

Effect of Dynamic Process Conditions on Nitrogen Oxides Emission from a Nitrifying Culture

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Nitric oxide (NO) and nitrous oxide (N₂O) emissions from nitrifying ecosystems are a serious threat to the environment. The factors influencing the emission and the responsible microorganisms and pathways were studied using a laboratory-scale nitrifying reactor system. The nitrifying culture was established at growth rates relevant to wastewater treatment plants (WWTPs). During stable ammonia oxidation, 0.03% of ammonium was emitted as NO and 2.8% was emitted as N₂O. Although mixed cultures were used, clear responses in emission of ammonia oxidizing bacteria (AOB) could be detected and it was concluded that the denitrification pathway of AOB was the main source of the emissions. Emissions of nitrogen oxides in the system were strongly influenced by oxygen, nitrite, and ammonium concentrations. Steady state emission levels greatly underestimate the total emission, because changes in oxygen, nitrite, and ammonium concentrations induced a dramatic rise in NO and N₂O emission. The data presented can be used as an indication for NO and N₂O emission by AOB in plug-flow activated sludge systems, which is highly relevant because of the atmospheric impact and potential health risk of these compounds.

Introduction

Gaseous nitric oxide (NO) and nitrous oxide (N₂O) emissions can be the result of the activity of different groups of microorganisms in wastewater treatment plants (WWTPs): ammonia-oxidizing bacteria (AOB) (1), nitrite-oxidizing bacteria (NOB) (2), denitrifying microorganisms (3), and methanotrophic microorganisms (4). NO and N₂O emissions resulting from microbial activity have been detected in many man-made and natural environments, such as wastewater treatment plants, soils, and sediments. The release into the environment is small compared to the overall microbial nitrogen conversion, but may have a significant environmental impact. N₂O is a significant greenhouse gas, having approximately a 300-fold stronger effect than carbon dioxide

(5). NO plays a key role in chemical reactions that cause depletion of the ozone layer (6). Improved nitrogen removal from wastewater as a result of stricter legislation has increased the emission of N₂O into the atmosphere, due to introduction of anoxic zones and low dissolved oxygen concentrations in WWTPs (7). The emission of NO is less frequently reported but is expected to follow the same trend as N₂O, because it is produced in the same biological pathways. The contribution of WWTPs to the anthropogenic N₂O emissions was estimated to amount to 2.3% in The Netherlands, whereas the contribution to NO emissions was suggested to be negligible (8). Still, a thorough investigation into the potential contribution of WWTPs to NO and N₂O emissions and the role of the individual processes in the emissions seems to be lacking.

Significant N₂O and NO emissions from pure ammonia-oxidizing and denitrifying cultures have been measured repeatedly (9). However, pure culture cultivation conditions are generally strongly different from those prevailing in natural environments and WWTPs. Direct extrapolation of emission data from pure culture studies to emissions in full-scale WWTPs is troublesome due to the presence of a complex microbial community and different operational and environmental parameters compared to those of controlled laboratory-scale cultivations. The conditions in WWTPs we consider most important in this respect are (i) the low growth rate (high solids retention time, SRT), (ii) dynamic variations in the concentrations of ammonium, nitrite, and nitrate and the exposure of biomass to alternating aerobic and anaerobic conditions, (iii) the presence of a mixed microbial population, (iv) the variable wastewater temperature, and (v) an around neutral pH. In general, the NO and N₂O emission by nitrifying microorganisms seems to be induced by suboptimal growth conditions. Especially oxygen limitation, low pH, high nitrite and nitrate concentrations, and low SRT have been recognized to promote NO and N₂O production by nitrifiers (10). In field studies the impact of these factors has not been determined independently.

The combined emission of NO and N₂O from nitrifying mixed cultures has, to our knowledge, not been reported. Because NO is a known signaling compound, having not only an important impact on the metabolism of AOB (11), but also on many other groups of prokaryotes and eukaryotes (12), the focus of this study was on NO emission. Whenever possible N₂O emission was also measured, allowing a better correlation with previous investigations on emissions from nitrifying cultures which generally focused on N₂O.

The objective of this study was to investigate the impact of dynamic changes in ammonium, nitrite, and oxygen concentration on the NO and N₂O emission in an open mixed culture of nitrifying bacteria.

