

Model assisted identification of N₂O mitigation strategies for full-scale reject water treatment plants

M. Beier , I. Feldkämper and A. Freyschmidt


ABSTRACT

In a 3-year research project, a new approach to forecast biological N₂O formation and emission at high-strength reject water treatment has been developed (ASM3/1_N₂O_{ISAH}). It was calibrated by extensive batch-tests and finally evaluated by long-term measurement campaigns realized at three wastewater treatment plants (WWTPs) with different process configurations for nitrogen removal of reject water. To enable a model application with common full-scale data, the nitrification-connected supplementary processes that are responsible for N₂O formation are not depicted in the model. Instead, within the new model approach the N₂O formation is linked to the NH₄-N oxidation rate by defining specific formation factors $[N_2O-N_{form}/NH_4-N_{ox}]$, depending on the concentrations of NO₂ and O₂ as well as the NH₄ load. A comparison between the measured and the modeled N₂O concentrations in the liquid and gas phase at the full-scale treatment plants prove the ability of the proposed modelling approach to represent the observed trends of N₂O formation, emission and reduction using the standard parameter set of kinetics and formation factors. Thus, enabling a reliable estimation of the N₂O emissions for different operational conditions. The measurements indicate that a formation of N₂O by AOB cannot completely be avoided. However, a considerable reduction of the formed N₂O was observed in an anoxic environment. Applying the model, operational settings and mitigation strategies can now be identified without extensive measurement campaigns. For further enhancement of the model, first results for kinetics of N₂O reduction kinetics by denitrification processes were determined in laboratory-scale batch tests.

Key words | ASM, denitrification, greenhouse gas emission, modelling, nitrous oxide, reject water

HIGHLIGHTS

- A practical oriented model for the estimation of N₂O emission has been developed relying on N₂O formation factors.
- The model is successfully validated based on several data sets from full scale WWTP with different process configurations.
- Operational mitigation strategies were evaluated applying the new model.
- The measurement results show a significant N₂O reduction potential of the denitrification process.

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INTRODUCTION

Motivation and state of the art

Nowadays, anaerobic treatment for carbon removal and deammonification for nitrogen removal (partial nitrification + anaerobic ammonium oxidation) are two well-established processes that allow energy-efficient wastewater treatment. These two processes can be combined for the valorization of carbon to biogas and a maximum of energy saving. For the evaluation of the overall CO₂ equivalent emissions, the direct release of N₂O has to be additionally addressed due to its high global warming potential (=298 CO₂ eq; IPCC 2013). Depending on the nitrification process, specific N₂O emissions, described as a percentage of emitted N₂O-N per eliminated NH₄-N, are observed in a wide range from 0 to 25% (Beier & Seyfried 1994; ReLaKo 2015). Especially, in the case of high strength industrial wastewater treatment, increased N₂O emissions are linked to high NH₄-N conversion rates, high temperatures and high NO₂ concentrations. Depending on the energy demand of the process being operated, the share of these CO_{2,eq} emissions can reach up to 70% of total emissions (Trautmann *et al.* 2013). Therefore, direct N₂O emissions are an important factor that should be taken into consideration while designing and operating biological treatment plants for high strength wastewaters.

The various processes of N₂O formation and emission have been extensively researched over the last decade. Thus, increasing the understanding of the bio-catalytic pathways and the enzyme-based mechanisms. However, the transfer of these findings to practical wastewater treatment plant (WWTP) operation is still scarce. The present study aims to provide a dynamic model-based approach for the evaluation of plant-specific emissions, their causes, and the identification of operational strategies for efficient N₂O reduction.

N₂O formation during nitrification

During nitrification, N₂O is formed by the activity of ammonium-oxidizing bacteria (AOB) (Kampschreur *et al.* 2008); in particular, the autotrophic denitrification and the incomplete hydroxylamine oxidation have to be named as causative metabolism pathways (Guo *et al.* 2018). Nitrite-oxidizing bacteria (NOB) and anaerobic ammonium-oxidizing bacteria (AAOB) do not contribute to N₂O formation (Starkenburger *et al.* 2008; Kartal *et al.* 2011).

Autotrophic denitrification is reported to be induced by limited O₂ availability as well as high nitrite concentrations.

The specific impact of dissolved oxygen (DO) on N₂O formation cannot definitively be characterized. Schneider (2013) did not detect an increase in N₂O formation at low DO concentrations in the nitrification zone. However, other authors observed a high concentration of N₂O in the liquid phase, when available DO is limited. Chandran *et al.* (2011) describe that limitation of oxygen supply as well as high nitrite concentrations would support N₂O formation via autotrophic denitrification. These authors also report that under aerobic conditions, a sudden increase of substrate supply leads to N₂O production from hydroxylamine.

N₂O generation due to high NO₂-N concentrations is affected by adaption processes. While N₂O formation in low-loaded systems can occur at nitrite concentrations below 15 mg NO₂-N/L (Wunderlin *et al.* 2012), high-loaded systems can deal with remarkably higher NO₂-N concentrations. Investigations of Schneider (2013) did not reveal N₂O formation at concentrations below 100 mg NO₂-N/L in high-strength wastewater. However, up to concentrations of 150 mg NO₂-N/L, a sharp increase in N₂O formation can be observed before reaching a constant level.

In general, N₂O formation is correlated with NH₄ conversion (Schneider 2013). An increase in ammonium concentrations results in higher ammonium oxidation rates, which lead to high N₂O formation factors. It should be highlighted that higher ammonium concentrations themselves do not induce any changes in the specific N₂O formation factor [N₂O-N_{formed}/NH₄-N_{ox}] (Schneider *et al.* 2014). Also, the authors reported that the pH-related NH₃ concentration is the critical factor in terms of N₂O formation. On the other hand, Law *et al.* (2011) did not report any distinct relation between N₂O formation and concentrations of NH₃ or HNO₂. However, their findings indeed confirm that N₂O enrichment occurs under an increase of pH from 7 to 8 resulting in an increased ammonium oxidation rate. At pH values above 8, N₂O formation decreases, supposedly caused by inhibition of AOB (again in accordance with Schneider 2013). Additionally, unstable operational conditions, like an increase in NH₄ load or a decrease of the sludge retention time, are linked to high N₂O generation (Kampschreur *et al.* 2008; Sander *et al.* 2013; ReLaKo 2015).

N₂O formation during denitrification

N₂O is an intermediate product of heterotrophic denitrification, which is formed during the metabolic reduction of

nitrate to dinitrogen by using organic carbon as an energy source. Each step is catalyzed by a specific enzyme (Eitinger 2017). Accumulation of N₂O during heterotrophic denitrification can be influenced by the inhibition of one or several sub-processes. Current measurements proved that N₂O enrichment during denitrification is usually negligible. Contrarily, N₂O formed during nitrification can be reduced during the denitrification step (ReLaKo 2015; Beier *et al.* 2016).

The evaluation of the specific reduction rates based on the literature reveals that the degradation kinetic of nitrate is lower than nitrite. However, the nitrogen reduction rate for nitrate covers a wide range (Table 1). The literature survey showed the scarcity of data for nitrite and nitrous oxide. Scientific investigations of the last decades have mostly focused on the complete reduction of nitrate to dinitrogen and the evaluation of the influence of different C-sources. Only a few researchers have examined the reduction of process intermediates like nitrite and nitrous oxide (Zhou *et al.* 2008; Pan *et al.* 2013). The range of reduction rates for nitrate, nitrite and nitrous oxide are shown in Table 1.

N₂O modelling

In recent years, increasing efforts have been made to model N₂O formation and reduction within the framework of biological processes and to use these models as a forecasting tool. Depending on the challenges faced, the approaches mainly differ in the N₂O formation pathways and the intermediates included (e.g. autotrophic denitrification, heterotrophic denitrification). In addition, the individual models are characterized by the employed database (batch experiments, large-scale measurements) and derived kinetic parameters. The model expansions were predominantly based on ASM models describing the conversion of COD and N.

