Ambiente & Água - An Interdisciplinary Journal of Applied Science
ISSN: 1980-993X
ambi-agua@agro.unitau.br
Universidade de Taubaté
Brasil

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Ambiente & Água - An Interdisciplinary Journal of Applied Science, vol. 11, núm. 2, abril-junio, 2016, pp. 250-267
Universidade de Taubaté
Taubaté, Brasil

Ambiente & Água - An Interdisciplinary Journal of Applied Science
ISSN 1980-993X – doi:10.4136/1980-993X
www.ambi-agua.net
E-mail: ambi.agua@gmail.com

Advances in wastewater nitrogen removal by biological processes: state of the art review

doi:10.4136/ambi-agua.1772

Received: 06 Oct. 2015; Accepted: 28 Mar. 2016

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ABSTRACT

The paper summarizes the state-of-the-art of the most recent advances in biological nitrogen removal, including process design criteria and technological innovations. With reference to the Modified Ludzck Ettinger (MLE) process (pre-denitrification and nitrification in the activated sludge process), the most common nitrogen removal process used nowadays, a new design equation for the denitrification reactor based on specific denitrification rate (SDNR) has been proposed. In addition, factors influencing SDNR (DO in the anoxic reactor; hydrodynamic behavior) are analyzed, and technological solutions are proposed. Concerning technological advances, the paper presents a summary of various "deammonification" processes, better known by their patent names like ANAMMOX®, DEMON®, CANON®, ANITA® and others. These processes have already found applications in the treatment of high-strength wastewater such as digested sludge liquor and landfill leachate. Among other emerging denitrification technologies, consideration is given to the Membrane Biofilm Reactors (MBfRs) that can be operated both in oxidation and reduction mode.

Keywords: biological processes, deammonification, denitrification, nitrogen removal.

Avanços na remoção biológica de nitrogênio das águas residuais: uma revisão

RESUMO

O artigo resume o estado da arte dos mais recentes avanços na remoção biológica de nitrogênio, incluindo os critérios para o cálculo do processo e as inovações tecnológicas. Em relação ao processo Ludzck Ettinger modificado (pré-desnitrificação, nitrificação, no sistema de lodo activados), o qual é amplamente utilizado na remoção biológica de nitrogênio, foi proposta uma nova equação para calcular o reactor de desnitrificação, com base na taxa de desnitrificação (SDNR-Specific Denitrification Rate). Além disso, foram analisados os fatores que influenciam o SDNR (oxigênio dissolvido no reator; comportamento hidrodinâmico do reator) e foram propostas as soluções tecnológicas. Quanto à evolução tecnológica, o artigo apresenta um resumo dos vários processos de remoção biológica de amônia, que são mais conhecidos com os nomes patenteados, tais como: ANAMMOX®, DEMON®, CANON®, ANITA® e outros. Estes processos já encontraram aplicações industriais no tratamento de águas residuais de alta concentração, tais como o licor de lodo digerido e o percolado de aterro sanitário. Entre as tecnologias emergentes de desnitrificação foram considerados os bioreatores com membranas (MBfR), os quais podem ser operados tanto na fase aeróbia quanto na fase anóxica.

Palavras-chave: desnitrificação, remoção biológica de amônia, remoção de nitrogênio.

1. INTRODUCTION

Nitrogen removal from wastewater can be achieved by means of physico-chemical or biological processes. As far as the former are concerned, many technologies are available, as discussed elsewhere (Raboni et al., 2013b; Capodaglio et al., 2015).

Biological processes for nitrogen removal are generally applied to the denitrification of municipal wastewaters, and often to food and beverage processing industries, tanneries, fertilizer and petrochemical plants, as well as extensive livestock farm wastewaters. At present, the dominant technology is pre-denitrification in activated sludge systems, where an anoxic stage (DEN) is located upstream of an aerobic oxidation-nitrification (OX-NIT) stage.

Research in this field is mainly focused on evaluating optimal sizing criteria for the denitrification reactor. One of the key factors is represented by dissolved oxygen (DO) inhibition on the denitrification rate; thus, technological improvements that can reduce DO presence in the anoxic stage are among the most relevant (Raboni et al., 2014a; Torretta et al., 2014; Urbini et al., 2015). Recent studies have highlighted the limits of denitrification efficiency in small communities' plants due to strong fluctuations of incoming loads (Raboni et al., 2013b). Equally important is the effect of the denitrification reactor hydrodynamic behavior on process performance (Raboni et al., 2015).

Among the most recent developments in biological denitrification, the "deammonification" process (better known by its patented versions ANAMMOX®, DEMON®, CANON®, ANITA® and others) carried out by aerobic ammonia-oxidizing bacteria capable of degrading ammonia to nitrogen, has already found widespread application in the treatment of high-strength wastewaters such as digested sludge liquor and landfill leachate (US-EPA, 2009; 2010).

2. ADVANCES IN DESIGN OF DENITRIFICATION REACTORS IN ACTIVATED SLUDGE PLANTS

2.1. SDNR (Specific denitrification rate) for pre-denitrification design

Design of biological pre-denitrification has been usually carried out on the basis of the denitrification rate r_{DEN}, represented by a zero-order kinetics (for both NO_3-N and organic substrate) corrected for the effects of process temperature according to Arrhenius (Equation 1; Ekama, 2011):

$$(r_{DEN})_T = (r_{DEN})_{20°C} \cdot \theta^{T-20} \tag{1}$$

where:

$(r_{DEN})_{20°C}$ is the denitrification rate at 20°C in denitrification tank (DEN), usually equal to 2.9÷3.0 $gNO_3\text{-N}\ h^{-1}\ kgMLVSS^{-1}$ (Raboni et al., 2014b). This approach is still widely used nowadays; however, since it was observed that denitrification rates can be significantly reduced by the presence of DO, its inhibitory effect was taken into account by including an inhibition term $K'_O/(K'_O+DO)$ in r_{DEN} expression (US-EPA, 2009; 2010) (Equation 2).