Experimental Section

Continuous Operation of Sequencing Batch Reactors (SBRs). The study was conducted in two open laboratory-scale reactors containing a nitrifying culture with a working volume of 2 L at 20 °C. The first reactor (A) was running for more than 9 months when the experiments were carried out and was inoculated with activated sludge from the nitrification stage of Rotterdam Dokhaven WWTP. The second reactor (B) was started with sludge from reactor A and was running for more than 3 months prior to the experiments. The SBR systems were operated identically in cycles of 6 h: 10 min fill phase, 307 min reaction phase, 3 min excess sludge removal, 20 min settling, and 20 min effluent discharge. One liter of influent (consisting of 35 mL of concentrated medium and

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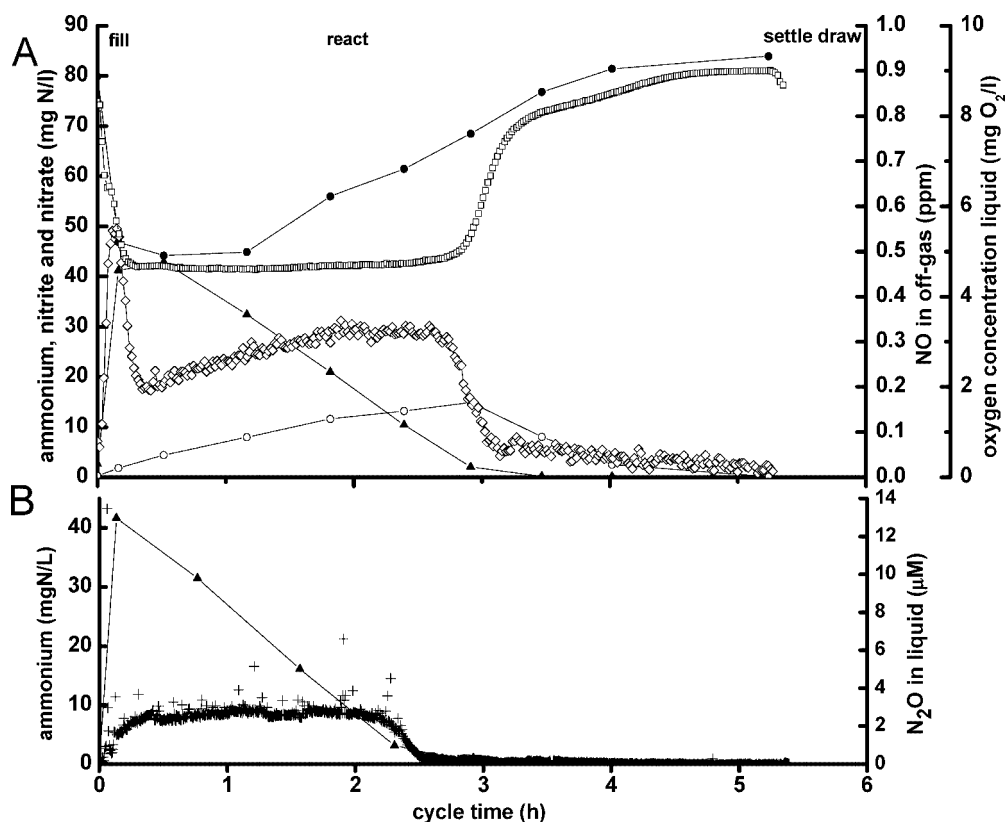


FIGURE 1. (A) Concentrations of ammonium (\blacktriangle), nitrite (\circ), nitrate (\bullet), oxygen (\square), and NO in the gas (\diamond) during one typical SBR cycle (6 h) after the establishment of a steady-state N-conversion. (B) N_2O in liquid (+) and ammonium (\blacktriangle) during another cycle (for practical reasons NO and N_2O could not be determined in the same cycle).

965 mL of water) was fed during the fill period and removed during the effluent discharge period, resulting in a hydraulic retention time of 0.5 days. During the fill and reaction phase, aeration was provided ($Q_G = 0.8$ L/min), stirring speed was 220 rpm.

The nitrogen load of the reactors was $0.6 \text{ kg N m}^{-3} \text{ day}^{-1}$. The concentrated medium contained NH_4Cl (11.45 g/L), NaCl (5.26 g/L), $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (2.07 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.985 g/L), $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ (0.147 g/L), KCl (0.835 g/L), yeast extract (10 mg/L), and trace elements solution (described previously (13); 0.3 mL/L). When yeast extract was omitted, poorly settling sludge was obtained. pH control at 7.0 was established using Na_2CO_3 (0.25M) as a base (and carbon source) and HCl (0.5M) as acid.

The two reactors were continuously monitored by online measurement of dissolved oxygen (DO), pH, Na_2CO_3 dosage, and NO and NO_2 concentration in the off-gas (by Rosemount Chemiluminescence NO_x analyzer). During several cycles MLSS (mixed liquor suspended solids), ammonium, nitrite, and nitrate were analyzed off-line, by respective standard filtration methods and standard spectrophotometric methods (Dr. Lange spectrophotometry kit). Particle size distribution of the sludge was analyzed as in ref (14) and N_2O was measured in the liquid phase by a Unisense N_2O microsensors and in the gas phase via Agilent 6890 GC system. The gas compounds were separated with a HP Porapak Q Column (1 m \times 2 mm i.d.) and nitrogen was applied as carrier gas at a flow of 25 mL min^{-1} . The separated compounds were quantified via an electron capture detector. The temperatures of the injector, column, and detector were 125, 60, and 300 $^\circ\text{C}$, respectively.

