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Published in:
Environmental Science & Technology

Link to article, DOI:
[10.1021/acs.est.9b04889](https://doi.org/10.1021/acs.est.9b04889)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Chen, X., Mielczarek, A. T., Habicht, K., Andersen, M. H., Thornberg, D., & Sin, G. (2019). Assessment of Full-Scale N₂O Emission Characteristics and Testing of Control Concepts in an Activated Sludge Wastewater Treatment Plant with Alternating Aerobic and Anoxic Phases. *Environmental Science & Technology*, 53(21), 12485-12494. <https://doi.org/10.1021/acs.est.9b04889>

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Environmental Processes

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Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.9b04889 • Publication Date (Web): 08 Oct 2019

Downloaded from pubs.acs.org on October 10, 2019

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Assessment of Full-Scale N_2O Emission Characteristics and Testing of Control Concepts in an Activated Sludge Wastewater Treatment Plant with Alternating Aerobic and Anoxic Phases

Xueming Chen^{1,*}, Artur Tomasz Mielczarek², Kirsten Habicht³, Mikkol Holmen Andersen³, Dines Thornberg², Gürkan Sin^{1,*}

¹Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.

²Biofos A/S, Refshalevej 250, 1432 Copenhagen, Denmark.

³Unisense Environment A/S, Tueager 1, DK-8200 Aarhus N, Denmark.

*Corresponding authors:

Dr. Xueming Chen, Phone: +45 8194 8080, E-mail: xuem.chen@hotmail.com

Dr. Gürkan Sin, Phone: +45 2381 1148, E-mail: gsi@kt.dtu.dk

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ABSTRACT

This work aims to obtain full-scale N₂O emission characteristics translatable into viable N₂O control strategies and conduct full-scale testing of the proposed N₂O control concepts. Data of a long-term monitoring campaign was firstly used to quantify full-scale N₂O emission and probe into the seasonal pattern. Then trends between N₂O production/emission and process variables/conditions during typical operating cycles were revealed to explore the dynamic N₂O emission behavior. A multivariate statistical analysis was performed to find the dependency of N₂O emission on relevant process variables. The results show for the first time that relatively low/high N₂O emission took place in seasons with a decreasing/increasing trend of water temperature, respectively. Aerobic phase contributed to N₂O production/emission probably mainly through the hydroxylamine pathway. Comparatively, heterotrophic bacteria had a dual role in the anoxic phase and could be responsible for both net N₂O production and consumption. Incomplete denitrification might contribute mainly to the N₂O production/emission in the anoxic phase and the accumulation of N₂O to be significantly emitted in the following cycle due to the competition between different denitrification steps for electron donors. Therefore, properly extending the length of anoxic phase could serve as a potential control means to regulate N₂O accumulation in the anoxic phase. The full-scale testing not only verified the efficacy of reduced dissolved oxygen set-point in reducing N₂O emission by 60%, but also confirmed the proposed concepts of control over the aerobic and anoxic phases collectively.

1. INTRODUCTION

Wastewater treatment plants (WWTPs) have been regarded as an important contributor to the global greenhouse gas stock through the production and emission of nitrous oxide (N_2O)^{1, 2}, which has a global warming effect 265 times stronger than carbon dioxide on a 100-year time horizon³. Moreover, N_2O is a scavenger of ozone and therefore plays an important role in the destruction of the stratospheric ozone layer⁴. Therefore, on one hand, N_2O emission should be accurately quantified to assess the performance of WWTPs comprehensively. On the other hand, timely measures should be taken to mitigate full-scale N_2O emission.

Despite the fixed, low level of 0.035% suggested by IPCC³, a significantly varied N_2O emission factor has been reported for full-scale WWTPs, ranging from 0 to 14.6% relative to incoming nitrogen^{2, 5-8}. This variability is due to the existence of multiple N_2O production contributing pathways (i.e., the nitrifier denitrification pathway and the hydroxylamine pathway mediated by ammonium-oxidizing bacteria (AOB) during nitrification⁹ and the sequential heterotrophic denitrification which is carried out by heterotrophic bacteria (HB) and involves N_2O as an obligate intermediate¹), which are regulated by a number of affecting process variables (e.g., dissolved oxygen (DO), carbon source, NO_2^- , pH, and temperature¹⁰).

Long-term measurement campaigns are therefore needed to clearly demonstrate full-scale N_2O emission characteristics under various process conditions. Analyses of long-term data to reliably deduce the relationships between N_2O emission and relevant process variables¹¹ serve as the basis of developing viable N_2O control strategies. Although long-term measurement campaigns and relevant analyses have been reported¹², the insights obtained are largely dependent on the plant/process design applied and the control strategy involved and cannot be generalized to all types of WWTPs. With respect to N_2O control strategies, only limited work

based on model simulations has been reported¹³. The numerous N₂O models available are seldom calibrated or verified using highly dynamic full-scale data in the long term¹¹, thus bringing high uncertainty to the validity of the model-based N₂O control strategies. In this case, data-driven development of N₂O control strategies, which is rarely performed, serves as a reliable alternative. Despite all the reported efforts, to the best of our knowledge, no full-scale testing has been conducted to verify the efficacy of the N₂O control strategies proposed based on the analyses of long-term measurements of N₂O as well as other relevant process variables.

