

# Influence of Nitrogen Cycle Bacteria on Nitrogen Mineralisation, Water Quality and Productivity of Freshwater Fish Pond: A Review

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## Abstract

Nitrogen is one of the prime influential macronutrients which directly stimulate the productivity of pond ecosystems. The steps involving chemical transformations of nitrogen bound in complex organic matters are dominated by a variety of nitrogen cycle bacteria responsible for mineralisation of organic nitrogen into inorganic forms. Application of excess nitrogen in the form of fertiliser may cause deterioration of water quality by accumulation of toxic nitrogenous compounds such as ammonia and nitrite. Over-stimulation of nitrogen beyond a pond's assimilatory capacity may be detrimental to the aquatic organisms and needs special attention to keep water quality conducive for fish growth. Seasonal variations of physicochemical parameters of water and sediments directly influence the nitrogen cycle through population dynamics of nitrogen cycle bacteria. Potentials of surplus nitrogen deportation by denitrifiers create opportunities for management of nitrogen cycle in ponds. The magnitude of loss of ammonia is proportional to the abundance of the nitrifiers, which is positively correlated with phytoplankton productivity, thereby enhancing fish growth. Organic fish farming is the best way for enumeration of nitrogen cycle bacteria for sustaining the quality of aquatic ecosystems. The present review focused on nitrogen biogeochemistry of aquaculture ponds with issues on use of nitrifiers as bioaugmentors for management of aquatic resources and use of essential micronutrients to a certain limit to enhance the efficacy of nitrifiers for increasing productivity. Further investigations are required to establish region-specific cost-effective methods to monitor the nitrogen cycle, thereby intensify the abundance of nitrifiers for increasing aquaculture production in a sustainable manner.

**Keywords:** nitrogen cycle bacteria, N-mineralisation, N-transformations, water quality, productivity

## Introduction

Nitrogen cycle of an aquatic ecosystem is the prime nutrient cycle because nitrogen is the primary productivity limiting nutrient in such ecosystems. Nitrogen transformations in sediments include assimilation, mineralisation, nitrification and denitrification (Rysgaard et al., 1993). In the aquatic systems, bacteria play a vital role in the mineralisation of organic nitrogen by decomposition and utilisation of nitrogenous organic matters (Botan et al., 1960). Nitrogen-fixing bacteria take the responsibility of direct conversion of atmospheric N<sub>2</sub> into biomass, whereas other organisms (plants and bacteria) use dissolved inorganic-N (NH<sub>4</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N) or any organic forms of the element (Wallace et al., 1991).

Assimilation refers to the direct use of a substance which is coupled with the process of ammonification, a crucial step in the nitrogen cycle that causes microbial remineralisation of the organic nitrogen. The process of ammonification includes oxidative deamination in which the amine group (NH<sub>2</sub>) is removed from the amino acid of organic matters in the form of ammonia (NH<sub>3</sub>). Ammonia toxicity is dependent on the rate of ionisation according to the pH of water (Hargreaves, 1998) as unionised ammonia is more toxic to aquatic life than ionised one (Fernandes et al., 2010). Ammonia excreted by fish, other aquatic organisms, and also liberated by the decomposition of uneaten fish feed and different organic matters or metabolites, may be transformed into different forms through algal assimilation, ammonification and

nitrification (Shilo and Rimon, 1982; van Rijn et al., 1984; Diab and Shilo, 1986). Thus, the nutrients being liberated in the culture system can be transformed into a plant or other biomass, which in turn can be easily removed and may be a valuable by-product (van Rijn, 1996). Although the unionised form of ammonia is toxic because of its ability to diffuse across the cell membrane and in comparison to ammonia and nitrite, nitrate is relatively non-toxic to fish and other aquatic species (van Rijn, 1996).

The oxidation of ammonia to nitrite is carried out by ammonia-oxidising bacteria (AOB) through the process of nitrification which is further oxidised to nitrate by the help of nitrite-oxidising bacteria (NOB) (Kumari et al., 2011). That is why the abundance of AOB and NOB demands special attention to get rid of surplus ammonia in the aquatic system. While during oxidation of nitrogen, more carbon is fixed by AOB than NOB (Feliatra and Bianchi, 1993). Currently, different nitrifiers are already commercially available as bioaugmentors for wastewater treatment (Krishnani et al., 2009). The *amoA* gene, mostly found in the nitrifiers can be used as an essential tool to isolate different nitrifiers from the aquatic system to use them as bioaugmentors (Krishnani et al., 2009). The present review tries to focus on the use of nitrogen cycle bacteria, especially nitrifiers, with a broader range in aquaculture as well as in wastewater treatment to avoid ammonia toxicity. The study also reveals how organic farming intensifies the natural nitrification process of the ecosystem, thereby enhancing the natural productivity along with providing sustainable development. Different mineral nutrients (boron, copper, zinc, iron, manganese, molybdenum, nickel, cobalt) which influence biological nitrogen fixation, need special attention to improve soil quality (Weisany et al., 2013). Although the use of micronutrients in agricultural fields is common, the current review presents a comprehensive study on the similar use of micronutrients to enhance nitrification in aquatic system.

Phytoplankton, the most important primary producer in the aquatic ecosystem, is interconnected with nitrifying bacteria. Nitrifying bacteria produces nitrate by using ammonia which in turn is used by the phytoplankton (Kumari et al., 2011). The activity of the nitrogen cycle bacteria, in aquaculture ponds are tightly coupled with several environmental factors and management practices. The present review aims to focus on the relationship between nitrogen cycle and pond productivity with reference to the abundance of different types of nitrogen cycle bacteria and the availability of different species of nitrogen. The study also focuses on the use of organic manures which are beneficial over inorganic fertilisers since they maintain the nitrogen and other essential nutrients in proper proportions, thereby enhancing productivity. The role of nitrifiers in different management practices has also been investigated. Further study is needed to ensure an improved version for the usage of nitrifiers

as bioaugmentors.

## Organic Decomposition, Nitrogen Mineralisation and Assimilation in Pond Ecosystem

Nitrogen cycle plays an important role in organic matter recycling which includes nitrogen fixation, ammonification, nitrification and denitrification, carried out by a group of microorganisms (Abraham et al., 2004). Various microbial decomposers are engaged in the transformation of organic matters into the form of nutrients to make them available for uptake by plants and thereby depict recycling of nutrients known as internal cycling. Availability of nitrogen in the soil system is a rate limiting factor of absorption by plants and also has a direct impact on primary productivity. Organic N mineralisation is much more in the sediments of the pond than overlying water (Blackburn et al., 1988). Decomposers may be present as aerobic or anaerobic bacteria responsible for the decomposition of animal matters (Kristensen et al., 1995), whereas fungi are major decomposers of plant materials. In the sediments of the aquatic ecosystem, as oxygen levels are depleted anaerobic bacteria carry out most of the decomposition (Smith and Smith, 2006).

The transformation of organic nitrogen into mobile inorganic forms by the decomposers is known as mineralisation. At the same time, decomposers also require some nitrogen for their growth known as immobilisation. The difference between the rate of mineralisation and immobilisation is known as net mineralisation rate, which determines the net nitrogen content of the ecosystem. The microbial community holds the most crucial role in the transformation of deposited organic matters in the pond sediment, which in turn offers a source of remineralised nutrients vital for plankton growth (Burford and Longmore, 2001).

