

Effect of substrate availability on nitrous oxide production by deammonification processes under anoxic conditions

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Summary

Due to its high global warming potential, nitrous oxide (N₂O) emissions from wastewater treatment processes have recently received a high degree of attention. Nevertheless, there is still a lack of information regarding the microbiological processes leading to N₂O production. In this study, two lab-scale sequencing batch reactors were operated with deammonification biomass to investigate the role of denitrification and the influence of substrate availability regarding N₂O formation during the anoxic phase of deammonification. Three different operational phases were established: within the first phase conversion by anammox was favoured and after a transition phase, denitrification activity was promoted. Low nitrous oxide production was observed during stable operation aiming for anammox conversion. Pulsed inflow of the wastewater containing ammonium (NH₄⁺) and nitrite (NO₂⁻) led to increased N₂O production rates. Within the period of denitrification as dominating nitrogen conversion process, the nitrous oxide concentration level was higher during continuous inflow conditions, but the reaction to pulsed inflow was less pronounced. The results indicated that denitrification was responsible for N₂O formation from the deammonification biomass. Operational settings to achieve suppression of denitrification processes to a large extent were deduced from the results of the experiments.

Introduction

Nitrous oxide is a strong greenhouse gas {global warming potential 300 times higher than of carbon dioxide (CO₂)

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[Intergovernmental Panel on Climate Change (IPCC), 2007]] that has been found to be produced at wastewater treatment plants (WWTP) mainly during biological nitrogen elimination and sludge handling. Recent measurements in lab- and full-scale (Ahn *et al.*, 2010; Foley *et al.*, 2010; van Loosdrecht, 2011) have revealed that nitrous oxide can be produced from conventional nitrification/denitrification plants mainly during events of nitrite accumulation or transient anoxia. However, the percentage of nitrous oxide emissions from innovative nitrogen removal processes such as nitritation/denitritation or deammonification (nitritation/anammox) has been found to be even higher (Kampschreur *et al.*, 2009a; Desloover *et al.*, 2011). This is of special interest as high emissions would be opposed to the primary goal of these processes, which is energy-efficient (and thus CO₂-saving) nitrogen elimination. Due to the limited number of full-scale applications on the one hand and to the difficulty of measuring and quantifying volatile emissions on the other hand, till now, limited reliable data are available concerning nitrous oxide emissions and the responsible biological conversions for innovative nitrogen elimination processes. Concerning deammonification, recent studies on N₂O emissions have focussed on the nitritation step due to the high emission potential caused by permanent stripping during aeration. In contrast, to our knowledge there is no explicit study on the fate of nitrous oxide during the anoxic phase of the deammonification process.

To contribute to this research field, this study deals with the investigation of N₂O formation in deammonification biomass during anoxic conditions focusing on the role of heterotrophic denitrifiers and their interaction with anammox bacteria. Based on results from previous work (Schneider *et al.*, 2011), the following hypotheses were set up to be tested in this study:

- The production of N₂O during the anoxic phase of deammonification is related to incomplete denitrification.
- The formation of N₂O by denitrification depends on substrate availability.

For that purpose, two lab-scale sequencing batch reactors (SBR) were operated under anoxic conditions with deammonification biomass. With the aim to separate the

processes of anammox and denitrification to the largest extent possible, the operation was divided into the following phases:

- (i) Phase 1 – conditions favourable for anammox (organic carbon limitation)
- (ii) Phase 2 – shifting the main nitrogen conversion to denitrification
- (iii) Phase 3 – conditions favourable for denitrification (ammonium limitation, organic carbon supply)

The dynamic behaviour of N₂O formation and depletion was monitored online during phases 1 and 3 with constant or pulsed inflow. The main interest of the study was to observe trends of N₂O production and clarify the possible causes and identify responsible conversion steps rather than actual emissions, which are highly dependent from reactor design, the aeration system (*k_La*-value) and more. Accordingly, the development of N₂O concentrations and net production rates are discussed in this paper and N₂O emissions are only given for comparison with earlier studies.

Results

In order to emphasize the relation of overall nitrogen conversion performance and nitrous oxide formation, the results are described ordered by operational phases. In contrast, results are organized by parameter in the tables and figures for comparison of the different phases. Nitrous oxide was only monitored in reactor 1, while reactor 2 served as a reference reactor for overall nitrogen conversion.

Phase 1 – promoting anammox

Overall nitrogen conversion. In reactor 1, stable mean effluent concentrations of 308.5 mg NH₄⁺-N l⁻¹ and 31.5 mg NO₃⁻-N l⁻¹ were reached within the first week of

reactor operation and maintained throughout phase 1. Nitrite was almost completely eliminated during one cycle resulting in low effluent concentrations below 0.6 mg NO₂⁻-N l⁻¹ and was the limiting factor in all cycles of phase 1. The mean conversion rates for ammonium and nitrite were 20.5 ± 13.7 mg NH₄⁺-N/(g VSS-d) and 8.1 ± 1.6 mg NO₂⁻-N/(g VSS-d) with a mean nitrate production of 4.4 ± 5.3 mg NO₃⁻-N/(g VSS-d). The chemical oxygen demand (COD) was removed at a mean rate of 4.0 ± 4.2 mg O₂/(g VSS-d), accounting for 23% of the total COD load. The mean dissolved oxygen concentration during this phase was 0.12 ± 0.07 mg O₂ l⁻¹ and pH remained almost stable during all cycles with a mean value of 7.44 ± 0.3.

