CONVENTIONAL AND NEW CONCEPTS OF BIOLOGICAL TREATMENT PROCESSES FOR THE NITROGEN REMOVAL FROM WASTEWATER

PROCESSOS BIOLÓGICOS CONVENCIONAIS DE TRATAMENTO E NOVAS CONFIGURAÇÕES APLICADAS PARA REMOÇÃO DE NITROGÊNIO DE ÁGUAS RESIDUÁRIAS

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Abstract

Nitrogenous compounds are predominant in many wastewater and need treatment prior to discharge in order to prevent oxygen depletion and eutrophication of surface water bodies. Nitrogen removal is usually accomplished through sequential nitrification and denitrification processes, by nitrification under aerobic conditions plus denitrification under anoxic conditions. Here, it is presented a review of the conventional biological nitrogen removal by nitrification-denitrification and also some processes that make use of new concepts, as: Anammox, Sharon, and Simultaneous nitrification and denitrification (SND).

Key words: nitrification; denitrification; C:N ratio; wastewater treatment.

Resumo

Compostos nitrogenados são predominantes em várias águas residuárias que necessitam tratamento prévio à sua descarga em corpos hídricos, visando impedir a depleciação de oxigênio e a eutrofização. A remoção do nitrogênio normalmente é realizada através de processos sequenciais de nitrificação e desnitrificação, sendo a nitrificação em condições aeróbias e a desnitrificação em condições anóxicas. É apresentada uma revisão do processo biológico convencional pela nitrificação-denitrificação e também alguns processos baseados em novas configurações, como: Anammox, Sharon e Nitrificação e desnitrificação simultâneas.

Palavras-chave: nitrificação; desnitrificação; relação C:N; tratamento de águas residuárias.

1.INTRODUCTION

Industrial wastewater is characterized by presenting a huge variety of pollutants, both in kind and composition, as in volume and concentrations. Among this sector, some agro-industries are included. These industries are characterized by disposing effluents with high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations, suspended and dissolved solids, settleable solids, and nutrients (usually nitrogen and phosphorus), etc.

The principal chemical species containing nitrogen which are of important trace in wastewater are ammonia, organic, nitrite, and nitrate nitrogen (BENEFIELD & RANDALL, 1980).

Metcalf & Eddy (2003) affirm that the chemistry of nitrogen is complex, because of the several oxidation states that nitrogen can assume and the fact that changes in the oxidation state can be brought about by living organisms. The most common and important forms of nitrogen in wastewater and their corresponding oxidation states in the water/soil environment are ammonia (NH_3, -III), ammonium (NH_4^+, –III), nitrogen gas (N_2, 0), nitrite ion (NO_2^-, +III), nitrate ion (NO_3^-, +V), and the oxidation state of nitrogen in most of the organic compounds is –III.

The nitrogen compounds such as ammonia and nitrite can be toxic to aquatic life if presented at sufficiently high concentrations, while nitrate is known to cause "blue baby syndrome" and is therefore a potential public health threat. Furthermore, nutrient such as nitrogen and phosphorus are known to stimulate growth of algae and other photosynthetic aquatic life, which leads to excessive eutrophication, excessive loss of oxygen resources, and undesirable changes in the aquatic ecosystem (JANG *et al*., 2004).

The removal of nitrogen from wastewater is usually accomplished using conventional processes, like: ponds, activated sludge process, trickling filters, rotating biological contactors, etc. These traditional biological processes, by nitrification plus denitrification, involve separate aerobic and anaerobic phases that are generally carried out in separate reactors or by different aeration intervals (METCALF & EDDY, 2003).

New concepts have been studied for nutrient removal, novel and promising alternatives to conventional nitrogen removal systems are able to treat nitrogenous compounds at lower cost, saving requirement of nitrogen and organic matter. For instance systems, like: the Anammox process (Anaerobic ammonia oxidation), Sharon process (Single reactor high activity) and SND (Simultaneous nitrification and denitrification).

