**A review on nitrous oxide (N2O) emissions during biological nutrient removal from municipal wastewater and sludge reject water**

Theoni Maria Massara**a,b**, Simos Malamis**c**, Albert Guisasola**d**, Juan Antonio Baeza**d**, Constantinos Noutsopoulos**c**, Evina Katsou**a,b,\***

**a** Department of Mechanical, Aerospace and Civil Engineering, Brunel University London, Uxbridge Campus, Middlesex, UB8 3PH, Uxbridge, UK.

**b** Institute of Environment, Health and Societies, Brunel University London, Kingston Lane, Middlesex, UB8 3PH, Uxbridge, UK.

**c** Department of Water Resources and Environmental Engineering, School of Civil Engineering, National Technical University of Athens, 5 Iroon Polytechniou St., Zographou Campus, 15780, Athens, Greece.

**d** GENOCOV. Departament d’Enginyeria Química, Biològica i Ambiental, Escola d’Enginyeria, Universitat Autònoma de Barcelona, Cerdanyola del Vallés (Barcelona), 08193, Barcelona, Spain.

**\*Corresponding Author:**

Dr Evina Katsou

E-mail address: [Evina.Katsou@brunel.ac.uk](mailto:Evina.Katsou@brunel.ac.uk)

Full postal address: Office 234, Howell Building, Department of Mechanical, Aerospace and Civil Engineering, Brunel University London, Uxbridge Campus, Middlesex, UB8 3PH, Uxbridge, UK.

Tel.: +44 (0)1895 265721

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| **Nomenclature** |  |
| AMO | Ammonia monooxygenase |
| Anammox | Anoxic ammonium oxidation |
| AND | Alternating nitrification denitrification |
| AO | Anoxic Oxic |
| AOB | Ammonia oxidizing bacteria |
| AOR | Ammonia oxidation rate |
| AS | Activated sludge |
| ASM | Activated sludge model |
| ASMN | Activated sludge model for nitrogen |
| BNR | Biological nutrient removal |
| C | Carbon |
| CF | Carbon fibers |
| COD | Chemical oxygen demand |
| DFBBR | Denitrifying fluidized bed bioreactor |
| DO | Dissolved oxygen |
| F/M | Food to microorganisms ratio |
| FNA | Free nitrous acid |
| GHGs | Greenhouse gases |
| HAO | Hydroxylamine oxidoreductase |
| HRT | Hydraulic Retention Time |
| IC | Inorganic carbon |
| IPCC | Intergovernmental panel on climate change |
| IWA | International Water Association |
| MLE | Modified Ludzack-Ettinger |
| MLSS | Mixed liquor suspended solids |
| MLVSS | Mixed liquor volatile suspended solids |
| N | Nitrogen |
| NaR | Nitrate reductase (for denitrifiers) |
| NERC | Natural environment research council |
| NiR | Nitrite reductase (for denitrifiers) |
| NirK | Nitrite reductase (for AOB) |
| NLR | Nitrogen loading rate |
| NOB | Nitrite oxidizing bacteria |
| Nor | Nitric oxide reductase (for AOB) |
| NOR | Nitric oxide reductase (for denitrifiers) |
| N2OR | Nitrous oxide reductase (for denitrifiers) |
| OD | Oxidation ditch |
| PHAs | Polyhydroxyalkanoates |
| PNA | Partial nitritation anammox |
| SBR | Sequencing batch reactor |
| SND | Simultaneous nitrification denitrification |
| SS | Suspended solids |
| TN  TKN | Total nitrogen  Total Kjeldahl nitrogen |
| UK | United Kingdom |
| US | United States |
| vNLR | volumetric Nitrogen Loading Rate |
| VSS | Volatile suspended solids |
| WWTPs | Wastewater treatment plants |

**ABSTRACT**

Nitrous oxide (N2O) is an important pollutant which is emitted during the biological nutrient removal (BNR) processes of wastewater treatment. Since it has a greenhouse effect which is 265 times higher than carbon dioxide, even relatively small amounts can result in a significant carbon footprint. Biological nitrogen (N) removal conventionally occurs with nitrification/denitrification, yet also through advanced processes such as nitritation/denitritation and completely autotrophic N-removal. The microbial pathways leading to the N2O emission include hydroxylamine oxidation and nitrifier denitrification, both activated by ammonia oxidizing bacteria, and heterotrophic denitrification. In this work, a critical review of the existing literature on N2O emissions during BNR is presented focusing on the most contributing parameters. Various factors increasing the N2O emissions either per se or combined are identified: low dissolved oxygen, high nitrite accumulation, low chemical oxygen demand to nitrogen ratio, slow growth of denitrifying bacteria, uncontrolled pH and unmonitored temperature. However, there is no common pattern in reporting the N2O generation amongst the cited studies, fact that complicates its evaluation. When simulating N2O emissions, all microbial pathways along with the potential contribution of abiotic N2O production during wastewater treatment at different dissolved oxygen/nitrite levels should be considered. The undeniable validation of the robustness of such models calls for reliable quantification techniques which simultaneously describe dissolved and gaseous N2O dynamics. Thus, the choice of the N-removal process, the optimal selection of operational parameters and the establishment of validated dynamic models combining multiple N2O pathways are essential for studying the emissions mitigation.

**Keywords: BNR, N2O emission, nitritation/denitritation, Anammox, nitrifier denitrification, modeling**

1. **INTRODUCTION**

Wastewater treatment plants (WWTPs) are constructed to serve sanitary purposes and remove undesirable contaminants from sewage. Nevertheless, environmental concerns are raised over their energy consumption and emissions to the atmosphere (Hospido et al., 2004). Within the European Union alone, there is an important number of WWTPs with a total treatment capacity of over 400 million population equivalents. Thus, efforts for minimizing the related energy requirements (mainly linked to aeration) are essential (Mamais et al., 2015). The widely applied activated sludge (AS) process has been criticized as energy intensive (Hassani et al., 2011). Moreover, greenhouse gases (GHGs), i.e. nitrous oxide (N2O), carbon dioxide (CO2) and methane (CH4), are emitted during wastewater treatment (Lijó et al., 2017). In that sense, it is of particular interest to identify where and how the GHGs are generated within a WWTP. Regarding CO2, indirect emissions are estimated based upon the energy requirements of the plant, while direct emissions are calculated based on the processes occurring during all stages of the WWTP. CH4 is mainly emitted in the sewerage compartments as well as during the sludge treatment works. N2O is particularly significant considering that its greenhouse effect is much higher than the respective one of CO2 and its steady-state lifetime in the atmosphere is 114 years (IPCC, 2007). According to the Intergovernmental Panel on Climate Change (IPCC, 2013), CH4 and N2O have a global warming potential which is 28 and 265 times higher respectively than CO2 in a 100-year period. N2O reacts with the ozone of the stratosphere inducing the depletion of the ozone layer (Portmann et al., 2012). In addition, direct N2O emissions during wastewater treatment contribute by 26% to the total global warming potential of the water cycle (i.e. considered as the sum of drinking water production, wastewater treatment and effluent discharge, sludge processing and disposal) (Frijns et al., 2008). N2O emissions can be attributed to several processes taking place in a WWTP and present significant variation depending on the nitrogen (N) load, influent characteristics and operational/environmental conditions (Kampschreur et al., 2009; Yang et al. 2009; Ahn et al., 2010; Law et al., 2012a; Wang et al., 2014b; Li et al., 2015).

Conventional N-removal from wastewater takes place through the bioprocesses of nitrification and denitrification. Most of the N-amount entering the plant finally takes a gaseous form and is released into the atmosphere. Advanced biological processes for N-removal include nitritation/denitritation and the completely autotrophic N-removal over nitrite (NO2-). During these bioprocesses, increased amounts of N2O are likely to be produced leading to a rise in the atmospheric N2O concentration and the carbon (C) footprint of the WWTP (Tallec et al., 2006; Zhu et al., 2008; Yang et al., 2009).