The effluent concentrations of SBR 1 during phase 1 and phase 3 are shown in Fig. 1 and influent and effluent concentrations of both SBR 1 and 2 are compiled in Table 1. This table shows that reactor 2 – used as a reference reactor for overall nitrogen conversion – achieved similar performance as reactor 1, thus confirming the representativeness of obtained results.

Nitrous oxide concentration profiles. For all cycles operated with constant inflow, similar development of the dissolved N₂O concentration was observed: N₂O accumulated in the liquid during the first 16 h of the feed and reaction phase, afterwards a slight decrease of the concentration was observed. N₂O concentration dropped further during the final settling phase, probably due to entrapment of the gas to settled sludge flocs. Most of the gas remained dissolved until the beginning of the following cycle, as indicated by elevated N₂O concentrations at the beginning of every cycle. Figure 2 shows a typical dissolved N₂O concentration profile with an increase from 0.087 mg N₂O-N l⁻¹ up to max. 0.161 mg N₂O-N l⁻¹. Here, the increase of N₂O concentration indicates accumulation due to biological production rates that exceed N₂O emission (no aeration leading to low gas transfer rates).

Table 1. Inflow and effluent nitrogen concentrations in both reactors during phases 1 and 3 (mean values, standard deviation in parentheses).

Phase	Parameter	Inflow conc. [mg N l ⁻¹]	Effluent conc. [mg N l ⁻¹]	
			Reactor 1	Reactor 2
1	NH ₄ ⁺ -N	476.1 (±77.8)	308.5 (±70.7)	299.7 (±61.0)
	NO ₂ ⁻ -N	60.2 (±5.4)	0.27 (±0.14)	0.32 (±0.18)
	NO ₃ ⁻ -N	157.8 (13.7) ^a n.d.	31.5 (±6.0)/ 56.7 (±4.4) ^a	27.2 (±6.4) 43.6 (±14.3) ^a
3	NH ₄ ⁺ -N	20.6 (±3.1)	0.54 (±0.3)	2.0 (±4.5)
	NO ₂ ⁻ -N	63.4 (±11.4)	0.31 (±0.26)	0.14 (±0.17)
	NO ₃ ⁻ -N	159.0 (±12.7) ^a n.d.	66.0 (±8.8) 135.9 (±42.0) ^a	69.9 (±19.3) 124.67 (±17.6) ^a

a. Pulsed inflow.

n.d., below detection limit (0.13 mg NO₃-N l⁻¹).

