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Title

**Nitrous oxide production in intermittently aerated Partial Nitrification-Anammox reactor: oxie N<sub>2</sub>O production dominates and relates with ammonia removal rate.**

### Authors

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### Abstract

Emissions of the greenhouse gas nitrous oxide from the Partial Nitrification-Anammox process are of concern and can determine the carbon footprint of the process. In order to reduce nitrous oxide emissions intermittent aeration regimes have been shown to be a promising mode of operation, possibly due to an effective control of accumulation of nitrogen intermediates. However, due to frequent changes of redox conditions under intermittent aeration regimes, nitrous oxide production and emissions are dynamic. In this study the production and emission dynamics of nitrous oxide in an intermittently aerated sequencing batch reactor were monitored in high temporal resolution, the contribution of different redox conditions to overall nitrous oxide production was quantified and the most relevant factors for nitrous oxide production were identified. The average fraction of nitrous oxide produced (per unit ammonium removed) was  $1.1 \pm 0.5\%$ . Cycle-averaged approx. 80% of nitrous oxide was produced during aerated phases, the remaining 20% were produced during non-aerated phases. Yet, the intra-cycle dynamics of nitrous oxide were substantial. The net-production rate of nitrous oxide during aerated phases correlated with the ammonia removal rate, whereas the concentration of nitrite determined the production during non-aerated phases. While aerated phases contributed predominantly at the beginning of reactor cycles, non-aerated phases became the dominant source of nitrous oxide at the end. Particularly low net-production rates were observed at ammonia removal rates below  $5 \text{ mg NH}_3\text{-N} \cdot \text{gVSS}^{-1} \cdot \text{L}^{-1}$ , when the fraction of nitrous oxide produced was  $0.011 \pm 0.004\%$  (per ammonia removed). Based on the nitrous oxide dynamics and correlations, reactor operation at relatively low nitrogen loadings (below  $100 \text{ mg NH}_4^+\text{-N} \cdot \text{L}^{-1}$ ), ammonia removal rates of approx.  $5 \text{ mg NH}_3\text{-N} \cdot \text{gVSS}^{-1} \cdot \text{L}^{-1}$  and nitrite concentrations below  $1 \text{ mg NO}_2^-\text{-N} \cdot \text{L}^{-1}$  appears as beneficial for low emission of nitrous oxide.

### Keywords

aerobic granules, nitrogen removal, biofilm, anammox, nitrous oxide

### 1. Introduction

The removal of ammonium ( $\text{NH}_4^+$ ) from municipal wastewater streams is essential to protect receiving water bodies from eutrophication. One process to accomplish this task is the Partial Nitrification-Anammox (PNA) process [1]. To date, most PNA processes are applied in the treatment of  $\text{NH}_4^+$ -rich water streams at mesophilic temperatures (sidestream). Essentially, the PNA process consists of two biological conversion steps, a) Partial Nitrification (PN) and b) anaerobic ammonium oxidation (anammox). During PN  $\text{NH}_4^+$  is converted aerobically to nitrite ( $\text{NO}_2^-$ ) by ammonia oxidizing bacteria (AOB). During anammox  $\text{NH}_4^+$  is, together with  $\text{NO}_2^-$ , converted anaerobically to the desired product dinitrogen gas ( $\text{N}_2$ ) by anammox bacteria (AnAOB) [2]. PNA can be obtained via single-stage (one reactor) or two-stage (two reactors) operation. The majority (88% in 2014) of full-scale applications are single-stage systems in which AOB and AnAOB co-exist in bio-granules to metabolize  $\text{NH}_4^+$  to environmentally inert dinitrogen gas ( $\text{N}_2$ ) [1,3]. Both continuous and

intermittent aeration strategies have been implemented in PNA systems to achieve efficient  $\text{NH}_4^+$  removal [3]: While some studies show stable operation under continuous aeration [4], others find increased performance under intermittent aeration regimes and claim benefits of periodic anoxic phases for community metabolism, the suppression of undesired microbial groups (e.g. NOB) and energy savings due to a reduction of overall aeration time [5,6].

Emissions of nitrous oxide ( $\text{N}_2\text{O}$ ) from PNA systems are of concern, as  $\text{N}_2\text{O}$  is an ozone depleting agent and a potent greenhouse gas with a global warming potential ( $\text{GWP}_{100}$ ) of 298 [7]. Due to its large  $\text{GWP}_{100}$ , already low amounts of emitted  $\text{N}_2\text{O}$  can substantially contribute to the carbon footprint of PNA [8]. A better understanding of the biological turnover processes of nitrogen compounds during PNA and relevant parameters for the mitigation of  $\text{N}_2\text{O}$  emissions are needed to improve process sustainability.

Nitrous oxide originates mainly from three biochemical pathways during PNA [2,9–12]: 1) In the hydroxylamine oxidation pathway (HO),  $\text{N}_2\text{O}$  is produced by AOB during the oxidation of hydroxylamine ( $\text{NH}_2\text{OH}$ ), 2) in the nitrifier denitrification (ND) pathway,  $\text{N}_2\text{O}$  is produced by AOB during the reduction of  $\text{NO}_2^-$ , and 3) during anaerobic reduction of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  by heterotrophic denitrification (HD),  $\text{N}_2\text{O}$  is produced as an intermediate before the final reduction to  $\text{N}_2$  [10].  $\text{N}_2\text{O}$  produced during anammox is considered negligible [13].

The contribution of the different pathways to the total  $\text{N}_2\text{O}$  production depends on the activity of different microbial groups and varies with process conditions, e.g. concentration of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ , DO and pH [2,14,15]. The production of  $\text{N}_2\text{O}$  by HO increases at high ammonia removal rates (AOR) and  $\text{NH}_4^+$  concentrations [16], whereas  $\text{N}_2\text{O}$  production by ND is favored at high  $\text{NO}_2^-$  and low DO concentrations [14,17]. The availability of organic carbon is critical for the contribution of HD, and HD can become the dominant  $\text{N}_2\text{O}$  production pathway under stoichiometric sub-optimal carbon loadings [11].

Dynamics of  $\text{N}_2\text{O}$  production in PNA systems have been investigated in intermittently aerated and continuously-fed reactors [4,18] and also in continuously aerated sequencing batch reactors (SBRs) [8,19]. Yet, few studies have investigated the dynamics of  $\text{N}_2\text{O}$  production in intermittently aerated SBRs, which potentially combine the advantages of SBRs, e.g. high volumetric loading rates and low effluent concentrations, with advantages of intermittent aeration regimes, e.g. control of accumulation of N-intermediates and potential reduction of aeration energy [20,21]. In an earlier study,  $\text{N}_2\text{O}$  production was reduced in a PNA SBR at high frequencies of aeration intermittency [20]. The authors speculated that a decline in net- $\text{N}_2\text{O}$  production rates at high aeration frequency was probably caused by lower transient  $\text{NO}_2^-$  accumulation and lower AORs. However, AORs were determined by extant AOB activity assays, which likely overestimated AOB activity.

This study monitored the AORs and  $\text{N}_2\text{O}$  dynamics of an intermittently aerated PNA SBR at a high temporal resolution. The combination of intermittent aeration and continuous stripping experiments together with  $\text{N}_2\text{O}$  micro-sensor measurements and off-gas  $\text{N}_2\text{O}$  analysis on a short time scale allowed an unique quantification of the contribution of different redox conditions to the overall  $\text{N}_2\text{O}$  production and enabled an in depth analysis of the effect of AORs and  $\text{NO}_2^-$  on net- $\text{N}_2\text{O}$  production. To study the contribution of different redox conditions, AORs and  $\text{NO}_2^-$  concentrations we designed a series of experiments a) to identify the main phases of  $\text{N}_2\text{O}$  production during SBR cycles, b) to correlate the  $\text{N}_2\text{O}$  production with process parameters, c) to examine the effect of fluctuating pH as

a driver for N<sub>2</sub>O dynamics and d) to assess the potential contribution of heterotrophic bacteria to N<sub>2</sub>O production.