N₂O formation in the context of nitrification was depicted firstly as a one-step process (Ni *et al.* 2011; Mampaey *et al.* 2013;

Snip *et al.* 2014). With the advancement in the understanding of metabolic processes, newer models distinguishing the two alternative metabolic pathways via hydroxylamine and autotrophic denitrification are suggested (Ni *et al.* 2014). Furthermore, the integration of N₂O accumulation in heterotrophic denitrification can be implemented as a three- (Schulthess & Gujer 1996) or four-step process (including NO) (Kampschreur *et al.* 2007; Hiatt & Grady 2008; Ni *et al.* 2011). Other approaches integrate an indirect coupling of electrons like ASM_ICE by Pan *et al.* (2015).

For all models, uncertainties result from insufficient knowledge about the biological formation processes. Moreover, kinetic parameters still need to be evaluated and more process data sets for calibration and validation are required (Sweetapple *et al.* 2013; Ni & Yuan 2015).

Objective

The main objective of this study is to transfer the theoretical knowledge of N₂O formation and emission processes to a full-scale practical application by developing a dynamic model. Since complex measurement technology is required for N₂O measurement, calibration of the model can be difficult to realize in practice. For this reason, N₂O emissions are calculated only relying on parameters usually measured at WWTPs. To evaluate the performance of the model when using the standard parameter set, three selected WWTPs with different side-stream treatment procedures were modeled without changing the parameter set of kinetics and formation factors. The simulation outcomes were compared with the results from full-scale measurements. For the minimization of N₂O emissions, different constructional and operational measures were developed and tested.

MATERIALS AND METHODS

Basic model

The presented concept for the determination of N₂O emissions is embedded in an expanded ASM model that was developed at the Institute of Sanitary Engineering and Waste Management to depict the deammonification (ASM3/1_DEAM_{ISAH}). The conventional ASM1 and ASM3 models do not distinguish between nitrate-nitrogen and nitrite-nitrogen as well as nitrite-oxidizing bacteria and nitrate-oxidizing bacteria. However, for the modelling of the deammonification process, it is necessary to describe nitrification as a two-step process. Therefore, the SNO

Table 1 | Literature-based reduction rates for nitrate, nitrite and nitrous oxide

Parameter	Reduction rate [mg N/ (g VSS ⁻¹ h)]	References
NO ₃	0.18–49	Prakasam & Loehr (1972); Lee & Welander (1996)
NO ₂	0.4–74	Lee & Welander (1996); Vogel (2018); Pan <i>et al.</i> (2013)
N ₂ O	3.9–349	Zhou <i>et al.</i> (2008); Pan <i>et al.</i> (2013)

fraction is divided into a SNO₂ fraction and a SNO₃ fraction. Moreover, the nitrite-oxidizing bacteria group (X_AOB) and the nitrate-oxidizing bacteria group (X_NOB) replace the autotrophic bacteria group XA. The suppression of NOB growth due to intermittent aeration is implemented by introducing a factor f_{LAG} describing the deactivation of NOB within anoxic phases and the delay in reactivation within the aerobic phase depending on the oxygen concentration. Additionally, inhibiting effects of HNO₂ and NH₃ on the autotrophic metabolism are taken into account by introducing inhibition factors. However, these factors are not fixed for different bacteria groups but have to be confirmed by measurements due to adaption processes in dependence on operational conditions. These implementations enable to use ASM3/1_DEAM_{ISAH} for investigation of (insufficient) NOB suppression, too. The process of anaerobic ammonium oxidation is included by adding a fraction X_{AAOB} (anammox bacteria). The respective conversion rate comprises inhibiting effects of nitrite. Due to the division of the SNO fraction, there are two possible electron acceptors for the heterotrophic processes. For this reason, the associated process rates are modified, so that both nitrogen oxides can be employed for the heterotrophic metabolism. The decay rates of the autotrophic microorganisms are defined for an aerobic as well as an anoxic environment. According to the conventional ASM1, the decay of these bacteria induces an increase of the XI, XS, and SNH fractions due to the release of COD and nitrogen, thus enabling endogenous denitrification in contrast to the ASM3 approach, where the decayed nitrifiers are 'deactivated'. This turned out to be a very important factor to be included in modelling autotrophic dominated reactor systems like reject water treatment. Due to the low level of easily biodegradable COD in process water, denitrification processes are mainly driven by endogenous respiration, and processes like COD storage and adsorption (included in ASM3) are not necessarily to be included. The model used thus represents a hybrid of ASM1 and ASM3. The detailed Petersen matrix of the model is given in the supplementary material.

Growth and decay rates are adapted to the new bacteria groups and substances. All required kinetic parameters are derived from laboratory-scale batch tests operated with high nitrogen loaded sludge liquor from municipal WWTP (see supplementary material and Table 3 for denitrification kinetics). In this study, no extra calibration or variation of the kinetic parameters was performed when modelling the individual plants.

The software SIMBA# (ifak, Magdeburg, Germany) was used for modelling.

Gas transfer model

Additionally to the formation processes, transport processes have to be depicted for the modelling of the N₂O emissions, too. The formation of N₂O initially occurs in the liquid phase. Since dissolved N₂O can be further converted to N₂ by denitrification processes, the integration of the gas transfer into the process model is crucial for reliable calculations of N₂O concentrations in the liquid phase and the N₂O emissions itself. Modelling WWTP, both processes diffusion and stripping have to be considered for the implementation of the gas transfer. Equation (1) shows how this is transferred to the model:

Diffusion into the gaseous phase is implemented according to Henry's law as a function of the saturation concentration, depending on temperature, salinity and N₂O partial pressure. However, the phase transition is a complex procedure driven by diffusive and convection forces. Summarizing both processes, the mass transfer coefficient k_L is introduced. Additionally, the diffusion rate is influenced by the concentration gradient between the liquid and gas phases, and by the ratio between the surface of the boundary layer between both phases, and finally the volume of the water body. If the N₂O concentration in the atmosphere is above the specific saturation concentration (e.g. when the reactor is covered), diffusion from the gaseous phase to the liquid phase takes place. This relevant inversion of the gas transfer direction from emission to immission is covered by the model as well.

The gas-stripping-driven N₂O emissions are described by adding the term f_A . In this factor, the aeration-related influences like the intensity of bubble aeration (influences the ratio of the boundary layer surface) are summarized, including that the bubble-induced flow promotes the transportation of gaseous N₂O to the water surface. Consequently, introducing higher air volume flows increases the transport capacity and thus N₂O emission.

$$GTR_{N2O} = k_{L,N2O} \times (c_{sat,N2O} - c_{liquid,N2O}) \times \frac{A_{reactor} \times (1 + f_{A,aer})}{V_{reactor}} \quad (1)$$

GTR_{N2O} : gas transfer rate [g/(m³*d)];

$k_{L,N2O}$: mass transfer coefficient of N₂O [m/d];

$c_{sat,N2O}$: saturation concentration of N₂O in the liquid phase [g/m³];

$c_{liquid,N2O}$: present concentration of N₂O in the liquid phase [g/m³];

$A_{reactor}$: reactor surface [m²];

$V_{reactor}$: reactor volume [m³];

$f_{A,aer}$: = 0, if aeration is switched off; =100, if aeration is switched on.

Investigated processes and characteristics of the WWTPs

The full-scale measurements for model evaluation were carried out at three large WWTPs in Germany representing three different types of part stream nitrogen removal systems.

