A critical review on nitrous oxide (N_2O) emissions during wastewater treatment processes

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Disclaimer *– All these conference papers have been submitted as partial fulfilment for the project requirement for the BE(Hons) degree. Although they have been assessed, no errors or factual information have been corrected or checked.*

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Glossary of Terms

WWTPs – Wastewater Treatment Plants
WWTS – Wastewater Treatment System
N_2O - Nitrous Oxide
GHG – Greenhouse Gas
CO_2 – Carbon Dioxide
CH_4 – Methane
NO – Nitric Oxide
BNR - Biological Nitrogen Removal
AOB - Ammonia-oxidising Bacteria
HDN – Heterotopic Denitrifying Bacteria
DO – Dissolved Oxygen
COD – Chemical Oxygen Demand
NH_2OH - Hydroxylamine
NO_3^- - Nitrate
NO_2^- - Nitrite
FNA – Free Nitrous Acid
NLR – Nitrogen Loading Rate
SBR – Sequencing Batch Reactors
N_2OR - Nitrous Oxide Reductase
SRT – Solid Retention Time

Abstract

In recent decades, wastewater treatment emissions have slowly risen, accounting for 3.4% of the global nitrous oxide (N_2O) pollution budget. At the rate of current N_2O pollution, the ozone capacity is anticipated to deplete within the next 100 years. We undertook this critical literature review to identify and evaluate the contributing factors and protocols associated with N_2O emissions in order to develop mitigation strategies to decrease N_2O pollution. N_2O production is at its greatest during the denitrification processes, and the operational parameters that contribute most to N_2O emissions are dissolved oxygen, nitrite concentration, ammonia oxidation rate and aeration. To design mitigation measures, we determined that a suitable surveillance system capable of monitoring and assessing N_2O emissions from WWTPs must first be established. We have identified effective analytical devices and summarised existing protocols to assist with building a surveillance system. Additionally, a simplified protocol flowchart was developed to guide users in deciding which operations to follow for N_2O collection and analysis. Establishing a surveillance strategy will produce useful information that will enable operators to make changes in the WWTP processes, allowing optimisations of critical parameters such as DO and nitrite, which we believe is the most effective means of reducing N_2O emissions from WWTPs.

1. INTRODUCTION

Nitrous oxide (N_2O) is the third most common greenhouse gas (GHG) in the atmosphere and is considered the most impactful ozone sink. Worldwide N_2O emissions have increased by 43% since 1970, and N_2O accounts for 6.2% of total anthropogenic GHG emissions (Duan et al., 2017). Furthermore, N_2O atmospheric lifespan is approximately 114 years longer than methane (CH_4), and its global warming potential is 310 times that of carbon dioxide (CO_2) (Chen et al., 2020). In addition to its impact on the atmosphere, N_2O can react with atomic oxygen to create nitric oxide (NO), which is detrimental to the stratosphere (Duan et al., 2017). N_2O is currently the most significant anthropogenic ozone-depleting agent being released, according to comparative analysis with other ozone-depleting substances (Ravishankara et al., 2009); given these findings, N_2O is expected to severely increase ozone depletion capacity within the next hundred years (Revell et al., 2015).

Wastewater treatment plants (WWTPs) are a noteworthy source of N_2O emissions (Desloover et al., 2012; Law et al., 2012). N_2O is primarily released during biological nitrogen removal (BNR) by performing nitrification and denitrification in various reaction areas. Autotrophic nitrifying bacteria, which are mainly ammonia-oxidising bacteria (AOB), is a recognised obligatory intermediate in the heterotrophic denitrification pathway, also known to emit N_2O as a by-product (Law et al., 2012). Elevated concentration of N_2O is likely to be released during these bioprocesses, increasing atmospheric N_2O concentration and the WWTPs carbon (C) footprint (Tallec et al., 2006; Yang et al., 2009).

Wastewater treatment emissions have been rising every year and are responsible for 3.4% of the global N_2O pollution. Considerable research has been undertaken to find mitigation solutions to reduce N_2O pollution in the atmosphere. Through a “black box” approach, studies initially focused on investigating the impact of critical parameters contributing to N_2O pollution (Duan et al., 2017). However, the ‘black box’ methodology led to inaccurate or system-specific assumptions, limiting the general applicability of findings. Process-based pathway expertise is needed to improve understanding of the impact of operational conditions on N_2O emissions and ensure the transferability of results from one WWTP to another (Duan et al., 2017). By devoting time and energy to researching N_2O pathways, mitigation techniques will become more prevalent, and the surveillance off N_2O in WWTPs will be even more feasible.

2. RESEARCH AIM AND OBJECTIVES

This review aims to critically assess the contributing factors and protocols associated with Nitrous Oxide emission during wastewater treatment processes in order to develop mitigation strategies that can be used to combat N_2O emissions.

To realise the research aim, the following objectives have been formulated:

a) Investigate critical parameters that contribute to Nitrous Oxide emissions.
b) Establish a protocol to capture Nitrous Oxide emissions.
c) Collect and analyse data from research journals to evaluate the effects critical parameters have on N_2O emissions.
d) Define mitigation strategies to reduce N_2O emissions.

3. EXTENDED LITERATURE REVIEW

This report is an extended literature review where a wide variety of literature was analysed to evaluate the N_2O lifecycle in WWTPs, determine which operational parameters contribute most to N_2O emissions, explore the methodologies and protocols reported in previous research to quantify N_2O emissions, and finally to propose suitable N_2O mitigation strategies.

4. N_2O PRODUCTION PATHWAYS IN WWTPs

Physical, biological, and chemical methods are used in wastewater treatment plants to remove dissolved and suspended contaminants (Frutos et al., 2018). N_2O is primarily produced in wastewater treatment systems (WWTS) by biological nitrogen removal processes such as nitrification and denitrification (Duan et al., 2017). In the production of N_2O, three major microbial pathways and two classes of bacteria are involved (Fig. 1). Autotrophic ammonia-oxidising bacteria (AOB) releases N_2O through the hydroxylamine (NH_2OH) oxidation pathway during nitrification (Chen et al., 2020). Both autotrophic and heterotrophic AOB release N_2O during denitrification via the nitrifier denitrification pathway and via the heterotrophic denitrification pathway (Fig. 2) (Kampschreur et al., 2009; Law et al., 2012). The heterotrophic denitrifiers are responsible for converting nitrate (NO_3^-) / nitrite (NO_2^-) to nitrogen gas (N_2) during denitrification; N_2O is an intermediate product of denitrification, and coincidently, the N_2O production rate is at its highest during denitrification. AOB are believed to have genes encoding enzymes that catalyse NO_2^- and nitric oxide (NO) reductions. However, the genes needed to synthesise the enzyme that catalyses the reduction of N_2O to N_2 are absent, implying that at the final reduction stage during nitrifier denitrification, N_2O is produced rather than N_2. (Casciotti and Ward, 2001; Cantera and Stein, 2007). Poughon et al. (2001) theorised that N_2O could also be generated through the nitrosyl radical (NOH) chemical breakdown, which is an intermediate in the oxidation step NH_2OH.

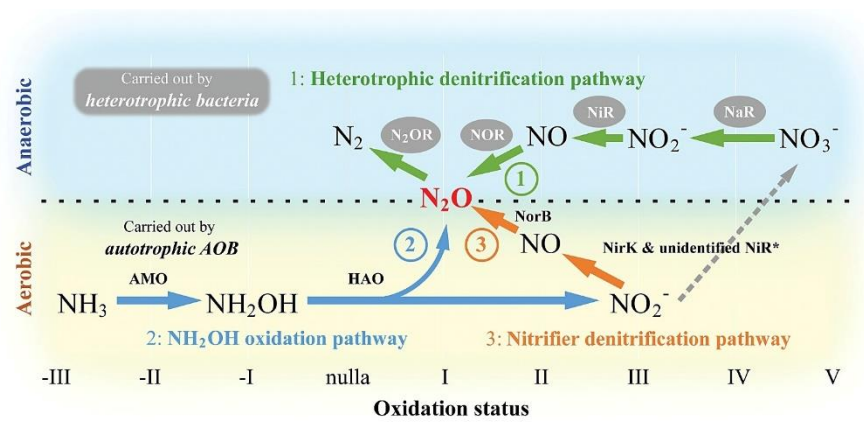

Figure 1: N_2O produced in WWTPs during BNR through three main microbial pathways attained from Duan et al. (2017)

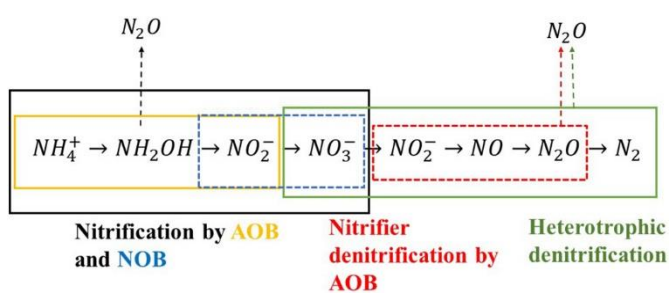

Figure 2: Biological pathways of N_2O production in denitrification-nitrification processes during wastewater processes attained from Frutos et al. (2018)

Evaluating N_2O emission from activated sludge systems is difficult due to the complex mixture of microorganisms and metabolic pathways during sludge treatment. Future advancements in research and treatment are needed to research individual N_2O pathways during active sludge treatment (Duan et al., 2017). Contrarily, well-engineered WWTPs working under optimal conditions offers a promising potential to mitigate N_2O pollution once the pathways and the necessary influencing conditions are identified (Law et al., 2012). Table 1 below provides a summary of the N_2O pathways.

Table 1: Summary of N_2O pathways in wastewater treatment based on finding from (de Haas & Ye, 2021)

N_2O Pathway	Sub-pathway	Dominant Microorganisms	Conditions leading to N_2O formation	Biochemical mechanism leading to N2O production
Autotrophic nitrification	Nitrifier denitrification	Primarily ammonia oxidising bacteria (AOB)	Low DO concentration and nitrite accumulation	AOB oxidises ammonia to nitrite, via hydroxylamine, but lack of N_2O reductase enzyme (to N_2), leads to N_2O production and emission.
	Hydroxylamine oxidation		High DO, high ammonium loading rates and accumulation of hydroxylamine	The formation of an unstable intermediate (nitrosyl radical, NOH) during oxidation of hydroxylamine to NO_2^- produces N_2O due to the unstable breakdown of NOH.
Heterotrophic denitrification	N/A	Heterotrophic denitrifies	Anoxic conditions, low pH, presence of hydrogen sulphide, and high accumulation of nitrite and nitric oxide	Partial inhibition of N_2O reductase (enzyme) leads to N_2O emmisons.
Chemical	N/A	Abiotic	High hydroxylamine and nitrite concentrations and low pH	Chemical reaction

5. KEY OPERATIONAL PARAMETERS THAT CONTRIBUTE TO N_2O EMISSIONS

Latest analyses of N_2O emissions from WWTPs have stated that the main operational parameters responsible for N_2O generation include but are not restricted to: i) dissolved oxygen (DO), ii) aeration, iii) NO_2^- / free nitrous acid (FNA), iv) organic substrate, v) nitrogen loading rate, vi) ammonia oxidation rate, vii) temperature and viii) pH. Coincidentally, these critical operational parameters can complement each other, and any minor changes (e.g., change in DO level) can affect N_2O production rate (Vasilaki et al., 2019).