## 2. Material and Methods

### 2.1. Reactor operation

A 4L lab-scale reactor (Biostat A Plus, Sartorius, Göttingen, DE) was operated as an intermittently aerated sequencing batch reactor. Temperature was controlled at 30°C. The reactor was inoculated with biomass from a carrier based PNA pilot plant (AnoxKaldnes™, Sweden). Biomass was scraped off the carriers before transfer to the reactor. The reactor was fed with synthetic digester liquor medium, which was based on van de Graaf et al. [22] and contained per 1 L deionized water: 1694 mg NH<sub>4</sub>HCO<sub>3</sub> (= 300 mg NH<sub>4</sub>-N/L), 360 mg NaHCO<sub>3</sub> (CO<sub>3</sub><sup>2-</sup>:NH<sub>4</sub><sup>+</sup> = 1.2), 170 mg KH<sub>2</sub>PO<sub>4</sub> (1.25 mM), 750 mg MgSO<sub>4</sub>\*7H<sub>2</sub>O (3.05 mM), 450 mg CaCl<sub>2</sub>\*2H<sub>2</sub>O (3.07 mM), 10 mg FeSO<sub>4</sub>\*7H<sub>2</sub>O (36 μM), 10 mg EDTA (34 μM), 4 mg EDTA-Na<sub>2</sub> (11 μM), 0.43 mg ZnSO<sub>4</sub>\*7H<sub>2</sub>O (1.5 μM), 0.24 mg CoCl<sub>2</sub>\*6H<sub>2</sub>O (1 μM), 1 mg MnCl<sub>2</sub>\*4H<sub>2</sub>O (5 μM), 0.25 mg CuSO<sub>4</sub>\*5H<sub>2</sub>O (1 μM), 0.24 mg NaMoO<sub>4</sub>\*2H<sub>2</sub>O (1 μM), 0.19 mg NiCl<sub>2</sub>\*6H<sub>2</sub>O (0.80 μM), 0.2 mg NaSeO<sub>4</sub>\*10H<sub>2</sub>O (0.57 μM). The daily reactor load was 900 mg NH<sub>4</sub>-N L<sup>-1</sup> d<sup>-1</sup>. One SBR cycle included a feeding phase (t<sub>feed</sub>), reaction phase (t<sub>react</sub>), settling phase (t<sub>settle</sub>) and effluent phase (t<sub>effluent</sub>) (nomenclature adapted from [23]). For experiments 1 and 3, a long cycle configuration was applied (Table 1). For experiment 2, a short cycle configuration was applied. The short cycle mimicked the last third of the long cycle operation. During t<sub>react</sub> aeration was switched on and off 15 times (f<sub>redox</sub>=15) with each aerated and non-aerated phase lasting for 15 minutes. For the short cycle configuration, f<sub>redox</sub> was 5. The length of aerated/non-aerated phases was fixed and guaranteed a full depletion of NO<sub>2</sub><sup>-</sup> during non-aerated phases to prevent accumulation of NO<sub>2</sub><sup>-</sup> over cycle time. Air was supplied at Q<sub>air</sub>= 2.0 L min<sup>-1</sup> during aerated phases through a diffuser at the bottom of the reactor. Stirring took place with 80 rpm during t<sub>feed</sub> and t<sub>react</sub> and was stopped during t<sub>settle</sub> and t<sub>effluent</sub>.

**Table 1 - Key parameters of long and short cycle operation of SBR.**

		configuration	
		long cycle	short cycle
phase	$t_{\text{cycle}}$ [min]	480	160
	$t_{\text{feed}}$	10	2
	$t_{\text{react}}$	450	150
	$t_{\text{settle}}$	13.5	5.5
	$t_{\text{effluent}}$	6.5	2.5
	$f_{\text{redox}}$	15	5
	T [°C]	30	30
	pH	7-8	7
	HRT [hr]	16	16
	$V_{\text{ex}}$ [%]	50	17
N-species monitored	N-load [mg $\text{NH}_4^+\text{-N} \cdot \text{L}^{-1} \cdot \text{cycle}^{-1}$ ]	300	100
	[mg $\text{NH}_4^+\text{-N} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ ]	900	900
	$\text{NH}_4^+\text{-N}$	yes	yes
	$\text{NO}_2^-\text{-N}$	no	yes
	$\text{NO}_3^-\text{-N}$	yes	yes
	$\text{N}_2\text{O}_{(\text{g})}$	yes	yes
$\text{N}_2\text{O}_{(\text{aq})}$	no	yes	

## 2.2. Process control & monitoring

The SBR was controlled by a digital control unit (Sartorius, Göttingen, DE), which itself was accessed by a customized LabView (National Instruments, Austin, US) protocol to manage cycle operation. Temperature, pH and DO were monitored continuously (every 10 seconds) using a Pt-100 temperature sensor, an EasyFerm plus K8 pH electrode (Hamilton, Bonaduz, CH) and an OxyFerm 235 FDA DO electrode (Hamilton, Bonaduz, CH), respectively. Air flow was controlled by an EL-FLOW mass-flow controller, which was regulated by FlowDDE software (Bronkhorst, Ruurlo, NL).

## 2.3. Monitoring of N-species

### 2.3.1. $\text{NH}_4^+$ , $\text{NO}_3^-$ and $\text{NO}_2^-$

Bulk  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  concentrations were measured every minute by Varion Plus 700IQ ion-selective electrodes (WTW, Weilheim, DE).

Grab samples for  $\text{NO}_2^-$  measurements were filtered (0.2  $\mu\text{m}$ ) before measurement.  $\text{NO}_2^-$  concentrations were determined by a colorimetric assay based on sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride with absorbance measurement at 540 nm in a 96 well plate reader (Synergy Mx, BioTek, Winooski, US) (adapted from [24]).

### 2.3.2. N<sub>2</sub>O

The N<sub>2</sub>O concentration in the reactor off-gas was measured and logged every minute with a gas filter correlation N<sub>2</sub>O analyzer (Teledyne API, San Diego, CA, US). The off-gas N<sub>2</sub>O analyzer collected off-gas at a constant rate of 0.4 L\*min<sup>-1</sup> from the headspace of the reactor. Calibration was performed after manufacturer's instruction with 200 ppm N<sub>2</sub>O in N<sub>2</sub> as span gas and N<sub>2</sub> as zero gas.

The N<sub>2</sub>O concentration in the bulk liquid was measured every 30 seconds by a N<sub>2</sub>O-R micro-sensor (Unisense, DK). Calibration was performed in biomass free medium with spikes of saturated N<sub>2</sub>O solution after manufacturer's instruction.

### 2.4. Particle size analysis

15 ml mixed liquor samples were withdrawn in an aerated phase (maximum turbidity) at three different positions of the reactor (top, middle, bottom), 5 ml each. The samples were pooled and triplicate particle size measurements were performed in a Mastersizer 2000 laser diffractometer equipped with a liquid sample loading and dispersion unit (Malvern Instruments Ltd., Malvern, UK). The refraction index was set to 1.5.

### 2.5. DNA extraction and quantitative PCR (qPCR)

To quantify the abundance of bacteria involved in the nitrogen transformations, triplicate biomass samples were taken in the first aerated phase of SBR cycles. DNA was extracted from 3 mg of biomass (dry weight) using a Fast DNA™ SPIN Kit for soils (MP Biomedicals, USA) according to manufacturer's instruction. The quantity and quality of extracted DNA was measured and checked by its 260/280 ratio with NanoDrop (ThermoFisher Scientific, Rockwood, TN, USA). DNA was stored at -80°C before further analysis. Biomass sampled during experiment 1 was subject to qPCR analysis. The relative abundance of ammonia oxidizing bacteria, anammox bacteria, nitrite oxidizing bacteria and total bacteria was determined based on 16S rRNA genes, following the procedure by [25]. Primers and conditions for quantification of each target organism are listed in Table 2.