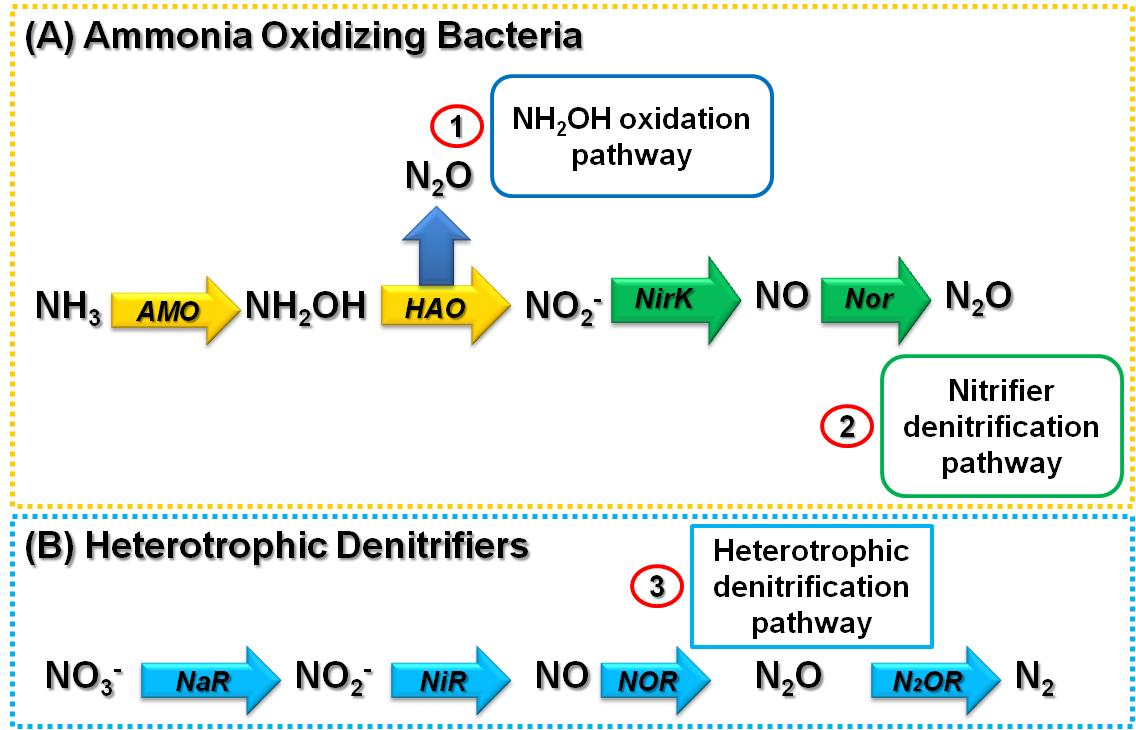
Hence, the aim of this work was: i) to introduce the mechanisms involved in N2O production during biological N-removal, ii) to review past studies which reveal the operational parameters (e.g. dissolved oxygen (DO), temperature, pH, concentration of N2O precursors, etc.) with the greatest impact on the N2O emissions during the biological nutrient removal from municipal wastewater and sludge reject water, iii) to outline methods concerning the N2O quantification, iv) to report the relevant mathematical modeling which has been developed for the simulation of N2O during the biological nutrient removal (BNR) processes, and v) to propose potential mitigation strategies.

Based on recent literature covering circa the last 5 years, this review provides a holistic and comprehensive perspective on the real on-field N2O emissions during the BNR in WWTPs. Our work acts as a continuation of reviews in the field (e.g. Kampschreur et al., 2009; Desloover et al., 2012; Law et al., 2012a; Ni and Yuan, 2015), covering all important aspects of N2O in BNR processes.

1. **MECHANISMS OF N2O PRODUCTION DURING BIOLOGICAL NITROGEN REMOVAL FROM SEWAGE**

There are several alternatives for N-removal from sewage, which involve different operational conditions and bacterial populations, leading to different N2O emission levels (Xavier et al., 2007; Ahn et al., 2008). During the conventional biological process of nitrification, ammonium (NH4+) is firstly oxidized to NO2- by the autotrophic ammonia oxidizing bacteria (AOB) and, afterwards, to nitrate (NO3-) by the autotrophic nitrite oxidizing bacteria (NOB). During denitrification, NO3- is reduced to NO2-, then NO2- to nitric oxide (NO), NO to N2O and, lastly, N2O to diatomic molecule N2 by heterotrophic bacteria (Kampschreur et al., 2009; Malamis et al., 2015).

Nitritation is the oxidation of ΝΗ4+ to NO2- and, denitritation is the reduction of NO2- to N2 (Turk and Mavinic, 1987; Frison et el., 2013). During the completely autotrophic N-removal, or deammonification as it is alternatively called, part of the entering ΝΗ4+ (around 60%) is oxidized to NO2- (Strous et al., 1998). The N-removal takes place via the anoxic ammonium oxidation (anammox) process; the anammox bacteria oxidize ΝΗ4+ (with NO2- as electron acceptor) to N2 and a part to NO3- in an anoxic environment (Malamis et al., 2015).



**Figure 1:** A simplified presentation of the biological N2O generation by: (A) AOB (NH2OH oxidation and nitrifier denitrification pathways), and (B) heterotrophic denitrifiers (heterotrophic denitrification pathway) (adapted from Ni and Yuan, 2015).

Based on the current knowledge of the processes, the biological pathways leading to the N2O generation are principally three: hydroxylamine (NH2OH) oxidation, nitrifier denitrification and heterotrophic denitrification (Wunderlin et al., 2012, 2013; Ni and Yuan, 2015). These processes are schematically presented in Figure 1. Regarding the N2O production by AOB: the first step is the oxidation of NH3 to NH2OH with the reduction of molecular oxygen; this process is catalyzed by ammonia monooxygenase (AMO). Afterwards, NH2OH is oxidized to NO2- by the aid of the hydroxylamine oxidoreductase (HAO) with oxygen functioning as the primary electron acceptor. At this point, N2O can be produced if NH2OH is not completely oxidized to NO2- (Figure 1: NH2OH oxidation pathway). The AOB-related enzymes also include an amount of nitrite reductase (NirK) and nitric oxide reductase (Nor). NirK catalyzes the NO2- reduction to NO and Nor the NO conversion to N2O (Figure 1: nitrifier denitrification pathway). Although nitrifier denitrification is not an important bioprocess in terms of total N (TN) removal, it can be critical in terms of N2O emissions (Hooper et al., 1997; Poughon et al., 2000; Chandran et al., 2011; Stein, 2011a,b; Law et al., 2012a; Ni and Yuan, 2015).

As shown in Figure 1, N2O is also an intermediate product of heterotrophic denitrification (von Schulthess and Gujer, 1996; Pan et al., 2012, 2013a; Ni and Yuan, 2015). It is a 4-step chain of reactions with three intermediate products: NO2-, NO and N2O (Figure 1: heterotrophic denitrification pathway). The steps are catalyzed by the following enzymes: the nitrate reductase (NaR), the nitrite reductase (NiR), the nitric oxide reductase (NOR) and the nitrous oxide reductase (N2OR) (Ni and Yuan, 2015).

The heterotrophic denitrification, if not disturbed and stopped at the N2O formation stage, serves as a mechanism of N2O consumption. The two AOB metabolic routes are regarded as the major N2O hotspots and can occur simultaneously. The conditions which determine the AOB predominant pathway in each case should be examined.

Chemical N2O and NO production is also possible. However, the percentage of N2O emitted due to chemical processes in biological systems is still under research and is considered to depend upon various factors (e.g. influent N-content, pH). In any case, the knowledge acquired through the current research suggests that most of the N2O emitted in WWTPs is generated during biochemical processes. Therefore, this review does not focus on the purely chemical pathways as they are not regarded as significant N2O and NO contributors in these scenarios (Schreiber et al., 2012).

1. **PARAMETERS WHICH IMPACT ON N2O EMISSIONS**

This section includes a comprehensive review on the lab-, pilot- and full-scale N2O emissions reported in literature in order to better understand the causes of these emissions and obtain some guidelines on the potential strategies for their mitigation. The N2O emissions in a WWTP are subjected to variations due to numerous factors, such as the nitrogen loading rate (NLR) (e.g. Frison et al., 2015), the bioreactor configuration characteristics along with the applied operational conditions (e.g. Pan et al., 2016), the choice upon the BNR processes (conventional or advanced) (e.g. Ahn et al., 2011), the DO (e.g. Tumendelger et al., 2014), the C-source (e.g. Zhu and Chen, 2011), the pH (e.g. Li et al., 2015), the temperature (e.g. Adouani et al., 2015), etc., which are analyzed below. A synthetic description of the effect of each parameter along with short illustrations of relevant references are provided in the text; all the chosen references are summarized in Table 1.

**3.1 Nitrogen loading rate**

Here the potential influence of the NLR on the N2O emission is explored. Quan et al.(2012) utilized three lab-scale aerobic granular SBRs for the treatment of a mixture of municipal wastewater and liquid pig manure digestate. N2O was measured in 3 10-day operational periods with NLRs of 448.5, 321.2 and 224.2 mg L-1 d-1, corresponding to Chemical Oxygen Demand to Nitrogen (COD:N) ratios of 1:0.22, 1:0.15 and 1:0.11, respectively. The respective maximum N2O emission factors were 8.2%, 7% and 4.4%, suggesting that lowering the NLR or, equivalently, raising the COD:N provoked less inhibition to the heterotrophic denitrification process. As a result, N2O consumption through heterotrophic denitrification was enhanced.

Frison et al. (2015) studied a pilot-scale nitritation-denitritation SBR treating the reject water produced from the anaerobic co-digestion of sewage sludge and the organic fraction of municipal solid waste. Two different NLRs were tested; one 35% higher than the systems N-removal capacity (2,077 mg L-1 d-1) and one close to the system’s treating capacity (1,080 mg L-1 d-1). The corresponding N2O emission factors were 1.49% and 0.24%. The authors noted that the application of an NLR respecting the system’s N-removing potential can contribute to the decrease of NH4+ and NO2- accumulation and, finally, to lower N2O production via the nitrifier denitrification pathway.