In aquaculture ecosystem, mostly in still, open water of ponds, particulate organic matter (POM) is constantly mineralised until much of the organic matter settles down as humic compounds at the bottom. In shallow waterbodies, bottom-dwelling detritivores such as crab, snail, and molluscs take much of the responsibilities together with the bacteria that work on the bottom (Covich et al., 1999). A few centimetres below the surface sediment where oxygen supply exhausted, some of the bacteria which are competent to undergo anaerobic respiration take over decomposition at a much slower rate than the bacteria of the aerobic environment in the surface sediments (Smith and Smith, 2006). Significant sources of dissolved organic matter (DOM) are free-floating macro-algae, phytoplankton and zooplankton that eat bacteria and in turn excrete nutrients as exudates and faecal pellets. Quality and the quantity of organic matter as a source of food for the decomposers directly influence the rate of

decomposition as well as mineralisation of nitrogen (Smith and Smith, 2006). Autolysis of the phytoplankton cells or hydrolysis of other particulate organic nitrogen (PON) can produce dissolved organic nitrogen (DON) which is further mineralised by proteolytic or other heterotrophic bacteria (HB) into dissolved inorganic substances.

Nitrogen assimilation by microbial communities may serve as an N reservoir in an ecosystem. Therefore, nitrogen cycle bacteria play an essential role in regulating pond productivity. Microbial biomass nitrogen is the chief component of organic nitrogen in an ecosystem. Lysis of microbial cells causes the subsequent release of nitrogen (depolymerisation and ammonification) which in turn supply bioavailable nitrogen (Isobe and Ohte, 2014). Benthic mineralisation of particulate organic nitrogen also diffuses dissolved inorganic nitrogen to overlying water which fuels primary productivity (Stief, 2013).

## Nitrogen fixation

Nitrogen enters into the aquatic ecosystem via two pathways of which one is atmospheric deposition such as precipitation while the other is via nitrogen fixation, which itself has two ways such as high energy fixation and biological fixation. In tropical fish ponds, during the dry season, nitrogen fixation ranges from 6 to 23 mg N.m<sup>-2</sup> d<sup>-1</sup> while during the rainy season it varies from 21 to 57 mg N.m<sup>-2</sup> d<sup>-1</sup> and inhibition of N<sub>2</sub> fixation is inversely correlated to the concentration of ammonia (Hargreaves, 1998). According to Vass et al. (2017), the magnitude of nitrogen fixation in freshwater fish ponds is around 5–8 kg N<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>. Studies by Hargreaves (1995) elucidate the role of ammonia and species composition of phytoplankton bloom as well as a significant proportion of heterocystic cyanobacteria in addition to nitrogen to aquaculture ponds. Nitrogen fixation in the presence of high energy splits N<sub>2</sub> in two atoms of free N which in turn combines with hydrogen to form ammonia. Plants are capable of using mineral nitrogen, but animals can derive it only from plant materials or acquire after conversion to other nitrogenous compounds.

Nitrogen fixation is carried out by a group of diversified bacterial strains reported by different researchers (Anderson, 1955; Kuznetsov, 1970; Niewolak, 1972; Mulder, 1975; Alexander, 1978; Sprent, 1979; Jones and Simon, 1981). Bacterial nitrogen fixation is usually attributed to the aerobic azotobacters (*Azotobacter agilis*, *Azotobacter chroococcum*), facultatively anaerobic bacilli group (*Bacillus polymyxa* and *Bacillus macerans*), facultatively anaerobic klebsiellas (*Klebsiella pneumoniae*), anaerobic saccharolytic clostridia (*Clostridium butyricum*, *Clostridium butylicum*, *Clostridium pasteurianum*, *Clostridium pectinovorum*), and also some anaerobic sulphate reducing bacteria (*Desulphovibrio* and *Desulphotomaculum*) (Jana and

Roy, 1986). Nitrogen fixation is catalysed by dinitrogenase reductase, which is encoded by *nifH* gene (Krishnani and Kathiravan, 2015). According to Fernandez et al. (2020), microbes carrying *anfH* can also participate in nitrogen fixation and methanogenesis. The key enzymes that catalyse nitrogen fixation are nitrogenases which may be present in three different forms, where molybdenum-iron nitrogenase (*nifH*) is the main nitrogenase gene carried by all nitrogen fixers. Vanadium nitrogenase gene (*vnfH*) and iron-only nitrogenase gene (*anfH*) may also be present as an alternative nitrogen-fixing enzyme (Fernandez et al., 2020). The iron-only nitrogenase, *anfH*, is capable of transforming N<sub>2</sub>, CO<sub>2</sub>, and H<sub>2</sub> into NH<sub>3</sub> and CH<sub>4</sub> in a single enzymatic step (Zheng et al., 2018), directly linking N fixation to CH<sub>4</sub> cycle. Presence of *nifH*, *vnfH*, *anfH* can be used as indicators of nitrogen fixation potential.

Nitrogen-fixing bacteria (NFB), present in water and sediment samples of fish ponds are abundant with maximum growth in summer and decline in winter showing their significant adaptation to elevated temperatures of tropical ponds. The optimum pH and water temperature for the growth of NFB range between 6.0–7.8 and 18–34 °C, respectively (Jana and Roy, 1986). The affluence of nitrogen fixers also has an inverse and direct correlation with the amount of nitrate whereas the ammonia level of bottom water exerts a direct influence on the growth kinetics of NFB occurring in sediments (Jana and Roy, 1986). A positive correlation is present between dissolved organic matter and nitrogen-fixing bacteria (Pandey and Shreelu, 2006). Elevated temperature, low pH range may account for the greater availability of nitrogen fixers during the summer than that of winter, although it is possible only when adequate amount of dissolved oxygen (DO) is present in water.

*Azotobacter* spp. and *Clostridium* spp. are the most popular among all the nitrogen fixers with significant potential to fix nitrogen. Abundant population densities of *Azotobacter* sp. and *Clostridium pasteurianum* have been observed to be about 10<sup>2</sup> cfu. cm<sup>-3</sup> and 10<sup>2</sup> MPN.100 cm<sup>-3</sup> respectively in the river Drwęca (Gołaś et al., 2008) indicating the opulence of those particular bacterial strains. The cell count of nitrogen-fixing bacteria in the sediment of the water-body was reported to be 7390 cell.g<sup>-1</sup> while 4600 cell.mL<sup>-1</sup> was reported from the water column (Jana and Roy, 1986) which indicates the number of nitrogen fixers is much more in the sediments of the pond than that of surface water which was also reported by Pandey and Shreelu (2006). The load of dissolved organic matter is substantially higher in the sediment than that of overlying water which implies the significant positive correlation between the nitrogen-fixing bacteria and dissolved organic matter in the pond sediment. Since nitrogen is one of the prime limiting factors of the aquatic ecosystem after carbon, nitrogen stock in the system has direct control over the population dynamics of the

ecosystem as well as ecological processes. After fixation of sufficient amount of mineral nitrogen in sediment, the rate of biological fixation significantly reduces, a dynamic equilibrium is established.

## Ammonification

Ammonification is the process whereby organic N is mineralised to  $\text{NH}_4^+$ . It is the first step in organic N decomposition and is often referred to as N mineralisation that causes the transformation of nitrogen contained in compounds of living organisms into inorganic ammonia ( $\text{NH}_3$ ) or as ammonium ion ( $\text{NH}_4^+$ ) (Hopkinson and Giblin, 2008). A group of proteolytic bacteria may breakdown organic compounds into simpler proteins are intermediated by a group of ammonifying bacteria to liberate ammonia.

Unionised ammonia ( $\text{NH}_3$ ) is more toxic than ionised ammonia ( $\text{NH}_4^+$ ) because it is able to diffuse across cell membranes (Fernandes et al., 2010). The transition between the two forms is greatly affected by pH. At pH 7.3 about 1 % is unionised; at pH 8.3 about 10 % of ammonia is unionised, and at pH 9.3 about 50 % of ammonia is unionised (Hargreaves 1998). Elevated levels of ammonium ions ( $\text{NH}_4^+$ ) can displace potassium ( $\text{K}^+$ ) and depolarise neurons. This leads to the activation of the NMDA (N-methyl-D-aspartate) type glutamate receptor, which causes an influx of calcium ions ( $\text{Ca}^{2+}$ ) and results in cell death in the central nervous system of vertebrates (Lu et al., 2015).