**Table 2 - Primers and conditions used for the quantification of bacterial numbers by qPCR based on 16S rRNA.**

Target Organism	Primers	Sequence (5'-3')	Annealing Temp.	Reference
All Bacteria	1055f 1392r	ATG GCT GTC GTC AGC T ACG GGC GGT GTG TAC	55	[26,27]
β-proteobacterial AOB	CTO189fa/b CTO189fc RT1r	GGA GRA AAG CAG GGG ATC G GGA GGA AAG TAG GGG ATC G CGT CCT CTC AGA CCA RCT ACT G	60	[28,29]
NOB <i>Nitrobacter</i>	FGPS872f FGP1269r	CTA AAA CTC AAA GGA ATT GA TTT TTT GAG ATT TGC TAG	50	[30]
NOB <i>Nitrospira</i>	Nspra675f Nspra746r	GCG GTG AAA TGC GTA GAK ATC G TCA GCG TCA GRW AYG TTC CAG AG	58	[31]
Anammox	Amx809f Amx1066r	GCC GTA AAC GAT GGG CAC T AACGTCTCACGACACGAGCTG	67	[32]

## 2.6. Experimental Design

The experiments of the study are summarized in Table 3.

**Table 3 – Summary of experimental design and objective of experiments**

experiment	configuration	feature	objective
1	a	long cycle standard operation	detailed monitoring of N <sub>2</sub> O emissions over the course of SBR cycles
	b	long cycle continuous sparging of gas results in continuous supply of gas to off-gas N <sub>2</sub> O analyzer	insights into non-aerated phases with off-gas N <sub>2</sub> O analyzer
2	short cycle	constant pH, pH = 7.0	monitoring of N <sub>2</sub> O production at constant pH for comparison with fluctuating pH in exp. 1
3	long cycle	stop of aeration after $\frac{1}{2} * t_{\text{cycle}}$ , operation under anoxic condition for 275 min.	assessment of activity of heterotrophic denitrifiers

### 2.6.1. Experiment 1: Analysis of temporal dynamics of N<sub>2</sub>O production and emission during SBR cycle

Experiment 1 was designed to monitor the N<sub>2</sub>O emissions during a SBR cycle in order to analyze the off-gas N<sub>2</sub>O profile in respect to other measured process parameters (pH, concentration profiles of N-species, DO, aerated/non-aerated phases). The SBR was operated under long cycle configuration, as described in 2.1. Due to the nature of the aeration regime, i.e. flow of gas during aerated phases and no flow of gas during non-aerated phases, no information about N<sub>2</sub>O production is available from the off-gas analyzer in the non-aerated phases. Therefore, we measured one additional cycle of experiment 1 with a constant flow of gas throughout  $t_{\text{react}}$  and supplied N<sub>2</sub> gas at a flow rate of  $Q_{\text{N}_2} = 2 \text{ L} \cdot \text{min}^{-1}$  during non-aerated phases. Consequently, N<sub>2</sub>O<sub>(aq)</sub> was stripped at approx. the same rate during both aerated and non-aerated phases. Thus, information on the N<sub>2</sub>O production dynamics during non-aerated phases was available from the off-gas N<sub>2</sub>O analyzer. Due to constant stripping, it was assumed that N<sub>2</sub>O emitted  $\approx$  N<sub>2</sub>O produced. Yet, we define the reactor operation without constant stripping as our reference condition, as it reflects more realistically how a full-scale SBR would be operated. We will refer to the measurement under standard operation with intermittent aeration as experiment 1.a. The measurements with constant flow of gas during  $t_{\text{react}}$ , is termed experiment 1.b.

### 2.6.2. Experiment 2: N<sub>2</sub>O emission profile at constant pH

The pH during a SBR cycle under standard operation fluctuated. To differentiate between a potential effect of pH (H<sup>+</sup> concentration) and the effect of varying NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> concentrations on N<sub>2</sub>O emissions (also as a result of varying pH), the system was operated at constant pH of 7.0 during experiment 2. The NH<sub>4</sub><sup>+</sup> source was changed from ammonium bicarbonate to ammonium chloride. Aliquots of concentrated sodium bicarbonate (83 g NaHCO<sub>3</sub>·L<sup>-1</sup>) were added to maintain pH. The cycle was operated at short cycle configuration. In comparison to experiment 1 and 3, in

experiment 2 additionally bulk liquid  $\text{N}_2\text{O}$  were measured in situ (see 2.3.2.) (micro-sensor, Unisense, DK) and  $\text{NO}_2^-$  concentrations were obtained from grab-samples (see 2.3.1.)

### **2.6.3. Experiment 3: Assessment of heterotrophic denitrification activity**

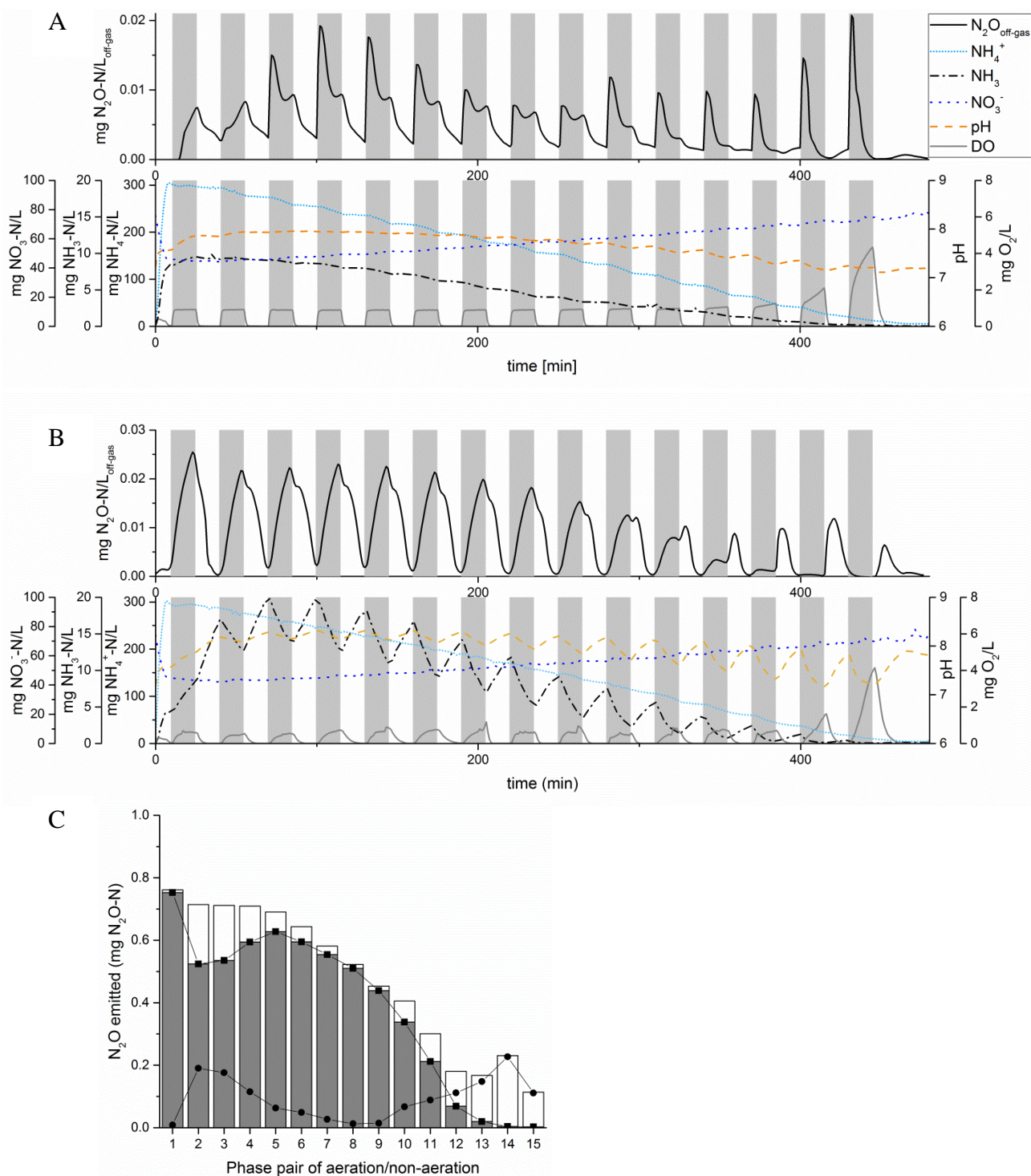
Although the system was only fed with carbonate as carbon source, activity of heterotrophic bacteria was likely. To assess their potential activity, the reactor was operated for four hours under anoxic conditions (as in experiment 1 under long cycle configuration, with the exception that aeration was stopped at  $t=205$  min.). The reactor remained under anoxic conditions with excess of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  for 275 minutes. The  $\text{NO}_3^-$  removal rate was used as an indicator of the activity of heterotrophic denitrifiers.