$$r_{DEN} = \left(\frac{1-1.42\,Y}{2.86}\right)\cdot\left(\frac{K\cdot S\cdot X}{K_S+S}\right)\cdot\left(\frac{NO_3\text{–}N}{K_N+NO_3\text{–}N}\right)\cdot\left(\frac{K'_O}{K'_O+DO}\right)\cdot\eta \qquad (2)$$

where:

r_{DEN} is the denitrification rate (NO_3-N removal by dissimilation; mgNO_3-N L^{-1} h^{-1});

Y is the heterotrophic bacteria synthesis yield (mgVSS mg^{-1} substrate consumed);

K is the maximum specific rate of substrate utilization (h^{-1});

X is the biomass concentration (mgMLVSS L^{-1});

S is the soluble degradable substrate concentration (mg L^{-1});

K_S is the substrate utilization half-velocity coefficient (mg L^{-1});

NO_3-N is the nitrate concentration, as N (mgNO_3-N L^{-1});

K_N is the nitrate half-velocity coefficient (mgNO_3-N L^{-1});

K'_O is the DO inhibition constant for nitrate reduction (mgO_2 L^{-1}); and

η is the fraction of heterotrophic bacteria that use nitrate in lieu of oxygen (dimensionless).

Tchobanoglous et al. (2003) proposed a similar equation, adding an assimilative (cell synthesis) term to the dissimilative one as Equation 3.

$$r_{DEN}' = r_{DEN} + \left(\frac{NO_3\text{–}N}{K_N+NO_3\text{–}N}\right)\cdot\left(\frac{K'_O}{K'_O+DO}\right)\cdot\left(\frac{1.42}{2.86}\right)\cdot K_d\cdot X\cdot\eta \qquad (3)$$

where:

K_d is the endogenous decay coefficient (h^{-1}).

K'_O varies in a wide value range (0.02-0.2 mg L^{-1}) depending on floc size and sludge structure (Tchobanoglous et al., 2003). In any case, the mere presence of 0.2 mgDO L^{-1} could theoretically induce a 40% decrease in denitrification, compared to uninhibited conditions (Tchobanoglous et al., 2003). Other studies showed the effects of inhibition (Oh and Silverstein, 1999; Plòsz et al., 2003); in particular, significant effects of DO inhibition at concentrations as low as 0.09 mg L^{-1}, with up to 35% denitrification reduction, were observed (Oh and Silverstein, 1999).

For the practical calculation of the denitrification reactor volume a semi-empirical relationship (Equation 4;Tchobanoglous et al., 2003) was proposed, which correlates *SDNR* (Specific DeNitrification Rate) with the sludge load in DEN ($F{:}M_{DEN}$; gBOD_5 applied d^{-1} gMLVSS^{-1}):

$$SDNR_{20°C} = 0.029 + 0.03\cdot F{:}M_{DEN} \text{ (at about 20°C)} \qquad (4)$$

where:

$SDNR = Q\cdot\Delta NO_3\text{-}N\ V_{DEN}^{-1}\ X_{DEN}^{-1}$ (gNO_3-N gMLVSS^{-1} d^{-1});

V_{DEN} is the DEN volume (m^3); and

X_{DEN} is the biomass concentration in DEN (gMLVSS m^{-3}).

US-EPA (2010) proposes the same formula applying a correction factor to the F:M ratio in order to take into account the deviation of the active fraction of biomass in the mixed-liquor from the reference value (30%). Values of *SDNR* observed in pre-anoxic reactors at full-scale installation range from 0.04 to 0.42 gNO_3-N $gMLVSS^{-1}$ d^{-1} (Tchobanoglous et al., 2003), while US-EPA (2010) reports a more narrow range, 0.05 to 0.15 gNO_3-N $gMLVSS^{-1}$ d^{-1} at 20°C.

Recently, the strong dependence of *SDNR* in DEN on DO and $F{:}M_{DEN}$ has been highlighted (Raboni et al., 2014b; Torretta et al., 2014). Experimental results, supported by theoretical evaluations, allowed the representation of $SDNR_{20°C}$ (Equation 5).

$$SDNR_{20°C} = 0.0864 \left(\frac{K_O^{'}}{K_O^{'} + DO} \right) + 0.05\ F{:}M_{DEN} \cdot \eta_{BOD} \cdot \left(\frac{DO}{0.2 + DO} \right) \tag{5}$$

where:

K'_O=0.18 mg L^{-1}; η_{BOD} = 0.90-0.95 depending on $F{:}M_{DEN}$ in the range 0.4-0.2 $kgBOD_5$ d^{-1} $kgMLVSS^{-1}$.

2.2. Limiting DO concentration in denitrification processes

As mentioned, small levels of DO are constantly present in biological pre-denitrification reactors, inducing adverse effects on the kinetics of nitrogen removal, and consequently on denitrification performance.

Normally, a great part of the oxygen supplied to the anoxic reactor can be attributed to the mixed-liquor recycle, while a small contribution is given by raw sewage, and that of sludge recycle can be considered negligible. In addition, surface dissolution exchanges from the atmosphere should also be considered. As a result, daily average concentrations measured in real scale facilities are mostly in the range 0.2-0.4 mg L^{-1}, with higher peaks during the day, especially in small plants, characterized by strong fluctuations of flow rate and quality (Raboni et al., 2013a).

Minimizing DO in the mixed-liquor recycle stream seems relevant for limiting DO additions to denitrification. Addition of a post-anoxic reactor (after the pre-denitrification and oxidation-nitrification steps) has been proposed (Urbini et al., 2015) as a possible means of increasing denitrification performance.

A further strategy for DO control is based on the addition of reducing agents that can react with it. A recent study evidentiated that the most suitable reagent for this purpose is a salt of ferrous iron (Fe^{2+}), that proved to be very efficient in controlling DO while rapidly oxidizing to ferric ion (Viotti et al., 2015). At the same time ferric ion reacts with phosphorous, precipitating as ferric orthophosphate. Tests proved that a dosage of 6 $mgFe^{2+}$ L^{-1} can lower the mean DO concentration from 0.45 mg L^{-1} to 0.28 mg L^{-1}, increasing denitrification efficiency from 64.8% to 77%. This efficiency was progressively increased to 89% dosing ferrous ion at 9 mg L^{-1} (50% over stoichiometric for P removal), achieving average DO of 0.1 mg L^{-1}. This also confirmed the strong influence of DO on *SDNR* suggesting that maintaining DO values lower than 0.2 mg L^{-1} may achieve high denitrification efficiency.