The microbial population was characterized by fluorescence in situ hybridization (FISH). The used probes were NSO190 (15), NTSPA662 (16), NSE1472 (17), NIT1035 (18),

and a mixture of EUB338, EUB338-II, and EUB338-III (19) at the appropriate formamide concentrations.

Calculations. The NO and N_2O emission rates ($\mu\text{g N/h}$) were calculated from the gas flow rates (Q_G) and the gaseous concentrations (C_G) as $Q_G C_G$. The gas-liquid mass transfer coefficient, $k_{l,a}$, was $16.0 \pm 2.4 \text{ h}^{-1}$ ($n = 4$), calculated from the measured oxygen uptake rate and the actual oxygen concentrations in gas ($C_{O_2,G}$) and in liquid ($C_{O_2,L}$). The oxygen penetration depth in the floc, δ_{O_2} (μm), due to combined diffusion and reaction is calculated by $\delta_{O_2} = \sqrt{2D_{O_2} C_{O_2,L} / q_{O_2}^{\max} C_X}$ assuming a flat geometry and zero-order substrate uptake kinetics (20), where $q_{O_2}^{\max}$ is the maximum specific oxygen uptake rate, derived from the specific ammonium and nitrite oxidizing activities, D_{O_2} is the oxygen diffusion coefficient ($2 \times 10^{-9} \text{ m}^2/\text{s}$), and C_X is the biomass concentration in the floc (assumed 50 g dw/L).

Results

Reactor Performance. A nitrifying mixed culture of bacteria was established in a SBR-type bioreactor. The operational conditions (SRT, N-concentration, temperature) were chosen to mimic as much as possible the cultivation conditions of a nitrifying population in a plug-flow activated sludge sewage treatment plant. Within one month after inoculation stable conversions of ammonium and nitrite were established. The two identically operated reactors were similar in nitrifying activities. Figure 1 shows a typical example of the concentration profiles measured during one SBR cycle, after stable N-conversions were achieved. The ammonium typically was completely oxidized after 3 h. Nitrite accumulated up to 15 mg N- NO_2^-/L after 3 h and was completely oxidized to nitrate after 4 h. The operational performance of the system was very stable and all process

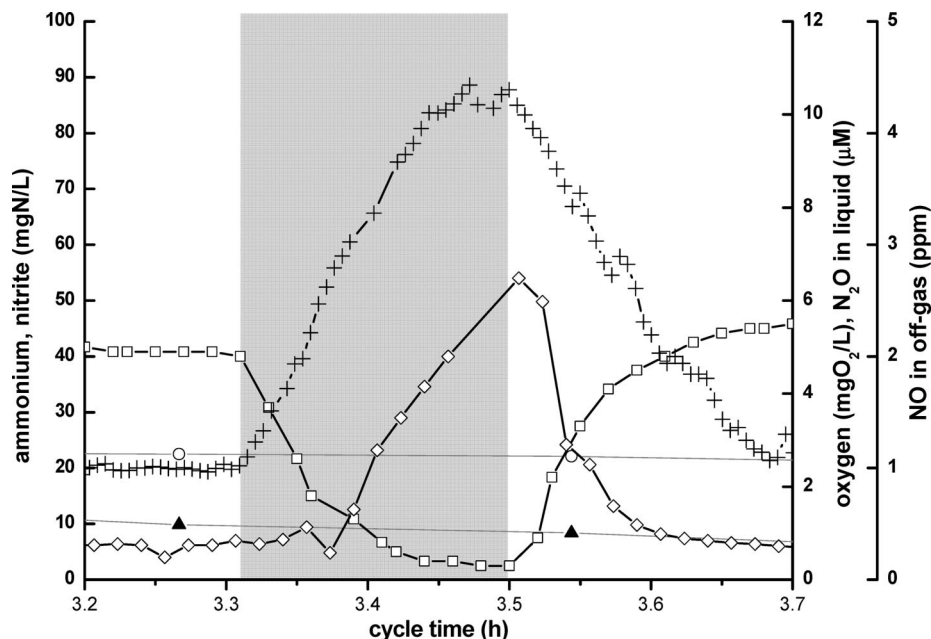


FIGURE 2. Increased NO/N₂O emissions in an oxygen depletion experiment (the shaded area depicts the period in which air was replaced by N₂). Symbols: concentration of ammonium (▲), nitrite (○), oxygen (◇), and NO (◊) and N₂O (+). Separate off-gas N₂O analyses yielded 200 ppm when oxygen was depleted, compared to 25 ppm N₂O at stable normal aeration.

rates were comparable throughout the experimental period of more than 8 weeks described here.