## **3. Results**

### **3.1. System performance**

System performance was stable for 3 month prior to the  $\text{N}_2\text{O}$  measurement campaign. Biomass concentration was  $1.8\pm 0.1$  gVSS/L at a median diameter of bio-granules of approx. 275  $\mu\text{m}$  during the measurement campaign (Fig.SI.1 in Supplementary Material). The microbial community was composed of ~18% AOB, ~30% AnAOB, <0.2% NOB and ~52% other bacteria (Fig.SI.2 in Supplementary Material). Under standard operation  $300\pm 3$  mg  $\text{NH}_4^+$ -N/L per cycle were removed with almost 99% removal efficiency (Fig. 1). Total N-removal efficiency was 89%. The effluent concentrations of  $\text{NH}_4^+$ -N,  $\text{NO}_2^-$ -N and  $\text{NO}_3^-$ -N were  $4.1\pm 0.6$  mg  $\text{NH}_4^+$ -N/L,  $0.7\pm 0.7$   $\text{NO}_2^-$ -N/L and  $75.4\pm 2.8$  mg  $\text{NO}_3^-$ -N/L. The fraction of  $\text{N}_2\text{O}$  emitted per  $\text{NH}_4^+$  removed was  $1.1\pm 0.5\%$ . The average  $\text{NH}_4^+$  removal rate was  $23.9\pm 2.6$  mg  $\text{NH}_4^+$ -N\*gVSS<sup>-1</sup>\*h<sup>-1</sup>.





**Figure 1 – Reactor performance and N<sub>2</sub>O profiles during experiment 1 at long cycle operation. Concentration profiles show average concentrations of three SBR cycles. For better visibility standard deviations are not shown, but are displayed in Fig.SI.3 in Supplementary Information. (A) Concentration profiles of off-gas N<sub>2</sub>O and bulk NH<sub>4</sub><sup>+</sup>, NH<sub>3</sub>, NO<sub>3</sub><sup>-</sup>, pH and DO over SBR cycle time under standard intermittent aeration regime and (B) continuous gas supply regime. Shaded areas represent aeration phases. (C) N<sub>2</sub>O emitted per phase pair of aeration/non-aeration under continuous gas supply regime. Grey bars and squares represent N<sub>2</sub>O emitted from aeration phases, white bars and circles represent non-aeration phases.**

### 3.2. Intra-cycle dynamics of N<sub>2</sub>O emissions

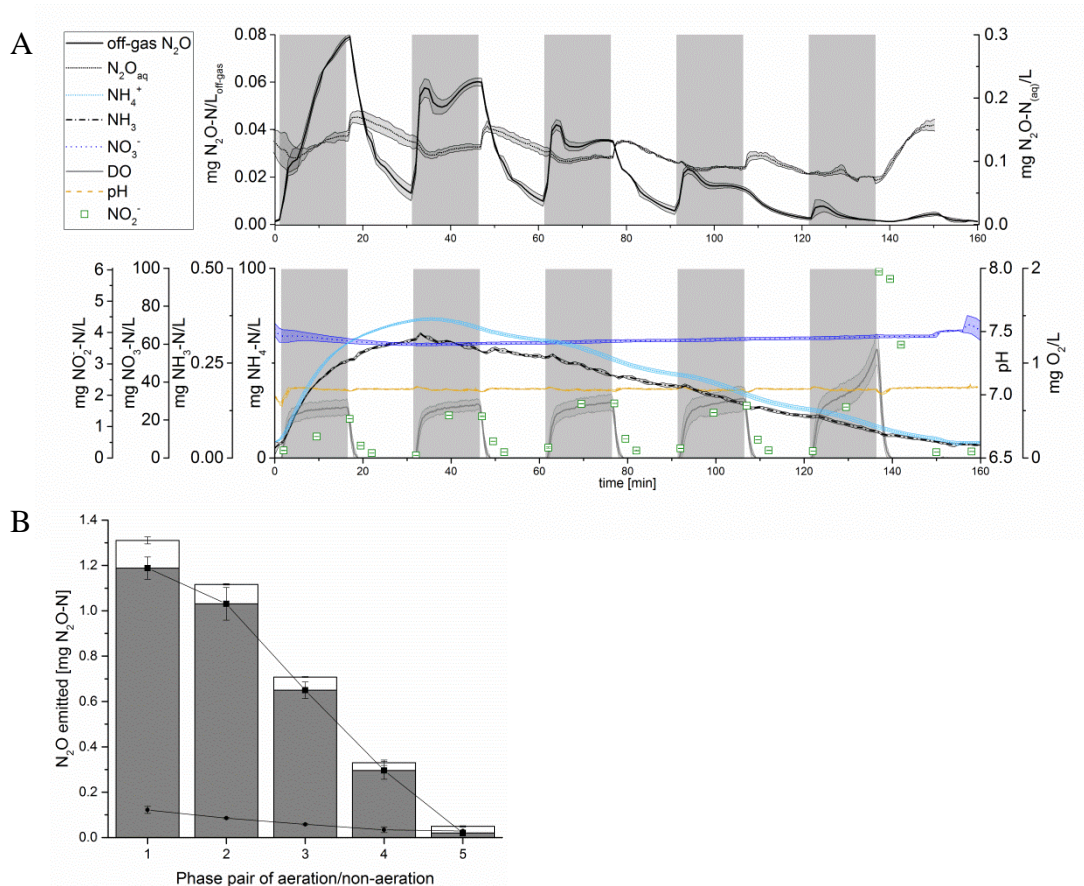
N<sub>2</sub>O emissions from the system were highly dynamic during a SBR cycle (Fig. 1; note: for better visibility only average concentration profiles of n=3 cycles are plotted. The standard deviations are presented in Fig.SI.3 in the Supplementary Information). Under the standard aeration regime (Experiment 1.a: no flow of gas during non-aerated phases) the vast majority (approx. 96%) of total N<sub>2</sub>O was emitted during aerated phases (Fig. 1.A). Except for aerated phases 1 and 2, the off-gas N<sub>2</sub>O concentrations peaked transiently at the onset of aerated phases, after which they gradually decreased to a transient minimum before they increased again as aeration continued. When aeration stopped at the end of an aerated phase, off-gas N<sub>2</sub>O concentrations decreased gradually to near background concentrations.