5.1. Dissolved oxygen

AOB's deep relations with O_2 allows it to flourish in low-DO environments, magnifying NO_2^- accumulation. Chen et al. (2020) research supports this claim, as shown by their findings, which indicate that the highest N_2O emission factors occur at low DO levels. For example, at 0.35 mg/L of DO, Chen et al. (2020) found a maximal emission factor of 4.32%. Under oxygen-limited conditions (DO < 0.5 mg/L), N_2O production can be triggered by the AOB denitrification pathway, where AOB uses NO_2^- as a terminal electron acceptor to reserve O_2 for NH_3 oxidation to

NH_2OH. In terms of heterotrophic denitrification, high levels of DO inhibit nitrous oxide reductase (N_2OR) activity, and since N_2OR is more vulnerable to O_2 than other reductases, N_2O to N_2 conversion cannot occur. Keeping DO levels to a minimum should be a priority for regulating N_2O output, particularly during the nitrification process.

5.2. Aeration

Castro-Barros et al. (2015) investigated aeration's effect on N_2O emissions in a granular sludge reactor. Based on his intensive research, he found that N_2O production was at its peak at high aeration levels, primarily due to the intensive gas stripping in the reactor. Additionally, N_2O typically formed in the liquid phase at low aeration periods, which then turned into gaseous N_2O as aeration increased. In an isolated experiment, gas (excluding fresh air) was fed into the reactor, which led to the consumption of N_2O; however, N_2O production was not apparent. Castro-Barros et al. (2015) ultimately concluded that the transition from low levels of aeration to high levels of aeration resulted in the highest N_2O formation. Based on this research, it is evident that controlling the aeration rate can be a suitable solution in minimising N_2O emissions.

5.3. Nitrite (NO_2^-) & Free Nitrous Acid (FNA)

The formation of N_2O is thought to be induced by the accumulation of NO_2^- (Chen et al., 2020). The driving forces of AOB denitrification under aerobic conditions are high NO_2^- concentrations and low DO levels (Law et al., 2013). It's also concluded that large amounts of NO_2^- can be used to regulate N_2O output (Chen et al., 2020). Furthermore, under anoxic conditions, the denitrification rate decreases as NO_2^- accumulates, eventually leading to N_2O formation (Kampschreur et al., 2009).

NO_2^- can be translated into FNA depending on the pH and temperature conditions. FNA and free ammonia (FA) promotes AOB production, resulting in NO_2^- accumulation and N_2O development. Recently, it was found that FNA can limit the growth of microorganisms in WWTPs, as well as prevent the formation of N_2OR (Zhou et al., 2011; Zhao et al., 2019). In essence, it is possible to maintain low NO_2^- levels to prevent the impact of FNA on microorganisms and thereby reduce N_2O output (Chen et al., 2020).

5.4. Organic substrate

Incomplete denitrification will occur in the absence of substrate because there are insufficient amounts of N_2OR present in the system. The carbon/nitrogen (C/N) ratio relates to the amount of substrate in the system; typically, a low C/N ratio implies a lack of substrate. Because of its inhibitory effect on autotrophic biomass, a low C/N ratio will increase pH and FA and thus, increasing N_2O generation in WWTS (Mannina et al., 2017). It has been stated that a C/N ratio of 12:1 results in the lowest N_2O flux and the highest N-removal rate (Li et al., 2017). Ultimately, a high C/N ratio results in high NO_2^- concentration, it's recommended to keep the C/N ratio at 1.5 to keep N_2O production at a minimum (Chen et al., 2020).

The structure of the substrate also influences heterotrophic denitrification. (Butler et al., 2009; Zhang et al., 2016). According to research, denitrifying bacteria in wastewater treatment facilities tend to feed on volatile fatty acids rather than complex organic molecules (Chen et al., 2020). It was also discovered that the substrate affects the composition of the microbial culture. To ensure low N_2O production rates, suitable amounts of carbon substrate should be used during treatment.

5.5. Nitrogen loading rate

Several researcher papers investigated the impact of the nitrogen loading rate (NLR) on N_2O emissions. For example, Quan et al. (2012) calculated the emission factors of three independent batch reactors containing ascending amounts of NLR. They found that the maximum N_2O emission factors were 8.2%, 7%, and 4.4%, respectively, indicating that reducing the NLR encouraged less inhibition of the HDN process. Thereby enabling greater N_2O consumption, thus reducing overall N_2O emissions (Massara et al., 2017).

Frison et al. (2015) investigated a pilot-scale nitrification/denitrification SBR that treated wastewater from anaerobic co-digestion of sewage sludge. Two separate NLRs were evaluated, one of which was 35% higher than the system's N-removal capability, and the other remained unaffected. The N_2O emission factors were 1.49% and 0.24%, respectively (Massara et al., 2017). Frison et al. (2015) found that using an NLR that recognises the system's N-removing capability will reduce NH_4^+ and NO_2^- accumulation and, ultimately, lower N_2O generation through the nitrifier denitrification pathway.

5.6. Ammonia Oxidation Rate

Law et al. (2012) investigated what effects ammonia (NH_3^+) oxidation rate has on N_2O generation during partial ammonium oxidation. Based on their intensive research, Law et al. (2012) concluded that the N_2O production rate had a positive exponential relationship with ammonia oxidation rate (AOR). The reason for this relationship is attributed to the process of N_2O generation through chemical decomposition of nitrosyl radical (NOH), it was found that NH_3^+ oxidation rate significantly increased the rate at which NOH is formed, thus increasing N_2O production. Ultimately, a lower concentration of NH_3^+ will reduce N_2O production by AOB pathways (Law et al., 2012).

5.7. Temperature

Adouani et al. (2015) investigated the influence of temperature on N_2O production in municipal WWTPs. Adouani et al. (2015) concluded that temperature is a critical key parameter for nitrogen reduction. At low temperatures (5 °C), the denitrification enzyme activities are slowed down considerably, resulting in minimal NO and N_2O biological reactions, thus limiting N_2O and NO emissions. Therefore, operating WWTS at low temperature should decrease the rate at which N_2O is produced.

5.8. pH

pH is an essential factor correlated with the production of N_2O by the AOB pathways in aerobic conditions (Law et al., 2011). At pH 6-8.5 the N_2O generation levels varied significantly, with minimal generation at pH 6 and maximum generation at pH 8 (Law et al., 2011). Law et al. recommends applying a slow-feeding strategy at the main reactor to limit pH levels between 6-7; this will significantly reduce N_2O production rate in the aerobic phases (Law et al., 2011).

6. ANALYTICAL METHODS FOR CAPTURING N_2O EMISSIONS IN WWTPs

Currently, there is no standardised approach to measure N_2O emission in WWTPs. However, several researchers have created their unique method to quantify N_2O emissions from WWTPs. Some of the analytical techniques used are as follows:

i) Measurement techniques using sensors and instruments
ii) Sampling techniques
iii) Gas chromatography/mass spectrometry (GC/MS)

iv) Continuous infrared analysis
v) Laser-absorption spectroscopy
vi) Fourier-transform infrared spectroscopy

This section aims to analyse the practicality and limitations of the listed methods.

6.1. SBR lab testing

The bulk of the articles studied used lab-scale sequencing batch reactors (SBR) to perform their investigations. Since it is performed in a lab environment, this approach has the benefit of monitoring all situations. However, as there is no standardised approach to carry out batch experiments for measuring N_2O emissions, the protocol for conducting lab testing is severely different across relevant journal papers. This method also involves building lab models of SBR plants, running months-long trials, planning optimal batch experiments, and processing samples at regular intervals for data collection and analysis. As a result, SBR lab testing is deemed out of scope for this study and will not be pursued further.

6.2. Capturing N_2O emissions using sensors and instruments

Baresel et al. (2016) used the Teledyne analytical instrument (Model GFC-7002E) (Fig. 3) to measure N_2O concentrations. The sample was taken from the ventilation system and injected into the instrument using an air compressor. Using floating hood devices for collecting N_2O concentrations have also become an industry standard.

Figure 3: Picture of Teledyne analytical instrument (Model GFC-7002E), attained from (GFC-7002T, n.d.).

Baresel et al. (2016) also placed multiple Clark-type microsensors to measure the dissolved N_2O in the water phase. The N_2O water sensor was mounted at various positions in the basin during the measurement campaign, measuring both aerated and non-aerated areas. The water sensor measured the dissolved N_2O and water temperature. However, collecting short-term concentration measurements at different treatment processes creates considerable uncertainty in emission estimations. As a result, more sophisticated measurement methods, such as the use of floating chambers to collect all emissions from a given surface region, should be used (Baresel et al., 2016).

6.3. Limitations for measurements using instruments and sensors

Despite improvements in measuring instruments and equipment for quantifying N_2O emissions, exhaustive usage of the device and or costly automated analyses usually limit the capacity of measurements. Readings taken from instruments typically only provide limited information about emissions. More straightforward and effective measurement methods could thus vastly improve N_2O emission measurement and increase awareness and mitigation measures to minimise emissions from WWTPs (Baresel et al., 2016).

6.4. Sampling methods

Duan et al. (2020) studied one of five SBR plants in the WWTP in Adelaide, Australia. Duan et al. (2020) placed multiple floating hoods (capable of online measurements) around the specified sampling locations to capture the possible variations in N_2O emissions. The sampling station can be seen in Fig. 4. The hoods collected the released gas and then passed the gas on to the central analysing facility. Three gas storage devices were implemented to store the gas obtained from the floating hoods, each equipped with a gas measurement instrument, a temperature detector, and a pressure reader. The N_2O analyser (Horiba VA-3000 & VS-3002) was used to test the extracted gas. Duan et al. (2020) also used analytical devices such as the Shimadzu GC-MA gas chromatograph (GC) to measure N_2O emissions of the SBR systems.