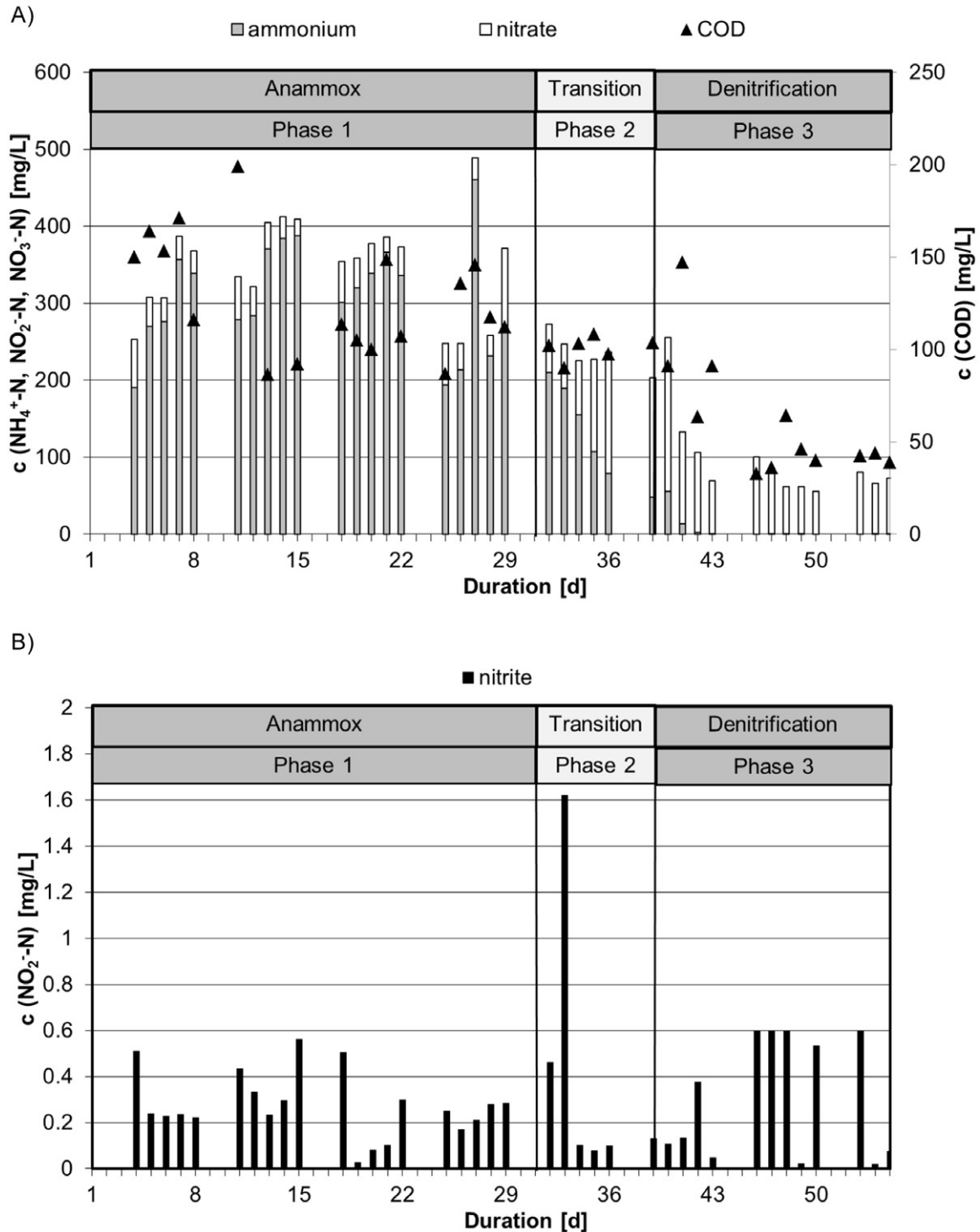


Fig. 1. Inflow and effluent nitrogen concentrations in reactor 1 during phases 1 and 3 (ammonium, nitrate and COD in Fig. 1A, nitrite in Fig. 1B).

Repeated cycles with application of pulsed inflow showed significantly different N_2O concentration profiles compared with constant inflow as shown in Fig. 3. Throughout the cycle, the N_2O concentration increased up to 120% of the initial concentration (e.g. initial: 0.139 mg

$\text{N}_2\text{O-N l}^{-1}$, max. 0.168 mg $\text{N}_2\text{O-N l}^{-1}$) and dropped back to this initial level about 3 h after the last feeding period. To be precise, the intermittent feeding pattern resulted in likewise intermittently increasing and decreasing nitrous oxide concentrations. The concentration profile of each

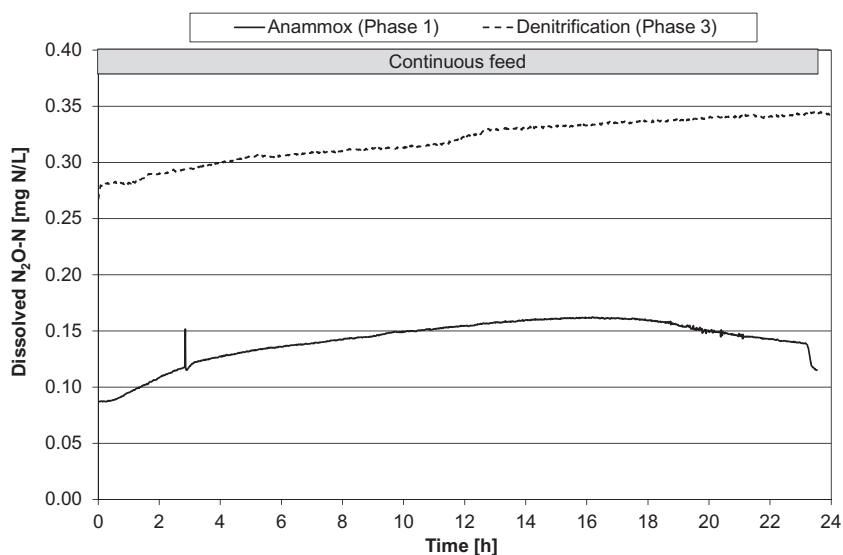


Fig. 2. Typical concentration profile of dissolved N_2O during phases 1 and 3 (continuous inflow).

4 h period, covering 1 h feeding phase and 3 h reaction phase without feeding, resembled the concentration profile during complete cycles with constant inflow.

Nitrous oxide production rates. For operation with continuous inflow, the mean N_2O production rate (increase during 16 h) as a mean value from all evaluated cycles was $0.03 \text{ mg } N_2O\text{-N}/(\text{g VSS}\cdot\text{d})$. The use of pulsed inflow resulted in significantly higher N_2O production rates. The mean N_2O production rate increased to $0.32 \text{ mg } N_2O\text{-N}/(\text{g VSS}\cdot\text{d})$ but was followed by significant N_2O depletion (emission and biological conversion) at a mean rate of $0.028 \text{ mg } N_2O\text{-N}/(\text{g VSS}\cdot\text{d})$. Mean production rates are depicted in Fig. 4. The average calculated N_2O emission

per cycle in phase 1 was 0.13% of the total nitrogen load during operation with constant inflow and 4.6% during pulsed inflow (Fig. 5).

Phase 2 – transition

During phase 2, the nitrite load of $80 \text{ mg } NO_2\text{-N day}^{-1}$ was completely eliminated during one cycle, resulting in decreasing ammonium and increasing nitrate concentrations. At the end of phase 2, reactor 1 contained an ammonium concentration of $56 \text{ mg } NH_4\text{-N l}^{-1}$ and a nitrate concentration of $200 \text{ mg } NO_3\text{-N l}^{-1}$. The development of nitrous oxide was not evaluated during this phase.