Hence, the aim of the present study is to carry out a review in order to compare the conventional biological nitrogen removal by nitrification-denitrification and the new concepts of biological treatment processes applied on wastewater treatment.

2. CONVENTIONAL BIOLOGICAL NITROGEN REMOVAL

During biological transformation of organic nitrogen into molecular nitrogen (N_2), autotrophic and heterotrophic bacteria are present, under aerobic and anaerobic conditions, and the nitrogen removal takes place through three basic mechanisms: ammonification, nitrification and denitrification.

2.1. Ammonification and assimilation

Ammonification is the conversion of soluble organic nitrogen into ammonia-N that occurs as bacteria consume soluble organic matter containing nitrogen (GRADY *et al*., 1999).

During the reaction of ammonification, organic nitrogen is converted into ammonia nitrogen, while in the assimilation process the opposite occurs.

Ammonia nitrogen exists as either the ammonium ion (NH_4^+) or ammonia gas (NH_3), depending on wastewater pH and temperature. According to Metcalf & Eddy (2003), at pH levels below 7, the ammonium ion is predominant, whereas at pH levels above 11, practically all ammonia nitrogen appears as ammonia gas. Since the distribution of the ammonia is a function of the pH, the percentage of each species can be determined using the following relationship:

$$NH_3, \% = 100/(1 + [H]^+/K_a) \quad \text{(eq. 1)}$$

Where: $K_a = 5.62 \times 10^{-10}$ (acid ionization constant).

Using the proposed equation (1), it is possible to calculate the pH at which both species of ammonia nitrogen exist in the same relation, being this pH value 9.25.

2.2. Nitrification

In agreement with Li & Irving (2007), nitrification is a two-step reaction: ammonium (NH_4^+) is first oxidized to nitrite (NO_2^-) by autotrophic ammonia oxidizers, nitrite is then oxidized to nitrate (NO_3^-). The microorganisms involved are the autotrophic species *Nitrosomonas* and *Nitrobacter*, which carry out the reaction in two steps, as it is shown in the equations (2) and (3) (Eckenfelder, 2000):

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \; (\textit{Nitrosomonas}) \quad \text{(eq. 2)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \; (\textit{Nitrobacter}) \quad \text{(eq. 3)}$$

Thus, the total oxidation equation is expressed by:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \; \text{(Nitrifiers)} \quad \text{(eq. 4)}$$

From the equations (2), (3) and (4), it can be noticed that the nitrification process does not remove nitrogen from the wastewater, instead, it only changes the nitrogen compounds oxidation states.

Based on the total oxidation reaction (4), Metcalf & Eddy (2003) affirm that the oxygen required for complete oxidation of ammonia is 4.57 g O_2/g N oxidized with 3.43 g O_2 / g used for nitrite production and 1.14 g O_2/g NO_2 oxidized.

Grady *et al.* (1999) show the mass-based stoichiometric equations for nitrification.

For *Nitrosomomas*, when NH_4^+ is the basis, the equation is:

$NH_4^+ + 2.457\ O_2 + 6.716\ HCO_3^- \rightarrow 0.114\ C_5H_7O_2N + 2.509\ NO_3^- + 1.036\ H_2O + 6.513\ H_2CO_3$ (eq. 5)

When NO_2^- is the basis, the equation for *Nitrobacter* is:

$NO_2^- + 0.001\ NH_4^+ + 0.014\ H_2CO_3 + 0.003\ HCO_3^- + 0.339\ O_2 \rightarrow 0.006\ C_5H_7O_2N + 0.003\ H_2O + 1.348\ NO_3^-$ (eq. 6)

Furthermore, combining the two reactions reveals that the overall stoichiometry is:

$NH_4^+ + 3.300\ O_2 + 6.708\ HCO_3^- \rightarrow 0.129\ C_5H_7O_2N + 3.373\ NO_3^- + 1.041\ H_2O + 6.463\ H_2CO_3$ (eq. 7)

From these, it is seen that a large amount of alkalinity (HCO_3^-) is consumed during the oxidation of ammonia to nitrate: 6.708 g HCO_3^-/g NH_4^+ removed. Which is equivalent to 8.62 g HCO_3^-/ g NH_4^+-N removed or 7.07 g $CaCO_3$/g NH_4^+-N removed by nitrifying bacteria, considering that 1 g of alkalinity as $CaCO_3$ = 1.22 g HCO_3^- (DUNCAN, 2004). According to Metcalf & Eddy (2003) the value 7.07 g of alkalinity as $CaCO_3$/g ammonia nitrogen was calculated without considering the conversion of some of the ammonia to cellular nitrogen.

