FLORE
Repository istituzionale dell'Università degli Studi di Firenze

Multi-point monitoring of nitrous oxide emissions in three full-scale conventional activated sludge tanks in Europe

Questa è la Versione finale referata (Post print/Accepted manuscript) della seguente pubblicazione:

Original Citation:

Multi-point monitoring of nitrous oxide emissions in three full-scale conventional activated sludge tanks in Europe / Giacomo Bellandi,
Jose Porro, Elisa Senesi,
Cecilia Caretti,
Simone Caffaz,

Availability:

This version is available at: 2158/1127947 since: 2018-05-09T09:56:16Z

Published version:

DOI: 10.2166/wst.2017.560

Terms of use:

Open Access

La pubblicazione è resa disponibile sotto le norme e i termini della licenza di deposito, secondo quanto stabilito dalla Policy per l'accesso aperto dell'Università degli Studi di Firenze (https://www.sba.unifi.it/upload/policy-oa-2016-1.pdf)

Publisher copyright claim:

(Article begins on next page)

19 April 2024

Multi-point monitoring of nitrous oxide emissions in three full-scale conventional activated sludge tanks in Europe

Giacomo Bellandi, Jose Porro, Elisa Senesi, Cecilia Caretti, Simone Caffaz, Stefan Weijers, Ingmar Nopens and Riccardo Gori

ABSTRACT

The large global warming potential of nitrous oxide (N_2O) is currently of general concern for the water industry, especially in view of a new regulatory framework concerning the carbon footprint of water resource recovery facilities (WRRFs). N_2O can be generated through different biological pathways and from different treatment steps of a WRRF. The use of generic emission factors (EF) for quantifying the emissions of WRRFs is discouraged. This is due to the number of different factors that can affect how much, when and where N_2O is emitted from WRRFs. The spatial and temporal variability of three WRRFs in Europe using comparable technologies is presented. An economically feasible and user-friendly method for accounting for the contribution of anoxic zones via direct gas emission measurements was proven. The investigation provided new insights into the contribution from the anoxic zones versus the aerobic zones of biological WRRF tanks and proved the unsuitability of the use of a single EF for the three WRRFs. Dedicated campaigns for N_2O emissions assessment are to be advised. However, similarities in the EF magnitude can be found considering treatment strategy and influent water composition.

Key words | activated sludge, GHG emissions, mixing, N_2O, off-gas

Giacomo Bellandi (corresponding author)
Elisa Senesi
Cecilia Caretti
Riccardo Gori
Department of Civil and Environmental Engineering,
University of Florence,
Via di S. Marta 3, 50139 Florence,
Italy
E-mail: *giacomo.bellandi@dicea.unifi.it*

Giacomo Bellandi
Jose Porro
Ingmar Nopens
BIOMATH, Department of Mathematical Modelling, Statistics and Bioinformatics,
Ghent University,
Coupure Links 653, B-9000 Gent,
Belgium

Jose Porro
Cobalt Water Global,
81 Prospect Street, Brooklyn, NYC 11201,
USA

Simone Caffaz
Publiacqua SpA,
Via Romania snc, 50055 Lastra a Signa, Firenze,
Italy

Stefan Weijers
Waterschap De Dommel,
Bosscheweg 56, 5283 WB Boxtel,
The Netherlands

INTRODUCTION

Biological processes in wastewater treatment contribute to global warming through direct emission sources over the whole water resource recovery facility (WRRF) area. Nitrous oxide (N_2O) can represent by itself 78% of the activated sludge (AS) plant carbon footprint (Daelman *et al.* 2013). Research efforts have focused on unravelling the specific bio-chemical processes responsible for N_2O production (Kampschreur *et al.* 2009; Schreiber *et al.* 2012) and the WRRF design and operational factors impacting its emission (*inter alia:* Kampschreur *et al.* 2008; Guo *et al.* 2013).

Biological formation of N_2O can mainly result from the activity of heterotrophic bacteria (heterotrophic denitrification), and of ammonia oxidizing bacteria (AOB) which seem often to be the most effective contributors in N_2O production due to their double production pathway (i.e. the nitrite (NO_2^-) reduction pathway (nitrifier denitrification) and the incomplete hydroxylamine (NH_2OH) oxidation pathway), and their ability to shift depending on the local conditions in the tank (*inter alia:* Peng *et al.* 2014). Dissolved oxygen (DO) and NO_2^- concentrations appear to be the key influencing factors for N_2O production pathways (*inter alia:* Peng *et al.* 2015). During nitrification at low DO, the

This is an Open Access article distributed under the terms of the Creative Commons Attribution Licence (CC BY 4.0), which permits copying, adaptation and redistribution, provided the original work is properly cited (http://creativecommons.org/licenses/by/4.0/).

doi: 10.2166/wst.2017.560

presence of NO_2^- can inhibit nitrite-oxidizing bacteria (NOB) (Buday *et al.* 1999) and trigger N_2O production via AOB denitrification as AOB can utilize NO_2^- as the electron acceptor rather than oxygen (*inter alia:* Bock *et al.* 1995). Higher DO levels have been linked to higher ammonia oxidation and higher N_2O production by AOB via NH_2OH oxidation (Chandran *et al.* 2011; Law *et al.* 2012). As for denitrification, it is mechanistically known that high NO_2^- concentrations can provide faster renewal for the NO_2^- reductase and reduction of NO_2^- to N_2O, while the presence of DO can inhibit heterotrophic denitrification (Nos enzyme), also leading to N_2O production (Von Schulthess *et al.* 1994). Free nitrous acid (FNA) and free ammonia (FA) have been observed to inhibit NOB activity already at 0.1–1.0 mg/L and 0.2–2.8 mg/L, respectively (Anthonisen *et al.* 1976). Svehla *et al.* (2014), significantly exceeding NOB-inhibiting concentrations of FA and FNA, observed adaptation in a continuous stirred-tank reactor (CSTR) as compared to a sequencing batch reactor (SBR) showing NOB-inhibition. On the other hand, FNA is used for sludge treatment, and it has been reported that the enzymes relevant to nitrifier denitrification were inhibited, decreasing the microbial community diversity, but increasing the abundances of AOB and denitrifiers, ultimately reducing N_2O emissions (Wang *et al.* 2016). Finally, despite an increase in external carbon source, excluding the anaerobic phase of an SBR promoted heterotrophic denitrifiers to be responsible for aerobic nitrogen removal instead of AOB, reducing N_2O production by heterotrophic denitrification (Chen *et al.* 2014).