In the pilot-scale oxidation ditch (OD) implemented by Zheng et al. (2015), the effect of imposing an initial NH4+ concentration 5 times higher than usual was examined. Under normal conditions, the N2O emission factor was as low as 0.027%. The N-overloading caused an abrupt increase of the emissions by 39.31% (monitoring zone with DO stabilized at 0.81 mg L-1 after the shock) and 113.06% (monitoring zone with DO stabilized at 0.17 mg L-1 after the shock). The NH4+ overload provoked a DO decline in each affected zone along with NO2- accumulation. Thus, N2O production through nitrifier denitrification was favored to a different extent at each zone depending on the local DO. Considering all these findings, the NLR can indeed constitute a factor hindering the completion of the nitrification and denitrification processes if it exceeds the system’s N-removal capacity. N overloading can enhance the N2O generation under the combination of other crucial parameters related to the activation of the N2O production pathways, such as the low DO.

**3.2 Bioreactor configuration characteristics and operational conditions**

In this section, the attention is drawn on the effect that the bioreactor configuration characteristics along with the applied operational conditions can have on the N2O emissions.

*3.2.1 Suspended-growth systems*

Sun et al*.* (2014) explored the magnitude of N2O emissions in three different full-scale wastewater treatment processes which included an anoxic-oxic (AO) process, a sequencing batch reactor (SBR) and an OD. In all cases, DO control at a proper level during nitrification in addition to the enhanced organic C utilization during denitrification were suggested as mitigation measurements ensuring the completion of nitrification-denitrification and, consequently, the minimization of NO2- and N2O accumulation. The authors observed significantly lower N2O emission in the OD process (0.25% of influent N), compared to the SBR (2.69% of influent N) and the AO process (1.37% of influent N). The OD process was found to be optimal in terms of N2O emissions. The latter is in accordance with the study by Zheng et al. (2015) where the implemented pilot-scale OD had remarkably low N2O emissions (0.027% of influent N). The taxonomic analysis within the OD revealed the abundance of NOB and denitrifying bacteria. The significant NOB population was a sign of avoided short-cut nitrification and less NO2- accumulation; consequently, there was less N2O production through the NO2- pathway. Furthermore, the considerable number of denitrifiers facilitated the N2O consumption through denitrification.

In the AOB-enriched lab-scale SBR system by Xie et al. (2012), nitrifier denitrification with NO2- as the terminal electron acceptor was the N2O hotspot under low DO conditions; the emissions accounted for 0.44% of the total NH4+ oxidation. The high NLR of the reactor (2,000 mg N L-1 d-1) in this low-DO environment (DO<0.5 mg L-1) with a microbial population enriched in AOB created the conditions for nitritation and the subsequent NO2- accumulation. Pijuan et al. (2014) studied a continuous granular airlift nitritation reactor at high DO (>4.5 mg L-1). The pilot-scale reactor was operated as a SBR towards the end of the study to evaluate the potential difference in N2O emissions. An important increase in the emissions was observed when shifting from airlift (2.2% of the oxidized N) to SBR operation (19.3±7.5% of the oxidized N). This significant difference was probably related to the operational mode of the SBR; the feeding phase was fast (only 6 min of duration) and induced high N2O emissions during the first part of the cycle. These emissions were possibly linked to the NH2OH oxidation pathway because they appeared during periods with high NH4+ concentration in the reactor. In the full-scale AS system studied by Tumendelger et al. (2014), high DO (2.5-3 mg L-1) favored the NH2OH oxidation pathway for the N2O generation and the N2O emission factor was 0.14% of the influent NH4+. In lower DO (1.5-2 mg L-1), N2O was mainly produced through the nitrifier denitrification pathway and the N2O emission factor was reduced to 0.03% of the influent NH4+. The study by de Mello et al. (2013) in a full-scale AS WWTP with a sequence of aerated and non-aerated stages showed that N2O was produced through nitrifier denitrification and emitted in both phases. However, the emission was higher during the aeration (0.1% of influent TN) because N2O was transferred from the liquid phase to the atmosphere due to stripping. Finally, Pan et al. (2016) investigated the N2O emission of a full-scale two-step plug-flow reactor treating domestic-municipal wastewater. The N2O emission factor of the 1st and the 2nd step differed significantly: 0.68%±0.09% (1st step) and 3.5%±0.49% (2nd step). The wastewater introduced as influent in the 2nd step caused dilution and resulted in a 40% lower mixed liquor volatile suspended solids (MLVSS) content. The higher specific ammonia oxidation rate (AOR) in the 2nd step facilitated the N2O production through the AOB pathways.

*3.2.2 Attached-growth systems*

Eldyasti et al. (2014) found that the increased biofilm thickness in a lab-scale fluidized bed bioreactor led to decreased N2O emission: the emissions were 0.53% of the influent TN for a biofilm thickness of 680 μm and 1.57% of the influent TN for a biofilm thickness of 230 μm. With the greater biofilm thickness, the slow growth denitrifiers were better retained and N2O reduction to N2 was enhanced.

In granular systems, both autotrophic nitrifiers and heterotrophic denitrifiers can be hosted. As a result, simultaneous nitrification-denitrification (SND) is possible (Quan et al., 2012). In the lab-scale study by Quan et al.(2012) three aerobic granular SBRs were set up for the treatment of a mixture of municipal wastewater and liquid pig manure digestate at three aeration rates (0.2, 0.6 and 1.0 L air min-1) and three COD:N ratios (1:0.22, 1:0.15 and 1:0.11). With NO2- as the only N-source, the specific N2O generation rates via denitriﬁcation were 1.7, 1.6 and 1.3 mgN2O g-1SS min-1 at the aeration rates of 0.2, 0.6 and 1.0 L air min-1, respectively, which were 40.9%, 44.8%, 39.9% higher than the respective ones with NO3- as the only N-source. The results indicated that: 1) the spatial structure of the granules created the conditions for incomplete denitrification which resulted in N2O production, 2) the N2O generation through the aerobic granules was mostly affected by NO2-, 3) the NO3- reduction rates were lower than those with NO2-, explaining the lower N2O production with NO3- as the only oxidized component.

It cannot be fully guaranteed that the aerobic granular structure provides the anoxic or, at least, the micro-aerobic conditions required for the completion of denitrification (e.g. study by Quan et al., 2012). There are certain bioreactor types which can potentially reinforce the denitrifiers’ activity and facilitate N2O consumption through denitrification such as the biofilm reactors or the ODs (e.g. studies by Eldyasti et al. (2014) and Sun et al. (2014)). However, it appears that selecting the optimal operational conditions (e.g. DO, aeration rate, phase duration, etc.) upon a certain configuration is more important. DO, for example, has an outstanding effect on the nitrification process, thus affecting the AOB-related N2O production pathways. Moreover, the aeration regime impacts on the N2O stripping and the overall C-footprint of the plant. Hence, mitigation strategies lie principally in the optimal combination of the operational parameters rather than in the configuration type.

**3.3 Biological nitrogen removal alternatives**

Here the emphasis is placed on how the choice upon the biological N-removal process impacts on the operational conditions (e.g. DO levels, NO2- concentration, etc.) and, thus, on the final N2O production. Various different processes are debated: nitrification-denitrification, SND, nitritation-denitritation and partial nitritation-anammox.

*3.3.1 Nitrification-denitrification*

Regarding the conventional biological N-removal process, nitrification is likely to constitute a N2O hotspot as it was shown in the studies by Shen et al. (2013), Sun et al.(2014) and Li et al. (2015). Sufficient DO is critical to ensure the completion of the nitrification process and, subsequently, the avoidance of N2O accumulation. N2O is also an intermediate product of denitrification highly generated in the event of DO presence, low temperatures, high NO2- levels, etc. The extension of an anoxic phase can give the heterotrophic denitrifiers enough time to use the available C and successfully perform all the denitrification steps; in this way, heterotrophic denitrification serves as a way to consume N2O. The safe completion of both processes (i.e. nitrification and denitrification) reduces the possibility of N2O being accumulated as an intermediate product and then being emitted (Gabarro et al.,2014; Sun et al.,2014; Adouani et al., 2015). As far as the AOB pathways are concerned, nitrifier denitrification is predominant under low DO, high NO2- levels and possible N-overloading of the system (Kampschreur et al.,2008; Wang et al., 2014a; Peng et al., 2015a, 2015b; Zheng et al., 2015). Nevertheless, determining the predominance between the two AOB pathways (i.e. NH2OH oxidation and nitrifier denitrification) is not always possible (Law et al., 2011; Rodriguez-Caballero et al., 2015).