In view of the aforementioned, this work aims to obtain full-scale N₂O emission characteristics that could be translated into viable N₂O control strategies and conduct full-scale testing of the proposed N₂O control concepts. To this end, data of a long-term monitoring campaign at a large Danish WWTP was firstly used to quantify full-scale N₂O emission and probe into seasonal pattern. Then trends between N₂O production/emission and process variables during typical operating cycles were revealed to probe into the dynamic N₂O emission behavior. A multivariate statistical analysis was performed to find the dependency of N₂O emission on relevant process variables. Based on the analysis results, potentially viable N₂O control strategies were proposed and then tested directly at the studied WWTP. Given the technical constraints on site and the crucial role of the studied WWTP in the public sector, not all process variables of interest were measured or could be made available. Therefore, the analyses conducted in this work followed the rule of making the most of the data readily available to obtain insightful but practical information. Overall, this work goes beyond the current state of the art by integrating full-scale data analyses with control concept testing in order to mitigate N₂O production/emission from WWTPs.

2. MATERIALS AND METHODS

2.1. Plant/Process Description and Data Origin

This work was based on the on-line data recorded at Avedøre WWTP (Copenhagen, Denmark), with a capacity of serving approximately 350,000 PE by treating around 70,000 m³ of wastewater daily under dry weather conditions. Combined with screening, grit removal, primary settlement, and secondary clarification in the mainstream treatment, activated sludge process is applied in four Carrousel reactors working in parallel, each consisting of two compartments alternatively fed and intermittently aerated as aerobic (aerated) or anoxic (non-aerated) zones (Fig. 1). Aeration is achieved with bubble diffusers mounted at the bottom of compartments and is activated when DO concentration is lower than the DO set-point which is determined based on in-situ NH₄⁺ concentration and is pre-set in the STAR Control[®] implemented (see Fig. S1A in the Supplementary Materials). The STAR Control[®] also regulates the entering/exiting of aerobic/anoxic phase of each compartment through the pre-set relationship between in-situ NH₄⁺ and NO₃⁻ concentrations (see Fig. S1B in the Supplementary Materials), thus ensuring good nitrogen removal performance. As a result, the average effluent quality reached 4.40 g m⁻³ for total nitrogen (TN) in 2017. For the side-stream treatment, anaerobic digestion is applied to produce biogas from primary and secondary sludge, which is then harvested for electricity generation.

NH₄⁺-N, NO₃⁻-N, DO, and water temperature are constantly monitored in both compartments of all four reactors, while liquid-phase N₂O is additionally measured using Unisense[®] N₂O sensors in two reactors (i.e., Reactor 1 and Reactor 3). As indicated in Fig. 1, the measuring sensors are placed in the same location with respect to the water flow direction in each compartment. All sensors are frequently calibrated and tested, thus ensuring the accuracy of measurements. Instead of the direct measurement of airflow rate into each compartment, the opening of aeration valve in each compartment and the total airflow into all reactors are

monitored. Influent flowrate into each reactor is also recorded online using a flow meter. Composite samples over 24 h are analyzed every one or two weeks to indicate influent quality.

2.2. Data Processing

The 12-month monitoring period spanned from March 2018 to February 2019. Similar to Vasilaki et al.¹¹, the data, after being extracted from the supervisory control and data acquisition (SCADA) system, was firstly processed to ensure its quality through i) data synchronization under the same time-stamp and ii) removal of unreliable measurements (e.g., significantly negative readings due to sensor drifting, readings outside the sensor measurement range, and readings taken when sensors were not in service). The on-line data for all process variables investigated in this work was aggregated into averages every 5 minutes for subsequent analyses and interpretations and is provided in the Supplementary Data.