In full-scale plants, local conditions are highly variable depending on process conditions and hydrodynamics, which will dictate which of the N_2O pathways will be dominant. The high variability of N_2O emissions has provided a fertile ground for the scientific debate on the correct definition and use of an emission factor (EF). Temporal variations are known to significantly impact (seasonally and daily) the assessment of EFs (Daelman *et al.* 2015). Spatial variations are also important and discrepancies in recognizing the highest emitting section of a WRRF often occur (Chandran 2010; Caivano *et al.* 2017; Marques *et al.* 2016), due to the fact that N_2O emissions are very specific to the treatment technology used, how the process is controlled, and the wastewater composition (Kampschreur *et al.* 2008; Ahn *et al.* 2010a). Nonetheless, local differences in substrate concentration within the same tank can lead to important variabilities (Rehman 2016), warranting key considerations for designing an *ad-hoc* sampling strategy for a correct EF assessment.

Aerated compartments are considered the greatest contributors to N_2O in WRRF (Chandran 2010); however, although more troublesome to measure, anoxic zones represent a central source (Ahn *et al.* 2010b). The Global Water Research Coalition (GWRC) (2011) reported 12 studies revealing an important contribution of anoxic zones to both production and emission of N_2O. At present, we can affirm that the monitoring of both aerobic and anoxic sections is normally suggested (Marques *et al.* 2016).

In order to contribute to the critical topic of the production dynamics and discrepancies among WRRFs, the biological tanks of three WRRFs (in Italy and the Netherlands) having similar configurations but different hydrodynamics were monitored for N_2O emissions from different points simultaneously.

The multi-point simultaneous monitoring allowed the capture of N_2O emission dynamics in both time and space domains. Spatial heterogeneities were highlighted for the three plants over time, helping to understand which location was more responsible for N_2O production at a given moment.

Spatial and temporal shifts in N_2O production were investigated to gain insights into the design of sampling strategies and to tackle the most timely issues in the assessment of the extent of N_2O emissions from WRRFs using AS biological nitrogen removal. Having a better understanding of this will facilitate strategies to reduce N_2O production and emissions.

MATERIALS AND METHODS

Three WRRFs were investigated, in Florence, Rome and Eindhoven. A schematic representation of each biological reactor is shown in Figure 1.

WRRF in Florence

The WRRF in Florence treats urban wastewater with a capacity of 600 k IE and a flowrate of approximately 200 k m^3/d. It is a municipal conventional activated sludge WRRF with a modified Ludzak-Ettinger denitrification-nitrification configuration. The biological treatment is carried out in 12 identical tanks working in parallel and grouped in three lanes, between which the influent is divided. Aeration is provided by fine-bubble diffusers (ABS, PIK300) with EPDM membranes placed 6.5 m deep in three equal zones along the aerated area with decreasing density of aerators towards the tank outlet, i.e. 44.0%, 30.5% and 25.5% of the aerators in each section. Aeration is balanced by an NH_4^+-DO cascade control.

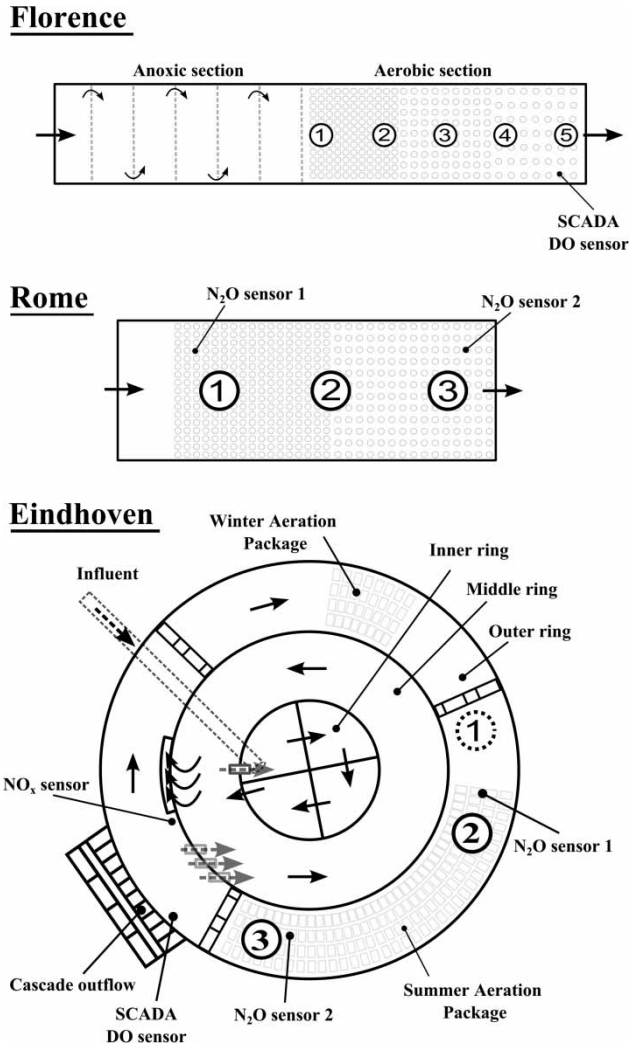

Figure 1 | Schematic overview of the biological tanks in the three plants. Locations of hoods are numbered in the direction of the flow. The circle with the dotted line is the anoxic hood.

Grab samples were taken hourly by means of an automatic sampler just before the entrance to the biological reactors and at the outlet of the AS tank, and analysis of ammonia nitrogen (NH_4^+-N) and NO_2^--N were carried out with standard kits (Hach). Since the primary sedimentation is bypassed due to the diluted influent character, the samples taken can be considered to also represent the influent concentration of the plant. The pH of the mixed liquor ranges around neutral (i.e. 6.9 ± 0.16).

Five floating hoods were distributed along the length of one of the aeration tanks (Figure 1) and numbered according to the flow direction. The first four hoods had an area of 0.35 m^2, while the fifth hood covered 0.7 m^2 of the tank surface.

Due to the very low expected concentrations of N_2O in the liquid, measurements were performed on AS grab samples from the pre-denitrification zone and from the aeration zone according to the head space gas method suggested by Kimochi *et al.* (1998), measuring the gas extracted from the headspace using gas chromatography with electron capture detector (GC-ECD).

WRRF in Rome

The WRRF in Rome treats 900 k IE (280 k m^3/d) municipal wastewater and is divided in two treatment lines. The largest treatment line (600 k IE) was in maintenance during this measurement campaign, therefore the smaller line was investigated. This treatment line, similar to the WRRF in Florence, is operated by bypassing primary sedimentation due to the high amount of infiltration diluting the raw wastewater. The influent, after a first coarse screening and sand trap, is directly split between the three parallel AS tanks. There is no pre-denitrification, and the influent directly enters the aerated volume after a mixing section. The pH varies around 7.6 ± 0.14. The aerated tank is equipped with EPDM membrane disk diffusers (ABS, PIK 300) at 5.5 m depth. The first half of the tank has 56.6% of the diffusers while in the second half are placed 43.4% of the diffusers.