Ammonia is considered as a preferred form of N substrate for phytoplankton when its concentration is depleted below  $0.03 \text{ mg N.L}^{-1}$ , which causes significant quantities of nitrate assimilation (Syrett, 1981; McCarthy, 1981). Nitrate assimilation, as well as its incorporation, is an energetically less-favourable process for N nutrition of phytoplankton as enzymatic-reduction within the phytoplankton cell takes place before the incorporation of ammonia into cellular amino acid. Both autochthonous and allochthonous types of organic matter, excretion of ammonium and amino acids by zooplankton (Brezonik, 1972), phytoplankton die-offs, excretion by fishes (Guerin-Ancy, 1976; Morii et al., 1978) and direct autolysis after cell death are chief sources of ammonia in fish ponds.

A number of micro-organisms (*Escherichia coli*, *Aerobacter cloacai*, *Pseudomonas* sp., *Proteus vulgaris*, *Bacillus subtilis* and *Flavobacterium* sp.) are responsible for the hydrolysis of proteins into simpler compounds such as peptides, urea and amino acids which in turn are mineralised by ammonifying bacteria (AB) to liberate ammonia or ammonium sulphate (Jana and Roy, 1985b). Regarding general ecological theories, it is tempting to assume that a variety of bacterial strains with small differences between their

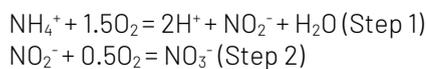
kinetic patterns use various amino acids separately to carry out aerobic mineralisation (Sepers, 1981).

Ammonification potential highly depends on the abundance of ammonifying (AB) and heterotrophic bacteria (HB) which is also a substrate-dependent procedure. Jiao et al. (2009) have investigated the role of nitrogen cycle-related bacteria in the Buji stream in Shenzhen (China), which was reported to be severely polluted with  $\text{NH}_4\text{-N}$ . Furthermore, the number of AB was much higher than AOB and NOB, which indicates that the abundance of ammonifiers (AB) is substrate-dependent. However, research based on the rate of natural ammonification by using peptone as a substrate, found that ammonification varies from  $7.2$  to  $14.6 \text{ mg.L}^{-1}$ , which is relatively higher in winter than in summer (Jana et al., 1997). The abundance of proteolytic bacteria as ammonifiers (AB) is maximum during winter with a decline during summer having a positive correlation with DO (Jana and Roy, 1985b; Jana et al., 1997). The contradictory results reported by the other researchers may be due to different climatic conditions. The number of ammonifying bacteria (AB) in treated sewage was reported to be the lowest in February, and the number gradually increased with increasing temperature and maximum abundance ( $7500 \text{ MPN.mL}^{-1}$ ) was found in November (Wielgosz et al., 2010).

There are significant positive correlations between  $\text{NH}_4\text{-N}$ , DO and AB, and too much acidic or alkaline pH inhibits the growth of AB, but excessive temperature fall also exerts a negative impact on the growth of AB. Zhao et al., (2015) reported a low abundance of AB during the winter months due to temperature fall, and the number increased in spring. Jiao et al., (2009) also found a significant correlation [ $r = 0.9076$  ( $P < 0.01$ )] between the most probable number (MPN) of AB and BOD of water indicating that the ammonifying bacteria plays the most crucial role in nitrogen mineralisation. Furthermore, nitrogen-related bacteria in sediments are 100 times higher than those in overlying water as sediments are rich in organic matters. Sudthikaran et al. (2007) reported the abundance of ammonifiers in upper and lower sediments of intensive shrimp culture pond within a range of  $3.3 \times 10^2 \text{ MPN.g}^{-1}$ –  $6.7 \times 10^5 \text{ MPN.g}^{-1}$  and  $9.2 \times 10^2 \text{ MPN.g}^{-1}$ –  $6.1 \times 10^5 \text{ MPN.g}^{-1}$  dry weight respectively. That corroborates the observation of Jiao et al. (2009) having similar findings of greater abundance of ammonifiers in sediment. It indicates the fact of substrate dependency of ammonifying bacteria. Since higher amounts of substrates are available in the sediments than the overlying water, the bacterial abundance is proportionate to substrate availability.

## Nitrification

Nitrification is two stepped oxidation of ammonium to nitrate (Kim et al., 2005; Abeliovich, 2006). The steps are:



The combined equation is:  $\text{NH}_4^+ + 2\text{O}_2 = \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$  (Stefanakis et al., 2014).

Nitrate typically comprises most of the increased dissolved inorganic nitrogen, and it is the most preferred non-toxic form of nitrogen incorporated in the aquatic ecosystem (Richard and John, 1996). Fish can excrete metabolic nitrogen in the form of urea (Gregory, 1977; Wright and Land, 1998). Urea can be transformed into ammonia by hydrolysis. Ammonia, in turn, is transformed into nitrite and nitrate by the process of nitrification. A nitrate concentration above 10 mg  $\text{NO}_3\text{-N L}^{-1}$  can have an adverse effect on freshwater invertebrates, fishes and amphibians by long-term exposures (Camargo et al., 2005). The main toxic action of nitrate and nitrite is their capacity to bind with haemoglobin, thereby forming methaemoglobin which makes it incapable of carrying oxygen (Hargreaves, 1998). Nitrate is relatively less toxic than ammonia and nitrite because of its low branchial permeability. Nitrite is a potential toxicant of fish which can disrupt a variety of physiological processes such as respiratory, ion regulatory, cardiovascular, endocrine and excretory system in them (Serezli et al. 2016). The extent of damage depends on pH, temperature, cations, anions, oxygen concentration, exposure time and fish size (Kroupova et al. 2005). Freshwater fish and crustaceans are hyperosmotic and as a result, requires active uptake of ions from their surrounding environment to compensate ion loss (Kroupova et al. 2005). Whenever nitrite is present in the environment, a part of  $\text{Cl}^-$  uptake is shifted to  $\text{NO}_2^-$  uptake due to high branchial permeability of nitrite. Nitrites can accumulate in liver and retina, and long-term exposure (24-48 h) to nitrite causes rise in extracellular  $\text{K}^+$  resulting in loss of  $\text{K}^+$  from RBC and skeletal muscles (Kroupova et al., 2005). Further, this leads to the depolarisation of excitable tissues resulting in severe problems like heart failure and nerve malfunction.

Nitrification is a two-step microbiological process guided by two groups of nitrifiers. In the first step, ammonia is oxidised to nitrite by ammonia-oxidising bacteria (AOB) (Fig. 1) which is subsequently oxidised to nitrate (Fig. 1) by nitrite-oxidising bacteria (NOB) (Kim et al., 2005; Abeliovich, 2006; Kumari et al., 2011; Daims et al., 2016). Research on Buji stream in Shenzhen (China) pointed towards the fact that very low proportion of nitrifiers (AOB and NOB) is the major cause of nitrogen toxicity (Jiao et al., 2009). Enrichment of the nitrifiers enhances the rate of nitrification, which is a vital step of the nitrogen cycle to balance the nitrogen budget of the particular ecosystem.