2.3. Data Analyses

2.3.1. N₂O emission rate calculation

To calculate N₂O emission rate, equations were differentiated for aerobic (aerated) and anoxic (non-aerated) zones as follows^{14, 15}:

$$\text{Aerobic (aerated) zone: } r_{N_2O} = H_{N_2O} \times S_{N_2O} \times \left[1 - e^{-\frac{K_L a_{N_2O}^{Aerated} V_R}{H_{N_2O} Q_A}} \right] \times \frac{Q_A}{V_R} \quad (1)$$

$$\text{Anoxic (non-aerated) zone: } r_{N_2O} = K_L a_{N_2O}^{Non-aerated} \times \left[S_{N_2O} - \frac{C_{N_2O,air}}{H_{N_2O}} \right] \quad (2)$$

r_{N_2O} : Volumetric N₂O emission rate (g-N m⁻³ d⁻¹), H_{N_2O} : Dimensionless Henry's constant, S_{N_2O} : liquid-phase N₂O concentration (g-N m⁻³), $K_L a_{N_2O}^{Aerated}$: Aerated N₂O mass transfer coefficient (d⁻¹), V_R : Volume of aerated part (m³), Q_A : Airflow rate (m³ d⁻¹), $K_L a_{N_2O}^{Non-aerated}$:

Non-aerated N_2O mass transfer coefficient (d^{-1}), $C_{N_2O,air}$: N_2O concentration in air equilibrium
($g-N\ m^{-3}$).

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$K_L a_{N_2O}^{Aerated}$ was determined according to Eq. 3¹⁵ while $K_L a_{N_2O}^{Non-aerated}$ was calculated following
Eq. 4¹⁶. Q_A into each compartment was calculated by proportioning the total airflow
measurement based on the ratio of the valve opening measurement in the compartment to the
sum of all valve opening measurements.

$$K_L a_{N_2O}^{Aerated} = \left[\frac{D_R}{0.815} \right]^{-0.49} \times 34500 \times \left[\frac{Q_A}{S_A} \right]^{0.86} \times 1.024^{(T-20)} \quad (3)$$

$$K_L a_{N_2O}^{Non-aerated} = 2 \times 1.024^{(T-20)} \quad (4)$$

D_R : Depth over diffusers (m), S_A : Size of aeration field (m^2), T: Temperature ($^{\circ}C$).

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2.3.2. Pearson's partial correlation analysis

A clear understanding of the impacts of process variables on N_2O emission is the key to the
reliable formulation of N_2O control strategies. To this end, Pearson's partial correlation which
measures the degree of association between two variables with the effect of other affecting
variables removed was used in this work. More details about Pearson's partial correlation
analysis are provided in the Supplementary Materials. Using N_2O emission rate as the outcome
variable and relevant process variables (NH_4^+-N , $NO_3^- -N$, DO, water temperature, influent, etc.)
as the affecting variables, Pearson's partial correlation analysis, which accounted for the
contributions of other affecting variables while indicating the individual contribution of the
process variable studied to N_2O emission, returned a coefficient (PCC) in the range from -1 to
1. The value -1 (1) conveyed a perfect negative (positive) linear relationship between N_2O
emission rate and the affecting variable investigated, while the value 0 indicated that there was

no linear relationship. Pearson's partial correlation analysis was conducted using a MATLAB function in Statistics and Machine Learning Toolbox called `partialcorri`, which also returned a p-value for testing the hypothesis of no partial correlation against the alternative that there was a non-zero partial correlation. Despite the criticisms about using the p-value¹⁷ mainly in the cases with relatively small data samples, similar criticisms haven't been found in the context of engineering analysis that typically features a large volume of data. Therefore, the p-value, which has been widely applied to assess statistical significance in similar works^{8, 11}, was directly adopted in this work. If the p-value was less than 0.05, then the partial correlation was regarded as significantly different from zero. It should be acknowledged that other significance tests not relying on the p-value such as the surrogate model based sensitivity analysis¹⁸ can also be used in the future for comparative analysis.

3. RESULTS AND DISCUSSION

3.1. Seasonal N₂O Emission Pattern

As depicted in Fig. 2A, a distinct relationship between monthly average of N₂O emission rate and water temperature has been observed for Reactor 3 over the monitoring period. The average N₂O emission rate was 0.77 g-N m⁻³ d⁻¹ at the average water temperature of 10.7 °C in March 2018 when the monitoring campaign commenced. With the increasing water temperature, the N₂O emission rate increased, reaching its peak monthly average of 2.30 g-N m⁻³ d⁻¹ at the average water temperature of 16.4 °C in May 2018. Further increase in the water temperature brought down the N₂O emission rate, arriving at 0.45 g-N m⁻³ d⁻¹ at the average water temperature of 20.6 °C in August 2018. Afterwards, the water temperature entered a decreasing stage. In September 2018 when the average water temperature was 19.8 °C, the minimum monthly average of N₂O emission rate was achieved at 0.01 g-N m⁻³ d⁻¹. Continuous decrease in the water temperature towards 12.1 °C in February 2019 (i.e., the end of the monitoring

campaign) led to the gradual recovery of the N_2O emission rate. With a high Pearson's correlation coefficient of 0.93 ($p=0.00$), the analogous results for Reactor 1 in Fig. 2B prove the reproducibility of the seasonal pattern of N_2O emission observed for Reactor 3 (i.e., Fig. 2A). In fact, this seasonal pattern of N_2O emission with respect to water temperature in the range between 10 °C and 20 °C is similar to the one observed for a Dutch WWTP which had a different plant/process design and was subject to a diverse control scheme⁸. However, Kosonen et al.¹⁹ reported a distinct, less pronounced seasonal pattern of N_2O emission in relation to water temperature for a Finnish WWTP which was also configured and controlled differently compared to the WWTP investigated in this work.