The WWTP Potsdam Nord (**WWTP A**) (90,000 PE) operates a two-step deammonification ($V_{reactor} = 140 \text{ m}^3$ both) with separated sludge cycles (TERRAMOX[®] technology) receiving sludge liquor from dewatering of digested sludge. The process water treatment step is designed for a maximal NH₄-N load of 160 kg NH₄-N/d and a volumetric flow of 3.5 m³/h. NOB are suppressed by intermittent aeration leading to time-controlled aerobic and anoxic phases in the nitrification reactor. Characteristic for two-step processes are the high NO₂-N concentrations in the first reactor (159–208 mg NO₂-N average in Potsdam Nord).

At WWTP Rodgau (**WWTP B**) (85,000 PE), process water from sludge dewatering is treated in a one-step deammonification reactor, realized as a partly covered sequencing batch reactor ($V_{reactor} = 300 \text{ m}^3$). One treatment cycle includes five phases of (i) filling, (ii) aeration (constant or intermittent), (iii) mixing, (iv) sedimentation, and (v) discharging. A specificity of WWTP B is its distinct seasonal variation of NH₄ concentrations in the sludge liquor (summer: 800 mg NH₄-N/L, winter: 1,100 mg NH₄-N/L). Effluent concentrations are in a range of 6.2–12.5 mg NO₂-N/L and 98–150 mg NH₄-N/L. During the measurement campaigns, the system was very low loaded (0.1–0.2 kg/m³/d). However,

it has to be considered that the AOB concentration is relatively low due to poor settleability of the activated sludge, leading to a high bacteria specific sludge load.

The WWTP Rheda-Wiedenbrück (**WWTP C**) receives municipal wastewater (94,000 PE) as well as wastewater from a meat-processing plant (600,000 PE). Although the industrial wastewater stream is pre-treated separately, all sludge streams are dewatered in the same centrifuge. Due to high nitrogen concentrations of up to 3,000 mg N/l, sludge liquor is treated with partial augmented nitrification denitrification with alkalinity recovery (PANDA) procedure including a two-step nitrification/denitrification with external carbon dosage ($V_{nitrification} = 2,014 \text{ m}^3$; $V_{denitrification} = 1,694 \text{ m}^3$), a sedimentation basin, and an internal recirculation (Hartwig et al. 2010).

The general characteristics of the three investigated WWTPs are summarized in Table 2.

Full-scale measurements

N₂O in the liquid and gas phase was determined by employing N₂O wastewater system (Clark-type sensor with an internal reference, a cathode, and a backstop, which contains oxygen reducing medium to prevent oxygen interfering with the nitrous oxide measurements) including automatic temperature correction when calibration temperature and temperature during measurement differ from each other (Unisense, Denmark).

Direct gaseous N₂O emissions were estimated by using a buoyant gas hood ($V = 97 \text{ l}$, $A = 0.39 \text{ m}^2$) immersing approximately 10 cm into the water phase to prevent gas from escaping during turbulences. N₂O concentration inside the gas hood was determined via an N₂O microsensor (Clark-type sensor, Unisense, Denmark). The resulting N₂O emissions were calculated by multiplying this N₂O concentration with the gas volume flow measured with a gas meter at the

Table 2 | General characteristics of the studied WWTPs

WWTP	Process water treatment technology	Inflow		Reactor NO ₂ -N mg/L	Nitrification reactor/phase Aeration O ₂ conc. during aeration
		NH ₄ -N mg/L	COD mg/L		
A	Two-stage deammonification	344–611	305–443	159–208 (nitrification)	Intermittent >2 mg/L
B	One-step deammonification, SBR	800 ^a 1,100 ^b	520 682–850	6.2–12.5 (after aerated phase)	Intermittent 0.6 mg/L Intermittent >1.5 mg/L
C	Nitrification/denitrification (separated spatially)	≥2,000	120–280	464 (nitrification)	Constant >4 mg/L

^asummer period.

^bwinter period.

hood's outlet. Alternatively, the volume of the gas hood was applied, when the outflowing gas volume flow was not determinable (unaerated areas or phases). Overall emissions were computed as a function of the reactor's water surface.

Besides N₂O emissions, N₂O formation and conversion were investigated, too, as these are the actual processes simulated in the biological model and therefore crucial for the validation. However, the determination of formation and conversion rates requires a discontinuous operation mode, which cannot be realized at all sites in full-scale operation. Therefore, bypass measurements were carried out at all WWTPs with continuous aeration. For this purpose, sludge was pumped in a separate covered lab-scale chemostat reactor ($V = 3.6$ l) with intermittent aeration. Concentrations of N₂O (liquid and gas phase), NH₄-N, NO₃-N, and O₂ as well as pH value were continuously measured. If possible, on-site installed measurement devices were used. Otherwise, pH meter SCHOTT HandyLab pH12 and oximeter SCHOTT HandyLab Ox12 were applied. In comparison to bypass measurements, the discontinuous operation mode of the deammonification plant at WWTP B and the intermittent aeration of the nitrification reactor at WWTP A (enables the determination of N₂O formation and conversion rates as well as concentration profiles directly in the tanks). The minimum duration of the respective measurement campaigns was one week.

Batch tests

Due to the high relevance of the denitrification process regarding the reduction of N₂O emissions, the investigations include batch tests with artificial dosing of nitrogen oxide (nitrate, nitrite or nitrous oxide) under non-carbon-limiting conditions to assess the N₂O removal potential by heterotrophic denitrification.

The batch tests were performed in a 3.6 L gas-tight batch reactor. During the batch tests, 3 L of activated sludge from a municipal wastewater treatment plant (NH₄-N = 1.2 mg/L; NO₃-N = 0.3 mg/L; COD = 220 mg/L, SS = 3.9 g/L, VSS = 75%) were continuously mixed. The temperature was set at 20–21 °C and 29 °C. Temperature, pH value and oxygen concentration were continuously recorded by a WTW multimeter hand-held instrument. The N₂O concentration was measured via N₂O microsensors from Unisense A/S in the liquid and gas phases. Additionally, the nitrogen and COD parameters were determined using cuvette tests from Hach.

At the beginning of the experiments, an optimal initial environment (pH = 8, c(O₂) = 0 mg/L) was set in each reactor to avoid an accumulation of nitrous oxide. Allylthiourea

(ATH) was dosed to suppress the activity of AOB. To set an anoxic environment, the reactor was flushed with nitrogen gas. After the preparation of the inoculum setting, the respective nitrogen oxide (NO₃, NO₂, N₂O) was dosed. After 1 h, the substrate acetate was added to enhance maximum heterotrophic denitrification. The concentration gradient was determined based on the linear decrease of the current concentration curve. Only experiments in which no intermediates accumulated were considered.

RESULTS AND DISCUSSION

Extended model for the calculation of N₂O emissions

The ASM3/1_DEAM_{ISAH} model does not include N₂O reduction as a sub-process of denitrification (pathway 2 in Figure 1). Moreover, N₂O formation by AOB and N₂O emission due to diffusion and stripping-related effects could not be depicted so far. With regard to the objectives of this study, these processes were added and a simplified description of the N₂O formation is defined (Figure 1).

N₂O formation by AOB (1)

In contrast to existing modelling approaches, the identified microbiological processes (autotrophic denitrification and the reaction pathway via hydroxylamine oxidation) are not displayed in detail. The main conversion pathways of nitrification and denitrification are maintained, but intermediates are limited to those fractions that are available by on-site measurements on WWTP. Hence, NO and NH₂OH are not included in the presented model approach; neither are the microbiological side pathways of AOB. Instead, N₂O formation linked to AOB activity is described by a 'black-box' model and N₂O formation factors are implemented as a percentage of the converted NH₄-N depending on three influencing factors:

- F_{R,NH_4} : basic formation factor depending on the sludge load and AOB activity
- F_{R,NO_2} : NO₂-related formation factor depending on NO₂-N concentration
- F_{R,O_2} : O₂-related formation factor depending on O₂ concentration

The functions for computing the individual N₂O formation factors found by evaluating several measurement results are shown in Figure 2.