The initial peaks disappeared, when the reactor was operated under the continuous gas-flow regime (Experiment 1.b: N<sub>2</sub> gas was supplied during non-aerated phases) (Fig. 1.B), indicating that the initial peaks in aerated phases observed in experiment 1.a were due to stripping of N<sub>2</sub>O that accumulated during non-aerated phases. Instead, under continuous gas flow regime, the off-gas N<sub>2</sub>O concentration increased continuously during aeration and reached its maximum at the end of an aerated phase (Fig. 1.B). During non-aerated phases, off-gas N<sub>2</sub>O concentrations declined slightly to a plateau within the first five minutes, before concentrations declined to background concentrations. These plateaus emerged as distinct peaks towards the end of the cycle.

Integration of the N<sub>2</sub>O profiles during aerated and non-aerated phases revealed that ~80% of the N<sub>2</sub>O was produced during aerated phases, whereas ~20% was produced during non-aerated phases (Fig. 1.C.). Generally, N<sub>2</sub>O emissions were highest at the beginning of a cycle (0.76 mg N<sub>2</sub>O-N from phase pair 1) and declined with successive aerated/non-aerated phases (0.11 mg N<sub>2</sub>O-N from phase pair 15). In the beginning of the cycle the fraction of N<sub>2</sub>O produced in aerated phases exceeded the N<sub>2</sub>O produced in non-aerated phases. Towards the end of the cycle this ratio turned and most N<sub>2</sub>O was produced during non-aerated phases.

### 3.3. N<sub>2</sub>O emission profile at constant pH

The pH declined with cycle time by approx. one unit from ca. pH 8 at the beginning of a cycle to ca. pH 7 at the end (Fig.1). In order to exclude that the decline of N<sub>2</sub>O production observed in experiment 1 was caused by the decline of pH, the reactor was operated at constant pH=7.0 during experiment 2 (Table 3). The pH did not affect the shape of the off-gas N<sub>2</sub>O profile (Fig. 2.A) and the decline of N<sub>2</sub>O emissions with cycle time prevailed under operation at constant pH (Fig. 2.B). However, the cycle-integrated fraction of N<sub>2</sub>O emitted per NH<sub>4</sub><sup>+</sup> removed was substantially larger at pH 7 with 2.7%, than 1.1% under standard operation.



**Figure 2 - Reactor performance and  $N_2O$  profiles during experiment 2 at short cycle operation with standard deviations. (A) Concentration profiles (avrg.  $\pm$  STD, n = 3 SBR cycles) of off-gas  $N_2O$  and bulk  $N_2O_{(aq)}$ ,  $NH_4^+$ ,  $NH_3$ ,  $NO_2^-$ ,  $NO_3^-$ , pH and DO over SBR cycle time under standard intermittent aeration regime at constant pH. Shaded areas represent aeration phases. (B)  $N_2O$  emitted per pair of aerated/non-aerated phases. Grey bars and squares represent  $N_2O$  emitted from aeration phases, white bars and circles represent non-aeration phases (avrg.  $\pm$  STD, n = 3 SBR cycles).**

In experiment 2 additionally to the measured parameters of experiment 1, bulk concentrations of  $NO_2^-$  and  $N_2O$ - $N_{(aq)}$  were monitored.  $NO_2^-$  accumulated during aerated phases in response to DO to  $1.5 \pm 0.2$  mg  $NO_2^-$ -N/L in aerated phases 1-4 and approx. 6 mg  $NO_2^-$ -N/L in aerated phase 5 (Fig. 2.A). The accumulated  $NO_2^-$  was consumed within the first five minutes of the following non-aerated phase. Additionally, a specific  $N_2O$  consumption rate of  $0.1 \pm 0.01$  mg  $N_2O$ - $N_{(aq)}$ \*gVSS $^{-1}$ \*h $^{-1}$  was detected during non-aerated phases based on the  $N_2O$ - $N_{(aq)}$  profile.

### 3.4. Assessment of heterotrophic activity

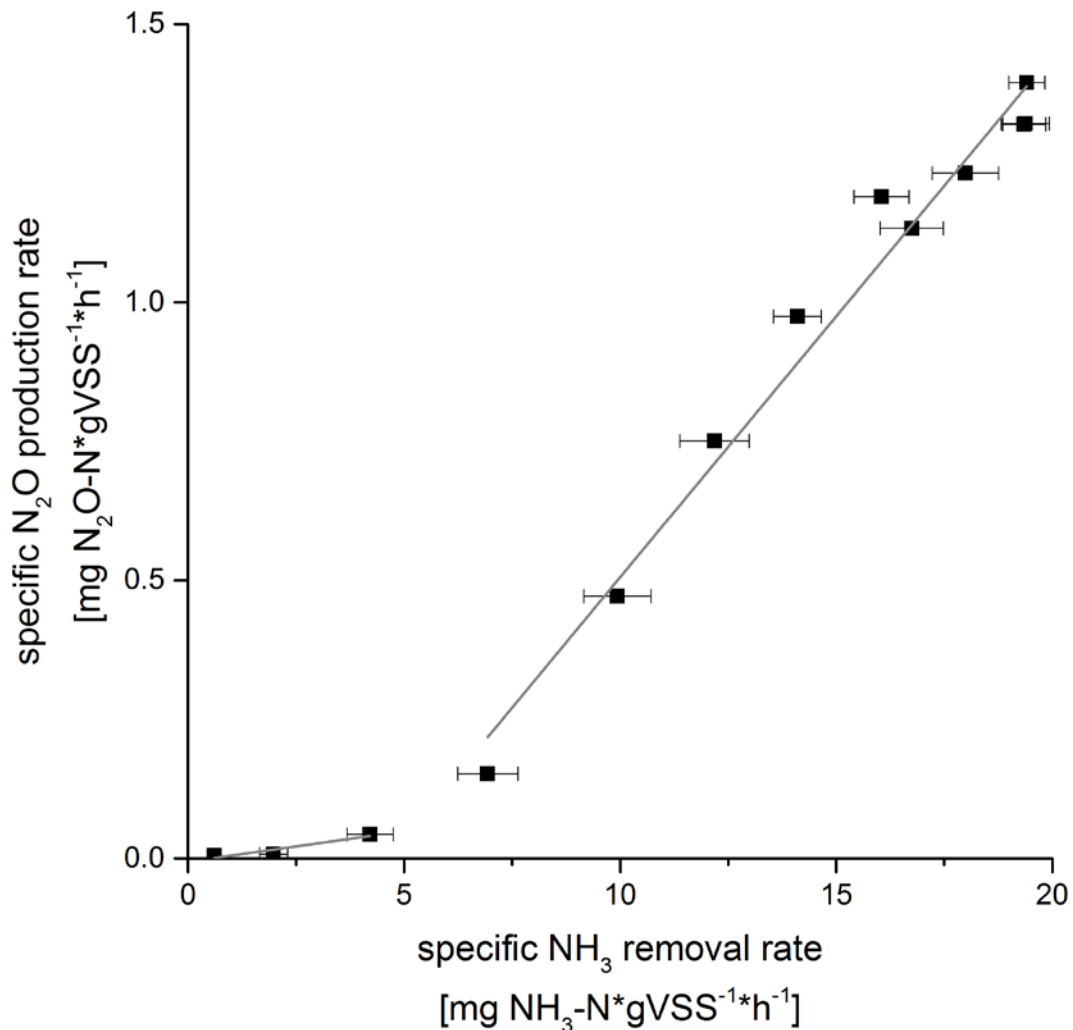
We used the anaerobic  $NO_3^-$  reduction rate of the system as a proxy of the activity of heterotrophic denitrifiers. In experiment 3 the system was exposed to four hours of anoxic conditions with approx. 53 mg  $NO_3^-$ -N/L present. The specific  $NO_3^-$ -N consumption rate was very low with  $0.71 \pm 0.01$  mg  $NO_3^-$ -N\*gVSS $^{-1}$ \*h $^{-1}$ . Generally, all parameters remained constant under anoxic conditions (Fig.SI.4 in Supplementary Material), which indicates a shut-down of activity in the system under prolonged phases of anoxia.