2.3. Influence of denitrification reactor hydrodynamics

Pilot plant studies highlighted the influence of hydrodynamics of DEN on denitrification efficiency (Raboni et al., 2015). In particular, Retention Time Distribution (RTD) influences the concentrations of the denitrification reactants (NO_3-N and BOD), but has also an influence on residual DO, and consequently on denitrification efficiency. A denitrification reactor composed of 4 reactors in series has an oxygen consumption capacity greater than a single complete mixing reactor (which allows, at equal conditions, a residual DO of 0.18-0.30 mg L^{-1}). The study showed a noticeable difference in *SDNR* values between the two reactor models. In fact, the model with four reactors in series has demonstrated a higher efficiency of 16.6% (compared to the complete mixing model) at $F{:}M_{DEN} = 0.2$ $kgBOD_5$ d^{-1} $kgMLVSS^{-1}$; this percentage has increased to 25% at $F{:}M_{DEN} = 0.4$ $kgBOD_5$ d^{-1} $kgMLVSS^{-1}$. Hence, hydrodynamic configuration plays an important role in the efficiency of the anoxic reactor, conditioning residual DO concentration and, consequently, denitrification performance. For the same reasons, the right selection of the operating F:M ratio in denitrification is of great importance.

2.4. Denitrification efficiency in small treatment plants

Large variations in quantity and quality of sewage, typical of small communities, make it hard to achieve high biological denitrification efficiencies (i.e. $\eta \geq 90\%$). In a specific study of a pre-denitrification plant, fed by sewage of a community of 15,000 inhabitants, an average efficiency of only 60.2% was achieved, with isolated peaks of 75% (Raboni et al., 2013a). Two factors affected this result:

- Great variability of the BOD_5/NO_3-N ratio in denitrification, implying a shortage of carbon (BOD_5) for denitrification at different times of the day;
- Accumulation of DO in DEN, in the periods of lower BOD_5 inflow at night time and early in the day, reaching peak values of 1.2 mgO_2 L^{-1}, enough to determine inhibitory effects on the denitrification rate.

It was shown that, by adding supplemental carbon, it was possible to overcome the difficulties encountered and achieve denitrification efficiencies greater than 90%. A practical solution could likely consist of the introduction of an equalization tank before the treatment. The choice of a simultaneous denitrification process (achieving complete mixing conditions), also appears of interest.

3. ADVANCES IN DENITRIFICATION PROCESSES AND TECHNOLOGIES

3.1. Deammonification

3.1.1. Deammonification cycle vs. conventional processes

Deammonification is a biological treatment process used to convert ammonia to nitrogen gas. It includes both nitritation and the anaerobic ammonia-oxidizing (*anammox*) processes. Nitritation, aerobic oxidation of ammonia-N (NH_4-N) to nitrite-nitrogen (NO_2-N) is carried out by autotrophic, Aerobic Ammonia-Oxidizing Bacteria (AerAOB): a well-known process in wastewater treatment which forms the initial step of biological nitrification of NH_4-N to nitrates (NO_3-N).

The *anammox* reaction is carried out by *Planctomycetales*, autotrophic, Anaerobic Ammonia-Oxidizing Bacteria (AnAOB) which use NO_2-N as electron acceptor to anaerobically oxidize NH_4-N to N_2 (Strous et al., 1999). In the overall process, about 89% of the inorganic nitrogen (NH_4-N + NO_2-N) ends up as N_2 gas and about 11% as NO_3-N. Deammonification and the complete denitrification reaction are illustrated in Figure 1.

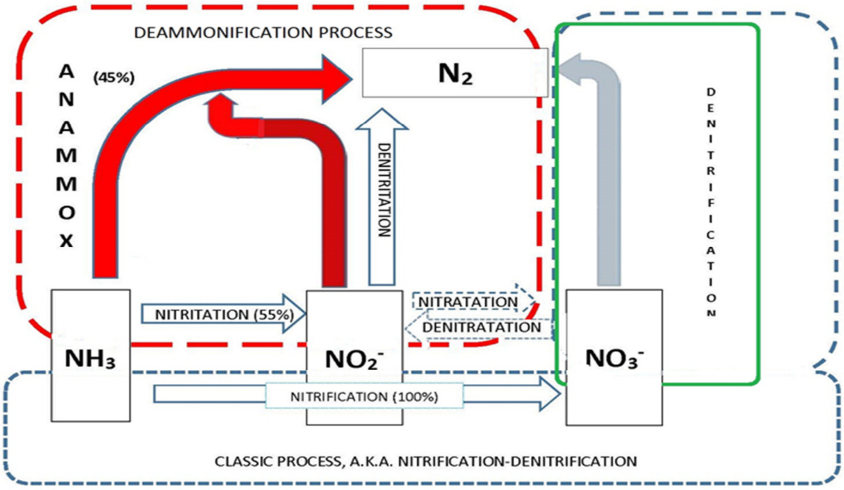

Figure 1. Simplified scheme of the Deammonification and nitrification/denitrification processes.

In order to facilitate the understanding throughout the paper, we introduce Table 1 with the major acronyms and units used in the discussion.

Table 1. Nomenclature of terms and symbols.

