Chapter VI

DIGESTATE TREATMENT
Nitrogen Removal

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Introduction

Digestate is often poor in biodegradable organic matter, but it is an effluent rich in nitrogen and phosphorus, as most of the biodegradable organic matter has already been consumed in the anaerobic digestion process. Thus, this nutrient-rich effluent has great potential to negatively impact the environment when its agricultural use is limited or inadequate.

Problems associated with excess nutrients in the aquatic environment are worrying. High concentrations of ammoniacal nitrogen may lead to serious ecological implications, for instance, strongly influencing dissolved oxygen dynamics in the medium, as 4.6 mg of O_2 are required to oxidize 1 mg of NH_3.

Moreover, nitrogen and phosphorus in aquatic environments can cause eutrophication both in lentic and lotic environments, as well as

nutrient accumulation in the soil, entering a vicious circle of difficult environmental recovery if not stagnant at release (Hauck et al., 2016).

Nitrogen compounds in the different oxidation states can pose serious risks to human health from a public health point of view. Nitrate can cause methemoglobinemia (blue baby syndrome), the result of the reduction of NO_3- to NO_2- by bacteria in the intestinal tract and consequent oxidation of Fe^{2+} to Fe^{3+} of hemoglobin, forming methemoglobin, which is unable to bind to O_2, thus preventing the gas exchange in the human organism (Knobeloch et al., 2000).

Nitrite can also be combined with secondary amines from the diet, forming nitrosamines, which have known carcinogenic and mutagenic potential (Hu et al., 2012; Sadeq et al., 2008). Table 1 summarizes the major impacts that nutrients from digestate can cause when inappropriately released into the environment.

Table 1. Impacts caused by the most common forms of nitrogen in liquid effluents.

Oxidation state	Effect on the environment	Consequence
Reduced forms such as ammonia and ammonium	Increased oxygen requirement	The oxidation of ammonia that is released into the aquatic environment reduces O_2 concentration in the liquid medium
	Aquatic toxicity	Ammonia in the non-ionic form is toxic to many aquatic organisms
Oxidized forms such as nitrite and nitrate	Effects on human health	Nitrite can cause methemoglobinemia, known as blue baby syndrome
	Eutrophication	Nutrients cause excessive algae growth, which reduces O_2 overnight and produce organic compounds that cause odor and taste to water

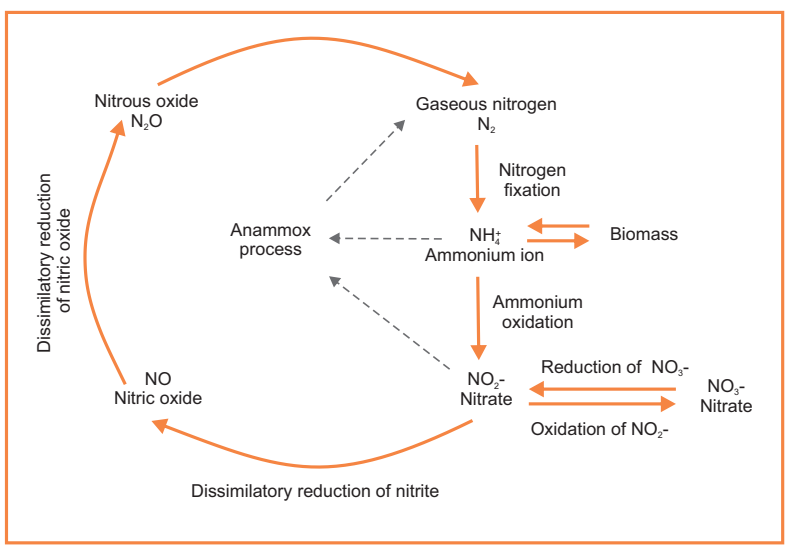
Given this scenario and facing environmental risks, digestates need to meet strict nitrogen and phosphorus concentration standards to be discarded at the end of treatment. Currently, few treatment systems contemplate nutrient removal, being associated with activated sludge systems in which, in the best scenario, nitrogen is only converted into nitrate without worrying about the environmental impact that it can cause.

The requirements regarding the management criteria for effluents from digesters have been increasing, making them significantly more restrictive and entailing the need for evolution in the effluent treatment processes that lead to a satisfactory reduction in nutrient concentration (Brasil, 2011; Fatma, 2014).

Nitrogen in the digestate and its main chemical transformations

Nitrogen is a nutrient present in the digestate in two main forms and oxidation states, being dissolved and particulate organic nitrogen and ammoniacal nitrogen (NH_3/NH_4^+).

The nitrogen cycle is carried out by a complex combination of various microorganisms and chemical reactions. Figure 1 shows the transformations of nitrogen compounds in the nitrogen cycle, resulting from microbial metabolism in the processes of fixation, nitrification, dissimilatory reduction of nitrite, denitrification, and anammox (anaerobic ammonia oxidation bacteria).



Source: Adapted from Ye; Thomas (2001).

Figure 1. Representation of reactions involved in the nitrogen cycle.

Figure 1 shows that nitrogen goes through several transformations, changing its oxidation state from the most reduced form to the most oxidized form. Table 2 shows the different chemical species of nitrogen that appear in the digestate and other effluents.

Table 2. Impacts caused by the most common forms of nitrogen in liquid effluents.

Chemical species	Description	Number of nitrogen oxidation (Nox)	Observation
$NH_3 + NH_4^+$	Total ammoniacal nitrogen (TAN)	-3	Independent of the medium pH
NH_3	Ammonia or free ammonia	-3	Varies depending on the medium pH
NH_4^+	Ammonium ion	-3	Varies depending on the medium pH
$NH_3 + NH_4^+ + N_{organic}$	Kjeldahl total nitrogen (KTN)	Indefinite	Total ammoniacal nitrogen added to the nitrogen present in organic matter
NO_2^-	Nitrite	+3	Generated through TAN oxidation
NO_3^-	Nitrate	+5	Generated through NO_2^- oxidation

The main source of ammoniacal nitrogen comes from metabolic reactions of bacteria that degrade organic substances, mainly urea, generating NH_3/NH_4^+. In contrast, gaseous nitrogen (N_2) can be converted into another form, mainly NH_3, by nitrogen-fixing bacteria (Hocking, 1985).

The formed ammonia can be anaerobically oxidized (together with nitrite) by bacteria with anammox activity or oxidized to nitrite by aerobic processes, which occurs with some frequency in effluents in the presence of oxygen. Nitrite can also be oxidized to nitrate or directly converted to gaseous nitrogen via nitric and nitrous oxide. Nitrate is the most oxidized form of nitrogen in nature and is often found in rivers and lakes, with the incorporation of oxygen from water movement (Galloway et al., 2008; Ye; Thomas, 2001).

Nitrogen in the ammoniacal form can still be assimilated by bacteria or oxidized to nitrite, which occurs with some frequency in effluents in the presence of oxygen. Other reactions of the microbiological nitrogen cycle, which is shown in Figure 1, commonly occurring in effluents with high ammoniacal nitrogen concentrations consist of the oxidation of nitrite to nitrate and dissimilatory reduction of nitrate to nitrite (Bailey et al., 2002; Gerardi, 2003; Ye; Thomas, 2001).

The pH and temperature influence the form that nitrogen is found in the digestate. The relationship between the concentrations of the two forms of ammoniacal nitrogen, ammonia and ammonium, and the relationship between nitrite and nitrous acid concentrations vary with the medium pH and temperature. The dissociation equilibria between these forms are described in Equations 1 and 2.

$$NH_4^+ \rightleftharpoons NH_3 + H^+$$ *Equation 1*

$$NO_2^- + H^+ \rightleftharpoons HNO_2$$ *Equation 2*

This equilibrium between the concentrations of ammoniacal nitrogen forms in an aqueous medium at 25 °C occurs at a pH of 9.25, in which 50% of both forms are observed. There is a predominance of ammonium ions at pH below the equilibrium point. However, the equilibrium is shifted to the formation of ammonia at pH values above 9.25, as shown in Figure 2.

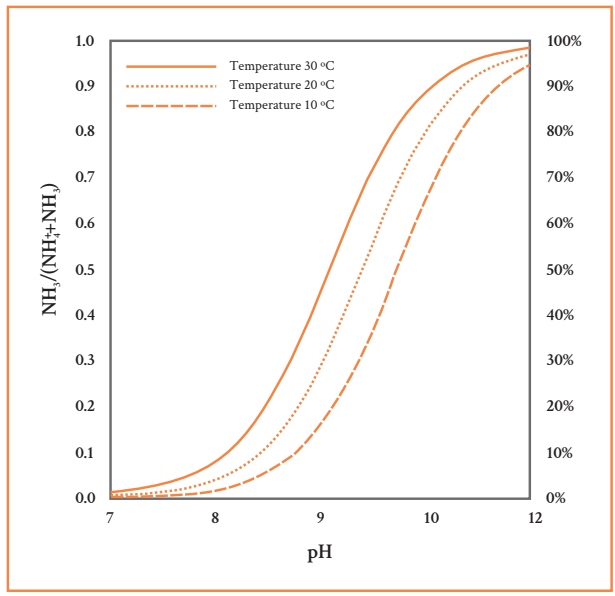


Figure 2. Influence of temperature and pH value on the equilibrium of NH_3 and NH_4^+ species.

Equation 3 is used to calculate ammonia and ammonium concentrations at any pH and temperature.

$$[NH_3\text{-}N] = \frac{[TAN]}{(1+K_{d,\,NH_3} \cdot 10^{pH})} \qquad \textit{Equation 3}$$

In which:

$$K_{d,\,NH_3} = e^{\frac{6344}{(273+T)}} \qquad \textit{Equation 4}$$

Where:

T = Medium temperature (°C)

$[NH_3\text{-}N]$ = Ammonia concentration

[TAN] = Total ammoniacal nitrogen

pH = Potential of hydrogen of the medium

Similarly, nitrous acid formation is a function of the temperature and pH of the medium. Figure 3 shows NO_2- and HNO_2 concentrations as a function of pH and temperature.

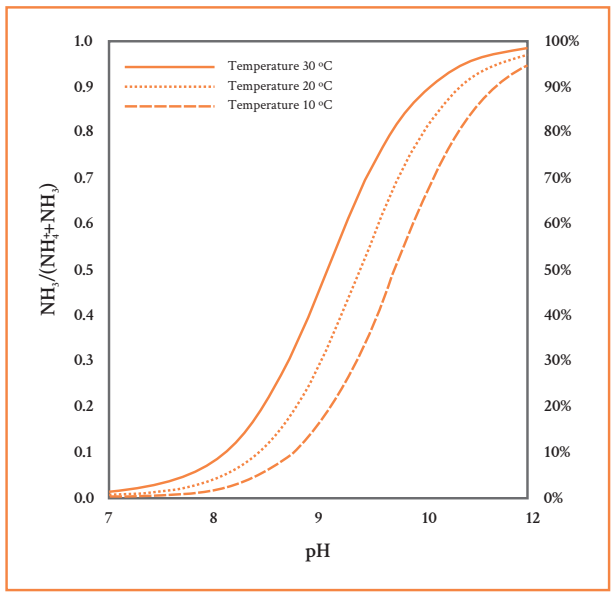


Figure 3. Influence of temperature and pH value on the equilibrium of NO_2- and HNO_2 species.