The combined effect of DO and NO2- on an enriched nitrifying culture was investigated by Peng et al. (2015a); nitrifier denitrification was predominant for a wide range of DO and NO2- levels. On the other hand, the NH2OH oxidation pathway was dominant only at a combination of high DO (e.g. 3.5 mg L-1) along with low NO2- concentration (e.g.<10 mg L-1). Chen et al. (2014) examined the nitrification-denitrification processes in a lab-scale SBR treating municipal wastewater with an alternation of anaerobic-oxic-anoxic phases. N2O emissions occurred mostly in the oxic phase due to the AOB nitrifying activity. By cancelling the anaerobic phase and extending the idle phase (i.e. the phase between the removal of the treated effluent and the beginning of the subsequent cycle) in the SBR, the authors observed the heterotrophic denitrifiers being benefited and the N2O emissions decreasing by 42%; N-removal was mostly happening through heterotrophic denitrification, and N2O emissions decreased by their consumption to produce N2. Rodriguez-Caballero et al.(2015) analyzed aeration strategies in a full-scale SBR treating municipal wastewater in order to achieve the N2O mitigation and the minimization of the plant’s C-footprint. The implementation of a cycle configuration with intermittent aeration (with a sequence of 20-30 minute oxic followed by short anoxic phases) was found to be an effective mitigation strategy both in terms of N2O emissions and energy requirements. The emissions dropped because N2O was consumed along with its predecessors (i.e. NO and NO2-) through the heterotrophic denitrification taking place between the aerobic phases. On the contrary, long oxic periods enhanced N2O emission. However, it should be noted that the authors operated the SBR at very high hydraulic retention time (HRT) (1.5 days) for municipal sewage, resulting in a low N-loading rate.

The denitrifying bacteria can denitrify even in a micro-aerobic environment. Thus, if the conditions allow it, nitrification and denitrification can simultaneously happen, especially in aggregates such as the activated sludge where aerobic and micro-aerobic conditions can exist (Krul and Veemingen, 1977; Ahn et al., 2010). Consequently, the SND systems have low DO in addition to low NO2- or NO3- levels. According to past studies, the presence of NH4+ and NO2- under SND conditions increased the N2O emissions; in these cases, N2O emissions were likely to occur through all the possible pathways (NH2OH pathway, nitrifier denitrification and heterotrophic denitrification) (Kim et al., 2010; Wunderlin et al., 2012; Lochmatter et al.,2013).

*3.3.2 Nitritation-denitritation*

A very common alternative for BNR processes is short-cut nitrification (nitritation) during which the AOB oxidize the NH4+ only to NO2-; then, the NO2- should be further denitrified (denitritation), thus by passing the formation of NO3-. The advantages of this process compared to conventional nitrification-denitrification are theoretically 25% lower oxygen consumption in the aerobic phase, 40% lower COD demand in the anoxic stage, lower sludge production and a faster denitrification rate (1.5-2 times quicker) (Gustavsson, 2010). As a consequence, short-cut nitrification reduces energy and external C-requirements, enables N-removal in wastewaters with low COD:N ratios and requires a smaller anoxic tank (Turk and Mavinic 1987). The fact that these systems can operate at low DO enables the AOB population to prevail over the NOB. They also present high NO2- concentration as a product of nitritation. In other words, nitritation-denitritation systems constitute a priori an unattractive alternative in terms of N2O emissions. Ahn et al. (2011) compared nitrification and nitritation in terms of N2O and NO generation in a lab-scale bioreactor treating high-strength wastewater (reactor fed with a nutrient medium containing 500 mg L-1 of NH4+). Both the N2O and the NO emissions were statistically higher during nitritation because low DO (1.1±0.38 mg L-1) conditions and high NO2- concentration (91±5.8% NH4+ conversion to NO2-) promoted the NirK and Nor enzymes’ activity and, thus, the N2O production through the nitrifier denitrification pathway. Wang et al. (2014b) worked on a lab-scale nitritation system treating anaerobic sludge digestion liquor. The NO2- produced during the nitritation was a key factor for the N2O production; it was later reduced to N2O through heterotrophic denitrification. By imposing an optimal DO (i.e. around 1 mg L-1), the authors were able to minimize the denitrification of NO2- to N2O. Pijuan et al. (2014) studied a pilot-scale continuous granular airlift nitritation reactor with focus on the DO effect. N2O emissions decreased from 6% to 2.2% of the oxidized N when DO increased from 1 to 4.5 mg L-1. However, at DO>4.5 mg L-1 N2O emissions remained constant at 2.2%, suggesting two different mechanisms behind N2O production, one DO-dependent (e.g. nitrifier denitrification) and one not (possibly chemical). Surprisingly, other changes related to NH4+, NO2-, ammonia (NH3) and nitrous acid (HNO2) concentrations had no effect on N2O emissions within the range of concentrations tested. In a review of full-scale BNR plants, Desloover et al. (2012) put the accent on NO2-; NO2- accumulated after nitritation was regarded as a key factor triggering the N2O generation via the nitrifier denitrification pathway in a low-DO environment. Frison et al. (2015) put into practice a pilot-scale nitritation-denitritation process for the treatment of reject water produced from the anaerobic co-digestion of sewage sludge and the organic fraction of municipal solid waste. In accordance with Desloover et al. (2012), Frison et al. (2015) noticed that avoiding excessive NO2- accumulation in the mixed liquor and maintaining the DO at 1.5 mg L-1 or above during the nitritation process were important operating factors in order to limit the N2O emissions. Gabarro et al. (2014) applied nitritation-denitritation for the treatment of industrial wastewater in a pilot-scale SBR under alternating anoxic-aerobic conditions. They concluded that N2O was mainly produced during the anoxic phases through the heterotrophic denitrification pathway.

*3.3.3 Partial nitritation-anammox*

In the partial nitritation-anammox system, AOB partially oxidize NH4+ to NO2-. Then the anammox bacteria oxidize the remaining NH4+using NO2- as electron acceptor. A major drawback of this process is that the growth kinetics of anammox bacteria is very slow and highly dependent on environmental changes. Anammox bacteria are inhibited by several parameters including DO, temperature, free NH3 and NO3- concentrations (Malamis et al., 2015; Ibrahim et al., 2016; Laureni et al., 2016). Partial nitritation-anammox process can be applied in one or two stages. The step which is mostly prone to N2O generation is partial nitritation, during which part of the NH4+ is oxidized to NO2-, usually under low DO conditions. Although it can be initially considered as an energy-saving option due to the reduced aeration requirements, the potential high N2O production (through nitrifier denitrification after the NO2- accumulation) can be finally translated into a high overall C-footprint of the process. The anammox step is the ΝΗ4+ oxidation (with NO2- as electron acceptor) partly to N2 and partly to NO3-; thus, it is not expected to be a primary N2O hotspot in the process. Therefore, anammox can be considered as a potential N2O emission mitigation actor since it consumes NO2-, the starting point of many N2O production mechanisms. In a full-scale two-stage partial nitritation-anammox system treating reject water, Kampschreur et al.(2008) observed lower N2O generation in the anammox reactor (0.6% of influent N) compared to the nitritation reactor (1.7% of influent N). Considering that AOB originating from the nitritation reactor were possibly transferred to the anammox reactor, it was suspected that nitrifier denitrification, under the presence of NO2- and low DO, was the most likely N2O pathway for both reactors. Castro-Barros et al. (2015) examined the application of partial nitritation-anammox at a full-scale scheme for the treatment of sludge digestion reject water with a special attention to the effect of different aeration patterns. During the non-aerated period, significant ΝΗ4+ accumulation (up to 0.107 kg N m-3) occurred. When the aeration started to resume, the ΝΗ4+ oxidation rate increased and the highest N2O formation rate was observed (0.064 kg N m-3 d-1). It was suggested that aerobic conditions along with the high ΝΗ4+ availability triggered the NH2OH oxidation pathway.

It can be concluded that even when an advanced N-removal process (e.g. nitritation-denitritation or partial nitritation-anammox) is applied with the view to reducing the energy demands, N2O emissions still occur and can even be higher than the ones observed during conventional nitrification-denitrification. The N-removal via NO2- has lower oxygen and C-source requirements and, thus, a lower energy demands. However, it also results in increased N2O emissions compared to the conventional processes. The latter implies that the final overall C-footprint of an advanced N-removal process can be higher than the respective one of a conventional process. It seems that it is necessary to optimize the operation of an advanced N-removal scheme to ensure the emission mitigation and the maintenance of the total C-footprint at the desired levels.