Symbol	Unit of measurement	Description
DO	$mgO_2\ L^{-1}$	Dissolved oxygen concentration
$\Delta NO_3\text{-}N$	$mgNO_3\text{-}N\ L^{-1}$	Nitrate concentration removed, as N
η	-	Fraction of heterotrophic bacteria that use nitrate in lieu of oxygen
η_{BOD}	-	BOD removal efficiency
K	h^{-1}	Maximum specific substrate utilization rate
K_D	h^{-1}	Endogenous decay coefficient
K_N	$mgNO_3\text{-}N\ L^{-1}$	Nitrate utilization half-velocity coefficient
K_S	$mg\ L^{-1}$	Substrate utilization half-velocity coefficient
K'_O	$mgO_2\ L^{-1}$	Oxygen inhibition constant
$F{:}M$	$gBOD_5\ d^{-1}\ gMLVSS^{-1}$	Sludge load
$MLVSS$	-	Mixed-Liquor Volatile Suspended Solids
$NO_3\text{-}N$	$mgNO_3\text{-}N\ L^{-1}$	Nitrate concentration, as N
Q	$m^3\ d^{-1}$	Flow rate
r_{DEN}	$gNO_3\text{-}N\ h^{-1}\ kgMLVSS^{-1}$	Denitrification rate
$r_{DEN'}$	$gNO_3\text{-}N\ h^{-1}\ kgMLVSS^{-1}$	Denitrification rate proposed by Tchobanoglous et al. (2003)
S	$mg\ L^{-1}$	Soluble degradable substrate concentration
$SDNR$	$gNO_3\text{-}N\ gMLVSS^{-1}\ d^{-1}$	Specific DeNitrification Rate
T	°C	Temperature
V_{DEN}	m^3	Denitrification reactor volume
θ	-	Coefficient of the Arrhenius equation
Y	$mgVSS\ mg^{-1}$ substrate consumed	Heterotrophic bacteria synthesis yield
X	$mgMLVSS\ L^{-1}$	Biomass concentration in the activated sludge
	Subscripts and superscripts	
T	°C	Temperature
20°C	-	At 20°C

In the conventional denitrification process, NH_4-N is oxidized first to NO_2-N and then to NO_3-N by autotrophic bacteria. The NO_3-N is then biologically reduced to N_2 by heterotrophic bacteria (denitrification) with the consumption of organic substrate in anoxic conditions (blue process path in Figure 1). Aerobic biological nitrification is well known and is accomplished by Nitroso-bacteria (*Nitrosomonas*) for NH_4-N oxidation to NO_2-N and by Nitro-bacteria (*Nitrobacter*) for NO_2-N oxidation to NO_3-N.

In the nitritation/denitritation process, NH_4-N is oxidized to NO_2-N, and then biologically reduced to N_2 by heterotrophic bacteria with the consumption of organic substrate in anoxic conditions (red process path in Figure 1). In the deammonification process, aerobic nitritation of NH_4-N to NO_2-N, accounts for the transformation of about 55% of the NH_4-N fed. The remaining NH_4-N is anaerobically oxidized with NO_2-N to N_2 gas in the *anammox* process. In practice, the process skips the need for complete NO_2-NO_3 oxidation, and subsequent NO_3 denitrification to nitrogen gas (green path in Figure 1). Compared to conventional nitrification and denitrification, the aeration and the carbon-source demand is reduced by over 50% and 100%, respectively. It should be noted that specific biomass production is higher in the conventional process than in the deammonification process (20 g $COD_{biomass}$ vs. 3 g $COD_{biomass}$ per mole of NH_4^+ degraded).

In the absence of dissolved oxygen, heterotrophic bacteria can oxidize organic substrate with NO_2-N or NO_3-N as electron acceptor to reduce oxidized nitrogen to N_2 gas. These reactions for NO_2-N and NO_3-N reduction are termed *denitritation* and *denitratation*, respectively. The stoichiometry of biological denitritation and denitratation reactions with acetate consumption and heterotrophic biomass growth are:

Denitritation *(nitrite reduction by heterotrophic bacteria)* (Equation 6).

$$1.0\ NO_2^- + 1.0\ H^+ + 0.24\ NH_4^+ + 0.975\ CH_3COO^- \rightarrow 0.5\ N_2 + 0.24\ C_5H_7O_2N + 0.015\ CO_2 + 0.735\ HCO_3^- + 1.235\ H_2O \quad (6)$$

Denitratation *(nitrate reduction by heterotrophic bacteria)* (Equation 7).

$$1.0\ NO_3^- + 1.0\ H^+ + 0.33\ NH_4^+ + 1.45\ CH_3COO^- \rightarrow 0.5\ N_2 + 0.33\ C_5H_7O_2N + 0.13\ CO_2 + 1.12\ HCO_3^- + 1.62\ H_2O \quad (7)$$

Therefore, 6.6 g of acetate COD are needed per g of NO_3-N denitrified (Equation 7). For denitritation of NO_2-N, about 30% less is needed, at 4.5 g acetate COD per g of NO_2-N denitrified (Equation 6).

Anammox involves the exoenergetic reaction of NH_4-N oxidation by NO_2-N, and uptake of CO_2 and nutrients by autotrophic *anammox* bacteria for biomass growth. The overall reaction, accounting for cell synthesis, was described in Equation 8 by Strous et al. (1998).

$$1.0\ NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (8)$$

During anaerobic oxidation of ammonia (Equation 8), some nitrates are formed from nitrites, which may provide reducing power for fixation of carbon dioxide (Schmidt et al., 2002). Eq. (8) also indicates that removal of 1.0 mole of NH_4-N requires 1.32 moles of NO_2-N and produces 0.26 moles of NO_3-N. Combination of nitritation and deammonification reactions gives the overall deammonification reaction (Equation 9).

$$1.0\ NH_4^+ + 0.804\ O_2 + 0.071\ HCO_3^- \rightarrow 0.436\ N_2 + 0.111\ NO_3^- + 0.009\ C_5H_7O_2N + 0.028\ CH_2O_{0.5}N_{0.15} + 1.038\ H^+ + 1.46\ H_2O \quad (9)$$

The stoichiometric ratio of nitrate production to ammonium consumption for the *anammox* bacteria is 1:0.382. In real applications, deammonification has demonstrated ammonia reductions of 90-95% and total nitrogen reductions of 80-85% (WERF, 2014).

Table 2 summarizes the benefits of the deammonification process in comparison to the conventional denitrification process.

Table 2. Comparison between deammonification process and conventional denitrification process (WERF, 2014).

Parameter	Deammonification	Nitritation/Denitritation (η=89%)	Nitrification/Denitrification (η=89%)
O_2 demand	1.84 gO_2 gNH_4-N^{-1} removed	2.65 gO_2 gNH_4-N^{-1} removed	3.3 gO_2 gNH_4-N^{-1} removed
Acetate-COD demand	0	4.5 g acetate COD gNO_2-N^{-1} removed	6.6 g acetate COD gNO_3-N^{-1} removed
Biomass production	0.12 gVSS gNH_4-N^{-1} removed	1.5 gVSS gNH_4-N^{-1} removed	1.93 gVSS gNH_4-N^{-1} removed

3.1.2. Deammonification technologies

Several technical solutions have been developed for the deammonification process. Table 3 shows the reported number of installations (as of 2014's end) under various technical proprietary solutions commercially available. These differ in terms of the method to grow and retain *anammox* bacteria, number of stages, configuration, control strategies, and include granular sludge blanket reactors (GSBRs), suspended growth sequencing batch reactors (SBRs), moving bed biofilm reactors (MBBRs), and rotating biological contactors (RBCs).