Equations 5 and 6 can be used to calculate NO_2- and HNO_2 concentrations at any pH and temperature.

$$[HNO_2\text{-}N] = \frac{[N - NO_2]}{(1 + K_{d,\,HNO_2} \cdot 10^{pH})} \qquad \textit{Equation 5}$$

In which:

$$K_{d,\,HNO_2} = e^{\frac{-2300}{(273+T)}} \qquad \textit{Equation 6}$$

Where:

T = Medium temperature (°C)

[HNO_2-N] = Nitrogen concentration in the form of nitrous acid

[NO_{2-} -N] = Nitrogen concentration in the form of nitrite

pH = Potential of hydrogen of the medium

The actual NH_3 and HNO_2 concentrations are of paramount importance for the control of biological processes. Firstly, both ammonia and nitric acid are believed to be the actual electron donors, that is, they are effectively the substrates involved in the nitrogen oxidation processes by microorganisms in the aqueous medium, requiring less energy to be transported into the cell compared to ionized forms (Wiesmann et al., 2007).

In addition to the substrate, the importance of knowing NH_3 and HNO_2 concentrations is related to the toxic potential of these two nitrogen species to ammonia-and nitrite-oxidizing microorganisms (De Prá et al., 2016).

The data have shown that there may be inhibition of microorganisms by the presence of excess ammonia or nitrous acid depending on the concentration of ammoniacal nitrogen and nitrite in the medium, even at a pH close to neutrality (Anthonisen et al., 1976).

Case study 1 – Fractions of ammoniacal nitrogen in effluents

A digester operating under continuous flow rate is fed with 250 $m^3.day^{-1}$ of swine manure. The digestate of this digester has a TAN concentration of 1,450 $mg.L^{-1}$ and is at a temperature of 26 °C and pH 8.4. Considering the equilibrium between ammonia and ammonium, determine the distribution of ammoniacal nitrogen fractions, according to the chemical equilibrium between the species.

- [TAN] = 1,450 $mg.L^{-1}$
- T = 26 °C
- pH = 8.4

The nitrogen concentration in the form of ammonia present in the sample can be calculated by Equation 7, which was obtained by substituting Equation 4 in Equation 3.

$$[NH_3\text{-}N] = \frac{[\text{TAN}]}{\left(1 + e^{\frac{6344}{(273+T)}} \cdot 10^{-pH}\right)} \qquad \textit{Equation 7}$$

$$[NH_3\text{-}N] = \frac{1450}{\left(1 + e^{\frac{6344}{(273+26)}} \cdot 10^{-8.4}\right)}$$

$$[NH_3\text{-}N] = \frac{1450}{(1 + 16.39 \cdot 10^{8} \cdot 10^{-8.4})}$$

$$[NH_3\text{-}N] = \frac{1450}{(1 + 6.525)}$$

$$[NH_3\text{-}N] = \frac{1450}{7.525}$$

$$[NH_3\text{-}N] = 192.7 \text{ mg } L^{-1}$$

$$[NH_3\text{-}N] = \frac{192.7}{1450} \cdot 100 = 13.29\%$$

$$[NH_4^+\text{-}N] = \frac{1450 - 192.7}{1450} \cdot 100 = 86.71\%$$

Case study 2 – Free nitrous acid concentration

An activated sludge reactor treating digestate from a digester is at a temperature of 33.4 °C and pH 6.42. A sample collected from the liquid medium of the reactor had a concentration of 572.3 mg.L^{-1} of -NNO_2-N. Based on these data, calculate the HNO_2 concentration present in the medium at the time of collection.

- $[NO_2\text{-}] = 572.3 \text{ mg.}L^{-1}$
- T = 33.4 °C
- pH = 6.42

Equations 5 and 6 allow calculating the HNO_2 concentration in the collected sample. Equation 8 is obtained by substituting Equation 6 in Equation 5.

$$[HNO_2\text{-}N] = \frac{[N\text{-}NO_2]}{\left(1+e^{\frac{-2300}{(273+T)}} \cdot 10^{pH}\right)} \quad \textit{Equation 8}$$

$$[HNO_2\text{-}N] = \frac{[N\text{-}NO_2]}{\left(1+e^{\frac{-2300}{(273+T)}} \cdot 10^{pH}\right)}$$

$$[HNO_2\text{-}N] = \frac{572.3}{\left(1+e^{\frac{-2300}{(273+33.4)}} \cdot 10^{6.42}\right)}$$

$$[HNO_2\text{-}N] = \frac{572.3}{(1+5.495 \cdot 10^{-4} \cdot 10^{6.42})}$$

$$[HNO_2\text{-}N] = \frac{572.3}{(1+1445)}$$

$$[HNO_2\text{-}N] = \frac{572.3}{1446}$$

$$[HNO_2\text{-}N] = 0.3958 \text{ mg.L}^{-1}$$

Consolidated technologies for biological nitrogen removal

Currently, there are numerous alternatives (biological and physicochemical) for removing nitrogen compounds. Biological processes are usually low cost and require less labor than others, which is why they have been widely used for treating digestates.

Among the main biotechnological processes for nitrogen removal are: nitrification-denitrification, known as the conventional process, of which the first studies date back to 1890 (Khin; Annachhatre, 2004);

partial nitrification process, one of the most recently proposed alternatives to nitrification (Hellinga et al., 1998); anammox process, anaerobic oxidation of the ammonium ion (Mulder et al., 1995); and combined deammonification processes, which aim to combine the last two processes in a single reactor (Third et al., 2001).

Nitrification process

Conventional nitrification is a microbiological reaction of nitrogen oxidation with oxygen as the final electron acceptor. This reaction occurs in two steps. The first step is where ammonia-oxidizing bacteria (AOB) oxidize NH_3-N (-III) to NO_2-N (III), with hydroxylamine as an intermediate product (Equation 9). The genus Nitrosomonas is often referred to in the literature as the most common AOB genus found in the environment. In the second step, nitrite-oxidizing bacteria (NOB) oxidize NO_{2^-}-N (III) to NO_{3^-}-N (V) (Equation 10). At this step, Nitrobacter is the genus of NOB most commonly found in the environment (Grady et al., 2011).

$$NH_4^+ + \frac{3}{2}O_2 \xrightarrow{\text{ammonia-oxidizing bacteria}} NO_2^- + H_2O + 2H^+ + \Delta G$$

Equation 9

$$NO_2^- + \frac{1}{2}O_2 \xrightarrow{\text{nitrite-oxidizing bacteria}} NO_3^- + \Delta G = -76\ kJmol^{-1}$$

Equation 10

The growth of AOB is more favored than NOB. It proves that cell growth is proportional to the energy released in the reaction. The ammonia oxidation reaction can be approximately 3.8 times more thermodynamically favored than the nitrite oxidation (Wiesmann et al., 2007).

According to Henze (2010), the equation that determines the oxidation reaction of ammonia to nitrate, as a single step, is shown in Equation 11.

$$NH_4^+ + 1.86O_2 + 1.98HCO_3^- \rightarrow 0.02C_5H_7NO_2 + 0.98NO_3^- + 1.88H_2CO_3 + 1.04H_2O$$

Equation 11

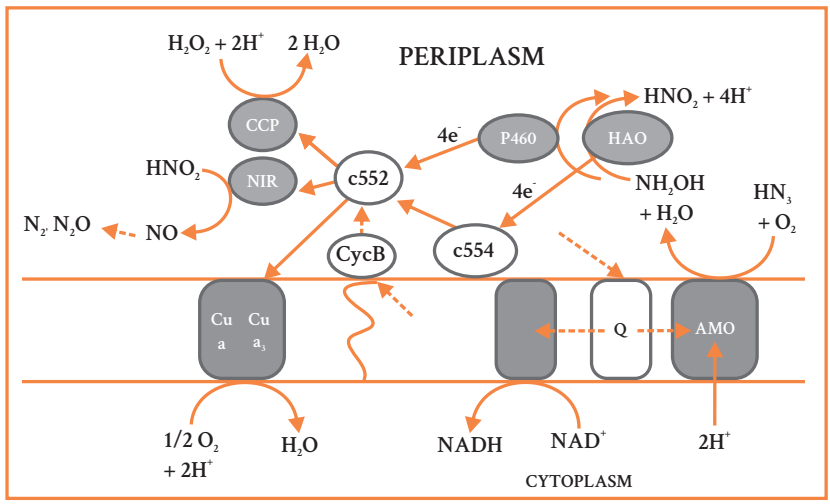
Biochemistry of nitrification

The reactions in the different steps of the NH_3-N oxidation (Nox -3) to NO_{3^-} -N (Nox +5) are catalyzed by specific enzymes. The most complex reactions occur in the first step, that is, the nitritation or NH_3 -N oxidation to NO_{3^-} -N, in which intermediates such as NH_2OH (hydroxylamine) appear. Two enzymes that participate in these reactions are the most important: ammonia monooxygenase, which acts to convert NH_3 to NH_2OH, and hydroxylamine oxidoreductase, which acts to convert NH_2OH to HNO_2.

Other enzymes catalyze reactions in the cell wall region of ammonia-oxidizing bacteria (Figure 4), such as nitrite reductase, which reduces HNO_2 to NO, nitric oxide reductase, which catalyzes the reduction of NO to N_2O, and nitrous oxide reductase, which catalyzes the reduction reaction of N_2O to N_2 (Hooper et al., 1997; Klotz; Stein, 2008; Bock; Wagner, 2013).

The oxidation of nitrite-N (III) to nitrate-N (V) occurs in a second step (Figure 5), that is, nitratation. Bacteria of the genus Nitrobacter participate in this step. The reaction is catalyzed by the enzyme nitrite oxidoreductase (NXR). This enzyme is found inside the cell wall and acts both in the oxidation of nitrite to nitrate and the reduction of nitrate to nitrite. Thus, the reaction is reversible.

Cytochrome a3 HCO (heme-copper oxidase) is another enzyme that plays an important role in this reaction. It is a group of proteins with a copper atom in the heme group that are part of the electron transport system of mitochondria and act as intermediate coenzymes in the cellular respiratory chain (Hooper et al., 1997; Klotz; Stein, 2007; García--Horsman et al., 1994).



Source: Adapted from Hooper et al., 1997(HOOPER et al., 1997).

Figure 4. Components of the nitrogen oxidation and electron transport system in Nitrosomonas. AMO – ammonia monooxygenase; HAO – hydroxylamine oxidoreductase; P460 – cytochrome P460; Q – ubiquinone-8; CycB – tetra-heme cytochrome c of the membrane; c552 – cytochrome c552; cp – di-heme c553 peroxidase; NiR – nitrite reductase; NOR – nitric oxide reductase; N_2OR – nitrous oxide reductase. Solid lines represent known mechanisms and dotted lines represent mechanisms not completely known, therefore, hypothetical.

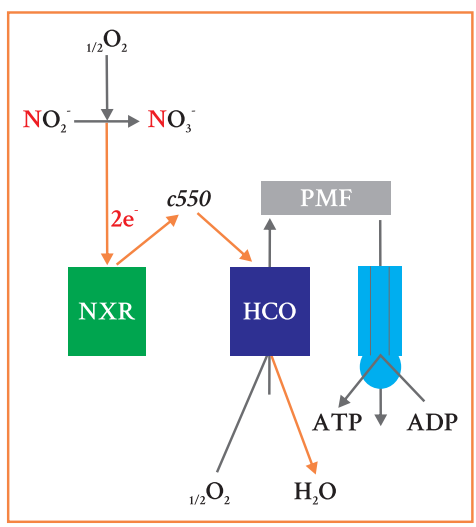


Figure 5. Components of the nitratation reaction system and their corresponding enzymes. NXR – nitrite oxidoreductase; c550 – cytochrome c550; HCO – heme-copper oxidase; PMF – proton motive force; ATP – adenosine triphosphate; ADP – adenosine diphosphate.