Nitrifying bacteria capable of oxidising ammonia-N to nitrite-N in the first step is largely confined to the genus *Nitrosomonas*, *Nitrosococcus*, *Nitrospira* and

*Nitrosolobus* and nitrite to nitrate in the next step is confined to genus *Nitrobacter* (Jana and Roy, 1985a). Santos et al. (2019) validate the same and have mentioned about *Nitrosovibrio* as AOB and *Nitrococcus* and *Nitrospina* as NOB. The presence of some chemoautotrophic ammonia oxidisers such as *Nitrosomonas europaea* as well as nitrite oxidisers such as *Nitrobacter winogradskyi* in large numbers can cause autotrophic nitrification in inland waters (Jana and Roy, 1985a). *Nitrosomonas europaea* is considered as the best autotrophic ammonia oxidiser by Wrage et al., 2001. Autotrophic AOB are Gram-negative and are traditionally placed within the *Nitrobacteriaceae* (Prosser, 2005) which are aerobes and many of them are obligate autotrophs (Wrage et al., 2001). *Nitrosococcus oceanus* has been placed in  $\gamma$ -proteobacteria and *Nitrospira*, *Nitrosococcus mobilis*, *Nitrosomonas europaea*, *Nitrosomonas communis*, *Nitrosomonas marina*, *Nitrosomonas cryotolerans*, *Nitrosomonas oligotropha* and *Nitrosomonas* sp. have been placed in  $\beta$ -proteobacteria. While in case of NOB most of the species of *Nitrobacter* are placed in  $\alpha$ -proteobacteria (Prosser, 2005). Recently two phototrophic NOB strains were isolated from sewage, namely *Rhodospseudomonas* sp. and *Thiocapsa* sp. which are capable of using  $\text{HS}^-$ ,  $\text{H}_2$ ,  $\text{Fe}^{2+}$  and many other organic compounds as reductants (Isobe and Ohte, 2014).

In autotrophic ammonia oxidisers, two enzymes play the pivotal role for energy conservation during the oxidation process; namely ammonia monooxygenase (AMO) which catalyses the oxygenation of ammonia to hydroxylamine and hydroxylamine oxidoreductase (HAO) which catalyses the oxidation of hydroxylamine to nitrite (Bothe et al., 2000). The AOB, *Nitrosomonas marina* is an obligatory, aerobic, Gram-negative halophile proteobacterium (Burrell et al., 2001) whereas *Nitrobacter* is facultative chemoautotrophic and mainly found in the terrestrial and aquatic environment. *Nitrococcus mobilis* is an obligate halophile which oxidises nitrite to nitrate and is found in lower numbers than other nitrifying bacteria (Santos et al., 2019). *Nitrospira moscoviensis* and *Nitrospira marina* are reported to be obligated chemoautotrophic (Santos et al., 2019). Some species of *Pseudomonas* are heterotrophic bacteria which are responsible for the reduction of nitrate and phosphate. *Nitrospira* spp. and *Nitrosomonas oligotropha* are the dominant ammonia-oxidisers in environments having low  $\text{N-NH}_4^+$  concentrations, but *Nitrosomonas europaea* and *Nitrosococcus mobilis* are mostly predominate in  $\text{N-NH}_4^+$  rich environment (Limpiyakorn et al., 2005). Nitrification is also a pH dependent procedure which varies from species to species. *Nitrosomonas europaea* is capable of hydrolysing urea at a pH value of 8.0 whereas *Nitrospira* can utilise urea as sole nitrogen source at pH 5.5 (Allison and Prosser, 1991). Most of the AOB belongs to  $\beta$  subdivision of class *proteobacteria* except *Nitrosococcus*, which belongs to  $\gamma$  subdivision (Burrell et al., 2001). *Nitrobacter winogradsky* is

autotrophic nitrite-oxidising bacteria which belong to  $\alpha$  subdivision of the *Proteobacteria* and many other chemolithoautotrophic nitrite-oxidising *Proteobacteria* may belong to  $\alpha$ ,  $\delta$  and  $\gamma$  subdivisions (Hovanec and DeLong, 1996). The *amoA* gene codes for ammonia monooxygenase that catalyses the oxidation of ammonia into hydroxylamine (Krishnani et al., 2009). It is very specific for nitrifying bacteria, thus creating interest to study genetic differences among different species of AOB. PCR amplification of this *amoA* is now being used as an excellent tool to analyse different ammonia-oxidisers for efficient ammonia removal from different ammonia-polluted aquaculture systems. On the other hand, *nxrA* and *nxB* are reported as a useful phylogenetic marker to identify uncultured NOB (Daims et al., 2016).

Nitrification is a complex process affected by a variety of interrelated factors such as DO concentration, temperature, substrate concentration, pH, nutrients availability, number of nitrifying bacteria and their availability of surfaces (Kumari et al., 2011). In comparison to NOB, AOB grows extremely slowly ( $\mu_{\max} = 0.5 \text{ d}^{-1}$ ) as its only source of energy is ammonia oxidation and source of carbon is  $\text{CO}_2$  fixation (Kim et al., 2005). Ammonia monooxygenase (AMO) is a membrane-integrated enzyme of AOB which catalyses the oxidation of ammonia to hydroxylamine known to be sensitive to environmental factors (Kim et al., 2005). Thus, the activity of the ammonia-oxidising bacteria shows seasonal variation.

Oxygen penetration is the key regulating factor for both the ammonia and nitrite oxidation process as nitrification takes place above the DO concentration of  $2 \text{ mg.L}^{-1}$  (Rysgaard et al., 1994). As a result, during the summer, nitrification in the sediments of aquaculture pond is most likely to be limited by the depth of oxygen penetration which is typically 1 to 5 mm (Hargreaves, 1998). Dissolved oxygen acts as a controlling factor in nitrification, introduces a Michaelis-Menten function (with half-saturation constant  $k_{O_2}$ ) where the decreasing rate of ammonium oxidation occurs with declining oxygen concentrations (Yool, 2011). During summer, the rate of nitrification decreases along with decreased oxygen penetration which indicates a positive correlation of DO with the abundance of AOB and NOB. Ammonia oxidation is inhibited by light, and the competition of AOB with phytoplankton and heterotrophic bacteria for using ammonium also exerts a negative impact on ammonia oxidation, both of which are minimised during winter. Therefore the abundance of ammonia-oxidiser is highest during winter. But during summer, higher abundance of AOB and NOB (Christman et al., 2011) may be due to temperature extremities in the polar regions.

Although nitrite-oxidisers are considered to be obligate aerobes, many can utilise nitrate as an electron acceptor in the absence of oxygen. Nitrite-oxidisers can grow in totally anaerobic environments

such as at the bottom of deep anaerobic wastewater reservoirs, deep in the mud, even in the absence of both nitrate or oxygen where significant concentrations of sulphides are present (Abeliovich, 2006). Nitrifying bacteria can stay alive for several months in the anaerobic sediments of aquatic systems and can resume their activity rapidly once oxygen is supplied (Cavari, 1977). Investigation on the abundance of AOB in aquaculture ponds by quantitative PCR of the *amoA* gene (encoding alpha subunit of AMO) showed maximum abundance of AOB during winter and autumn, and the number decreases during summer (Lu et al., 2015). The abundance of the nitrifiers (*viz.* AOB, NOB) has a positive correlation with DO and different species of nitrogen such as ammonium-N, nitrite-N, nitrate-N and inversely correlated with temperature. The abundance of nitrifiers are within the range of  $720\text{--}1848 \text{ cells.mL}^{-1}$  of AOB in water and it lies within the range of  $780\text{--}2325 \text{ cells.g}^{-1}$  in sediments with seasonal peaks during winter in both water and sediments (Jana and Roy, 1985a). Remarkable temporal variation of abundance of ammonia-oxidisers in different ponds of Lucknow (India), in surface and bottom water, showed minimum values in summer and maximum in the rainy season (Kumari et al., 2011). Christman et al. (2011) mentioned the occurrence of peak nitrifiers in summer.

