7 DO 5.8-6.3 mg L^{-1} Alc 81 mg L^{-1} $CaCO_3$ Hrd 3200 mg L^{-1} $CaCO_3$	Chen & Lei (1990)
Oreochromis niloticus	Larvae: 0.056±0.008 g / --- Fingerlings: 10.11±0.045 g / ----	---/1.007-1.01 [B] ---/7.390-7.41 [B]	---	23±1	pH 8.0 ± 0,2 DO 7.2 mg L^{-1} NO_2 0.001 mg L^{-1}	Karasu Benli & Koksal (2005)
Litopenaeus vannamei	--- / 22.0±2.4 mm	59.72/2.95 [A] 66.38/2.93 [A] 68.75/2.78 [A] 40.58/2.00 [B] 48.83/2.16 [B] 53.84/2.18 [B] 32.15/1.59 [C] 43.17/1.91 [C] 44.93/1.82 [C] 24.39/1.20 [D] 35.40/1.57 [D] 39.54/1.60 [D]	15 25 35 15 25 35 15 25 35 15 25 35	23±1	pH 8.05 DO 6.2±0.4 mg L^{-1}	Lin & Chen (2001)
Penaeus pauliensis	Egg stage Nauplius Zoea Mysis Postlarvae	6.25/--- [A] 102.3/--- [A] 41.80/--- [B] 25.59/--- [C] 22.93/--- [A] 14.53/--- [B] 10.94/--- [C] 9.39/--- [D] 74.87/--- [A] 41.80/--- [B] 32.85/--- [C] 21.98/--- [D] 24.19/--- [A] 8.59/--- [B]	28	25±1	pH 7.71-8.24	Ostrensky & Wasielesky (1995)

		5.65/---[C] 5.49/---[D]				
	Juvenile 5.45±0.4 g	51.87/---[A] 43.11/---[B] 40.05/---[C] 38.72/---[D]				
	Adult 31.43±1.3 g	61.63/---[A] 50.51/---[B] 46.57/---[C] 42.49/---[D]				
Cyprinius carpio	570±0.0 g / ---	123/45.5[A]	---	20-22	pH 7.0-7.2 DO 6-7 mg L^{-1}	Peyghan & Takamy (2002)

Exposure time: ([A]) 24 hours; ([B]) 48 hours; ([C]) 72 hours; ([D]) 96 hours. (Sal) salinity; (Tº) temperature; (DO) dissolved oxygen; (Alc) alkalinity; (Hrd) hardness; (---) data not available.

Table 2

Lethal concentration (CL) of nitrite (NO_2^-) in aquaculture species of warmwater

Species	*Weight/length*	*CL NO_2 (mg L^{-1})*	*Mort (%)*	*Sal (‰)*	*Other water contitions*	*Author*
Prochilodus magdalenae	0.5-1.8 g / ---	0.0 [E] 0.5 [E] 2.0 [E]	10 22.5 25	---	pH 6.72-7.69 Tº 27±1ºC NH_3 0.0002-0.0028	Ceballos et al (2001)
Penaeus monodon	4.87±1.4 g / 91.0±8.0 mm	218 [A] 193 [B] 171 [D]	50	20	pH 7.57 Tº 24.5 ºC Alc 1.6 mg L^{-1} Hrd 3180 mg L^{-1} $CaCO_3$	Chen et al (1990)
Penaeus monodon	0.27±0.06 g / 35.4±2.2 mm	215.85 [A] 185.33 [B] 88.54 [C] 54.76 [D]	50	20	pH 7.7 Tº 27 ºC DO 5.8-6.3 mg L^{-1} Alc 81 mg L^{-1} $CaCO_3$ Hrd 3200 mg L^{-1} $CaCO_3$	Chen & Lei (1990)
Prochilodus magdalenae	0.4–6.6 g / ---	4.2 [D] 13.3 [D] 22.3 [D]	25 88 100	---	pH 7.4-7.6 Tº 25.2-26.2 ºC Alc 24-27.6 mg L^{-1} $CaCO_3$ Hrd 21-26 mg L^{-1} $CaCO_3$ Chloride 6-9 mg L^{-1}	Gonzalez et al (2005)
Litopenaeus vannamei	3.96±1.42 g / 56.0±9.6 mm	187.9 [A] 274.1 [A] 521.2 [A] 142.2 [B] 244.0 [B] 423.9 [B] 92.5 [C] 224.8 [C] 375.0 [C] 76.5 [D] 178.3 [D] 321.7 [D]	50	15 25 35 15 25 35 15 25 35 15 25 35	pH 8.02 Tº 18 ±1ºC DO 6.8±0.2 mg L^{-1}	Lin & Chen (2003)
Piaractus brachypomus	Juvenile	35 [D] 50 [D] 65 [D]	7 27 33	---	---	Ochoa et al (2002)
Piaractus brachypomus	39.5±0.09 g / ---	4 [B] 8 [B] 16 [B] 32 [B]	22 44 12 56	0.05±0.006	pH 5.7±0.04 Tº 24±0 DO 7.7±0.2 mg L^{-1}	Torres-Tabares et al (2007)
Brycon amazonicus	69.1±13.9 g / ---	0.6 [D]	50	0.05±0.006	pH 5.7±0.04 Tº 24±0 DO 7.7±0.2 mg L^{-1}	Torres-Tabares et al (2007)

Exposure time: ([A]) 24 hours; ([B]) 48 hours; ([C]) 72 hours; ([D]) 96 hours; ([E]) 45 days. (Mort) mortality; (Sal) salinity; (Tº) temperature; (DO) dissolved oxygen; (Alc) alkalinity; (Hrd) hardness; (---) data not available.

As shown in Tables 1 and 2, the lethal concentration of ammonia and nitrite is very variable, studies have shown that the susceptibility to these molecules can change depending on the species (Arthur et al 1987; Lin & Chen 2003), the size or the stage of development (Chen et al 1990), temperature (Barbieri & Vigliar Bondioli 2015), salinity (Crawford & Allen 1977; Barbieri 2010) and pH (Jensen 2003; Baldisserotto 2013). The toxicity of nitrite can also depend on the ionic composition of water (Tomasso et al 1979), mainly on the concentration of chloride ions (Jensen 2003) and calcium (Crawford & Allen 1977). In studies with tilapia it was observed that fish begin to die when the concentrations of non-ionized ammonium reach values of 2 mg L^{-1} and the concentrations of nitrites exceed 5 mg L^{-1} (Rakocy 1989). Other studies report for larvae of the same species an average lethal concentration of 1 mg L^{-1} of ammonia and values close to 7 mg L^{-1} of nitrite for fingerlings for 96 hours (Karasu Benli & Koksal 2005). In the pink shrimp (*P. pauliensis*), the direct relationship of ammonia resistance with age has also been reported (Ostrensky & Wasieleski 1995), and in tiger shrimp (*P. monodon*) and other penaeides tolerance to nitrite has shown the same behavior (Lin & Chen 2003). Peyghan & Takamy (2002) reported in carps of 570 g that the lethal ammonium concentration was 150 mg L^{-1} and that the addition of zeolite at a concentration of 10 mg L^{-1} prevents fish poisoning and death. Barbieri & Vigliar Bondioli (2015), found for their part in pacú fish (*Piaractus mesopotamicus*), maintained at 15ºC, that the susceptibility to un-ionized ammonia decreases in comparison to that reported at 25ºC.