### 3.5. Two distinct trends of N<sub>2</sub>O emissions

The obtained off-gas N<sub>2</sub>O profiles revealed two distinct trends of N<sub>2</sub>O emissions in the course of a SBR cycle, both under standard operation and operation at constant pH: a. decline of N<sub>2</sub>O production with successive aerated phases (Fig. 1.C. squares, Fig. 2.B, squares); b. no clear trend of N<sub>2</sub>O production with successive non-aerated phases (Fig. 1.C., circles; Fig. 2.B., circles).

#### 3.5.1. Correlation of N<sub>2</sub>O production rate during aerated phases with AOR

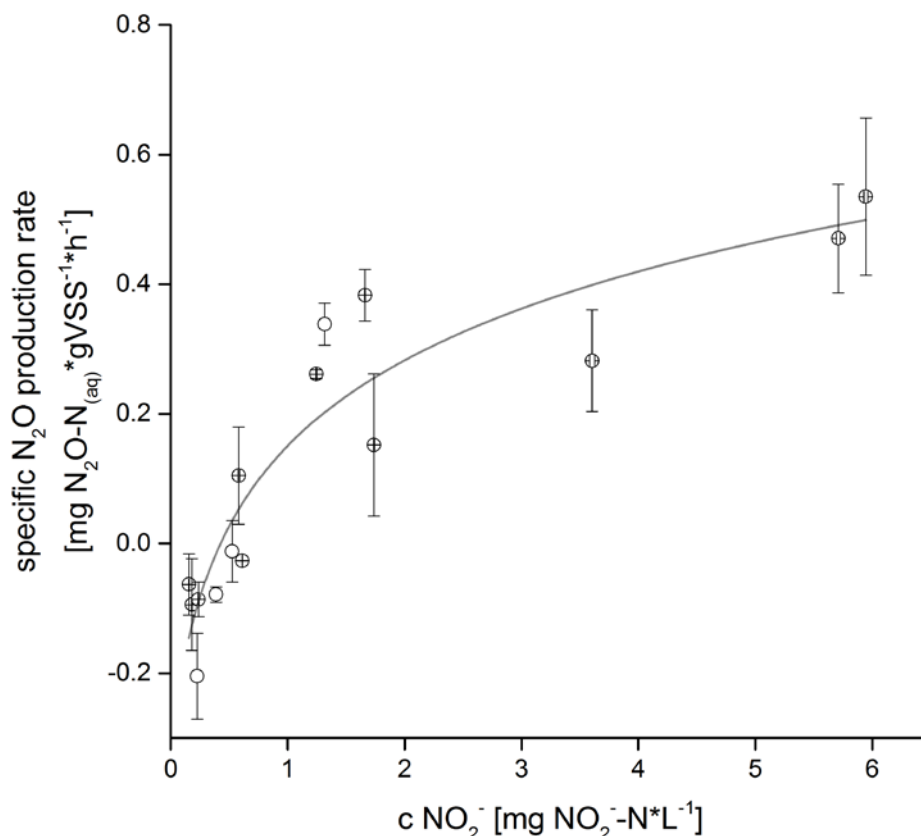
The specific N<sub>2</sub>O production rate during aerated phases was positively correlated with the specific NH<sub>3</sub> removal rate (AOR)(Fig. 3) and showed two distinct linear correlations: above specific AORs of 5 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup> the ratio of N<sub>2</sub>O produced per NH<sub>3</sub> removed was substantially higher with 0.09±0.01 mg N<sub>2</sub>O-N\*mg NH<sub>3</sub>-N<sup>-1</sup> (R<sup>2</sup>=0.97, p<0.05 (t-test)), than below specific AORs of 5 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup>, when the ratio of N<sub>2</sub>O produced per NH<sub>3</sub> removed was 0.01±0.004 mg N<sub>2</sub>O-N\*mg NH<sub>3</sub>-N<sup>-1</sup> (R<sup>2</sup>=0.78, p=0.2 (t-test)).



**Figure 3 – Net N<sub>2</sub>O production rates as a function of NH<sub>3</sub> removal rate. Data are based on aerated phases during experiment 1. Error bars represent standard errors of linear regression of NH<sub>3</sub> removal rates (n = 3 cycles). The solid grey lines represent the linear trend line: i) < 5 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup>: 0.01±0.004 mg N<sub>2</sub>O-N\*mg NH<sub>3</sub>-N<sup>-1</sup> (R<sup>2</sup>=0.78, p=0.2 (t-test)); ii) > 5 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup>: 0.09±0.01 mg N<sub>2</sub>O-N\*mg NH<sub>3</sub>-N<sup>-1</sup> (R<sup>2</sup>=0.97, p<0.05 (t-test)).**

### 3.5.2. Correlation of N<sub>2</sub>O production rate during non-aerated phases with NO<sub>2</sub><sup>-</sup>

The specific N<sub>2</sub>O-N<sub>(aq)</sub> production rate during non-aerated phases positively correlated with the concentration of NO<sub>2</sub><sup>-</sup> at the onset of non-aerated phases ( $R^2=0.86$ ,  $p<0.05$  ( $t$ -test))(Fig. 4). At NO<sub>2</sub><sup>-</sup> concentrations below 1 mg NO<sub>2</sub><sup>-</sup> N/L, the specific N<sub>2</sub>O consumption rate exceeded the specific N<sub>2</sub>O production rate and resulted in negative specific net-N<sub>2</sub>O production rates. Once NO<sub>2</sub><sup>-</sup> was depleted, no increase of N<sub>2</sub>O-N<sub>(aq)</sub> was measurable (Fig. 2.A).



**Figure 4 – Net N<sub>2</sub>O<sub>(aq)</sub> production rate in first five minutes of non-aerated phases as function of NO<sub>2</sub><sup>-</sup> concentration during operation at constant pH=7. Negative net-production rates occur, when N<sub>2</sub>O consumption rate is larger than N<sub>2</sub>O production rate. Grey curve represents logarithmic fit ( $R^2=0.86$ ,  $p<0.05$  ( $t$ -test)). Error bars present standard errors of N<sub>2</sub>O production rates and NO<sub>2</sub><sup>-</sup> concentrations of three consecutive SBR cycles.**

## 4. Discussion

In order to develop of N<sub>2</sub>O mitigation strategies for Partial Nitritation-Anammox systems, it is imperative to identifying the causes of N<sub>2</sub>O emission and production. The temporal dynamics of N<sub>2</sub>O production and emission during cycles of an intermittently aerated PNA SBR were studied and the contribution of different redox conditions was quantified. The N<sub>2</sub>O emission factor (N<sub>2</sub>O-N produced per NH<sub>4</sub><sup>+</sup>-N removed) determined in this study ( $1.1\pm 0.5\%$ ) is typical for PNA systems (0.1 to 2.4 %) (reviewed in [8]). With a cycle-averaged ammonium removal rate of  $0.9 \text{ kg NH}_4^+ \text{-N} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$  the studied system outperformed typical lab-scale systems ( $0.4\text{-}0.7 \text{ kg NH}_4^+ \text{-N} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ ) [8] and matched ammonium removal rates reported for pilot- and full-scale applications ( $0.6\text{-}1.5 \text{ kg NH}_4^+ \text{-N} \cdot \text{L}^{-1} \cdot \text{d}^{-1}$ ) [4,33]. The performance indicators introduced by Mutlu et al. [23],  $R_{\text{AmmTot}}=1.11$  and  $R_{\text{NatTot}}=0.11$ , indicated a balanced PNA process with low heterotrophic

denitrification activity. The microbial community composition was similar to other reports on PNA systems and efficient suppression of NOB was achieved (see 3.1.) [8,34,35].