**3.4 Carbon source**

At this section, the potential effect of the C-source composition and availability on the N2O generation is discussed. Zhu and Chen (2011) compared the effect of two different C-sources, sludge fermentation liquid and acetic acid, on the N2O generation in an anaerobic-aerobic lab-scale process. N2O emissions were decreased by 68.7% with sludge fermentation liquid used as C-source in comparison to the observed N2O generation with acetic acid as C-source. The presence of copper ion (Cu2+) along with propionic acid in the sludge fermentation liquid affected the denitrifying enzymes activity and induced the decrease of the following ratios of activity: NiR/NOR and NOR/N2OR (see Figure 1). Thus, both the NO and the N2O production during denitrification decreased. Hu et al. (2013) implemented 3 lab-scale anoxic/oxic SBRs to investigate the effect of 3 different carbon sources (i.e. sodium acetate, glucose and soluble starch) on the N2O emission. The N2O conversion ratio (defined as the percent of the TN removed which is converted to N2O) was 8.8% with sodium acetate, 5.3% with glucose and 2.8% with soluble starch, respectively. The microbial analysis showed that the denitrifiers diversity was significantly lower in the SBR with sodium acetate, thus facilitating N2O emission through the heterotrophic denitrification pathway. The comparison between a methanol-fed and an acetate-fed lab-scale anoxic/aerobic AS system in the study by Song et al. (2015) revealed lower N2O emissions in the acetate case. The N2O emission factor was 2.3% of influent N for the methanol fed process, and 1.3% of influent N for the acetate fed process. The results were explained through a microbial analysis which showed that the biomass became more abundant in bacteria capable of reducing N2O with acetate as C-source. In the lab-scale partial nitrification SBR system of Zhang et al. (2016), the effect of using mannitol instead of sodium acetate as carbon source was evaluated. The N2O conversion rate was 21% and 41% with mannitol and sodium acetate, respectively. Microbial analysis showed that mannitol lowered the N2OR enzyme (see Figure 1) inhibition caused by the high NO2- concentration in the partial nitrification system, thus enhancing the completion of heterotrophic denitrification.

According to Peng et al. (2015b), the alkalinity, mostly attributed to the inorganic carbon (IC), must be considered for the N2O emission mitigation. Under restricted IC presence, the carbonic anhydrase (enzyme) is activated and is utilized by *Nitrosomonas europaea* to produce ΝΟ from NO2-. Consequently, a limited IC provision can lead to greater NO production (this stage is catalyzed by the carbonic anhydrase) and, subsequently, to higher N2O generation after the reduction of NO to N2O (this stage is catalyzed by the ΝΟ reductase NOR) (Jahnke et al., 1984; Peng et al., 2015b). Conversely, Peng et al. (2015b) observed a linear relationship between the N2O production and the IC concentration (up to an IC concentration equal to 14 mmol L-1), which was expressed by the lower N2O generation under limited IC conditions. The different AOB population with lower content in *Nitrosomonas europaea* can justify the poor carbonic anhydrase activity in this study.

Finally, when the availability of external substrate is limited, internally stored compounds (e.g. polyhydroxyalkanoates (PHAs)) can be used instead; this can potentially induce increased N2O emissions during denitriﬁcation (Schalk-Otte et al., 2000). In the lab-scale SBR study by Zhou et al. (2012), the effect of different COD:N ratios and carbon source types (i.e. external: sodium acetate; internal: PHAs) on N2O consumption through denitrification was explored. Generally higher N2O reduction rates during denitrification were observed at higher COD:N ratios. The latter was slightly more noticeable with the use of external carbon source. The COD:N ratio increase from 0.625 to 1.25 induced an N2O reduction rate increase from 0.036 to 0.04 mg N2O-N min-1 g biomass-1 with the utilization of internal carbon source. Under the provision of external carbon source, N2O was hardly reduced (N2O reduction rate: 0.0025 mg N2O-N min-1 g biomass-1) at the lowest COD:N ratio (i.e. 0.625). The COD:N ratio rise to 1.25 or, equivalently, the gradually higher (external) carbon source availability for denitrification, led to a more pronounced increase in the N2O reduction rate (0.02 mg N2O-N min-1 g biomass-1). As soon as the COD:N reached 1.875, comparable N2O reduction rates (i.e. 0.045 mg N2O-N min-1 g biomass-1) were noted for both carbon source types. All in all, the system presented the trend of achieving a sharper increase in the N2O reduction rate under higher COD:N ratios and the use of external carbon source.

All in all, it is clear that C-source composition and amount can play a significant role in the N2O emission mitigation. For example, a C-source rich in Cu2+ (e.g. sludge fermentation liquid) or the use of mannitol instead of sodium acetate boost the denitrifying enzymes’ activity. Moreover, certain types of C-source (e.g. acetate in comparison to methanol) appear to be more beneficial to the denitrifying bacterial growth. In addition, it is essential to provide an adequate amount of external C-source so that the denitrifying bacteria are able to perform denitrification till the final stage (i.e. N2 formation) without having to resort to the consumption of internal carbon source (e.g. PHAs). The latter is important because the utilization of internally stored compounds can potentially lead to a lower N2O consumption rate during denitrification. Lastly, the role of IC is still not clear, as contradictory results have been reported.

**3.5 pH and temperature**

This section explains the impact of pH and temperature on the generation of N2O. Law et al. (2011) investigated the N2O production in a partial nitritation SBR with an enriched AOB culture treating sludge reject water under a pH fluctuating from 6.0 to 8.5. The minimum N2O generation was observed at a pH ranging between 6.0 and 7.0 (0.15±0.01 mg N2O-N h-1 g-1 VSS), whereas the maximum (0.53±0.04 mg N2O-N h-1 g-1 VSS) at pH=8.0. The authors discovered a linear relation between the AOR and the N2O production within the tested pH range. Under the low DO of the study (0.55±0.05 mg L-1) and the increasing AOR, it is possible that more electrons became available for N2O production through nitrifier denitrification. Li et al. (2015) investigated the pH effect on the N2O accumulation for a domestic-municipal SBR performing nitrification. At a stable DO (3 mg L-1), the maximum N2O accumulation (0.26 mg g-1 MLSS L-1 h-1) occurred at the lowest applied pH for a tested range of 6.0-8.5. On the contrary, the AOR increased with the pH increase, reaching its maximum value of 3.8 mg g-1 MLSS L-1 h-1 at the maximum applied pH (pH=8.5). In contrast to what was reported by Law et al. (2011), Li et al. (2015) explained their results by assuming that the electrons which became available with the AOR increase were mainly used for the reduction of O2 to H2O and less for nitrifier denitrification. Thus, it is possible to observe no correlation between the NH4+ oxidation and the N2O accumulation if the AOR is strongly influenced by the pH.

Adouani et al. (2015) studied the impact of temperature on N2O emissions during denitrification in a batch reactor fed with a synthetic solution containing acetate, NO3- and AS. Their results showed that the N2O generation increased as the temperature was decreasing; the N2O emissions rose from 13% to 40% and then to 82% of the total denitrified N at 20ºC, 10ºC and 5ºC respectively. The low temperatures decelerated all denitrification enzyme activities and, more importantly, the NO and N2O reductase activities. Consequently, significant amounts of N2O were produced because of the incomplete denitrification. Poh et al. (2015) conducted batch experiments on mixed liquor to see the effect of increasing temperature on the N2O accumulation during denitrification. As soon as the temperature rose from 25ºC to 35ºC, the specific NO3-, NO2- and N2O reduction rates showed an increase of 62.06% (from 5.8 to 9.4 mg N g-1 VSS h-1), 61.22% (from 4.9 to 7.9 mg N g-1 VSS h-1) and 41.25% (from 8 to 11.3 mg N g-1 VSS h-1), respectively. However, at 35ºC, N2O became less soluble in the mixed liquor, which meant that stripping was happening more intensively. The authors noted that it is difficult for N2O gas to be re-dissolved. As a result, the amount of dissolved N2O available for denitrification by denitrifiers was continuously decreasing as the experiment was proceeding. Thus, although higher temperatures are applied to enhance the denitrification kinetics, they are likely to generate more emissions in the end; the latter under the combination of higher N2O production with limited N2O solubility.

With the view to mitigating N2O emissions, it can be initially decided to maintain a pH≥6 in order to enhance nitrification along with a high temperature (>20⁰C) so as to boost the denitrifying enzymes’ activity. However, an uncontrolled pH and temperature augmentation might finally produce an adverse result. For example, a pH increase further than 7 is likely to bring about a higher AOR and a resulting N2O accumulation through the AOB pathways. Similarly, a temperature increase over 25⁰C renders the N2O produced during denitrification less soluble and facilitates its conversion to gas or, equivalently, its emission. On the whole, a pH controlled around 7 and a temperature of approximately 20⁰C are conditions which ensure the completion of nitrification and the N2O consumption through denitrification.

1. **N2O QUANTIFICATION AND EMISSION**

As described above, significant Ν2Ο amounts can be generated during the N-removal at lab-, pilot- and full-scale WWTPs. The study by Law et al. (2012a) emphasizes the significance of N2O measurement methods, the complexity of the N2O emission quantification in full-scale WWTPs and the lack of standardized methods for this purpose. This makes the comparison of Ν2Ο emissions from different WWTPs difficult. Thus, the N2O emissions’ well founded quantification along with the measurement standardization in full-scale schemes are a necessity (Ye et al., 2014).

**4.1 N2O quantification**

In this section different N2O quantification techniques are presented. In fully-covered WWTPs, the emitted N2O can be determined by using the outflow N2O gas concentration and the total gas flow rate. However, the majority of full-scale WWTPs are open systems where N2O is quantified by capturing the emitted N2O flux with a floating chamber (Law et al., 2012a; Marques et al., 2016). Then, the N2O grab samples can be analyzed either offline (e.g. with gas chromatography) or online (gas analyzers). Reliable online measurements require a controlled gas flow and samples free of humidity (Lim and Kim, 2014; Ye et al., 2014; Marques et al., 2014, 2016). For example, in the studies by Law et al. (2011), Wang et al. (2014a) and Peng et al. (2015a,b), a suitable filter was installed at the inlet of the implemented gas analyzers to prevent moisture from entering. Marques et al.(2016) conducted an N2O measurement campaign in a full-scale SBR with gas-phase microelectrodes combined with a conventional online gas analyzer in order to estimate both liquid and gaseous N2O dynamics in a single experimental layout. Their set-up proved to be more accurate compared to a commercial analyzer which generated results underestimated by 14%.