The chemolithoautotrofic bacteria are responsible for oxidation of ammonia nitrogen to nitrate in many biological wastewater treatment processes. These bacteria, collectively called nitrifiers, consist in the genera *Nitrosomonas* and *Nitrobacter*. Other autotrophic bacteria genera that obtain energy from the oxidation of ammonia to nitrite can also be present, like *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosorobrio* (METCALF & EDDY, 2003). Besides *Nitrobacter*, the nitrite is oxidized to nitrate by other autotrophic bacteria, such as *Nitrospira*, *Nitrococcus* and *Nitrosocystis* (HENZE *et al.*, 2001).

The cell yield for *Nitrosomonas* has been reported as 0.05 to 0.29 mg volatile suspended solids (VSS) / mg NH_3-N and for *Nitrobacter* 0.02 to 0.08 mg VSS / mg NH_3-N, a value of 0.15 mg VSS / mg NH_3-N is usually used for design purposes (ECKENFELDER, 2000). The growth rate of *Nitrobacter* is significantly higher than *Nitrosomonas*. As a result, nitrite typically does not accumulate in large concentration and the growth rate of *Nitrosomonas* generally controls the overall rate of nitrification (WEF *et al.*, 2005).

The nitrifying bacteria are characterized by a low growth rate (HENZE *et al.*, 2001). Admitting that bacterial biomass can be represented by the empirical formula $C_5H_7O_2N$, for every g of NH_4^+ removed only 0.129 g of biomass will be formed.

According to Hammer *et al.* (2007) the nitrification process does not remove the nitrogen, but converts it to the nitrate form. Only Nitrification-Denitrification reduces the total nitrogen content, converting the nitrate to gaseous nitrogen.

2.3. Denitrification

Biological denitrification involves the biological oxidation of many organic substrates in wastewater treatment using nitrate or nitrite as the electron acceptor instead of oxygen. This conversion is carried out by facultative heterotrophic bacteria under anoxic conditions (BENEFIELD & RANDALL, 1980). The nitrate reduction reactions involve the following reduction steps from nitrate (NO_3^-) to (NO_2^-), to nitric oxide (NO), to nitrous oxide (N_2O) and then nitrogen gas (N_2), as it is shown in the equation (8) (METCALF & EDDY, 2003).

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ (eq. 8)

The three last products in the equation are released in the gaseous form, but only the reduction to N_2 is able to avoid environmental damages to hydric resources, as rivers and streams.

Lemaire *et al.* (2006) affirm that a number of factors have been suggested to cause N_2O accumulation during denitrification process, like low COD: N ratio (2.6; 3.5), low pH (6.5), nitrite accumulation and oxygen gradient.

The denitrification, according to Hammer *et al.* (2007), has several benefits, including the recovery of approximately 60% of the energy dispended during the nitrification and about 50% of the alkalinity consumed by nitrification.

The process consumes approximately 3.7 g COD per g NO_3^--N reduced and produces 0.45 g VSS and 3.57 g alkalinity per g NO_3^--N reduced. This amounts to one-half the alkalinity that is consumed during nitrification (ECKENFELDER, 2000).