Climatic differences among different places are the probable reasons for contradiction because in the cooler region, the winter temperature may fall beyond the optimum temperature range required for the growth of nitrifiers and nutrient limitation is also a major factor for the decrease of the abundance of nitrifiers. However, in the temperate region, winter temperature offers suitable conditions for the growth of nitrifiers which may be the probable cause of winter peak. The spatial variation of the count of ammonia-oxidisers is substantial with higher values in bottom water ( $1297 \pm 3.6$  to  $1673.23 \pm 0.36 \text{ mL}^{-1}$ ,  $1074 \pm 1.07$  to  $1372.17 \pm 4.6 \text{ mL}^{-1}$  and  $435.05 \pm 15.7$  to  $547.54 \pm 2.12 \text{ mL}^{-1}$ ) compared to surface water ( $720.5 \pm 8.1$  to  $955.3 \pm 10.8 \text{ mL}^{-1}$ ,  $515 \pm 10.1$  to  $678 \pm 11.8 \text{ mL}^{-1}$  and  $218.7 \pm 7.3$  to  $368.4 \pm 9.32 \text{ mL}^{-1}$ ) during rainy, winter and summer seasons respectively and similar distribution pattern is also followed by nitrite-oxidising bacteria (Kumari et al., 2011). The reason may be a higher nutrient load in the bottom water than that of the surface water. Among the nitrifiers, ammonia-oxidisers occupy a greater proportion than that of nitrite-oxidisers. Ammonia-oxidisers (AOB) are within a range of  $0\text{--}240 \text{ MPN.g}^{-1}$  dry weight in the upper and lower layers of sediments, whereas, nitrite-oxidisers (NOB) are within a range of  $0\text{--}2.9 \text{ MPN.g}^{-1}$  dry weight, detected only in the upper sediment layer of intensive shrimp culture pond (Sudthikaran et al., 2007). Nitrite-oxidisers are more sensitive to environmental factors than that of ammonia-oxidisers (Dvir et al., 1999). It implies the greater abundance of the ammonia-oxidisers than nitrite-oxidisers.

The optimum ranges of temperature, pH and DO for nitrification are 20–30 °C, 7–8 and 5–10 mg.L<sup>-1</sup>, respectively (Diab et al., 1993). Nitrification tends to be inhibited below 5 °C with a lower pH, and complete inhibition takes place at pH 5.5 (Eddy and Williams, 1987) while the activity of *Nitrosomonas* decreases with increasing pH above the optimum range (Feliatra, 2001). Rate of ammonia oxidation drops during the night due to decrease in pH values and temperature, and as a result, in the early morning, the dominant product of nitrification is nitrate (Dvir et al., 1999). Nitrification potential (NP) is directly proportional to the number of nitrifying bacteria. Once ammonia concentration is depleted, it indicates a subsequent increase in the level of nitrite and thereby an increase in nitrate concentration (Cavari, 1977). The concentration of ammonia is a factor which influences the activity of nitrifiers (*viz.* AOB, NOB) (Jana et al., 1997; Stefanakis et al., 2014) and ammonia concentration below 5 µg atom.L<sup>-1</sup> ceases nitrification in the ocean (Wada and Hattori, 1971).

At the surface of the water, the nitrification activity is minimal because of the competition between ammonia-oxidisers and plankton to consume ammonia. While researchers using allylthiourea as AOB inhibitor and chlorate as NOB inhibitor have found that in the absence of nitrifiers (*viz.* AOB, NOB) the ammonia concentration becomes so toxic that it cannot support shrimp survival (Feliatra, 2001). Nitrification benefits on production system are observed when systems with nitrifiers are compared with systems without nitrifiers (Mahanta et al., 2014). When using nitrifying bacterial inoculant in bioremediation, the concentration of ammonia and nitrite are under control with aeration while the rate of nitrification is slower without aeration (Barik et al., 2018). This indicates the beneficial role of the nitrifiers in maintaining water quality of ponds.

## De-nitrification

Denitrification plays a significant role in lowering total nitrogen concentration or continuous emission of surplus nitrogen from aquatic systems which are necessary to maintain the conducive water quality of the systems. Denitrification is the next step in the nitrogen cycle chain, where ammonium-N includes the reduction of the transformed nitrite and nitrate to nitric oxide, nitrous oxide, and N<sub>2</sub> gas by denitrifying bacteria (DNB) (Wrage et al., 2001). Denitrification causes the transformation of oxidised inorganic nitrogenous compound to elemental nitrogen (van Rijn. et al., 2006). Ammonification and nitrification contribute to changes in the forms of nitrogenous compounds, while denitrification offers the elimination of NO<sub>3</sub>-N through their reduction to gaseous nitrogen (Fig. 1) and permits a decrease in the content of total nitrogen (Wielgosz et al., 2010).

Denitrification is higher in a nutrient rich system where denitrifiers (DNB) play a crucial role in

maintaining the cycling of nitrogen, thereby maintaining the integrity of the ecosystem by preventing eutrophication (Sousa et al., 2013). Denitrification is a reductive process to get rid of excess nitrate and nitrite which can be produced as a by-product of excess feed and different types of decayed organic wastes from animals in aquaculture ponds and thereby produce toxic effects to animals at higher concentrations. So denitrification is the conversion of nitrate (NO<sub>3</sub>) into atmospheric nitrogen (N<sub>2</sub>) through nitrite (NO<sub>2</sub>), nitric oxide (NO), and nitrous oxide (N<sub>2</sub>O) (Kathiravan and Krishnani, 2014) using the intermediate products as alternative e<sup>-</sup> acceptors for energy production in anaerobic condition (Vass et al., 2017).

Bacteria such as *Pseudomonas*, *Micrococcus*, *Bacillus*, *Thiobacillus*, *Propionibacterium* and many others are able to convert nitrate (Wrage et al., 2001). Facultative heterotrophic bacteria such as *Achromobacter*, *Aerobacter* and *Bacillus* also take part in denitrification (Wielgosz et al., 2010). The entire denitrification process requires the involvement of four enzymes such as nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (Chen et al., 2011). Most of the denitrifying bacteria possess either *nirS* gene that encodes cytochrome cd1 (cd1 NIR) or *nirK* gene that encodes Cu-containing enzyme for nitrite reduction (Cu NIR) (Bothe et al., 2000). The genes *narG* and *napA* code for nitrate reductase, *nirK*/*nirS* codes for nitrite reductase, *nor* codes for nitric oxide reductase and *nosZ* codes for nitrous oxide reductase (Krishnani and Kathiravan, 2015). The PCR analysis of these genes can be used for isolating DNB from the environment.

Most of the denitrifying bacteria are facultative anaerobes and can use nitrate as e<sup>-</sup> acceptor in place of oxygen to cope up with low O<sub>2</sub> or anaerobic conditions (Wrage et al., 2001). The N<sub>2</sub>O, a regular intermediate of denitrification is released at maximum level when pH is low because N<sub>2</sub>O reductase is inhibited at this low pH although NO<sub>3</sub><sup>-</sup> is preferred over N<sub>2</sub>O as e<sup>-</sup> acceptor (Wrage et al., 2001). At high O<sub>2</sub> concentration, aerobic respiration of the DNB inhibit NO<sub>3</sub><sup>-</sup> reduction (Wrage et al., 2001). Apart from that C<sub>2</sub>H<sub>2</sub> may be considered as a specific inhibitor of denitrification (Yoshinari et al., 1977).

Factors which play a vital role in controlling the rate of denitrification mainly include nitrate concentration, availability of organic carbon, DO, temperature and pH (Rathsack et al., 2014). Denitrification has three limiting factors, such as nitrate concentration, organic carbon and anoxic conditions (Stefanakis et al., 2014). Sediments are oxygen deficient and rich in organic matter which makes it preferable for denitrification than that of the overlying water. Researchers detected DNB within a range of 0–670 MPN.g<sup>-1</sup> dry weight in the upper layer, and 0–1271 MPN.g<sup>-1</sup> dry weight in the lower layer in intensive shrimp culture pond (Sudthikaran et al., 2007). The probable

cause of this result may be related to the decreasing concentration of DO from the upper layer of water to the lower layer. Denitrification decreases with an increase in DO concentration in water from 0-2 mg.L<sup>-1</sup>

while a further increase of DO does not exert any decreasing effect (van Kessel, 1977). Wielgosz et al. (2010) mentioned that the process of denitrification increases with sufficient supply of NO<sub>3</sub>-N and readily