The first full-scale facility was built in 2001, and their number has rapidly increased since 2007, operating on side stream dewatering flows and some industrial applications. The DEMON® process has the most installations. The largest facilities are two 12,000 kgN d^{-1} ANAMMOX® plants in China. A larger 13,500 kgN d^{-1} DEMON® installation is under construction in the USA (WERF, 2014).

Table 3. Active deammonification facilities worldwide.

Technology Trade Name	Number*	Size [kgN d^{-1}]		First installation (year)
		Smallest	Largest	
ANAMMOX®	22	50	12,100	2002
ANITA™Mox	6	110	350	2010
DeAmmon®	3	130	2,455	2001
DEMON®	37	50	13,500	2004
Terra-N®	5	90	750	2008

*In operation or construction as of Oct. 2014 (WERF, 2014).

3.1.2.1. ANAMMOX® Granulated Sludge Blanket Reactor (GSBR)

Anammox bacteria can grow in granules readily settling by gravity. GSBRs take advantage of this by employing a high rate clarifier that captures and retains *anammox* granules, while flushing out competing bacterial flocs. Two arrangements are used: two-step and single-step.

Two-Step ANAMMOX® Process:

Developed by the Paques company, it uses the SHARON process (Single reactor system for High Activity Ammonium Removal Over Nitrite), a stable, partial nitrification to nitrite instead of nitrate (Hellinga et al., 1998), to convert ammonia to nitrite, followed by the *anammox* process (Figure 2A).

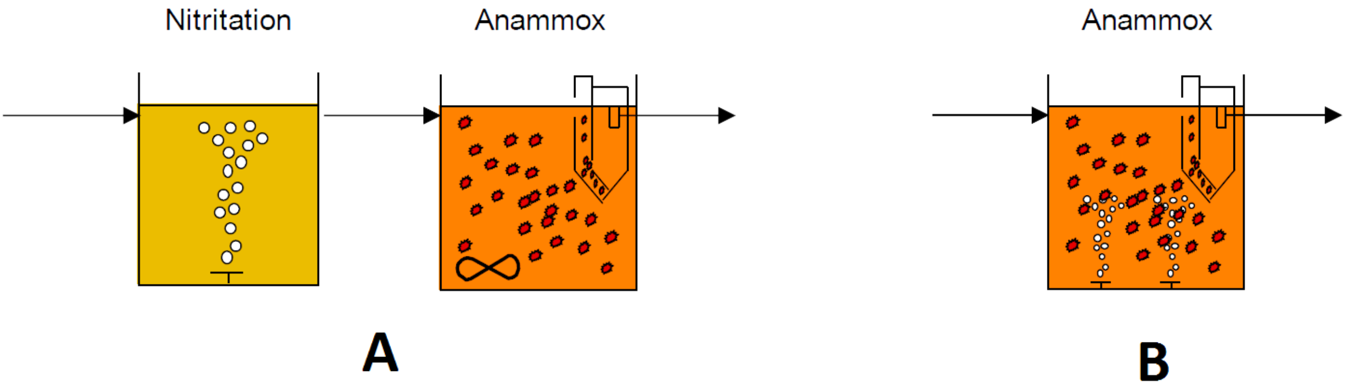

Figure 2. Two-step (A) and Single-step (B) *anammox* process.

This is optimized by controlling pH, DO, and by taking advantage of the higher growth rate of ammonia-oxidizing bacteria at higher temperatures (Van Loosdrecht and Jetten, 1998). Granular *anammox* bacteria are retained in the second reactor using up flow clarification. Their conversion capacity is high, up to 4.8 kgTN m^{-3} d^{-1}. This system was introduced in the Netherlands at several treatment plants, achieving 80% ammonia conversion to nitrogen gas at loading rate of 1.2 kgN m^{-3} d^{-1} (van Dongen et al., 2001). A system receiving 1,000-1,300 mgNH_4-N L^{-1} could produce an effluent with 5-10 mg NO_2-N L^{-1}, 60-130 mg NH_4-N L^{-1}, and about 130 mg NO_3-N L^{-1} (Abma et al., 2007).

The advantage of this arrangement is that the two main reactions (nitritation and deammonification) occur in separate reactors, allowing better control of each process. Its disadvantage is that reactor volumes are significantly larger due to the large nitritation reactor needed.

Single-Step ANAMMOX® Process:

In this process, nitritation and deammonification (*anammox*) occur in the same reactor (Figure 2B), making process control (pH, DO, and Oxidation-Reduction Potential - ORP) essential to prevent bacterial competition for nitrite (heterotrophic denitritation, and in particular autotrophic Nitrite Oxidizing Bacteria - NOBs), while maintaining a long solid retention time (SRT) for *anammox* bacteria. Ammonia reduction of 95%, with over 80% total nitrogen reduction, have been reported (Abma et al., 2010).

The advantage of this process is that single reactors are very compact due to high-granule concentration. Granular sludge growth protects the process against high nitrite levels. A disadvantage consists of requiring online control to achieve stable nitritation and deammonification in one reactor. Operational experience with this system has been quite positive in reported cases; therefore, single step ANAMMOX® is the most commonly used.

Incorporation of Anammox in mainstream processes

Incorporating *anammox* in mainstream processes is still an emerging technology. Figure 3 illustrates a possible process flowsheet including *anammox* return sludge treatment in a conventional WWTP (Wett et al., 2010).