In the case of nitrite, Tomasso et al (1979), found that in *Ictalurus punctatus* exposed to this metabolite increases the formation of methaemoglobin (42.5±3.8%), but if sodium chloride (NaCl) is added in a ratio of 16 Cl^- to 1 NO_2^-, it is possible to inhibit the formation of methaemoglobin and return to the normal condition in a time of 24 hours, further propose that the bicarbonate ion at concentrations of 1 mg L^{-1}, as well as the monovalent anions present in water may contribute to the inhibitory action of NO_2^-. Crawford & Allen (1977) report that the mortality caused by exposure to nitrite may be due to a different factor than methemoblobinemia in *Oncorhynchus tshawytscha*, since the LC50 of nitrite during 48 hours was 19 mg L^{-1} in exposed animals to 100 mg L^{-1} of NO_2^-, thus in artificial sea water without calcium the nitrite had a toxic effect but did not induce the formation of methemoglobin, whereas after adding calcium to water, the acute toxicity of nitrite decreased.

Nitrite, by oxidizing the iron in hemoglobin and forming methaemoglobin incapable of transporting oxygen in erythrocytes, causes physiological damage and death of the fish; but it has also been implicated in the decrease in growth at low and prolonged concentrations. Colt et al (1981) reported that 31 day exposure of channel catfish (*I. punctatus*) at nitrite concentrations between 1.60 and 2.61 mg L^{-1} resulted in decreased growth in about 20% and that mortality was significant at concentrations higher than 3.71 mg L^{-1}. Significant differences in length and mortality were also reported for the bocachico (*Prochilodus magdalenae*), exposed for 45 days at a concentration of 2 mg L^{-1} of NO_2^- (Ceballos et al 2001). In BFT systems where dominance of chemoautotrophic microorganisms is present, the crop species may be exposed to nitrite concentrations greater than 2 mg L^{-1} in a prolonged manner, which may intervene in the normal development of species sensitive to this compound.

On the other hand, the mechanism of toxicity of nitrate (NO_3^-), is still unknown, it is known that after its uptake it becomes NO_2^- and subsequently its toxic effect is triggered (Baldisserotto 2013). Unlike the effect of ammonia and nitrite, fish and other aquatic organisms tolerate high concentrations of nitrate; Rakocy et al (2000) reported that in tilapia concentrations of 600 or 700 mg L^{-1} can affect the consumption of food.

The effects of prolonged exposure to compounds such as ammonia or nitrite are relevant in the use of BFT since the culture species may be continuously exposed to high concentrations of both metabolites.

Nitrogen dynamic in Biofloc Technology. Microorganisms are a fundamental component of aquatic ecosystems, they are omnipresent forms of bacteria, fungi, algae, protozoa, nematodes, among others, that form trophic webs that interact in a wide range, thus being responsible for the movement of nutrients (Sigee 2005). The study of

these organisms has made it possible to classify them according to the trophic and metabolic relationships they carry out, basically they are grouped according to the process by which they obtain their energy and also depending on the carbon source with which they produce their biomass. In BFT the carbon source can be controlled to promote the microbial communities that are to be developed according to the conditions required by the crop species (Collazos-Lasso & Arias-Castellanos 2015), thus determining the C:N relationship prevalence is given to communities specifies that they remove the ammonium from the system by one of the following routes: assimilation by algae, oxidation by chemoautotrophic bacteria and assimilation by heterotrophic bacteria (Ray & Lotz 2014). Table 3 shows the main characteristics of each one of these nitrogen removal pathways.

Photoautotrophic organisms. Also known as phytoplankton, they are the primary producers of aquatic ecosystems and comprise most of algae suspended in water (microalgae), and photosynthetic bacteria that are capable of making their own food by converting solar energy into chemical energy through photosynthesis and producing its biomass from CO_2 and water (Avnimelech 2015). The algae are the main control organisms of inorganic nitrogen in extensive crops where they produce oxygen and absorb ammonia nitrogen in the presence of light, but in BFT systems these primary producers are not the most important in relation to these aspects and can generate instability in the system, mainly on cloudy days, where the overgrowth of algae causes ammonia accumulation, or at night and in the early morning hours when the CO_2 production is high and the pH increases equal that the oxygen consumption (Hargreaves 2013). For the above in the BTF seeks to control the "excessive" development of algae, limiting the incidence of sunlight to prevent these photoautotrophic microorganisms prevail causing descompensation of the system and competition for oxygen with the crop species (Ebeling et al 2006).

Chemoautotrophic organisms. Are those that obtain their energy from the oxidation of inorganic compounds such as ammonium, which undergoes biological oxidation to nitrite and later to nitrate through the process of nitrification with inorganic carbon consumption. Nitritation, conversion of ammonium (NH_4^+) to nitrite (NO_2^-), occurs under increased oxygen consumption, and is a limiting reaction in the nitrification process. It occurs with the intervention of bacteria of the genus *Nitrosomonas, Nitrosococcus, Nitrospira, Nitrosobulus* and *Nitrosovibrio* (Ray 2012). These bacteria, which consume ammonia as their only source of energy and produce nitrites, are Gram-negative, have a size between 0.4 and 0.6 microns, are aerobic autotrophic and need oxygen as an oxidation vehicle (Sigee 2005). The passage from nitrite to nitrate, called nitratation, occurs in the presence of bacteria of the genera *Nitrobacter, Nitrococcus, Nitrospira* or *Nitrospina* (Ray 2012), also Gram-negative, aerobic autotrophic, transform nitrites (NO_2^-) into nitrates (NO_3^-) (Sigee 2005).

The process of denitrification is subsidiary to nitrification and is a way that helps to reduce the concentration of nitrate dissolved in water, however it is not an autotrophic process such as nitrification, but heterotrophic (Sigee 2005). Denitrifying bacteria get energy for their growth from the conversion of nitrate to nitrogen gas, but require an organic carbon source for cell synthesis. This process is anaerobic heterotrophic respiration of the anoxic type, where the reduction of nitrate (NO_3^-) to nitrogen gas (N_2), follows a series of steps that involve the activity of different enzymes (Sigee 2005). Another way to reduce the concentration of nitrate in water is the assimilation of this compound by algae, which assimilate it to form biomass.

Heterotrophic organisms. They are the most abundant group in the BTF as well as in the natural aquatic ecosystems where they develop dependent on the photosynthetic organisms, take the organic matter that these produce or discard and use it as food and source of energy to grow (Sigee 2005). In the BTF where the organic matter and the ammonia are abundant, the growth of these bacteria is accelerated and helps in the decomposition of the organic matter generating large amounts of bacterial biomass that

later in some proportion becomes food for the culture species (Avnimelech 2015). Different studies document the benefits generated by the dominance of heterotrophic bacteria in terms of water quality and their efficient role in the degradation of organic substances (Ebeling et al 2006; Avnimelech 2015; Ray 2012; Hargreaves 2013).