Nitrifying bacteria are autotrophic and, therefore, cannot incorporate exogenous organic compounds, obtaining energy from the oxidation of inorganic compounds. Many of the equations that define the growth kinetics of nitrifying bacteria do not consider that carbon dioxide is the only required carbon source. Moreover, the maximum growth rate of nitrifying bacteria is much lower than the growth rate of heterotrophic bacteria (Grady et al., 2011).

Although the nitrification process is autotrophic, it can also occur through the action of heterotrophic bacteria, which use organic carbon and oxidize ammonia to nitrate, such as *Arthrobacter* and *Thiosfera pantotropha* (Bitton, 2005).

Dissolved oxygen, pH, and growth of nitrifying biomass

The growth rate of nitrifying biomass is low and depends on growing conditions. Several parameters influence the nitrification performance of populations of nitrifying bacteria, such as dissolved oxygen (DO), pH, temperature (T), hydraulic retention time (HRT), and cell retention time (CRT). Among them, DO and pH are the most important (Wiesmann et al., 2007).

DO must be monitored in a reactor where complete nitrification is aimed, mainly because it can present a form of selection of different populations. It happens naturally, regardless of the goal.

Populations of nitrite-oxidizing bacteria are easily inhibited by the limitation of dissolved oxygen. This event is evidenced in Table 3, which shows that the ratio between the cell concentration of populations of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria (X_{NS}/X_{NB}) increases considerably when the DO of the medium is restricted (Canziani et al., 2006).

Table 3. Calculated and measured parameters of populations of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria. μNS – specific population growth rate of ammonia-oxidizing bacteria; μNB – specific population growth rate of nitrite-oxidizing bacteria; DO – dissolved oxygen in the medium.

μ_{NS} (d^{-1})	μ_{NB} (d^{-1})	X_{NS}/X_{NB}	Observations
0.625	0.555	2.96	DO > 2.0 $mg.L^{-1}$
0.450	0.129	16.54	DO between 0 $mg.L^{-1}$ and 0.5 $mg.L^{-1}$
0.468	0.192	25.02	
0.474	0.256	42.43	
0.632	0.395	31.66	DO between 0.5 $mg.L^{-1}$ and 1.5 $mg.L^{-1}$
0.582	0.275	18.97	

Source: Adapted from Canziani et al. (2006).

Regarding the oxygen requirement in the aerobic oxidation process of ammonia to NO_3, Equation 11 shows the need for 1.86 moles of oxygen for the complete oxidation of 1 mole of ammonium into nitrate. Converting the values in moles into mass in grams, the complete nitrification requires 4.25 g of oxygen per gram of NH_4^+-N.

The calculation of oxygen requirement in aerobic reactor designs aiming at nitrification to nitrate requires the concentration and load of nitrogen in the form of ammonium at the inlet of the aerobic reactor.

Case study 3 - Daily requirement of oxygen (Rd_{O2})

A digester generates 178 $m^3.d^{-1}$ of digestate, which is sent to an aerobic nitrifying reactor. The total ammoniacal nitrogen concentration is 1,385 $mg.L^{-1}$ and BOD (biochemical oxygen demand) is 3,630 $mg.L^{-1}$. Calculate the daily oxygen requirement needed in the reactor for the oxidation of all nitrogen in the form of ammonium to nitrate.

Initially, we need to know the nitrogen load at the inlet of the aerobic reactor. Therefore:

$$\boldsymbol{C = [TAN].Q} \qquad \textit{Equation 12}$$

Where:

c = Nitrogen load ($kg.d^{-1}$)

[TAN] = Total ammoniacal nitrogen ($kg.m^{-3}$)

Q = Flow rate ($m^3.d^{-1}$)

The daily nitrogen load at the inlet of the aerobic reactor can be calculated using Equation 12.

$$C = [TAN].Q$$

$$C = 1385\ \frac{mg}{L}.\ \frac{g}{1000\ mg}\ .\ 178\ \frac{m^3}{d}$$

Considering:

$$\frac{g}{L} = \frac{kg}{m^3}$$

$$C = 246.5\ kg\ d^{-1}$$

The nitrogen load allows calculating the daily oxygen requirement (Rd_{O2}) in the aerobic reactor as a function of Equation 12.

$$Rd_{O_2 \rightarrow NAT} = 246.5\ \frac{kgNAT}{d}\ .\ 4{,}25\ \frac{O_2}{NAT}$$

$$Rd_{O_2 \rightarrow NAT} = 1047.63\ \frac{kgO_2}{d}$$

The calculation of the oxygen requirement for the oxidation of organic matter can be carried out analogously. The calculation of oxygen requirement is equal to the daily BOD load.

$$C = 3630\ \frac{mg}{L}.\ \frac{g}{1000\ mg}\ .\ 178\ \frac{m^3}{d}$$

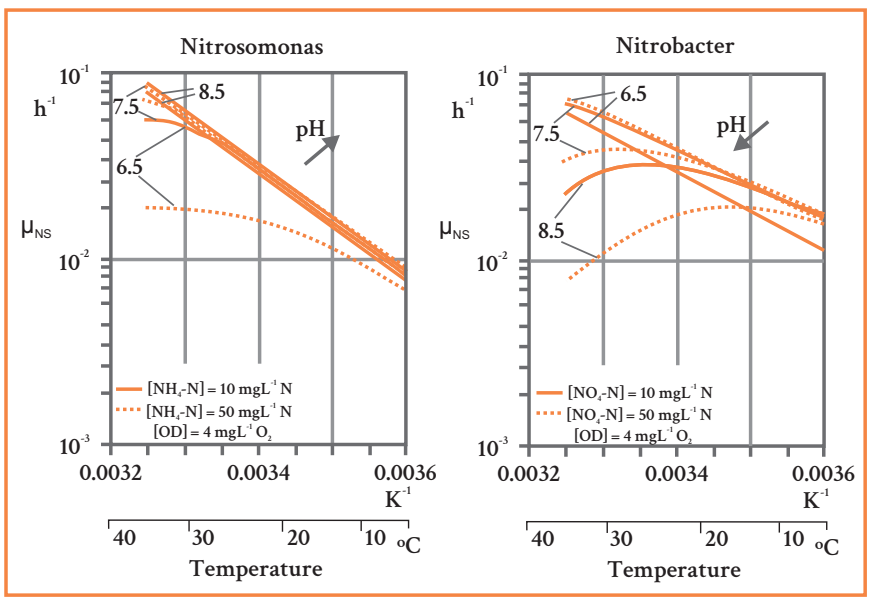
$$l = 646.1\ \frac{kg}{d}$$

$$Rd_{O_2 \rightarrow BOD} = 646.1\ \frac{kgO_2}{d}$$

In addition to the oxygen requirement necessary for the complete nitrogen oxidation in the nitrification process, pH has significant importance in terms of cell growth and stability of the nitrification process, as shown in Figure 6.

In the operation of nitrifying reactors, the pH contributes to the availability of the actual substrates of the process in addition to governing the equilibrium of the forms of ammonia (NH_3) and ammonium (NH_4), as well as nitrite (NO_2) and nitrous acid (HNO_2), as previously seen.

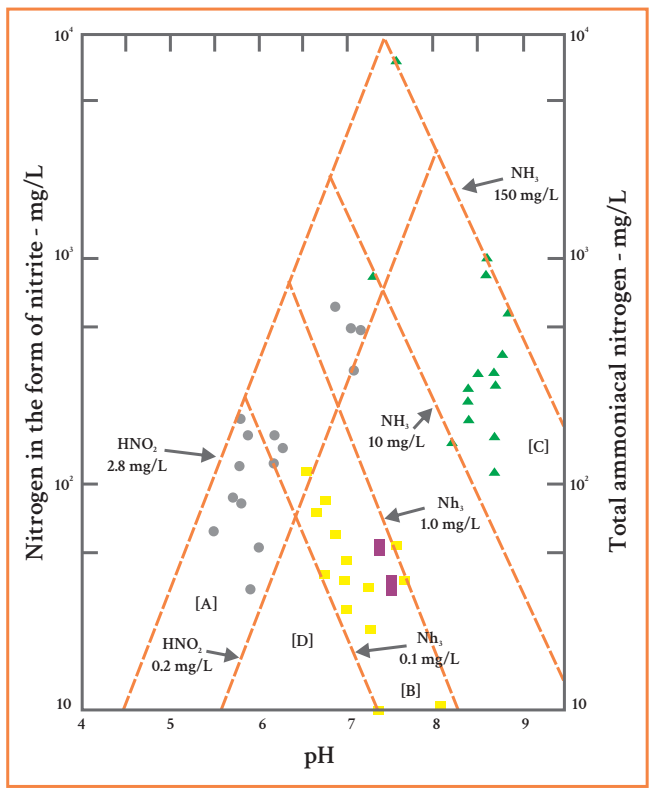
Only ammonia and nitric acid are effective substrates, as the cell uses less energy to transport these forms through the cell wall than the ionized forms ammonium and nitrite (Wiesmann et al., 2007).



Source: Adapted from Wiesmann et al., (2007).

Figure 6. The specific growth rate of bacteria of the genera Nitrosomonas (μ_{NS}) and Nitrobacter (μ_{NB}) as a function of pH, temperature, and concentration of the feeding medium.

The inhibitory effect of pH can be observed even at values close to neutrality, depending on the concentration of total ammonia and nitrite in the medium. The inhibitory effect of ammonia and nitrous acid on populations of *Nitrosomonas* (main population ammonia-oxidizing bacteria) and *Nitrobacter* (main population of nitrite-oxidizing bacteria) as a function of pH variation is shown in Figure 7.



Source: Adapted from Anthonisen et al., (1976).

Figure 7. Behavior of bacteria of the genera *Nitrosomonas* and *Nitrobacter* at different concentration ranges of free ammonia and nitrous acid as a function of pH.

The area identified as [A] in Figure 7, between 0.2 $mg.L^{-1}$ and 2.8 $mg.L^{-1}$, indicates the beginning of inhibition by HNO_2 for ammonia and nitrite-oxidizing bacteria, with a complete inhibition above 2.8 $mg.L^{-1}$ of HNO_2. The area [B] (0.1 $mg.L^{-1}$ to 1.0 $mg.L^{-1}$ of NH_3) identifies the beginning of inhibition of nitrite-oxidizing bacteria, while area [C]

(10 mg.L^{-1} to 150 mg.L^{-1} of NH_3) identifies the beginning of inhibition of ammonia-oxidizing bacteria (Anthonisen et al., 1976).

The pH control is essential for the maintenance of the nitrification process. The pH fluctuations can be minimized through a minimum amount of alkalinity, which acts by increasing the buffering power of the medium.

The alkalinity requirement can be determined by the stoichiometry of the TAN oxidation reaction. The stoichiometric requirement of bicarbonate (HCO_3-) is 2 mols for oxidation and 1 mol of NH_4^+, which corresponds to 4.36 grams of HCO_3 per gram of TAN (Galí et al., 2007).

