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2013

Link to publication

Citation for published version (APA):
Stenström, F., Baresel, C., & la Cour Jansen, J. (2013). *Nitrous oxide production under varied C/N-ratio and DO in an SBR treating digester supernatant*. Paper presented at 13th Nordic Wastewater Treatment Conference (NORDIWA2013), Malmö, Sweden.

Total number of authors:
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Nitrous oxide production under varied C/N-ratio and DO in an SBR treating digester supernatant

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Abstract
Nitrous oxide (N_2O) is a greenhouse gas formed during biological wastewater treatment under aerobic and anoxic conditions. Two reasons for high N_2O emissions are low oxygen concentration during nitrification and low carbon/nitrogen-ratio during denitrification. In this full-scale study of N_2O emissions from a sequencing batch reactor for treating digester supernatant, the oxygen concentration was reduced stepwise and the carbon/nitrogen-ratio was varied to investigate how N_2O emissions were influenced. N_2O concentrations were measured online in water and off-gas. A distinct relationship was found between low oxygen concentrations and high N_2O emissions. N_2O was formed in water during both nitrification and denitrification. Decreased oxygen concentration during nitrification led to increased nitrite concentration, which in turn led to increased N_2O concentration in the subsequent denitrification phase. When nitrification resumed, accumulated N_2O was stripped off to the atmosphere. A too low carbon/nitrogen-ratio resulted in increased N_2O emissions in off-gas under longer-term conditions and increased risk of separate peak emissions. Very high N_2O concentrations, over 76,000 ppm, were measured in the off-gas. Furthermore, the maximum N_2O emitted during one cycle represented 217.9% of the total nitrogen load (36.2% of total nitrogen present in the bulk liquid at cycle start), among the highest emission levels measured from a full-scale municipal plant treating digester supernatant.

Keywords: biological wastewater treatment, digester supernatant, nitrous oxide, C/N-ratio, sequencing batch reactor

Introduction
Greenhouse gases (GHGs) have attracted increasing attention in recent years. One GHG of special concern in wastewater treatment is nitrous oxide (N_2O). N_2O is the third largest contributor to climate change, after carbon dioxide and methane (Forster *et al*., 2007), and has been identified as the single most important ozone-depleting gas emitted in the twenty-first century (Ravishankara *et al*., 2009).

To prevent eutrophication in coastal zones, wastewater treatment plants (WWTPs) are required to remove nitrogen from wastewater. The most common method for doing this uses biological processes, but many of the bacterial groups involved in nitrogen removal are found to produce N_2O. Since N_2O is 298 times more potent as a GHG than is carbon dioxide, based on a time horizon of 100 years (Forster *et al*., 2007), even small emissions can greatly affect the carbon footprint of a WWTP.

N_2O can form under both aerobic and anoxic conditions. Nitrification is the oxidation of ammonium (NH_4^+) by, for example, autotrophic bacteria, via hydroxylamine (NH_2OH) to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB), which is further oxidized to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB). AOB and NOB use NH_4^+ and NO_2^-, respectively, as the electron donor (i.e. energy source), oxygen as the electron acceptor, and carbon dioxide as the carbon source. Denitrification is the stepwise reduction of NO_3^- via NO_2^-, nitric oxide (NO), and N_2O to nitrogen gas (N_2) by heterotrophic denitrifiers. Heterotrophic denitrifiers use organic carbon as both the electron donor and carbon source, and NO_3^- and NO_2^- as the electron acceptor.