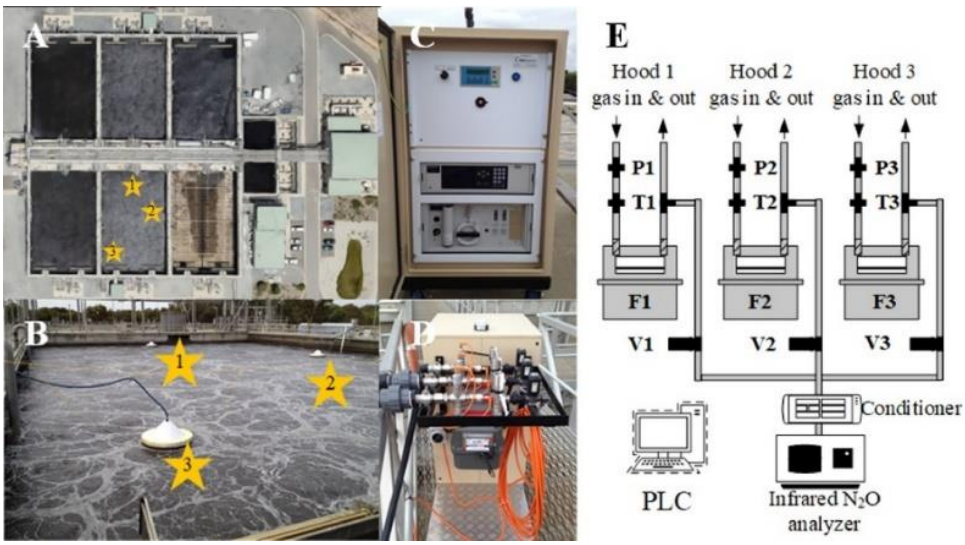

Figure 4: The N_2O online measuring/monitoring system was applied by Duan et al. (2020).

6.5. Gas chromatography/mass spectrometry (GC/MS)

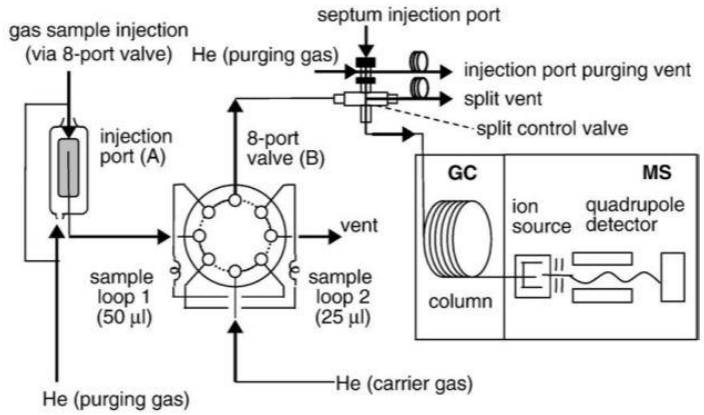

Figure 5: Modified GC/MS system attained from Isobe et al. (2011)

Modified GCMS instruments can be used for simultaneous quantification for a variety of gasses, including N_2O. Isobe et al. (2011) equipped a quadrupole GC/MS system with numerous modifications to deviate the risk of contamination on the sample; this included covering sensitive ports with a jacket and implementing a built-in filtration device. The complete schematic of the modified GC/MS device can be seen in Figure 5.

Isobe et al. (2011) modified GC/MS system was able to analyse and quantify the production of N_2O accurately within 2.5 minutes. Another key benefit for the GC/MS system is its ability to quantify a range of gas species simultaneously; this means trends, overlap, or any other relationships between species can be identified and highlighted during the investigation.

6.6. Continuous infrared analysis

Infrared (IR) analysers are capable of taking continuous on field measurements of N_2O (Montgomery et al., 1989). An IR analyser is relatively inexpensive and can be easily set up, which makes it a suitable apparatus for low level N_2O analysis, the schematic for a simple IR device is shown in Figure 6. IR analysers can identify the infrared absorption peaks at any given time during continuous measurements. We can extrapolate the concentration (typically given in ppm) of N_2O based on the IR absorption peaks. Montgomery et al. (1989) determined that 20-25% absorption levels approximate to 400ppm of N_2O.

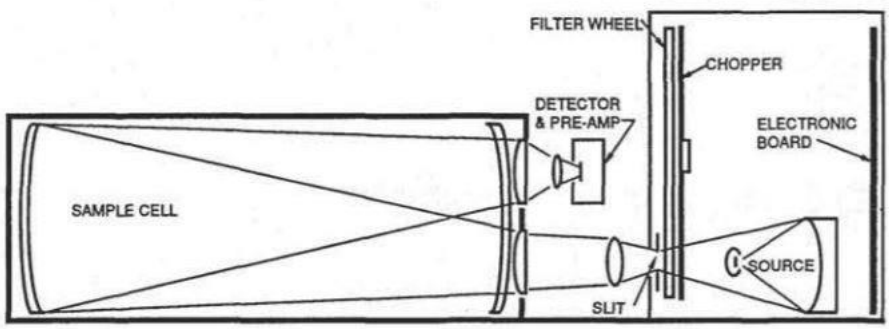

Figure 6: Schematic of a simple IR analyser attained from Montgomery et al. (1989).

6.7. Laser-absorption spectroscopy

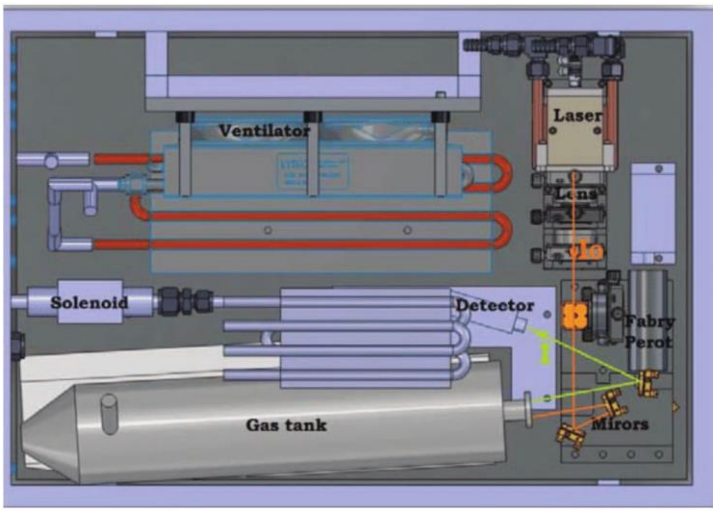

Figure 7: Quantum cascade laser configuration attained from (Mappé et al., 2013)

Laser spectroscopy is considered one of the fastest methods for analysing atmospheric gasses; this is because, in laser spectroscopy, only an individual wavelength is analysed instead of the entire spectrum (Rapson & Dacres, 2014). Laser spectroscopy can be differentiated by the type of laser used, such as the lead salt laser, which is the most common laser used for N_2O analysis provides rapid and accurate measurements (accuracy within 1ppb) while minimising interference with other gasses. The quantum cascade lasers offer more accurate results (accuracy within 0.05ppb) and other advantages such as portability, faster measurements, and the functionality to conduct direct isotopic analysis; however, the quantum lasers are expensive and inflexible (Rapson & Dacres, 2014).

6.8. Fourier-transform infrared spectroscopy (FTIR)

FTIR technique analyses the entire IR spectrum instead of an individual wavelength; this means that analysis is more thorough; however, the method is slower than that of laser spectroscopy. The optical frequencies are logged concurrently and stored in the interferogram, allowing simultaneous measurements of numerous gasses. Low-res FTIR is used to evaluate the total concentration of N_2O, while high-res FTIR is used to quantify all N_2O isotopomers simultaneously (Rapson & Dacres, 2014). The mechanism of FTIR is depicted in Figure 8.

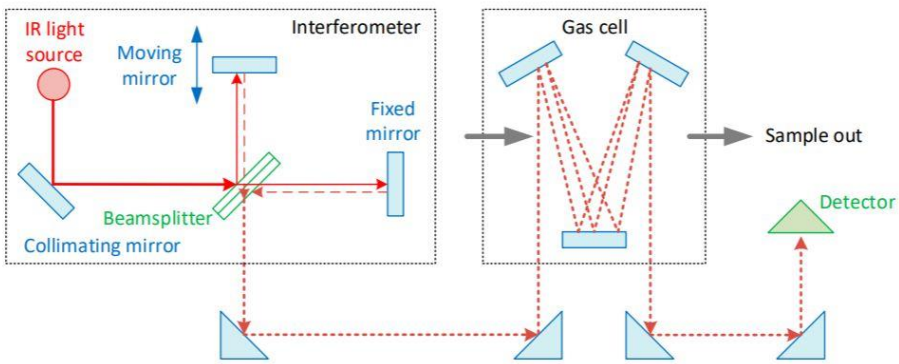

Figure 8: The mechanism of FTIR attained from Giechaskiel and Clairotte (2021)

FTIR has become a standard trace-gas analysis method due to the commercially available parts and portability, making it optimal for field testing. Additionally, multiple sources detailing FTIR are available online and can be referred to if required.

7. PROTOCOLS FOR MEASURING N_2O FROM WWTPs

This section of the report will investigate N_2O protocols curated by fellow researchers. This involves summarising existing protocols, comparing the advantages and disadvantages between protocols and finally presenting our simplified protocol that acts as a general guideline in deciding which operations to follow for N_2O collection and analysis. The global cooperation for the exchange and generation of water knowledge (GWRC) published a report detailing the different protocols other countries use to measure N_2O. Foley et al. (2015) are the leading researchers paving the way for establishing a standardised N_2O protocol; he and his team have published multiple protocols for capturing N_2O that vary in complexity. This section of the report will focus on summarising their findings.

7.1. Review of N_2O protocols - USA

Chandran, Kartik. (2011) developed a protocol to attain consistent and accurate data on N_2O emissions from open-surface wastewater treatment bioreactors in the USA. The protocol can be split into three sections: sampling procedures, data analysis and standardisation of protocol, and protocol adaptation. These sections are evaluated in the following pages.

7.1.1. Sampling procedures

N_2O headspace gas measurements: Measuring N_2O fluxes from the headspace involve combining an EPA device with the South Coast Air Quality Management District (SCAQMD) tracer methods. This is a specialised method capable of measuring high surface flux rates, making it more suitable for measurements in WWTPs. Figure 9 depicts the surface-emission isolation flux chamber (SEIFC) used for capturing the amount of N_2O fluxes in the treatment device. Sampling should be undertaken at multiple locations around the WWTS. More importantly, the SEIFC device should be placed in the centre of the anoxic and aerobic zone to attain encompassing data representative of the treatment facility. For discrete measurements, Chandran, Kartik. (2011) recommends taking 30 measurements of N_2O and one measurement of aqueous N_2O over 30 minutes. During continuous measurement, gaseous N_2O concentrations should be measured every minute, while aqueous concentrations should be measured every 4 hours in a 24-hr period. N_2O in the aqueous phase can be measured using a Clark-type sensor (Chandran, Kartik, 2011). Continuous real-time measurements of N_2O were performed using Infrared (IR) analysers as mentioned in section 6.6.