Aeration is run with a fixed air flow rate and adjusted once per day according to manual DO measurements and AS characteristics, i.e. mixed liquor concentration and retention time. There is no online monitoring or logging; however, offline measurements were performed by the WRRF laboratory on daily composite samples. Therefore, hourly grab samples were taken by means of an automatic refrigerated sampler right before entering the AS tank and NH_4^+-N measurements with standard kits (Hach) were carried out. Similarly to the case of Florence, the samples taken can also be considered to represent the influent concentration of the plant. Information on NO_2^-N concentration in the bioreactor was only available from analysis of a daily composite sample.

Three floating hoods were distributed along the length of one of the aeration tanks and numbered in the flow direction (Figure 1). The hoods had an area of 2 m^2, 0.7 m^2 and 0.35 m^2 respectively.

Liquid measurements of N_2O were performed by means of two Clark-type sensors (Unisense Environment, Denmark) placed at the beginning and at the end of the aerated zone.

WRRF in Eindhoven

The WRRF in Eindhoven treats 750 k IE (250 k m^3/d) municipal wastewater with three parallel treatment lines designed with a modified University of Cape Town (UCT) layout for

the AS tanks. Each line is equipped with one biological tank consisting of three concentric rings, i.e. one covered anaerobic tank (inner ring), one covered anoxic tank (middle ring) and one open air aerobic/anoxic tank (outer ring) (Figure 1). After passing the anaerobic ring, the wastewater is directed to the anoxic compartment where impellers assure the circulation of the liquid. An overflow allows the mixed liquor to pass to the outer ring after the aerated zone. The aerobic zone (7 m deep) is equipped with 168 plate aerators evenly distributed. The rest of the tank is non-aerated with the sole exception of a small compartment (winter package) only used in winter conditions or exceptional cases and was not active while performing measurements.

Aeration is provided by a feedback NH_4^+-DO cascade control which reduces the airflow when the effluent ammonia from the bioreactor is below 1 mg/L.

Three floating hoods were positioned on the tank surface and numbered from upstream to downstream. The first hood was positioned on the anoxic zone, before entering the aerated compartment. The second and third hoods were positioned at the beginning and at the end of the aeration compartment respectively (Figure 1).

Liquid N_2O measurements were performed with one Clark-type sensor placed close to hood number 2. A second sensor was not available. The location of the sensor was chosen in order to monitor the amount of liquid N_2O at the entrance of the aeration compartment at the first location where stripping could occur and where the ammonia concentration was expected to be highest.

Grab samples were taken every two hours during the day at three locations for offline measurements of NH_4^+-N and NO_2^-N.

N_2O measurements and emissions calculation

All hoods were connected via a Teflon tube (4 mm in diameter) to a multiplex sampler, allowing automatic switching between the different locations and control of the monitoring time spent on each hood. Two gas analyzers using the non-dispersive infrared technique were used at the WRRFs of Rome (Thermo Scientific^{TM}, Model 46i) and Eindhoven (Teledyne API^{TM}, Model T320), while a photoacoustic IR (LumaSense, Inc., INNOVA 1412i) was used at the Florence WRRF. For all the WRRFs, due to the difference in diffuser distribution along the length of the aeration tanks, N_2O readings were corrected according to the locally supplied airflow. Also, pressure and temperature corrections were applied in order to provide comparable values among WRRFs. More details about the sampling hoods can be found in the Supplementary material (SM), available with the online version of this paper.

Emissions from anoxic zones

Gaseous emissions of N_2O from the anoxic compartment were monitored in the WRRFs in Florence and Eindhoven with the use of a modified version of the Lindvall gas hood system (Lindvall *et al.* 1974) first implemented for this purpose by Desloover *et al.* (2011). This channel allows a clean sample of ambient air to travel in a confined space at the surface of a tank (SM). In this way, the air sample along the length of the exchange chamber is enriched with the compounds that are released from the water surface as would naturally happen. In order to be able to measure very low emissions from non-aerated surfaces, the sampling method was modified using a suction flow of 1 L/min. The inlet of the exchange channel was connected to a long tube to make sure the incoming air was unbiased ambient air. Knowing the ambient N_2O concentration, the size of the channel and the sampling airflow, it is possible to calculate the exchanged N_2O at the interface of non-aerated areas.

EF calculation

The N_2O EF was calculated as the N_2O emitted per unit NH_4^+-N removed (SM), which not only can account for N_2O production from AOB, but also from heterotrophic denitrification.

RESULTS AND DISCUSSION

Florence

It must be pointed out that the diluted character of the influent of the WRRF in Florence shows very limited NH_4^+-N concentrations already at the entrance of the plant (Figure 2, bottom) due to a constant infiltration of groundwater in the sewer, which is most probably the reason why influent peaks are known to be uncommon for this WRRF. Due to sensor failure, only 6 of the 24 hours of gas sampling are shown in Figure 2. NO_2^--N measurements in the bioreactor had no significant variation during the 24 hours (0.04 ± 0.02 mg/L).

In terms of temporal variation, the data from the off-gas measurements show that at the beginning of the measurements, N_2O emissions were higher, as were the DO concentration peaks. This is most likely a result of the

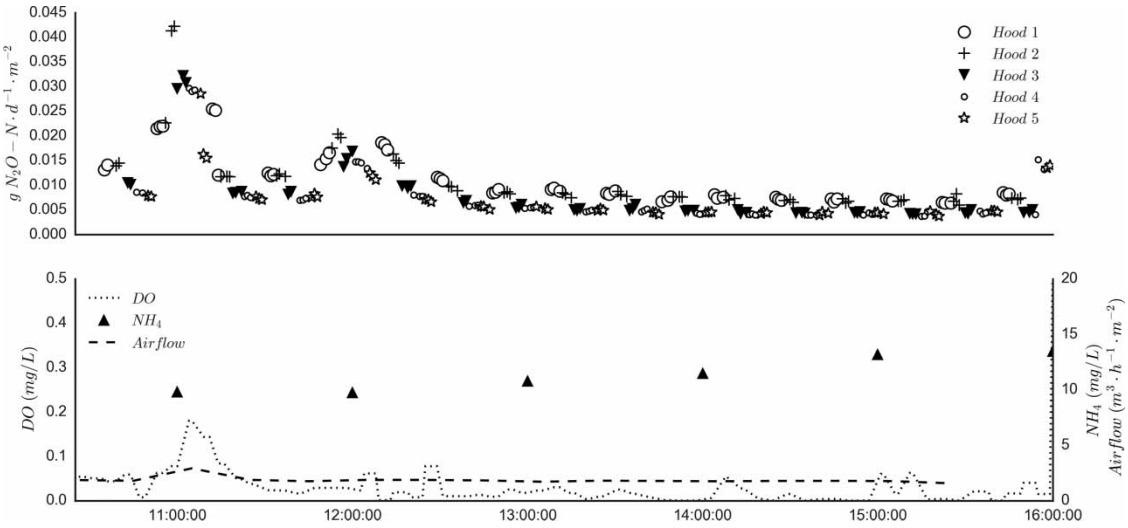

Figure 2 | N_2O emissions (top), total air flow and concentrations of DO from SCADA in the bioreactor and NH_4^+-N from automatic sampler before the AS tank (bottom) at the WRRF in Florence.