Microbial and Kinetic System Characterization. A constant sludge concentration in the reactors was established by sludge removal, resulting in a SRT (corrected for effluent suspended solids) of both reactors of 9.2 ± 2 days. The sludge content of reactors A and B throughout the experimental period was, respectively, 0.20 ± 0.04 g dw/L ($n = 7$) and 0.18 ± 0.06 g dw/L ($n = 8$). The biomass had a tendency to attach to the reactor walls. Biofilm formation was minimized by periodic mechanical removal, which did not influence reactor performance. The sludge predominantly grew in the form of flocs with a particle size distribution in the range of 1–100 μm , with a mass-based average diameter of 46 μm ($n = 1500$).

Characterization of the biomass composition using FISH and microscopy revealed that the culture contained AOB, NOB, and a minor amount of other eubacteria (see Supporting Information). The AOB are most likely ammonium-oxidizing *beta*-proteobacteria. The NOB most likely belong to the cluster of *Nitrospira*-like organisms.

In nitrifying mixed cultures like those described in this paper, the presence of heterotrophic microorganisms cannot be excluded based solely on the use of autotrophic growth medium. The organic carbon necessary for heterotrophic bacteria may originate from yeast extract in the influent. The estimated maximum amount of biomass that may originate from growth on yeast extract is limited to 0.0032 g dw/L, representing no more 1.5% of the total biomass in the system. Additionally, heterotrophic growth can occur on decaying biomass and influent impurities.

Both the ammonium and nitrite oxidation rates were constant during one SBR cycle (the substrate decrease and product increase was linear). Exceptions were the short lag phase just after feeding and a slower conversion at the end due to low substrate concentrations. The volumetric nitrite oxidation rate was lower than the ammonium oxidation rate, which explains the nitrite accumulation in nitrifying SBRs as observed also by others (21).

To allow comparison with other bioreactor studies, values for the biomass specific maximum substrate uptake rates (q^{max} values) and yields (Y) for AOB and NOB were roughly estimated (see Supporting Information) at $q_{\text{AOB}}^{\text{max}} =$

$120 \text{ mg N} \cdot \text{g}_{\text{dw}}^{-1} \cdot \text{h}^{-1}$, $Y_{\text{AOBN}} = 0.07 \text{ g } X_{\text{dw}} \cdot \text{g N}^{-1}$ and $q_{\text{NOB}}^{\text{max}} = 247 \text{ mg N} \cdot \text{g}_{\text{dw}}^{-1} \cdot \text{h}^{-1}$, $Y_{\text{NOBN}} = 0.02 \text{ g } X_{\text{dw}} \cdot \text{g N}^{-1}$.

NO and N₂O Emissions. The NO emission from the bioreactors during an SBR cycle showed a regular pattern: a NO peak occurred upon addition of the influent, which was followed by a lower constant NO production (Figure 1). The NO emission seemed to increase slightly upon increase in nitrite concentration. As soon as ammonium was consumed the NO emission dropped. The NO emission rate during stable ammonium oxidation was 0.025–0.035% of the ammonium uptake rate. The measured NO concentration during stable ammonium conversion ranged between 0.2 and 0.4 ppm. This corresponded to a calculated liquid NO concentration of 0.15–0.30 $\mu\text{g N/L}$. The liquid concentrations for NO were derived using a steady-state liquid mass balance (during the reaction phase the gas–liquid transfer equals the NO production rate). The NO gas concentration increased proportionally when the gas flow was decreased to 0.5 L/min. NO₂ was always below the detection limit (0.05 ppm) in these experiments.

Periodic analysis of the off-gas for N₂O showed a stable N₂O concentration of 25 ppm during ammonium oxidation. Like NO, the N₂O production ceased when ammonium was depleted (Figure 1). The N₂O emission rate during stable ammonium oxidation yields 2.8% of the ammonium oxidation rate. The liquid N₂O concentration during stable ammonium oxidation was approximately 65 $\mu\text{g N/L}$.

Factors Influencing NO Emission from Nitrifying Cultures. *Oxygen Concentration.* Oxygen limitation due to diffusion limitations in sludge flocs is known to promote NO emission from nitrifiers (10). We calculated the penetration depth for oxygen in the sludge flocs, indicating that during normal reactor operation oxygen limitation was not likely to occur in the major fraction of the sludge flocs. The penetration depth was estimated to amount 65 μm , which is larger than the average floc radius of 23 μm .