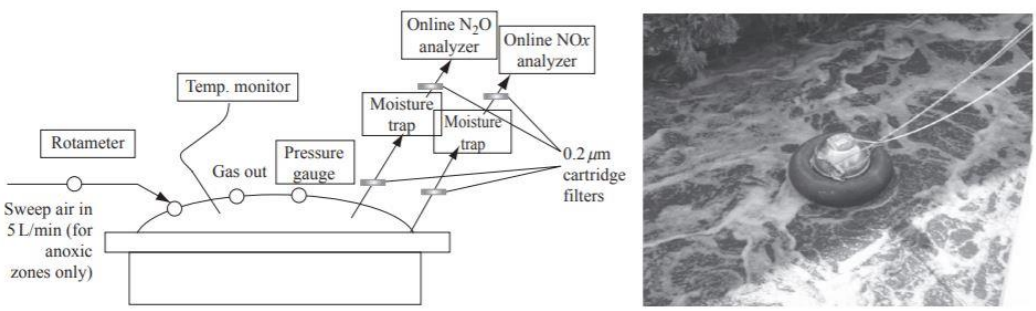

Figure 9: Schematic of the SEIFC device and the device functioning in a WWTP (Chandran, Kartik, 2011)

Measuring the advective gas flow rate (Q_e) in a flux chamber is imperative as it can be later used to determine the flux of N_2O. Additionally, a gas tracer is needed in the system to determine Q_e, in this case Chandran, Kartik. (2011) used helium as the gas tracer ($C_{He-tracer}$), the flow rate of the helium tracer is represented by $Q_{He-Tracer}$. Off-gas helium concentrations in the flux chamber ($C_{He-Offgas}$) were estimated using field gas-chromatograph equipment. Chandran, Kartik. (2011) equation for Q_e in aerated zones is depicted in Eq. (1).

$$Qe = \frac{Q_{He-Tracer} * (C_{He-tracer} - C_{He-Offgas})}{C_{He-Offgas}} \qquad (1)$$

For non-aerated zones, Chandran, Kartik. (2011) recommended introducing a sweep gas ($Q_{sweep} = 5\ L/\text{min}$). Q_{sweep} is required to overcome the low advective gas flow rate in the non-aerated zones. The sweep gas will encourage the mixing of contents in the SEIFC, allowing measurements to be taken in non-aerated zones. Chandran, Kartik. (2011) equation for Q_e in non-aerated zones is depicted in Eq. (2).

$$Qe = \frac{Q_{He-Tracer} * (C_{He-tracer} - C_{He-Offgas})}{C_{He-Offgas}} - Q_{sweep} \qquad (2)$$

7.1.2. Data analysis and standardisation of protocol

Chandran, Kartik. (2011) states that the net nitrogen flux (Flux) in the system is dependent on the advective gas flow rate (Q_e), gas concentration (C) and cross-sectional area of the SEIFC (A). The formula is depicted in Eq. (3).

$$Flux\left(\frac{mg}{min * m^2}\right) = \frac{Q_e(L/min) * C(mg/L)}{A(m^2)} \qquad (3)$$

Determining N_2O Emission Fractions: Once the net nitrogen flux has been calculated for each specific zone, it is possible to translate the finding to determine N_2O emission fractions (unitless). Chandran, Kartik. (2011) did this by normalising the net flux from each specific zone by the daily influent of TKN loading as shown in Eq. (4).

$$Emmison\ fraction = \frac{\sum_{i=1}^{n} Flux_i(\frac{kg\ N_2O - N}{m^2 d}) * Area_i(m^2)}{TKN\ load\ (kg - N)} \qquad (4)$$

Where:

- Flux is the net flux calculated for the *i*th zone
- The area is the surface area of the specific zone
- n is the number of zones
- TKN loading (influent Q * influent TKN concentration) over a 24-hour period

Every protocol must be validated, in Chandran, Kartik. (2011) study, two teams from the Columbia University Water Environment Research Foundation (WERF) were tasked with validating Kartik's protocol. The research foundation followed Kartik's protocol and conducted several experiments, including off-gas measurements using FTIR analysis and gas-filter correlation techniques and successive-dilution methods to test the validity of Kartik's protocol. The validation process was a resounding success, and importantly, no form of "biasing" was found. The final output, using Kartik's protocol, can be seen in Figure 10.

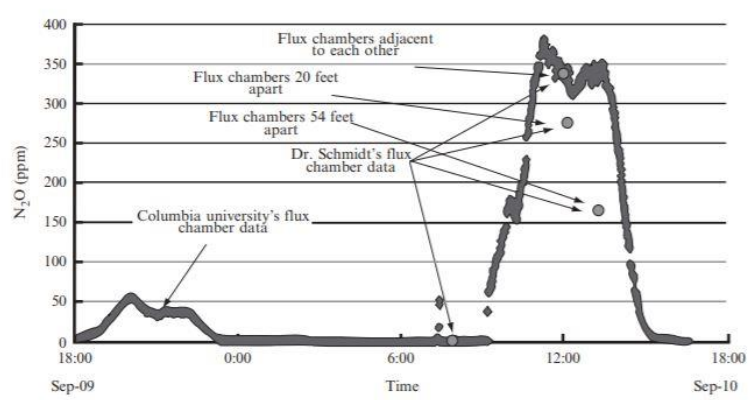

Figure 10: Illustration of the behaviour of N_2O concentrations in an aerobic zone during real-time online monitoring attained from (Chandran, Kartik, 2011).

7.1.3. Protocol adaptation for lab and field-scale measurements

Miniature versions of the SEIFC device can be implemented to fit lab-scale bioreactors, which scales down the protocol to facilitate lab and field-scale measurements. The procedure for gas and liquid phase measurements remains the same. However, the sampling frequency of liquid-phase variables may vary depending on the experimental setup (Chandran, Kartik, 2011).

7.2. Review of N_2O protocols – Australia

7.2.1. Sampling procedure

Sampling was conducted at multiple full-scale BNR WWTPs in Australia. Each WWTP in Australia differed in configurations, sizes, and effluent qualities, the variability in the wastewater treatment processes may help to highlight the factors contributing to N_2O production (Foley et al., 2015).

Data on the COD, TN, and other operational parameters were collected for the N_2O mass balances during each sampling session. The field data were collected using the following methods (Foley et al., 2015):

- Collection of wastewater samples
- Measurement of critical operational parameters (DO, pH, temperature) via water quality meter
- Determination of aqueous N_2O concentrations using a Clark-type microsensor

7.2.2. Data analysis

In the Australian protocol, determination of N_2O emission was done through COD and TN mass balances at every WWTP location. A combination of the analytical data as well as plant data were collected to perform the mass balances. The mass balance approach differs from other protocols; however, this approach encompasses the entire WWTP process, thus resulting in a very accurate estimation of the N_2O emissions.

Foley et al. (2015) took an iterative approach when determining the final N_2O mass balance equations. He and his team had to take multiple factors into accounts, such as the varying configuration of independent WWTPs and the individual zones for each WWTP. Once all the factors were considered, the mass balance equation was complete; however, it's rather complicated; refer to Foley et al. (2015) for the entire workings.

The mass of emitted nitrogen is determined by Eq. (5).

$$M_{N,AtmDN} = M_{n,inf} - M_{n,Eff} - M_{N,S} \qquad (5)$$

The emission factor for each zone in the WWTS is given in Eq. (6).

$$EF_R = \frac{Tr_{N_2O-N,R}}{M_{N,AtmDN}} \qquad (6)$$

Where:

- EF_R represents the N_2O emission factor for each treatment zone
- $Tr_{N_2O-N,R}$ represents the net generation of $N_2O - N$ emissions
- $M_{N,AtmDN}$ is the mass of emitted nitrogen

7.3. Review of N_2O protocols – France

7.3.1. Sampling procedure

France has two separate sampling techniques, one for aerated zones and the other for non-aerated zones. For aerated zones, a 1x1 meter wooded sampling box equipped with a gas mass counter is used to quantify the flow rate 'Q' (m^3/h). For non-aerated zones, the Odoflux dynamic flow chamber is used to quantify the flow rate. The sampling devices are shown in Figure 11.

Figure 11: Sampling device for aerated zones (left-hand figure) and sampling device for non-aerated zones (right hand figure) attained from (Foley et al., 2015)

The sampling methods must be calibrated based on GHG recommendations. To satisfy 'pseudo-isokinetic' conditions, flow in must equal flow out (Q1=Q2); the flow must be limited between 3 to 10 L/min; to meet 'static chamber' (uninterrupted liquid) conditions.

7.3.2. Data analysis

Steps for N_2O data analysis is depicted in Figure 12.

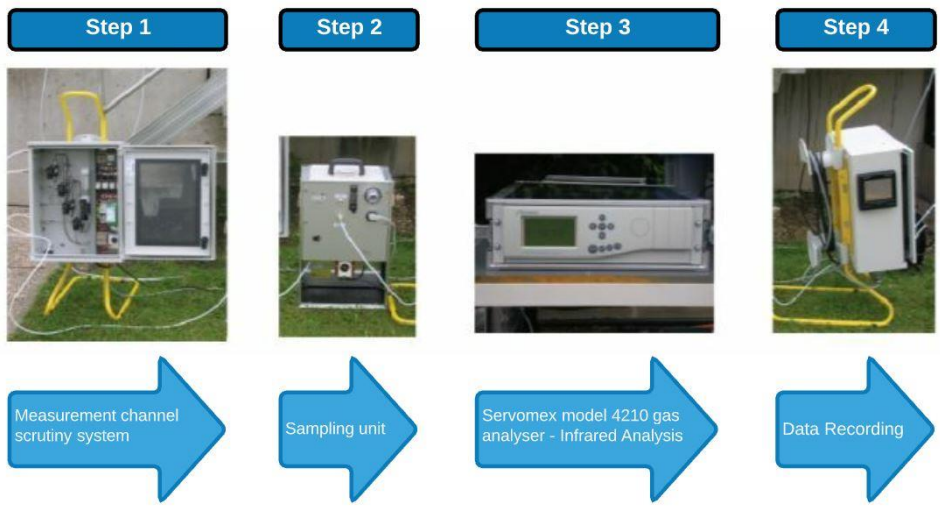

Figure 12: Steps to quantifying N_2O concentrations based on findings from Foley et al. (2015)

Step 1**:** The collected gas is fed through the mobile analysis system (sequencing box). The sequencing box is part of the preliminary stage to quantify N_2O concentrations.