slight increase in aeration and higher stripping. However, this could also be from higher N_2O production. As the DO concentrations were generally low, it is unlikely that N_2O production was due to incomplete hydroxylamine oxidation based upon the DO concentrations reported by Peng *et al.* 2014. This means that N_2O production may have been via AOB denitrification in addition to heterotrophic denitrification, given that NO_2^--N and DO were consistently low and NH_4^+-N was relatively constant until the end of the campaign. Also, due to the difference in oxygen half-saturation indices between AOB and NOB (Mota *et al.* 2005), which results in higher NO_2^- with lower DO, the peaks in N_2O corresponding to peaks in air flow and DO are likely not due to peaks in NO_2^- at those moments, but rather due to more stripping of N_2O resulting from the baseline NO_2^- concentrations and related AOB denitrification prompted by low DO conditions. DO has been seen to inhibit heterotrophic denitrification at 0.21 mg O_2/L (Kester *et al.* 1997), and particularly the Nos enzyme responsible for reducing N_2O to N_2, which would result in incomplete heterotrophic denitrification and accumulation of N_2O (Von Schulthess *et al.* 1994). Since there is removal of ammonia, it is most likely that N_2O is being produced from both AOB denitrification and heterotrophic denitrification with the low DO conditions. Therefore, the temporal variation is most likely due to diurnal variation of DO, substrate, and corresponding variation in the degrees of AOB denitrification and heterotrophic denitrification N_2O production. As for spatial variation, location 1 and 2 generally appear to be emitting more compared to the other locations. Seeing that location 1 and 2 are at the start of the aeration tank, which would be the locations with a higher expected substrate availability, it makes sense that generally this area of the tank emits more than the rest. However, this seems to be valid for only low emission periods. When emissions are higher (Figure 2, top between 10:00 and 12:00), location 3, 4 and 5 gain importance, emitting more than location 1. One possibility could simply be different local mixing conditions leading to significantly different DO concentrations at the different locations, keeping in mind that the DO data are from a sensor located at the end of the aeration tank (more representative of location 4 and 5). Another possibility is different DO concentrations due to the diffuser grid layout. The normally very low DO conditions (at the limit of anoxia) of the locations close to the outlet of the aeration tank are likely prompting both AOB and heterotrophic denitrification N_2O production and overall greater N_2O production, which is not fully stripped at the downstream locations until the aeration increases. Airflow, as well as local DO and liquid N_2O data at each location, could confirm which of these possibilities is most likely; however, the objective of the study was identifying the temporal and spatial variations and understanding possible factors for each.

Assessing the EF for each location separately using the respective average value results in very different estimates. In particular, as compared to location 1, estimating the EF in location 5 would result in an underestimate of about 37.5%. EFs estimated in location 3 and 4 both show 28.1% deviation from the EF calculated in location 1. On the

other hand, location 2 has a similar EF to location 1, showing 0.8% increase.

Rome

During low loading periods of the plant, there seems to be no relevant difference among the three locations in terms of N_2O emissions (Figure 3, top). However, discrepancies among hoods start to increase when a peak load enters the AS tank and location 2 and 3 gain more importance than location 1 (the one closer to the inlet). This observation was confirmed by the liquid N_2O measurements (Figure 3, bottom). The two probes, located close to the entrance (N_2O liquid sensor 1) and close to the outlet (N_2O liquid sensor 2) of the aeration zone, detected very low or zero N_2O concentration in the liquid during periods of low gaseous N_2O emissions. Interestingly, the N_2O liquid sensor 2 consistently detected higher concentrations than sensor 1 and this difference increased during the peak of N_2O emissions in the gas, confirming that the production in the second half of the tank was higher.

The constant aeration flow rate characterizing this plant facilitates the understanding of DO fluctuations, allowing them to be directly connected to influent load dynamics. The DO concentrations in Figure 3 (bottom), recorded at hood 1, show that increasing DO concentrations (around 4:00) did not influence N_2O emissions and production in the liquid phase. However, as soon as the decrease in DO occurs (after 9:00), probably due to an increased biological activity resulting from the higher incoming load, N_2O production in the liquid and relative gaseous emissions start to increase. Interestingly, the DO concentration at which the N_2O production has its maximum rate is when it reaches below 1 mg/L, in accordance with literature results (Tallec *et al.* 2008). As DO approaches limiting conditions during the highest N_2O concentrations, N_2O is most likely produced via the AOB denitrification pathway. The daily composite sample of NO_2^--N (i.e. 0.21 mg/L) may indeed suggest that NOB-inhibition concentration can be reached. The maximum emissions are registered from hood 2 and 3, which are located further downstream towards the outlet, further confirming this last observation as NO_2^--N concentration may increase and DO is likely to maintain limiting values. In addition to this, since the diffuser density is lower in this area than in location 1, DO is likely lower, potentially resulting in greater N_2O production from AOB denitrification.

Eindhoven

NH_4^+-N loads (Figure 4, bottom) and relative fluctuations are more prominent for this plant as compared to the others since the sewer experiences measurably lower infiltrations of groundwater. The temporal variation in N_2O emissions (Figure 4 top) appears to be mainly due to the diurnal effect of varying ammonia and corresponding DO concentrations. From the control, as NH_4^+-N increases, DO is increased until NH_4^+-N is lowered, when DO is lowered again. This pattern repeats throughout the day. The highest N_2O emissions occur when the daily ammonia peak arrives.