The effect of oxygen limitation during ammonium oxidation on NO and N₂O emission was tested by replacing the air with nitrogen gas. NO and N₂O increased 7-fold and 5-fold, respectively, upon oxygen depletion (Figure 2), while am-

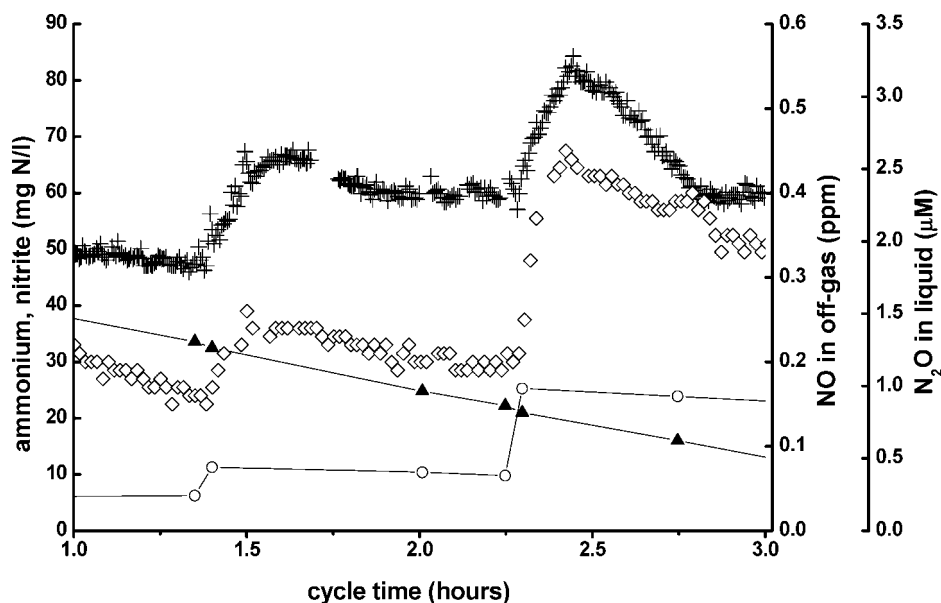


FIGURE 3. Nitrite pulses (5 and 15 mg N NO_2^-/L) in the aerobic ammonium oxidation phase lead to increased NO and N_2O emissions. Symbols: concentrations of NO in gas (\diamond) and N_2O (+), ammonium (\blacktriangle), and nitrite (\circ) in liquid. The oxygen concentration was constant at 50% (4.3 mg dissolved O_2/L).

monium and nitrite concentrations remained almost constant. The N_2O level in the liquid increased immediately after switching to N_2 , whereas the NO emission occurred only when the oxygen concentration reached a significantly lower level. Moreover, the response of NO and N_2O production upon changes of both nitrite and ammonium concentration differed for aerobic and anaerobic conditions, as shown by the experiments presented in the next paragraphs.

Ammonium Concentration. Ammonium is an important factor determining the NO and N_2O emissions, both under aerobic and anaerobic conditions. Under aerobic conditions ammonium needed to be present for NO emission from nitrifying cultures, as could be concluded from the cease in NO emission as soon as ammonium was depleted, but oxygen, nitrite, and nitrate were still available (Figure 1). Gradual changes in the ammonium concentration did not influence the NO emission; the decrease in ammonium concentration during ammonium oxidation was not reflected in the NO emission profile until the ammonium concentration was very low. However, it was found that pulse-wise ammonium addition after its absence resulted in a high NO peak, as it is the case at the beginning of the SBR cycle (Figure 1). The peak appeared relatively slowly, with the highest level being reached after 8 min. The height of the NO peak increased with the amount of pulse-wise added ammonium and with the nitrite concentration present during the pulse (Figures II and III in Supporting Information). Notably, when ammonium was absent in aerobic conditions, no NO was emitted upon nitrite pulses. Stripping the liquid phase prior to ammonium addition showed that the NO peak was not caused by NO accumulation during the settling phase. Measurements showed also an increase in N_2O production upon an ammonium pulse (data not shown).

Anaerobic conditions and the presence of ammonium and nitrite stimulate NO production as can be seen from Figures 3 and 4. The anaerobic NO emission was approximately 15 times higher compared to aerobic NO emission, in presence of ammonium (see Figures 3 and 4). Ammonium presence resulted in a four times higher NO emission in anaerobic conditions (see Figure 4). The response pattern of the NO emission when nitrite pulses were supplied in anaerobic conditions also depended strongly on the presence of ammonium. In presence of ammonium, a nitrite pulse resulted in a NO peak and a slightly higher NO level

after the peak. In absence of ammonium, NO production decreased first and subsequently increased to a slightly higher level (Figure 4).

