Strategies for enhanced deammonification performance and reduced nitrous oxide emissions

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Abstract

Deammonification's performance and associated nitrous oxide emissions (N_2O) depend on operational conditions. While studies have investigated factors for high performances and low emissions separately, this study investigated optimizing deammonification performance while simultaneously reducing N_2O emissions. Using a design of experiment (DoE) method, two models were developed for the prediction of the nitrogen removal rate and N_2O emissions during single-stage deammonification considering three operational factors (i.e., pH value, feeding and aeration strategy). The emission factor varied between $0.7 \pm 0.5\%$ and $4.1 \pm 1.2\%$ at different DoE-conditions. The nitrogen removal rate was predicted to be maximized at settings of pH 7.46, intermittent feeding and aeration. Conversely, emissions were predicted to be minimized at the design edges at pH 7.80, single feeding, and continuous aeration. Results suggested a weak positive correlation between the nitrogen removal rate and N_2O emissions, thus, a single optimizing operational set-point for maximized performance and minimized emissions did not exist.

Abbreviations

AOB: aerobic ammonium oxidizing bacteria; anammox: anoxic ammonium oxidation; AnAOB: anoxic ammonium oxidizing bacteria; ANOVA: Analysis of Variance; β : coefficient; BNR: biological nitrogen removal; C_0 : center point; COD: chemical oxygen demand; DO: dissolved oxygen concentration; DoE: design of experiment; ϵ : error of the model; FA: free ammonia; n: amount of influencing factors; MLR: multiple linear regression; NDIR: nondispersive infrared; FNA: free nitrous acid; N_2O : nitrous oxide; NOB: nitrite oxidizing bacteria; Q^2 : goodness of the prediction; R^2 : coefficient of determination; RSM: response surface methodology; SBR: sequencing batch reactor; SRT: sludge retention time; TOrC: trace organic compounds; TSS: total suspended solids; VSS: volatile suspended solids; WWTP: waste water treatment plant; X_j : influencing factor j; Y_i : response variable i

Keywords

Design of experiment (DoE); nitrogen removal; nitrous oxide (N₂O) emissions; N₂O mitigation strategies; single-stage deammonification

1. Introduction

With increasingly strict legal requirements for effluent concentrations and initiatives for a reduced energy consumption of wastewater treatment systems, the deammonification process comprising a partial nitrification and an anoxic ammonium oxidation (anammox) step by aerobic and anoxic ammonium oxidizing bacteria (AOBs and AnAOBs) holds promise to meet both criteria. Firstly, it is a completely autotrophic nitrogen removal process and the organic carbon compounds required for denitrification during conventional biological nitrogen removal (BNR) processes can instead be used to enhance methane production during digestion (Kartal et al., 2010; Ma et al., 2016). Additionally, the energy consumption can be reduced by more than 50% in comparison to conventional BNR because ammonium is only oxidized partially to nitrite and no further oxidation to nitrate is necessary (Fux et al., 2002). The deammonification process has been successfully applied as side-stream treatment in wastewater treatment plants (WWTPs), whose inflow usually is characterized by high ammonium concentrations and high temperatures (Lackner et al., 2014). Recently, deammonification attracts more interests to be applied as a mainstream treatment for nitrogen removal to replace the conventional BNR process (Cao et al., 2017; Gilbert et al., 2015; Laurenzi et al., 2016; Ma et al., 2011; Winkler et al., 2012).

For a high deammonification performance, favorable conditions for both sub-processes of partial nitrification-anammox should be provided, such as a low dissolved oxygen concentration (DO) for the suppression of nitrite oxidizing bacteria (NOBs) (Ciudad et al., 2006) and nitrite, which acts as both substrate, but also as an inhibitor for AnAOBs (Lotti et al., 2012). Favoring conditions can be either maintained by altering the process conditions time-