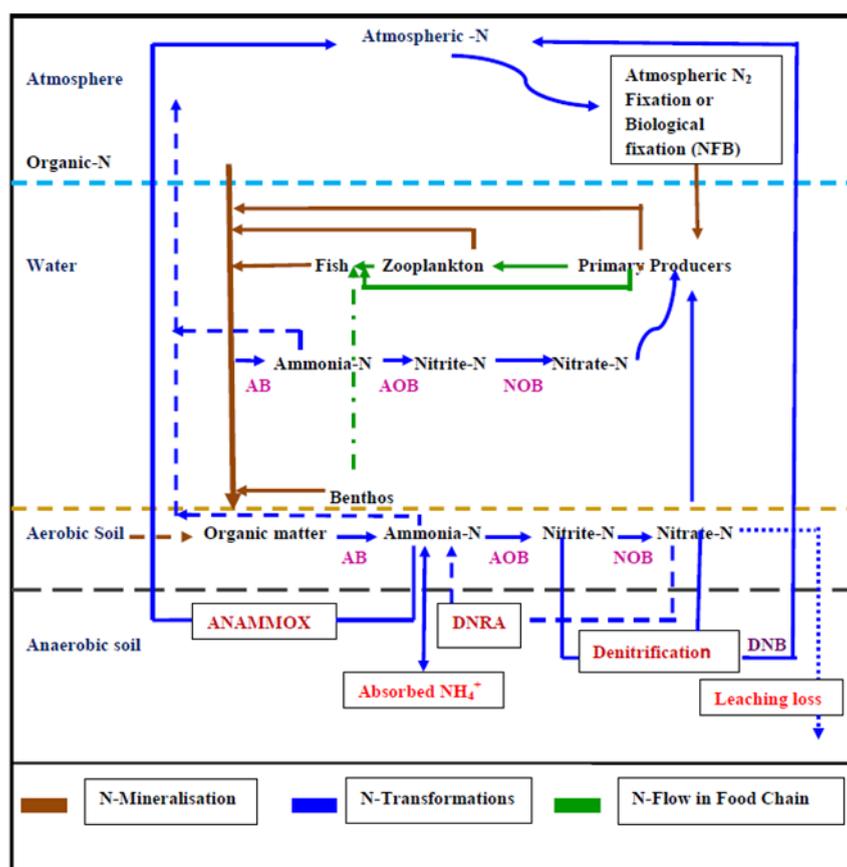


Fig. 1. Schematic representation of nitrogen transformations in aquatic system (Adapted from: Herbert, 1999; Wongkiew et al., 2017).

available carbon substrates if oxygen concentration does not exceed 0.5 mg O<sub>2</sub> dm<sup>-3</sup>.

Further increase in oxygen concentration slows down denitrification since the bacteria start to utilise DO as a source of oxygen and not the oxygen contained in NO<sub>3</sub>-N. Maximum abundance of DNB during spring and lowest number during winter (Zhao et al., 2015) emphasises the fact that DNB may have a negative correlation with DO. Researchers observed the highest number of DNB during November in treated sewage (Wielgosz et al., 2010). Contradictory results have also been reported, for example, in fish farming ponds, the maximum abundance of DNB was observed during summer (10<sup>4</sup> cells.g<sup>-1</sup>) and minimum during winter (10<sup>3</sup> cells.g<sup>-1</sup>) (Jana and Patel, 1985). Thus, the seasonal peak of the DNB changes according to the climate.

Denitrification is also temperature-dependent and shows a positive correlation with temperature. Below 5 °C the entire procedure of nitrification ceases due to inhibition of bacterial growth, and low temperatures also have a negative impact on denitrification (Wielgosz et al., 2010). Rate of

denitrification highly depends on the rate of transport of nitrate from overlying water to sediment and also on the nitrate concentration of the overlying water. At lower nitrate concentration, it follows first-order kinetics but gradually as the nitrate concentration increases, it becomes independent of the nitrate concentration and follows zero-order kinetics (van Kessel, 1977). Low DO and organic compounds are beneficial for the process of denitrification and the entire procedure is largely restricted by the low concentration of nitrite and nitrate (Jiao et al., 2009). Most probable number of the denitrifying bacteria has a positive correlation with BOD and the concentration of nitrogenous compound with correlation coefficients of 0.6642 and 0.6636 ( $P < 0.05$ ) respectively (Jiao et al., 2009).

Denitrification causes continuous emission of valuable nitrogen from the system resulting in nutrient loss. Procyanidins from *Fallopia* spp. have the ability to inhibit bacterial denitrification termed as biological denitrification inhibition (BDI). *Pseudomonas brassicacearum* responds by expressing membrane bound NO<sub>3</sub>-reductase and procyanidins inhibit membrane-bound NO<sub>3</sub>-reductase

encoded by *narG* gene induce conformational changes of the enzyme through membrane disturbance (Bardon et al., 2016). Further study should be carried on for the inhibition of  $\text{NO}_2^-$ -reductase encoded by the *nirS* gene to prevent nitrogen loss through denitrification, although denitrification is an important nitrogen budget limiting process and is beneficial up to a certain level.

### ***N-loss by anammox and ammonia volatilisation***

Supply of available substrates may regulate nitrogen loss in the ecosystem. In the sub-oxic zone and oxygen-minimum zones, N-loss mainly occurs through anammox (anaerobic ammonia oxidation/ammonia-dependent denitrification) reaction and this anammox (Fig. 1) may also regulate ammonia production known as dissimilatory nitrate reduction to ammonium (DNRA) (Isobe and Ohte, 2014). Anammox is carried out by bacteria belonging to the phylum Planctomycetes which oxidise ammonia to produce gaseous nitrogen by using nitrite as the electron acceptor (Yadav, 2014). Anammox was first discovered in wastewater treatment plant although it is considered as a major factor for most of the N-loss in ocean whereas in other systems denitrification is more responsible for N-loss (Fig. 1). Denitrification is related to nitrate ammonification and may be related to the anaerobic ammonia oxidation termed as anammox reaction. Nitrate ammonification is typical for the *Enterobacteriaceae*, such as *Wolinella succinogenes* and *Sulfurospirillum deleyianum* (Bothe et al., 2000). The first step causes the reduction of nitrate to nitrite which is catalysed by a nitrate reductase which has similarities with the enzyme in denitrifying bacteria (Bothe et al., 2000). In the second step, a cytochrome c-dependent nitrite reductase catalyses the reduction of nitrite to ammonia. *Escherichia coli* contains at least two different membrane-bound nitrate reductases which are periplasmic nitrate reductases (Bothe et al., 2000). The genes, *narG* and *napA* encoding two distinct nitrate reductases (NAR and NAP), mediate the reduction of nitrate to nitrite in both denitrifying bacteria and DNRA. The subsequent reactions are carried out by *nirS* and *nirK* gene in denitrifying bacteria but in case of DNRA, nitrite is reduced to ammonium by enzymes encoded by *nrfA* gene (Dong et al., 2009).

Ammonia in aquatic ecosystems is available in ionised ( $\text{NH}_4^+$ ) or in unionised gaseous ( $\text{NH}_3$ ) form depending on the pH and temperature of the water (Zhou and Boyd, 2015). Elevated pH and high temperature generally favours the unionised gaseous form of ammonia (Camargo Valero and Mara, 2010). Depending on the pH, temperature and partial pressure of ammonia in atmosphere above the water surface, volatilisation of ammonia takes place when the equilibrium between ammonia ( $\text{NH}_3$ ) and ammonium

( $\text{NH}_4^+$ ) is shifted towards the free form (Camargo Valero and Mara, 2010).