Under **aerobic** conditions, N_2O can be formed biologically by AOB and heterotrophic nitrifiers. Aerobic N_2O generation by AOB can occur through two mechanisms: (1) as a byproduct of nitrification when hydroxylamine is oxidized to NO_2^- (Hooper & Terry 1979) or (2) by the aerobic denitrification of NO_2^- (so-called nitrifier denitrification). The latter is an important mechanism for N_2O production under aerobic conditions (Kim *et al*., 2010; Wunderlin *et al*., 2012). In this N_2O production pathway, NO_2^- is reduced stepwise to NO, N_2O, and N_2 (Wrage *et al*., 2001), whereas NO_2^- is used instead of oxygen as the electron acceptor. Some of these denitrifying nitrifiers lack the enzyme for the last denitrification step, which implies that the end product is N_2O instead of N_2; this phenomenon has been found for the nitrifying bacteria *Nitrosomonas europaea* (reviewed in Hooper *et al*., 1997). Some heterotrophic bacteria can nitrify under aerobic conditions and simultaneously produce N_2O (Papen *et al*., 1989). These bacteria are also often capable of

denitrifying under aerobic conditions (Robertson *et al.*, 1989) with N_2O being included as an intermediate. The formation of N_2O under aerobic conditions is stimulated by low dissolved oxygen (DO) concentrations and high NO_2^- concentrations (Kampschreur *et al.*, 2009). Lemaire *et al.* (2011) observed a positive correlation between concentrations of free nitrous acid (HNO_2) at the end of an aeration phase and N_2O emissions. Furthermore, Yu *et al.* (2010) observed that N_2O is produced by AOB during the recovery to aerobic conditions after an anoxic period.

Under **anoxic** conditions, N_2O can be formed biologically by AOB (Ritchie & Nicholas 1972; Remde & Conrad 1990) and heterotrophic denitrifiers. Anoxic N_2O generation by AOB occurs via the same pathway as mentioned above, i.e. nitrifier denitrification. The various steps of the process of denitrification by heterotrophic denitrifiers are catalyzed by enzymes. If the last reaction in the denitrification process is inhibited, the reduction ends with N_2O, which can cause the accumulation of N_2O in the water and N_2O emissions in the off-gas. The formation of N_2O under anoxic conditions could be caused by a low carbon/nitrogen ratio (Itokawa *et al.*, 2001), high NO_2^- concentration (Schulthess *et al.*, 1995), and the presence of oxygen (Otte *et al.*, 1996).

Moreover, N_2O formation is stimulated by rapidly changing conditions, for example, low–high concentrations of DO, NO_2^-, or NH_4^+ (Kampschreur *et al.*, 2008).

In general, higher N_2O emissions are found in lab-scale than in full-scale studies (Barton & Atwater 2002). Studies performed in full-scale WWTPs demonstrate that N_2O emissions vary significantly from plant to plant and depending on the process configuration. In a study of 25 activated sludge plants, Wicht and Beier (1995) found that N_2O emissions ranged from 0% to 14.6% of nitrogen load, while in a study of 7 WWTPs with various configurations, Foley *et al.* (2010) found that N_2O emissions ranged from 0.6% to 25.3% of denitrified nitrogen. The maximum N_2O emissions found in these two studies are among the highest ever measured from full-scale WWTPs treating municipal wastewater. There are limited reports of N_2O emissions from the full-scale treatment of digester supernatant; however, owing to higher concentrations of nitrogen species and higher transformation rates than in the mainstream process, considerably higher N_2O emissions could be expected.

The present work examines the N_2O emissions from a sequencing batch reactor (SBR) treating NH_4^+-rich digester supernatant with nitrification and denitrification. The DO concentration and carbon/nitrogen-ratio was varied during the study. More details from campaign 2 of the study are presented in Stenström *et al.* (submitted).

Materials and methods

Process design and normal SBR operation at Slottshagen WWTP

Slottshagen WWTP mainly treats municipal wastewater from the city of Norrköping, Sweden. It is designed for 200,000 population equivalents (PE), defined as 70 g BOD_7/(PE × day), a total nitrogen (TN) load of 2240 kg d^{-1}, and a flow of 2000 m^3 h^{-1}. The actual load corresponds to 135,000 PE, a TN load of 1650 kg d^{-1}, and a flow of 1900 m^3 h^{-1}. Ferric chloride is used for chemical precipitation of phosphorus. The sludge is pumped to two mesophilic digesters. The sludge is dewatered in centrifuges and the digester supernatant is piped to a buffer tank with a water volume of 200 m^3, from which it is pumped into the SBR. The treated water from the SBR is directed to the biological step of the mainstream process.