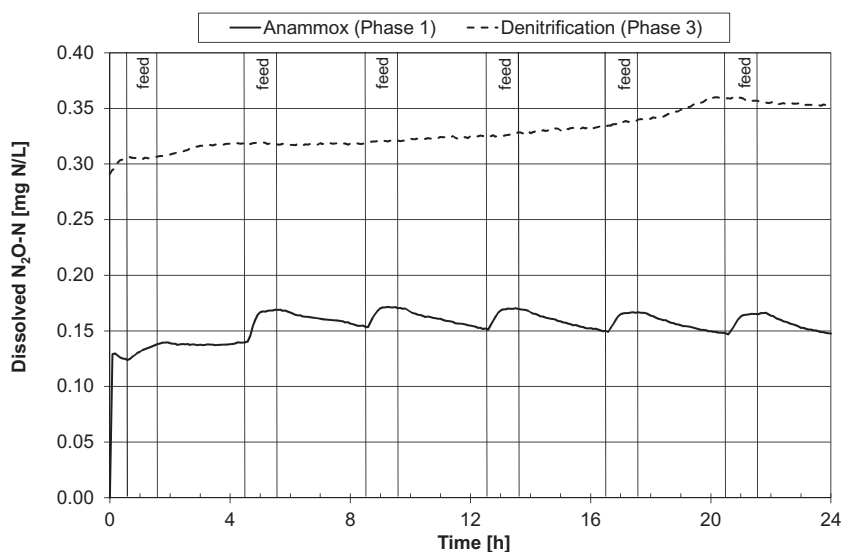


Fig. 3. Typical concentration profile of dissolved N_2O during phases 1 and 3 (pulsed inflow).

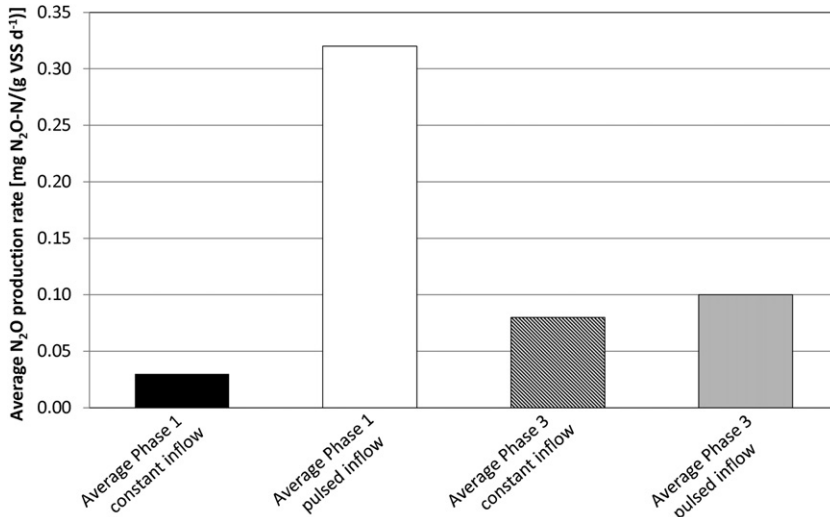


Fig. 4. Calculated average N₂O production rates for phases 1 and 3 for continuous and pulsed inflow.

Phase 3 – promoting denitrification

During phase 3 (switch to primary effluent as inflow) ammonium concentrations at the end of the cycles dropped quickly to a mean effluent concentration of 0.54 ± 0.3 mg NH₄⁺-N l⁻¹. The nitrite load contained in the inflow (60 mg NO₂⁻-N day⁻¹) remained to be removed within one cycle and nitrite remained the performance limiting compound. The mean COD removal rate during this phase was 27 ± 18.1 mg O₂/(g VSS·d). Despite this removal performance, strikingly high nitrate concentrations were accumulated in the reactor and the nitrogen balance showed that nitrate was produced in significant amounts [12.6 ± 9.5 mg NO₃⁻-N/(g VSS·d)] compared with nitrite and ammonium elimination [10.7 ± 4.8 mg N/(g VSS·d)]. During phase 3, the mean dissolved oxygen concentration was 0.16 ± 0.08 mg O₂ l⁻¹ and pH remained almost stable during all cycles with a mean value of 6.99 ± 0.64 .

The effluent concentrations of SBR 1 during phase 1 and phase 3 are shown in Fig. 1 and influent and effluent concentrations of both SBR 1 and 2 are compiled in Table 1. This table shows that reactor 2 – used as a reference reactor for overall nitrogen conversion – again achieved similar performance as reactor 1, confirming the representativeness of results.

Nitrous oxide concentration profiles. The dissolved N₂O concentration increased constantly until the beginning of the settling phase during cycles with constant inflow. During the exemplary cycle depicted in Fig. 2, N₂O concentration increased from initially 0.266 mg N₂O-N l⁻¹ to 0.344 mg N₂O-N l⁻¹. In comparison with the N₂O concentration during phase 1, a higher concentration level (three-fold) was reached due to accumulation of N₂O, as it was not depleted during the reaction nor the settling phase of the cycles. During operation with pulsed inflow,