dependently (during single-stage deammonification) or spatially (during two-stage deammonification/single-stage deammonification as plug flow reactor or by utilizing prevailing spatial substrate gradients in biofilms, granules, or flocs). However, these factors amongst others were also reported to influence the production of dissolved nitrous oxide (N_2O) potentially being emitted into the atmosphere due to diffusion and stripping induced by aeration (Kampschreur et al., 2009b). Different N_2O production pathways have been reported, including nitrifier denitrification (or also termed ‘aerobic nitrous denitrification’ as proposed by Weißbach et al. (2017)) and hydroxylamine oxidation by AOBs as well as incomplete heterotrophic denitrification due to inhibition of N_2O reductase (Ding et al., 2016; Law et al., 2012; Rathnayake et al., 2015). In contrast, the metabolic pathway of AnAOBs is not known to be capable of N_2O production (Kartal et al., 2007). N_2O is both an ozone-depleting substance and a strong greenhouse gas with a global warming potential 298 times higher than that of carbon dioxide based on a 100-year time horizon (Intergovernmental Panel on Climate Change, 2013). Therefore, effective strategies for the mitigation of N_2O emissions are desirable to minimize adverse effects on the environment, even though a complete avoidance of N_2O formation will be hardly feasible, as N_2O is not only biologically, but also abiotically produced (Schreiber et al., 2012).

Ideally, high nitrogen removal rates would simultaneously come along with low N_2O emissions to create an incentive for WWTP operators to voluntarily implement N_2O mitigation strategies. However, previous studies regarding autotrophic nitrogen removal processes suggested a positive correlation. Simulation studies mimicking autotrophic nitrogen removal conditions altering DO concentration, granule size, temperature, and the ammonium load revealed that maximal N_2O emissions caused by AOB denitrification correlated with optimum conditions for AnAOBs (Van Hulle et al., 2012). Castro-Barros et al. (2015) also observed an increased N_2O formation and emission with increased ammonium conversion rates in a single-stage granular sludge reactor promoting the partial nitrification-anammox

process by changes of aeration intensities. Other factors influencing deammonification performance and its N_2O emissions are conceivable, which is why more studies are needed to elucidate their effect in such systems.

For this reason, the aim of this study was two-pronged: a) to investigate the correlation between the nitrogen removal rate and N_2O emissions for the single-stage deammonification process in side-stream under varying operational conditions and b) to determine the optimal operational settings to achieve a maximized nitrogen removal rate at minimized N_2O emissions. A design of experiment (DoE) approach was adopted to evaluate the direct effects and interactions of the influencing factors simultaneously. This approach also enabled a less intensive, less costly, and more reliable experimental design via a reduced number of pre-designed tests, compared with traditional univariate approaches (Ferreira et al., 2007).

The influencing factors chosen in the DoE design followed two rules: 1) easily to be adjusted by WWTP operators and 2) sensitive enough to affect both nitrogen removal rate and N_2O production. Based on these two rules, the cycle's initial pH value, the feeding strategy, and the aeration strategy were closely investigated. The pH value does not only influence the activity of the microorganisms (Jetten et al., 1999; Van Hulle et al., 2007), which perform necessarily as a symbiosis in a single-stage deammonification process, but also the concentration of free ammonia (FA, NH_3) and free nitrous acid (FNA, HNO_2) having an inhibitory effect on microorganisms (Anthonisen et al., 1976; Hellinga et al., 1999; Kim et al., 2012). In addition to influencing the performance of the deammonification process, pH was also reported to affect N_2O production (Kampschreur et al., 2009b; Law et al., 2012; Rathnayake et al., 2015). The feeding strategy, which can be varied from single to continuous feeding, has a direct influence on the ammonium concentration or load, on the pH value, as well as on the FA concentration. It can, therefore, affect both the performance and the N_2O formation due to varying substrate availabilities and changing ammonium concentrations (Domingo-Félez et al., 2014), possible ammonia shock loads (Burgess et al., 2002), and

changing microbial activities. The aeration strategy, comprised of the aeration intensity as well as the aeration interval, influences both the DO concentration (including transient changes in anoxic and oxic phases) and stripping effects. Thus, it affects not only the nitrite production and consumption, but also the FNA concentration and the conditions for N_2O production influenced by the nitrite and DO concentration (Kampschreur et al., 2009b; Kampschreur et al., 2008b; Peng et al., 2015). Furthermore, N_2O emissions are enhanced by active aeration (Law et al., 2012). While studies reported in the literature generally agree on an increase of N_2O production upon the presence of nitrite (Foley et al., 2010; Kampschreur et al., 2009b; Kampschreur et al., 2008a,b; Peng et al., 2015; Wunderlin et al., 2012), contradictory observations have been reported regarding the influence of DO on N_2O production, including both negative and positive correlations (Castro-Barros et al., 2015; Kampschreur et al., 2009b; Peng et al., 2014, 2015; Pijuan et al., 2014). Some of these studies were based on one-dimensional investigations or changed only one influencing factor at a time. In contrast, the DoE method has the advantage that a set of influencing factors can be investigated jointly by changing them all at a time creating a more comprehensive and precise picture of operational conditions these systems can experience.