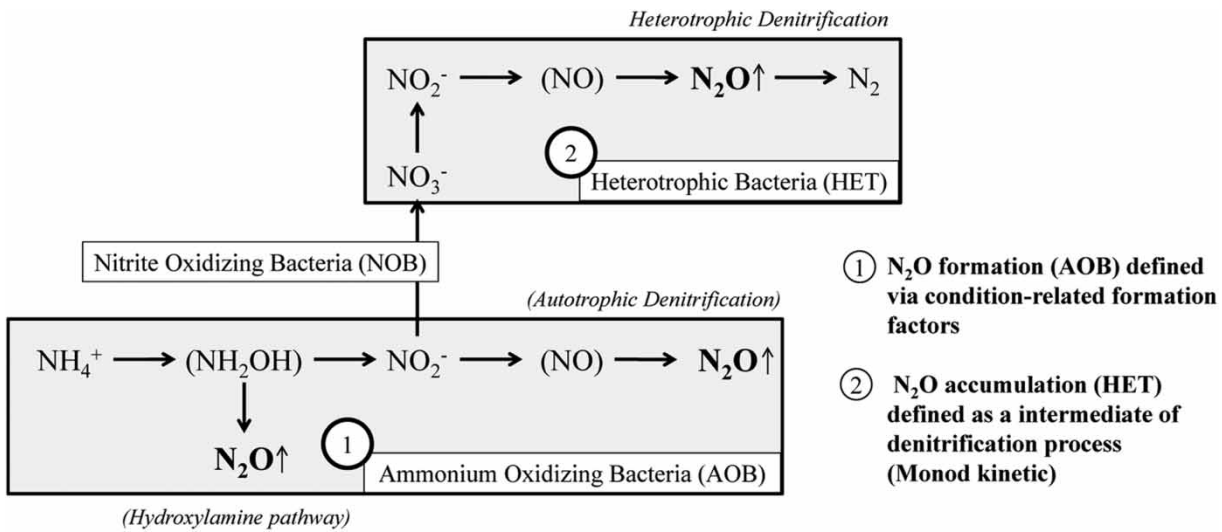


Figure 1 | Scheme of the extended biological model describing the implemented bacteria groups, process parameters and the N₂O formation model concept.

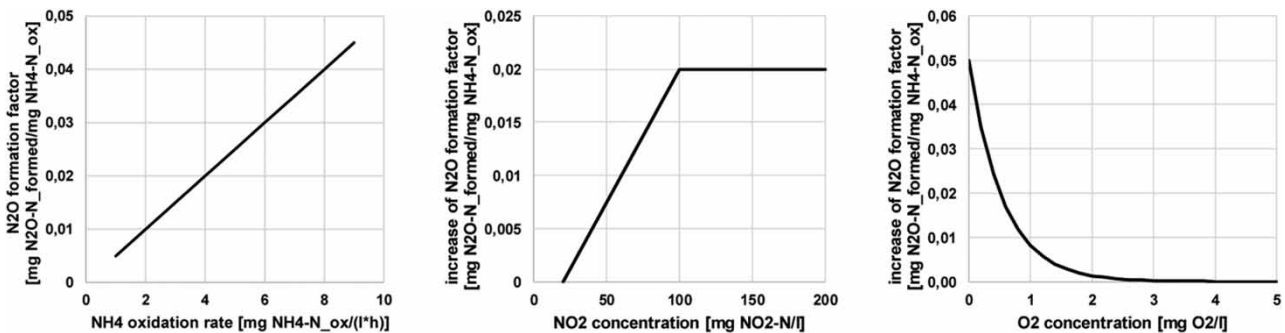


Figure 2 | Functions defined for the description of the N₂O formation depending on NH₄ oxidation rate (left), NO₂ concentration (middle) and O₂ concentration (right).

One basic advantage of this approach is that the formation factors can directly be derived from on-site measurement data. By linking the functions with the ammonium oxidation rate and the concentrations of oxygen and nitrite, the system-specific formation factors are dynamically calculated by summing up the individual formation factors. The model concept using predefined formation factors represents a generally valid approach to determine N₂O emissions applicable at different WWTPs without the need for extensive calibration data.

Denitrification based N₂O enrichment (2)

A three-step denitrification (NO₃ → NO₂ → N₂O → N₂) was implemented neglecting the NO fraction as it usually cannot be measured in lab- or full-scale due to its high reactivity. In the model, it is assumed that NO is immediately converted to N₂O. Based on own lab-scale results, maximum process rates are ranked (N₂O-R > NO₂-R > NO₃-R) and integrated

into process rate equations along with Monod functions for substrate dependency and inhibition effects (inhibition of N₂O-R by HNO₂ and oxygen limitation by low COD/NO_x-N ratios). This enables the calculation of short time N₂O accumulation induced by peak loads. All other denitrification processes (aerobic growth and decay of the heterotrophs) remain unchanged and are still modeled as single-step processes. Contrary to other models, the concentration of HNO₂ instead of NO₂ was implemented to depict the associated inhibitory effect on the N₂O reduction during denitrification. The minimal HNO₂ concentration causing N₂O accumulation was determined to approximately 1.3 µg HNO₂-N/L in own lab-scale tests (Vogel et al. 2016). At this concentration, the N₂O reduction rate corresponded to the NO₂-N reduction rate, whereas N₂O was immediately formed and reduced again. The values for denitrification rates of the sub-processes given in literature vary over a wide range and are not applicable for optimized process control and advanced plant engineering. Therefore, kinetic

parameters of the sub-processes are derived from the parameters of the one-step denitrification process (ASM3/1_DEAM_{ISAH}) by splitting the maximal growth rate to the sub-processes according to the respective share of COD demand (Table 3). Otherwise, the heterotrophic growth rate would be tripled (compared to conventional ASM). Subsequently, the yield factors of the individual sub-processes were iteratively determined to assure that the total biomass yield corresponds to the results from one-step denitrification.

The model was calibrated by modelling laboratory-scale batch tests. Nitrification and denitrification were calibrated separately. Kinetic parameters taken from literature or own laboratory-scale experiments were employed (see supplementary material).

Model evaluation using data from full-scale measurements

The subsequently presented N₂O measurement data from three wastewater treatment plants form the basis for the model evaluation of ASM3/1_N2O_{ISAH}. This data is compared with the calculated concentrations.

WWTP A (two-step deammonification, intermittent aeration): Figure 3 shows the measured N₂O concentration in the nitritation reactor. The N₂O-stripping-effect during the aerated phase as well as the increase of N₂O concentration in the unaerated phase (no stripping) can clearly be

Table 3 | Conversion rates of denitrification sub-processes

Process	Max. growth rate [1/d]	Yield coefficient [g VSS/g N]
NO ₃ -N reduction	0.76	1.89
NO ₂ -N reduction	0.74	1.85
N ₂ O-N reduction	0.31	0.77

identified. During aerated phases, an average N₂O formation factor of 0.05 mg N₂O-N_{form}/mg NH₄-N_{ox} (=5%) was derived from measurement results. For the anoxic phase, a formation factor of 33% was determined. For the whole process, this results in a total formation factor of 27% of oxidized NH₄-N. Even though the denitrification capacity is 10 fold lower than the nitrification capacity, denitrification is the dominant N₂O-formation process. Applying the new ASM3/1_N2O_{ISAH} model, the unexpectedly high N₂O formations during the anoxic phase can be explained as an inhibition effect of the N₂O denitrification process caused by HNO₂ concentrations that occur up to 70 µg HNO₂-N/L. A contrary N₂O formation factor was found in the anammox reactor, even though the reduction of COD in the anammox reactor indicates the presence of heterotrophic bacteria and thus simultaneous denitrification, N₂O was not detected. This agrees again with the stated impact of the HNO₂ concentration. With NO₂-N concentrations below 20 mg/L and a pH around 7, the HNO₂ concentration in the anammox reactor is below the N₂O reductase inhibition threshold and no N₂O enrichment occurs.