Table 3

Removal of nitrogen through micro-organisms developing in an aquatic system (adapted from Ebeling et al (2006))

Photoautotrophic	*Chemoautotrophic*	*Heterotrophic*
Energy source: solar radiation (photosynthesis)	Energy source: inorganic compounds	Energy source: organic matter
Nitrogen source: NH_4^+ NO_3^-	Nitrogen source: NH_4^+ NO_2^-	Nitrogen source: NH_4^+
Carbon source: inorganic	Carbon source: inorganic	Carbon source: organic
C:N approx 10:1	C:N approx 15:1	C:N approx 20:1
N biological reactions: $\underline{16\ NH_4^+} + 92\ CO_2 + 92\ H_2O + 14\ HCO_3^- + HPO_4^{2-} \rightarrow C_{106}H_{263}O_{110}N_{16}P + 106\ O_2$ $\underline{16\ NO_3^-} + 124\ CO_2 + 140\ H_2O + HPO_4^{2-} \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138\ O_2 + 18\ HCO_3^-$	N biological reactions: $\underline{NH_4^+} + 0.094\ CO_2 + 1.83\ O_2 + 0.024\ \underline{HCO_3^-} \rightarrow 0.024\ C_5H_7O_2N + 0.977\ \underline{NO_3^-} + 0.953\ H_2O + 1.95\ H^+$	N biological reactions: $\underline{NH_4^+} + 1.18\ C_6H_{12}O_6 + 2.06\ O_2 + HCO_3^- \rightarrow C_5H_7O_2N + 6.06\ H_2O + 3.07\ CO_2$
Consumptions: 3.13 g alkalinity / g NH_4^+ 4.02 g alkalinity / g NO_3^- 18.07 g CO_2 / g NH_4^+ 24.40 g CO_2 / g NO_3^-	Consumptions: 7.05 g alkalinity / g NH_4^+ 4.18 g O_2 / g NH_4^+	Consumptions: 3.57 g alkalinity / g NH_4^+ 4.71 g O_2 / g NH_4^+ 15.17 g carbohidrates / g NH_4^+
Products: 15.40 g O_2 / g NH_4^+ 19.71 g O_2 / g NO_3^- 15.85 g VSS / g NH_4^+ y NO_3^-	Products: 5.85 g CO_2 / g NH_4^+ 0.976 g NO_3^- / g NH_4^+ 0.20 g VSS / g NH_4^+	Products: 9.65 g CO_2 / g NH_4^+ 8.07 g VSS / g NH_4^+
Assimilation: Dependent on sunlight	Assimilation: Slow	Assimilation: Fast
Disadvantages: - daytime function except on cloudy days; - may be flowering and accidents; - possible variation in dissolved O_2, pH and ammonium	Disadvantages: - consumption of oxygen - decrease of pH by consumption of alkalinity for nitrification	Disadvantages: - high consumption of oxygen

(VSS) volatile suspended solids; (C) carbon; (N) nitrogen.

Table 3 shows that heterotrophic bacteria to fix 1 gram of ammonia (NH_4^+) require about 4.71 g of oxygen (O_2), 15.17 g of carbohydrates and generate 8.07 g of suspended volatile solids (SSV). On the other hand, the chemoautotrophic bacteria require around 7.05 g of inorganic carbon, 4.18 g of O_2 and produce 0.20 g of SSV for fixing the same amount of NH_4^+ (Ebeling et al 2006). The requirements of these two groups of microorganisms, the most common and efficient in the dynamization of nitrogen in the BFT, as noted, present notable differences, mainly in the consumption of oxygen, in the source of carbon assimilated and in the generation of biomass (Ebeling et al 2006; Avnimelech 2015), in addition heterotrophic bacteria take about half the time that chemoautotrophs need to grow (Timmons & Ebeling 2010). It is important to clarify that the chemoautotrophic bacteria assimilate inorganic carbon from the carbonates dissolved

in water (Sigee 2005), however it has been reported that the C:N ratio 15:1, allows that in addition to these, heterotrophic bacteria develop that do not they come to dominate the system but they help in the ammonium sequestration, mainly in the first days of establishment of the crop when the bacteria just start to implant (Avnimelech 2015). Therefore, it is essential to verify the requirements of the species of consumption to be cultivated before defining the C:N relationship to be used (Ebeling et al 2006).

C:N ratio. In the natural environment, C:N ratios are usually found in the 5-10:1, where the substrate is rich in organic carbon and poor in nitrogen, which forces the bacteria to grow and multiply at a moderate rate (Goldman et al 1987). In intensive and super intensive cultivation systems, such as BFT, the high feed rate with a high percentage of protein (30-50%), produce large amounts of ammonium and then nitrite, which can be assimilated by the microorganisms under high C:N ratios, so it is necesary to adds carbohydrates. Relationships in BFT usually reported are approximate to 10:1 that favors photoautotrophic microorganisms, approximate to 15:1 that favors the chemoautotrophs and to 20:1 that favors the heterotrophic microorganisms (Table 3) (Ebeling et al 2006). The C:N relationship can also be influenced by the assimilation capacity of N by the cultivated species (Nur Syuhada et al 2015), and by the characteristics of the food and inputs that are added to the culture waters (Ebeling et al 2006), so as an example it is known that the C:N ratio and the protein content are inversely proportional (Table 4). According to the above, selecting the concentrated food that meets the needs of the crop species and then balancing this relationship is essential to promote the dominance of the desired microorganisms and allow the capture of ammonium and nitrite while keeping the comfort conditions under the comfort range (Table 5) (Avnimelech 2015).

Table 4

C:N ratio of fish feed (adapted from Avnimelech (2015))

Protein content (%)	*C:N ratio*
15	21.5
20	16.1
25	12.9
30	10.8
35	9.2
40	8.1

Table 5

Water quality parameters in biofloc technology (adapted from Emerenciano et al (2017))

Water quality parameters in BFT	*Values*
Oxygen	> 4 mg L^{-1}
Saturation	> 60%
Temperature	28-30ºC (for tropical species)
pH	6.8-8.0
NAT	< 1 mg L^{-1}
Nitrite	< 1 mg L^{-1}
Nitrate	0.5-20 mg L^{-1}
Alkalinity	> 100 mg L^{-1} $CaCO_3$
Salinity	0-50 ppt
Settleable solids (SS)	5-15 mL L^{-1} shrimps
	5-20 mL L^{-1} tilapia fingerlings
	20-50 mL L^{-1} juveniles and adult tilapia
Total suspended solids (TSS)	< 500 mg L^{-1}

(ppt) parts per thousand.

The benefits of the dominance of heterotrophic microorganisms in the biofloc have been documented by means of C:N approximate to 20:1 ratios (De Schryver et al 2008; Hargreaves 2013; Ray & Lotz 2014; Avnimelech 2015), as these have been shown to

help to efficiently reduce the concentration of nitrogenous compounds when they are added easily assimilated carbon such as sugars, avoiding the formation of nitrite and nitrate and generating microbial biomass more quickly than the chemoautotrophic communities (C:N approximate to 15:1), and in conditions more stable than those dominated by photoautotrophic communities (C:N approximate to 10:1). However, other studies (Perez-Fuentes et al 2016), reported that for tilapia fed with different C:N ratios, the 10:1 ratio presented better survival and growth under reduced illumination (70%); also, Avnimelech (2015), reported favorable results in productivity and survival in systems under the relation C:N 15:1.