Given that the direct impact of water temperature might be coupled with those of process design and operation, only the overall impact of water temperature on N_2O emission was studied in this work. On the whole, the monitoring period could be divided into two sub-periods: sub-period with relatively high N_2O emission and sub-period with relatively low N_2O emission. As indicated in Fig. 2, the sub-period with relatively high N_2O emission was characterized by an increasing trend of water temperature (i.e., between March 2018 and August 2018), while the sub-period with relatively low N_2O emission was featured with a decreasing trend of water temperature (i.e., from August 2018 to February 2019). Despite the real-time N_2O emission dynamics, the general correlation between the seasonal N_2O emission and the changing trend of water temperature should be largely attributed to the various responses of functional microorganisms involved in nitrogen conversion reactions (including AOB, nitrite-oxidizing bacteria (NOB), and HB) to changes in temperature. Although biological activities are known to accelerate with increasing temperature within a suitable temperature range, each type of functional microorganisms has its own specific temperature dependency. This could be one of the reasons the maximum and minimum N_2O emission rates didn't correlate directly with the

extremum water temperature conditions (see Fig. 2A). This proposed hypothesis regarding microbial responses to temperature changes in this work warrants future investigations. A higher-resolution snapshot of the dynamic profiles of process variables in the two parallel reactors (Fig. S2 in the Supplementary Materials) shows the existence of periods with similar concentrations and behavior of NH_4^+ , NO_3^- , and DO but different ranges and responses of N_2O in the two parallel reactors. On the whole, the notably different magnitude of N_2O emission rates between the two parallel reactors operated under the same conditions in Figs. 2A and 2B, especially in the sub-period with relatively high N_2O emission, might also be the result of the different amount of biomass as well as the diverse local microbial community structures shaped after long-term operation. For example, in June 2018 when N_2O emission was relatively high, the mixed liquor suspended solids (MLSS) concentration in Reactor 1 was $2.02 \pm 0.58 \text{ kg m}^{-3}$ while it was $2.60 \pm 0.52 \text{ kg m}^{-3}$ in Reactor 3. Therefore, monitoring on one reactor might not reflect the N_2O emission from other parallel reactors. In other words, reliable quantification of the plant-wide N_2O emission might necessitate respective monitoring on all different reactors. There is no doubt that this argument would be further verified upon the future availability of additional N_2O emission results of the other two parallel reactors. Although there were also two parallel Carrousel reactors being monitored in the study of Daelman et al.⁸, the respective N_2O emission from the two reactors was not distinguishable. Therefore, whether difference existed between the two reactors in terms of N_2O emission couldn't be validated.

Based on the seasonal N_2O emission pattern observed in this work (i.e., Fig. 2), control strategies are particularly needed in seasons with an increasing trend of water temperature (i.e., spring and summer) to mitigate N_2O emission effectively, while their implementation in autumn and winter will further strengthen the abatement performance. As viable N_2O control strategies need to deal with N_2O emission dynamics under various process conditions, their

development should be based on high-resolution analyses looking into the cyclic patterns associated with N_2O emission in the reactors as well as the correlations between N_2O emission and relevant process variables, which are presented in the following sections.

3.2. Recurring Cyclic Patterns in High N_2O Emission Seasons

Fig. 3 delineates three types of recurring cyclic patterns in high N_2O emission seasons, i.e., the sub-period with relatively high N_2O emission identified in the previous section. In this work, a cycle was defined to consist of a preceding aerobic (aerated) phase and a following anoxic (non-aerated) phase, regulated by the STAR Control[®].

Cycles 1 to 4 in Fig. 3A represent the first type of recurring cyclic patterns. Upon the start of aeration in the aerobic phase, NH_4^+ firstly increased without significant formation of NO_3^- . This was due to the higher supply of NH_4^+ compared to its consumption through nitrification under limited DO conditions. NO_2^- , which was not specifically monitored at the studied WWTP, might to some extent accumulate. Therefore, the absence of N_2O production therein might indicate the insignificant role of the nitrifier denitrification pathway, which is typically triggered under low DO and/or high NO_2^- conditions²⁰⁻²⁴. However, the potential contribution of the coexisting HB as the sole N_2O scavenger in the reactors to N_2O consumption could not be ruled out. Once DO reached a sufficiently high level, nitrification was enhanced. As a result, NH_4^+ started to decrease, accompanied by the formation of NO_3^- . The co-occurring N_2O production under ample DO conditions might originate from the hydroxylamine pathway^{9, 22, 25}. However, contrary to the reported simultaneous changes of nitrogenous compounds^{22, 25}, there existed an apparent lag phase between the emergence of N_2O and the conversions of NH_4^+ and NO_3^- . This lag phase might be caused by the remaining heterotrophic N_2O consumption under ample DO conditions, which was weakened with the formation of NO_3^- due to the