The analytical methodology for total alkalinity in water and effluents determines the alkalinity in calcium carbonate ($CaCO_3$) (Rice et al., 2012) and, therefore, the alkalinity value in HCO_3- needs to be converted into Ca_2CO_3. Thus, the alkalinity requirement for complete oxidation of one gram of TAN is 7.14 grams of $CaCO_3$.

Case study 4 - Alkalinity requirement calculation

A nitrifying reactor is fed with digestate containing 2,190 mg.L^{-1} of N-NH_4+ at a flow rate of 135 $m^3.d^{-1}$. A concentration of 6,450 mg. $CaCO^3.L^{-1}$ was found after quantifying the total alkalinity in the digestate. Based on the data, calculate the daily alkalinity supplementation requirement in $CaCO_3$ for complete oxidation of NH_4^+ to NO_3-.

Initially, the daily load (L) of nitrogen and alkalinity present in the digestate that feeds the nitrifying reactor needs to be calculated before determining the daily requirement, using Equation 12.

$$C\left(\frac{kg}{d}\right) = [TAN] \,.\, Q$$

$$C = 2190 \frac{mg}{L} . \frac{g}{1000\ mg} . 135\ \frac{m^3}{d}$$

$$C = 2.19 \frac{g}{L} . 135\ \frac{m^3}{d}$$

$$C = 295.65 \frac{kg\,TAN}{d}$$

Analogous to calculating the nitrogen load, we can calculate the alkalinity load in $CaCO_3$ that feeds the nitrifying reactor daily.

$$C\left(\frac{kg}{d}\right) = [CaCO_3] \,.\, Q$$

$$C = 6450\frac{mg}{L} . \frac{g}{1000\ mg} . 135\ \frac{m^3}{d}$$

$$C = 6.45\frac{g}{L} . 135\ \frac{m^3}{d}$$

$$C = 870.75\frac{kgCaCO_3}{d}$$

Considering that 7.14 grams of $CaCO_3$ per gram of NH_4^+-N are needed, we can calculate the required daily alkalinity load through the product of the nitrogen load and alkalinity requirement.

$$\text{Alkalinity requirement} = 295.65\frac{kg\,TAN}{d} . 7.14\frac{kgCaCO_3}{kgTAN}$$

$$\text{Alkalinity requirement} = 2110.9\frac{kgCaCO_3}{d}$$

The daily alkalinity deficit for nitrification is obtained by subtracting the daily alkalinity load present in the digestate from the alkalinity requirement based on the TAN load that feeds the nitrifying reactor.

$$\text{Alkalinity deficit} = \text{Daily alkalinity load} - \text{Daily alkalinity requirement}$$

$$\text{Alkalinity deficit} = 870.75\frac{kgCaCO_3}{d} - 2110.9\frac{kgCaCO_3}{d}$$

$$\text{Alkalinity deficit} = -1240.15\frac{kgCaCO_3}{d}$$

Therefore, the alkalinity present in the digestate is not sufficient for the nitrification of this effluent, making it necessary to complement the alkalinity so that the pH of the system does not decrease to inhibitory levels.

Part of the alkalinity in processes with denitrification returns to the system and, when the processes are coupled, the alkalinity generated in the denitrification offsets part of the nitrification requirement, as discussed below.

Denitrification process

Denitrification is part of the nitrogen cycle. It consists of the transformation of NO_3 to N_2 under O_2 absence conditions. It is a reductive process and, therefore, a type of respiration. It occurs in four stages, according to Equation 13. Microorganisms oxidize an organic substrate as an energy source, producing numerous reduction equivalents.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \textit{Equation 13}$$

Each step in this reaction is catalyzed by specific enzymes. The nitrogen reduction steps have been widely studied. The structures of these enzymes have been visualized in high resolution, except for nitric oxide reductase. Furthermore, it is already known that there may be more than one type of reductase per step.

In general, the enzymes required for denitrification are only produced under or close to anaerobic conditions, but they are readily inhibited if anaerobic growing cells are exposed to O_2. Furthermore, the reactions are catabolic, that is, governed by heterotrophic microorganisms, requiring organic matter as a source of carbon for their cellular synthesis (Richardson et al., 2007; Mendonça, 2002; Tchobanoglous et al., 2013).

The microorganisms most frequently found in nature capable of reducing oxidized nitrogen consist of the genera *Pseudomonas* and *Alcaligenes*. However, other microorganisms have been described in the literature, such as *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Arthrobacter, Bacillus, Brevibacterium, Chromobacterium, Corynebacterium, Flavobacterium, Hyphomicrobium, Moraxella, Neisseria, Paracoccus, Propionibacterium, Rhizobium, Rhodopseudomonas, Spirillum,* and *Vibrio* (Tchobanoglous et al., 2013).

Biochemistry of denitrification

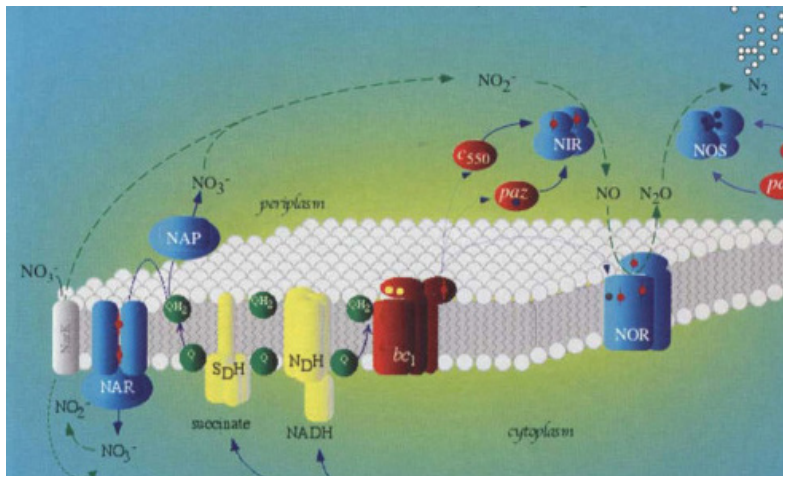
The enzymes responsible for denitrification in most bacteria receive electrons from the currents of the respiratory systems in the cytoplasmic membrane. In other words, denitrification is a form of respiration and a part of respiration with the electron transport system.

Denitrification occurs with the participation of specific components, such as ubiquinol/ubiquinone. The reduction reaction of ubiquinone to ubiquinol occurs using electrons from reductants such as NADH, volatile organic acids, and succinate. In denitrification, ubiquinol is directly oxidized in the cytoplasmic wall by nitrate reductase. There is a corresponding crystalline structure for this enzyme, commonly known as Nar, thus allowing knowing in detail how the enzyme works.

In summary, ubiquinol is oxidized towards the periplasmic membrane surface, with the release of H^+ into the periplasm, but electron transfer occurs across the membrane to the active site, which is located in a globular domain projected into the cytoplasm. The key point to note here is that electron transfer by Nar, together with the release of H^+ and absorption on both membrane sides, generates a driving force of protons across the membrane.

The location of the NO_3- reduction site on the cytoplasmic side of the membrane requires a NO_3- transport system, as shown in Figure 8. This task is believed to be the function of the NarK protein, which is a transporter both from the outside to the inside of the cell and the inverse. Normally, the NarK protein is the fusion of two proteins. Evidence suggests that one of these proteins catalyzes the entry of NO_3- into the cell with one or more H^+. It would allow NO_3- to enter the cell to initiate respiration.

In the steady-state, the import of NO_3- would be in exchange for the export of NO_2- to the periplasm, a process that would be the neutral exchange of electrons, thus neither affecting nor dissipating the proton motive force. The export of NO_2- to the periplasm is necessary because it is where nitrite reductase (NIR in Figure 8) is located in denitrifying systems (Moir; Wood, 2001; Spanning et al., 2007).



Source: Adapted from Spanning et al., (2007).

Figure 8. Scheme of the complete denitrification process in *Paracoccus denitrificans*. Dashed lines: transport of nitrogen oxides; solid lines: transport of electrons. SDH, succinate dehydrogenase; NDH, NADH dehydrogenase; Q, quinone; bc_1, cytochrome bc_1 complex; c_{550}, cytochrome *c*; paz, pseudoazurin; NAR, nitrate reductase of the membrane; NIR, cd_1-type nitrite reductase; NOR, *BC*-type nitric oxide reductase; NOS, nitrous oxide reductase; NarK, NO_3-/NO_2- transporter.

Electrons are delivered to cytochrome cd_1 by a mono-heme cytochrome c, cytochrome c_{550}, or the cupredoxin protein known as pseudoazurin. These two periplasmic, water-soluble proteins are reduced by the integral membrane complex, called the cytochrome *bc*1 complex, which in turn is reduced to ubiquinol. This complex is not specific for denitrification, occurring in several respiratory systems in all bacteria and mitochondrial electron transfer.

Nitric oxide is generated by nitrite reductase, but at low concentrations due to its toxicity. However, it is still an intermediary free from denitrification. Nitric oxide reductase is an enzyme present in the cell membrane, participating in the reduction of nitric oxide to nitrous oxide. It is believed, but not yet proven in the laboratory, it is provided by pseudoazurin or cytochrome c_{550} in common with nitrite reductase. The final step of denitrification is catalyzed by nitrous oxide reductase, another periplasmic enzyme, and acts to reduce nitrous oxide to gaseous nitrogen.

According to Wrage et al. (2001), the microorganisms responsible for denitrification are facultative anaerobes. That is, they are capable of using both oxygen and NO_3- and NO_2-. Therefore, the denitrification process is inhibited even at low dissolved oxygen concentrations.

Regarding the intermediates in the denitrification process, NO and N_2O are gaseous and accumulate in the medium when their enzymes are mainly inhibited at acidic pH.

Organic carbon and alkalinity in the denitrification

Denitrification is a heterotrophic process and needs a source of organic carbon to be carried out. There are two main forms in which denitrifying microorganisms obtain the organic carbon needed for reactions. It is called endogenous when the source comes from the cellular material. The other form is through an exogenous source, that is, an organic substrate, organic effluent, acetate, methanol, among others.

The organic carbon found in the composition of natural effluents comes basically from proteins, carbohydrates, and fats (Gerardi, 2002). In general, effluents after anaerobic treatment show the prevalence of short-chain carboxylic acids, such as acetic, propionic, and butyric acid (Miller; Varel, 2003; Ziemer et al., 2009).

An extra carbon source is required when there is not enough organic carbon present in the effluent. Methanol is commonly used in this role in industrial effluent treatment processes (Tchobanoglous et al., 2013). The denitrification reactions from acetate (Equation 14) and methanol (Equation 15) are shown below.

$$5CH_3COOH + 8NO_3 \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$$

Equation 14

$$5CH_3OH + 6NO_3 \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$$

Equation 15

Organic carbon availability in a given effluent is measured by the ratio between the available organic carbon mass and the nitrogen mass to be reduced in the denitrification. This ratio is usually called the C/N (carbon/nitrogen) ratio.

The C/N ratio has a great influence on the competition between the dissimilar reduction of nitrate to gas products (denitrification) and ammonification (Yoon et al., 2015). In laboratory tests with adapted biomass, the denitrification efficiency is not compromised when the C/N ratio is above 1 using methanol and above 2 using acetic acid as a carbon source (Her; Huang, 1995).