Step 2**:** The output from the sequencing box is fed through a sampling unit via a 4/6mm Teflon pipe. The sampling unit ensures the samples are at optimal condition before infrared analysis.

Step 3**:** The sample is fed through the Servomex model 4210 gas analysers equipped with a filiation device for N_2O quantifications.

Step 4**:** The N_2O concentration (ppm) data is stored and exported to excel for practicality.

N_2O emissions are expressed as $mg * h^{-1} * m^{-2}$, it was determined by the output concentration measured by the sampling system. During the analysis process, key parameters such as DO, ammonia and nitrates were monitored to gain greater insight into N_2O formation.

7.4. Comparisons – Advantages & Disadvantages between N_2O protocols

Below are tables that summarise the points discussed in the prior sections. Note that protocols for the majority of WWTPs around the world were not detailed in this review, as there is limited information available.

Table 2: Summary of N_2O collection methods undertaken by various countries attained from Foley et al. (2015)

Country	Sample collection	Period	Time	Location in WWTP	Liquid / gas phase	Gas hood / Total off gas
Australia	Grab samples	Winter / Spring 2008; Ideally 4 rounds.	Per round: 2-4 hours per day for 2 days	Anaerobic, anoxic, aerobic zones	Liquid	-
France	Continuously	*Information not available*	*Information not available*	Anaerobic, anoxic, aerobic zones	Gas	Gas hood
USA	Continuously / Grab samples	Summer / early Fall & Winter / early Spring	1 day (1/min; gas phase) 4-5x day (liquid)	Anaerobic, anoxic, aerobic zones Geometric center	Liquid (grab) & Gas (grab and continuous)	Gas hood
Netherlands	Continuously	Fall / Spring / Winter	7 days	-	Gas	Total off gas

Table 3: The methodology used to quantify N_2O concentrations in the gas/liquid phase attained from Foley et al. (2015)

Country	N_2O gas phase	N_2O liquid phase
Australia	-	Clark-type microsensor (N_2O 25 with 70µm outside tip diameter), Unisense A/S, Aarhus, Denmark
France	Servomex model 4210 gas analyser	Via liquid phase sampling*
USA	Infrared	Miniaturised Clark-type sensor with internal reference and guard cathode; Unisense A/S, Aarhus, Denmark
Netherlands	Infrared; Emerson Process management Rosemount Analytical	-

Note. Reprinted from "N_2O and CH_4 emission from wastewater collection and treatment systems."
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Table 4: Outline of the advantages and disadvantages of the reviewed protocols

	USA	Australia	France
Advantages	• Highly effective N_2O protocol, verified by WERF. • The protocol is scalable. • Utilises online monitoring, produces a wide range of data. • Capable of taking gas & liquid phase N_2O measurements	• The Australian protocol considers the differences in configuration resulting in an accurate representation of the data. • Different treatment zones (anoxic, aerobic, etc.) are included in the data analysis.	• A simple and effective method • Operationally feasible • It can be used for smaller-scale WWTPs • Satisfies GHG sampling conditions
Disadvantages	• This is an expensive procedure that requires high-cost equipment and helium tracers. • N_2O protocol is extensive, may not be operationally feasible for some WWTPs.	• Applicable to only Full-Scale WWTPs. • Extensive sampling procedure • Approach widely differs from other protocols. • Incapable of measuring gaseous N_2O.	• May not give the most accurate representation of the data set. • Cannot take liquid phase N_2O measurements.

7.5. Simplified N_2O protocol flowchart

The flowchart below summarises the high levels procedures that must be taken to Quantity N_2O emissions. It also acts as a general guideline in deciding which operations to follow for N_2O collection and analysis. For example, if analysis of only aqueous N_2O is needed, the suggested sampling strategy would be to use Clark type sensors; additionally, if exact measurements are desired, implementing procedures reported by the USA or Australia would be recommended.

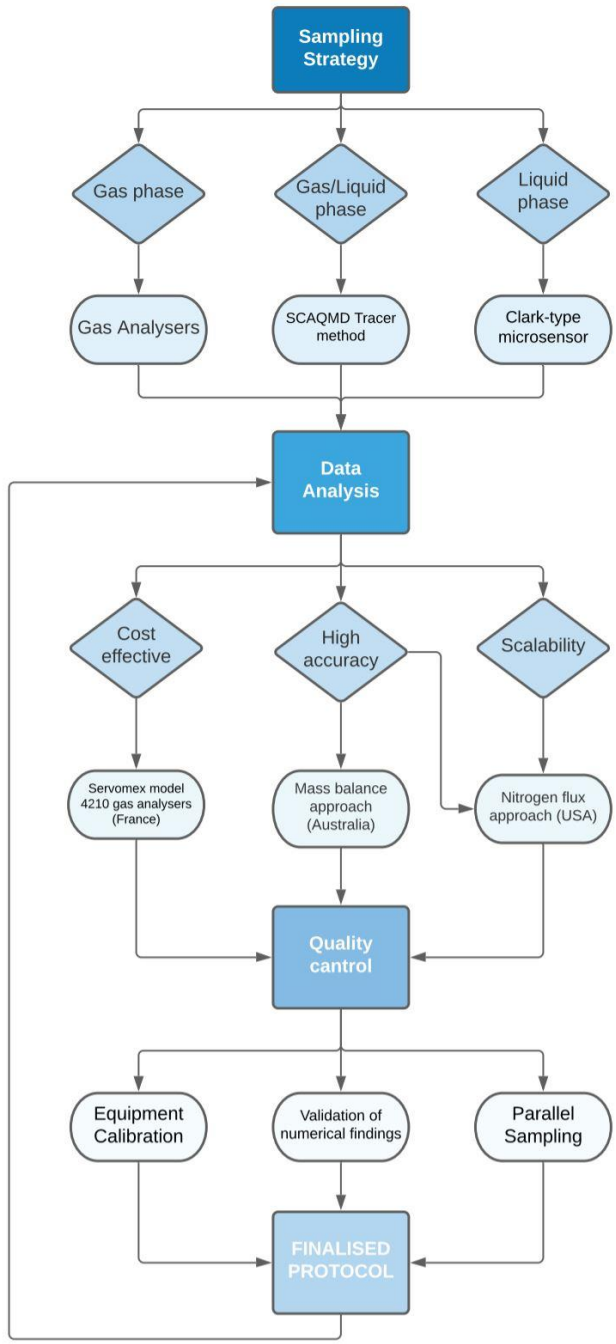

Figure 13: Flowchart depicting the N_2O protocol operations

8. RESULTS & DISCUSSION

This section of the review will evaluate the effects key operational parameters (identified in section 5 of the report) have on N_2O production using findings from existing literature. The following critical parameters were assessed to gain a greater understanding of the relationship it has on N_2O production:

- Dissolved Oxygen (DO)
- Aeration
- Nitrite concentration
- Ammonia Oxidation rate

The results were attained from existing literature; unfortunately, due to COVID-19 restrictions, we were unable to produce our own dataset for evaluation. However, we have summarised the main N_2O findings from WWTPs worldwide; refer to the appendices at the end of the report.

8.1. The effect of varying DO concentrations on N_2O emissions

Pijuan et al. (2014) investigated the effects varying DO concentrations have on N_2O emissions. His main findings were as follows:

- The N_2O emission factor is at its highest at DO levels below 2mg O_2/L, as seen in figure 13.
- At high DO levels (4.5+ mg O_2/L), N_2O emission factors remained constant at 2.2% of N^- oxidised.
- High DO concentration (6+ mg O_2/L) did not have any effect on the N_2O emission factor.
- When changing the reactor's operation to SBR mode, a high DO concentration (5+ mg O_2/L) increased N_2O emissions significantly. This goes against the trend of low DO levels means low N_2O emission factor, signalling more research is needed for this topic.

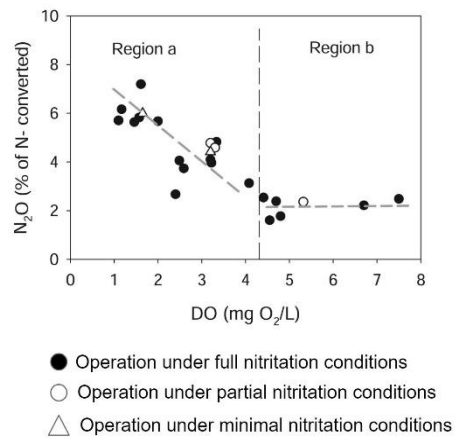

Figure 14: Relationship between DO and N_2O emission factor attained from Pijuan et al. (2014).

8.1.1. Different N_2O pathways under varying DO concentrations

Multiple researchers have claimed that lower levels of DO will result in increased N_2O emissions. This statement is clear; however, what is not clear is what levels of DO concentration should be maintained in wastewater treatment to minimise the production rate of N_2O emissions. This is because different levels of DO for WWTPs worldwide result in different amounts of N_2O production, so no conclusive statement can be made.

Pijuan et al. (2014) took a different outlook; they proposed that there are two distinct processes behind N_2O generation, one reliant on DO concentration and one independent of it. As mentioned in section 4 of the report, the two main pathways of N_2O in WWTPs are the nitrifier denitrification pathway and the hydroxylamine oxidation pathway. Recent investigations suggest that DO concentrations have different effects depending on the N_2O pathway. N_2O produced through the hydroxylamine oxidation pathway are more susceptible to DO; as a result, high DO concentration will increase the N_2O production. In contrast, low DO concentrations lead to higher N_2O production during the nitrifier denitrification pathway (Sutka et al., 2006).

8.2. The effect of aeration on N_2O emissions

Castro-Barros et al. (2015) investigated the effect of varying aeration rates on N_2O emissions. The following are the results they have found during their investigation:

- Gas stripping was at its maximum at high aeration rates, which accelerated the N_2O formation rate, resulting in high amounts of N_2O emissions, as depicted in Figure 15.
- During the steady-state part of the experiment (consistent gas flow rate), the formation of N_2O equalled that of the N_2O that was stripped (emissions).
- Interestingly during low aeration phases, some N_2O failed to transition into the gas phase to compensate N_2O accumulation in the liquid phase increased exponentially.
- This pattern depicted in Figure 15 remained identical during the next interval of the experiment, suggesting that the behaviour of aeration is relatively consistent.