competition between different denitrification steps for electron donors^{26, 27}. At the end of the aerobic phase, NH_4^+ arrived at the bottom level while NO_3^- reached its peak. Once entering the anoxic phase, NO_3^- decreased immediately due to denitrification, while NH_4^+ increased as a result of the consistent supply on top of the suspended nitrification process. Nevertheless, a different, non-monotonic behavior has been noticed for N_2O production/emission. N_2O firstly continued to increase for around 15 minutes, which clearly indicated the heterotrophic contribution to N_2O production. When NO_3^- decreased to a low level at the end of the anoxic phase, N_2O started to be consumed and was depleted quickly. In the anoxic phase when the whole compartment was not aerated, DO, an obligate electron acceptor for nitrification, became and remained at 0. The suspension of the nitrification activity in the anoxic phase due to the unavailability of DO was further supported by the observation that nitrate didn't reappear after depletion while ammonium increased consistently due to the influent supply (see the higher-resolution example Fig. S3 in the Supplementary Materials). Therefore, the observed trend between N_2O and NO_3^- was likely the result of the higher preference of HB for reducing NO_3^- than N_2O in the competition for limited electron donors^{27, 28} or/and the presence of slowly biodegradable organics as electron donors which has been found to induce elevated N_2O emission during denitrification^{10, 29}.

Fig. 3B shows the second type of recurring cyclic patterns. Compared to cycles 1 to 4 in Fig. 3A, the lowered NH_4^+ loadings in cycles 5 to 7 led to the lowered N_2O production probably through the hydroxylamine pathway during nitrification based on recent literature findings. However, no N_2O was actually detected in the aerobic phase. This phenomenon could only be caused by the heterotrophic N_2O consumption as HB were the sole N_2O scavenger in the reactors. Consequently, N_2O production/emission only occurred in the anoxic phase. Despite the difference in magnitude, NO_3^- and N_2O had a correlation similar to the one observed in the

first type of recurring cyclic patterns (i.e., Fig. 3A), further confirming the dual role of HB in N_2O turnover in the anoxic phase.

The third type of recurring cyclic patterns which covered all the cycles with N_2O emission peaks in high N_2O emission seasons are exemplified by cycles 8 to 16 in Fig. 3C. Cycle 8 was largely analogous to the first type of recurring cyclic patterns presented in Fig. 3A, where N_2O was produced and emitted in both the aerobic and anoxic phases. The major difference lied in the disappearance of N_2O consumption in the anoxic phase (i.e., incomplete denitrification), which might be explained by the relatively high NO_3^- level at the end of the anoxic phase, thus placing the heterotrophic N_2O consumption in a disadvantageous position in the competition for electron donors. This incomplete denitrification actually resulted from the inadequate length of anoxic phase determined by the STAR Control[®]. Once entering the following cycle (i.e., cycle 9), the N_2O accumulated in the liquid phase got stripped immediately, thus generating high N_2O emission. This phenomenon of significantly higher N_2O emission from reactors with intermittent aeration or compartments switching between aerobic and anoxic phases has also been documented in other types of activated sludge systems^{5, 30}. Despite the high N_2O emission in the aerobic phase, the N_2O level remained relatively stable, which might be due to i) the continuous N_2O production during nitrification and ii) the depressed heterotrophic N_2O consumption in the presence of the relatively high NO_3^- level. On account of the sole heterotrophic N_2O production contribution, the accumulation of N_2O continued until the end of the anoxic phase. The accumulated N_2O got carried over to the next cycle (i.e., cycle 10), thus causing significantly high N_2O emission in the aerobic phase. This cyclic pattern persisted for several cycles until cycle 14, where the heterotrophic N_2O consumption was observed in the anoxic phase (i.e., complete denitrification) due to the reduced NO_3^- level compared to the preceding cycles. Consequently, N_2O was removed from the liquid phase

before entering the next cycle, which resembled the first type of recurring cyclic patterns (i.e., Fig. 3A) and therefore only incurred relatively low N₂O emission (see cycles 15 and 16 in Fig. 3C).