The magnitude of organic carbon is often expressed in the literature as the chemical oxygen demand (COD), which indirectly expresses the amount of organic matter present in the sample, accounting for the need for oxygen to oxidize the present organic carbon. Also, the relationship between the magnitudes TOC and COD is a function of factors such as the composition of organic matter and the presence of inorganic compounds, which consume oxygen in the oxidation of organic matter by the COD method, which may not occur in TOC determination methods.

The differences observed regarding the denitrification efficiency when comparing different substrates are due to the carbon bioavailability in each substrate. Studies have indicated the preference of denitrifying microorganisms for the use of short-chain carboxylic acids as a carbon source in denitrification (Elefsiniotis; Wareham, 2007; Adouani et al., 2010; Ahn et al., 2010).

Besides the preference for short-chain carboxylic acids and the high affinity of denitrifying microorganisms mainly for acetic acid, the denitrification rate using this acid as the main carbon source is more than twice the denitrification rate using propionic acid (Elefsiniotis; Wareham, 2007). It suggests that the use of longer-chain carboxylic acids by denitrifying microorganisms is complex and difficult.

Therefore, the C/N ratio must be observed considering only the soluble organic carbon, discarding the particulate material, when using the MLE (modified Ludzak and Ettinger) process aiming at the removal of nitrogen, regardless of the form in which the organic carbon is found in the digestate.

The organic carbon requirement is approximately 1.1 g per gram of nitrogen in the form of nitrate-based, considering the stoichiometry of the denitrification reaction (Equation 13). However, organic carbon is also consumed for cell synthesis and endogenous respiration in the denitrification process (Henze, 2010). In this sense, the denitrification reaction will occur without limitations if the digestate shows a C/N ratio above 2 or a COD/N ratio above 5 (Velho et al., 2017; Kishida et al., 2004; Chung et al., 2004).

Case study 5 - C/N ratio calculation

The concentration of TOC of 3,350 $mg.L^{-1}$ and total ammoniacal nitrogen (TAN) of 1,200 $mg.L^{-1}$ was observed in a digestate from a digester. Suppose the digester feeding flow rate is 10 $m^3.h^{-1}$ and 8 $m^3.d^{-1}$ of digester sludge is discarded. Calculate the C/N ratio and say if the denitrification process can be applied to this case.

Ideally, the carbon and nitrogen load are calculated and, subsequently, the relationship between the loads is verified to reduce the possibility of errors when calculating the C/N ratio.

Therefore:

$$C_{TOC}\left(\frac{kg}{d}\right) = Q\frac{m^3}{d} \,.\, [TOC]\frac{kg}{m^3}$$

In this case, specifically, the digester feeding flow rate is not the same as the outlet flow rate, as there is sludge disposal. Therefore, the outlet flow rate is the feeding flow rate minus the sludge disposal flow rate.

$$C_{TOC} = \left[Q_{feeding}\frac{m^3}{d} - Q_{sludge\ disposal}\frac{m^3}{d}\right] \,.\, [TOC]\frac{mg}{L}$$

$$C_{TOC} = \left[10\frac{m^3}{h} . \frac{24\ h}{d} - 8\frac{m^3}{d}\right] \,.\, 3350\frac{mg}{L}\frac{g}{1000\ mg}$$

$$C_{TOC} = \left[240\frac{m^3}{d} - 8\frac{m^3}{d}\right] \,.\, 3.35\frac{kg}{m^3}$$

$$C_{TOC} = 232\frac{m^3}{d} \,.\, 3.35\frac{kg}{m^3}$$

$$C_{TOC} = 777.2\frac{kg}{d}$$

The daily nitrogen load is calculated analogously.

$$C_{TAN} = \left[Q_{feeding}\frac{m^3}{d} - Q_{sludge\ disposal}\frac{m^3}{d}\right] \,.\, [TAN]\frac{kg}{m^3}$$

$$C_{TAN} = \left[Q_{feeding}\frac{m^3}{d} - Q_{sludge\ disposal}\frac{m^3}{d}\right] \,.\, [TAN]\frac{mg}{L}$$

$$C_{TAN} = \left[10\frac{m^3}{h}.\frac{24\ h}{d} - 8\frac{m^3}{d}\right] \,.\, 1200\frac{mg}{L}\frac{g}{1000\ mg}$$

$$C_{TAN} = 232\frac{m^3}{d} \,.\, 1.2\frac{kg}{m^3}$$

$$C_{TAN} = 278.4\frac{kg}{d}$$

The daily nitrogen load is calculated analogously.

$$\frac{C}{N}ratio = \frac{C_{TOC}}{C_{TAN}}$$

$$\frac{C}{N}ratio = \frac{777.2\frac{kg}{d}}{278.4\frac{kg}{d}}$$

$$\frac{C}{N}ratio = 2.79$$

The C/N ratio is 2.79, and it is expected that the denitrification process will not have its efficiency impaired due to the need for carbon because the amount of nitrogen is adequate.

The alkalinity equilibrium in the nitrogen removal process is a sensitive step. The generation of alkalinity equivalents can be observed in the complete denitrification cycle (Equations 14 and 15). Eight hydroxyl ions are generated for every 5 moles of acetate, that is, 3.57 g of alkalinity (as $CaCO_3$) is generated by reducing 1 g of NO_3-N(Tchobanoglous et al., 2013; Van Rijn et al., 2006). However, the values are lower than those from the stoichiometry on a real scale, that is, from 2.95 (Jeris; Owens, 1975) to 2.89 mg (Horstkotte et al., 1974) of $CaCO_3$ per mg of reduced nitrogen.

Thus, each gram of reduced nitrogen generates 3 grams of alkalinity in the form of $CaCO_3$, considering the stoichiometric value and the values observed in real-scale reactors for denitrifying reactor designs (Scheible et al., 1993).

A significant advantage of this excess alkalinity generated in denitrification is observed when thinking about the global nitrogen removal process via nitrification and denitrification. Given that alkalinity is consumed in the nitrification and alkalinity is generated in the denitrification, there is a compensation for the total alkalinity requirement when the processes are coupled.

Combined nitrification and denitrification process

The first configuration aiming at nitrogen removal consisted of the process proposed by Ludzak and Ettinger (1962), which was later modified and called modified Ludzak and Ettinger (MLE), shown in Figure 9. It is one of the most used processes for nitrogen removal in effluent treatment. The process consists of an anoxic tank before the aerobic tank, where the nitrification occurs. The nitrate produced in the aerobic tank returns to the anoxic tank.

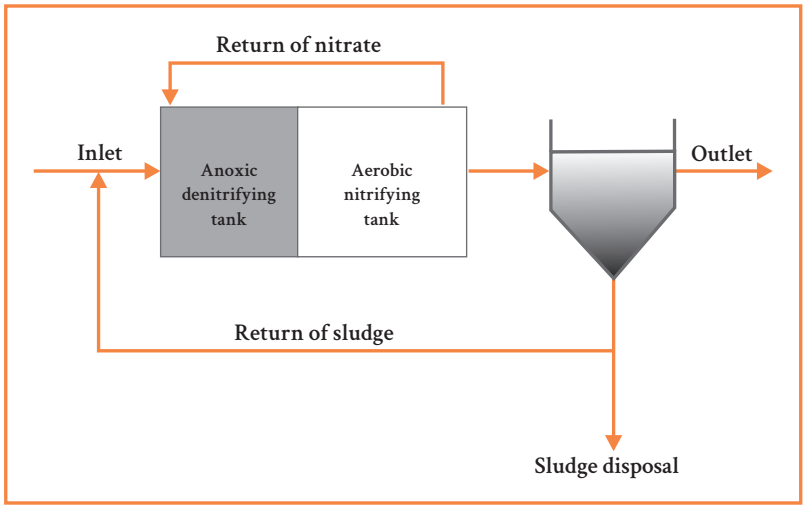


Figure 9. Representation of a complete mix reactor system using the modified Ludzak--Edinger process.

Heterotrophic microorganisms and the largest amount of organic carbon that will serve as electron donors in the nitrate reduction are in the anoxic tank. The process is also known as anoxic pre-denitrification because the anoxic tank precedes the aeration tank (Tchobanoglous et al., 2013; Wiesmann et al., 2007).

The recirculation rate of the liquid medium from the nitrifying reactor to the denitrifying reactor must be controlled. The higher the recirculation rate is, the higher the nitrogen removal. The overall system efficiency can be calculated by Equation 16.

$$E = 1 - \frac{[N_T]_s}{[N_T]_e} \qquad \textit{Equação 16}$$

Where:

$[N_T]_e$ = Total nitrogen concentration at the MLE system inlet ($mg.L^{-1}$)

$[N_T]_s$ = Total nitrogen concentration at the MLE system outlet ($mg.L^{-1}$)

Both reactors in the MLE system are full-mix and continuous flow rate, and the nitrogen removal efficiency is dependent on the ratio of the total recirculation flow rate from the nitrifying reactor to the denitrifying reactor and the system feeding flow rate (Equation 17). The total recirculation flow rate is the sum of the recirculation flow rate between the nitrifying and denitrifying reactor and the recirculation flow rate of the sludge from the sludge settler to the denitrifying agent (Equation 18).

$$R_T = \frac{Q_{RT}}{Q_{feeding}} \qquad \textit{Equation 17}$$

Where:

R_T = Total recirculation ratio

Q_{RT} = Recirculation flow rate ($m^3.d^{-1}$)

$Q_{feeding}$ = Feed flow rate of the MLE system ($m^3.d^{-1}$)

$$Q_{RT} = Q_{R\text{-}ND} + Q_{R\text{-}L} \qquad \textit{Equation 18}$$

Where:

$Q_{R\text{-}ND}$ = Recirculation flow rate between the nitrifying and denitrifying reactor ($m.d^{-1}$)

$Q_{R\text{-}L}$ = Sludge recirculation flow rate ($m^3.d^{-1}$)

Therefore, the maximum theoretical nitrogen removal efficiency for the MLE reactor system configuration is directly dependent on the total recirculation ratio (QRT) between reactors, as shown in Equation 19.

$$\frac{[N_T]_s}{[N_T]_e} = \frac{1}{1 + R_T} \qquad \textit{Equation 19}$$

The total recirculation ratio influences the system efficiency, as it is the basis of the nitrogen removal process. It can be used as a process control parameter. However, the ideal Q_{RT} value ranges between 3 and 6 times the feeding flow rate (Tchobanoglous et al., 2013; Chung et al., 2004), usually being set at 4.5 times the feeding flow rate ($Q_{feeding}$). The

sludge recirculation ratio is fixed at 1, as the sludge recirculation flow rate ($Q_{R\text{-}S}$) has the sole purpose of preventing excessive loss of biomass from the system.

The MLE process is very versatile, and results have demonstrated an efficiency above 90% of nitrogen removal from swine effluents. However, these effluents have a high concentration of total suspended solids, which can cause disturbances in the reactor operation, evidencing the attention that must be paid regarding this factor to avoid the fixed solids accumulation in the reaction tanks.

It is based on the nitrogen removal process via nitrification and denitrification and, therefore, there is a need for equilibrium between the amount of organic carbon and nitrogen, as previously mentioned, with the C/N ratio being essential in this process.

Most animal manure has enough carbon for denitrification because there is a large amount of available organic carbon, that is, a high C/N ratio. However, the amount of organic carbon available in the digestate is lower than that found in raw manure. The C/N ratio may decrease 20 times after the effluents and/or animal manure go through an anaerobic digestion process (Rico et al., 2011), which may remove nitrogen from the digestate by the MLE process unfeasible. A situation in which there is a lack of organic carbon available for denitrification requires the carbon supplementation or bypass from the digester directly to the denitrifying reactor.