The combined investigation of pH, feeding strategy, and aeration strategy (including also their interactions) influencing both the nitrogen conversion rate and N_2O emission has, to the best of our knowledge, never been reported before. The developed models enable the prediction of both response variables and thus allow to target a desired combination of the plant's performance and N_2O emission as effective process control strategies. Thus, the models can be used as a helpful tool for WWTP operators to establish a more efficient and/or environmentally friendly deammonification process.

2. Materials and Methods

2.1 Experimental Setup

All experiments of the DoE were conducted in two identical gas-tight sequencing batch reactors (SBR) with a working volume of 10 L. The reactors were temperature-controlled via a circulation thermostat at 32°C. A gas meter (Dr.-Ing. Ritter Apparatebau GmbH & Co. KG, Bochum, Germany) connected to the headspace of the reactor was used for the manual control of aeration intensities adjusting a needle valve ahead of initiating the experiments. The gas meter enabled the measurement of the total off-gas volume, which was comprised of the aeration gas and gas production by the process itself, to precisely balance the N₂O emissions of each cycle. The off-gas was collected in a gas bag for subsequent N₂O analysis.

Additionally, the reactors were equipped with a stirrer, a peristaltic pump, and *in situ* probes for process monitoring. The reactors were time- and signal-controlled by a supervisory control and data acquisition (SCADA) system. A detailed description of the experimental setup can be found in Leix et al. (2016). Additionally, a flow schematic is provided in the supplementary information (SI; Figure SI-1).

2.2 Design of Experiment (DoE)

A Box-Behnken design with three influencing factors (X_j) on three levels (1-, 0, +1), namely the cycle's initial pH value (X_1) at the beginning of a cycle, the feeding strategy (X_2), and the aeration strategy (X_3), has been developed to investigate which factors - including also their interactions - had a significant influence on the two response variables (Y_i), such as the nitrogen removal rate (Y_1) and N₂O emission (Y_2). The Box-Behnken design including one center point (C_0) was comprised of 13 DoE, each with a different combination of influencing factors, as shown in Table 1. To avoid a falsification of effect estimations with respect to the influencing factors due to a trend or other undetected changes of results, the order of DoE runs was randomized. Every DoE run was repeatedly studied. The statistical significance of

main effects, two-way and squared interactions of influencing factors was determined with an Analysis of Variance (ANOVA) at a 95 % confidence level ($p < 0.05$). Based on these results, two mathematical models were fitted using multiple linear regression (MLR) described by the following quadratic polynomial equation in a n -dimensional design space:

$$Y_i = \beta_0 + \sum_{j=1}^n \beta_j X_j + \sum_{j=1}^n \beta_{jj} X_j^2 + \sum_{j=1}^{n-1} \sum_{k=j+1}^n \beta_{jk} X_j X_k \pm \varepsilon \quad (1)$$

with Y_i being the respective response variable, β_0 , β_j , β_{jj} , and β_{jk} the coefficients of the intercept, the linear, squared, and two-way effect, respectively, n the amount of influencing factors (here $n=3$), $X_{j/k}$ the respective influencing factor, and ε the error of the model. To improve the quality of the model (Q^2 : measure for the goodness of the prediction), the statistically significant influencing factors were included into the model exclusively.

Response surface methodology (RSM) was used to determine statistically optimum conditions for both the nitrogen conversion rate (maximized) and the N_2O emission (minimized) independently as well as their combination. Data were analyzed with the software MODDE Pro 11.01 (MKS Data Analytics Solutions, Malmö, Sweden).