The SBR operates only from November to May, to ensure that the legislatively permitted TN level in the effluent from the mainstream process is fulfilled during the cold period of the year. The covered reactor has an effective volume of 1000 m^3 and is operated with pre-denitrification. It is run with a cycle length of 8 h; a normal cycle with its various phases is shown in Figure 1.

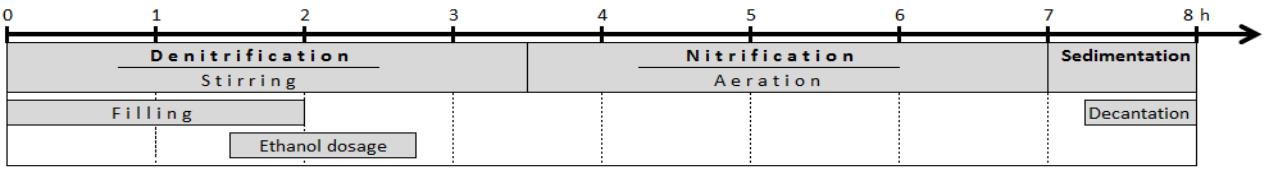


Figure 1. The phases of a normal SBR cycle. During decantation, there is a short period (several seconds) for the withdrawal of excess sludge.

The typical composition of the digester supernatant in the SBR influent is: TN, 1200 mg L^{-1}; NH_4^+-N, 1000 mg L^{-1}; NO_3^--N, 5 mg L^{-1}; COD, 2000 mg L^{-1}; PO_4^{3-}-P, 40 mg L^{-1}; HCO_3^-, 90 mM (5500 mg L^{-1}); pH, 8.0; and temperature, 28°C. In an ordinary SBR cycle, the influent flow is 70 m^3.

Under normal operating conditions, the SBR has a temperature of 30°C, a concentration of mixed liquor suspended solids (MLSS) of 3000 mg L^{-1}, a pH range of 1.3 units (typically 6.2–7.5), and a DO set point during nitrification of 2.0 mg L^{-1}. The hydraulic retention time (HRT) is 4.7 ± 0.3 d. The sludge retention time (SRT) is 15 ± 3 d. Removal of TN and NH_4^+-N are typically 80% and 95%, respectively. In the SBR, these parameters normally fluctuate in the following ranges: NH_4^+-N, 30–90 mg L^{-1}; NO_2^--N, 10–25 mg L^{-1}; and NO_3^--N, 90–150 mg L^{-1}.

Two stirrers ensure good mixing during denitrification, using a total power input of 13.2 W m^{-3}. A variable-frequency drive blower with a maximum capacity of 2400 Nm^3 h^{-1} provides air for nitrification; the air is distributed through fine bubble membrane diffusers.

Chemical analysis and instrumentation

Grab samples for analysis were taken at various time intervals. Grab samples for the analysis of soluble parameters were immediately filtered through a 0.45-µm filter. All samples were kept cold and analyzed within 18 h. All chemical analyses were performed using a commercial cuvette test kit (Hach Lange, Düsseldorf, Germany) and a Xion 500 spectrophotometer (Hach Lange). Grab samples for analysis were taken as follows: for NO_2^--N (Hach Lange test kit no. LCK 341), every 30 min; for soluble fractions of COD (LCK 114), TN (LCK 138), NH_4^+-N (LCK 303), and NO_3^--N (LCK 339), every 60 min; and for COD, TN, MLSS, mixed liquor volatile suspended solids (MLVSS), alkalinity, total phosphorous (LCK 348), and soluble PO_4^{3-}-P (LCK 348), three times per cycle. MLSS, MLVSS, and alkalinity were determined according to the Swedish standard methods SS-EN 872:2005, SS 028112-3, and SS 028139-1, respectively.