Nitrate conversion into gas nitrogen is realized by several bacteria, including those in the genera: *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Chromobacterium*, *Corynebacterium*, *Flavobacterium*, *Hypomicrobium*, *Moraxella*, *Neisseria*, *Paracoccus*, *Propionibacterium*, *Pseudomonas*, *Rhizobium*, *Rhodopseudomonas*, *Spirillum* e *Vibrio*, being *Pseudomonas* the most common (METCALF & EDDY, 2003). And the genera: *Achromobacter*, *Alcaligenes*, *Micrococcus*, *Pseudomonas* and *Thiobacillus* (DUNCAN, 2004). These are facultative heterotrophic bacteria which use the nitrate as electron receptor in anoxic conditions.

Wang *et al.* (2007), when studying aerobic denitrification process on sequencing batch reactors, managed to isolate and characterize bacteria belonging to four genera: *Pseudomonas*, *Delftia*, *Herbaspirillum* and *Comamonas*.

In addition to these microorganisms, four enzymes are involved in the processes of denitrification: nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase (GRADY *et al.*, 1999).

2.4. Parameters influencing nitrification and denitrification

According to Henze *et al.* (2001) there are many factors that affect nitrification and denitrification processes, such as temperature, pH, oxygen, alkalinity and energy sources.

Alkalinity

In agreement with Grady *et al.* (1999), while the nitrification process consumes alkalinity, denitrification generates it. If both processes occur in the same tank, as in a SBR (sequencing batch reactor) system, the effluent alkalinity is the overall result of alkalinity consumed in nitrification and alkalinity generated in denitrification. Theoretically, the difference between influent alkalinity and effluent alkalinity (as $CaCO_3$) is 7.14 – 3.57 = 3.57 mg·L^{-1} per mg·L^{-1} N removed (LI & IRVIN, 2007).

The amount of alkalinity that may be added depends on the initial alkalinity concentration and the amount of NH_4-N to be oxidized. Alkalinity may be added as lime, soda ash, sodium bicarbonate and carbonate; sodium, calcium and magnesium hydroxide, depending on costs and chemical handling issues (METCALF & EDDY, 2003; WIESMANN *et al.*, 2007). Alkalinity of secondary treatment wastewater is normally regulated as higher than 80-100 mg·L^{-1} to keep sufficient buffer capacity (LI & IRVIN, 2007).

Temperature

WEF *et al.* (2005) affirm that nitrification has been shown to occur in wastewater temperatures from 4 to 45°C, with an optimum growth rate occurring in the temperature range 35 to 42°C. However, studies carried out by Fontenot *et al.* (2007), testing different temperatures (22, 28, 37 and 45°C) on shrimp wastewater treatment using a SBR system, showed that the temperature range of 22-37°C worked well and removed more than 89% of all nitrogen species (nitrite, nitrate, ammonia nitrogen) and carbon.

Researchers reported by Eckenfelder (2000) reveal that the nitrifiers were less tolerant to variations in influent composition and temperature than were the heterotrophic organisms responsible for BOD removal and denitrification.

According to Henze *et al.* (2001), denitrification rate is very low below 5°C and increases with increasing temperature until 35°C. However, most wastewater treatment plants operate with liquid temperature between 20 and 30°C.

pH

Metcalf & Eddy (2003) suggest that the nitrification rate decline significantly at pH values below 6.8; and at pH values near 5.8 to 6.0 the rates may be 10 to 20 percent of the rate at pH 7.0.

In accordance with Wiesmann *et al.* (2007) the optimum pH for the growth of nitrifying bacteria is generally assumed to be pH 7.2-8.0. And if the pH value drops below pH 5.5 or goes above pH 9.0, a significant decrease in nitrification occurs as a result of protein damage. WEF *et* *al.* (2005) consider the optimum pH range for nitrification between 6.5 and 8.0.

In agreement with Benefield & Randall (1980) typical design parameters for a biological nitrification/denitrification process employing separate-stage nitrification apply pH values varying from 6.0 to 8.0. Metcalf & Eddy (2003) recommend that 6.5 to 7.5 is the optimum pH range for denitrifying bacteria.

The denitrification process may be carried out at higher levels of pH, since the production of toxic nitric oxides will be increased when pH values decline below 7.0 (HENZE *et al.*, 2001).