2.3 Influencing Factors and Reactor Operation

For the development of applicable mathematical models, the three levels of influencing factors needed to cover realistic conditions for the deammonification process. Following the summarized operational strategies of full-scale partial nitrification-anammox processes as suggested by Lackner et al. (2014), the pH values at the cycle beginning were adjusted to pH 7.2, pH 7.5, and pH 7.8, the feeding strategy from single feeding to intermittent to highly intermittent feeding (i.e., 1, 13, 25 feeding intervals per cycle), and the aeration strategy from continuous to intermittent to highly intermittent aeration (i.e., 1, 25, 49 aeration intervals per cycle) according to the Box-Behnken design (Table 1, Table SI-1). The desired initial pH value was adjusted using sodium hydroxide (NaOH) or hydrochloric acid (HCl). Process water was dosed by a time controlled pump either directly at the beginning of the cycle for

single feeding or evenly distributed over the entire cycle duration for (highly) intermittent feeding. A constant aeration volume of 120 L for every DoE run was provided, while aeration intensities and aeration intervals were adjusted, again evenly distributed over the whole cycle for (highly) intermittent aeration (also see Table SI-2).

Apart from changing the influencing factors for every DoE run, all other conditions were kept constant as far as possible. The cycle duration (including feeding and the reaction phase without settling) and the exchange volume were fixed to six hours and 10 %, respectively. During feeding and reaction phases, the reactors were constantly stirred to guarantee homogeneity. An effluent concentration of 60 mg $\text{NH}_4\text{-N/L}$ was targeted to provide comparable starting conditions after settling and decant phase for every DoE run. Gas bags for the collection of one cycle's off-gas volume were evacuated, flushed with N_2 and evacuated again, before they were connected to the gas meter. Before sealing the reactor gas-tightly and starting a new cycle, the reactor headspace was flushed with ambient air to assure that no N_2O was trapped in the headspace falsifying the results.

2.4 Inoculation

To guarantee comparable starting conditions for every new DoE run with a microbial community not being influenced by protraction of the previous DoE run, every new combination of operational parameters was freshly inoculated with 10 L of suspended sludge originated from a pilot-scale deammonification reactor with a 130 L working volume. This reactor was exclusively operated to provide inoculum of constant quality. As the nitrogen removal rate as well as the nitrogen degradation were relatively stable over the entire experimental period for the model development as well as for the validation of results, the relatively constant composition of the microbial community was assumed during the course of this study (Figure SI-2).

2.5 Process Water

Process water of the WWTP Garching/Munich was used as feed water. To avoid naturally occurring compositional fluctuations of the process water over time, one charge of process water required for both the model calibration and validation of the DoE was stored frozen in canisters at -20°C. For every new DoE run, process water was gently thawed. The process water during the model calibration was characterized as follows: 1,270 mg NH₄-N/L, 1,280 mg/L COD, and pH 8.3. The Environment Protection Authority (2000) recommended a maximal storage time of 28 days until analysis. Despite the study duration being longer than this recommended storage time, freezing the process water for keeping its composition as stable as possible to mimic realistic wastewater conditions was still believed to be the best compromise. Nevertheless, the most severe change was observed for the COD concentration until the validation experiments could be conducted. During this time, the process water was characterized by the following water quality: 1,245 mg NH₄-N/L, 800 mg/L COD, and pH 8.2.

2.6 Analytical Methods

Ammonium, nitrite, nitrate, and COD concentrations were determined using photochemical testing kits (LCK303, LCK341 and LCK342, LCK340, LCK514; Hach Lange GmbH, Düsseldorf, Germany). To prevent a falsification of analyzed nitrate concentrations by disruptive nitrite ions, nitrite was eliminated by adding sulfamic acid (Wu et al., 1997). Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed according to Standard Methods (APHA, 2012). The gaseous N₂O concentration was analyzed via a gas analyzer using a nondispersive infrared sensor (NDIR) (X-STREAM XEGP, Emerson Process Management GmbH & Co. OHG, St. Louis, USA).