The off-gas N<sub>2</sub>O profiles under standard operation were dynamic and strongly fluctuating with a high emission peak at the onset of aeration, followed by a smaller emission peak towards the end of aerated phases. Similar N<sub>2</sub>O emission profiles were reported from a full-scale PNA system (Fig.1) [4]. The initial emission peak could either be instantaneously produced N<sub>2</sub>O at the onset of aeration or N<sub>2</sub>O that was produced in the previous non-aerated phase and abruptly stripped from the liquid phase at the onset of an aerated phase [4,18]. Experiment 1 b (operation under continuous stripping) verifies that rapid instantaneous N<sub>2</sub>O production was not the cause. Instead, aerobic N<sub>2</sub>O production increased steadily with ongoing aeration (Fig.1.B). The change from anoxic to oxic conditions itself did not trigger an abrupt peak in N<sub>2</sub>O production, which contrasts a study with *N. europaea* that identified peak N<sub>2</sub>O production at the onset of oxic conditions [12,36]. The study hypothesized a link between N<sub>2</sub>O production and high specific activity at high nitrogen fluxes during rapid changes of redox conditions. The absence of elevated N<sub>2</sub>O production during the change from anoxic to oxic conditions in this study may be attributed to a metabolic adaptation of the microbial community to high NH<sub>4</sub><sup>+</sup> concentrations and frequent switches of redox conditions [36]. The second, smaller emission peak is then the result of a steadily increasing N<sub>2</sub>O production rate with aeration time [20]. The observed complex emission pattern is therefore the result of an overlay of two processes: stripping of anoxically produced N<sub>2</sub>O and aerobically produced N<sub>2</sub>O.

#### **4.1. N<sub>2</sub>O production in aerated phases: Correlation of specific N<sub>2</sub>O production rate with specific AOR**

The highest N<sub>2</sub>O emission was at the beginning of a SBR cycle (Fig.1C), when also the NH<sub>4</sub><sup>+</sup> concentration was highest (300 mg NH<sub>4</sub><sup>+</sup>-N/L). High concentrations of NH<sub>4</sub><sup>+</sup> and oxic conditions have been described to promote N<sub>2</sub>O production, when high nitrogen oxidation rates occur [37–39]. The linear correlation between the specific N<sub>2</sub>O production rate and the specific AOR observed in this study, matches findings from a AOB enrichment cultures (Fig.3) [40,41]. In the reported AOB enrichment cultures the specific N<sub>2</sub>O production rate correlated linearly with AORs below 100 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup>. At higher AORs the specific N<sub>2</sub>O production rate of the AOB enrichment culture increased exponentially. The exponential increase at higher AORs was hypothesized to be caused by a chemical breakdown of nitrosyl radicals at high turnover of hydroxylamine dehydrogenase [40]. However, AORs over 100 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup> are not typically achieved in full-scale applications [8]. A linear correlation between the specific N<sub>2</sub>O production rate and AOR has further been supported by extant AOB activity assays for an intermittently aerated PNA lab-scale system that identified a correlation of 0.4 mg N<sub>2</sub>O-N/mg NH<sub>3</sub>-N [20]. Therefore, the correlation between the specific N<sub>2</sub>O production rate and the AOR can likely be considered linear at AORs commonly achieved in applied PNA systems (below 100 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup>).

Relatively high AORs at the beginning of SBR cycles (approx. 20 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup>) may also lead to a transient accumulation of NH<sub>2</sub>OH [42]. NH<sub>2</sub>OH can react abiotically with free nitrous acid (HNO<sub>2</sub>) to generate N<sub>2</sub>O at concentrations as low as 0.06 mg NH<sub>2</sub>OH-N/L [43]. A fraction of N<sub>2</sub>O produced during aerated phases in this study (AORs of approx. 20 mg NH<sub>3</sub>-N\*gVSS<sup>-1</sup>\*h<sup>-1</sup> and NO<sub>2</sub><sup>-</sup> concentrations of approx. 2 mg NO<sub>2</sub><sup>-</sup>-N/L) may therefore be attributed to an abiotic reaction. However, the reaction kinetics of abiotic N<sub>2</sub>O production strongly depend on pH, which determines the concentration of HNO<sub>2</sub>, the reaction partner. At pH 7.6 the abiotic NH<sub>2</sub>OH depletion rate is reported to be 30-times lower, than at acidic pH of 4.3 [43]. Considering the relatively high pH of approx. 8.0-7.2 in this study, the abiotic N<sub>2</sub>O production rates were likely low in the studied system.

## 4.2. N<sub>2</sub>O production in non-aerated phases: Correlation of specific N<sub>2</sub>O production rate with NO<sub>2</sub><sup>-</sup> concentration

Production of N<sub>2</sub>O only occurred in the first minutes of a non-aerated phase, when NO<sub>2</sub><sup>-</sup> was present (Fig. 2.A). N<sub>2</sub>O production during non-aerated phases remained relatively constant throughout the cycle and can be attributed to the reoccurring accumulation of approx. 1.5 mg NO<sub>2</sub><sup>-</sup> N\*L<sup>-1</sup> during aerated phases throughout the cycle. The NO<sub>2</sub><sup>-</sup> was then available at the onset of non-aerated phases for denitrification.

A stimulating effect of NO<sub>2</sub><sup>-</sup> on N<sub>2</sub>O production has been described before [14,16,39]. NO<sub>2</sub><sup>-</sup> had a large effect on the N<sub>2</sub>O production rate at low DO concentrations (0.35-1.5 mg O<sub>2</sub>\*L<sup>-1</sup>)[14]. The strong effect of NO<sub>2</sub><sup>-</sup> at low DO concentrations has been assigned to an increased nitrifier denitrification activity [16,44]. The correlation found in this study shows a similar effect of NO<sub>2</sub><sup>-</sup> on N<sub>2</sub>O production at low DO (Fig. 4). The almost instantaneous decline of DO below detection limit in the first minutes of non-aerated phases together with the available NO<sub>2</sub><sup>-</sup> created conditions that foster ND. Due to the direct effect of NO<sub>2</sub><sup>-</sup> on N<sub>2</sub>O production at anoxic conditions, low concentration of NO<sub>2</sub><sup>-</sup> at the onset of non-aerated phases would result in lower N<sub>2</sub>O production and subsequently less stripping of N<sub>2</sub>O. The accumulation of NO<sub>2</sub><sup>-</sup> during aeration can be effectively controlled by the length of aerated phases and decreases with increasing aeration frequency [20]. A high frequency of aeration that maintains NO<sub>2</sub><sup>-</sup> concentrations below 1 mg NO<sub>2</sub><sup>-</sup> N\*L<sup>-1</sup> appears as a suitable strategy to mitigate N<sub>2</sub>O production during non-aerated phases without reducing the cycle-average total oxygen load [20,45]. In the presented system the length of non-aerated phases was fixed, yet NO<sub>2</sub><sup>-</sup> was depleted already after approx. the first third of non-aerated phases (Fig. 2). Thus, the anammox process was substrate limited for the remaining time of non-aerated phases. A dynamic aeration control that is based on NO<sub>2</sub><sup>-</sup> concentration could reduce the length of non-aerated phases and thus increase the total time of aeration. With an overall longer aeration time during each SBR cycle, aeration rates could be reduced while maintaining cycle-averaged oxygen load. Lower aeration rates reduce the AOR, which in turn would be beneficial for lower N<sub>2</sub>O production (see. 4.1. and Fig.3).