Alternatively, quantification occurs by combining liquid N2O data with the concept of mass transfer from liquid to gas. N2O in the liquid phase is produced during the BNR and can be converted to gas either due to over-saturation or because of stripping (Marques et al., 2016). Henry’s coefficient for N2O is 0.024 M atm-1 (Kampschreur et al.,2009); thus, it is relatively soluble in water and its stripping is not fast. The emission rate can be calculated by using the dissolved N2O concentration and the volumetric mass transfer coefficient (*kLa*), which is composed by the global transfer coefficient *kL* and the interfacial area *a* (interphase transport between liquid and gas per unit of reactor volume). Practically, the *kLa* estimation is difficult because wastewater treatment takes place under conditions varying in time and space. Furthermore, dissolved N2O can be measured via grab samples which provide measurements discrete in time (Ye et al., 2014; Marques et al., 2016). Responding to this need, Mampaey et al. (2015) developed a tool for measuring the dissolved N2O concentration; it consisted of a gas stripping device able to indirectly measure dissolved N2O under aerated and non-aerated conditions on a minute time scale.

The sampling strategy effect on the N2O quantification was investigated by Daelman et al. (2013). The authors applied different monitoring scenarios on a 16-month dataset of a fully covered WWTP to conclude to the most accurate and cost-effective one. The estimation of the average annual N2O emission required long-term, either online or grab samples (including nightly and weekend sampling), in order to capture the seasonal dynamics. On the contrary, short-term campaigns unraveling the diurnal patterns demanded high-frequency online sampling.

Different quantification protocols and techniques were described amongst the various studies cited in Table 1, in different length and detail. Several of them were built upon the concept of describing both the liquid and the gaseous N2O dynamics during all the treatment phases and along the whole treatment line for extended periods. For example, De Mello et al. (2013) measured N2O in a full-scale WWTP by collecting air bubbles (aerated phases) and N2O fluxes at the liquid-air interface (non-aerated phases). They continued measuring during the phase alternation throughout the whole campaign to cover possible temporal variations. Similarly, in the pilot and full-scale SBRs implemented by Pijuan et al. (2014) and Rodriguez-Caballero et al. (2015), respectively, emissions were continuously monitored for 33 days (143 cycles) with nonstop offgas collection from the reactor. In the case of SBR operation in specific, potential peaks in the N2O emission are likely to be missed if the measurements are discrete in time.

Tumendelger et al. (2014) sampled in 7 different points covering the whole length of the full-scale WWTPs under investigation to ensure that their measurements were representative of any spatial variability. In the pilot-scale OD study by Zheng et al. (2015), 14 sampling points corresponding to 12 intensive sampling zones were determined for measurements under steady-state operation. Likewise, in the lab-scale SBR study by Chen et al. (2014), data reporting started after steady state conditions were obtained. Thus, N2O quantification covering the whole treatment line after ensuring stable plant operation is significant for the acquisition of accurate and representative results.

**4.2 N2O emission factor**

To determine the N2O emitted during biological wastewater treatment, emission factors are calculated as the amount of N2O emitted relative to the influent N. In this section, the suitability of using a standard N2O emission factor in all cases (regardless of scale) following the IPCC 2006 guideline is discussed. According to this guideline, the older (standard) emission factor (i.e. 1% of the influent N-content) should decrease to 0.5% of the influent N-content. However, both factors are still in use. Based on limited data for emissions from soils, it is doubtful whether they can successfully represent full-scale emission data from wastewater treatment (Kampschreur et al., 2009; Law et al., 2012a).

Studies have demonstrated that there is a wide variation in the N2O emission in WWTPs. For example, the data presented by Kampschreur et al.(2009) revealed a remarkable difference in the N-fraction emitted as N2O: 0-95% of the influent N (lab-scale) and 0-14.6% of the influent N (full-scale). The repetition of more strategic measurement campaigns in the examined WWTPs was recommended for the acquisition of more accurate and, possibly, less variable results. Largely different N2O emission factors can be noted even among systems of similar scale. For example, Law et al.(2012a) reported an emission factor range of 0-25% amongst different full-scale WWTPs. This variation can be attributed to the increasingly dynamic conditions in WWTPs, as well as on the different configurations and operational conditions in each case (Law et al., 2012a; Marques et al., 2016). For instance, Ahn et al. (2010) mentioned that systems designed for performing complete nitrification within a year (under controlled NH4+ concentration in the bioreactor and the effluent) are supposed to have higher N2O emissions at higher temperatures; the latter because of improved kinetics in the whole N-activity (oxidative and reductive). Moreover, Daelman et al. (2013) reported N2O emission data from a 16-month online monitoring campaign in a full-scale WWTP. Seasonally, the emissions were correlated with the daily maximum NO2- concentrations. Thus, the NO2- daily peaks, as result of nitrifier denitrification under suboptimal DO, were suggested as a diagnostic tool for the estimation of potential seasonal emission peaks.

In addition, the N2O emission factors can be influenced by the N2O quantification method (Law et al., 2012a; Lim and Kim*,* 2014). After examining 12 different full-scale WWTPs in the U.S. employing a variety of BNR and non-BNR processes (e.g. separate-stage BNR, step-feed non-BNR, OD, 4-stage Bardenpho etc.), Ahn et al. (2010) noted emission factors from 0.01 to 1.8% when normalized to the influent TKN. They chose different sampling locations (aerated and non-aerated) in each treatment process. At each one of these, online gaseous N2O measurements were taken for over a day to examine diurnal variability. The variability in the reported emission factors was correlated with the diurnal variations of the influent N-loading. Similarly, Rodriguez-Caballero et al. (2014) examined the N2O dynamics in a full-scale WWTP; gas data were recorded online in both the aerated and non-aerated zones. Due to instable nitrification in the bioreactor, emissions presented significant variability within the same day; the N2O emission factor was reported to decrease from 0.116 to 0.064% of the influent Total Kjeldahl Nitrogen (TKN). In both cases, continuous online reporting of the data was a way to obtain a clear picture of the changing temporal N2O dynamics. Foley et al. (2010) studied 7 full-scale BNR WWTPs in Australia with various configurations (e.g. OD, SBR, etc.), concluding to an N2O emission factor range of 0.6-25.3% of the denitrified N. In this case, grab samples during four intended sampling rounds were collected. However, not all of the four intended rounds were executed in all WWTPs; the latter can question how comparable and representative the results from the different plants were. The authors recommended online monitoring of the biological compartments for the in-depth understanding of influent dynamics and process characteristics in WWTPs.

At this point, it should be noted that there is no common pattern for reporting the N2O production and emission amongst the studies presented in Table 1, which renders direct comparisons difficult. The calculation of the N2O emission factor (as the N2O emitted relative to the influent N) is essential, enables comparison with other works and can serve as concrete proof of whether the specific conditions of each study significantly contributed to the N2O emission mitigation.

Taking all these aspects into consideration, the use of one standard emission factor in all cases is probably unlikely to ensure the realistic quantification of N2O emissions. N2O emissions are influenced by a number of parameters such as the scale of the study, the various operational features and design options, the temporal and/or spatial trends as well as the N2O quantification methods applied in each system. The choice upon the optimal quantification method also determines the time scale of the measurements as well as whether they can be taken from the whole extent of the system operation. In that sense, it inevitably impacts on the final results (i.e. measurements) and, subsequently, on the N2O emission factor calculation. All in all, future research should focus on the successful full-scale application of standardized set-ups which simultaneously estimate liquid and gaseous N2O dynamics as the most holistic approach concerning the accurate N2O quantification.

1. **MODELING OF N2O EMISSIONS**

In this section, the mathematical modeling of N2O produced during the BNR is examined. N2O models can be developed as an extension of the existing activated sludge models (ASM), introduced by the International Water Association (IWA) task group (Henze *et al.,* 1987, 2000). The various ASM versions constitute a widely accepted tool for the description of various processes: e.g. organic matter oxidation and nitrification/denitrification (ASM1), biological phosphorus removal (ASM2 and ASM2d), internal storage and endogenous respiration (ASM3). Nevertheless, the ASM considers nitrification as an one-step process and the accumulation of NO2- as an intermediate product is ignored. The latter is deficient when operational failures occur or advanced BNR processes (e.g. nitritation/denitritation or the completely autotrophic N-removal) are applied in a WWTP (Gujer et al., 1999; Henze et al., 2000; Iacopozzi et al., 2007). Several ASM modifications including two-step nitrification-denitrification have been developed (e.g. Iacopozzi et al., 2007; Guerrero et al., 2011; Ostace et al., 2011) in an attempt to estimate the NO2- accumulation. As discussed in Section 3.3, the NO2- accumulation is critical for the N2O formation. In that sense, such models serve as a tool to predict the dissolved NO2- concentration in the mixed liquor. However, they need to be expanded so as to include N2O and other intermediates dynamics and, hence, allow the calculation of the N2O emissions to the atmosphere.