Regarding dimensioning, the MLE system needs attention relative to two points, in addition to those already discussed: the volumetric nitrogen load (L_{vN}) and the hydraulic retention time (HRT).

The volumetric organic nitrogen load expresses the mass of nitrogen that feeds the MLE system per day as a function of the nitrifying reactor volume, according to Equation 20. The volumetric nitrogen load influences the nitrogen removal efficiency from the system. The MLE system operates with a nitrogen removal efficiency above 95% and L_{vN} ranging from 0.26 $kg.m^{-3}.d^{-1}$ and 0.41 $kg.m^{-3}.d^{-1}$. Therefore, an L_{vN} of 0.35 $kg.m^{-3}.d^{-1}$ is recommended to be used for the dimensioning of MLE systems (Bortoli, 2014; Vanotti et al., 2009; Park et al., 2004; Chung et al., 2004).

$$L_{vN} = \frac{[TAN]\frac{kg}{m^3} \cdot Q\frac{m^3}{d}}{V_{nitrifying\ reactor\ (m^3)}} \qquad \textit{Equation 20}$$

The same volume of the nitrifying reactor is usually used for the dimensioning of the denitrifying reactor, which can be dimensioned with a volume up to 20% smaller than that of the nitrifying reactor, with no efficiency loss (Park et al., 2004; Chung et al., 2004).

Finally, the calculation of the sludge settler (Equation 21) is performed using the hydraulic loading rate (HRR), which is based on the flow rate applied by the settler area. Conventionally, values between 1.5 $m^3.m^{-2}.h^{-1}$ and 4.33 $m^3.m^{-2}.h^{-1}$ are adopted. The sludge decanter volume is recommended to be between 5% and 10% of the nitrifying reactor volume as a safety parameter.

Considering that the system sludge is largely biomass and that its sedimentation is rapid, the sludge settler is established to not exceed three hours of HRT, when possible, to avoid sludge flotation and biomass loss (Wiesmann et al., 2007).

$$S_{settler} = \frac{Q_{feeding}}{SRR} \qquad \textit{Equation 21}$$

Case study 6 - Dimensioning of nitrifying/ denitrifying reactors

A piglet production unit with 4,800 sows has a digester for biogas generation and uses biofertilizer in arable areas belonging to partners close to the property. The need to treat the digestate for discharge into the receiving water body was highlighted in the new stage of environmental licensing. Considering that each sow produces an average of 32 L of manure per day and based on the digestate characteristics data presented below, determine the nitrifying reactor volume, the denitrifying reactor volume, and the dimensions of the sludge settler. Moreover, express the flow rates of feeding, recirculation from the nitrifying reactor to the denitrifying reactor, and sludge recirculation from the settler to the nitrifying reactor. Finally, determine the HRT of each reactor and sludge settler.

Digestate characteristics

Total ammoniacal nitrogen (TAN) ($mg.L^{-1}$) 2,200

Total organic carbon (TOC) $mg.L^{-1}$ 6,000

The calculation of volumes requires the establishment of some assumptions based on the literature.

1. The total recirculation ratio (RT) will be 5.0.
2. The nitrogen load should not exceed 0.35 $kg.m^{-3}.d^{-1}$.
3. The denitrifying reactor must be 20% smaller than the nitrifying reactor.
4. Surface runoff rate (TES) of 4 $m^3.m^{-2}.d^{-1}$, with a maximum HRT of 1 hour.

First, the manure flow rate is calculated.

$$Q = \text{No. of animals} \cdot \text{Manure production per animal}$$

$$Q = 4800 \cdot 32 \frac{L}{d}$$

$$Q = 153{,}600 \frac{L}{d} \frac{m^3}{1000L}$$

$$Q = 153.6 \frac{m^3}{d}$$

The C/N ratio in the digestate needs to be verified to assess the feasibility of using the nitrification and denitrification process.

$$\frac{C}{N}\text{ratio} \geq 2$$

$$\frac{C}{N}\ ratio = \frac{C_{TOC}}{C_{TAN}}$$

$$\frac{C}{N}\text{ratio} = \frac{Q.[TOC]}{Q.[TAN]}$$

$$\frac{C}{N}\ \text{ratio} = \frac{[TOC]}{[TAN]}$$

$$\frac{C}{N}\ \text{ratio} = \frac{6000\ \text{mg/L}}{2200\ \text{mg/L}}$$

$$\frac{C}{N}\text{ratio} = 2.72$$

There is the potential to use the coupled nitrification/denitrification process (MLE) because the C/N ratio is higher than 2.

Subsequently, Equation 20 is used to calculate the nitrifying reactor volume.

$$C_{vN} = \frac{[TAN]\frac{kg}{m^3}\ .\ Q\frac{m^3}{d}}{V_{\text{nitrifying reactor}}\ (m^3)}$$

Rearranging Equation 20, we have:

$$V_{\text{nitrifying reactor}} = \frac{[TAN]\frac{kg}{m^3}\ .\ Q\frac{m^3}{d}}{L_{vN}}$$

$$V_{\text{nitrifying reactor}} = \frac{2200\frac{kg}{m^3}\ .\ 153.6\frac{m^3}{d}}{0.35\ kg/m^3.d}$$

$$V_{\text{nitrifying reactor}} = \frac{2200\frac{kg}{m^3}\ .\ \frac{kg}{1000\ g}.\ 153.6\frac{m^3}{d}}{0.35\ kg/m^3.d}$$

$$V_{\text{nitrifying reactor}} = 965.5\ m^3$$

From the calculation of the nitrifying reactor volume and considering the established assumption, the denitrifying reactor volume will be 20% smaller.

Therefore:

$$V_{\text{denitrifying reactor}} = V_{\text{nitrifying reactor}} - (V_{\text{denitrifying reactor}} \,.\, 0.20)$$

$$V_{\text{denitrifying reactor}} = 965{,}5\ \text{m}^3 - (965.5\ \text{m}^3 . 0.20)$$

$$V_{\text{denitrifying reactor}} = 965.5\ \text{m}^3 - 193.1\ \text{m}^3$$

$$V_{\text{denitrifying reactor}} = 772.4\ \text{m}^3$$

Finally, the sludge settler volume of the system is calculated using the surface area value, according to Equation 21.

$$S_{\text{settler}} = \frac{Q_{\text{feeding}}}{\text{SRR}}$$

$$S_{\text{settler}} = \frac{153.5\ \text{m}^3/\text{d}}{4\ \text{m}^3/\text{m}^2.\text{d}}$$

$$S_{\text{settler}} = 38.4\ \text{m}^2$$

Setting the volume through the maximum HRT of three hours, the maximum volume will be:

$$V_{\text{settler}} = Q_{\text{feeding}} \,.\, \text{HRT}$$

$$V_{\text{settler}} = 153.5\ \frac{\text{m}^3}{\text{d}} . 3\ \text{h}$$

$$V_{\text{settler}} = 153.5\ \frac{\text{m}^3}{\text{d}} . 3\ \text{h} \,.\, \frac{d}{24\ \text{h}}$$

$$V_{\text{settler}} = 19.18\ \text{m}^3$$

Because the calculated settler volume value is lower than 5% of the nitrifying reactor volume, the highest value is adopted for safety reasons. Therefore, the sludge settler will have a volume of:

$$V_{dec} = V_{\text{nitrifying reactor}} . 5\%$$

$$V_{dec} = 965.5\ m^3.5\%$$

$$V_{dec} = 48.3\ m^3$$

Dividing the volume by the surface area, we have the height of the settling bed.

$$h = \frac{V_{dec}}{S_{dec}}$$

$$h = \frac{48.3\ m^3}{38.4\ m^2}$$

$$h = 1.26\ m \quad \rightarrow \quad h = 1.3\ m$$

Finally, the settler diameter is calculated from the surface area, considering a circular settler.

$$S_{dec} = \pi . r^2$$

$$r = \sqrt{\frac{S_{dec}}{\pi}}$$

$$r = \sqrt{\frac{38.4}{3.14}}$$

$$r = 3.5 \ \rightarrow \ D = 7.0\ m$$

Thus, from the volumes, the dimensioning of the finished system is shown in Table 4.

Table 4. Dimensioning of nitrifying/denitrifying reactors and sludge settler.

	Volume (m^3)	HRT (d)
Nitrifying reactor	965.5	6.3
Denitrifying reactor	772.4	5
Sludge settler	48.3	0.3

Nitrification and denitrification via nitrite

Unlike the conventional nitrification/denitrification process via nitrate, the nitrification/denitrification process via nitrite is mediated by the presence of NO_2-. This process is based on the fact that nitrite is the intermediate product of both the nitrification and denitrification process, thus being produced during nitrification and, subsequently, reduced to N_2 during the subsequent denitrification (Ciudad et al., 2005; Ruiz et al., 2006).

Figure 10 shows the paths of nitrification/denitrification via nitrate and denitrification via nitrite. The denitrification via nitrite reduces the ammonia oxidation pathway, making the oxidation of NO_2- to NO_3- unnecessary. An advantage is the 25% reduction in oxygen consumption in the aerobic phase, which implies a 60% energy saving in the entire process.

Moreover, the demand for electron donors, that is, organic carbon for denitrification, is 40% lower, and the denitrification rate via nitrite is 1.5 to 2 times higher than the denitrification rate via nitrate, which is technically feasible and economically favorable, especially when it comes to effluents with a high ammonia concentration or a low C/N ratio (Yang; Yang, 2011). Therefore, the ideal C/N ratio changes from 2 for the conventional denitrification to a C/N ratio of 1.2 for the denitrification via nitrite.

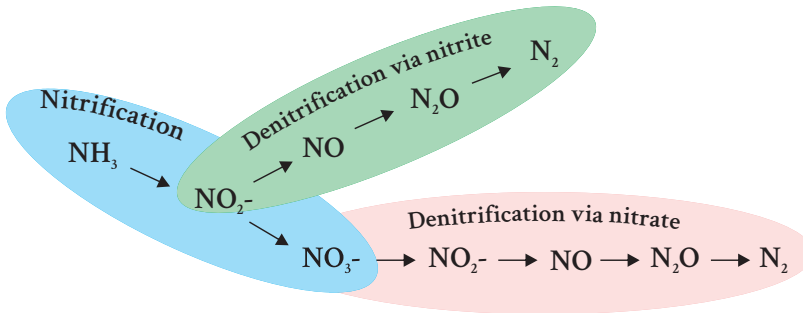


Illustration: Marcelo Bortoli

Figure 10. Representation of the of nitrification, denitrification via nitrite, and denitrification via nitrate paths.

Case study 7 - Calculation of the daily requirement of nitritation oxygen ($Rd_{O_2\text{-}NO_2^-}$) and C/N ratio for nitritation/denitritation

A biodigester generates 26 $m^3.h^{-1}$ of digestate, operating for eight hours a day. The digestate is sent to an aerobic reactor with the nitritation and denitrification process via nitrite. The total ammoniacal nitrogen concentration is 1,640 $mg.L^{-1}$ and the total organic carbon concentration (TOC) is 2,000 $mg.L^{-1}$. Calculate the daily oxygen requirement needed in the reactor for the oxidation of all nitrogen in the form of ammonium to nitrite to occur and calculate the C/N ratio, highlighting whether it would meet the denitrification process via nitrite.