In BFT culture systems with a C:N 20:1 ratio, the organic carbon supplement in the presence of high levels of TAN, increases oxygen demand due to the rapid assimilation of TAN by heterotrophic bacteria, aspect that can affect the crop especially in species sensitive to different levels of dissolved oxygen (Schveitzer et al 2013; Ray & Lotz 2014). Another important aspect in the ratio 20:1, is the rapid generation of solids product of the accelerated growth of microorganisms, which generates an increase in the floc volume that must be controlled to avoid high oxygen consumption, the generation of anoxic zones in the tank for excess solids and the affectation of the crop species (Hargreaves 2013). In O. niloticus at different ratios C:N, Perez-Fuentes et al (2016) reported an increase in solids of 200% in the ratio 20:1 with respect to the 10:1 control, and a considerable decrease in oxygen concentration dissolved from 3.2 to 1-1.5 mg L^{-1}, when molasses was added at a concentration higher than 0.12 g L^{-1}. To keep the levels of NAT and dissolved oxygen stable, it is essential to maintain adequate aeration and carry out the addition of carbohydrates based on the feed provided, this allows to maintain stable the C:N ratio and the development of the biofloc (Luo et al 2017).

Organic carbon. Thanks to the metabolic diversity of microorganisms (Sigee 2005), continuous studies look for raw materials rich in organic carbon (Table 6), available and inexpensive that can be harnessed by biofloc bacteria (Hargreaves 2013), for the uptake of ammonia nitrogen without the aerobic metabolism of microorganisms decreasing dissolved oxygen levels (De Schryver et al 2008), preventing the lethal effect in hypoxia-sensitive culture species (Landman et al 2005), or stress conditions in species more resistant to it (Avnimelech 2015).

In general, it has been reported that the addition of soluble carbon sources with simple structure allow a faster removal of ammonium than carbon sources with more complex structure, mainly in heterotrophic biofloc (De Schryver et al 2008). This explains that in most studies glucose, glycerol and sucrose are the most efficient sources (Ray & Lotz 2014; Wei et al 2016), however, more complex carbon sources such as cellulose have also shown efficiency in the removal of ammoniacal nitrogen, without having to be added with the same frequency as sugars due to its slower assimilation (Serfling 2006). Additionally, sources rich in cellulose can help fix the bacteria that make up the biofloc, particularly in the first weeks of establishment of the same (De Schryver et al 2008).

Table 6 shows some studies reporting different carbon supplements in BFT culture systems with different species. As already mentioned, better microbial assimilation of ammonium is reported with: glucose (Wei et al 2016), sucrose (Harini et al 2016), glycerol (Wei et al 2016), molasses (Castro et al 2016), tapioca (Ahmad et al 2016), dextrose (Suita et al 2015), corn flour (Day et al 2016), rice flour (Kumar et al 2017) and recently beet (Najdegerami et al 2016; Green & McEntire 2017). Other studies verify that the nature of the carbon source can affect the biofloc formation and its structure (De Schryver et al 2008), its composition and stability (Hollender et al 2002; Oehmen et al 2004) and the microbial community that makes it up (Wei et al 2016).

Table 6

Carbon sources and C/N ratio used in fish farming

Carbon source	*Species*	*C/N ratio*	*Author*
Tapioca*, wheat, corn, sugar bagasse	*Labeo rohita*	15:1	Ahmad et al (2016)
Molasses*	*Litopenaeus vannamei*	10:1	Azhar et al (2016)
Tapioca, tapioca by product, Rice brand		15:1* 20:1*	
Molasses, coffees remain, dry *Moringa*	*Carasius auratus*	20:1	Castro et al (2016)
Molasses	*Oreochromis niloticus*	15:1	Cavalcante et al (2016)
Glycerol*, glucose, acetate	*Macrobrachium rosenbergii*	10:1	Crab et al (2010)
Maize meal	*Oreochromis mossambicus* *Oreochromis andersonii* *Oreochromis niloticus*	20:1	Day et al (2016)
Molasses, tapioca, tapioca by product*, rice brand	*Litopenaeus vannamei*	15:1	Ekasari et al (2014)
Wheat brand (10%) + sugar cane molasses (90%)	*Farfantepenaeus pauliensis*	20:1	Emerenciano et al (2011)
Glucose, sucrose, molasses, cornflour	*Artemia* sp.	20:1	Gao et al (2017)
Rice brand*, molasses	*Litopenaeus vannamei*	20:1	Gomes Vilani et al (2016)
Molasses*, beet pulp	*Ictalurus punctatus*	Photoautotrophic	Green & Mc Entire (2017)
Sugar	*Pseudotropheus saulosi*	15:1	Harini et al (2016)
Molasses*, starch, wheat flour, mixture of them	*Litopenaeus vannamei*	15:1	Khanjani et al (2017)
Rice flour*, molasses	*Penaeus monodon*	10:1	Kumar et al (2017)
Poly-B-hydroxybutyric acid, glucose	*Oreochromis niloticus*	15-20:1	Luo et al (2017)
Molasses, molasses+rice powder	*Oreochromis niloticus*	20:1	Maya Gutierrez et al (2016)
Beet molasses	*Cyprinus carpio*	20:1	Najdegerami et al (2016)
No specification	*Piaractus brachypomus*	Heterotrophic	Poleo et al (2011)
Dextrose*, molasses	*Litopenaeus vannamei*	6:1	Suita et al (2015)
De-oiled oil palm kernel meal	*Litopenaeus vannamei*	15:1	Syamala et al (2017)
Glucose*, glycerol, starch	No specification	15:1	Wei et al (2016)

(*) best results in each report

Wei et al (2016) supplemented biofloc with glucose, glycerol and starch in a 15:1 approximate ratio and found significant differences in the structure, nutritional composition and community of microorganisms that made up the biofloc in each one treatments. The biofloc constituted when starch was used as a carbon source showed a more compact structure, with a lower amount of protein (31.5±0.6%) and ash (12.4±0.2%) and a higher quantity of lipids (8.5±0.2%), carbohydrates (47.6±0.3%) and crude energy (18.7±0.2%), compared to treatment with glycerol (35.5±1.2%, 15.2±0.3%, 4.2±0.1%, 45.1±0.2%, 17.5±0.3% respectively) and glucose (41.2±0.8%; 15.0±0.1%; 6.1±0.1%; 37.7±0.2%; 18.2±0.3% respectively), thus the Essential Amino Acids Index (IAAE) also varied, which was recorded at 0.93; 0.99 and 0.98 for the starch, glucose and glycerol respectively. In terms of water quality, biofloc supplemented with starch had a higher concentration of ammonium (0.20±0.03 mg NH_4^+ L^{-1}) and nitrite (0.13±0.02 mg NO_2 L^{-1}), which the one supplemented with glucose (0.13±0.01 mg NH_4^+ L^{-1} and 0.04±0.02 mg NO_2 L^{-1}) and glycerol (0.12±0.03 mg NH_4^+ L^{-1} and 0.02±0.01 mg NO_2 L^{-1}); the most abundant microorganisms in the three types of biofloc were Bacterioides and Proteobacteria, Cyanobacteria being the most abundant in the biofloc

supplemented with starch, other groups present in smaller amounts were Planctomycetes, Actinobacteria, Verrucomicrobia and Firmicutes among others.