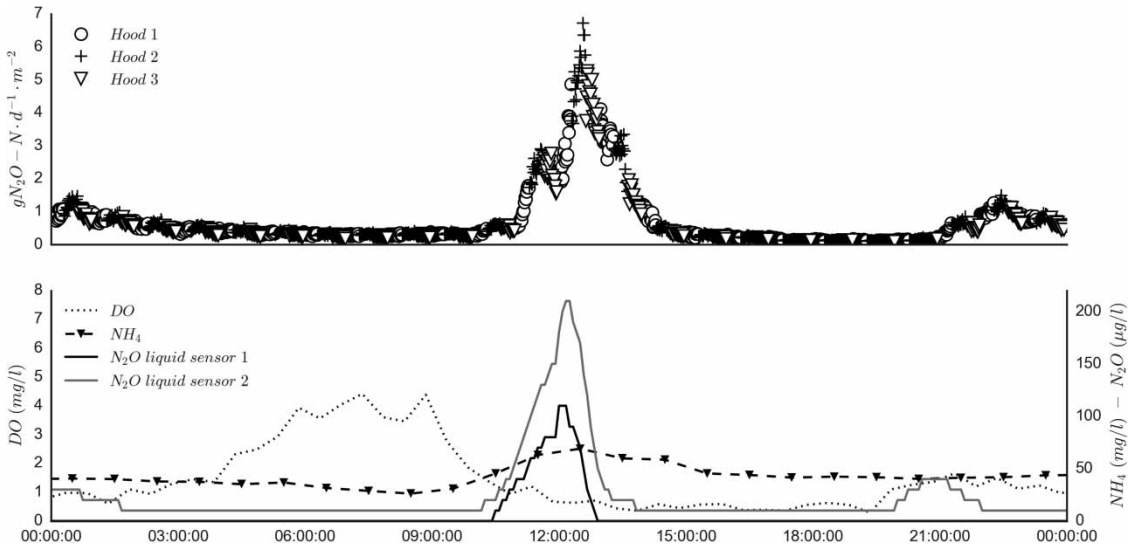

Figure 3 | N_2O gas emissions (top graph) and liquid measurements of DO, NH_4^+, and N_2O (bottom graph) at the WRRF in Rome.

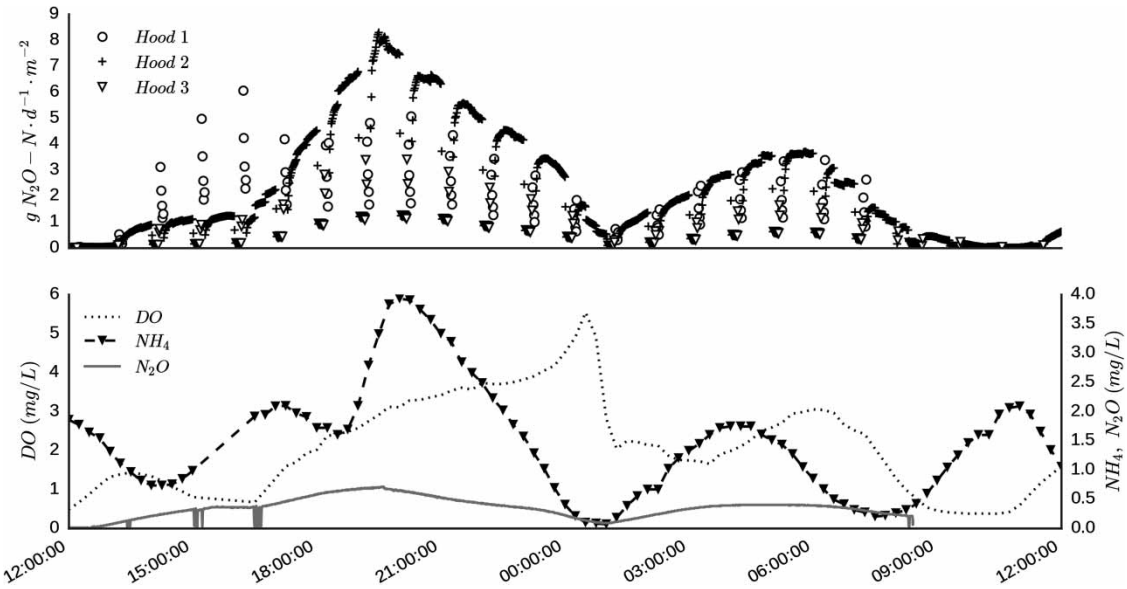

Figure 4 | N_2O gas emissions and air flow (top graph) compared with liquid concentrations of DO, NH_4^+-N NO_x–N and N_2O-N (bottom graph) at the WRRF of Eindhoven. Due to unavailability of influent data, measurements of NH_4^+ are from the SCADA sensor in the bioreactor.

NO_2^--N at the three locations showed similar values throughout the day (0.25 ± 0.03 mg/L) and, although, it cannot provide specific information for the single location, may suggest the NOB-inhibitory effect in favor of the AOB denitrification pathway where DO is limiting.

In terms of spatial variation, measurements from hood 1 (Figure 4, top) were generally lower than the ones observed in location 2, but higher than the ones registered from location 3. However, in the last part of the time series, there are missing data points from location 1. The N_2O emission from the anoxic zone appears to be fluctuating more (within the same group of measurement samples) than from other locations. This could be due to the fact that, unlike in the aerated zone, there is no constant stripping in the anoxic zone, and the occurrence of recirculations from deeper zones and eddies at the surface provide more variable instantaneous emissions. These variations from the anoxic zone are consistent but do not repeat similar patterns within the same cloud of data, reinforcing the previous observation.

Emissions from hood 1 were of the same magnitude as the ones registered from the other hoods even though location 1 was in the anoxic zone. Therefore, only diffusion at the surface could account for comparable emissions to the ones occurring for active stripping.

Peaks in N_2O emission from location 1 seemed to occur when relatively high NH_4^+ peaks appeared and DO values were close or even below 1 mg/L (17:00 and 5:00). The highest N_2O emission recorded from hood 1 occurred at 17:00 when DO was below 0.5 mg/L. These observations suggest that emissions from location 1, and thus production from the anoxic zone, are most likely to happen either due to the AOB denitrification pathway, or from incomplete heterotrophic denitrification.

Emissions from location 2 registered the highest peaks at the same time as significant NH_4^+ peaks. Interestingly, the first peak in N_2O emission from location 2 corresponds with a first peak of location 3 (Figure 4, bottom at 20:00) and, since at the same time N_2O emission from location 1 seems too decrease, it is likely that an important part of this production takes place in the aerobic compartment. This is also confirmed directly by the increase in liquid N_2O concentration. The rising DO corresponding to these peaks (at 20:00 and 8:00), indicates that the dominant pathway at this particular time could be the hydroxylamine oxidation, especially considering DO is non-limiting, approximately 3 mg O_2/L.

N_2O measurements in the liquid (sensor placed at location 2) (Figure 4, bottom), seem to corroborate this hypothesis. The highest rate of N_2O emission (steepness of the N_2O curves) for location 2 and 3 occurs in those moments when both increasing NH_4^+-N availability and increasing DO values above 1 mg/L occur. The differences in emissions between location 2 and 3, both within the aerobic zone, but at the beginning and end, respectively, are most likely attributable to different $NH4^+$-N and DO concentrations.

Literature studies on the same WRRF confirm our observations. A qualitative comparison with the findings of the modelling work of Rehman (2016), performed on the same plant, corroborates the distribution of emissions measured in this work. In particular, the anoxic zone corresponding to location 1 in this work is shown to have double the N_2O concentration in the liquid as compared to the beginning and the end of the aerated zone (location 2 and 3 respectively in this work). Also, the beginning of the aerated zone shows higher N_2O concentration as compared to the end (Guo *et al.* 2013; Rehman 2016).