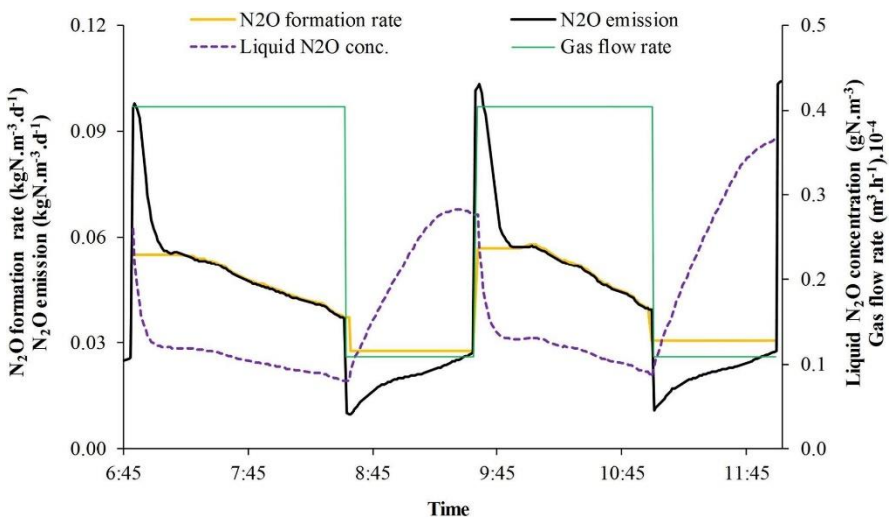

Figure 15: N_2O production rate depending on aeration levels attained from Castro-Barros et al. (2015)

8.2.1. The effect of no aeration (anoxic conditions) on N_2O emissions

The effect of aeration is known, but what happens if there is no aeration in the system? Castro-Barros et al. (2015) conducted an experiment to test this situation. Aeration was completely stopped for 2 hours which decreased the rate of N_2O stripping, thus reducing N_2O emissions; when the air was reintroduced into the reactor, an N_2O emission peak was detected. This suggests that during anoxic conditions, N_2O was consumed since no fresh air was added, preventing emissions from developing from the reactor.

8.3. The effect of nitrite (NO_2^-) concentration on N_2O emissions

The Global Water Research Coalition (GWRC) concluded that high nitrite concentrations accelerate the formation of N_2O during denitrification and nitrification, increasing N_2O emissions. The nitrite concentration is believed to be dependent on various parameters such as (Kampschreur, 2009):

• SRT
• toxic compounds
• Extreme temperatures
• Increased ammonium (NH_4^+) concentrations

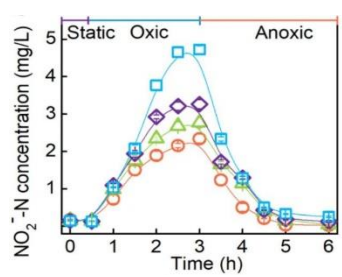

Figure 16: NO_2^- concentration at different cycles attained from Chen et al. (2020)

The nitrite concentration is at its peak around 2 – 3 hours during the experiment, as seen in Figure 17; coincidently, the highest N_2O emission rate was obtained within that timeframe, suggesting that nitrite is one of the critical parameters that dictate the rate at which N_2O emissions are produced.

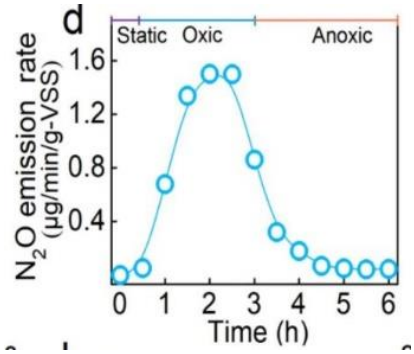

Figure 18: N_2O emission rate at different time intervals attained from Chen et al. (2020)

8.3.1. The effect of carbon sources on NO_2^- concentrations

Ge et al. (2012) used glucose as the primary carbon source. Glucose was fed into the reactor, which resulted in high rates of NO_2^- accumulation. More specifically, glucose was responsible for 23% of the NO_2^- accumulation in the system, more than any other carbon source (methanol and benzoic acid). With Ge et al. (2012) findings, we have found yet another factor that can contribute greatly to N_2O emissions, which signifies the importance of continuous research and highlights the amount of research that is still yet to be done.

8.4. The effect of ammonia oxidation rate (AOR) on N_2O emissions

Law et al. (2012) investigation on the relationship between AOR and N_2O emissions resulted in the following findings:

- AOR has an exponential relationship with N_2O production during the hydroxylamine oxidation pathway, as seen on the left-hand graph in Figure 19.
- AOR has a positive linear relationship with N_2O production during the nitrifier denitrification pathway, as seen on the right-hand graph in Figure 19.
- DO and NH_4^+ accelerates AOR, resulting in increased N_2O production.
- The transition from aerobic to anoxic conditions resulted in the greatest N_2O production.

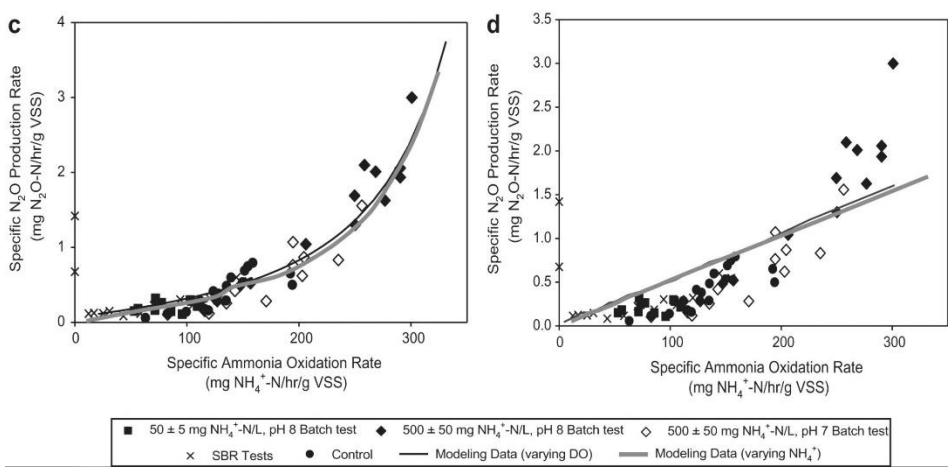

Figure 19: AOR vs N_2O production attained from Law et al. (2012)

The exponential relationship between AOR and N_2O production goes against trends found in previous studies, which reported only a linear relationship between AOR and N_2O. However, past studies were not as advanced and used outdated methodologies and smaller sample sizes to conduct their experiments, which hindered the data integrity. Nonetheless, the discovery of potential exponential relationships provides new insight into the mechanisms involved in N_2O production.

9. N_2O MITIGATION STRATEGIES

The key to developing appropriate mitigation tactics is first to establish an effective surveillance strategy capable of accurate N_2O monitoring. An effective surveillance strategy involves picking a suitable analytical device and following an optimal protocol (reported in sections 6 and 7 of the report). With proper N_2O monitoring, developing effective mitigation strategies will become much more feasible, which will, in turn, be greatly beneficial to our environment. Nevertheless, a variety of new and exciting methods developed to mitigate N_2O production is outlined within this section. Fig. 20 below highlights why regulating metabolic pathways and enzymatic is critical to mitigate N_2O production.

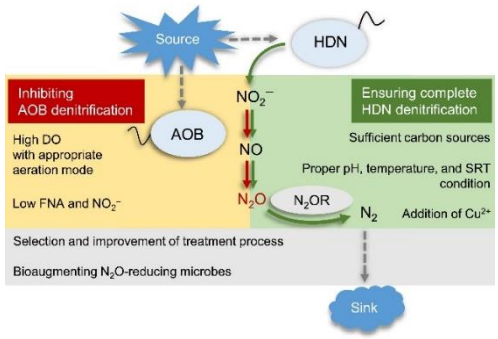

Figure 20: The significant strategies used to mitigate N_2O productions in WWTPs attained from Chen et al. (2020)

9.1. Optimisation of effective parameters

In laboratory experiments, it has been demonstrated that reducing N_2O production by optimising operational parameters is feasible (Chen et al., 2020). As discussed in Section 5, the Accumulation of DO and NO_2^- significantly impacts N_2O production. As a result, further minimising NO_2^- accumulation and regulating DO would effectively reduce the N_2O produced by AOB. Additionally, determining the optimal aeration input for WWTS is an effective technique to reduce N_2O emissions.

Other operating parameters, such as temperature, pH, solid retention time (SRT), and so on, would also significantly affect N_2O production (Chen et al., 2020). As a result, mitigation techniques dependent on these factors must be acknowledged. Avoiding acidic conditions during denitrification helps reduce N_2O, with a neutral pH being ideal. Lowering pH levels at given intervals under aerobic conditions can effectively inhibit N_2O development (Chen et al., 2020). Furthermore, in the nitrogen removal process, increasing the SRT will decrease the rate at which N_2O is produced (Li and Wu, 2014; Zhou et al., 2019).

9.2. Regulation of microbial community structure

The composition of the microbial community has a strong influence on nitrification and denitrification performance (Chen et al., 2020). Bacteria activities play an essential role in lowering N_2O levels. A common strategy for controlling microbial activity is achieved through the addition of Alcaligenes faecalis, a dominant bacterium (Chen et al., 2020). Alcaligenes faecalis is a potent bacterium used in WWTS to control N_2O production whilst improving nitrogen removal efficiency.

9.3. Copper additive

Cu^{2+} is used to slow down the microbial process of reductases (NirK and N_2OR) thus, reducing the N_2O to N_2 production, which will ultimately decrease the N_2O emission factor. Additionally, Cu^{2+} can be added to liquid sludge acting as an additional form of carbon substrate, thus decreasing N_2O and NO output while not affecting nitrogen and phosphorus removal performance (Chen et al., 2020).

10. CONCLUSIONS

This review explored and evaluated key concepts that relate to N_2O production from WWTPs. We began by identifying the main N_2O production pathways responsible for N_2O emissions. The production pathways are as follows, i) Autotrophic nitrification through the hydroxylamine oxidation pathway, ii) Autotrophic nitrification through the nitrifier denitrification pathway and iii) the heterotrophic denitrification pathway carried out by the heterotrophic bacteria. However, it is believed that several primary and intermediate pathways involved in N_2O production has not yet been found due to insufficient amounts of research and data availability.

The second evaluation step involved identifying and assessing the key operational parameters that contribute most to N_2O emissions. Our investigation concluded that roughly nine operational parameters play a role in N_2O production; however, out of the ten parameters, four were evaluated to be the most critical. The four parameters are as follows, i) dissolved oxygen, ii) nitrite concentration, iii) ammonia oxidation rate and lastly iv) aeration, which by itself is not very impactful; however, aeration can amplify the influence of the aforementioned parameters, which will ultimately accelerate the rate at which N_2O is produced.