Online sensors were installed at the SBR to measure DO (LDO; Hach Lange), pH (1000 TR; Elmacron, Norrköping, Sweden), MLSS concentration (Solitax; Hach Lange), ethanol dosage (Mass 2100; Siemens, Munich, Germany), blower airflow (ST50-EF32BN0A; Fluid Components International, San Marcos, CA, USA), SBR water level (7060; MJK Automation, Säffle, Sweden), and temperature (MCR-SL-PT100-UI-200; Phoenix Contact, Blomberg, Germany).

Measurement of N_2O in water and off-gas

A microsensor (N_2O-R; Unisense, Aarhus, Denmark) was used for the online measurement of N_2O concentration in water. When measuring N_2O in the off-gas, a 0.81-m^2 floating gas hood was used to collect the gas from the water surface. An extra air inlet was added to the hood to enable gas flow to the off-gas analyzer during periods without aeration. The total air flow was measured using a mass flow meter (model 6441; Testo, Lenzkirch, Germany) and a variable area meter, i.e. a rotameter (model 6441; Brooks Instrument, Hatfield, PA, USA). A dilution system with a maximum dilution of 40 times was used to measure high N_2O concentrations in the off-gas. An ultra-trace nitrous oxide analyzer (model GFC-7002E; Teledyne Analytical Instruments, City of Industry, CA, USA) and a single-beam infrared spectrophotometer (MIRAN 1B; Foxboro Co., Foxboro, MA, USA) were used for the online measurement of N_2O concentration in off-gas.

Experimental plan

Measurements were made from February through May 2012 in three campaigns and in a longer-term study. Each campaign lasted approximately 36 h. Before campaigns 1 and 3, the process in the SBR was disturbed, which resulted in low pH and high NO_2^--N concentrations. Between and after the campaigns, the N_2O emissions in off-gas were measured without performing any chemical analyses, in order to study the longer-term effect of ethanol dosage on N_2O emissions.

Campaign 1

Campaign 1 was performed from February 29 through March 1. This campaign comprised four cycles in which DO was reduced stepwise during the nitrification phases. The DO set points in these successive cycles were 2.0, 1.2, 0.9, and 0.6 mg L^{-1}. The ethanol dosage to the SBR was accidentally interrupted for several days just before the campaign started. This resulted in low pH and high NO_2^--N concentrations, which in turn resulted in high N_2O emissions from the start of the campaign, before the DO was reduced. Therefore, the results from campaign 1 are only sparingly presented.

Campaign 2

Campaign 2 was performed on 20–21 March. This campaign comprised four SBR cycles, each with a different DO set point: cycle 1, 2.0 mg L^{-1} (normal DO set point at the plant); cycle 2, 0.9 mg L^{-1}; cycle 3, 2.0 mg L^{-1} (recovery); and cycle 4, 0.5 mg O_2 L^{-1} (Figure 2). The measurements included the online recording of N_2O levels in water and off-gas for all cycles. Grab samples were taken from the SBR throughout cycles 1, 2, and 4. Some grab samples were also taken at the beginning of cycles 3 and 5 to follow how the process was affected by the reduced DO level in the preceding cycles. Several grab samples were taken and mixed together to form a composite sample of the influent and effluent during cycles 1, 2, and 4 in order to calculate mass flows.

During the campaign, high DO levels were recorded during the anoxic and sedimentation phases. However, these artificially high DO concentrations registered by the sensor were caused by high NO_2^- concentrations that interfered with the DO sensor under anoxic conditions. To eliminate this phenomenon, the blower was routinely started and kept in operation for a few minutes. Though these DO peaks during anoxic phases have been excluded from the presented graphs, the values for N_2O in water and off-gas were nevertheless affected since N_2O in the water is stripped off to the air when the blower is in operation.