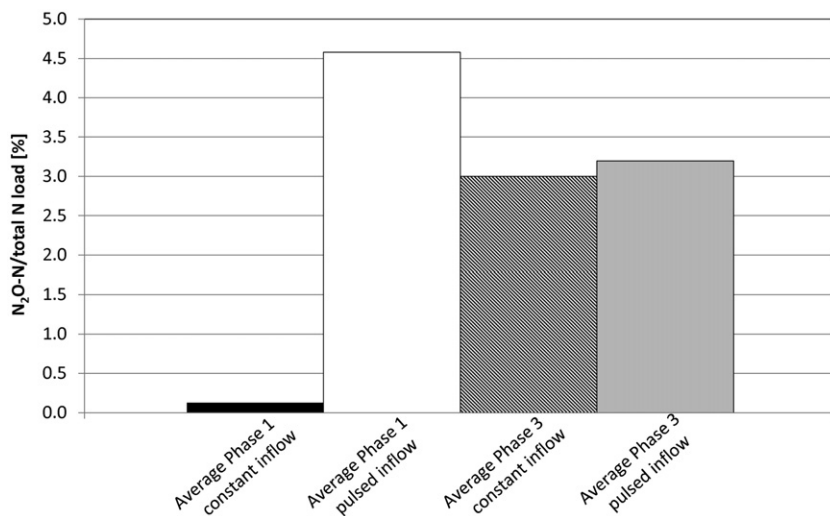


Fig. 5. Calculated N₂O emission expressed as fraction of the total nitrogen load for phases 1 and 3 and for continuous and pulsed inflow.

the N₂O concentration profile did not follow the inflow pattern, but showed a continuous increase up to 123% of the initial concentration (initial 0.291 mg N₂O-N l⁻¹, max. 0.358 mg N₂O-N l⁻¹) instead (Fig. 3).

Nitrous oxide production rates. The mean N₂O production rate during continuous inflow was 0.08 mg N₂O-N/(g VSS·d) and increased during periods with pulsed inflow up to 0.10 mg N₂O-N/(g VSS·d) (Fig. 4). The average calculated emission from reactor 1 was 3.0% of the total N load for constant inflow conditions and 3.2% during pulsed inflow (Fig. 5).

Discussion

Potential sources of N₂O

Generally, several bacteria groups that are present in deammonification biomass used here have been related to N₂O production under anoxic conditions in previous studies.

For denitrifying bacteria, the most important influencing factors for N₂O production are reported to be: limitation of organic carbon source (Chung and Chung, 2000), high nitrite concentrations (Alinsafi *et al.*, 2008), presence of oxygen (Anderson and Levine, 1986) and peak loads of readily degradable substrate like organic acids (Wicht and Beier, 1995).

For reactors operated with enrichments of anammox bacteria, N₂O emissions have been detected in both full- and lab-scale measurements (Kartal *et al.*, 2007; Kampschreur *et al.*, 2008; 2009a; Weissenbacher *et al.*, 2010). As no pure culture of anammox is available, the actual origin of N₂O in these processes might be other than anammox bacteria. Recent investigations of intermediates in the anammox pathway and the genome of anammox bacteria imply that N₂O is not part of the anammox metabolism (Jetten *et al.*, 2009; Kartal *et al.*, 2010). Kartal and colleagues (2010) suggested that chemodenitrification from NO could be a source for N₂O emissions.

Several studies have been published on the emission of nitrous oxide by aerobic ammonium oxidisers, including investigations of influencing factors and metabolic pathways (Yu *et al.*, 2010; Ahn *et al.*, 2011). Basically, nitrous oxide formation is supposed to result from autotrophic denitrification that is used as a detoxification process in case of high nitrite concentrations and low oxygen availability. In that case, oxidation of ammonium could be the electron source for reduction of NO₂⁻ to NO and finally N₂O. This explanation is in accordance with the results from studies on enzyme expression by AOB during unfavourable boundary conditions and implies that AOB do not produce N₂O under strictly anoxic conditions.

Attribution of N₂O production to denitrification (Hypothesis A)

During phase 1, nitrite and readily degradable COD were found to be the limiting factors for nitrogen conversion. Based on ammonium and COD elimination, the main nitrogen removal could be attributed to the anammox process (about 89%).

$$\text{NO}_2^- \text{-N}_{\text{amx}} = \text{NO}_2^- \text{-N}_{\text{total}} - \text{COD}_{\text{elim}} \times \text{COD}/\text{NO}_2^- \text{-N}_{\text{deni}}$$

NO₂⁻-N_{amx} – the nitrite potentially removed by anammox [mg day⁻¹]

NO₂⁻-N_{total} – total nitrite load [mg day⁻¹]

COD_{elim} – amount of COD removed [mg day⁻¹]

COD/NO₂⁻-N_{deni} – COD demand for denitrification of nitrite (=3) [mg mg⁻¹].

In comparison with the generally accepted stoichiometry of anammox conversion (NH₄⁺-N/NO₂⁻-N: 1/1.32) determined in earlier studies (Strous *et al.*, 1998), the ratio of eliminated NH₄⁺-N/NO₂⁻-N observed in our experiments was much higher (2.38/1). As the high rate of ammonium removal could not be explained by free ammonia stripping, it might have to be attributed to aerobic ammonium oxidation to nitrite before subsequent anammox conversion. Aerobic ammonium oxidation could have been promoted by O₂ intrusion during mixing and by dissolved O₂ in the influent, with fast oxygen uptake rates resulting in constant O₂ concentrations of 0.12 mg O₂ l⁻¹ in the reactor. The additional amount of eliminated ammonia was calculated as

$$\Delta \text{NH}_4^+ \text{-N} = \text{NH}_4^+ \text{-N}_{\text{elim}} - \text{NH}_4^+ \text{-N}_{\text{amx}} = \text{NH}_4^+ \text{-N}_{\text{elim}} - \text{NO}_2^- \text{-N}_{\text{amx}} \times \text{NH}_4^+ \text{-N}/\text{NO}_2^- \text{-N}_{\text{stoich}}$$