3. Results and Discussion

3.1 Nitrogen removal rates and N₂O emissions for model calibration

The 13 runs of experiments following the Box-Behnken design with the different settings of the influencing factors were conducted repeatedly and in randomized order as presented in Table 1, leading to a total of 55 analyzed cycles. Even though both response variables were investigated jointly, some experiments were chosen to be evaluated more often regarding their specific nitrogen removal rate or N₂O emission to reduce fluctuations, thus, the amount of analyzed repetitions differed in some instances. For the development of the two mathematical models, the single results of both response variables were taken into account. Results are shown in Table 1 and also include the N₂O emission factor related to the nitrogen removed. The emission factors observed in this study varied between $0.7\pm0.5\%$ and $4.1\pm1.2\%$. This is well within the range described by Kampschreur et al. (2009b) who reported 0–95% of the nitrogen load being emitted as N₂O in laboratory-scale studies and 0–15% in full-scale studies, respectively. These observations demonstrate that N₂O emission can vary largely between different studies which is why further investigations into N₂O emissions influenced by the process operation are crucial.

Table 1

3.2 Models and optimal set-points

3.2.1 Model development and maximization of the nitrogen removal rate

The model for the prediction of the nitrogen removal rate was developed by taking into account all significant factors revealed by the ANOVA. Apart from the interaction term of ‘pH·Aeration’ and ‘pH·Feed’, all other main effects, two-way, and squared interactions were found to be significant. The absolute value of the effect indicates the degree of the factor’s influence on the response variable. It can be seen that the squared effect of the aeration

strategy had the highest effect on the nitrogen removal rate, followed by the squared effect of the cycle's initial pH value and so forth (Table 2). In contrast, the feeding strategy (including the main effect and the two-way interaction) had the lowest effect.

Table 2

The model for the response variable of the nitrogen removal rate (Y_1) with the unit $\text{kg}_\text{N}/(\text{m}^3 \cdot \text{d})$ was estimated according to Table 2 as follows:

$$Y_1 = -50.8659 + 13.7265 \cdot X_1 + 0.0034 \cdot X_2 + 0.0091 \cdot X_3 - 0.9200 \cdot X_1^2 - 0.0003 \cdot X_2^2 - 0.0002 \cdot X_3^2 + 0.0001 \cdot X_2 X_3 \pm 0.0324 \quad (2)$$

with X_1 being the influencing factor of the pH value, X_2 representing the feeding strategy, and X_3 the aeration strategy. Further evaluation of the mathematical model for the prediction of the nitrogen removal rate is presented in Figure SI-4.

Applying this model enabled the generation of 2D-response contour plots visualizing the impact of the three influencing factors on the nitrogen removal rate (Figure 1). The corresponding 3D-response contour plots can be found in the SI (Figure SI-5).

Figure 1

The response contour plots revealed that moderate operational settings for all influencing factors investigated (pH value, feeding strategy, and aeration strategy) influenced positively the performance of the deammonifying process. Conversely, settings were neither on the low (-1), nor on the high level (+1) of the Box-Behnken design advantageous for the nitrogen removal rate. Thus, the edges of the influencing factors have been successfully chosen.

The optimum set-point for a maximized nitrogen removal rate within the examined frame of operational settings was calculated by using a simplex algorithm. An extrapolation of results

beyond this frame would contain an inestimable uncertainty which is why statements in this direction are not being discussed, also because they would cover rather uncommon operational set-points. A maximized nitrogen removal rate of $0.49 \pm 0.03 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$ was calculated to be achieved at a cycle's initial pH value of 7.46 combined with 30 aeration and 11 feeding intervals per cycle. In a survey by Lackner et al. (2014), volumetric nitrogen loads of eight deammonifying systems comparable to the system tested here have been summarized with a variation from as low as $0.04 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$ up to $0.65 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$. Assuming a maximal nitrogen removal of 89% according to the stoichiometry (Strous et al., 1998), minimal and maximal nitrogen removal rates of $0.03 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$ and $0.58 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$ could be achieved, respectively. Thus, the maximal predicted nitrogen removal rate of $0.49 \pm 0.03 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$ can be ranked into the upper level of nitrogen removal rates in comparison to other comparable deammonification systems.