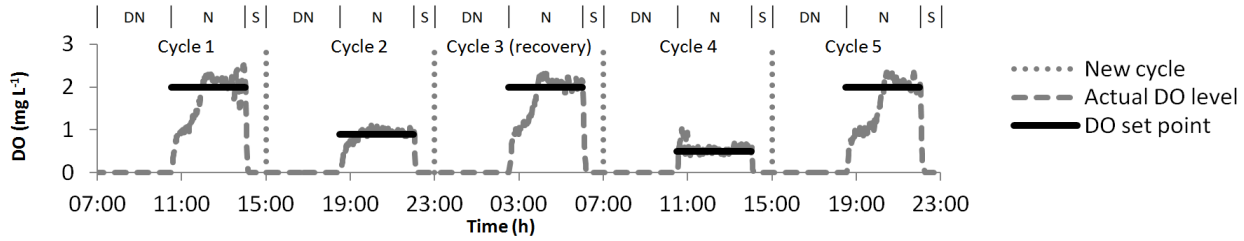


Figure 2. DO set point and actual DO during the campaign (DN = denitrification, N = nitrification, S = sedimentation).

Campaign 3

Campaign 3 was performed on 28–29 March. During the campaign, the ethanol dosage was varied in four successive cycles: 2.0 (normal ethanol dosage at the plant), 1.0, 2.0 (recovery), and 0 L m^{-3}. In addition, the process in the SBR was disturbed by a low influent volume to the SBR some days before the campaign started, which meant a lower ethanol dosage, in turn resulting in low pH and high NO_2^--N concentrations in the SBR.

Results and discussion

SBR operation during the study

Because the process conditions differed considerably before each campaign started, and because each campaign affected the process in a different way, there was great variation in the results and values for different parameters between campaigns (Table 1). Nonetheless, the SBR influent was consistent in quality throughout the campaigns. In some cycles, the amount of N_2O-N exceeded 100% of the TN in the influent, because the TN in the SBR's bulk liquid was also transformed into N_2O. This occurred both in campaign 2 when the DO was reduced and in campaign 3 when the ethanol dosage was reduced.

Table 1. Maximum, minimum, and average values for various SBR parameters in all three campaigns.

	Parameter:	pH	N_2O in off-gas (ppm)	N_2O-N in off-gas (% of TN in influent per cycle)	N_2O in water (µmol L^{-1})	TN (filtr.) (mg L^{-1})	NH_4-N (mg L^{-1})	NO_3-N (mg L^{-1})	NO_2-N (mg L^{-1})	COD (filtr.) (mg L^{-1})
Campaign 1	Max.	8.1	4903	8.9%	846	543	160	188	86	723
	Min.	5.6	1	3.5%	1	280	64	96	1	651
	Average	7.1	420	6.5%	200	431	109	143	45	690
Campaign 2	Max.	8.7	56,441	107.6%	1645	218	69	110	66	482
	Min.	6.2	0	5.1%	2	156	0	41	8	382
	Average	7.2	1608	32.5%	343	184	37	75	34	437
Campaign 3	Max.	8.3	76,219	217.9%	1187	365	123	120	124	701
	Min.	5.5	5	34.3	9	235	22	37	66	607
	Average	6.8	4275	83.5%	285	281	73	76	90	642

N_2O formation and carbon dosage

Between and after the three campaigns, the N_2O in the off-gas was measured without performing any chemical analyses. After the last campaign, the ethanol dosage was stepwise increased as part of a longer-term study to discern whether the N_2O in the off-gas would be affected. The original ethanol dose of 2.0 L m^{-3} digester supernatant in the SBR was increased in steps of 0.5 L m^{-3}, ending at 3.5 L m^{-3}. This corresponds to an approximate increment of COD from dosed ethanol of 2.3 to 4.1 g COD kg^{-1} TN in the influent. The increased ethanol dosage resulted in lower N_2O emissions in the off-gas and a more stable process without separate peak emissions (Figure 3).