On the other hand Luo et al (2017), using glucose as a carbon source in addition of two insoluble biological polymers, polibetahidroxibutiric acid (PHB) and polycaprolactone (PCL) in the culture of Nile tilapia in biofloc, reported similarities in bacterial biofloc communities, being that the dominant microorganisms were the Proteobacteria groups, followed by Bacerioides, Actinobacteria, Planctomycetes, Firmicutes, Fusobacteria, Tenericutes and Chlamydiae and obtaining final biomasses of 37.93±8.87 kg m^{-3}; 34.29±9.29 kg m^{-3} and 44.14±10.51 kg m^{-3} for PHB, PCL and glucose respectively. Other studies that have evaluated different carbon sources corroborate the good performance of glycerol (Crab et al 2010; Ray & Lotz 2014) and sucrose (Xu & Pan 2012), however, the management and the addition of other inputs is decisive in the results that can be obtained (Avnimelech 2015).

New ongoing studies are being focused on the search for cheaper sources of carbon such as by-products of the food industry, which are also low cost and promote the formation of biofloc with high content of protein, essential amino acids, carbohydrates and fatty acids and optimize the growth of the crop. The potential use of low-cost by-products, together with the reduction in the use thereof, make the BFT one of the most attractive production technologies in the future.

Inorganic carbon. In the BFT, in addition to the organic carbon supplementation, the addition of inorganic carbon in the form of carbonates is indispensable from the beginning of the activity, since the development of the heterotrophic and chemoautotrophic bacteria mainly requires such inputs. Carbonate supplementation is done by providing limes, which in addition to supplying the requirements for the development of bacteria, generate a buffer effect that prevents abrupt changes in pH, product of the respiration and assimilation reactions carried out in the culture tanks.

In intensive production systems the high CO_2 generation, product of the high rate of microbial respiration in addition to the fish or shrimp culture, and the dissociation of carbonate by bacterial metabolism, can destabilize the system rapidly (Ebeling et al 2006; Hargreaves 2013). When the alkalinity is gone, the system is acidified, since the combination of the two previous processes makes it necessary to add the necessary alkalinizers to protect the crops in progress (Martins et al 2017). Different studies have evaluated the use of alkalizing compounds capable of providing inorganic carbon to the microorganisms and controlling the pH under conditions of high CO_2 production. Furtado et al (2011) reported favorable results on the production of *Litopenaeus vannamei* using sodium bicarbonate ($NaHCO_3$) and calcium hydroxide ($Ca[OH]_2$), which provided alkalinity efficiently, compared with sodium carbonate (Na_2CO_3). The decrease in the zootechnical parameters of the species was observed when the alkalinity was less than 100 mg L^{-1} and the pH less than 7 during prolonged periods. Similar results were reported by Martins et al (2017), for *Oreochromis niloticus*, where they found that sodium bicarbonate followed by calcium hydroxide allowed greater productivity 23.52±0.49 kg m^{-3} and 21.64±0.89 kg m^{-3} respectively compared to calcium oxide ($CaCO_3$) 20.76±0.70 kg m^{-3}; additionally this study reports the adjustment of alkalinity and pH based on the food consumed: 14.64±0.49; 7.18±0.32 and 24.09±2.32% for sodium bicarbonate, calcium hydroxide and calcium oxide respectively, an important aspect to be taken into account for the practical adjustment of alkalinity in culture systems (Martins et al 2017).

BFT and other technologies. The richness of nutrients that are dissolved in the water product of the microbial transformation of organic substances into inorganic in BTF, allows this technology to be coupled with other production systems in search of the optimization of resources and to reduce environmental damage (Avnimelech 2015). In aquaponics, the production of plants in a nutrient-rich aqueous medium (hydroponics) is integrated with aquaculture, whereby BFT waters in continuous recirculation through the roots of vegetables can be exploited to the maximum allowing the two systems benefit, taking care of both water quality in more dynamic and stable ranges for organisms in culture (Rakocy 2012). Pinho et al (2017) collect the results of some aquaponics experiments with BFT waters showing comparatively better production than other waters

of different origins and explain that this may be due to the action of the dense and diverse microbial communities and its role in the productivity and nutrient recycling. Also shows that culture of *O. niloticus* in biofloc in densities of 9.2 kg m^{-3} together with 0.56 kg m^{-2} of lettuce butter, 1.36 kg m^{-2} of crispy lettuce and 0.56 kg m^{-2} of red lettuce, grown in its waters, compared to clear water controls with lower results in the production of *O. niloticus* 7.74 kg m^{-3} and 1.53 kg m^{-2}, 1.04 kg m^{-2} and 0.28 kg m^{-2} of butter lettuce, crunchy and red respectively in 21 days.

Trang et al (2017) evaluated for 25 days the rate of water recirculation (50%, 200%, and 400%), and the relation water quality vs production of biomass with two densities of *O. niloticus* (122 and 220 fish m^{-3}) in an aquaponic system with *Lactuca sativa, Ipomoea aquatica* and *Canna glauca*. The authors report that in the higher recirculation per day, the feed conversion rate can be decreased and the growth of fishes can be decreased in densities from 6 to 7 kg m^{-3} on a hydroponic surface of 4.2 m^{-2}.

Aquaponics with BTF waters has also been experienced in the cultivation of submerged plants, such as *Kappaphycus alvarezii*, a valuable red algae with high content of phenolic compounds, flavonoids and carotenoids (La Macchia Pedra et al 2017). In this study the growth of *K. alvarezii* cultivated in vitro with biofloc water from a culture of *Litopenaeus vannamei*, was compared with that enriched with nutrient solution (von Stosch), with commercial extract of *Ascophyllum nodosum* and with sterile water as control. Treatment with BFT and von Stosch solution showed higher growth rates (1.56±0.08% day^{-1} and 1.70±0.14% day^{-1}, respectively), compared with enrichment with *A. nodosum* (0.57±0.07% day^{-1}) and control (0.51±0.05% day^{-1}). In addition, the content of flavonoids (92.2±22.4 µg g^{-1}) and phenolic compounds (18.8±0.6 µg g^{-1}) obtained with the cultures enriched with water from the biofloc was higher than that of the other treatments. In other study Kuhn et al (2009) report the elaboration of microbial biofloc flour prepared from the effluent of a tilapia culture. The food was subsequently used in feeding *Litopenaeus vannamei*, for which significant weight gains were reported (8.07 to 8.18 g), in a total of 5 weeks compared to controls (4.42 to 5.46 g) with other sources of feed. The two previous studies highlight the importance of the use of nutrients in the generation of biomass and the potential of biofloc as a feed due to its high content of protein and other components (Bauer et al 2012; Emerenciano et al 2013).

Conclusions. In natural and extensive aquatic systems, the availability of nutrients and resources such as oxygen and light, restrict the populations of benefical microorganisms that compete for them, making the transformation processes of organic and inorganic substances slow; on the contrary, in current and controlled intensive and superintensive systems of aquaculture production, the high concentration of nutrients and oxygen promote that populations of profitable microorganisms develop rapidly, favoring a dynamic nutrient assimilation system. In the productive systems with BFT the main elements for the growth of the microorganisms are originated by the waste compounds from the high-density culture of the species of interest for human consumption, which generates a complex trophic network in which the nitrogen compounds become essential molecules for favourable bacterial development and food for the culture.

The proper functioning of this technology in the production of new species of commercial interest implies knowledge of the fundamentals of technology and its effect on cultivation, according to the characteristics of each species. The knowledge of new and efficient production systems that are implemented and can be articulated, would favor the sustainability of the production systems.

References

Ahmad H., Verma A. K., Babitha Rani A. M., Rathore G., Saharan N., Gora A. H., 2016 Growth, non-specific immunity and disease resistance of *Labeo rohita* against *Aeromonas hydrophila* in biofloc systems using different carbon sources. Aquaculture 457:61-67.