Estimation of an EF

In order to further illustrate how the use of a single EF for describing the extent of emissions of different (although similar in AS technology used) WRRFs is not valid, an overview of EF from all the different locations in the three WRRFs studied is provided (Figure 5).

The case of the WRRF in Florence is shown in the top graphs of Figure 5. Selecting only the peak of N_2O emission (Figure 5, top, graph a) the average values of location 3 and 4 gain importance over the rest of the hoods. However, deviation bars around the data in location 1 and 2 hamper the strength of this observation.

Considering only the period outside the peak of N_2O emission for the case in Florence (Figure 5, top, graph b), emissions seem to be consistently low with location 1 showing a slightly higher EF than the rest.

A boxplot of the entire dataset from Florence (Figure 5, top, graph c) shows the higher emission of location 1 and 2, but also considerable overlapping variations from the rest of the hoods. However, this overall statistical description is hiding the importance that location 3, 4 and 5 gain when DO and ammonia increase. Location 1 and 2 show a

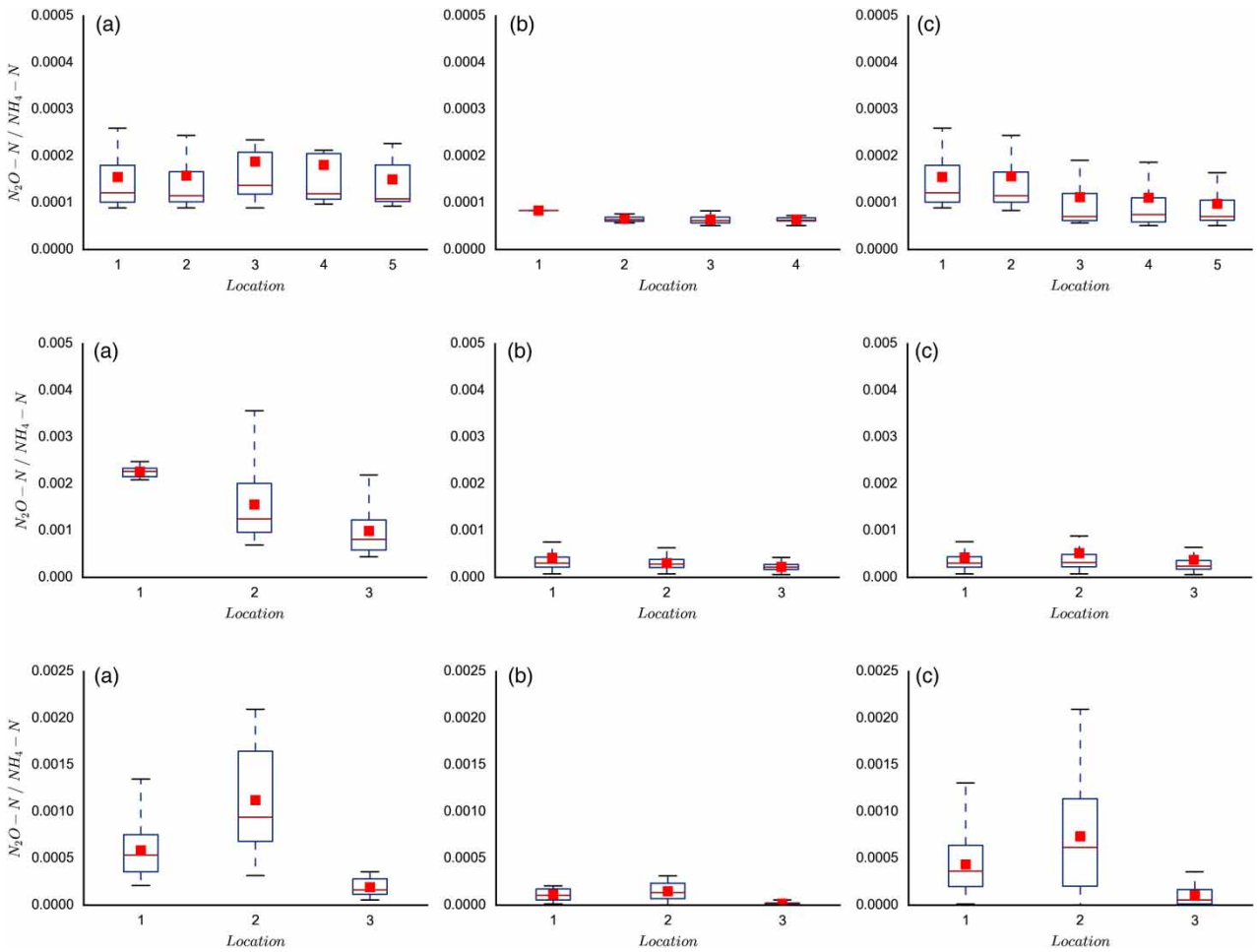

Figure 5 | Boxplots of peak emission periods (a), low emission periods (b), and overall emission (c) for the case of Florence (top), Rome (middle), and Eindhoven (bottom).

tendency to be the highest-emitting locations in comparison with 3, 4 and 5, from their mean values and their general behavior in the dataset. However, these initial locations also show the highest variability (up to 0.0002 N_2O-N/NH_4^+-N) and unsuitability for assessment of EF from a sampling campaign shorter than a full day.

Isolating the N_2O emission peak from the WRRF in Rome (Figure 5, middle, graph a) it is noticeable how the highest emission values reached by hood 2 is not able to drag its mean value higher than hood 1, thus remaining the highest-emitting location. Therefore, for the case of Rome, location 1 remains the highest emitter of the AS tank.

During low emissions (Figure 5, middle, graph b), all locations appear to contribute to the same extent to the release of gaseous N_2O. Similarly, looking at the boxplot over the whole dataset from Rome (Figure 5, middle, graph c), differences among locations do not stand out.

For the case of Eindhoven (Figure 5, bottom) the picture is rather different. Location 2 and 3 have very different emissions in all cases. During N_2O emission peak events (Figure 5, bottom, graph a), location 2 has EF values more than one order of magnitude higher than location 3. When low emission of N_2O occurs (Figure 5, bottom, graph b), the contribution of location 3 practically disappears. In the overall picture (Figure 5, bottom, graph c), even considering the whole data set, the contribution of location 3 is measurably lower than the rest, and hood 2 provides the highest emissions. Spatial differences along the aeration package are therefore very important.

In addition to this, the contribution of the anoxic compartment was also very relevant, comparable to location 2. This is due to the big surface available for exchange in the anoxic part of the outer ring of the WRRF in Eindhoven. As a matter of fact, overall hood 1 maintains EF values and deviations close to what occurs at hood 2.