## **Different Forms of Nitrogen and Pond Productivity**

Nitrifying bacteria have a unique correlation with phytoplankton with respect to their usage of ammonia and nitrite. Nitrate produced by nitrifiers (AOB and NOB) is used by phytoplankton, considered as important primary producers in the aquatic ecosystem (Kumari et al., 2011). Nitrate uptake by phytoplankton may enhance the productivity of zooplankton and the productivity of detritus (Yool, 2011), thereby offering more food for the fishes. Nitrogen can exist in a variety of dissolved and particulate forms in the aquatic system that can be used for phytoplankton production directly or indirectly and incorporation of nitrate may enhance the carbon uptake by phytoplankton depending upon the salinity of water (Montgomery et al., 1991). Nitrogen uptake by phytoplankton is an influential mechanism for the conversion of toxic inorganic N to relatively non-toxic organic N. Nitrogen demand of the phytoplankton depends on the rate of supply of inorganic nitrogen to the euphotic zone. The principal sources of nitrogen in the aquatic system which promote phytoplankton growth are molecular nitrogen by nitrogen fixation, surface runoff of ammonium and nitrate, turbulent diffusion of ammonium and nitrate from aphotic bottom water and sediments, and internally regenerated ammonia or urea discharged through excretion and mineralisation processes (Axler et al., 1981). The preferred N substrate for phytoplankton is ammonia because nitrate incorporation and assimilation is an energetically less-favourable pathway as enzymatic-reduction of ammonium is necessary within the phytoplankton cell for inclusion into cellular amino acids (Hargreaves, 1998). Measurement of nitrate uptake with radiotracer  $^{13}\text{N}$  and ammonia uptake with stable isotope  $^{15}\text{N}$  analysed that ammonia assimilation may have a positive relation with plankton productivity (Axler et al., 1981). Ammonia excreted by zooplankton can successfully be used by phytoplankton, thereby increasing their productivity. Nitrogen uptake by phytoplankton ranges from 151 to 453  $\text{mg N.m}^{-2} \text{d}^{-1}$  in temperate ponds, and from 750 to 1,500  $\text{mg N.m}^{-2} \text{d}^{-1}$  in tropical ponds and a net fish yield of 30–40  $\text{kg.ha}^{-1} \text{d}^{-1}$  (3–4  $\text{g.m}^{-2} \text{d}^{-1}$ ) is possible at a loading of 700–800  $\text{mg N.m}^{-2} \text{d}^{-1}$  to the aquaculture ponds in the form of organic or inorganic fertiliser (Hargreaves, 1995). Nitrogen budget of the aquaculture pond and the subsequent transformation of the nitrogenous compound to more favourable species of nitrogen is the prime regulator of pond productivity as well as fish growth. Nitrogen assimilation in the aquaculture ponds changes according to climatic differences among places.

## Nitrogen Mineralisation and Organic Fish Farming

Every ecosystem presents special characteristics regarding nitrogen compounds. Fish farming ponds which are fertilised by organic manures are one of such ecosystems whose eutrophy is under control, to increase fish productivity (Montuelle et al., 1992). The biological productivity of an aquatic ecosystem is generally judged through the qualitative and quantitative analysis of plankton, which serves as the natural food of fish. The input of animal wastes in the aquaculture ponds increase biological productivity of ponds which in turn increase fish production (Oribhabor and Ansa, 2006). Application of inorganic fertilisers in aquaculture ponds results in deficiency of quality and quantity of essential nutrients in the form of carbon, nitrogen and phosphorous (Jana et al., 2001a). While chemical fertilisers are often used for rapid solubilisation of nutrients though they are expensive and may cause some unfavourable effects on soil structure, composition, micro-flora, macro-flora and many other characteristics of the pond ecosystems (Jana et al., 2001a). However, organic manures contain almost all the essential nutrients that enrich the organic matter content of pond soil and the input of organic manures stimulate the degradation rate of sedimented particulate organic matter (Jana et al., 2001b).

Livestock farming, together with aquaculture provides a new dimension of sustainable development with a satisfactory amount of annual fish yield. With the application of chicken manure, 70 % of input N accumulates in the tropical fish pond (Hargreaves, 1998). Researchers detected the effect of different doses of mixed fertiliser composed of poultry droppings, cattle manure, single super phosphate and urea on the response of some biogeochemical cycling bacteria and observed that overall counts of HB ( $15-270 \times 10^3 \text{ mL}^{-1}$ ), cellulose decomposing bacteria (CDB) ( $15-237 \times 10^2 \text{ mL}^{-1}$ ), DNB ( $5-221 \times 10^2 \text{ mL}^{-1}$ ) and phosphate solubilising bacteria (PSB) ( $3-159 \times 10 \text{ mL}^{-1}$ ) in water and HB ( $32-249 \times 10^4 \text{ mL}^{-1}$ ), CDB ( $31-236 \times 10^3 \text{ mL}^{-1}$ ), DNB ( $26-186 \times 10^4 \text{ mL}^{-1}$ ) and PSB ( $13-162 \times 10^2 \text{ mL}^{-1}$ ) in sediment increased with increasing dose of the mixed fertilisers (Bhakta et al., 2006). Maximum bacterial growth is observed at a fertiliser dose of  $48000 \text{ kg} \cdot \text{ha}^{-1} \text{ yr}^{-1}$ , while further increase slows down bacterial activity (Bhakta et al., 2006).

Increase in the abundance of biogeochemical cycling bacteria causes a parallel increase in the productivity of plankton which is positively correlated with fish growth and survival. Manure application incorporates the essential nutrients in the system and also triggers the natural primary productivity, but nutrient overloading deteriorates water quality which may lead to eutrophication. That is why manure application up to a specific dose is supportive to fish growth and over stimulation shows an inhibitory effect on productivity. In a similar type of research with poultry

droppings, cattle manure, single super phosphate and urea, the maximum amount of nitrogen (ammonia, nitrite, nitrate) is found in the fish pond treated with poultry droppings followed by a combined fertiliser of poultry droppings and cow dung which conclude the same (Jana et al., 2001b). Organic manuring offers fish farming production intensification by balancing the proportion of carbon to other important nutrient parameters such as nitrogen and phosphorus (Mlejnkova and Sovova, 2012). Addition of organic carbon sources to the aquaculture ponds stimulates the growth of heterotrophic bacteria and induces nitrogen consumption to provide microbial protein to the aquaculture ecosystem. This results in the accumulation of different microorganisms, protozoa and algae, which generate microbial protein as feed (Qin et al., 2016). Therefore, in case of organic farming, input of the nutrients in the aquaculture pond is high that will lead to a subsequent increase in the microbial population which in turn will increase the productivity of phytoplankton, zooplankton and various other fish feed in the aquatic ecosystem.

Previous reports indicate that fish growth increases with the increase in fertiliser doses, having a positive correlation with bacterial loading. However, after a certain dose though the bacterial load increases, it does not influence the primary productivity and fish growth. So, from the above observation, it can be concluded that even in the case of organic farming application of the fertiliser should be dose-specific; otherwise, it can cause environmental pollution. Banerjee (1967) has studied soil and water properties of some freshwater fish ponds of India and has suggested that soluble inorganic N below  $0.1 \text{ mg} \cdot \text{L}^{-1}$  can deduct productivity of fish ponds while values above  $0.2 \text{ mg} \cdot \text{L}^{-1}$  provide favourable conditions. Application of nitrogen in the form of fertiliser should not exceed pond assimilatory capacity; otherwise, it may have an adverse effect on water quality by the accumulation of toxic nitrogenous compounds. Researchers have also stated that production of the fish farming ponds can be limited by a surplus of nitrogen and high stocking density (Jana and Roy, 1985a). Elevated concentration of ammonia in pond water causes gill damage, reduction in oxygen-carrying capacity of haemoglobin, lack or depletion of adenosine triphosphate (ATP) in the brain of fish (Zhou and Boyd, 2015) and in addition to that contributes to the eutrophication of water bodies.