Alabaster J. S., Lloyd R., 1982 Water quality criteria for freshwater fish. 2nd edition. Butterworths, London, pp. 85-97.

Arthur J. W., West C. W., Allen K. N., Hedtke S. F., 1987 Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bulletin of Environmental Contamination and Toxicology 38:324-331.
Avnimelech Y., 2015 Biofloc technology - a practical guide book. 3rd edition, The World Aquaculture Society, Baton Rouge, Louisiana, USA, pp 21-73.
Azhar M. H., Supriyono E., Nirmala K., Ekasari J., 2016. Organic carbon source and C/N ratio affect inorganic nitrogen profile in the biofloc-based culture media of Pacific white shrimp (*Litopenaeus vannamei*). Ilmu Kelautan 21(1):23-28.
Baldisserotto B., 2013 Fish fisiology applied to fish farming. 3rd edition, Ed. UFSM, Santa Maria, ISBN: 978.85-7391-198-5, pp. 139-156. [in Portuguese]
Barbieri E., 2010 Acute toxicity of ammonia in white shrimp (*Litopenaeus schmitti)* (Burkenroad, 1936, Crustacea) at different salinity levels. Aquaculture 306:329-333.
Barbieri E., Vigliar Bondioli A. C., 2015 Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus*, Holmberg, 1887) at different temperatures levels. Aquaculture Research 46(3):565-571
Bauer W., Prentice-Hernandez C., Tesser M. B., Wasielesky Jr. W., Poersch L. H. S., 2012 Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture 342-343:112-116.
Camargo J. A., Alonso A., 2006 Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environment International 32:831–849.
Castro M. G., Castro M. J., De Lara A. R., Monroy D. M. C., Ocampo C. J. A., Davila F. S., 2016 Length, weight and condition factor comparison of *Carassius auratus* (Linnaeus, 1758) juveniles cultured in biofloc system. International Journal of Fisheries and Aquatic Studies 4(6):345-350.
Cavalcante D., dos Santos Lima F. R., Tomaz Rebouças V., do Carmo e Sá M. V., 2016 Association between periphyton and bioflocs systems in intensive culture of juvenile Nile tilapia. Acta Scientiarum. Animal Sciences 38(2):119-125.
Ceballos S., Pinzon O., Gonzalez J. F., 2001 [Effect of two nitrite concentrations on growth and survival in Bocachico (*Prochilodus magdalenae*)]. Rev Med Vet Zoot 48:11-15. [in Spanish]
Chakraborty S. C., Chakraborty S., 1998 Effect of dietary protein level on excretion of ammonia in Indian major carp, *Labeo rohita* fingerlings. Aquaculture Nutrition 4: 47-51.
Chen J. C., Lei S. C., 1990 Toxicity of ammonia and nitrite to *Penaeus monodon* juveniles. Journal of the World Aquaculture Society 21(4):300-306.
Chen J. C., Chia P. G., 1995 Effects of unilateral eyestalk ablation on oxygen consumption and ammonia excretion of juvenile *Penaeus japonicus* Bate at different salinity levels. Journal of Crustacean Biology 15:434-443.
Chen J. C., Liu P. C., Lei S. C., 1990 Toxicities of ammonia and nitrite to *Penaeus monodon* adolescents. Aquaculture 89:127-137.
Clifford H. C., Brick R. W., 1978 Protein utilization in the freshwater shrimp *Macrobrachium rosenbergii*. Proceedings of the Annual Meeting - World Mariculture Society 9:195-208.
Collazos-Lasso L. F., Arias-Castellanos J. A., 2015 Fundamentals of bioflocs technology (BFT). An alternative for fish farming in Colombia: A review. Orinoquia 19 (1):77-86. [in Spanish]
Colt J., Ludwig R., Tchobanoglous G., Cech Jr. J. J., 1981 The effects of nitrite on the short-term growth and survival of channel catfish, *Ictalurus punctatus.* Aquaculture 24:111-122.
Crab R., Chielens B., Wille M., Bossier P., Verstraete W., 2010 The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquaculture Research 41:559-567.
Crawford R. E., Allen G. H., 1977 Seawater inhibition of nitrite toxicity to Chinook salmon. Transactions of the American Fisheries Society 106(1):105-109.

Day S. B., Salie K., Stander H.B., 2016 A growth comparison among three commercial tilapia species in a biofloc system. Aquaculture International 24(5):1309-1322.
De Schryver P., Crab R., Defoirdt T., Boon N., Verstraete W., 2008 The basics of bioflocs technology: the added value for aquaculture. Aquaculture 277:125-137.
Ebeling J. M., Timmons M. B., Bisogni J. J., 2006 Engineering analysis of the stoichiometry of photoautotrophic, autotrophic and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346-358.
Ekasari J., Hanif Azhar M., Surawidjaja E. H., Nuryati S., De Schryver P. Bossier P., 2014 Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish and Shellfish Immunology 41:332-339.
Emerenciano M., Ballester E. L. C., Cavalli R. O., Wasielesky W., 2011 Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp *Farfantepenaeus paulensis*: growth performance, floc composition and salinity stress tolerance. Aquaculture International 19:891-901.
Emerenciano M., Cuzon G., Arevalo M., Gaxiola G., 2014 Biofloc technology in intensive broodstock farming of the pink shrimp *Farfantepenaeus duorarum*: spawning performance, biochemical composition and fatty acid profile of eggs. Aquaculture Research 45(10):1713-1726.
Emerenciano M., Gaxiola G., Cuzon G., 2013 Biofloc technology (BFT): a rewiew for aquaculture application and animal food industry. In: Biomass now: cultivation and utilization. Matovic M. D. (ed), IntechOpen, DOI: 10.5772/53902.
Emerenciano M., Martinez-Córdova L. R., Martínez-Porchas M., Miranda-Baeza A., 2017 Biofloc technology (BFT): a tool for water quality management in aquaculture. In: Water quality. Tutu H. (ed), IntechOpen, DOI: 10.5772/66416.
FAO - Food and Agriculture Organization of the United Nations, 2016 [The state of world fisheries and aquaculture], pp. 23. [in Spanish]
Fromm P. O., Gillette J. R., 1968 Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). Comparative Biochemistry and Physiology 26:887-896.
Furtado P. S., Poersch L. H., Wasielesky Jr. W., 2011 Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture 321:130-135.
Gao M., Wang J., Ma G., Van Stappen G., Sui L., 2017 Carbon supplementation and microbial management to stimulate artemia biomass production in hypersaline culture conditions. Aquaculture Research 48(3):1240-1250
Goldman J. C., Caron D. A., Dennett M. R., 1987 Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio. Limnology and Oceangraphy 32:1239-1252.
Gomes Vilani F., Schveitzer R., da Fonseca Arantes R., do Nascimento Vieira F., Manoel do Espírito Santo C., Quadros Seiffert W., 2016 Strategies for water preparation in a biofloc system: effects of carbon source and fertilization dose on water quality and shrimp performance. Aquacultural Engineering 74:70-75.
Gonzalez J. F., Suarez R., Gil B., Torres G., 2005 [Acute exposure to three concentrations of nitrite in Bocachico juvenile (*Prochilodus magdalenae*)]. Rev Med Vet Zoot 52:36-43. [in Spanish]
Green B. W., McEntire M. E., 2017 Comparative water quality and channel catfish production in earthen ponds and a biofloc technology production system. Journal of Applied Aquaculture 29(1):1-15.
Hargreaves J. A., 2013 Biofloc production systems for aquaculture. Southern Regional Aquaculture Center SRAC Publication No. 4503, 12 pp.
Harini C., Rajagopalasamy C. B. T., Sampath-Kumar J. S., Santhakumar R., 2016 Role of biofloc in the growth and survival of blue morph, *Pseudotropheus saulosi*. Indian Journal of Science and Technology 9(8):1-7.
Hayatsu M., Tago K., Saito M., 2008 Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Science and Plant Nutrition 54(1):33-45.