ΔNH₄⁺-N – additional amount of NH₄⁺-N removed [mg day⁻¹]

NH₄⁺-N_{elim} – total amount of NH₄⁺-N removed [mg day⁻¹]

NH₄⁺-N_{amx} – amount of NH₄⁺-N removed removed by anammox [mg day⁻¹]

NO₂⁻-N_{amx} – amount of NO₂⁻-N removed by anammox [mg day⁻¹]

NH₄⁺-N/NO₂⁻-N_{stoich} – ratio of NH₄⁺-N to NO₂⁻-N according to anammox stoichiometry (1:1.32)

Assuming simultaneous aerobic and anaerobic (anammox) ammonium oxidation with complete removal of ΔNH₄⁺-N gives the amount of additional ammonium converted directly by anammox:

$$\Delta \text{NH}_4^+ \text{-N}_{\text{amx}} = \Delta \text{NH}_4^+ \text{-N} - \Delta \text{NH}_4^+ \text{-N} \times (\text{NO}_2^- \text{-N}/\text{NH}_4^+ \text{-N}_{\text{stoich}})/\text{n(N)}_{\text{amx}}$$

$\text{NO}_2^- \text{-N} / \text{NH}_4^+ \text{-N}_{\text{stoich}}$ – ratio of $\text{NO}_2^- \text{-N}$ to $\text{NH}_4^+ \text{-N}$ according to anammox stoichiometry (1.32:1)

$n(\text{N})_{\text{amx}}$ – amount of N-atoms needed for the anammox reaction (2.32)

Furthermore, comparing $\text{NO}_3^- \text{-N}$ produced to $(\text{NH}_4^+ \text{-N}_{\text{amx}} + \Delta \text{NH}_4^+ \text{-N}_{\text{amx}})$ eliminated gives a ratio of 0.2, which is in the range of typical values for the anammox process. This supports the assumption that the main removal of ammonium and nitrite was due anammox (and partial nitrification), while heterotrophic denitrification activity was limited by the availability of degradable carbon sources. To completely prevent denitrification activity, synthetic nutrient media without organic carbon sources could have been used, but for this study different types of real wastewater were intentionally used to mimic the boundary conditions at full-scale plants. Especially reject water as used in this study can be considered the most common type of water treated with deammonification processes at the moment.

The N_2O production determined in the first operational phase with emphasis on anammox conversion was in the range of previously reported results from lab- and full-scale studies of anammox reactors. During operation of a membrane bioreactor with free anammox cells (enrichment of 90% *Kuenenia stuttgartiensis*) less than 0.01% of the nitrogen converted was emitted as N_2O (van der Star *et al.*, 2008). Kartal *et al.* (2007) found that only 0.26% of labelled nitrate was bonded in N_2O by a physically purified *K. stuttgartiensis* culture with 80–90% enrichment. In contrast the N_2O emissions from a full-scale anammox reactor turned out to be 0.6% of the nitrogen load (Kampschreur *et al.*, 2008). The average emissions of 0.13% of the total nitrogen load found in this study are at the lower margin of the emissions range from earlier studies.

During phase 3, on average 30% of the nitrite load was removed by the anammox process, while denitrification accounted for depletion of the remaining nitrite. Nitrite remained to be the limiting factor for overall nitrogen conversion, while ammonium availability limited the contribution of anammox (and partial nitrification) to the overall nitrogen removal. Consequently, the high nitrate concentrations could not solely be formed as a by-product of the anammox process. The mechanism of this nitrate production could not be discovered during this study. However, increased nitrate production by anammox enriched biomass has also been observed during operation of an anoxic lab-scale SBR reactor succeeding a nitrification stage (N. Trautmann, pers. comm.). Despite this phenomenon, the main nitrate conversion during phase 3 can be attributed to denitrification activity. In comparison with phase 1, N_2O production during phase 3 was significantly higher. All in all, these observations support the hypoth-

esis that N_2O formation in the biomass used here can be attributed to incomplete denitrification.

Influence of substrate availability on N_2O production by denitrification (Hypothesis B)

N_2O emissions during the denitrification phase have to be compared with experiments with activated sludge rather than anammox biomass. Alinsafi and colleagues (2008) reported N_2O emissions of 4.5% of the total nitrogen load from denitrification with low COD/ $\text{NO}_3^- \text{-N}$ -ratio (= 3), while only 2.4% and 0.9% were emitted at COD/ $\text{NO}_3^- \text{-N}$ -ratios of 5 and 7 respectively. N_2O emissions were explained by inhibition of N_2O reductase due to nitrite accumulation related to COD limitation. Emissions increased immediately after pulse additions of nitrite, but the average emissions over the whole test duration of 10 h were only significantly higher when peak concentrations of nitrite > 20 mg $\text{NO}_2^- \text{-N l}^{-1}$ were applied, which was interpreted as an inhibitory effect of nitrite. Further reports from lab-scale experiments that were collected by Kampschreur and colleagues (2009b) reflect a range of 0–8% of the nitrogen load being emitted as N_2O in anoxic reactors with activated sludge. The main factors for elevated N_2O emissions that were proposed include low COD/N ratio and increased nitrite concentrations. Thus, the N_2O emissions observed in this study during the denitrification phase match the results of earlier studies. However, the factors mentioned so far do not explain the increase of N_2O emissions between phase 1 and phase 3 of this study, as no nitrite accumulation was observed and the COD/N ratio was increased and not limited.