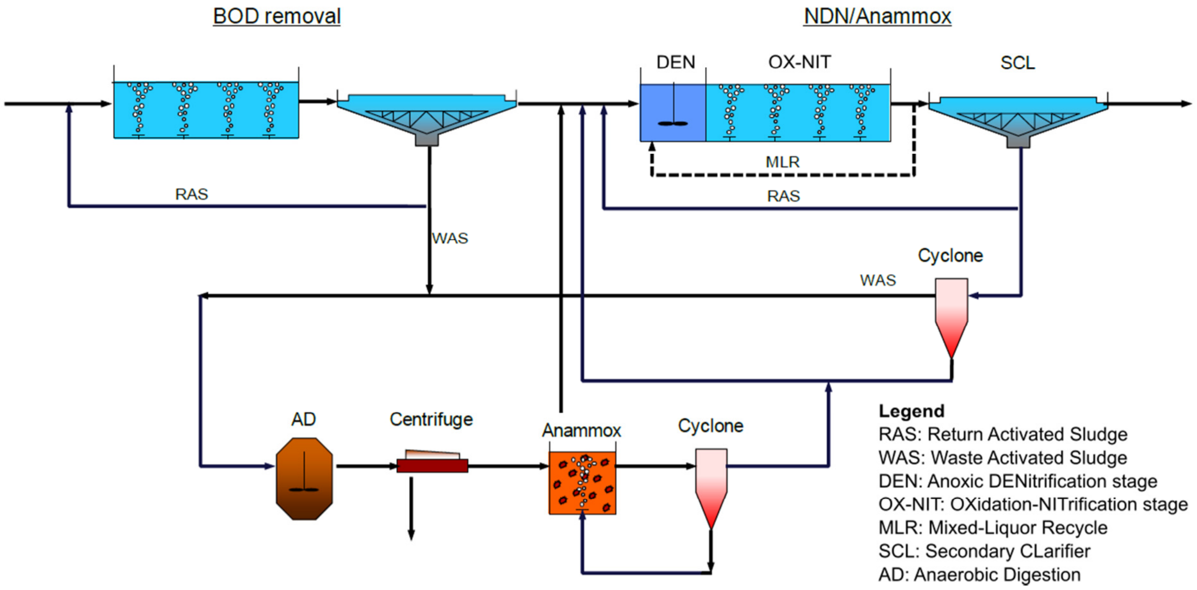

Figure 3. Integration of *Anammox* process in conventional processes (Wett et al., 2010).

3.1.2.2. DEMON® Sequencing Batch Reactor (SBR)

The DEMON® process (DE-amMONnification) employs aerobic nitrification to nitrite and anaerobic ammonia oxidation under controlled DO and pH conditions (Wett et al., 2010). The process uses a hydrocyclone to separate heavier granular *anammox* bacteria from floc-forming bacteria, allowing accumulation of NOBs, and performance stabilization.

Process control addresses three parameters: (1) time of filling and drawing the SBR (feed rate), (2) DO, and (3) pH, to balance the nitritation and *anammox* reactions (O'Shaughnessy et al., 2008). DO is set to a low value (around 0.3 mg L^{-1}) to prevent rapid nitrite production by AOB, and repress NOBs. Aeration cycles control also regulates pH: nitritation depresses the pH during aeration, while the *anammox* reaction increases pH in its absence. Aeration is initiated at the upper pH and stopped at the lower pH set points to maintain pH within 0.01 units (Wett et al., 2010). The nitritation step may suffer inhibition, leading to process instability.

3.1.2.3. Moving-Bed Biofilm Reactor (MBBR)

MBBR configurations using different types of support media are used for deammonification of high-strength, ammonium-rich recycle streams (Seyfried et al., 2001). AOB and *anammox* bacteria are established within the biofilm (Figure 4). The main technologies are DeAmmon®, ANITA™Mox and Terra-N® Process.

DeAmmon®

Developed by Purac/Läckeby AB (Sweden) in collaboration with the University of Hannover and Ruhrverband (the Ruhr River Association), consists of a single- or dual-train system with three stages per reactor, normally operated in series, with built-in flexibility to allow also parallel operation. For biofilm growth support, Kaldnes K1 filter media (AnoxKaldnes/Veolia) with active area of 500 m^2 m^{-3} is typically added to fill up to 40% of the reactor volume. To retain media within each stage, separating screens are placed between stages. Internal recirculation from the third to the first stage may be required for very high-strength streams, to limit ammonia concentration in the first stage. Intermittent aeration supporting partial nitritation and *anammox* reactions is provided by coarse bubble diffusers. Alternance of aeration/anoxic periods limits nitrite accumulation and restrict the growth of aerobic nitrite-oxidizing bacteria (Plaza et al., 2011; Thöle, 2007). DO concentration of

3 mg L^{-1} during aeration is considered appropriate for design and operation, but higher concentrations are avoided to prevent potential for NOB growth, and limit *anammox* inhibition. Mechanical mixers ensure well mixed conditions during non-aerated periods. Reactor pH is usually in the 7.3-7.7 range and not controlled. On-line conductivity measurements are a low-cost tool for performance monitoring and control (e.g. duration of aeration cycles).

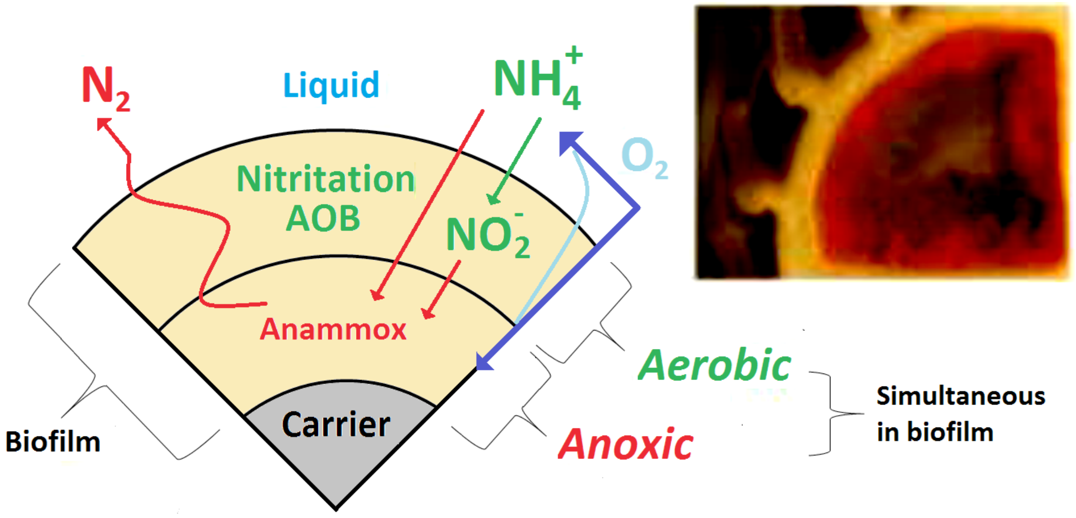

Figure 4. *Anammox* bacteria in biofilm (Source: Seyfried et al., 2001).