Hollender J., Van der Krol D., Kornberger L., Gierden E., Dott W., 2002 Effect of different carbon sources on the enhanced biological phosphorus removal in a sequencing batch reactor. World Journal of Microbiology and Biotechnology 18(4):359-364.
Ip Y. K., Chew S. F., Wilson J. M., Randall D. J., 2004 Defences against ammonia toxicity in tropical air-breatihng fishes exposed to high concentrations of environmental ammonia: a review. Journal of Comparative Physiology B 174:565-575.
Jensen F. B., 2003 Nitrite disrupts multiple physiological functions in aquatic animals. Comparative Biochemistry and Physiology Part A 135:9-24.
Karasu Benli A. C., Koksal G., 2005 The acute toxicity of ammonia on tilapia (*Oreochromis niloticus L.*) larvae and fingerlings. Turk J. Vet Anim Sci 29:339-344.
Kaushik S. J., 2000 [Factors that affect nitrogenous excretion in teleosts and crustaceans]. In: Avances en Nutrición Acuícola IV Civera-Cerecedo R., Pérez-Estrada C. J., Ricque-Marie D., Cruz-Suárez L. E. (eds), Memorias del IV Simposium Internacional de Nutrición Acuícola. Noviembre 15-18, La Paz, B.C.S., México. [in Spanish]
Khanjani M. H., Sajjadi M. M., Alizadeh M., Sourinejad I., 2017 Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: the effect of adding different carbon sources. Aquaculture Research 48(4):1491-1501.
Kuhn D. D., Boardman G. D., Lawrence A. L., Marsh L., Flick Jr. G. J., 2009 Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture 296:51-57.
Kumar S., Anand P. S. S., De D., Deo A. D., Ghoshal T. K., Sundaray J. K., Ponniah A. G., Jithendran K. P., Raja R. A., Biswas G., Lalitha N., 2017 Effects of biofloc under different carbon sources and protein levels on water quality, growth performance and immune responses in black tiger shrimp *Penaeus monodon* (Fabricius, 1978). Aquaculture Research 48(3):1168-1182.
La Macchia Pedra A. G., Ramlov F., Maraschin M., Hayashi L., 2017 Cultivation of the red seaweed *Kappaphycus alvarezii* with effluents from shrimp cultivation and brown seaweed extract: effects on growth and secondary metabolism. Aquaculture 479: 297-303.
Landman M. J., Van Den Heuvel M. R., Ling N., 2005 Relative sensitivities of common freshwater fish and invertebrates to acute hypoxia. New Zealand Journal of Marine and Freshwater Research 39(5):1061-1067.
Lin Y. C., Chen J. C., 2001 Acute toxicity of ammonia on *Litopenaeus* v*annamei* (Boone) juveniles at different salinity levels. Journal of Experimental Marine Biology and Ecology 259:109-119.
Lin Y. C., Chen J. C., 2003 Acute toxicity of anitrite on *Litopenaeus* v*annamei* (Boone) juveniles at different salinity levels. Aquaculture 224:193-201.
Luo G., Zhang N., Cai S., Tan H., Liu Z., 2017 Nitrogen dynamics, bacterial community composition and biofloc quality in biofloc-based systems cultured *Oreochromis niloticus* with poly-β-hydroxybutyric and polycaprolactone as external carbohydrates. Aquaculture 479:732-741.
Martínez-Córdova L. R., Martínez-Porchas M., López-Elías J. A., Campaña-Torres A., Miranda-Baeza A., Ballester E., Porchas-Cornejo M. A., 2010 [Natural food in aquaculture: an updated review]. In: [Advances in Aquaculture Nutrition X]. Cruz-Suarez L. E., Ricque-Marie D., Tapia-Salazar M., Nieto-López M. G., Villarreal-Cavazos D. A., Gamboa-Delgado J. (eds), Memorias del X Simposio Internacional de Nutrición Acuícola, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0. Universidad Autónoma de Nuevo León, Monterrey, México, pp. 668-699. [in Spanish]
Martinez-Lopez R., 2002 [Effect of diet and other factors on the excretion of ammonium and the use of nitrogen by dorada *Sparus aurata*, and its incidence in the crops of this species]. PhD Dissertation, Instituto de Ciencias del Mar de Barcelona, Universidad de Barcelona, pp. 119-151. [in Spanish]

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

Maya Gutiérrez S., Monroy Dosta M. C., Handam Partida A., Castro Mejía J., Rodríguez Montes de Oca G. A., 2016 Effect of two carbon sources in microbial abundance in a biofloc culture system with *Oreochromis niloticus* (Linnaeus, 1758). International Journal of Fisheries and Aquatic Studies 4(3):421-427.

Milhazes-Cunha H., Otero A., 2017 Valorisation of aquaculture effluents with microalgae: The Integrated Multi-Trophic Aquaculture concept. Algal Research 24:416-424 .

Najdegerami E. H., Bakhshi F., Lakani F. B., 2016 Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio* L.) fingerlings in zero-water exchange system. Fish Physiology and Biochemistry 42:(2)457-465.

Nur Syuhada A. B., Nurfarahana M. N., Fathurrahman L., Siti Hajar A. H., Su Shiung L., Ahmad J., 2015 Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish (*Clarias gariepinus*) utilizing bioflocs technology. International Biodeterioration and Biodegradation 102:100-106.

Ochoa D. M., Peña L. C., González J. F., 2002 Waterborne nitrite exposure on white cachama, *Piaractus brachypomus*. Dahlia 5:27-31.

Oehmen A., Yuan Z., Blackall L. L., Keller J., 2004 Short-term effects of carbon source on the competition of polyphosphate accumulating organisms and glycogen accumulating organisms. Water Science and Technology 50(10):139-144.

Ostrensky A., Wasielesky Jr. W., 1995 Acute toxicity of ammonia to various life stages of the Sao Paulo shrimp, *Penaeus paulensis* Perez-Farfante, 1967. Aquaculture 132: 339-347.

Perez-Fuentes J. A., Hernandez-Vergara M. P., Perez-Rostro C. I., Fogel I., 2016 C:N ratios affect nitrogen removal and production of Nile tilapia *Oreochromis niloticus* raised in a biofloc system under high density cultivation. Aquaculture 452:247-251.

Pertúz-Buelvas V., Roa-Lázaro A., Espinosa-Araujo J., Prieto-Guevara M., Atencio-García V., 2016 [Evaluation of the first feeding of Bocachico *Prochilodus magdalenae* using floc macroagregates]. Memorias del Séptimo Congreso Colombiano de Acuicultura, Universidad de Nariño, Pasto, Colombia in Revista Investigación Pecuaria suplemento, pp. 137-138. [in Spanish]

Peyghan R., Takamy G. A., 2002 Histopathological, serum enzyme, cholesterol and urea changes in experimental acute toxicity of ammonia in common carp *Cyprinus carpio* and use of natural zeolite for prevention. Aquaculture International 10:317-325.