Addition of acid to lower pH, application of an algacide to lessen phytoplankton photosynthesis and to reduce pH, or exchanging of water in ponds to flush out ammonia can be used as emergency percussions where the TAN concentration is too high (Zhou and Boyd, 2015). The concentration of ammonia-N above  $1.5 \text{ mg N} \cdot \text{L}^{-1}$  is toxic to commercially cultured fish and in case of stagnant water pond total ammonium nitrogen (TAN) accumulates due to insufficient nitrification (Crab et al., 2007). To get rid of those problems associated

with ammonia toxicity periodic aeration of the pond sediment by drainage, use of nitrifying filters in aquaculture applications and addition of carbohydrates to the pond to enhance bacterial growth and nitrogen uptake are beneficial for flushing out excess ammonia. Less than 10 % of ammonia is in toxic form when pH is less than 8, and that is the reason for adding acid to the ammonia polluted water. In addition, increase water exchange, a source of organic carbon, acid application, ion exchange materials and bacterial amendments can be used to dilute ammonia concentration and to maintain the pH (Hargreaves and Tucker, 2004). Carbon and nitrogen ratio in the system also plays a vital role in the conversion of ammonium (Crab et al., 2007). So, the addition of a source of organic carbon will reduce ammonia concentration. Addition of phosphorus increases algal growth, thereby causing an increase in ammonia uptake by algae (Hargreaves and Tucker, 2004). Nitrifiers take a vital role for immobilisation of ammonia strongly influencing nitrogen retention in the system. Various nitrifiers (AOB and NOB) are capable of forming biofilms for the treatment of ammonia polluted water (Hoang et al., 2016). Biofloc technology which is based on promoting aerobic microorganism growth in culture containers that use different molecules to grow and multiply can be used to recycle nitrogen thereby maintaining the water quality (Jiménez-Ojeda et al., 2018). Probiotics, which are biofriendly microorganisms are used in a sustainable form of aquaculture (Hasan et al., 2012). In addition, natural ion exchanger such as zeolite can be used for removing ammonia from water in aquaculture system.

## Usage of Nitrifying Bacteria in Aquaculture and its Future Prospects

Polluted water gives rise to different fish diseases and to overcome the problem, antibiotics are used indiscriminately. Antimicrobial use in aquaculture may develop resistance gene in bacteria which may be disseminated through horizontal gene transfer (Fečkaninová et al., 2017) to other animals and fish leading to serious health hazards to human consumers. Conversely,  $\text{NO}_2$  volatilisation may also present a serious environmental issue. Different oxides of nitrogen are dominant greenhouse gases and also cause photochemical smog. Nitrous oxide ( $\text{N}_2\text{O}$ ) is an important greenhouse gas (GHG) which has 300 times higher global warming potential than that of carbon dioxide ( $\text{CO}_2$ ) (Ahn et al., 2010). Subtropical coastal aquaculture ponds are critically responsible for  $\text{N}_2\text{O}$  emission causing an adverse effect on global warming. Currently,  $\text{N}_2\text{O}$  discharge from the aquaculture ponds is a serious problem to the environment and may have an adverse effect in the near future.

At low oxygen concentration and high ammonia concentration, nitrification may also enhance  $\text{N}_2\text{O}$

production (Bianchi et al., 1994). Disruptions of the nitrogen biogeochemical cycle may also cause acidification of aquatic system and may lead to biodiversity loss. Researchers should focus on devising satisfactory methods to minimise  $\text{N}_2\text{O}$  emission. Aquaponic and biofloc technology may be considered as useful branches of biotechnology to manage these problems. Different nitrifying bacteria are grabbing special attention because of their use in aquaculture to maintain the nitrogen budget of the aquatic ecosystem and also for sludge and wastewater treatment. Nitrifying bacteria can be used for purification of brackish water ponds (Feliatra, 2003). *Nitrosomonas nitrosa* and some other species of *Nitrosomonas* are already available in the GenBank to be used for ammonia removal from brackish water aquaculture systems (Krishnani et al., 2009). Polymerase chain reaction can be used to amplify the 16S rRNA genes of specific nitrifier groups to improve the sensitivity (Siripong et al., 2006). Water quality may deteriorate rapidly due to total ammonium nitrogen accumulation which may lead to decreased productivity.

The airlift reactor consisting of immobilised culture of nitrifying bacteria on polyvinyl alcohol beads is efficient for ammonia removal in both freshwater and saline water (Shan and Obbard, 2003). Nitrification activities of ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) cause nutrient removal in wastewater treatment plant. NOB and AOB at a ratio of 1.72–5.87 may cause ammonia uptake of  $3.25 \pm 0.52 \text{ mg NH}_4^+\text{-N (g VSS)}^{-1}\cdot\text{h}^{-1}$  and nitrite uptake of  $4.49 \pm 0.49 \text{ mg NO}_2^-\text{-N (g VSS)}^{-1}\cdot\text{h}^{-1}$  and are efficient for nutrient removal in wastewater treatment plant (Yao and Peng, 2017).

Chemoautotrophic nitrifying bacteria have a very slow growth rate, and that is why they hardly form biofilms. Nitrifiers having high biofilm-forming capacity can be used for the treatment of ammonia polluted aquaculture water. Different strains of *Nitrosococcus* and *Nitrosomonas* are efficient for ammonia removal (Hoang et al., 2016). Probiotics formed by *Nitrosomonas* and *Nitrobacter* are beneficial for ammonia removal in shrimp (*Litopenaeus vannamei*) culture (Karthik et al., 2015). Several studies have been done about the uses of nitrifying bacteria. Hence the enhancement of the activity of nitrifying bacteria within their natural system without introducing them from outside needs special attention.

Weisany et al. (2013) have mentioned the role of some micronutrients in the enhancement of the nitrification process. However, some of those micronutrients are also essential for fish growth. Application of cobalt in the form of  $\text{CoCl}_2$  at a dose of  $10 \text{ kg}\cdot\text{ha}^{-1}$  significantly increases primary productivity, Chlorophyll *a* production, plankton volume and growth of *Labeo rohita* (Hamilton, 1822) (Adhikari and Ayyappan, 2002). Cobalt is an important micronutrient

for fish growth, and at the same time, it is essential for nitrogen-fixing microorganisms, including cyanobacteria. Zinc is an essential nutrient because of its catalytic function over 300 proteins which greatly enhances fish growth, reproduction and development (Terech-Majewska et al., 2016) and at the same time also have a role in nitrogen fixation. Similarly, many other nutrients such as iron and copper also play a dual role both in enhancing fish growth and nitrogen cycle. So the application of these micronutrients to enhance the abundance of nitrifying bacteria will give a new dimension for increasing the productivity of the aquatic system as well as minimise N<sub>2</sub>O emission by continuous uptake of nitrogen by the primary producers.

## Conclusion

Nitrogen, as different species of dissolved inorganic nitrogen consisting of NH<sub>4</sub>-N, NO<sub>2</sub>-N and NO<sub>3</sub>-N, plays an important role in fish pond productivity. Variability of these inorganic forms of nitrogen along with other nutrients determines the water quality and pond productivity, thereby composition and abundance of flora and fauna. Various microbial decomposers are responsible for the conversion of organic matter and mineralisation of organic N into inorganic forms. The abundance of the nitrifiers is highly correlated with phytoplankton productivity as it prefers to use nitrogen in the form of nitrate. The potentiality of surplus nitrogen removal by DNB produces opportunities for the management of the nitrogen cycle in ponds. The nitrogen cycle is also affected by seasonal variations of O<sub>2</sub> penetration, pH, water exchange, aeration and circulation. Different methods have been proposed by the researchers to avoid nitrogen toxicity in fish farming ponds, thereby maintaining the pond water conducive for fish growth. Moreover, the usage of nitrifiers (viz. AOB, NOB) in the form of biofilm also enhances the productivity of the aquatic ecosystem. Application of system-specific suitable methods for utilisation of different nitrogen cycle bacteria will increase the efficiency of organic matter recycling for fish growth as well as reduces the emission of N<sub>2</sub>O. Therefore, further studies are needed to enhance the nitrogen mineralisation by avoiding its toxicity and getting optimum available N nutrients for increasing fish growth and production in organic fish culture systems considering economic feasibility of the culture methods and the environment.

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