Li and colleagues (2008) reported a positive correlation between N_2O emissions and the supply of organic carbon sources. In their experiments with addition of sodium acetate, glucose and sucrose, significant amounts of N_2O were produced from NO_3^- by activated sludge. Concurrent with other researchers, they attributed N_2O formation to nitrite accumulation causing inhibition of N_2O reductase and they reasoned that the addition of easily accessible carbon source caused an imbalance of the subsequent steps of denitrification. Their interpretation included increased nitrate reduction rates after addition of carbon sources, followed by nitrite accumulation and inhibition of N_2O , which is in general the rate limiting step of denitrification.

The results of the study presented here strongly suggest that nitrous oxide production from deammonification biomass has to be attributed to the denitrification activity. During phase 1 denitrification was limited by organic substrate availability and anammox bacteria were probably favoured over denitrifiers by the low nitrite concentrations due to their strong nitrite affinity (Trimmer *et al.*, 2005). During stable inflow conditions, very low N_2O

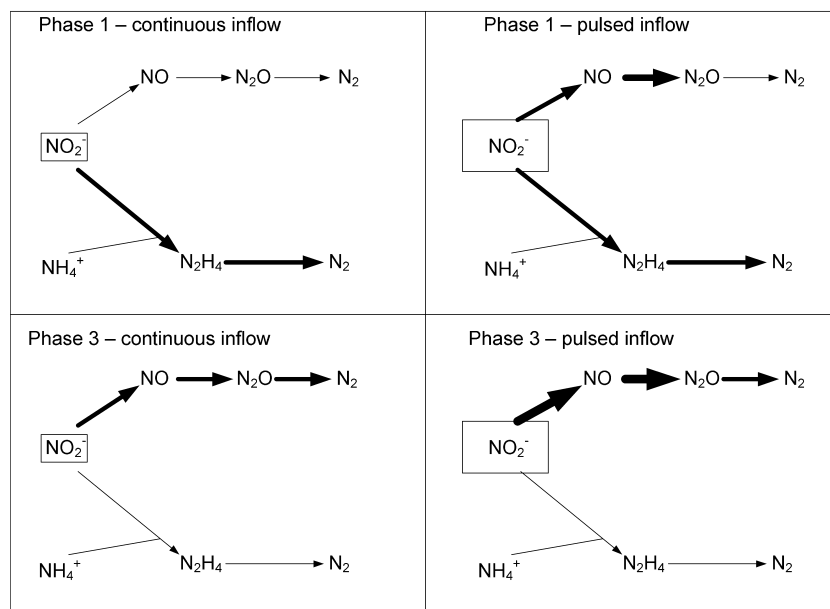


Fig. 6. Theoretical mechanism of N_2O accumulation in SBR experiments. The width of the arrows indicates activity of the particular reductase enzymes, the size of the square encircling NO_2^- indicates the NO_2^- availability, which increases temporarily during pulsed inflow.

emissions were observed. Nitrite did not accumulate during operation with continuous inflow. However, discontinuous nitrite supply during operation with pulsed inflow caused short-term peaks of N_2O production rates and emissions. During phase 3 with denitrification being the dominating nitrogen conversion process, the nitrous oxide production rate was higher throughout the operation, but more stable during varying inflow conditions. These effects might originate from the interaction of enzymes during the course of denitrification. During phase 1 with permanently low nitrite concentration and low nitrite reduction activity, subsequent NO and N_2O reduction activity is also kept at a low level. Short-term peaks of nitrite concentration can lead to a rapid increase in nitrite reductase activity, while especially the activation of nitrous oxide reductase will take hours or days longer. This effect has been described by Betlach and Tiedje (1981), who clarified kinetics of nitrite, NO and N_2O accumulation during denitrification, and is in line with the effect reported by Li and colleagues (2008) as discussed before. According to their results (Betlach and Tiedje, 1981), accumulation of intermediates originates from an imbalance of the diverse reduction rates. In our experiments, these explanations are supported by subsequent stabilization of the N_2O production rate and even N_2O consumption during idle periods. In contrast, higher substrate availability of nitrite and organic substrates during phase 3 might have promoted higher activity of all enzymes included in the denitrification pathway, which was probably maintained during the short phases without inflow during the weekends. Thus, pulsed nitrite addition did not lead to accumulation of N_2O . This theoretical pathway is depicted in Fig. 6.

As nitrifiers were obviously active during phase 1 of our experiments, N_2O formation from autotrophic denitrification cannot be completely excluded. But following the proposed N_2O production pathway stated above, emissions should have been higher during phase 1 with measurable ammonium conversion than in phase 3. This was not the case, thus autotrophic denitrification is not considered to be a significant pathway in this study.

Conclusions

Two SBR were operated with deammonification biomass under anoxic conditions to investigate the causes and responsible microbial conversion steps of nitrous oxide formation. By application of either carbon or ammonium limited inflow, anammox or denitrification dominated nitrogen elimination was established. N_2O concentration was monitored during operation with constant or pulsed inflow and N_2O production occurred in all SBR cycles but to variable extent. The results support hypothesis (A) that N_2O during the anoxic phase of deammonification is related to incomplete heterotrophic denitrification rather to activity of aerobic ammonia oxidizing bacteria or anammox bacteria. As stated in hypothesis (B), the availability of nitrite and organic carbon sources controlled the activity of denitrification enzymes and thus had a direct influence on the formation of N_2O via denitrification.