Establishing a surveillance strategy to monitor N_2O is essential for control emission rates from WWTPs. The third step of evaluation involved investigating standard analytical methods used to capture N_2O emissions from WWTPs. We studied six analytical methods that have been used to quantify N_2O. Out of the six, we believe that sensors (such as Clark type microsensors), infrared analysers and online sampling devices are the key analytical devices that should be implemented for N_2O surveillance. This is attributable to the fact that these devices can capture continuous measurements, which will offer a broad range of data that can be utilised to spot patterns and hence give helpful information that may be employed in various control strategies.

To implement a surveillance strategy, we must first establish a protocol for capturing N_2O emissions. In the fourth step of evaluation, we investigated and assessed protocols reported by other countries. Each protocol was inherently different because every WWTP is distinct; they come in various sizes and configurations and operate at different parameters. In our original research aim, we wanted to figure out a way to standardise a protocol; however, based on this review, we found that this is out of scope. So, in turn, we summarised each protocol and outlined its advantages and disadvantages. Next, we created a flow chart that acts as a guideline in what protocol should be followed depending on preferences. For example, if highly accurate measurements for N_2O in both gaseous and aqueous phases is desired; following the USA protocol would be the best option.

New mitigation strategies have emerged, such as regulating microbial communities and adding copper additives to slow down the microbial processes; however, the key to mitigating N_2O emissions from WWTPs rely on the surveillance strategy. The surveillance strategy will provide helpful information to make changes in the WWTP operation, allowing the optimisation of effective parameters such as DO and nitrite. By doing so, we will be one step closer to minimising N_2O emissions in the atmosphere, ultimately preventing the severe depletion of our ozone.

11. FUTURE RESEARCH

A considerable knowledge gap exists in the field of N_2O production from WWTPs. Additional research is needed to uncover the mechanisms behind the N_2O pathway in order to figure out new and effective mitigation strategies to limit N_2O emissions. The importance of continuous development and evaluation based on existing statistical models cannot be overstated. A key priority should be to combine existing information on AOB and HDN to identify the remaining N_2O production pathways. The limitations of current measuring instruments illustrate the need for more accurate methods to be developed. Early investments into long-term N_2O surveillance projects with machine learning capability should be made to expedite research and produce precise data models that can be utilised in N_2O control strategies for WWTPs worldwide.

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Reference table:

Number	Reference (hyperlinks)
1	Ahn et al., 2011
2	Law et al., 2011
3	Quan et al., 2012
4	Zhu and Chen, 2011
5	Xie et al., 2012
6	Zhou et al., 2012
7	Hu et al., 2013
8	Lochmatter et al., 2013
9	Chen et al., 2014
10	Eldyasti et al., 2014
11	Wang et al., 2014a
12	Wang et al. 2014b
13	Adouani et al., 2015
14	Peng et al., 2015a

15	Gabarro et al., 2014
16	Pijuan et al., 2014
17	Frison et al., 2015
18	Li et al., 2015
19	Zheng et al., 2015
20	Aboobakar et al., 2013
21	Spinelli et al., 2018
22	Sun et al., 2013
23	Yan et al., 2014
24	Rodriguez-Caballero et al., 2015
25	Kosonen et al., 2016
26	Brotto et al., 2015
27	Kampschreur et al., 2008
28	Weissenbacher et al., 2010
29	Gustavsson and la Cour Jansen, 2011
30	Stenström et al., 2014
31	Ahn et al., 2010

14. APPENDIX

The tables below summarise the main N_2O findings from WWTPs all around the world. Each **number** corresponds to the reference (given as a hyperlink); refer to the reference table above to go to the source.

Lab Scale								
Number	**Location**	**Methodology**	**Configuration**	**Water type/Characteristics**	**HRT/NLR**	**N20 Production Mian Finidngs**	**Hotspot**	**Main Findings**
1		-	1 bioreactor operated sequentially in nitrification & nitritation	• Synthetic • NH4-N = 500 mg L-1	• HRT= 1.1 d • NLR= 454.5 mg L-1 d-1	N20 emission: • 0.13 ± 0.24% of influent NH4-N (nitrification) • 0.57 ± 0.17% of influent NH4-N (nitritation) Average N20 emission: 0.55% of influent NH4-N	Nitrifier denitrification	**• Nitritation reduced operating costs & energy requirements • optimization needed against the C-footprint related to higher N20 emissions**
2	Brisbane, Australia	• SBR was operated to select for AOB performing partial nitritation • pH automatically adjusted using a programmable logic controller (PLC) system • Gaseous N2O concentration was analysed with an infraredanaly	SBR	• Synthetic with characteristics of an - aerobic digester liquor • NH4-N = 1000mgL-1	• HRT= 1 d • NLR= 1000 mg L-1 d-1	Average N20 emission: 0.55% of influent NH4-N	AOB pathways at pH 8 (no clear predominance between the two pathways)	**• pH increase enhanced the AOR of the AOB culture • 6.0 s pH s 7: minimum N20 production (0.15 ± 0.01 mg N20-N h-l g-1 VSS) • pH = 8: maximum N20 production (0.53 ± 0.04 mg N20-N h-l g-1 VSS)**
3	Beijing, China	• 3 aerobic SBR systems in 4 hour cycles • measurement of soluble N2O in the liquid phase with a picoammeter • N2O emissions from the aqueous phase to the atmosphere was mainly through air stripping across the water-air interface in the SBRs. Linear equations used to measure air stripping rates of N2O.	3 aerobic granular sludge SBRs	• Synthetic: mixture of municipal & pig manure digestate liquid • N20 measurement in 3 10-day operational periods: influent NH4-N = 148, 106 & 74 mg L-1 (corresponding to COD:N ratios of 1:0.22, 1:0.15 & 1:0.11)	• HRT = 0.33 d • NLR = 448.5 mg L−1 d−1 (influent NH4-N = 148 mg L−1), 321.2 mg L−1 d−1 (influent NH4-N = 106 mg L−1), 224.2 mg L−1 d−1 (influent NH4-N = 74 mg L−1)	N20 emission (relative to influent N) at the aeration rates of 0.2, 0.6 & 1.0 Lmin^{-} • 8.2%, 6.1% & 3.8% (COD:N =1:0.22) • 7.0%, 5.1% & 3.5% (COD:N = 1:0.15) • 4.4%, 2.9% & 2.2% (COD:N = 1:0.11)	• Low DO (0.5-1.5 mg LA): heterotrophic den itri-fication • DO>1.5mgL-l: AOB pathways	**• N20 emissions decreased with the increase of aeration rate & COD:N ratio**
4		No Accsess	1 SBR with acetic acid as C-source & 1 SBR with sludge fermentation liquid as C-source	• Municipal (real supplemented to attain the desired NH4-N, TN concentrations) • Average initial NH4-N = 30 mg L−1 • Average initial TN = 35.5 mg L−1	• HRT = 0.67 d • NLR = 53 mg L−1 d−1	N2O generation: • 0.507 ± 0.028 mg N2O-N mg−1 N-removed (acetic acid as Csource) • 0.121 ± 0.016 mg N2O-N mg−1 N-removed (sludge fermentation liquid as C-source)	Heterotrophic denitrification	**• Sludge fermentation liquid as C-source increased the number of bacteria reducing N2O to N2**
5	Hefei, China	• N2O concentration in the reactors was calculated with Henry's law • SBR with 6 hour cycle	AOB-enriched SBR system	• Synthetic • NH4-N = 1000mgL-1	• HRT = 0.5 d • NLR = 2000 mg L-1 d-1	N20 emission: 0.44% of total NH4-N oxidation	Nitrifier denitrification	**• Low DO (eg DO<O.5 mg L-1): AOB with N02^{-} as terminal electron acceptor responsi-ble for N20 emission**
6	Singapore	• Dissolved N2O (within the liquid phase) and emitted N2O (in the reactor headspace) samples were summed together • N2O was measured by gas chromatography • Helium was used as the carrier gas	SBR	• Synthetic • NH4-N = 20mgL-1	• HRT = 0.83 d • NLR = 24.1 mg L-1 d-1	N2O reduction rate (mg N2O min−1 g biomass−1) with COD:N ranging from 0.625 to 2.5: • 0.036–0.05 (internal carbon source: PHA) • 0.0025–0.05 (external carbon source: sodium acetate)	Heterotrophic denitrification	**• Lowest COD:N(=0.625): very limited N2O reduction with external carbon source • COD:N = 0.625–1.25: sharper increase in the N2O reduction rate with external carbon source • COD:N = 1.875–2.5: similar N2O reduction rates regardless of carbon source type**
7	Jinan, China	• 3 A/O SBRs with different carbon sources with 5 steps. • Off gases were collected into gas sampling bags to measure the N2O concentrations.	3 anoxic/oxic SBRs	• Synthetic mimicking urban • NH4-N = 55.4 mg L-1	• HRT = 0.6 d • NLR = 92.3 mg L−1 d−1	Percentage of TN-removed converted to N20: • 5.3% (carbon source: glucose) • 8.8% (carbon source: sodium aceate) • 2.8% (carlx)ll source: soluble starch)	Heterotrophic denitrification	**• Sodium acetate (compared to glucose & soluble starch): optimal nutrient removal but highest N20 emission (low denitrifiers diversity)**
8	Lausanne, Switzerland	• N2O was measured by gas chromatography • As the head-space gas was recirculated, the measured concentrations do not correspond to the real emissions. • A tracer gas was then used to determine the ratio between recirculated gas and gas leaving the system.	2 aerobic granular sludge SBRs	Synthetic • NH4-N= 50mg L-1	• HRT = 0.25 d • NLR = 200 mg L-1 d-1	Alternating high/low D highest N20 emission with the lowest COD loading rate (1.6 g COD LA (1-1) at the low-DO stage: 9% of influent N	Heterotrophic denitrification	**• N20 emissions significantly decreased with higher COD loads & under AND conditions**
9	Shanghai, China	N2O concentration in gas and liquid phases was determined by the Unisense microsensors.	1 SBR with a sequence of anaerobic-oxic-anoxic phases & I anaerobic phase-cancelled SBR	• Synthetic • NFL-N = 31 mg L-1	HRT = 0.46 d • NLR = 67.4 mg L−1 d−1	N20 generation: • 0.069 ± 0.002 mg N20-N mg-TN-removed (anaerobic-anoxic SBR) • 0.040 ± 0.003 mg N20-N mg-I TN-removed (anaerobic phase- cancelled SBR)	Nitrifier denitrification	**• N20 generation reduced by 42% in the an-aerobic phase-cancelled SBR by benefiting the heterotrophic denitrifiers' activity**
10	Ontario, Canada	• Operating anoxic denitrifying fluidized bed bioreactors (DFBBRs) at two different loading rates • Synthetic municipal wastewater and recycled effluent pumped into DFBBR • Purpose is to discover biofilm thickness' contribution to N20 emissions	FBBR (Fixed-Bed Bioreactor)	• Municipal (synthetic) • TN = 31 ± 1.5 mg (phases I & II) • TN = 51 ± 2.5 mg L-l (phase II)	• HRT = 0.025 d • NLR = 1240 mg L−1 d−1 (phases I & III), 2040 mg L−1 d−1 (phase II)	N20 emission: • 0.53% of influent TN (biofilm thickness = 680 um) • 1.57%ofinfluentTN (limited COD& biofilm thickness = 230 um)	Heterotrophic denitrification	**• N20 emissions decreased exponentially with biofilm thickness increase due to the slow growth denitrifiers retention & the limited N20 diffusivity**