3.3. Correlations between N₂O Emission and Process Variables

Different from the reported Pearson's correlation analysis by Daelman et al.⁸ and Spearman's rank correlation analysis by Vasilaki et al.¹¹, this work applied Pearson's partial correlation analysis to explore the degree of association between N₂O emission and each process variable, with the effects of other process variables removed. In this work, following common practice, a PCC between 0 and 0.3 (0 and -0.3) was defined to indicate a weak positive (negative) correlation, a PCC between 0.3 and 0.7 (-0.3 and -0.7) indicated a moderate positive (negative) correlation, and a PCC between 0.7 and 1.0 (-0.7 and -1.0) indicated a strong positive (negative) correlation. The two sub-periods identified in Fig. 2A were analyzed separately to probe into the change in the dependency of N₂O emission on relevant process variables. Considering their significant difference in operational conditions and control scheme, the aerobic and anoxic phases, each accounting for around 85% and 15% of N₂O emission on both monthly and yearly scale, were also analyzed separately in the partial correlation analysis. Even though a few full-scale studies reviewed by Vasilaki et al.¹² exhibited negligible N₂O emission in the anoxic phase without aeration, the significant relative contribution of the anoxic phase to N₂O emission (i.e., 15%) due to liquid-gas transfer was observed in this work. This might be caused by the differences between Avedøre WWTP and those WWTPs reviewed by Vasilaki et al.¹² in the affecting pathways as well as the specific operational conditions.

Fig. 4A illustrates the PCC between N₂O emission rate and process variables (including influent flowrate, DO, NH₄⁺, NO₃⁻, and water temperature) in the aerobic phase of the sub-

period with relatively high N_2O emission. Among all the process variables studied in this work, only NO_3^- had a moderate positive correlation ($\text{PCC}=0.33$, $p=0.00$) with N_2O emission rate, which might indicate the main contribution of the hydroxylamine pathway during nitrification to the N_2O production/emission. This was also supported by the weak positive correlations of N_2O emission rate with DO ($\text{PCC}=0.20$, $p=0.00$) and NH_4^+ ($\text{PCC}=0.11$, $p=0.00$), which have been shown to possess a positive impact on the hydroxylamine pathway^{9, 22, 25}. Water temperature also had a weak positive correlation ($\text{PCC}=0.13$, $p=0.00$) with N_2O emission rate, which might be due to the positive relationship between overall kinetics of nitrogen conversion processes and water temperature in the studied range (i.e., between 10 °C and 20 °C) and was consistent with Ahn et al.⁶. Influent flowrate was found to insignificantly correlate with N_2O emission rate ($\text{PCC}=0.08$, $p=0.14$). As full nitrification was pursued at the studied WWTP through implementing high DO set-point (i.e., lowest DO set-point of 0.5 g m^{-3} when NH_4^+ is 0 g-N m^{-3}) and existing criteria of aerobic phase based on the in-situ $\text{NH}_4^+/\text{NO}_3^-$ relationship in the STAR Control®, NO_2^- might not have a significant impact on N_2O emissions, although it has been found to highly correlate with N_2O emission in other activated sludge systems^{8, 11}. This hypothesis couldn't be validated in this work due to the upfront technical limitation in onsite NO_2^- measurement (i.e., unavailability), which is recommended as future work. Fig. 4B exhibits the PCC between N_2O emission rate and process variables (including influent flowrate, NH_4^+ , NO_3^- , and water temperature) in the anoxic phase of the sub-period with relatively high N_2O emission. Compared to the weakly correlated influent flowrate, NH_4^+ , and water temperature, NO_3^- had a moderate positive correlation ($\text{PCC}=0.40$, $p=0.00$) with N_2O emission rate, supporting the hypothesis that incomplete denitrification (caused by the competition between heterotrophic NO_3^- and N_2O reduction for electron donors) contributed mainly to the N_2O production/emission in the anoxic phase.

Distinct results were obtained in the counterpart analysis of the aerobic (Fig. 4C) and anoxic (Fig. 4D) phases of the sub-period with relatively low N_2O emission. The first major difference lied in water temperature which appeared to have an around moderate negative correlation with N_2O emission rate (i.e., PCC of -0.29 for the aerobic phase and -0.36 for the anoxic phase). The second major difference was associated with NO_3^- which was found to be weakly correlated or uncorrelated with N_2O emission rate. The distinct PCC results obtained for seasons with different magnitude of N_2O emission manifested the change in key factors affecting N_2O emission which led to the seasonality in N_2O emission, as observed in Fig. 2. The fact that none of the studied process variables in Fig. 4 had a strong correlation with N_2O emission rate was expected as strong correlations have been in fact rarely found in highly dynamic systems like WWTPs. This could be caused by some other process variables not accounted for in this work, which also gave rise to the significant discrepancy between Figs. 4A-B and Figs. 4C-D. For example, the microbial community structure might shift significantly in response to the declining trend in water temperature but remain relatively stable when facing the increasing water temperature, thus leading to the opposite PCC between water temperature and N_2O emission rate as well as the different significance of NO_3^- in affecting N_2O emission in Figs. 4A-B and Figs. 4C-D. Follow-up work in consideration of additional process variables such as microbial community dynamics is therefore highly desired in order to fully disclose the causes of the seasonal N_2O emission.