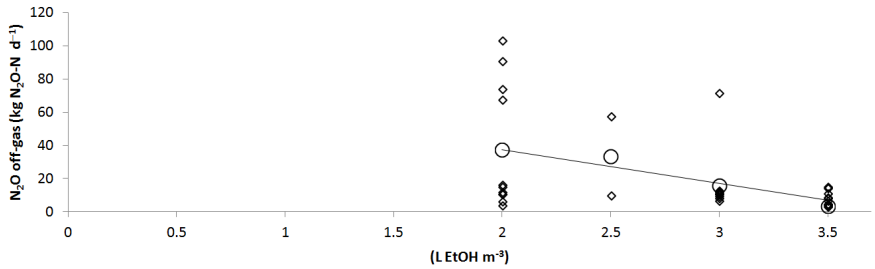


Figure 3. N_2O-N in off-gas from the SBR from 15 March to 2 May 2012, over different dosages of ethanol (diamonds) and with average values for each dosage (circles). Data affected by the modified operation during the campaigns are excluded. Emissions of 10 kg N_2O-N d^{-1} in the off-gas correspond to approximately 3.5% of the TN in SBR influent.

N_2O formation and DO

The immediate effect of decreased DO concentrations was revealed in campaign 2. When the DO concentration was reduced from 2.0 to 0.9 mg L^{-1} in cycles 1 and 2, the formation of N_2O increased in both water and off-gas. The increment of N_2O in water was 65.6%, comparing N_2O formed during the nitrification phases with TN in the influent (Figure 4). The vast majority of N_2O formed in these cycles was produced during the nitrification phases.

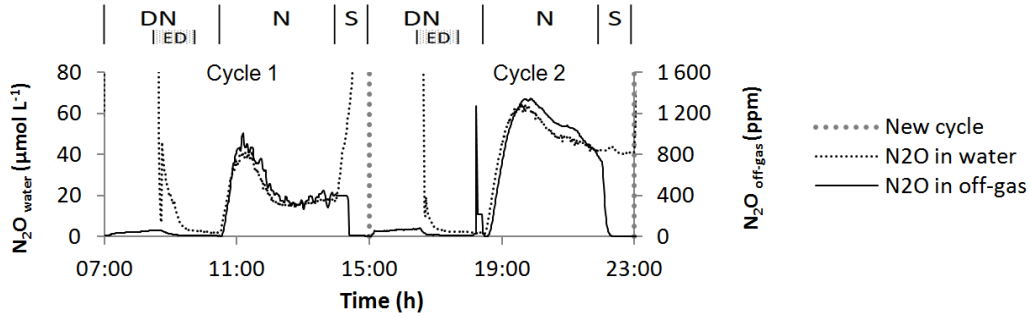


Figure 4. Online measurements of N_2O in water and off-gas during campaign 2, cycle 1 (DO set point, 2.0 mg L^{-1}) and cycle 2 (DO set point, 0.9 mg L^{-1}) (DN = denitrification, N = nitrification, S = sedimentation, ED = ethanol dosage). From Stenström *et al.* (submitted).

In cycles 3–5, much more N_2O was formed in water than in cycles 1 and 2. Nevertheless, the N_2O emissions in off-gas were still low during denitrification, because the formed N_2O accumulated in the water during denitrification and was stripped off when the nitrification started.

In campaign 2, stepwise reduction of the DO concentration revealed an obvious correlation between decreased DO and increased N_2O concentrations in the water (Figure 5). When the DO concentration was below 1.0–1.5 mg L^{-1}, the N_2O concentration in the water increased.