Figure 4 exemplarily shows the comparison of measured and simulated values for a short time period. It can be stated, that the simulated N₂O concentration in the liquid phase appropriately replicates the measurement results of the bypass reactor. Moreover, the timing and altitude of N₂O peak concentrations in the gas phase are well depicted. The sudden drop of the N₂O concentration in the gaseous phase when turning off the aeration is an artifact of the bypass measurement initiated by the expulsion of gaseous N₂O from the covered bypass reactor, which is induced by turbulence and unsteady feed. Therefore, the modeled values are more accurate since they only include the diffusion process of N₂O into the liquid phase for periods with zero gas stripping (no aeration).

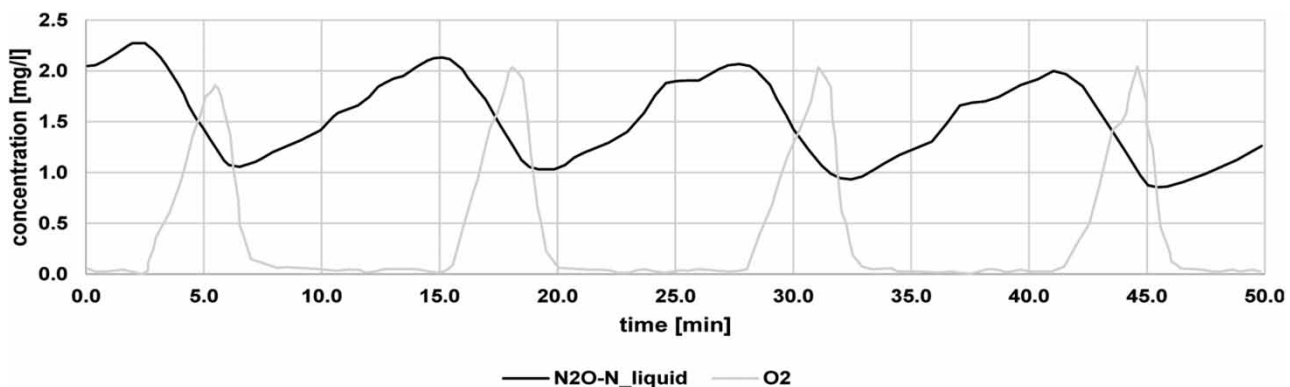


Figure 3 | Exemplary measurement results for N₂O concentrations at WWTP A (nitritation reactor with intermittent aeration).

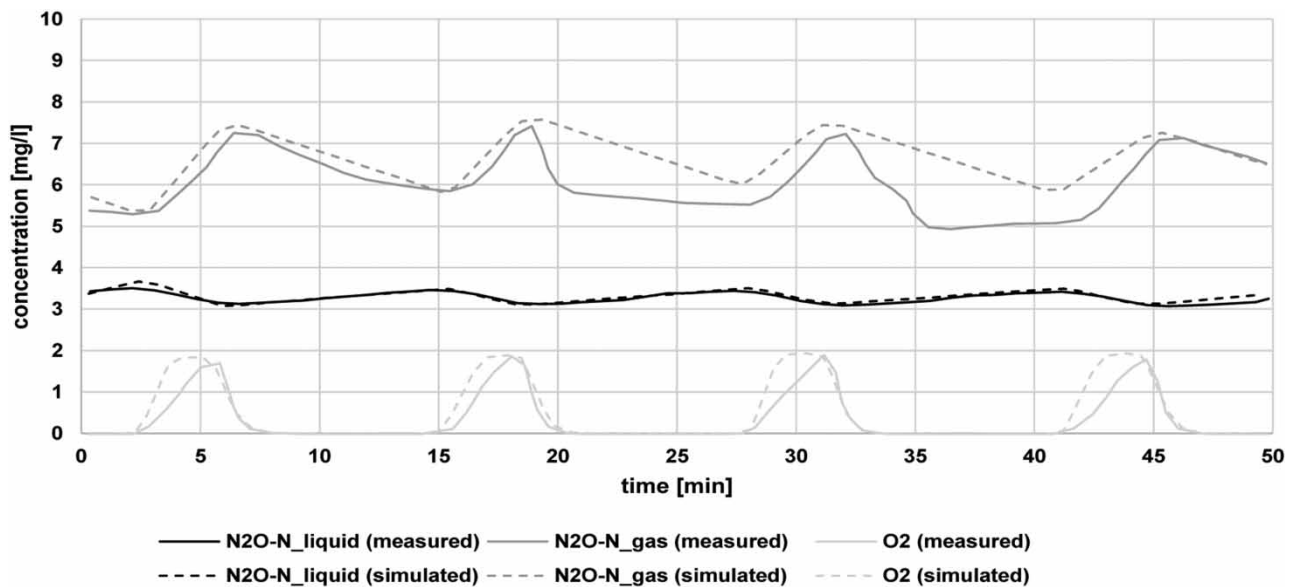


Figure 4 | Excerpt of measurement and model results for the covered bypass reactor at WWTP A (nitrification reactor, intermittent aeration).

WWTP B (one-step deammonification, intermittent aeration): **Figure 5** shows exemplary full-scale measurement results for two different load situations (a) 0.2 kg/m³/d; (b) 0.1 kg/m³/d). The diminishing N₂O concentration in the liquid phase during the anoxic phase indicates a high denitrification rate caused by the relatively high load of degradable COD related to the feed phase of the SBR cycle with the stop of aeration. Consequently, N₂O formation during the anoxic phase was not observed. However, N₂O is formed in a short period of time immediately after the aeration is turned off when there is still O₂ available (no stripping leads to a faster increase of the N₂O concentration in the liquid phase). After oxygen has been consumed, the concentration in the gas phase continues to rise as the saturation concentration in the water phase is exceeded. However, when the N₂O concentration in the liquid phase falls below a certain value (see **Figure 5**), re-transfer from the gas phase begins.

The activity of AOB forces an average N₂O formation of 0.08 mg N₂O-N_{form}/mg NH₄-N_{ox} during the low load period and of 0.12 mg N₂O-N_{form}/mg NH₄-N_{ox} during the high load operation. (The sludge load is classified as “high” in relation to the AOB concentration and not in relation to total bacteria concentration, even though the volume load is comparatively low with 0.1–0.2 kg/m³/d.) An accumulation of N₂O in the liquid phase during nitrification was observed in both periods, even though stripping led to N₂O transfer into the gas phase during aeration.

Regarding the N₂O concentration in the gas phase, the benefit of covering the reactor is evident. With an increase

of the N₂O partial pressure associated with a decreased concentration gradient, the diffusion of N₂O into the gas phase is initially reduced, compared to an open reactor. According to **Figure 5**, N₂O concentrations in the liquid phase below 0.5–0.6 mg/L (a) and 0.2–0.35 mg/L (b) are associated with an N₂O re-transfer to the liquid phase. Under the given boundary conditions (not gas-tightly covered reactor), the saturation concentrations are much higher than the saturation concentration of an open reactor. To emphasize the benefit of covering the reactor, the model was employed to compare the N₂O emissions of an open and covered reactor (**Figure 6**).