Comparing the different WRRFs, the EFs of the WRRF in Florence are in general one order of magnitude lower than for the other plants. This fact can be mainly attributed to its highly diluted influent, preventing sudden N peak loads to be converted. Interestingly, despite the known diluted influent character also for the case in Rome, higher EFs than the one in Eindhoven were observed.

One important detail to notice is that all EFs provided in Figure 5 represent the EF of the plant that one would have calculated measuring only from a specific hood. In this view, variations among the points represent the error that would have been made in judging EF by an operator measuring N_2O emissions from only a single location.

Adding the contribution of each location

Clearly, single point calculation measurements of EFs are usually not representative of the different contributions from all locations of a bioreactor. Given the availability of parallel measurements, it is possible to add up the contributions from each of the hoods and calculate a more refined EF for the three cases studied. In particular, this is possible by addressing a specific portion of the surface area of the tank under each hood based on their location. The main assumption is that the given surface area behaves in a similar fashion, which is still a better approach than assuming it for the whole reactor.

For the WRRF in Florence the overall EF calculated with the contributions from all hoods becomes 0.012% (±0.007%), which is not far from what already observed in the boxplots. However, the sole average might not be representative in this case as a consistent standard deviation is present.

For the case of Rome the three hoods contributed to give an average 0.06% (±0.07%) which is also in the range observed in the box plots. A relevant deviation is observable also in this case.

For the WRRF in Eindhoven the overall EF accounting for both contributions of aerobic and anoxic zones is 0.1% (±0.04%). Interestingly, if one would have neglected the contribution of anoxic zones the estimated mean EF would have been 0.03% (±0.03%). Therefore, neglecting the contribution of anoxic zones in the case of the WRRF in Eindhoven leads to an underestimation of the EF of 68.2%. However, as reported by Rehman 2016 location 1 might be the highest-emitting part of the whole AS tank, therefore leading us to an overestimation of the anoxic contribution of the EF. Nonetheless, this confirms the necessity of different sampling locations to reasonably represent the behavior of a tank.

CONCLUSIONS

The experimental method used allowed the simultaneous monitoring of different locations in full-scale AS tanks and highlighted the wide range in EF values from plant to plant and within the same facility. Spatial variabilities heavily influence emission results, and the use of a single EF describing the entire WRRF operation or classifying a treatment technology is simply not valid.

The EF measurements performed in WRRFs using similar AS tanks differed by more than one order of magnitude.

Therefore, when a WRRF needs to be evaluated in terms of its environmental impact, the use of an EF should be accompanied with information regarding its variability and potential extents of emissions (e.g. 75 percentiles of a cumulative emission) to better understand the WRRF potential and refine its classification.

The assessment of the contribution of anoxic zones should be a normal procedural approach. A method for accounting for the contribution of anoxic zones via direct gas emission measurements was proven. The anoxic hood effectively allowed the detection of N_2O emissions from non-aerated surfaces of an AS tank with an economic, practical and user-friendly approach.

Both the temporal domain, relative to influent dynamics, and the spatial domain, relative to hydrodynamics, are crucial for understanding N_2O emission evolution.

For the case of the WRRF in Florence the diluted influent (groundwater infiltration) is the most probable reason for which the EFs are so low.

The plant in Rome showed higher N_2O emissions in the locations closer to the outlet of the AS tank when a peak load was experienced. Higher DO levels may be maintained to hamper N_2O production.

The carrousel configuration of the AS tank of the WRRF in Eindhoven seems to result in anoxic N_2O emissions comparable to the ones from the aeration compartment. This validates with literature studies on hydrodynamics on the same plant. Tuning the DO control to lower DO levels during peak loads may reduce production from both anoxic (removing limiting DO conditions that favor AOB denitrification) and aerobic zones (removing high DO levels that may favor hydroxylamine oxidation).

To our knowledge, this is the first time that spatial N_2O emissions are made visible at this resolution comparing similar configurations of AS tanks from different WRRFs.

REFERENCES

Ahn, J. H., Kim, S., Park, H., Katehis, D., Pagilla, K. & Chandran, K. 2010a Spatial and temporal variability in atmospheric nitrous oxide generation and emission from full-scale biological nitrogen removal and non-BNR processes. *Water Environment Research: A Research Publication of the Water Environment Federation* **82** (12), 2362–2372. [online] http://www.ncbi.nlm.nih.gov/pubmed/21214030.

Ahn, J. H., Kim, S., Park, H., Rahm, B., Pagilla, K. & Chandran, K. 2010b N_2o emissions from activated sludge processes, 2008 – 2009: results of a national monitoring survey in the United States. *Environmental Science & Technology* **44** (12), 4505–4511. [online] http://pubs.acs.org/doi/abs/10.1021/es903845y.

Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S. & Srinath, E. G. 1976 Inhibition of nitrification by ammonia and nitrous acid. *Journal of the Water Pollution Control Federation* **48**, 835–852.

Bock, E., Schmidt, I., Stuven, R. & Zart, D. 1995 Nitrogen loss caused by denitrifying *Nitrosomonas* cells using ammonium or hydrogen as electron donors and nitrite as electron acceptor. *Archives of Microbiology* **163** (1), 16–20.

Buday, J., Drtil, M., Hutňan, M. & Derco, J. 1999 Substrate and product inhibition of nitrification. *Chemical Papers* **53**, 379–383.

Caivano, M., Bellandi, G., Mancini, I. M., Masi, S., Brienza, R., Panariello, S., Gori, R. & Caniani, D. 2017 Monitoring the aeration efficiency and carbon footprint of a medium-sized WWTP: experimental results on oxidation tank and aerobic digester. *Environmental Technology* **38** (5), 629–638. [online] https://www.tandfonline.com/doi/full/10.1080/09593330.2016.1205150.

Chandran, K. 2010 '*Protocol for the Measurement of Nitrous Oxide Fluxes From Biological Wastewater Treatment Plants*' *in Methods in Enzymology*. Elsevier Inc., New York, pp. 369–385. [online] http://dx.doi.org/10.1016/B978-0-12-381294-0.00016-X.

Chandran, K., Stein, L. Y., Klotz, M. G. & van Loosdrecht, M. C. M. 2011 Nitrous oxide production by lithotrophic ammonia-oxidizing bacteria and implications for engineered nitrogen-removal systems. *Biochemical Society Transactions* **39** (6), 1832–1837. [online] http://www.biochemsoctrans.org/content/39/6/1832.abstract.

Chen, Y., Wang, D., Zheng, X., Li, X., Feng, L. & Chen, H. 2014 Biological nutrient removal with low nitrous oxide generation by cancelling the anaerobic phase and extending the idle phase in a sequencing batch reactor. *Chemosphere* **109**, 56–63. [online] http://dx.doi.org/10.1016/j.chemosphere.2014.02.011.