Nitrite Concentration. An increase in nitrite concentrations resulted in increased NO formation if ammonium was present in the reactor medium. Step-wise addition of nitrite during aerobic ammonium oxidation gave higher NO and N_2O production (Figure 3). The response in NO formation seemed to be linearly proportional: the larger the nitrite pulse, the larger the corresponding NO peaks. Figure 5 summarizes the proportional relation between the nitrite concentration and the NO concentration in the off-gas, during both gradual nitrite accumulation during a normal cycle and nitrite pulse addition. The raised nitrite concentrations in the reactor did not have an inhibiting effect on the ammonium oxidation rate, since the ammonium concentrations decreased at the same rate as before (Figure 3). The influence of oxygen limitation on the effect of nitrite pulses was large. Similar to aerobic conditions, the NO production increased in response to nitrite pulses under anaerobic conditions, but the level of emission is higher (Figure 4).

Discussion

Which Microorganisms and Pathways Are Responsible for the NO and N_2O Emission in a Nitrifying Mixed Culture?

In theory, in a nitrifying mixed culture NO and N_2O could be produced by ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), and heterotrophic microorganisms. Ammonium presence appeared crucial for NO emission under aerobic conditions. When ammonium was depleted, NO emission immediately ceased and nitrite pulses only resulted in NO emission when ammonium was present. Also during anaerobic conditions the NO emission is much higher when ammonium is present. The importance of ammonium presence for NO emission indicates that AOB are the main source of NO production in these nitrifying cultures.

The next important issue that was addressed was the identification of the possible pathway in AOB responsible for NO emission. This could be either aerobic ammonium oxidation or (nitrifier) denitrification. Under aerobic conditions ammonium is oxidized via hydroxylamine to nitrite. During the oxidation of hydroxylamine by the hydroxylamine oxidoreductase low amounts of NO and N_2O can escape, possibly from unstable intermediates (22). On the other hand,

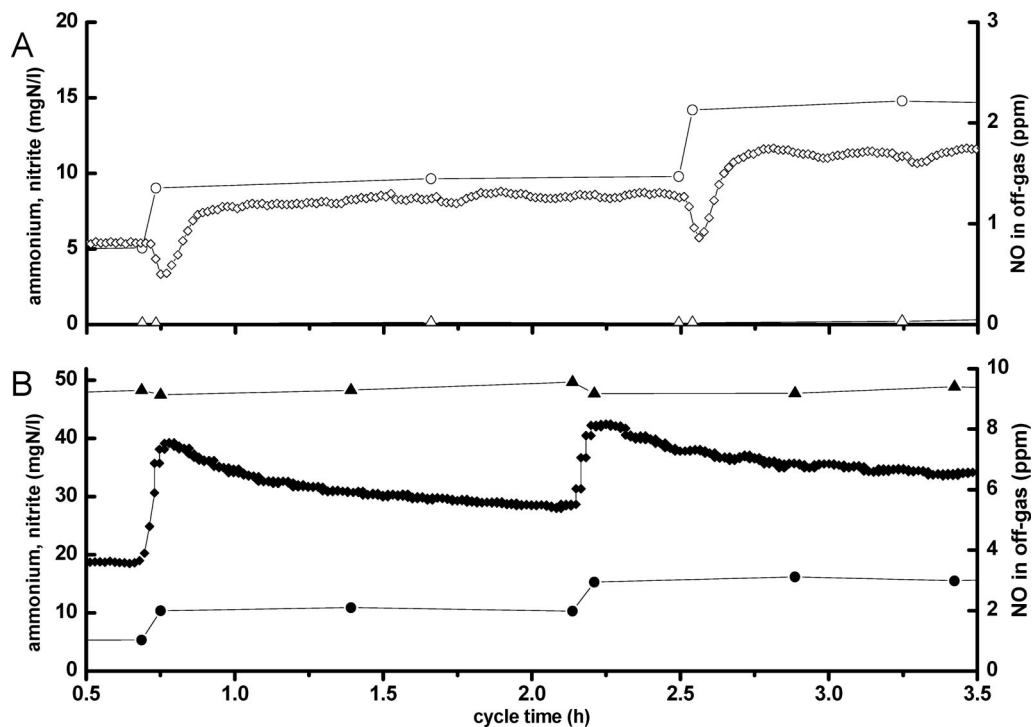


FIGURE 4. Nitrite pulses (5 mg N NO₂⁻/L) in anaerobic experiments with and without ammonium present both lead to increased NO emissions: (A) without ammonium, concentrations of ammonium (Δ), nitrite (○), and NO (◇); (B) with ammonium, concentrations of ammonium (▲), nitrite (●), and NO (◆). Note the different y-scales in the two subgraphs.