Carbon/nitrogen ratio

The carbon/nitrogen relation affects the nitrification and denitrification rates, the amount of nitrifiers decrease when the C/N ratio increases.

WEF *et al.* (2005) affirm that an excessive increase of the C/N ratio result in the heterotrophic biomass growth, sequestering nitrogen that could be nitrified.

Metcalf & Eddy (2003) suggests that in order to achieve a good performance in the nitrification process, the BOD/TKN ratio should be in the range of 3 to 5.

Fontenot *et al.* (2007) tested the effect of different C/N ratios (5:1, 10:1, 20:1, 30:1, and 40:1) on a sequencing batch reactor applied for shrimp wastewater treatment with both nitrification and denitrification occurring in the same reactor. They observed a performance of nitrogen reduction higher than 91% for all species of nitrogen (nitrite, nitrate and ammonia nitrogen) when working with C:N = 10, and similar removal nitrogen values with C:N = 5. The higher C:N ratios tested did not show satisfactory results.

Chiu *et al.* (2007), investigating different initial C/NH_4^+-N ratios (6.3; 11.1 and 19.7) in a SND-based SBR process treating synthetic wastewater, observed that 11.1 was the optimum initial C/N ratio, allowing the system to reach equilibrium between the nitrification and denitrification reactions and resulting in optimal removal of both nitrogen and organic carbon.

In agreement with Chang & Hao (1996), the complete removal of nitrogen from wastewater that contain high ammonia concentration or low C/N ratio is often limited by the lack of an available organic carbon source in the anoxic phase to sustain high denitrification. Consequently, it is a common practice to introduce an external organic carbon source in the anoxic phase, like ethanol (GUO *et al.*, 2007), methanol and acetate (WEF *et al.*, 2005).

Dissolved oxygen

Ferreira (2000) affirm that optimum nitrification rates can be obtained using DO levels higher than 4.0 mg·L^{-1}, provided that there is a sufficient population of nitrifying bacteria. Seixo *et al.* (2004) maintained, during the aerobic phase, a dissolved oxygen concentration around 5.5-6.0 mg·L^{-1}, and obtained satisfactory results. However, in practice, it is generally accepted that values higher than 2.0 mg·L^{-1} (WEF *et al.*, 2005) or 2-3.0 mg·L^{-1} (WIESMANN *et al.*, 2007) do not limit nitrification.

In denitrifying systems, the maintenance of low dissolved oxygen concentration is very important to obtain good nitrate removal rates. The denitrification can be completely inhibited at DO concentrations lower than 0.2 mg·L^{-1} (WIESMANN *et al.*, 2007). Ferreira (2000) recommends DO values in the range of 0.5-1.0 mg·L^{-1} for denitrification.

In activated sludge systems, the nitrification processes may be inhibited for DO concentrations raging from 0.3 to 1.5 mg·L^{-1}, due to the agglomerated growth of flock-shaped cells, as denoted by (U.S. EPA, 1993).

3. ALTERNATIVE PROCESS CONFIGURATIONS FOR BIOLOGICAL NITROGEN REMOVAL

Besides the conventional biological process, which has been commented for nutrient removal, there are some alternative configurations for biological nitrogen removal, fication (SND), Canon, NO_x process, etc.

3.1. Sharon (Single-reactor high-activity ammonia removal over nitrite)

In agreement with Metcalf & Eddy (2003) the Sharon process has been developed at Delft University of Technology in the Netherlands. It is based on the partial oxidation of ammonia to nitrite, being its main advantage the elimination of both the nitrite to nitrate oxidation step and the nitrate to nitrite reduction step, resulting in operational cost reduction through energy consumption reduction, which happens due to oxygen consumption reduction during the aerobic phase and exogenous carbon source demand reduction during denitrification.

The Sharon process, according to Department of Environmental Protection Pennsylvania - USA (2002), allows the system to preserve 25% in oxygen transference and 40% in carbon consumption, denitrifying with a bacterial growth comparative to conventional processes, working at temperatures above 15°C, specifically from 30°C to 40°C.