WWTP C (two-step nitrification/denitrification, high load and concentration): The average conversion and formation parameters are summarized in **Table 4**. While an N₂O formation of 0.06 mg N₂O-N_{form}/mg NH₄-N_{ox} was determined for nitrification, the formation factor for the denitrification differs between the two measurement campaigns. With high concentrations of NO₂ in campaign 1 (about 160 mg NO₂-N/L), an enrichment factor of 0.2 mg N₂O-N_{form}/mg NO_x-N_{red} was detected while lower nitrite concentrations around 60 mg NO₂-N/L in the denitrification tank enable a sufficient denitrification rate leading to zero-emission. The low N₂O reduction rate during the first measurement campaign can be traced back to the inhibiting effects of HNO₂, which is induced by high NO₂-N concentrations in combination with low pH (compare WWTP A). In both campaigns, the N₂O formation is originally related to the nitrification step (proved by COD removal and modeling analysis). Again, an inhibited denitrification process in

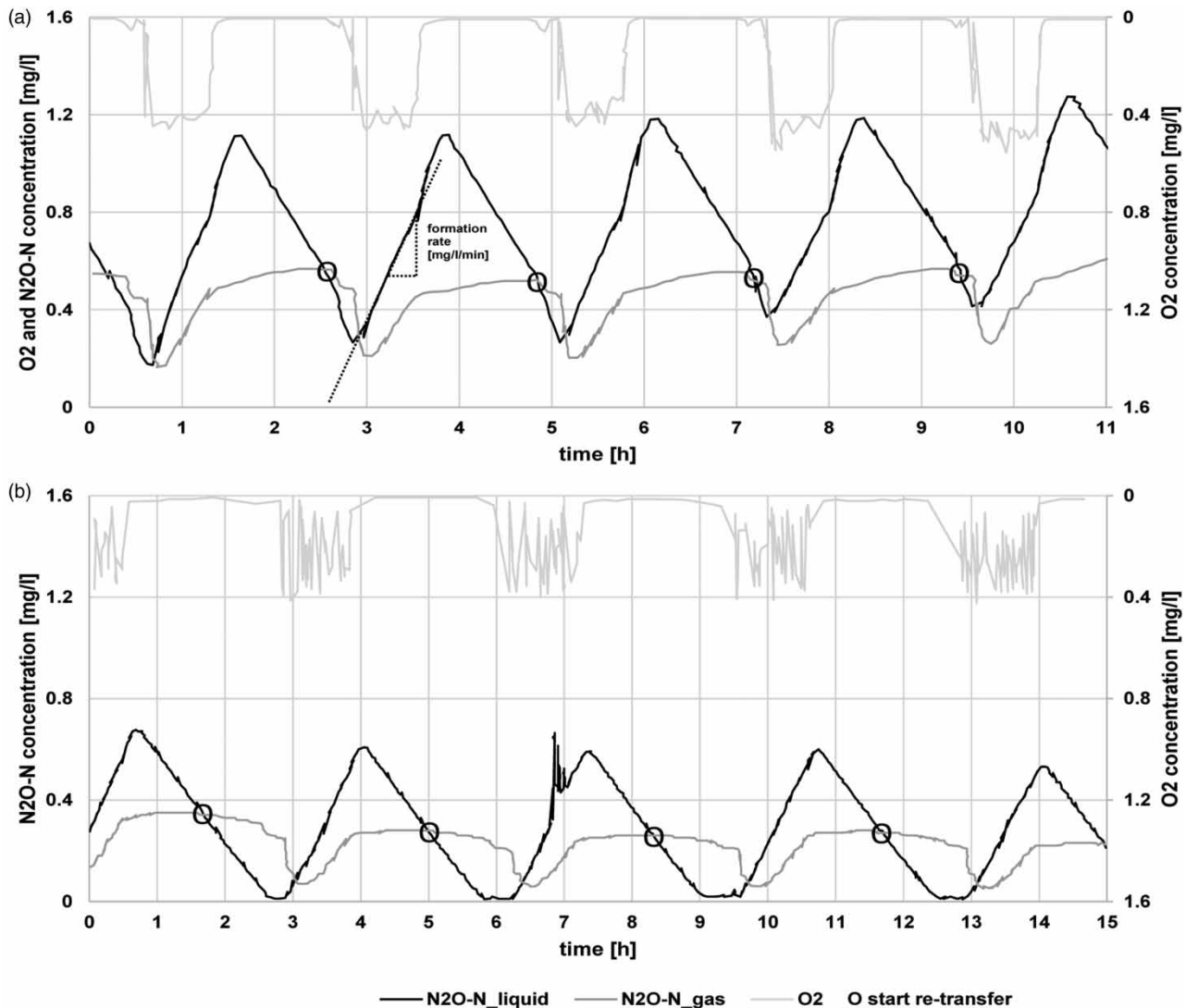


Figure 5 | Measurement results for N₂O concentrations at WWTP B (deammonification reactor with intermittent aeration) during (a) winter period (NH₄-N inflow = 1,100 mg/L) and (b) summer period (NH₄-N inflow = 800 mg/L).

the second reactor contributes 87% to the overall N₂O emissions, while undisturbed denitrification enables complete N₂O reduction with zero-emission.

Figure 7 exemplarily presents an excerpt from the N₂O measurement in the nitrification reactor as well as the modeling results. While the modeled concentrations remain almost constant, the measured N₂O concentration in the nitrification tank of WWTP C is characterized by a distinct cyclic behavior. This profile originates from varying aeration intensities that are necessary to achieve a constant oxygen concentration. As a result, N₂O stripping also differs over time. The model does not consider this effect, consequently, only mean concentrations are calculated. The measured N₂O concentration in the gaseous phase also shows a

slightly cyclic appearance, which is not depicted in the model. Because of its regularity, this inaccuracy of the gas transport description can be neglected when estimating average emission or formation factors.

Statistical evaluation of the model quality

The simulation results were correlated to the measured data to assess the quality of the model. The obtained correlations are presented in Figure 8. Table 5 summarizes the statistical evaluation parameters. Good model accuracy can be stated for N₂O-N concentrations in liquid phase. However, N₂O-N concentrations in gas phase is more inaccurate, because this concentration is more influenced by specific gas transfer

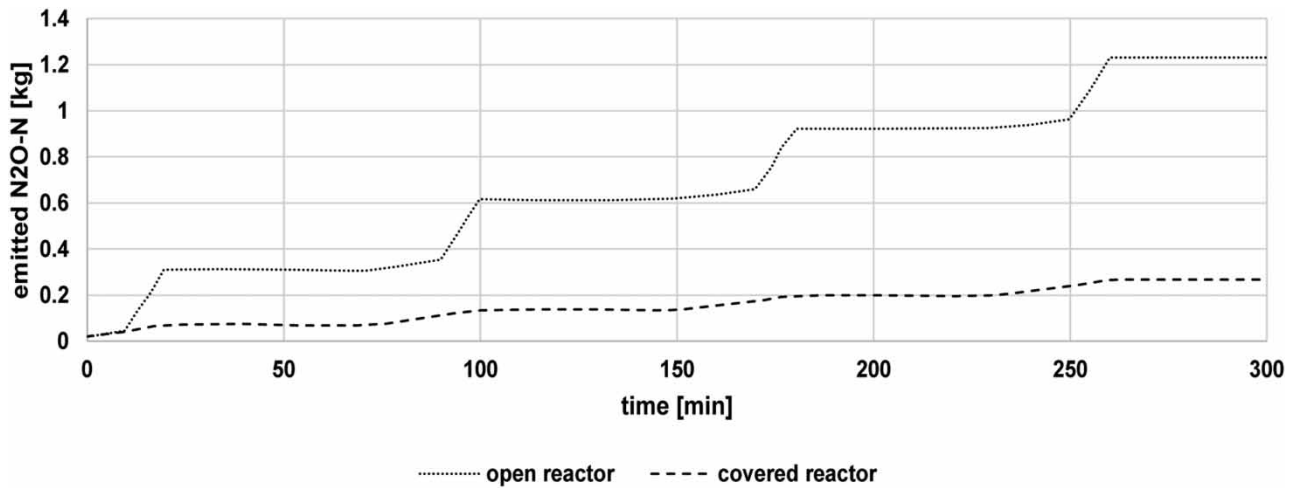


Figure 6 | Comparison of N₂O emissions for open and covered reactor (modelling results, WWTP B).