Inorganic nitrogen removal efficiency is usually in the range 70-85% and in the temperature range 25-30°C (Plaza et al., 2011). Minimum process operating temperature is 20°C, after which substantial performance deterioration occurs (Thöle, 2007). With Kaldnes K1 media, a design load of 0.6 kg-N m^{-3} d^{-1} can be assumed.

ANITA™Mox

ANITA™Mox is a single-stage deammonification MBBR system developed by AnoxKaldnes/Veolia. It uses *BiofilmChip™M*, a support medium with high specific surface area which allows achieving a volumetric ammonium-N removal rate of up to 1.2 kg-N m^{-3} d^{-1} with 40% media-filled volume in a temperature range of 27-30°C (Christensson et al., 2011). The specific ammonium removal rate is approximately 3 g-N m^{-2} d^{-1}, with ammonium-N and total inorganic nitrogen removal efficiencies of approximately 90% and 80%, respectively, at pH in the range 6.7-7.5. The MBBR is continuously aerated with DO concentration controlled in the range 0.5-1.5 mg L^{-1}. The reported energy consumption for the single-stage MBBR is 1.6-1.9 kWh kgN^{-1} removed (Christensson et al., 2011).

Terra-N® Process

Terra-N® Process, developed by Clariant/SÜD-Chemie AG (Munich, Germany), uses bentonite instead of plastic as support medium for biofilm growth. The process is designed as single-stage SBR, or as two-stages in series, with gravity clarification. The bentonite product contains a wide range of particle sizes, with mean 25-45 µm, and mean surface area of 60 m^2 g^{-1}, with lower active surface area for biofilm growth.

Bentonite is added at 10-12 g L^{-1} to the nitritation stage of the two-stage process, or in the single-stage SBR, resulting in a TSS concentration of 15-20 g L^{-1}, at full loading. Granulation of *anammox* bacteria in the second stage of the two-stage system eliminates the need for a support media in that stage. Second-stage biomass concentrations of 5-7 g L^{-1} have been reported.

The bentonite induces a high solids settling rate, and compact settled solids, resulting in insignificant losses of bentonite in the effluent. Biomass lost with the effluent by washout is sufficient to control the reactor solids concentration, so no additional solids wastage is normally necessary.

In SBR systems, aeration is intermittent and the aerobic/anoxic pattern is adjusted based on ammonium loading and reactor performance. Mechanical mixing is required to maintain solids in suspension during the SBR anoxic phases, and in the *anammox* stage of a two-stage system.

Many Terra-N® systems are retrofits of existing tanks, and operate at loading rates from 0.4 to 1.0 kg-N m^{-3} d^{-1} (WERF, 2014); however, with the SBR variant, higher loading rates up to 1.5 kg-N m^{-3} d^{-1} are possible. Inorganic nitrogen removal efficiencies are reported in the range 80-90%. Startup time for the SBR or the *anammox* stage (two-stage process) can be reduced to 60-90 d by seeding the reactors with *anammox*-enriched sludge from existing full-scale systems.

3.1.2.4. Benefits of deammonification

The benefits of deammonification can be summarized as:

- Energy savings: aeration energy needed for the process is about 55-60% of that needed for a conventional nitrification/denitrification process;
- No extra carbon is needed for nitrogen removal. Even if carbon is added to remove the nitrates produced from *anammox*, total carbon demand is still about 90% less than that used in the conventional nitrification/denitrification process;
- Greenhouse effects: the process is a net consumer of CO_2, compared to that released by heterotrophic bacteria in the conventional nitro/denitro process;
- Alkalinity demand for nitrogen removal is reduced by about 45%;
- The process sludge production is much less than in a conventional nitrification/denitrification plant.

3.2. Technologies for conventional denitrification with attached biological culture

3.2.1. Denitrification in submerged bio filters

Denitrifying filters have been utilized for wastewater treatment for a number of years. The combination of denitrification and solids removal was first patented in the 1970s. Since then, several companies have developed their own denitrifying filters. There are two main configurations for denitrification filters commercially available: down flow and up flow continuous backwash filters. Down flow denitrification filters operate in a conventional filtration mode and consist of media and support gravel laying on an underdrain. Manufacturers include Severn Trent Services (TETRA Denite®), F.B. Leopold Co. Inc. (elimi-NITE®), and Siemens Water Technology (Davco®). In up flow continuous-backwash filters, wastewater flows upward through the filter, countercurrent to the movement of the sand bed. Manufacturers include Parkson Corp. (DynaSand®), and Paques bv. (Astrasand®) (US-EPA 2007). Other configurations include Biologically Active Filter (BAF) with floating media, continuous backwashing moving bed filters, and nonbackwashing, and submerged filters. BAF with floating media includes systems with polystyrene, polypropylene, or polyethylene media, such as Kruger Biostyr®. These are backwashed using intermittent countercurrent flow, and are typically designed to also remove suspended solids to secondary levels. Continuous backwashing moving bed filters operate in up flow mode and consist of media heavier than water continuously moving countercurrent to the wastewater. Media is directed to a central air lift where it is scoured, rinsed, and returned to the top of the media bed. Well-known filters are DynaSand®, AstraSand®, and Centraflo®, typically designed to produce filter-quality effluent (<5 NTU). Nonbackwashing, submerged filters consist of submerged, static media and are often referred to as submerged aerated filters (SAF), although recent work applied this technology in anoxic conditions for denitrification. Solids are carried through the reactor and removed by a separate, dedicated solid-separation process (WERF, 2010).

Leopold elimi-NITE® 2.0 denitrification system reduces nitrate-nitrogen in wastewater effluent by converting it to nitrogen gas. The biological conversion is done in an attached growth, down flow, deep bed, mono-media filter. Oxygen is removed to create an anoxic environment for denitrification, and particulate matter as well as insoluble P is removed. The system adds a supplemental carbon source or microbiological food needed to metabolize nitrogen (Leopold, 2003).