Biological nitrogen removal via nitrite has become an attractive and suitable alternative to treat wastewater streams with high ammonium concentration since aeration costs and organic carbon demand are reduced in comparison with nitrogen removal via nitrate. When is necessary the methanol is the organic carbon source most frequently used (CLAROS *et al.*, 2011).

In a SBR, the nitrification via nitrite could be achieved working with a high ammonium concentration and an appropriate pH range (GALÍ *et al.*, 2007).

3.2. Anammox (Anaerobic ammonia oxidation)

The Anammox process is an alternative to remove nitrogen compounds from high nitrogen-loaded wastewater with low organic matter content, instead of the traditional combined nitrification/denitrification processes (ARROJO *et al.*, 2006).

In agreement with Jin *et al.* (2007), the Anammox process takes place under anoxic conditions; NH_4^+ is oxidized to gaseous N_2 using nitrite (NO_2^-) as electron acceptor with the production of meager amounts of NO_3^-, saving requirements of oxygen and organic matter compared with the conventional nitrification/denitrification process. The principal product is gaseous N_2, however close to 10% of influent nitrogen (ammonia and nitrite) are converted to nitrate.

The Anammox process is equivalent to the classical denitrification, but it uses ammonium, instead of organic compounds, as the electron donor to reduce nitrite (GALÍ *et al.*, 2007).

It does not require a carbon source for nitrogen removal, because it is a litho autotrophic process. According to Schmid *et al.* (2000), it is established that autotrophic bacteria belonging to the order *Planctomycetales* carry out anammox reactions, as it is shown in the equations (9) and (10).

$$NO_2^- + NH_4^+ \rightarrow N_2 + H_2O \quad \text{(eq. 9)}$$

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0,5}N_{0,15} + 2.03\ H_2O \quad \text{(eq. 10)}$$

From the industrial applicability point of view, the principal disadvantage of this process relies on the slow growth rate of the Anammox micro-organisms (ARROJO *et al.*, 2006).

3.4. Simultaneous nitrification and denitrification (SND)

The Simultaneous nitrification and denitrification consists in the oxidation of ammonia nitrogen under aerobic conditions, and the reduction of oxidized nitrogen compounds in the same reactor, under aeration conditions.

That is possible, according Chiu *et al.* (2007), because the nitrifiers are active in the areas of high dissolved oxygen (DO) concentration, whereas denitrifiers are active in areas of very low DO concentration. The uneven distribution of DO inside the biomass allows simultaneous proliferation of nitrifying and denitrifying bacteria.

According to (YANG, *et al.* 2010) the SND has become an attractive technology for nitrogen removal, due to its potential to eliminate the need for separate tanks, required in conventional treatment plants, inducing a simplified and smaller design. The traditional biological nitrogen removal processes involve the oxidation of ammonium to nitrate (nitrification) and then reduction with an organic carbon source to nitrogen gas (N_2) (denitrification). Both nitrification and denitrification involve nitrite as an intermediate. Hence, if SND is accompanied by the inhibition of the second step of nitrification (oxidation of nitrite to nitrate), theoretically many advantages over conventional SND could be achieved, as a reduction in aeration and the COD demand during denitrification and lower biomass yield during anaerobic growth.

4. CONCLUSIONS

Some conclusions can be drawn on this review:

The conventional biological nitrogen removal through nitrification/denitrification can be affected by many factors, such as temperature, pH, alkalinity, oxygen and energy sources (carbon). The system operation in the same reactor or in different reactors is very important to optimize biological nutrient removal.

Alternative process configurations, like Sharon process, have been developed meeting the need of treatment plants to handle, for instance, high nitrogen loaded wastewater. And others, such as Anammox, for saving requirements of oxygen and organic matter compared with conventional nitrification/denitrification process.

After these considerations, we believe there is no single process that best fits all situations of ammonia nitrogen removal from wastewater. In each case, an evaluation is necessary in order to choose the most suitable process.

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