Table 4 | Average conversion rates and formation factors measured at the part stream treatment step of WWTP C

Time period	Reactor	Average conversion rates [mg/(G VSS*h)]				N ₂ O formation factor		Share of total N ₂ O emission %
		NH ₄ -N	NO ₃ -N	NO ₂ -N	N ₂ O-N	[N ₂ O-N _{form} /NH ₄ -N _{ox}]	[N ₂ O-N _{form} /NO _x -N _{red}]	
1	Nitritation	-10.5	1.3	8.0	0.6	0.06	-	13
	Denitritation	5.1	-8.8	-12.0	4.1	-	0.2	87
2	Denitritation	6.8	-7.5	-13.1	-1.9	-	-	-

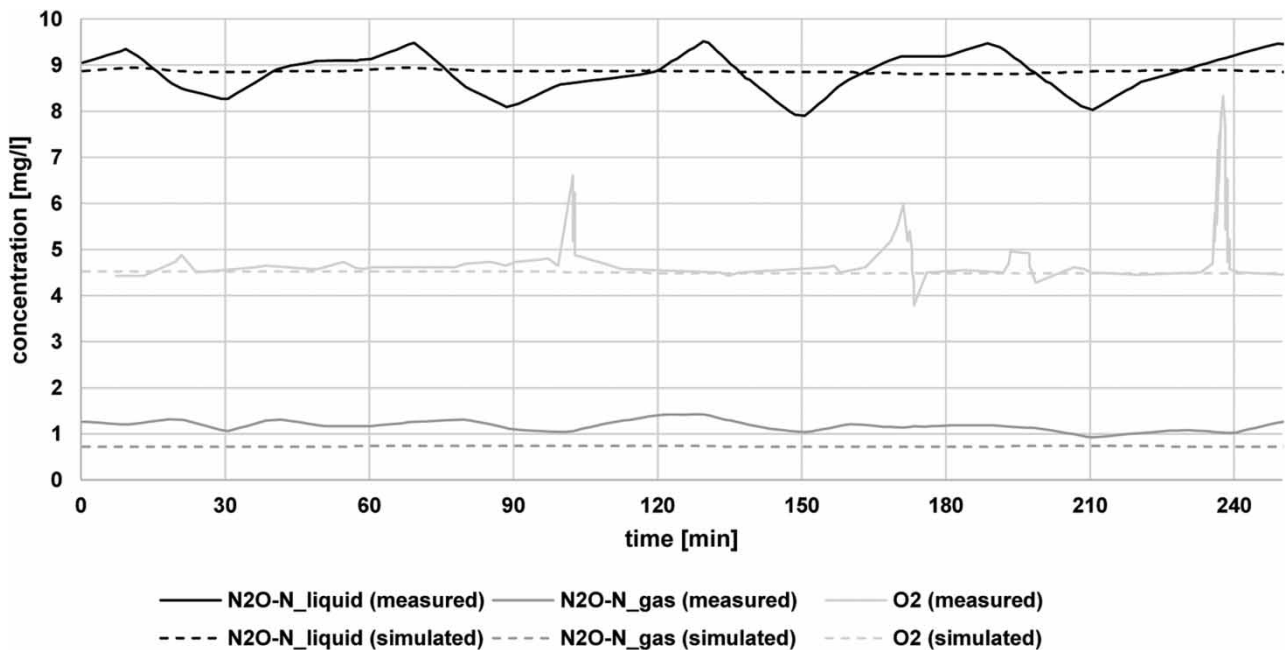


Figure 7 | Measurement results and simulated values at WWTP C (nitritation reactor, constant aeration).

effects and higher imprecisions in the measurement of gaseous N₂O (WWTP A, see above). WWTP B (partly covered reactor) was modelled using open and

covered reactor models, but only results for the open reactor are included in the statistical evaluations, as this is the more suitable approach. The differences between simulation and

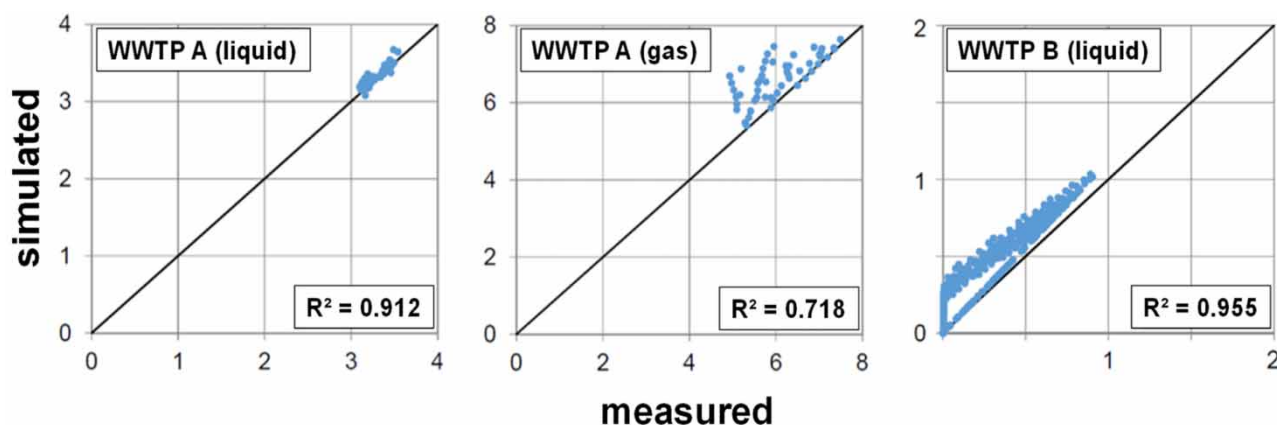


Figure 8 | Correlations of measured and simulated N₂O concentrations.

Table 5 | Statistical values for model evaluation on measurement campaign at WWTP A and WWTP B

Process	N ₂ O-N _{liquid} (WWTP A)		N ₂ O-N _{gas} (WWTP A)		N ₂ O-N _{liquid} (WWTP B)	
	Measured	Simulated	Measured	Simulated	Measured	Simulated
Average (mg/L)	3.31	3.34	6.05	6.64	0.30	0.44
Standard derivation (mg/L)	0.13	0.13	0.71	0.56	0.31	0.37
Median (mg/L)	3.32	3.33	5.92	6.69	0.22	0.922
Correlation coefficient, R ²	0.912		0.718		0.955	

measurement results at WWTP C lead to high statistic variance (data not shown), even though these discrepancies can be explained adequately (see above).

Altogether, in this study only high-loaded systems are investigated – for lower concentrations it has to be supplemented.

The previously described results confirm, that the new ASM3/1_N₂O_{ISAH} model is generally appropriate to depict N₂O formation and reduction and to estimate the resulting emission. Three independent measurement campaigns on different WWTPs were successfully modeled using the same set of ASM parameters. Furthermore, it was shown that the model allows to identify the plant-specific main cause of the detected N₂O emission and underlines the importance of the denitrification process for mitigation.

STRATEGIES TO MINIMIZE N₂O EMISSIONS

The modelling analysis with regard to plant-specific N₂O mitigation strategies identifies the denitrification process in the nitrification reactor as the main lever for emission reduction

for WWTP A. This can be done by shortening the unaerated phase or, focusing on the inhibition effect caused by the HNO₂ concentration, by elevating the pH value.

For WWTP B, the enhanced denitrification process during the anoxic periods leads to diminished N₂O concentrations. This effect can even be optimized by covering the reactor to avoid N₂O stripping. Based on simulation results, the effect of covering is shown in Figure 6. Accumulated N₂O can subsequently be denitrified during the anoxic phase. Moreover, re-transfer into the liquid phase is possible. At WWTP B, covering of SBR leads to a decrease of N₂O emissions up to 70%, compared to an open reactor.