Consequently, N_2O production can be restricted by limitation of incomplete denitrification processes. In applications of deammonification for reject water treatment, organic carbon sources are generally limited and as long as overload is avoided, anammox bacteria can successfully compete for the nitrite. Thus, both substrates for

Table 2. Experimental settings and nitrogen loads during the different experimental phases.

Phase	Days	Nitrogen load	Biodegradable organic COD load
1	1–31	750 mg NH ₄ ⁺ -N day ⁻¹ (weekday) 80 mg NO ₂ ⁻ -N day ⁻¹	30 mg COD day ⁻¹ (weekday)
2	32–38	80 mg NO ₂ ⁻ -N day ⁻¹	No external COD source
3	39–66	38 mg NH ₄ ⁺ -N day ⁻¹ (weekday) 80 mg NO ₂ ⁻ -N day ⁻¹	450 mg COD day ⁻¹ (weekday)

denitrification and N₂O production become limiting and the potential for N₂O emissions from deammonification is significantly lower than from conventional denitrification.

Experimental procedures

Description of SBR

Two SBR with a working volume of 3.0 l were operated in parallel under anoxic conditions for a period of 8 weeks. The acrylic glass reactors were equipped with magnetic stirring and closed gastight. The operating temperature was maintained between 25–26°C. Both reactors were inoculated with 1.5 l of sludge from a single-stage full-scale deammonification plant, which included nitrifiers (7%), anammox bacteria (19%) and heterotrophic bacteria (not quantified) as determined by FISH analyses and full-scale conversion rates (based on theoretical anammox stoichiometry). At the beginning of reactor operation, the biomass content of the SBRs was 3 g VSS l⁻¹ (at 100% filling). As the inflow to the reactors, two types of municipal wastewater, supplemented with NaNO₂, were used as specified below.

Operational settings

In order to investigate the effect of substrate peak loads, two different inflow patterns were applied: continuous inflow and pulsed inflow. To reduce the effects of general changes of conversion performance, these inflow patterns were used alternately with constant inflow for 5 days and pulsed inflow for 3 days. For operation with constant inflow, the reactors were operated in 24 h batch cycles with a feeding and reaction phase with mixing of 23.5 h, followed by a settling (0.25 h) and a decanting phase (0.25 h). To avoid temporary nitrogen overload of the reactor during pulsed inflow, the cycle duration was prolonged to 72 h. This resulted in a feeding and reaction phase of 71.5 h consisting of 18 alternating feeding (1 h) and idle phases (3 h), followed by a settling (0.25 h) and a decanting phase (0.25 h).

One point five litres of wastewater was pumped to the reactors during the feeding period using membrane dosing pumps (MAGNADOS, Fa. Jesco) at a flow rate of 1.4 ml min⁻¹. With these operational settings, the HRT was 2 day/6 day for operation with constant inflow and with pulsed inflow respectively. During the prolonged cycles, the ammonium and COD load to each reactor was reduced to 1/3 of short cycles. The incoming nitrite load was constant over the whole period of operation.

As stated in the introduction, reactor operation was divided into three main operational phases with the following objectives:

- Phase 1 – conditions favourable for anammox
- Phase 2 – shifting the main nitrogen conversion to denitrification
- Phase 3 – conditions favourable for denitrification

During phase 1, diluted reject water from WWTP Hannover-Guemmerwald containing high ammonium concentrations but low biodegradable COD content (BSB₅/COD = 8%) was used as substrate. In contrast, primary effluent (pre-settled water) from WWTP Hannover-Herrenhausen with low ammonium and high biodegradable COD content (BSB₅/COD = 89%) was fed to the reactors during phase 3. Both types of wastewater were supplemented with NaNO₂ as substrate for both, anammox and denitrification. During phase 2 only NaNO₂ stock solution was fed to the reactor to remove residual ammonium. The resulting loads during the different operational phases are summarized in Table 2.

Analyses and measurements

Dissolved inorganic nitrogen compounds (NH₄⁺-N, NO₂⁻-N, NO₃⁻-N) and filtered COD were analysed on a daily basis using cuvette tests (Hach-Lange, Duesseldorf). Temperature, pH and O₂ were monitored online in both reactors using a multi-parameter measurement system (KM2000, Sensortechnik Meinsberg) equipped with two pH and two O₂ electrodes (EGA 1731/MF 39 SMEK, Sensortechnik Meinsberg). Dissolved N₂O concentration in the liquid phase of reactor 1 was monitored online using a Clark type microsensor (N₂O100, Unisense AS) at a sampling rate of 1 min. TSS and VSS were determined according to standard methods (DIN EN 12880 and 12879 respectively).

Calculation of nitrous oxide production and emission rates

Transfer of N₂O from the liquid phase to the reactor headspace (i.e. N₂O emissions) was calculated according to Henry's law from the concentration in the liquid phase, temperature corrected Henry's constant for N₂O in water (6.27) and reactor specific k_La value (2.64 per day) for each time step (1 min). The net biological N₂O turnover rate r_{N_2O} was calculated as the change of dissolved N₂O concentration for each time step and corrected for dilution by inflow and the N₂O emission rate. Because the production rates are highly biomass specific and emission rates depend on the reactor configuration, these were only used for comparison with results from other studies and comparison of the different experimental phases within this study. They should not be generalized nor extrapolated to full-scale systems.

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