Initially, the nitrogen daily load at the aerobic reactor inlet needs to be found. Therefore:

$$C = [TAN] \cdot Q$$

Where:

$$C = \text{nitrogen daily load } (\frac{kg}{d})$$

$$[TAN] = \text{total ammonia nitrogen concentration } (\frac{kg}{m^3})$$

$$Q = \text{flow rate } (\frac{m^3}{d})$$

The daily nitrogen load at the aerobic reactor inlet can be calculated using Equation 12.

$$C = [TAN] \cdot Q$$

$$C = 1640 \frac{mg}{L} \cdot \frac{g}{1000\ mg} \cdot 26\ \frac{m^3}{h} \cdot 8\ \frac{h}{d}$$

$$C = 1.64\ \frac{g}{L} \cdot 208\ \frac{m^3}{d}$$

Considering:

$$\frac{g}{L} = \frac{kg}{m^3}$$

$$C = 341.1\ \frac{KgN\text{-}NH_4^+}{d}$$

The nitrogen load value allows the calculation of the daily oxygen requirement ($Rd_{O_2\text{-}NO_2^-}$) in the aerobic reactor, using Equation 12.

$$Rd_{O_2\text{-}NO_2^-} = 341.2\ \frac{kgTAN}{d} \cdot \left[3.43\ \frac{gO_2}{gTAN}\right]$$

$$Rd_{O_2\text{-}NO_2^-} = 1170.3\ \frac{kgO_2}{d}$$

The C/N ratio can be calculated as follows:

$$\frac{C}{N}ratio = \frac{C_{TOC}}{C_{TAN}}$$

$$\frac{C}{N}ratio = \frac{Q[TOC]}{Q[TAN]}$$

$$\frac{C}{N}ratio = \frac{26\frac{m^3}{h} \cdot 8\frac{h}{d} \cdot 2000\frac{mg}{L}}{26\frac{m^3}{h} \cdot 8\frac{h}{d} \cdot 1640\ \frac{mg}{L}}$$

$$\frac{C}{N}ratio = 1.22$$

Therefore, the denitrification process via nitrite will not have its efficiency impaired since the C/N ratio is 1.22.

Recent technologies for biological nitrogen removal

Fundamentals and mechanisms

Digestates from efficient anaerobic digestion systems have been increasingly showing low carbon concentrations, resulting in an effluent with a low carbon/nitrogen (C/N) ratio. The trend in the absence of carbon makes it difficult to remove soluble nitrogen by the conventional nitrification/denitrification process detailed in the previous sections. It occurs because effluents that have a low C/N ratio may not have enough (and necessary) bioavailable organic carbon to carry out denitrification. In these cases, the addition of an external source of organic carbon is often necessary, which implies an increase in the operating cost of the conventional nitrification/denitrification process.

Effluents highly concentrated in nitrogen and little concentrated in carbon lead to difficulties in the dimensioning and operation of conventional systems, as seen in the previous sections. For this reason, new proposals have emerged to carry out this task. Recent studies on nitrogen removal aim to improve efficiency and reduce costs by optimizing available treatment strategies or implementing new processes and possibly new microorganisms capable of converting ammoniacal nitrogen into gaseous nitrogen, its inert form.

All these new processes seek to carry out the nitrogen elimination using nitrite as an electron acceptor and not nitrate, as there is a clear saving of oxygen for ammonium oxidation. The volumetric oxygen transfer coefficient (kLa) for different values of hydraulic retention time is approximately 25% lower for oxidation to nitrite than for nitrate, which results in energy savings in this process (De Prá et al., 2013).

Nitritation (or partial nitrification) is necessary to ensure nitrite availability, preventing the subsequent oxidation of nitrite to nitrate. According to Wiesmann et al. (2007), the ammonia oxidation reaction

is 3 to 3.8 times more thermodynamically favorable (240 KJ.mol^{-1} to 350 KJ.mol^{-1}) than nitrite oxidation (65 KJ.mol^{-1} to 90 KJ.mol^{-1}). Considering that the cell growth of the bacteria involved in this process is proportional to the energy released in their reaction, we can say that the growth of ammonia-oxidizing bacteria (AOB) is more favored than that of nitrite-oxidizing bacteria (NOB), which is advantageous when the objective is to accumulate nitrite in the reactor.

Some difficulties regarding the establishment of these processes are found due to the required control, especially when dealing with long periods of operation. Also, the steady-state phase is often difficult to achieve. Thus, some attention must be paid to most of these processes regarding the possible elimination of remaining nitrite into the environment due to its considerable toxicity.

The new proposals for processes for nitrogen removal via nitrite present in the literature will be mentioned with details below.

Partial nitritation process

The partial nitritation process is a technology based on the selection and favoring of ammonia-oxidizing bacteria (AOB), working as a pre-treatment capable of producing an effluent with ideal characteristics for feeding reactors with Anammox activity (as presented in the next section).

The strategy for the effectiveness of this process is to stop the oxidation of ammonia to nitrite (preventing oxidation to nitrate) and, at the same time, control the proportion of oxidized ammonia so that a portion of residual ammonia is maintained. In microbiological terms, it means disfavoring NOB activity, standing out bacteria of the genus *Nitrobacter*, allowing only AOB activity, standing out bacteria of the genus *Nitrosomonas* (De Prá et al., 2013; Yamamoto et al., 2006).

In summary, partial nitritation must limit the amount of ammonia oxidized by AOB activity, in addition to preventing the conversion of NO_2- to NO_3- by inhibiting NOB. Only 50% of the ammoniacal nitrogen should be oxidized to nitrite to make the stoichiometry according to the Anammox reaction, as described in Equation 22.

$$NH_4^+ + 0.75\ O_2 \Rightarrow 0.5\ NO_2^- + 0.5\ NH_4^+ + 2\ H^+ + H_2O$$

Equation 22

In this context, the effluent of this reactor, containing NH_4^+ and NO_2- without the complete oxidation of NH_{4+} to NO_2-, estimating a conversion of only 50%, would be suitable for feeding a subsequent reactor with Anammox activity to complete the intended degradation (Yamamoto et al., 2011).

However, some difficulties regarding the establishment of this process are found due to selectivity, especially related to long periods of operation. The physiological differences between AOB and NOB become extremely important in the process stability due to this condition. In this context, some operational strategies can be used to influence nitrite generation by favoring AOB due to the higher AOB sensitivity to certain environmental conditions (De Prá et al., 2013; Volcke et al., 2005).

The main alternatives to favor nitrite accumulation in biological systems are based on the proper regulation of control parameters such as dissolved oxygen (DO), aeration time, temperature, hydraulic retention time (HRT), solids retention time (SRT), pH, free ammonia (FA), free nitrous acid (FNA), and chemical inhibitors (Cui, 2012; De Prá, 2013). However, the economic feasibility of the process, in addition to its advantages and limitations, needs to be evaluated when using these strategies.

In this sense, the strategies for AOB selectivity cannot be generalized regarding nitrogen conversion using digestates, as the physicochemical characteristics of the effluent vary according to the production process and are dependent on its origin. Therefore, the process can be more or less efficient depending on the type of production.

Anammox process

Based on methodologies for identifying microorganisms and the type of metabolism developed by specific populations, the existence of a new segment of the nitrogen cycle was discovered in the 1990s, known

as anaerobic ammonium oxidation (Anammox) (Mulder et al. al., 1995). The anammox process evolved over the next few years from a largely unexplored part of the nitrogen biological cycle to become a key part of the overall nitrogen cycle. It is currently seen as a revolutionary technology for wastewater treatment (Scheeren et al., 2011).

The process involves an alternative route that consists of the anaerobic ammonium oxidation via specific microorganisms directly to N_2, using nitrite as an electron acceptor, with a small nitrate production. The free energy for this reaction is in the same order of magnitude as the free energy of the aerobic nitrification process, demonstrating that the anaerobic ammonium oxidation process is as favorable as the aerobic nitrification process. The stoichiometry of the anaerobic ammonium oxidation is presented in Equation 23 (Jetten et al., 2009).

$$NH_4^+ + 1.31\,NO_2^- + 0.066\,HCO_3^- + 0.13\,H^+ \Rightarrow$$
$$1.02\,N_2 + 0.26\,NO_3^- + 0.066\,CH_2O_{0.5}N_{0.15} + 2.03\,H_2O$$

Equation 23

Anaerobic chemolithoautotrophic microorganisms are responsible for these reactions, easily adhering to any solid surface, not existing uniformly within bioreactors (Isaka et al., 2006). Also, the culture of anammox microorganisms has excellent granulation properties, which allows the use of upflow reactor technologies to work with intense cell recycling, providing shorter reactor startup times (Kartal et al., 2011; Lotti et al., 2015).

Strous et al. (1998) combined the maximum bacterial activity and the substrate-to-biomass conversion factor to estimate the doubling time of bacteria with anammox activity between 9 and 11 days. The process produces a small volume of sludge due to this low growth rate, in addition to preserving approximately 60% of the oxygen used in the process, reducing treatment costs compared to the conventional nitrification/denitrification method (Ali et al., 2015; Jetten et al., 2001; Wang et al., 2016).

Several processes using bacteria with anammox activity have been implemented to optimize autotrophic nitrogen removal in wastewater since the discovery of anaerobic ammonia oxidation. Casagrande et al. (2013) observed high nitrogen removal loads (up to 20 $kgN.m^{-3}.d^{-1}$) when working with reactors with anammox activity, reaching values 66 times higher than the conventional process. These results demonstrate the potential efficiency that these processes can achieve and justify the worldwide trend of using these microorganisms in the treatment of effluents concentrated in nitrogen.

Like any biological process, bacteria with anammox activity can be inhibited under certain operating conditions or the presence/absence of any specific compound. In addition to oxygen, the process can be affected by pH, temperature, shear stress, and concentration of substrates and products. Therefore, the control and optimization of the process when applied is extremely important for the overall efficiency of the nutrient removal system.

Combined of deammonification process

The discussions presented in the previous sections allow for reflection on the combination of partial nitritation and anammox processes in terms of a new technology proposal for nitrogen removal. Deammonification is any technology that operates simultaneously with both partial nitrification and anammox processes (De Prá et al., 2012; Dosta et al., 2015; Gilbert et al., 2015; Magrí et al., 2012).

This technology appears as a promising alternative for eliminating high nitrogen loads in digestates and can be carried out using two or even a single reactor. As mentioned before, the bacteria responsible for the partial nitritation process are aerobic and, therefore, need oxygen during their metabolic activity.

On the contrary, anaerobic bacteria are responsible for the anammox process, with their activity stagnant when subjected to certain dissolved oxygen concentrations. These two processes are usually operated separately due to this condition, aiming at greater operational control and efficiency in nitrogen removal.

However, with the evolution and development of new technologies, recent studies have proposed that both bacteria can coexist in a single reactor as long as the system is maintained under limited dissolved oxygen conditions (Wett et al., 2007).

A representation of combined processes of deammonification is reproduced in Figure 11.