Following these findings, the MiNzE concept (minimized-nitrous oxide-zero-emission) was developed to diminish N₂O emissions. The concept is based on three main principles:

- (i) Minimization of N₂O formation by equalizing inflow conditions and avoiding unfavorable process conditions like high NO₂ concentrations, low oxygen levels and unstable process conditions.
- (ii) Avoidance of N₂O transfer to the gaseous phase by implementing low/no-bubble aeration or enhancement of re-diffusion by covering the reactor.
- (iii) Reduction of N₂O via denitrification.

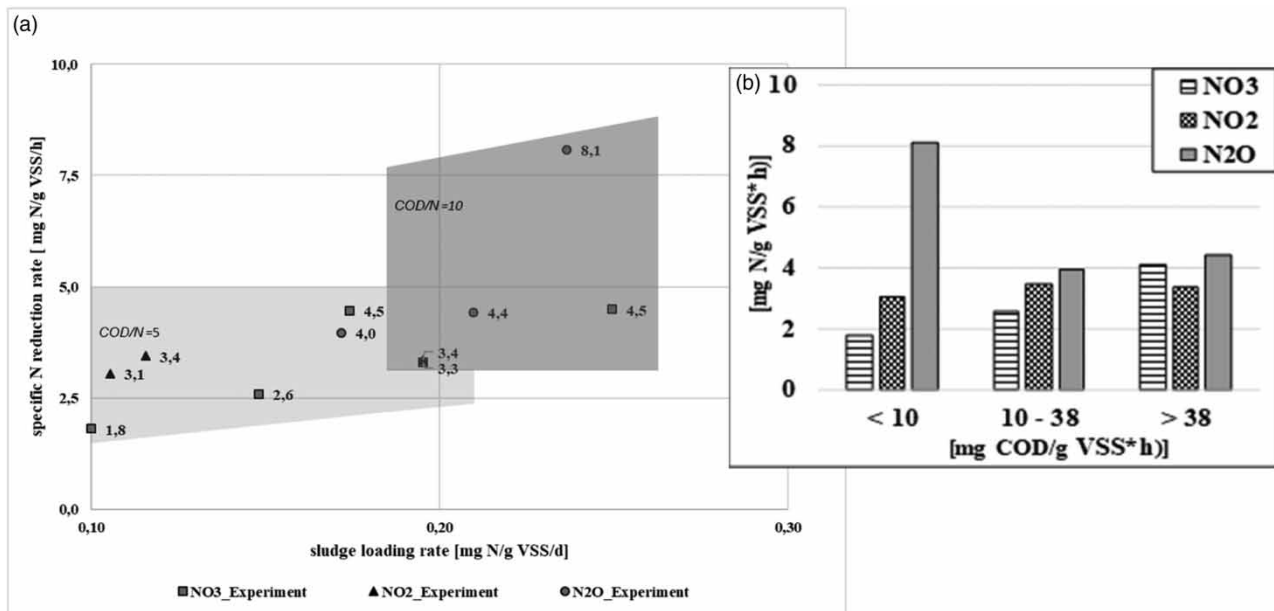


Figure 9 | Experimental reduction rates for intermediates of heterotrophic denitrification as a function of sludge loading rate (a) and clustered by denitrification rates as COD removal rate (b) (pH = 8, temperature 21 °C, substrate acetate, initial nitrogen oxide concentration 20–35 mg/L).

The modelling outcomes demonstrate the high relevance of denitrification concerning the reduction of N₂O emissions. However, the boundary conditions of denitrification are not optimal in part stream sludge liquor treatment (e.g. low C:N ratio); furthermore, N₂O reduction probably competes with nitrite or nitrate reduction. Referring to the important role of denitrification as a sink for N₂O, first denitrification batch tests were carried out to enhance the knowledge about the N₂O reduction kinetics compared to the reduction kinetics of nitrite and nitrate (Figure 9).

The large range of variation from the literature was not visible in the experiments (see Table 1). Although the measured nitrate reduction rates are still low, they are in line with the work of others (Gómez et al. 2000; Cherchi et al. 2009; Wunderlin et al. 2012), showing a range of 4.4–13.6 mg N/g VSS/d (T = 20 °C). The measured reduction rate of nitrate (1.8–4.6 mg N/(g VSS*h)) and nitrite (3.1–3.4 mg N/(g VSS*h)) indicate that the degradation kinetics for nitrate and nitrite are almost in a similar range. However, nitrous oxide exceeds the rates of the other nitrogen oxides with 4.0–8.1 mg N/g VSS/h.

For all substrates, the nitrogen consumption rate increased with the sludge loading rate. Even though peak values for N₂O reduction rate such as 349 mg N/g VSS/h (Pan et al. 2013) are not reached, the N₂O denitrification rate is consistently highest in comparison to NO₂ and NO₃ denitrification. Clustering the results by the COD

reduction rate shows a decreasing difference between the specific nitrogen denitrification rates with increasing activity. This suggests superior N₂O denitrification for low loaded systems. From the microbiological point, it seems to be possible to achieve N₂O reduction based on endogenous respiration and despite the continuous presence of NO₂, thus potentially enabling a combination with the anammox stage. For this approach, further research needs to be conducted on the competition of anammox and denitrification bacteria to broaden the empirical database.

CONCLUSION

With the ASM3/1_N2O_{ISAH} model, the ASM3/1_DEAM_{ISAH} model was successfully modified to enable the calculation of N₂O formation and emission for the biological treatment of ammonium-rich reject water without relying on extensive measurement campaigns. The statistical evaluation of measurement data and modelling results confirms that the proposed practice-oriented approach can correctly predict the N₂O formation and emission for relevant operational settings by applying N₂O formation factors in dependence of AOB activity as well as NO₂ and O₂ concentrations. Thus, the model can be employed as a useful and practicable tool for the development of operational strategies and plant design.

Concerning N₂O emissions, it has been shown that the formation of N₂O by AOBs cannot completely be avoided, especially in high-loaded systems. However, N₂O formation can distinctively be reduced by avoiding high activity of AOB, high and varying NO₂ concentrations and HNO₂ concentrations in the denitrification zone above 1 µg/L. Enhancing the denitrification capacity by the targeted use of the reduction potential of denitrification in the anoxic phases or downstream anoxic basins, a significant reduction of N₂O emissions can be achieved. Thus, the accumulation of N₂O in the liquid phase has to be pursued. In this context, covering the reactors was observed to be an effective measure. For example, covering the SBR reactor at WWTP B reveals a potential reduction of N₂O emissions up to 75% (876 kg N₂O-N/a). Employing the model, feasible emission reductions up to a factor of 10 were calculated. This could be achieved by applying the measures described above. Assuming these optimal conditions, the potential for N₂O emission saving at WWTP C was exemplarily calculated to 38% (1,595 kg N₂O-N/a).

The basic work was carried out in the frame of the German-Polish joint research project ReNeMo (Beier et al. 2017). This project also provides the background for the dissertation of Benjamin Vogel (Vogel 2018). The denitrification kinetic studies are part of the MiNzE project started in 2019. Both projects are financially supported by the German Federal Ministry of Education and Research. We would like to thank the ministry for the support and the opportunity to transfer the idea of the MiNzE concept into practice within the program 'BMBF innovative, practice-oriented research for SME'. The kinetic studies were enabled by the excellent equipped DFG funded research device and will be continued in the future in further detail.

Special thanks to the lab team of ISAH, the operators of the treatment plants for supporting our measurement campaigns and Aniruddha Bhalerao for translation support.

The process matrix and the model parameters of the ASM3/1_DEAM_{ISAH} model as well as the ASM3/1_N2O_{ISAH} model are published in the supplementary information.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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