Pinho S. M., Molinari D., de Mello G. L., Fitzsimmons K. M., Emerenciano M. G. C., 2017 Effluent from a biofloc technology (BFT) tilapia culture on the aquaponics production of different lettuce varieties. Ecological Engineering 103:146-153.

Poleo G., Aranbarrio J. V., Mendoza L., Romero O. 2011 [Culture of white cachama in high densities and in two closed systems]. Pesquisa Agropecuária Brasileira 46(4):429-437. [in Spanish]

Rakocy J. E., 1989 Tank culture of tilapia. Southern Regional Aquaculture Center Publication, No. 282, 4 pp.

Rakocy J. E., 2012 Aquaponics-integrating fish and plant culture. In: Aquaculture production systems. First edition, Tidwell J. (ed), John Wiley & Sons, Inc., pp. 343-344.

Rakocy J. E., Bailey D. S., Martin J. M., Shultz R. C., 2000 Tilapia production systems for the Lesser Antilles and other resource-limited tropical areas. In: Tilapia Aquaculture in the 21 Century: Proceedings from the Fifth International Symposium on Tilapia in Aquaculture. Fitzsimmons K., Filho J. C. (eds), Rio de Janeiro, Brazil pp. 651-662.

Ray A. J., 2012 Management of biological and chemical constituents for the advancement of intensive, minimal-exchange, biofloc-based shrimp (*Litopenaeus vannamei*) aquaculture. PhD Dissertation, The University of South Mississippi, 472 pp.

Ray A. J., Lotz J. M., 2014 Comparing a chemoautotrophic-based biofloc system and three heterotrophic-based systems receiving different carbohydrate sources. Aquacultural Engineering 63:54-61.
Regnault M., 1987 Nitrogen excretion in marine and fresh-water crustacea. Biological Reviews 62:1-24.
Remen M., Imsland A. K., Stefansson S. O., Jonassen T. M., Foss A., 2008 Interactive effects of ammonia and oxigen on growth and physiological status of juvenile Atlantic cod (*Gadus morhua*). Aquaculture 274:292-299.
Schmitt A. S. C., Santos E. A., 1998 Ammonia-N efflux rate and nutritional state of juvenile pink shrimp, *Penaeus paulensis* (Perez-Farfante), in relation to food type. Aquaculture Research 29:495-502.
Schmitt S. C., Hodds A., Uglow R. F., 1992 Salinity and temperature effects on ammonia effluxes in *Crangon crangon* (L.). Proc Eur Crustacean Conf, pp. 138-139.
Schveitzer R., Arantes R., Costódio P. F. S., do Espírito Santo C. M., Arana L. V., Seiffert W. Q., Andreatta E. R., 2013 Effect of different biofloc levels on microbial activity, wáter quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. Aquacultural Engineering 56:59-70.
Serfling S. A., 2006 Microbial flocs: natural treatment method supports fresh water, marine species in recirculating systems. Global Aquaculture Advocate 9:34-36.
Sigee D. C., 2005 Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment. John Wiley & Sons LTD, 544 pp.
Stein L. Y., Klotz M. G., 2016 The nitrogen cycle. Current Biology 26:94-98.
Suita S. M., Ballester E. L. C., Abreu P. C., Wasielesky Jr. W., 2015 Dextrose as carbon source in culture of *Litopenaeus vannamei* (Boone, 1931) in zero exchange system. Latin American Journal of Aquatic Research 43(3):526-533.
Syamala K., Lakra W. S., Chadha N. K., Sahu N. P., Prasad K. P., 2017 Effect of de-oiled oil palm kernel meal based biofloc system on growth and digestive enzyme activity of Pacific white shrimp, *Litopenaeus vannamei*. International Journal of Current Microbiology and Applied Sciences 6(6):1806-1816.
Timmons M. B., Ebeling J. M., 2010 Recirculating aquaculture. 2nd edition, Ed Cayuga Aqua Ventures, USA, pp. 300-307.
Tomasso J. R., Simco B. A., Davis K. B., 1979 Chloride inhibition of nitrite-induced methemoglobinemia in channel catfish (*Ictalurus punctatus*). Journal of the Fisheries Research Board of Canada 36:1141-1144.
Torres-Tabares A., Sierra-Fontecha F. A., Medina-Robles V. M., Cruz-Casallas P. E., Velasco-Santamaría Y. M., 2007 [Acute toxicity of nitrite ($N\text{-}NO_2$) in two fish species of the Colombian Orinoquia: Yamú (*Brycon amazonicus*) and white Cachama (*Piaractus brachypomus*)]. Jornada de Acuicultura, Universidad de Los Llanos XIII pp. 80-85. [in Spanish]
Trang N., Konnerup D., Brix H., 2017 Effects of recirculation rates on water quality and *Oreochromis niloticus* growth in aquaponic systems. Aquacultural Engineering 78:95-104.
Valbuena-Villarreal R. D., Vásquez-Torres W., 2011 [Body weight is inversely related to the excretion of ammonium in red tilapia (*Oreochromis* sp.)]. Revista Colombiana de Ciencias Pecuarias 24(2):191-200. [in Spanish]
Wei Y. F., Liao S. A., Wang A. L., 2016 The effect of different carbon sources on the nutritional composition, microbial community and structure of bioflocs. Aquaculture 465:88-93.
Williams E. M., Eddy F. B., 1986 Chloride uptake in fresh-water teleosts and its relationship to nitrite uptake and toxicity. Journal of Comparative Physiology B 156:867-872.
Wilson R. W., Taylor E. W., 1992 Transbranchial ammonia gradients and acid-base responses to high external ammonia concentration in rainbow trout (*Oncorhynchus mykiss*) acclimated to different salinities. Journal of Experimental Biology 166:95-112.
Wood C. M., 2001 Influence of feeding, exercise and temperature on nitrogen metabolism and excretion. Fish Physiology 20:201-238.

Wright P. A., Wood C. M., 2012 Seven things fish know about ammonia and we don't. Respiratory Physiology and Neurobiology 184:231-240.
Xu W. J., Pan L. Q., 2012 Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed. Aquaculture 356-357:147-152.

Received: 24 March 2018. Accepted: 30 June 2018. Published online: 27 July 2018.
Authors:
Yira Karina Jiménez Ojeda, Instituto de Acuicultura de los Llanos - IALL, Universidad de los Llanos, Villavicencio - Meta, Colombia, e-mail: yirajimenez@hotmail.com
Luis F. Collazos-Lasso, Instituto de Acuicultura de los Llanos - IALL, Universidad de los Llanos, Villavicencio - Meta, Colombia, e-mail: lcollazos@unillanos.edu.co
José Alfredo Arias Castellanos Instituto de Acuicultura de los Llanos - IALL, Universidad de los Llanos, Villavicencio - Meta, Colombia, e-mail: josealfredoariasc@hotmail.com

How to cite this article:
Jiménez-Ojeda Y. K., Collazos-Lasso L. F., Arias-Castellanos J. A., 2018 Dynamics and use of nitrogen in Biofloc Technology - BFT. AACL Bioflux 11(4):1107-1129.