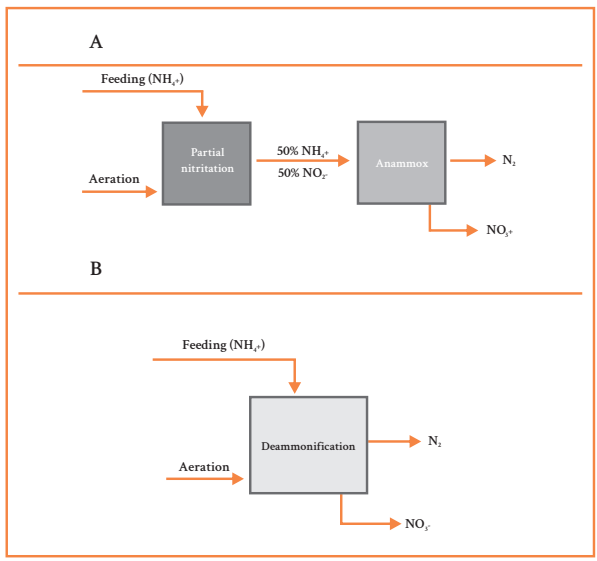


Figure 11. Representation of the partial nitritation + Anammox process using two (A) and a single (B) reactor for the operation of the deammonification technology.

In fact, it is easy to imagine a situation as shown in Figure 11A, where there is a first reactor operating under aerobic conditions, with only AOB activity, and a second reactor operating under anaerobiosis, with only anammox bacteria activity. However, this ammonia oxidation ratio in the first partial nitritation reactor can be difficult to maintain, leading to further problems in the anammox reactor (Cho et al., 2011). Thus, the overall nitrogen elimination efficiency in these systems is limited by the nitritation process of the first reactor. It occurs because there is a high requirement for operational oxygen control, in addition to ammonia oxidation, so that the partial nitritation process consumes all the dissolved oxygen from the liquid before entering the subsequent anammox reactor.

The overall nitrogen removal efficiency will be compromised if any imbalance occurs, and a higher oxygen concentration enters the anammox reactor. It demonstrates the importance of the operational process control to maintain the activity stability of ammonia-oxidizing bacteria not to reduce the nitrogen removal efficiency in the anammox process.

Although contradictory, the demand for operational control and technical demand decreases when we operate in a single reactor instead of two, as shown in Figure 11B. In this technology, aerobic ammonia-oxidizing bacteria are in symbiosis with the anammox anaerobic bacteria to form a single consortium for nitrogen elimination.

In terms of reaction, this process consists of the partial oxidation of ammonia to nitrite (by AOB activity) under limited oxygen conditions and, subsequently, the conversion of the nitrite produced together with part of the remaining ammonium to gaseous nitrogen (by the activity of anammox bacteria), forming a small amount of nitrate. The reaction combination of both processes results in the overall nitrogen removal reaction described in Equation 24.

$$NH_{4^+} + 0.85\ O_2$$

$$0.44\ N_2 + 0.11\ NO_{3^-} + 1.43\ H_2O + 1.14\ H^+$$

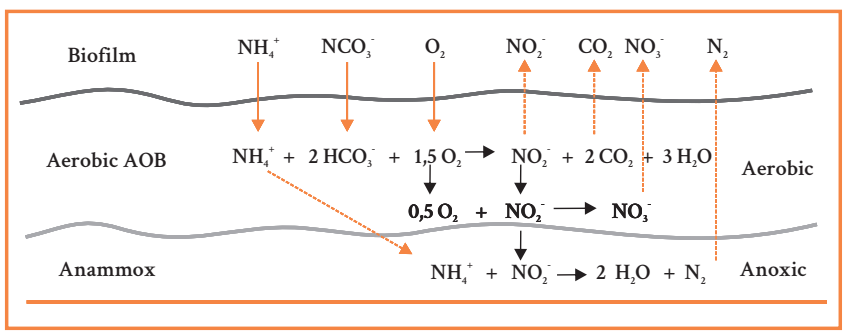
Equation 24

The Canon (completely autotrophic nitrogen removal over nitrite) process is a well-known single-step deammonification process initially proposed to operate sequencing batch reactors (SBR) at 35 °C (Figueroa et al., 2012; Third et al., 2001). However, new configurations have been proposed in recent years to perform nitrogen removal at lower operating temperatures (Chang et al., 2013; González-Martínez; Gonzalez-Lopez, 2016; Laureni et al., 2016; Veys et al., 2010), all aimed at reducing production costs and ease of operation to increase the process scale.

Reactor configuration

The deammonification process was originally proposed in sequencing batch reactors (SBR), but current technologies have evolved, and the proposal is also valid for continuous systems, with biofilm and airlift reactors (Egli et al., 2003; Leix et al., 2017; Reino et al., 2016).

Reactors in systems with biofilm eliminate nitrogen by forming a film, concentrating AOB in its external part and bacteria with anammox activity in its internal part. Thus, theoretically, the partial conversion of ammoniacal nitrogen to nitrite will occur on the biofilm surface and, subsequently, in its anoxic zone, with nitrite and the remaining residual ammonia being converted to N_2 by the anammox activity, as shown in Figure 12.



Source: Adapted from Zhu et al. 2008

Figure 12. Representation of the biochemical transformations of nitrogen in the deammonification process in biofilms.

In the same context as systems with biofilm, reactors that work with suspended biomass also have a concentration gradient of the substrate and DO, that is, the outside of the granule remains under aerobic conditions to perform the partial nitritation whereas the inside maintains anaerobic condition for anammox activity to occur (Figure 13).

This reactor configuration has been gaining prominence and preference for use due to its ability to reach higher N removal loads, related to the larger surface area for mass transfer. This condition opens the possibility for the deammonification application at low temperatures, without significantly losing efficiency in nitrogen removal compared to systems with biofilm at 30 °C -35 °C.

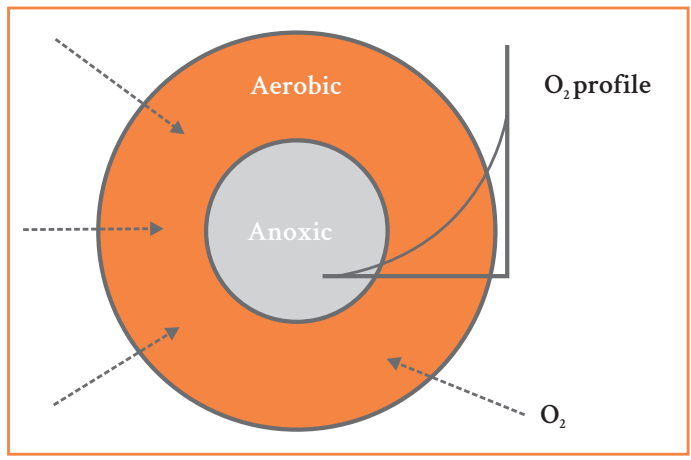


Figure 13. Representation of the oxygen concentration profile in a microbial flake.

Reactors that work with suspended biomass can be limited by resistance to mass transfer. Oxygen is the main factor for controlling the overall rate as the nitrite produced in the outer granule layer is consumed by bacteria found in the inner part, which can be attributed to diffusion in the granule or transfer of gas-liquid in the medium.

Control parameters

The control of the deammonification process follows almost entirely the parameters referring to the partial nitritation and anammox processes. The difference consists of the choice of the parameter used for selective inhibition of NOB and the effects it can cause to anammox bacteria. However, the deammonification process efficiency is usually directly related to three main factors: dissolved oxygen concentration, ammonia concentration, and control of the AOB population.

Dissolved oxygen is the electron acceptor in the partial nitritation process, being the main factor for controlling the global stoichiometry of the process, besides being directly related to the mass transfer and conversion of ammonia to nitrite. High dissolved oxygen concentrations can lead to the inhibition of the deammonification process for anammox bacteria (which are anaerobic) and AOB suppression, with excessive production of nitrite, which, in turn, is toxic for anammox activity depending on its concentration (De Prá et al., 2016).

Ammonia concentration is directly related to oxygen availability, but it can be critical for the process, as it serves as a substrate for both AOB and anammox. Thus, the process will substantially reduce efficiency if there is ammonia accumulation in the reactor or all ammonia is oxidized to nitrite due to the system imbalance. Many studies on the deammonification process have been conducted on a laboratory scale, but the applied volumetric load is lower than that applied to anammox due to operating conditions. However, significant savings have been observed as only one reactor is required, which can be advantageous depending on the effluent to be treated.

Regarding microbial populations, the interaction between aerobic and anaerobic bacteria present in the system plays an essential role in deammonification development. AOB require ammonia and oxygen as a source of substrate and electrons, while anammox bacteria require ammonia and nitrite. NOB require nitrite and oxygen and can interrupt the deammonification process if present in the medium due to competition for oxygen with AOB and nitrite with anammox bacteria. Therefore, maintaining the selectivity and interrelationship of microbial populations is essential in the deammonification process.

Usually, studies have been conducted mainly at high temperatures because temperatures above 25 °C favor anammox activity and act by expanding the differences between the growth rate of AOB compared to NOB. According to Veys et al. (2010), the ideal temperature for operating the deammonification process is 30 °C-35 °C, but recent studies have shown better advantages in operating reactors with lower applied loads but at room temperature (20 °C-25 °C) due to difficulties and energy costs (Chang et al., 2013; Cui, 2012; Wett et al., 2015).

This process has been a revolutionary technology for removing nitrogen. However, further investigations and research can contribute to this process to gain wide dissemination and become fully consolidated.

Trends and other processes in development

As already mentioned, the deammonification process has been one of the most innovative alternatives for the biological treatment of wastewater in recent years. An entirely new way of removing nitrogen was made available with its discovery in the 2000s. Many technologies have been developed and studied over the past few years for applicability in real effluents and several of them have already managed to transfer this technology to the full scale of operation.

Since the first full-scale anammox reactor for wastewater treatment in Dokhaven, Rotterdam, The Netherlands, set up and stabilized in 2002, there are 114 deammonification units reported around the world, including ten under construction and eight in the design phase (Lackner et al., 2014), and this number is increasing rapidly. Most plants (88 out of 114) were built in Europe, followed by China and North America, according to data from 2014.

The deammonification reactor capacity has been increasing rapidly despite the first anammox reactor was only 70 m^3 in volume (Lackner et al., 2014; Van Der Star et al., 2007). Full-scale plants with more than 142,000 m^3 of volume capacity are currently in operation, with the capacity to treat 134 tons of nitrogen load per day. Most of these full-scale treatment plants have been set up to treat municipal wastewater. However, they have not yet been applied on a full scale for the treatment of agro-industrial or agricultural effluents to date.

Initially, the plants have utilized the deammonification process at two stages and two reactors aiming at better operational control, using already consolidated partial nitritation systems. However, the focus has shifted mainly to single-stage deammonification with the setup experience and, since then, the trend towards single reactor setup has been increasing year after year. According to Lackner et al. (2014), approximately 88% of the full-scale plants are currently operated in a single-step deammonification configuration.

Several plants have implemented their own deammonification strategies, with differences mainly in the feeding cycle (intermittent vs. continuous), biomass disposal (suspended vs. fixed), and aeration con-

trol (intermittent vs. continuous). Another fact to be highlighted is that traditional technologies have also been modified and used efficiently for the application of the single-step deammonification process.

The deammonification process is likely to be implemented on a larger scale in the next few years due to cost savings, facility stability, and ease of control, associated with more stringent nitrogen removal requirements. In addition to application in municipal wastewater, industrial effluents have the potential for use.

Deammonification technologies will certainly be suitable for digestate within a technological package and aim to comply with current environmental legislation. Its application and demand for unit operations are directly related to the type of effluent and are paths to be scientifically explored to transform this technology into a reality on a large scale of operation in Brazil shortly.

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