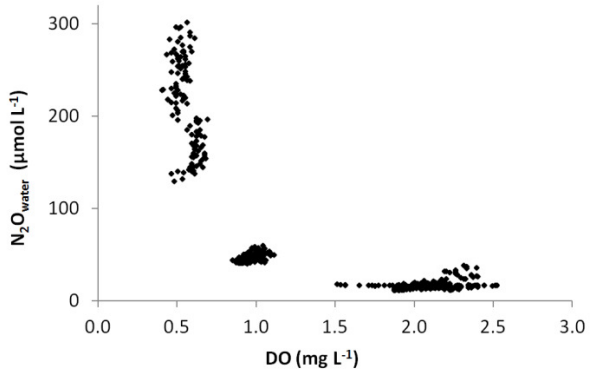


Figure 5. Concentration of N_2O in water over the last 2 h of the nitrification phase versus DO. The N_2O concentration in water increased when the DO concentration was reduced. The graph is based on data from cycles 1, 2, and 4, campaign 2. From Stenström *et al.* (submitted).

N_2O formation and nitrite concentration

When the DO concentration was reduced in campaign 2, the NO_2^- concentration increased and, in turn, the N_2O concentration increased in both water and off-gas (Figure 6). The correlation between simultaneously high concentrations of NO_2^- and N_2O has been observed before in several studies, as reviewed by Kampschreur *et al.* (2009).

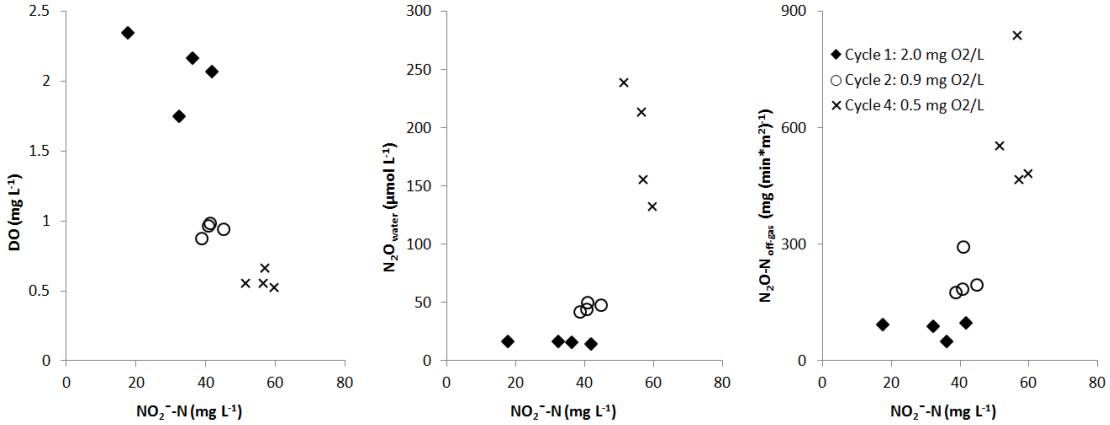
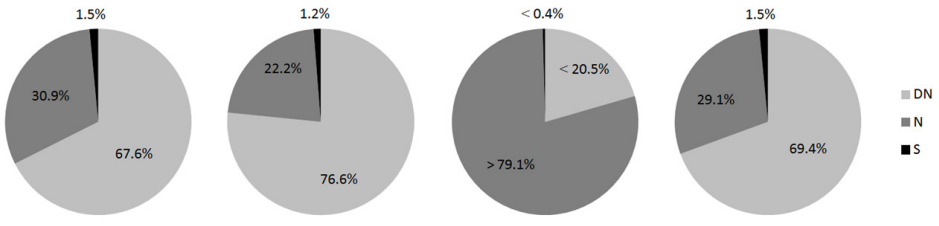


Figure 6. Concentrations of DO and N_2O in water and in off-gas versus NO_2^- over the last 2 h of the nitrification phase of cycles 1, 2, and 4, campaign 2. N_2O in off-gas is expressed in mg min^{-1} m^{-2} of water surface. From Stenström *et al.* (submitted).