11	Brisbane, Australia	• Measurements were taken from the Two-Lab scale SBR performing nitrification. The return activated sludge from the WWTP was the input. • Gas and liquid N20 phases were measured every 3-4 days through online gas analysers and liquid microsensors. • Batch experiments were carried out to investigate factors leading to higher N20 emissions.	2 SBRs: a control SBR & an experimental SBR achieving the NO2− pathway via an FNA treatment unit	• Synthetic: domestic wastewater & anaerobic digestion liquor • TKN = 50 mg L-1 • Anaerobic digestion liquor addition: additional 20% N-load	• HRT= 1 d • NLR=60mg L-1 d-1	N20 emission: • 1.52 ± 0.43% of influent TN (control reactor) • 0.52 ± 0.03% of influent TN (experimental reactor)	Nitrifier denitrification	**• FNA-based strategy for N02¯ pathway: TN-removal substantially improved without negatively affecting N20 emission**
12	Brisbane, Australia	The same methodology as the one above; however, SBR is fed with sludge reject water instead of return activated sludge.	2 SBRs	• 1 SBR fed with real sludge reject water (NH4-N = 861 ± 13 mg LA) & I SBR fed with synthetic reject water (NH4-N = 1000 mg L¯l)	• HRT= 1 d • NLR = 861 mg L-1 d-1 (real sludge reject water), 1000 mg L-1 d-1 (synthetic sludge reject water)	N20 emission: • 3.12 ± 0.16% of NH4-N oxidized (SBR with real digestion liquor) • 0.8 ± 0.09% of NH4-N oxidized (SBR with synthetic digestion liquor)	Heterotrophic denitrification	**• Nitritation systems with real anaerobic sludge digestion liquor. N20 emissions via heterotrophic denitrification aided by the organic C in the liquor • FNA hindering effect: heterotrophic N02¯ reduction IN)ssibly stopIRd at the N20 formation stage**
13	Mulhouse, France	• Experimentations were achieved in a batch reactor of 10 L of working volume. • Nitrates, nitrites, and COD concentrations in the liquidphase were determined by colorimetric methods according to French normalized standards. • The reactor headspace was periodically analyzed byusing an online gas chromatograph (GC Varian CP-4900) tomeasure N2O emissions.	Batch reactor	• Synthetic • NH4-N = 6mg L-1	Batch experiments	N20 emission: N20-N (N-denitrified) ¯ 1 • 13% (20°C) • 40% (10 °C) • 82% (5 °C)	Heterotrophic denitrification	**• Low temperatures slowed down all denitrification enzyme activities (eslm-ially NO and N20 reductase activities)**
14	Brisbane, Australia	• SBRs with a working volume of 8 L was operated in the laboratory at room temperature. The SBR was fed with ammonium with the aim to obtain an enriched culture of AOB and NOB. • N2O concentration in gas phase of the batch reactor was measured with a URAS 26 infrared photometer (Advance Optima Continuous Gas Analyser AO2020 series, ABB).	SBR	• Synthetic • NH4-N = 20 mg L-1	• HRT= 1 d • NLR = 20 mg L-1 d-1	• Highest N20 emission factors at lowest (0.35 mg L-1) & high NO2- (> 20 mg L-1): 20-22% • Lowest N20 emission factors at highest DO (3.5 mg L-1) & low N02- (< 5 mg L-1): 1.8-2%	Nitrifier denitrification	**• N20 emission factor increase with decreased & increased N02¯ concentration • Nitrifier denitrification predominant in a wide range of & NOX levels; NH20H oxidation dominant only at a high (e.g. 3.5 mg L¯l) with low N02¯ (eg < 10 mg L¯l)**
Pilot-scale								
15	Catalonia, Spain	• Continuous operation experiments were carried out in a PN sequencing batch reactor (PN-SBR) with a minimum and maximum volume of 111 L and 250 L, respectively. • The reactor was water jacketed to achieve a constant temperature (35 °C) by a thermostatic bath. • N20 was determined ccording to standard methods (APHA, 2005).	Partial nitritation SBR	• Industrial (real: raw landfill leachate) • NH4-N = 2000–2300 mg L−1	HRT = 0.5 d • NLR = 4000–4600 mg L−1 d−1	N2O production: 3.6% of influent TN	Heterotrophic denitrification	**N2O production: anoxic (60%) & aerobic (40%) • NO2− denitrification advancing faster than N2O denitrification; high N2O accumulation**
16	Catalonia, Spain	• The pilot plant consisted in a 150 L granular airlift reactor performing full partial nitrification of reject wastewater. • Off gas was collected continuously (at 0.5 L/min) from the reactor headspace which was covered with a plastic bag and connected via a gas tube to a gas conditioning unit (series CSS, M&C Tech group). The gas outlet from the conditioning unit was connected to the multicomponent online gas analyser (VA-3000, Horiba, Japan) which provided an online measurement of N2O and CH4 concentrations from the gas flow.	Continuous granular airlift nitritation reactor switched to SBR towards the end of study	• Sludge reject water produced in situ during the dewatering process of the anaerobic digester sludge from municipal • NH4-N = 726 ± 50 mg L−1	• HRT = 0.5 d • NLR = 1452 mg L−1 d−1	N2O emission: • 2.2% of oxidized N (airlift operation under DO N 4 • 19.3 ± 7.5% of oxidized N (when shifting to SBR operation at DO N 5 mg L−1)	• DO-dependent: nitrifier denitr • Non-DO-dependent: possibly chemical N2O production	**DO increase from 1 to 4.5 mg L−1: N2O emission decreased from 6% to 2.2% of Noxidized • Higher DO: N2O emission remained constant at 2.2% of N-oxidized • Two different mechanisms behind N2O production; one DO-dependent & one not**
17	Veneto Region in North Italy	• The gaseous emissions from the surface of the SBR tank were determined on line by the static chamber method, using the Bruel and Kjaer photo-acoustic analyzer (Type 1302, Copenhagen, Denmark). Specifically, a 0.2 m high floating container (chamber) was placed on the surface of the tank and the time variation of the gas concentration inside the chamber was measured on line, in order to determine its emission rate.	SBR	Anaerobic supernatant produced from the co-digestion of the organic fraction of municipal solid waste & waste AS • NH4-N = 559 ± 75 mg L−1	Acetic acid as C-source: • HRT = 0.75 d • NLR = 1080 mg L−1 d−1 Fermentation liquid as C-source: • HRT = 0.52 d • NLR = 2077 mg L−1 d−1	N2O emission: • 0.24% of influent N (SBR with DO = 1.5 mg L−1, vNLR = 0.81 kg N m−3, acetic acid as Csource) • 1.49% of influent N (SBR with DO = 0.95 mg L−1, vNLR = 1.08 kg N m−3, fermentation liquid as C-source)	Nitrifier denitrification	**N2O emission decreased by: • maintaining the DO ≥ 1.5 mg L−1 during the nitritation stage • applying a NLR respecting the system's Nremoval capacity • applying the aerobic/anoxic sequence**
18	-	-	SBR	• Domestic (real) • Average NH4-N = 64.37 mg L−1	• HRT = 0.625 d • NLR = 103 mg L−1 d−1	N2O accumulation ratio: • 29% (pH = 6 & DO = 1 mg L−1) • 5% (pH = 8.5 & DO = 1 mg L−1) • 12% (pH = 6 & DO = 3 mg L−1) • 2.9% (pH = 8.5 & DO = 3 mg L−1)	Nitrifier denitrification	**AOR increase with pH & DO increase**
19	Langfang, China	The channels were divided into 12 intensive samplingzones for gaseous and liquid samples collections, so in total therewere 14 sampling points including the bioselector and clarifie.	OD	• Synthetic • NH4-N = 50 mg L−1	• HRT = 0.58 d • NLR = 86.2 mg L−1 d−1	N2O emission: 0.027% of influent N	Nitrifier denitrification	**• High abundance of denitrifying bacteria & AOB inhibiting N2O production**

EF (%N-load)	DO (mg L-1)	Temp (*C)	HRT (hours)	SRT (days)	Influent. TN (mg NL-1)	Configuration (zones)	Number
0.036	1.5	17.8	5	10	50	Plug-flow 1-step feed in preanoxic/aerobic zone	20
0.738	4	17.7		10		1-step feed (anoxic/aerobic)	21
5.6	2	17.5			75	SBR: 1 hour feeding in aerobic/settling(anoxic) zone	22
0.11		range	15	8	40.3–98.8	Reversed A2/O:1-step feed (anoxic/anaerobic/oxic)	23
0.068		range	15	14	40.3–98.8	A2/O: 1-step feed (anoxic/anaerobic/oxic)	-
4.9	2.5	15.4	36.72	26	69	SBR: Continuous feeding during reaction phase in aerobic/anoxic alternations	24
1.9	3.5	1 year range	7.5			A/O: 1-step feed (anoxic/aerobic)	25
0.12	1	26-31	9	2	46	Non - BNR activated sludge: 1-strp feed in aerobic condition	26
1.7	2.5					Two-reactor nitritation-anammox process	27
0.17	0.3					PN and PN/A: (aerobic/anoxic)	28
3.8	1.3		2.3			Nitritation SBR	29
5.1	2		120	15		Nitrification-denitrification SBR	30
0.18		17			28.6	Step-feed non BNR process	31
1.8		26			28.6	Step-feed non BNR process	-
0.09		23			22.8	Aerobic plug-flow reactor 2	-
0.4		22			29.1	Aerobic plug-flow reactor 1	-
0.41		11			29.1	Aerobic plug-flow reactor 1	-
0.07		26			44.9	Modified Ludzack-Ettinger1	-
0.06		26			44.5	Modified Ludzack-Ettinger2	-
0.03		19			30.3	Oxidation ditch	-
0.03		15			21.6	Separate-stage BNR	-
0.05		19			36.1	Four-stage Bardenpho	-
1.5		29			41.5	Step-feed BNR	-
1.6		19			26.9	Step-feed BNR	-
0.62		25			26.9	Step-feed BNR	-