Daelman, M. R. J., van Voorthuizen, E. M., van Dongen, L. G. J. M., Volcke, E. I. P. & van Loosdrecht, M. C. M. 2013 Methane and nitrous oxide emissions from municipal wastewater treatment – results from a long-term study. *Water Science & Technology* **67** (10), 2350. [online] http://www.iwaponline.com/wst/06710/wst067102350.htm.

Daelman, M. R. J., van Voorthuizen, E. M., van Dongen, U. G. J. M., Volcke, E. I. P. & van Loosdrecht, M. C. M. 2015 Seasonal and diurnal variability of N_2O emissions from a full-scale municipal wastewater treatment plant. *Science of the Total Environment* **536**, 1–11. [online] http://linkinghub.elsevier.com/retrieve/pii/S0048969715303272.

Desloover, J., De Clippeleir, H., Boeckx, P., Du Laing, G., Colsen, J., Verstraete, W. & Vlaeminck, S. E. 2011 Floc-based sequential partial nitritation and anammox at full scale with contrasting N_2O emissions. *Water Research* **45** (9), 2811–2821. [online] http://linkinghub.elsevier.com/retrieve/pii/S0043135411000959.

Guo, L. S., Lamaire-chad, C., Bellandi, G., Daelman, M. R. J., Maere, T., Nous, J., Flameling, T., Weijers, S., Mark, C. M., Loosdrecht, V., Volcke, E. I. P., Nopens, I. & Vanrolleghem, P. A. 2013 'High frequency Field Measurements of Nitrous

oxide (N_2O) Gas Emissions and Influencing Factors at WWTPs under Dry and Wet Weather Conditions' in WEF/IWA Nutrient Removal and Recovery 2013.

GWRC 2011 *N_2O and CH4 Emission From Wastewater Collection and Treatment Systems*. Global Water Research Coalition, London.

Kampschreur, M. J., Tan, N. C. G., Kleerebezem, R., Picioreanu, C., Jetten, M. S. M. & van Loosdrecht, M. C. M. 2008 Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. *Environmental Science & Technology* **42** (2), 429–435. [online] http://pubs.acs.org/doi/abs/10.1021/es071667p.

Kampschreur, M. J., Temmink, H., Kleerebezem, R., Jetten, M. S. M. & van Loosdrecht, M. C. M. 2009 Nitrous oxide emission during wastewater treatment. *Water Research* **43** (17), 4093–4103. [online] http://linkinghub.elsevier.com/retrieve/pii/S0043135409001420.

Kester, R. A., De Boer, W. & Laanbroek, H. J. 1997 Production of NO and N_2O by pure cultures of nitrifying and denitrifying bacteria during changes in aeration. *Applied and Environmental Microbiology* **63** (10), 3872–3877. [online] http://www.ncbi.nlm.nih.gov/pubmed/16535707.

Kimochi, Y., Inamori, Y., Mizuochi, M., Xu, K.-Q. & Matsumura, M. 1998 Nitrogen removal and N_2O emission in a full-scale domestic wastewater treatment plant with intermittent aeration. *Journal of Fermentation and Bioengineering* **86** (2), 202–206. [online] http://www.sciencedirect.com/science/article/pii/S0922338X98801141.

Law, Y., Ni, B. J., Lant, P. & Yuan, Z. 2012 N_2o production rate of an enriched ammonia-oxidising bacteria culture exponentially correlates to its ammonia oxidation rate. *Water Research* **46**, 3409–3419.

Lindvall, T., Noren, O. & Thyseliu, L. 1974 Odor reduction for liquid manure systems. *Transactions of the Asae* **17** (3), 508–512.

Marques, R., Rodriguez-Caballero, A., Oehmen, A. & Pijuan, M. 2016 Assessment of online monitoring strategies for measuring N_2O emissions from full-scale wastewater treatment systems. *Water Research* **99**, 171–179. [online] http://www.sciencedirect.com/science/article/pii/S0043135416302494.

Mota, C., Head, M., Ridenoure, J., Cheng, J. & de los Reyes, F. 2005 Effects of aeration cycles on nitrifying bacterial populations and nitrogen removal in intermittently aerated reactors. *Applied Environmental Microbiology* **71** (12), 8565–8572.

Peng, L., Ni, B.-J., Erler, D., Ye, L. & Yuan, Z. 2014 The effect of dissolved oxygen on N_2O production by ammonia-oxidizing bacteria in an enriched nitrifying sludge. *Water Research* **66** (2), 12–21. [online] http://www.sciencedirect.com/science/article/pii/S0043135414005764.

Peng, L., Ni, B., Ye, L. & Yuan, Z. 2015 The combined effect of dissolved oxygen and nitrite on N_2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge. *Water Research* **73** (2), 29–36. [online] http://dx.doi.org/10.1016/j.watres.2015.01.021.

Rehman, U. 2016 Next generation bioreactor models for wastewater treatment systems by means of detailed combined modelling of mixing and biokinetics. [online] http://hdl.handle.net/1854/LU-8109100.

Schreiber, F., Wunderlin, P., Udert, K. M. & Wells, G. F. 2012 Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Frontiers in Microbiology* **3** (372). [online] http://journal.frontiersin.org/article/10.3389/fmicb.2012.00372/abstract.

Svehla, P., Bartacek, J., Pacek, L., Hrncirova, H., Radechovsky, J., Hanc, A. & Jenicek, P. 2014 Inhibition effect of free ammonia and free nitrous acid on nitrite-oxidising bacteria during sludge liquor treatment: influence of feeding strategy. *Chemical Papers* **68** (7), 871–878. [online] http://www.degruyter.com/view/j/chempap.2014.68.issue-7/s11696-014-0538-6/s11696-014-0538-6.xml.

Tallec, G., Garnier, J., Billen, G. & Gousailles, M. 2008 Nitrous oxide emissions from denitrifying activated sludge of urban wastewater treatment plants, under anoxia and low oxygenation. *Bioresource Technology* **99** (7), 2200–2209. [online] http://linkinghub.elsevier.com/retrieve/pii/S0960852407004452.

Von Schulthess, R., Wild, D. & Gujer, W. 1994 Nitric and nitrous oxides from denitrifying activated sludge at low oxygen concentration. *Water Science & Technology* **30** (12), 123–132.

Wang, D., Wang, Q., Laloo, A. E. & Yuan, Z. 2016 Reducing N_2O emission from a domestic-strength nitrifying culture by free Nitrous Acid-Based sludge treatment. *Environmental Science and Technology* **50** (14), 7425–7433.

First received 19 July 2017; accepted in revised form 23 October 2017. Available online 30 November 2017