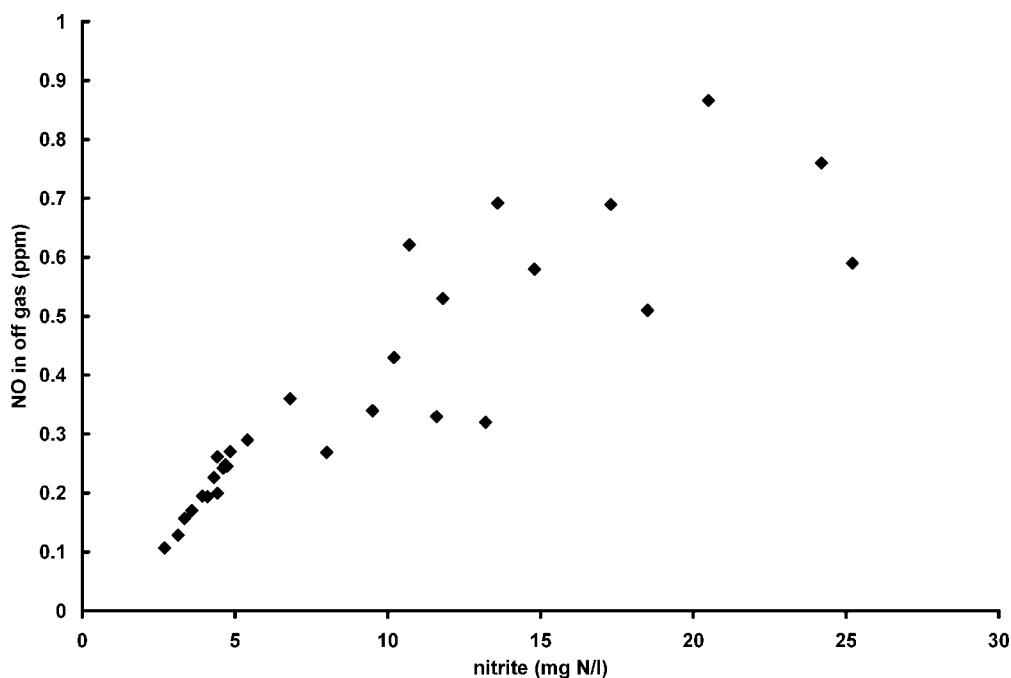


FIGURE 5. NO concentration in the off-gas increases with the nitrite concentration in the bulk liquid. Data shown are from six different SBR cycles spread over 4 months.

AOB possess denitrification enzymes from nitrite until N₂O, while the enzymes responsible for nitrate and N₂O reduction were not detected in their genomes (23). The electron donor for the denitrification pathway of AOB in the present study was ammonium. It has been described that the denitrification pathway of AOB is generally stimulated by oxygen limitation (24).

Our data supported the denitrification pathway in AOB as the main source of NO emission, because of (i) the increase in NO emission upon nitrite pulses (Figure 3), (ii) the increase in NO and N₂O emission upon oxygen

limitation (Figure 2), and (iii) the fact that nitrite presence (the substrate for denitrification) accelerates the appearance of the NO peak at the start of a SBR cycle (Figure III Supporting Information). In a separate paper we have used a modeling approach to demonstrate the dominant role of NO emission by denitrification in AOB (13). This coincides well with other studies which suggested that the denitrification pathway in AOB was the main source of the NO and N₂O emissions from AOB (10). It has furthermore been reported that the tolerance to nitrite of *N. europaea* was dependent on the activity of nitrite reductase NirK

(25), which was another indication that the NO peaks induced by nitrite pulses in this study could indeed be caused by nitrite reduction. In theory, NO and N₂O could also have been formed chemically from nitrous acid or hydroxylamine (26), but this explanation is highly unlikely because in such case no ammonium dependency would occur.

NO and N₂O Emission from Nitrifying Cultures in Response To Dynamic Process Conditions. The experiments have clearly shown that the metabolism of the nitrifying culture responded to rapid changes in concentrations of ammonium, oxygen, and nitrite by an elevated NO and N₂O emission, compared to emission during steady-state conversions. From Figure 1 it can be seen that the NO peak after substrate feeding corresponds to a lag period required to initiate ammonium degradation. Only approximately 30 min after supply of substrate, a stable ammonium oxidation rate was established. This suggests that AOB may rapidly lose their capacity to oxidize ammonium (27). The time between ammonium depletion and the supply of a next feed was approximately 3 h in the SBR systems investigated. The NO peak seemed to be correlated to the recovery of ammonium oxidation capacity after starvation, as described for *N. eutropha* by Zart et al. in ref (11). The peak appears relatively slowly when nitrite is absent, which indicates the nitrite first needed to be formed by ammonium oxidation. This is in good agreement with the observation that nitrite could increase the initial ammonium oxidation activity in ammonium deprived *N. europaea* (28). The increased NO formation when ammonium was present under anaerobic conditions (during which ammonium is actually not consumed), indicated that ammonium also might play a role in the regulation of nitrite reduction in AOB.