## 4.3. Potential N<sub>2</sub>O production pathways

It is well known that different N<sub>2</sub>O production pathways may contribute differently to the overall N<sub>2</sub>O production with varying redox conditions [8,16,17]. During nitrification NH<sub>4</sub><sup>+</sup> is oxidized to NO<sub>2</sub><sup>-</sup> via the intermediates NH<sub>2</sub>OH and NO [10]. Both intermediates are reactive and can be partly detoxified to N<sub>2</sub>O or react abiotically to form N<sub>2</sub>O [10,43]. During anoxic conditions denitrification of accumulated NO<sub>2</sub><sup>-</sup> is the main source of N<sub>2</sub>O [14]. NO<sub>2</sub><sup>-</sup> is either reduced by AOB to N<sub>2</sub>O, when N<sub>2</sub>O constitutes the end product of the ND pathway, or NO<sub>2</sub><sup>-</sup> is denitrified by heterotrophic bacteria towards N<sub>2</sub> [10]. During HD N<sub>2</sub>O is an intermediate, but can also constitute the end product, when incomplete denitrification occurs. The share of the HO, ND and HD pathway to the total N<sub>2</sub>O produced therefore varies with the state of aeration.

HO is expected to be the dominant source of N<sub>2</sub>O during aerated phases, as high NH<sub>4</sub><sup>+</sup> concentrations (100-300 mg NH<sub>4</sub><sup>+</sup>-N/L), low NO<sub>2</sub><sup>-</sup> concentrations (1-2mg NO<sub>2</sub><sup>-</sup> N/L) and moderate concentrations of DO (0.8 mg O<sub>2</sub>/L) create conditions that increase the share of HO to the total N<sub>2</sub>O production [38]. Elevated DO and NH<sub>4</sub><sup>+</sup> concentrations have been shown to increase the contribution of the HO pathway [8,17,37]. As the concentration of NH<sub>2</sub>OH is directly linked to the

oxidation rate of the ammonia monooxygenase (amoA) and vice versa, the AOR becomes the key parameter to control the concentration of  $\text{NH}_2\text{OH}$  and thus the  $\text{N}_2\text{O}$  production by AOB via HO [12]. That is reflected in the correlation of the  $\text{N}_2\text{O}$  production rate and the AOR (this study, [40]).

In non-aerated phases and in anoxic layers of the biofilm the contribution of HO decreases and  $\text{N}_2\text{O}$  is predominantly produced by ND [14,17]. ND is directly linked to the availability of  $\text{NO}_2^-$  [12,44,46] and as seen from Fig. 2 the production of  $\text{N}_2\text{O}_{(\text{aq})}$  ceases upon depletion of  $\text{NO}_2^-$ . The correlation of the  $\text{N}_2\text{O}$  production rate at the onset of non-aerated phases and the concentration of  $\text{NO}_2^-$  suggests that ND is the dominating  $\text{N}_2\text{O}$  production pathway during non-aerated phases.

In addition to the temporal changes of redox conditions in intermittently aerated PNA reactors, spatial stratification of bio-granules limits the availability of oxygen to the top layer of bio-granules (approx. 20  $\mu\text{m}$  oxygen penetration depth vs. approx. 275  $\mu\text{m}$  average particle diameter, Fig.SI.1, [8,39]). Consequently, changes of redox conditions may only apply to the top layer of bio-granules, while anoxic conditions prevail in the center of the granules throughout the SBR cycle. Clear stratification of the microbial guilds has previously been documented in PNA bio-granules [8]: Based on microelectrode measurements the oxic surface layer, where the majority of nitrifiers is located, was identified as the main  $\text{N}_2\text{O}$  producing layer (approx. 70% in the reported PNA bio-granules). Less  $\text{N}_2\text{O}$  production was observed in deeper layers of biofilm, where AnAOB and HB are dominant [8]. These findings align with the results from this study that the majority of  $\text{N}_2\text{O}$  production was assigned to oxic  $\text{N}_2\text{O}$  production (approx. 80%).

#### 4.4. Implications for PNA systems

High loaded PNA processes (1.5-2.0  $\text{kg NH}_4^+-\text{N}*\text{L}^{-1}*\text{d}^{-1}$ ) with high volumetric AORs (1.3-1.5  $\text{kg NH}_4^+-\text{N}*\text{L}^{-1}*\text{d}^{-1}$ ) suffer from  $\text{N}_2\text{O}$  production (0.9-2.7% per unit  $\text{NH}_4^+$  removed) that significantly impacts the carbon footprint of the process [4,8,33]. Mitigating  $\text{N}_2\text{O}$  emissions from PNA systems may require revision of the concept of high rate N-removal processes. In respect to the mitigation of  $\text{N}_2\text{O}$  production, a step forward for PNA applications appears to be single-stage operation at low specific AORs (approx. 0.12  $\text{g NH}_4^+-\text{N}*\text{gVSS}^{-1}*\text{d}^{-1}$ ; Fig. 3) and low concentrations of  $\text{NO}_2^-$  (below 1  $\text{mg NO}_2^-\text{N}*\text{L}^{-1}$ ; Fig. 4). In order to maintain commonly applied volumetric  $\text{NH}_4^+$  removal rates of approx. 1.4  $\text{kg N}*\text{m}^{-3}*\text{d}^{-1}$  [4,33], high biomass concentrations of approx. 12  $\text{g VSS}*\text{L}^{-1}$  would be required to keep specific AOR low. Alternatively, lower AORs can be achieved by lower aeration rates. An aeration control based on  $\text{NO}_2^-$  set-points would allow for longer total aeration time at lower aeration rates. Lower AORs can also be achieved by lower initial N-loading (below 100  $\text{mg NH}_4^+-\text{N}*\text{L}^{-1}$ , Fig. 1)[39]. Shorter, but more frequent cycles per day or intermittent feeding appear as a feasible strategy to decrease AORs and thus the relative fraction of  $\text{N}_2\text{O}$  produced [47].

## 5. Conclusion

In the presented study  $\text{N}_2\text{O}$  production and emission dynamics from an intermittently aerated partial nitrification anammox SBR were studied. The  $\text{NH}_4^+$  and total N removal efficiency of a lab-scale PNA SBR were 99% and 89%, respectively with a cycle-averaged volumetric  $\text{NH}_4^+$  removal rate of 0.9  $\text{kg NH}_4^+-\text{N}*\text{L}^{-1}*\text{d}^{-1}$ . The fraction of  $\text{N}_2\text{O}$  emitted per unit  $\text{NH}_4^+$  removed was  $1.1 \pm 0.5\%$  with a cycle-averaged specific  $\text{N}_2\text{O}$  production rate of 0.23  $\text{mg N}_2\text{O}-\text{N}*\text{gVSS}^{-1}*\text{h}^{-1}$ . Approx. 80 % of  $\text{N}_2\text{O}$  were produced during oxic phases and approx. 20 % during anoxic phases. Oxic  $\text{N}_2\text{O}$  production correlated with the ammonia removal rate, while anoxic  $\text{N}_2\text{O}$  production correlated with the



concentration of  $\text{NO}_2^-$ . Two distinct linear correlations of the specific  $\text{N}_2\text{O}$  production rate with AOR were identified. At low ammonia removal rates below  $5 \text{ mg NH}_3\text{-N} \cdot \text{gVSS}^{-1} \cdot \text{h}^{-1}$  the fraction of  $\text{N}_2\text{O}$  produced per  $\text{NH}_3$  removed was as low as  $0.011 \pm 0.004 \text{ mg N}_2\text{O-N} \cdot \text{mg NH}_3\text{-N}^{-1}$ , which was approx. 9 times lower than at AOR above  $5 \text{ mg NH}_3\text{-N} \cdot \text{gVSS}^{-1} \cdot \text{h}^{-1}$ . The study indicates that the operation of PNA SBRs with short cycle times, low volumetric N-loading (below  $100 \text{ mg NH}_4^+\text{-N} \cdot \text{L}^{-1}$ ), low specific AORs (approx.  $5 \text{ mg NH}_3\text{-N} \cdot \text{gVSS}^{-1} \cdot \text{h}^{-1}$ ) and high frequency of intermittent aeration are a feasible strategy to reduce  $\text{N}_2\text{O}$  emissions from PNA.

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