The development of mathematical models describing N2O emissions is essential in order to comprehend the effect of different operational parameters and design strategies to decrease the environmental impact of WWTPs. Both the AOB and the heterotrophic denitrifiers can be linked to the N2O generation/consumption and the development of models considering all these pathways can lead to efficient N2O estimation models and mitigation strategies (Kampschreur et al., 2009; Law et al., 2012b; Ni and Yuan, 2015). A basic description of models focusing on either one or more of the possible pathways of N2O generation is presented below.

*5.1 Models focusing on the nitrifier denitrification pathway*

In the model developed by Ni et al. (2011), nitrifier denitrification with NO2- as the final electron acceptor produces NO and, eventually, N2O (NH2OH functioning as the electron donor). The model was successful in describing how low DO levels (i.e. ≤1.5 mg L-1) inhibited nitrification leading, in this way, to NO2- accumulation and, finally, to increased N2O emissions through the nitrifier denitrification pathway. Mampaey et al. (2013) examined the scenario of nitrifier denitrification happening simultaneously with the NH4+ oxidation; electrons deriving from the NH4+ oxidation to NO2- became available for the AOB reduction of NO2- to NO and, finally, to N2O. In a continuously aerated partial nitrification (SHARON) process, their model predicted maximum N2O emissions for relatively low DO concentrations (i.e. DO≤1.5 mg L-1). Under an intermittent aeration regime (with aerobic and anoxic phases of equal length), resulting in a varying DO profile, the same model indicated that N2O production and emission was mainly observed during the aerated phases at relatively low DO concentrations (i.e. DO≤1.5 mg L-1). Both models linked N2O production via the nitrifier denitrification pathway with the DO level. Nevertheless, the following difference regarding the N2O formation was detected: NH2OH was a model variable for Ni et al. (2011) part of which was directly converted to N2O, whereas Mampaey et al. (2013) considered the NH4+ oxidation to NO2- as an one-step process without the NH2OH intermediate. The model by Guo and Vanrolleghem (2014) was developed after combining the work of Mampaey et al. (2013) along with the model of Hiatt and Grady (2008). The kinetics regarding the DO influence on the nitrifier denitrification were modified into a Haldane term. Moreover, two different growth reduction factors were applied for the two nitrifier denitrification steps of the study (1st: NO2-→NO; 2nd: NO→N2O) so as to consider that the AOB growth rate can differ in each step. The simulation results showed that increasing temperatures enhanced the nitrifier denitrification rates. Under these conditions, the N2O emissions presented an increasing trend. Furthermore, the Haldane term addition enabled the estimation of NO2- accumulation along with the subsequent N2O production (through nitrifier denitrification) at low DO levels.

*5.2 Models focusing on the NH2OH oxidation pathway*

The other AOB pathway, the NH2OH oxidation pathway, was the core of the Law et al. (2012b) and Ni et al. (2013) models. Law et al. (2012b) studied the relationship between the AOR and N2O production rate of an enriched AOB culture performing NH4+ oxidation to NO2- in sludge reject water. They found that the N2O production rate increased exponentially with the AOR. Amongst the different tested models, the experimental results were more accurately explained by the NH2OH/NOH pathway; according to this, N2O production occurs through the decomposition of the unstable nitrosyl radical (NOH) which is an intermediate product of the NH2OH oxidation. Although nitrifier denitrification has been linked with low DO levels due to decreased electron competition between O2 and NO2- (Anderson et al., 1993), it was probably less favored under the specific conditions of the study which included high NH4+ levels (500 mg L-1) combined with low DO levels (0.5-0.8 mg L-1). In an attempt to model full-scale data from an OD and a SBR plant, Ni et al. (2013) developed a model where NH4+ was first oxidized to NH2OH, and, then, NH2OH was sequentially oxidized to NO and NO2-. N2O production was attributed to the NO reduction by AOB with NH2OH as the electron donor. No DO inhibition was considered on the NO reduction; on the other hand, an anoxic correction factor was applied to describe the lower specific AOB reaction rate with NO as electron acceptor (instead of O2). In both plants, the model associated N2O emissions with high NH4+ concentrations within the aerated zones (OD) or during the aerated phases (SBR). The authors suggested that the NH2OH oxidation pathway was mainly responsible for the emissions in the OD after observing a decrease in the NH4+ concentration without a simultaneous NO2- increase in the aerated zones. The same pathway was considered as most likely for the SBR given that it operated under 3 basins (instead of 4) and, hence, there was significant NH4+ accumulation; this was translated into a high specific AOR and, finally, into the enhanced production of by-products such as NH2OH.

*5.3 Models focusing on both AOB pathways*

With regard to modeling both AOB pathways, Ni et al. (2014) developed a model which combines the complicated biochemical reactions and the electron transfer processes of the AOB metabolism via a three-step NH3 oxidation process (NH3→NH2OH→NO→NO2-) and three reductions (including NO2-→N2O and NO→N2O). Consequently, this model estimates the contribution of each AOB pathway to the total N2O production and determines the predominant one at different DO and NO2- levels. Data from an enriched nitrifying culture (culture I) performing nitritation in a lab-scale SBR fed with synthetic anaerobic digester liquor (previously studied by Law et al. (2013)), as well as from a nitrifying culture (culture II) performing full nitriﬁcation by both AOB and NOB were used for the model calibration and validation. The model successfully depicted the following experimental trends: (i) NH2OH oxidation pathway was predominant under extremely low/high NO2- concentrations with high DO, (ii) nitrifier denitriﬁcation dominated at low DO with moderate NO2- accumulation. In the two-pathway model by Pocquet et al. (2016), the following enzymatic oxidations were modeled: NH3→NH2OH→NO→NO2-. Moreover, they added the NO reduction to N2O along with NH2OH oxidation to NO2- (NH2OH oxidation pathway), and the HNO2 reduction to N2O coupled with NH2OH oxidation to NO2- (nitrifier denitrification pathway). They considered no AOB growth inhibition (e.g. by NH3 or HNO2), whereas they included DO inhibition for the nitrifier denitrification pathway by restricting the N2O production to a maximum rate with the DO decrease. Their simulations showed that, for a stable HNO2 concentration (0.7 mg L-1), the DO increase provoked a decrease in the N2O emission factor; in that case, the nitrifier denitrification predominance slightly decreased, whereas the contribution of NH2OH oxidation was relatively higher. This was in accordance with the experimental findings by Peng et al. (2014) (lab-scale domestic-municipal SBR with an enriched nitrifying sludge containing both AOB and NOB) where the DO increase from 0.2 to 3 mg L-1 resulted in similar changes in the pathway contribution: nitrifier denitrification from 95% to 73%, and NH2OH oxidation from 5% to 27%. The maximum predicted N2O emission factors were observed as a combination of high HNO2 concentrations (0.6-1 μg L-1) and low DO (0.5-1 mg L-1). This was consistent with long-term data collected by the authors for the model evaluation from a lab-scale SBR fed with NH4+-rich wastewater (403-445 mg L-1); the N2O emission peak was reported under the co-occurrence of high HNO2 (0.9 mg L-1) and low DO (1 mg L-1).

*5.4 Models focusing on the heterotrophic denitrification pathway*

The next point concerns the heterotrophic denitrification pathway. The activated sludge model for nitrogen (ASMN) developed by Hiatt and Grady (2008a) describes each one of the (four) denitrification steps (NO3-→NO2-→NO→N2O→N2) as a discrete reduction reaction with NO2-, NO and N2O as electron acceptors. Moreover, different heterotrophic specific growth rates were assigned to each denitrification step. The mathematical validation of the model was conducted by Hiatt and Grady (2008b) upon a modified Ludzak-Ettinger (MLE) process where the NO3- (electron acceptor) generated in the aerobic tank (nitrification) was returning to the anoxic tank (denitrification) through internal recirculation. According to their simulation results, one mitigation strategy was to regulate the internal recirculation; thus, the anoxic tank was receiving enough biodegradable organic substrate (electron donor) for performing complete denitrification. However, the validation described above was based on mathematical simulations and not on experimental data. An additional point to consider is that the ASMN ignores the fact that the NOx reduction and the C oxidation can be performed by different enzymes with different kinetics; therefore, whichever of the two bioprocesses can impact on the denitrification rate (Pan et al., 2013a). Responding to this need, Pan et al. (2013a) modeled the electron competition amongst the four denitrification steps by dissociating the C-oxidation and the N-reduction. They used electron carriers as a new component linking both processes; C-oxidation offering electrons to carriers and NOx reduction receiving the electrons from those carriers. Different affinity constants with reduced carriers were assigned to each denitrification step in order to represent each step’s ability to contend for electrons. Model calibration and validation was conducted upon data from a lab-scale SBR study on the electron competition during denitrification of a methanol- and NO3--fed denitrifying culture under variable C and NOx provision (Pan et al., 2013b). The model was successful in describing the following trends of the experimental data: (i) the highest NO3-, NO2-, N2O reduction rates were noted when the respective NOx functioned as the unique electron acceptor (less electron competition), and (ii) as the methanol feed changed from pulse to continuous, the reduced C provision induced increased electron competition; N2O accumulation increased with decreasing the C loading rate because less electrons became available for the N2O reductase (under the concurrent presence of NO3- and NO2-).