3.4. Implications for N_2O Emission Quantification/Control, Testing of Control Concepts, and Future Work

Using the off-line measured total Kjeldahl nitrogen (TKN) of composite samples to obtain the monthly average of nitrogen loading, the monthly N_2O emission factor for Reactor 3 over the monitoring period was calculated to vary between 0.01% and 3.55%, with a yearly level of

1.05% (Fig. 5). These full-scale N₂O emission factors resided well in the reported range from 0 to 14.6% organized by Daelman et al.⁸ but were largely (11 out of 12 monitored months, i.e., except September 2018) higher than the level of 0.035% proposed by IPCC³ (see Fig. 5). Therefore, guidelines provided by IPCC should be used with care in order to represent full-scale N₂O emission. Considering the shortage of reliable modelling techniques capable of predicting long-term N₂O emission as well as the rather complicated correlations between N₂O emission and process variables, monitoring campaigns are still needed to report full-scale N₂O emission. As discovered in Section 3.1, different N₂O emission levels were observed for different reactors. Consequently, separate monitoring on different reactors should lead to more reliable quantification of the plant-wide N₂O emission.

This work also provides useful insights for N₂O emission control. As unveiled in Sections 3.2 and 3.3, HB had a dual role in the anoxic phase and could be responsible for both net N₂O production and consumption, which tended to take place in sequence probably resulting from the competition between different denitrification steps for electron donors. Therefore, in order to avoid the accumulation of N₂O as a result of incomplete denitrification which would be carried over to the following cycles and therefore subject to high emission, sufficient usage of the heterotrophic N₂O consuming capability of the anoxic phase (i.e., complete denitrification) should be guaranteed through relevant control strategy such as extending the length of anoxic phase. Practically, the peak NH₄⁺ level and the ending NO₃⁻ level in the aerobic phase of a certain cycle could be used as indicators of the need for relevant control strategy in the following anoxic phase. As shown in Fig. 6, when the peak NH₄⁺ level and the ending NO₃⁻ level in the aerobic phase nearly double compared to the conventional levels, it would be highly likely that N₂O would accumulate at the end of the cycle and then get carrier over to the subsequent cycle, thus triggering high N₂O emission. In this case, the aforementioned control

strategy should be executed timely to prevent N_2O emission peaks (i.e., significantly high N_2O emission in the aerobic phase of cycles 9 to 14 in Fig. 3C). In fact, this finding regarding the important role of denitrification in mitigating N_2O emission should be applicable to other activated sludge reactors with intermittent aeration or compartments switching between aerobic and anoxic phases. In Europe, according to relevant statistics, there are over 80 WWTPs that are using the STAR Control[®] as their control solution. For these plants, when needed, the current control system could be readily modified to reduce N_2O emission through wisely controlling the anoxic phase.

Comparatively, control over the aerobic phase should be based more on the fact that the hydroxylamine pathway during nitrification was more likely to dominate the N_2O production. Therefore, for the WWTP investigated in this work, reduced DO set-point might help reduce the N_2O production through the hydroxylamine pathway. However, it remains unknown whether the reduced DO set-point would enhance the nitrifier denitrification pathway or not. To verify the efficacy of reduced DO set-point in mitigating N_2O production/emission, a full-scale testing was conducted at Avedøre WWTP. To be brief, after a good baseline between Reactor 1 and Reactor 3 was obtained, the DO set-point in Reactor 3 was reduced by 0.5 g m^{-3} (see Fig. S1A in the Supplementary Materials) to identify the disparities between Reactor 1 and Reactor 3 caused by the different DO set-points. To minimize potential threat to the stability of process operation, this full-scale testing only lasted for a week. As shown in Fig. 7, before the change in the DO set-point in Reactor 3 was made, the difference between Reactor 1 and Reactor 3 in terms of average N_2O emission rate was less than 5%. Moreover, as presented in Table 1, other process variables including MLSS and lengths of aerobic and anoxic phases were also quite comparable in both reactors. These similarities showed that both reactors should have comparable N_2O emission during this period and therefore set the baseline