BIOSTYR® is a process based on the biological filter principle to fix biomass in a biofilm combination. The bed is formed by special polystyrene balls with a diameter of 4.5 mm, kept inside the filter thanks to the filter-jets ceiling. Biomass development starts in the filter-bed, while removing nitrogen from sewage water. Periodical cleaning of the filter is made by purified wastewater stored over the filter ceiling. Dosing of an external substrate (methanol) is necessary for nitrate-nitrogen denitrification. Installation of a separation stage is not necessary, and the fixed biomass enables rather high volumetric loading. This technology is used for post denitrification, when a low concentration of TN is achieved and specific consumption of external substrate can be minimized.

Airlift reactors with biofilm attached on fixed supports exploit the different DO concentrations within the biofilm for nitrogen removal. In such systems, a Simultaneous Nitrification and Denitrification (SND) process occurs in the outer and inner part of the biofilm, respectively. The advantages of these reactors are the small volumes, a low-odor sludge due to high DO concentration, and low suspended solids content in the effluent (Luciano et al., 2012; Viotti et al., 2014).

3.2.2. Moving bed biofilm and fluidized bed biofilm reactors

A moving bed biofilm reactor (MBBR) is a process utilizing a basin filled with a carrier medium that promotes attachment and growth of biofilm carrying out biological treatment. MBBRs have been extensively used for BOD/COD removal, as well as for nitrification and denitrification in municipal and industrial wastewater treatment. Nitrification rates as high as 1.2 $gNH_4\text{-}N\ m^{-2}$ d were demonstrated in practical operation at low temperatures (11°C), while denitrification rates as high as 3.5 $gNO_3\text{-}N_{equiv.}m^{-2}\ d^{-1}$ have been reported. Depending on pretreatment needs, total hydraulic retention time (HRT) of a MBBR for N-removal will be in the range of 3-5 h (Ødegaard, 2006).

The carrier media is either in fixed form as sheets/rope or made by buoyant, loose plastic media requiring energy in the form of either aeration or mixing for buoyancy, showing at the same time durability by not so far requiring replacement as in first generation MBBRs built over 15 years ago (Rusten et al., 1996). The primary difference between BAFs and MBBRs is that an MBBR does not provide any filtration or require any backwash. Rather, a separation process downstream of the unit removes solids from the stream. Downstream separation technologies used include sedimentation, flotation, filtration, high-rate clarifiers, and membranes (WERF, 2010).

Fluidized Bed Biofilm Reactor (FBBR) is a treatment process that utilizes carrier media to promote attached growth like BAFs and MBBRs; similarly to MBBRs, FBBRs do not filter solids and require downstream solids separation. Carrier media selection governs process sizing: historically, carrier medium of choice has been either silica sand or granular activated carbon (GAC). (Coahelso et al., 1992). A FBBR operates in up flow mode with the media fluidized within the reactor as the drag force associated with the feed up flow exceeds the gravity force pulling down particles, and lifts the carrier media. This configuration (i) maximizes media exposure between attached biomass and the feed stream, (ii) avoids plugging, and (iii) provides scour to maintain a thin biofilm improving mass transfer. Despite small footprints, FBBRs suffer from scale-up issues and lack of commercial systems (Sutton and Mishra, 1994).

3.2.3. Membrane Biofilm Reactors (MBfR)

MBfR is an emerging technology for water and wastewater treatment, based on pressurized membranes that supply a gaseous substrate to a biofilm formed on the membrane's exterior. MBfR biofilms behave differently from conventional biofilms due to their counter-diffusion of substrates, and are suited for numerous treatment applications, including removal of carbon and nitrogen when oxygen is supplied, and reduction of oxidized contaminants (such as nitrate) when hydrogen is supplied. Major benefits include high gas utilization efficiency, low energy consumption, and small reactor footprints. MBfRs development still faces challenges, including biofilm management, design of scalable reactor configurations, and identification of cost-effective membranes (Martin and Nerenberg, 2012).

4. CONCLUSIONS

The paper presents some of the most recent advances in biological nitrogen removal. Both design criteria of conventional denitrification processes and technological innovations have been considered.

With reference to former, a new equation for SDNR applicable to reactor sizing was proposed, highlighting the influence of both Dissolved Oxygen (DO) and the F:M ratio in the denitrification reactor on process design. The adverse effect of DO in a conventional denitrification anoxic reactor is quite relevant, and its minimization, mainly by reduction in the mixed-liquor recycle, or by addition of a post-anoxic reactor (after pre-denitrification and oxidation-nitrification) should be pursued. An alternative for controlling DO in the denitrification phase is based on the addition of chemical reducing agents that can react with DO. Specifically, the most suitable reagent has proved to be a salt of ferrous iron, evidencing that such dosages can be very efficient in controlling DO, as it rapidly oxidizes ferrous to ferric ion, subsequently reacting with phosphorous and precipitating as orthophosphate.

Of great importance are also recent studies highlighting the limits of denitrification efficiency in small community plants, due to the strong load fluctuations. Effects of the hydrodynamic behavior of the denitrification reactor (complete-mixing or plug-flow) on the denitrification performance have been proven equally important.

The greatest technological innovation to date regards the increasing diffusion of "deammonification" processes, often better known by their patent names (ANAMMOX®, DEMON®, CANON®, ANITA® and others). Deammonification is achieved by bacteria capable of using nitrous ions as electron acceptors to degrade ammonia to nitrogen. The process reduces aeration and carbon-source demands by over 50% and 100%, respectively, compared with traditional nitrification-denitrification and has already found industrial applications in the treatment of high-strength wastewater such as digested sludge liquor and landfill leachate.

A promising new technology MBfRs is based on pressurized membranes supplying a gaseous substrate to a biofilm formed on the membrane's exterior. These can be used in both oxidizing (COD removal) and reducing (nitrate removal) processes depending on the substrate provided.

5. ACKNOWLEDGEMENT

Part of the work that led to this paper has been performed under the project No. LO1408 "AdMaS UP - Advanced Materials, Structures and Technologies" supported by the Czech Ministry of Education, Youth and Sports under the "National Sustainability Programme I".

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