Comparison of Data To Pure Culture and Field Studies. The nitrifying community that developed in the SBRs corresponded reasonably well to the organisms that dominated in WWTPs and soils. The AOB in the nitrifying reactors belong to the beta-subclass of Proteobacteria, including *Nitrosomonas* and *Nitrospira* species. AOB diversity surveys in WWTPs indicated that *Nitrosomonas* species were the dominant species (29). The NOB in this study belong to the *Nitrospira* genus, which were also found to be the dominant nitrite-oxidizing species in WWTPs and soils at low nitrite concentrations (30). The calculated maximum substrate uptake rates and yields for the AOB and NOB correlate well with other reported values (31). The actual SRT in the bioreactors was controlled at values relevant for conventional WWTPs.

In our studies the fraction of N converted that was emitted as NO (~0.03%) was low compared to pure culture AOB studies, which reported 0.32–0.87% of the ammonium oxidized to be converted to NO (1, 9). This can potentially be explained by the much higher nitrite concentrations in those pure culture studies (no NOB or denitrifiers present), which would result in increased NO production. However, the comparison is impeded by many other parameters that also differed such as temperature, ammonium concentration, pH, growth rate and, obviously, presence of other microorganisms.

The level of N₂O emission reported in pure culture AOB studies varied from 0.05 to 3.3% (10, 32) as compared to 2.8% found in this work. The N₂O emission from pure culture AOB was generally lower than the NO emission (1, 9), which differs from our findings in a nitrifying mixed culture.

It is difficult to find good data in the literature on NO and N₂O emissions from full-scale installations. In a full-scale nitrifying WWTP, the N₂O emission was 0.1–0.4% of the total ammonium conversion (7), which is smaller than our findings (~2.8%). However, as heterotrophic denitrification took place simultaneously in the WWTP, N₂O produced may have been consumed simultaneously. The NO emission in our studies

correlated well with the NO emission measured in a pilot WWTP, performing simultaneous nitrification and denitrification, where 0.02% of the converted ammonium was emitted as NO (33). Regrettably, no studies reported both NO and N₂O measurements during nitrification under WWTP-relevant conditions. When measured simultaneously in a denitrifying system, the overall emission of N₂O was larger than that of NO (34).

Environmental Impact and Translation To Practice. From the observations made in this study it can be concluded that NO and N₂O emission from systems where biomass is exposed to dynamic changes of nitrite, ammonium, and oxygen concentrations will be underestimated when emissions are estimated based on steady-state measurements only. Sharp concentration changes are established when biomass is transferred between reactor compartments (aeration tank, denitrification reactor, settler) or in plug-flow reactor systems. Denitrifying organisms also increased N₂O, and to a lesser extent NO, production upon changes in nitrite and dissolved oxygen concentration (34). Other potentially important sources of NO and N₂O emissions in WWTPs are the new treatment methods that have been developed recently for treatment of high ammonium wastewater often originating from sludge treatment (35). Due to the accumulation of nitrite and the high ammonium concentrations in these systems, significant NO and N₂O emissions are anticipated. Even though these nutrient removal strategies have improved the effluent quality and the treatment efficiency of WWTPs, we suggest that there is a potential risk of displacement of the environmental problem from water bodies to the atmosphere. Additional measurements in WWTPs that do consider the system dynamics are required to quantify this risk.

From the SBR-grown nitrifying cultures used in this study, 0.03% of ammonium was emitted as NO and 2.8% was emitted as N₂O during the stable ammonium conversion phase. The emission was predominantly generated in the denitrification pathway of AOB. Steady-state emission levels of NO and N₂O may lead to underestimation of the total emission of a WWTP because concentration changes of ammonium, nitrite, and oxygen have been shown to cause a dramatic rise in NO and N₂O. The NO emission increased up to 5-fold upon a sudden increase in ammonium and nitrite concentrations. Oxygen limitation boosted the NO and N₂O emission to a similar extent. The experimental conditions applied in this study are close to those encountered in plug-flow activated sludge treatment plants, and the NO and N₂O emissions measured here could be used as an indication for the emissions by AOB in these systems.

Acknowledgments

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Supporting Information Available

FISH image of a nitrifying sludge granule from reactor A; analysis of the biomass composition; estimation of kinetic parameters of nitrifiers; ammonium pulses at the start of an SBR cycle lead to increased NO emission; and increased nitrite concentrations lead to quicker and higher NO emission. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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