needed for testing the proposed N_2O control strategy. When the DO set-point in Reactor 3 was reduced by 0.5 g m^{-3} , the average N_2O emission rate in Reactor 3 was 60% lower than that in Reactor 1 (see Fig. 7). Compared to the baseline level, the observation of the increased N_2O emission rate in Reactor 1 with the default DO set-point might be the result of the different influent flowrate/composition conditions and the increasing trend of water temperature. As manifested in Table 1, the reduced DO set-point extended the length of the aerobic phase in Reactor 3 by 13%, compared to Reactor 1. This phenomenon was expected as a longer duration would be needed when the nitrification intensity was weakened by the reduced DO set-point. Additionally, the reduced DO set-point also indirectly affected the anoxic phase. Compared to Reactor 1, the length of the anoxic phase in Reactor 3 was extended by 33%. This observation was in line with the aforementioned control over the anoxic phase to reduce N_2O emission. In short, the proposed concepts of control over the closely related aerobic and anoxic phases were evaluated collectively by this full-scale testing of reduced DO set-point. In addition to proving the efficacy of properly reduced DO set-point in mitigating N_2O emission from WWTPs with similar plant/process configuration and control scheme to Avedøre WWTP, the results of this full-scale testing might also serve as practical evidence supporting the hypotheses on the N_2O producing pathways proposed based on recent literature findings.

Although Foley et al.¹⁵ claimed that the concentrations of nitrogenous compounds could be assumed to approach perfectly mixed conditions in a Carrousel compartment, the real uniformity of each process variable is barely possible to achieve at full-scale WWTPs. Potential spatial heterogeneity could be checked by placing a second set of measuring sensors in the compartment. However, the fact that the existing sensors are installed around the same place ensures the comparability between process variables, laying the solid foundation for the analyses done in this work. Besides the one-year data presented in this work, the availability

of more data is highly desired to clarify whether N_2O emission would differ from year to year. The hypotheses regarding the N_2O production pathways were proposed based on recent literature findings and could be proved/tested with the availability of on-line NO_2^- , hydroxylamine and COD measurements and biological measurements as well as the help of in-situ isotope tracing techniques³¹ and sophisticated mathematical modelling, which are the focus of future work.

ACKNOWLEDGEMENTS

This work was supported by VUDP (Vandsektorens Udviklings- og Demonstrationsprogram) through “Demonstration af en ny kontrol metode til at reducere lattergas udledningen fra renseanlæg” Project (ID No. 87.2016). Dr. Xueming Chen acknowledges the financial support by the European Union’s Horizon 2020 research and innovation programme through Marie Skłodowska-Curie Individual Fellowship under grant agreement No. 790231.

SUPPORTING INFORMATION

Supplementary Data: On-line data for all process variables (including influent flowrate, DO, NH_4^+ , NO_3^- , N_2O , and temperature) and calculated N_2O emission rates over the 12-month monitoring period.

Supplementary Materials: Details about Pearson’s partial correlation analysis; Settings of the default and tested STAR Control® (Fig. S1); Dynamic profiles of process variables in the two parallel reactors with similar concentrations and behavior of NH_4^+ , NO_3^- , and DO but different ranges and responses of N_2O (Fig. S2); Cyclic data showing lack of nitrification activity in the anoxic phase (Fig. S3).

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Table and Figure Legends

Table 1. Results of full-scale testing of reduced DO set-point.

Figure 1. Schematic diagram of Carrousel reactor comprising two alternatively fed and intermittently aerated compartments (Locations of measuring sensors with respect to water flow direction are marked).

Figure 2. Seasonal N₂O emission pattern in (A) Reactor 3 and (B) Reactor 1 (Highlighted box in (B) indicates months when N₂O was only monitored in one compartment or both N₂O sensors were not in service).

Figure 3. Three types of recurring cyclic patterns in seasons with relatively high N₂O emission.

Figure 4. Pearson's partial correlation coefficients between monitored process variables and N₂O emission rate in (A and C) aerobic and (B and D) anoxic phase of seasons with relatively (A and B) high and (C and D) low N₂O emission (Highlighted box indicates partial correlation coefficient not significantly different from zero, i.e., $p > 0.05$).

Figure 5. N₂O emission factors of Reactor 3 over monitoring period and comparison with IPCC proposed level.

Figure 6. Comparison of peak NH₄⁺ level in aerobic phase and NO₃⁻ level at the end of aerobic phase between cycles without and with N₂O carrying over to subsequent cycle in peak N₂O emission month.

Figure 7. Comparison of N₂O emission rate between (A) Reactor 1 and (B) Reactor 3 (Green region in B indicates period when DO set-point in Reactor 3 was reduced by 0.5 g m⁻³).

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Table 1. Results of full-scale testing of reduced DO set-point.

	Average MLSS (kg m ⁻³)		Average length of aerobic phase (min)		Average length of anoxic phase (min)		Average N ₂ O emission rate (g N m ⁻³ d ⁻¹)	
	Reactor 1	Reactor 3	Reactor 1	Reactor 3	Reactor 1	Reactor 3	Reactor 1	Reactor 3
Baseline: Default DO set-point	2.21	2.22	54.2	54.1	34.1	37.4	4.1±0.1	3.9±0.1
Testing: Reduced DO set-point in Reactor 3	2.18	2.28	98.2	111.1	31.3	41.7	10.1±0.2	4.0±0.1

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