*5.5 Modeling the biological N2O production pathways*

With the view to explaining the significant spatial variations in the N2O fluxes of a step-feed full-scale AS plant, Ni et al. (2015) combined the part referring to the AOB pathways from Ni et al. (2014) and the part related to heterotrophic denitrification from Ni et al. (2013) in an integrated model. The model was validated using real data obtained from different measurement campaigns in the step-feed AS plant. It was able to provide an explanation about the significant difference between the emission factors of each step (1st step: 0.68% of influent N, 2nd step: 3.5% of influent N). The specific AOR and the specific N2O production rate were significantly higher in the 2nd step. In the anoxic zone of the 2nd step, important NO2- accumulation along with high NO3- levels enhanced N2O production through heterotrophic denitrification. According to the authors, the latter was attributed to the fact that the 2nd step had about 70% less biomass compared to the 1st step under the applied sludge return; sludge from the secondary clarifiers was returning only to the (1st) anoxic zone of the 1st step. Increased sludge concentration in the 2nd step can result in a lower specific AOR. Therefore, recycling around 30% of the total return sludge stream to the 2nd step was suggested as a potential mitigation strategy for a decreased overall N2O emission.

*5.6 Modeling the coupling of biological with biologically-driven N2O production*

In a recent model by Domingo-Felez and Smets (2016), the three biological pathways for N2O production were coupled with two biologically-driven abiotic N2O production processes: (i) NH2OH produced during nitrification can form nitroxyl (HNO) which dimerizes (via hyponitrous acid (H2N2O2)) to N2O and H2O at high pH, and (ii) nitrosation of NH2OH (with HNO2 as nitrosating agent) can form N2O inside nitritation reactors at low pH. Even though biological N2O production is generally considered the main hotspot, the authors underlined that abiotic N2O production is possible in WWTPs and probably underestimated at the moment. For example, Harper et al. (2015) analyzed batch data acquired during partial nitrification regarding the abiotic N2O production resulting from reactions between NH2OH and NO2- at approximately neutral pH. They observed that the abiotic N2O generation rates were 1-3 orders of magnitude higher than the biological contribution, and that the abiotic N2O production rate was accelerated by the presence of AS. In this frame, the NH2OH concentration in a full-scale nitritation reactor treating reject water was measured between 0.03 and 0.11 mg L-1 in the study by Soler-Jofra et al. (2016). An emission factor (N2O emitted relative to the oxidized N during nitritation) equal to 1.1% was attributed to the abiotic N2O production; this was approximately one third of the total measured N2O emission rate within the reactor. Thus, although the study by Domingo-Felez and Smets (2016) lacks validation, it addresses the need for in-depth modeling of the abiotic N2O generation whose contribution to the N2O emissions is currently underrated and obscure.

*5.7 Conclusions and perspectives*

A brief overview of all the models presented in sections 5.1-5.6 is given in Table 2. Given to what was analyzed in previous sections as well as in the current one, the AOB pathways are likely to generate N2O emissions with the specific pathway contribution depending on the combination of DO and NO2-. Thus, the two-pathway AOB models (e.g. Ni et al., 2014; Pocquet et al., 2016) seem more suitable, especially if they already consider DO variations (e.g. Ni et al., 2014), DO inhibition on the maximum N2O formation levels (e.g. Pocquet et al., 2016) or the impact of different NO2- levels (e.g. Ni et al., 2014). Nevertheless, the AOB and the heterotrophic denitrifiers can act simultaneously and must be considered, especially in terms of full-scale modeling (Kampschreur et al., 2009; Law et al., 2012b; Ni and Yuan, 2015). In that sense, the contribution of models describing the kinetics of each denitrification step along with the effect of potential changes to the system (e.g. Pan et al., 2013a) is significant. It is clear that multiple pathway models have the ability to better describe the effect of an operational parameter (e.g. DO, NO2- concentration, return sludge stream etc.), unravel any possible spatial/temporal variations and, consequently, help the operators with designing the best mitigation strategy (e.g. Ni et al., 2015). Thus, it is necessary to develop even more integrated models for simulating full-scale WWTPs, including all the potential microbial pathways for N2O production without excluding the abiotic (although biotically triggered) N2O production routes which have recently been proposed as non-negligible N2O contributors (e.g. Harper et al., 2015). However, there is still place for more accurate modeling on the abiotic pathway.

Final aspects that should be considered for the complete modeling of N2O emissions include the following points. On one hand, modeling N2O transfer from liquid to gas under different gas flow patterns: e.g. the stagnant transfer of non-aerated processes with surface contact between gas and liquid, different aeration patterns (i.e. different aeration flows and blowers) in aerobic zones with high N2O liquid concentrations. Simple mass transfer models applying a *kLa* and a driving force based on the liquid N2O concentration can prove to be overly simplistic in some cases. The second potential aspect to be included in the models is the chemical N2O production. As described above, the significance of these processes in lab- and full-scale systems is currently a controversial issue and future research conducted on this topic will determine whether it is essential to be included in general N2O production models.

1. **N2O MITIGATION**

In this work, a number of past studies were critically reviewed in order to conclude on the principal influential factors regarding the N2O emission, quantification and modeling during the BNR in WWTPs. The synthesis of these conclusions can lead to mitigation strategies.

* + - 1. Select the optimal operational conditions. For example, DO has a significant effect on nitrification, thus, influencing the N2O production through the major hotspots (i.e. AOB pathways). It needs to be maintained at a proper level (e.g. at around 2 mg L-1) to ensure the completion of nitrification. Moreover, the aeration regime impacts on the N2O stripping and the overall C-footprint of the plant. Operators should ensure an optimal aeration rate which is of benefit to nitrification without causing intensive stripping or excessively increasing the energy requirements of the plant. Finally, they should ensure a plant operation which respects the system’s N-removal capacity in order to avoid NO2- accumulation.

1. Apply advanced N-removal (e.g. nitritation-denitritation or partial nitritation-anammox) after process optimization. Although such processes are implemented in order to reduce the system’s energy requirements, they generate higher N2O emissions. Thus, process optimization (e.g. through the operation at an optimal DO) is essential in order to maintain the system’s overall C-footprint within the accepted limits.
2. Ensure the sufficient provision of an (external) C-source promoting N2O consumption through denitrification. An adequate amount of external C-source is demanded to ensure that the denitrifiers perform complete denitrification without consuming internally stored compounds. The latter is significant because internal carbon source utilization has been associated with potentially higher N2O production. The C-source composition is also important since it can be beneficial to the denitrifiers growth.
3. Operate at an optimal combination of pH (e.g. 7) and temperature (e.g. 20⁰C). Thus, nitrification-denitrification are safely completed with minimum accumulation of N2O precursors and minimized N2O stripping.
4. Calculate a realistic, customized emission factor. The latter occurs by taking into account the scale of the study and the special operational characteristics of the scheme under examination. It will enable operators appreciate whether the plant operation leads to accepted levels of N2O emission and, subsequently, decide if changes should be made.
5. Accurately estimate the N2O production in the WWTP. Successful measurement campaigns should cover the whole length of the treatment line for extended periods in order to capture spatial and/or temporal variations. Furthermore, they should help operators evaluate the average N2O emitted load over a prolonged and representative observation period. Continuous online measurements provide the most accurate and representative estimations, but can be less cost-effective in the case of long-term monitoring. Future research on the precise N2O quantification should pay particular attention to the development of full-scale methods estimating both liquid and gaseous N2O dynamics.
6. Use multiple-pathway full-scale N2O production models to predict the N2O hotspots and influential conditions in a WWTP under examination. The AOB pathways under conditions of low DO or abrupt changes in the DO level in combination with NO2- accumulation are regarded as N2O hotspots. In full-scale cases however, all microbial pathways (the AOB pathways and the heterotrophic denitrification) in addition to abiotic pathways can contribute to the N2O emissions. Successful and holistic N2O modeling should include all possible pathways (microbial and abiotic) under changing conditions (e.g. different DO levels, varying NO2- concentration) in addition to N2O stripping. The modeling of abiotic N2O production, in specific, as well as its integration to existing microbial models is a research area which is currently under development.

All in all, the N2O emission occurring during the BNR in WWTPs involves a large number of influential parameters as it was demonstrated throughout this review. It seems that the development of a dynamic model which takes into account all pathways of N2O production and has been calibrated and validated upon real data originating from various pilot and full-scale BNR schemes with different operational characteristics is needed. Then, we will be able to predict the potential N2O emissions of a scheme under construction or directly interfere in the operation of an existing one so as to achieve the desired mitigation.

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