Apportionment of formed N_2O between nitrification and denitrification

The process had already been disturbed before campaign 3, in which the ethanol dosage was reduced, leading to high N_2O emissions in general. Nevertheless, the data collected were suitable for calculating the apportionment of N_2O formed between phases (Figure 7). The assumption applied during calculation was that all N_2O in water at the end of the denitrification phase was stripped off to the air during the subsequent nitrification phase. There was massive formation of N_2O in cycle 3, the N_2O in off-gas corresponded to over 217.9% of the TN in the SBR influent. During the nitrification phase of this cycle, the N_2O concentration in off-gas exceeded the calibration range of the instrument.

In three of the four studied cycles, most N_2O in water was formed during the denitrification phase. However, the opposite occurred in cycle 3, when most N_2O in water was formed during nitrification. Examining the mass of N_2O formed during the various phases revealed that the N_2O formation during denitrification in cycle 3 was greater by a factor of 1.3–2.0 than in the other cycles, whereas during nitrification it was greater by a factor of 13–18. Therefore, the vast majority of the increased N_2O formation in cycle 3 occurred during nitrification. The results from cycle 3 should perhaps be considered an extreme outlier, the reason for this very high N_2O formation has not been ascertained.



1	2	3	4	Cycle no
2.0	1.0	2.0	0	Ethanol dosage (L ethanol m^{-3})
106.7	88.9	88.7	86.4	TN in influent (kg TN $cycle^{-1}$)
36.7	40.3	>193.3	29.6	N_2O formation in water (kg N_2O-N $cycle^{-1}$)
36.6	40.5	>193.3	29.8	N_2O emissions in off-gas (kg N_2O-N $cycle^{-1}$)
34.3%	45.6%	>217.9%	34.5%	N_2O emissions in off-gas of TN in influent

Figure 7. Distribution of N_2O-N formed in water during the various cycles and phases of campaign 3 (light grey = denitrification, grey = nitrification, black = sedimentation).

Conclusions

This full-scale study of N_2O formation in water and off-gas from an SBR treating digester supernatant, under both nitrifying and denitrifying conditions and with varied DO levels and ethanol dosages, has demonstrated that:

- Reduced DO concentrations during nitrification (<1.0–1.5 mg L^{-1}) lead to increased NO_2^- concentrations, which in turn enhance N_2O formation in water and emissions in off-gas.
- The importance of a sufficient carbon dosage cannot be overstated, both to achieve as low long-term N_2O emissions as possible and to prevent separate peak emissions.
- Emissions of N_2O in off-gas can be considerable when the operating conditions have been adverse for several cycles. The highest emissions found in this study were 107.6% of TN in influent when the DO concentration was reduced, and 217.9% of TN in influent when the ethanol dosage was reduced (or 21.9% and 36.2%, respectively, of TN in bulk liquid at the beginning of the cycle).
- The immediate effect of a lowered DO level can be distinct. When the DO set point was reduced from 2.0 to 0.9 mg L^{-1}, the N_2O emitted in off-gas during nitrification increased by 65.6%.
- The apportionment of N_2O formed in water between nitrification and denitrification indicated that most N_2O was formed during denitrification in three of four cycles. In the cycle when most N_2O was formed during nitrification, the total share of formed N_2O was massive.
- During the first cycles of the three campaigns, when the operation of the process had not yet been changed from its normal mode, the N_2O emissions in off-gas were 8.9, 5.1, and 34.3% of TN in SBR influent. The emissions were substantial, indicating the importance of how a plant treating digester supernatant is run.
- N_2O formed in water during denitrification accumulates mainly in the water volume until aeration starts, when it is quickly stripped off to the atmosphere.

Acknowledgements

The authors would like to thank the staff of Slottshagen WWTP for being consistently helpful and supportive, Gertrud Persson at Lund University who performed all the chemical analyses, and Kåre Tjus at IVL for making the measurements of N_2O in off-gas. This work was partially funded by the Swedish Water and Wastewater Association (project no. 11-106).

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