Intermittent aeration with evenly distributed aerated ($\sim 5.5 \text{ min}$) and unaerated phases ($\sim 6.5 \text{ min}$) at a moderate aeration intensity ($\sim 44 \text{ L/h}$) seemed to be the most suitable condition for both the partial nitrification and the anammox process. Such an aeration strategy generally creates moderate DO concentrations in comparison to continuous and highly intermittent aeration, which would lead to a permanent low DO concentration or peaks with high DO concentrations, respectively. Mean, minimal, and maximal DO concentrations for the three different aeration strategies are summarized in Figure SI-3. Comparing different aeration strategies, it has been reported that the continuous aeration will lead to a higher performance of the deammonification process (Joss et al., 2009). This could not be confirmed by the model under the precondition of a constant aeration volume over a set cycle duration to render different aeration strategies comparable, but might be applicable to conditions where the aeration intensity is increased. Consequently, the intermittent aeration is not only more cost-effective compared to continuous aeration due to aeration savings observed here, but

creates also unaerated lag-phases to suppress the growth of NOBs (Gilbert et al., 2014; Kornaros et al., 2010).

Additionally, operation should be aimed for a pH value of approximately 7.5 to promote a high performing deammonification process as a symbiosis of partial nitrification and anammox. If deviations from this ideal set-point are not avoidable, pH values should be controlled to be rather below than above this target set-point as the nitrogen removal reduction is less pronounced at a lower pH-range (Figure 1). This observation is in line with favorable pH values observed before for AOBs and AnAOBs (pH 6.5-pH 8.0 for AOBs (Van Hulle et al., 2007) and pH 6.7-pH 8.3 for AnAOBs (Jetten et al., 1999)) as well as with the inhibitory effect of FA, as the FA concentration increases with an increasing pH value (inhibitory effect on AOBs starts at threshold values of 10 mg $\text{NH}_3\text{-N/L}$ with complete inhibition at 150 mg $\text{NH}_3\text{-N/L}$ (Anthonisen et al., 1976)). In comparison, no inhibition of AOBs by FNA could be assumed because nitrite did not accumulate during the single-stage deammonification process (inhibitory effect on AOBs at threshold values above 0.2 mg $\text{HNO}_2\text{-N/L}$ (Hellinga et al., 1999)).

As for the feeding strategy, again a lower loss of the plant's performance can be expected when operated rather below the optimal set-point represented by a moderate intermittent feeding than above. This impact became less conspicuous the further the aeration strategy approached continuous aeration conditions. Nevertheless, a change of the feeding strategy from a single to a highly intermittent feeding was not predicted to severely affect the nitrogen removal rate (Figure 1).

3.2.2 Model development and mitigation of the N_2O emissions

A model for the prediction of N_2O emissions was developed correspondingly to the model for the estimation of the nitrogen removal rate. The ANOVA revealed the linear and squared influencing factors to be statistically significant, apart from the linear influencing factor

‘aeration’ and the squared influencing factor ‘feed·feed’. However, their removal would have negatively influenced the quality of the model (Q^2), which is why these terms were retained in the statistical model with the squared pH value having the highest effect on the N_2O emissions, followed by the squared aeration strategy, and so forth (Table 2).

The model for the response variable of the N_2O emission (Y_2) with the unit mg_N/d was estimated according to Table 2 as follows:

$$Y_2 = -17363.60 + 4700.12 \cdot X_1 + 6.27 \cdot X_2 + 2.83 \cdot X_3 - 317.48 \cdot X_1^2 - 0.15 \cdot X_2^2 - 0.05 \cdot X_3^2 \pm 32.12 \quad (3)$$

Further evaluations of the mathematical model for the prediction of the N_2O emission are provided in the SI (Figure SI-6).

This model was applied for the generation of the 2D-response contour plots visualizing the impact of the three influencing factors on the N_2O emission (Figure 2). The corresponding 3D-response contour plots can be found in the SI (Figure SI-7).

Figure 2

Based on the model, the following correlations between the three influencing factors and the N_2O emission could be derived, again only being valid within the limits of the investigation area. A continuous aeration was predicted to be more favorable than (highly) intermittent aeration for the purpose of reduced N_2O emissions. With respect to the aeration regime, a previous study by Joss et al. (2009) observed the same correlation, while another study found no difference (Yang et al., 2013). Generally, N_2O formation was believed to be promoted by low DO concentration levels (Kampschreur et al., 2008b, 2009b). Oxygen limiting conditions can occur during continuous rather than (highly) intermittent aeration, thus continuous aeration would be counterproductive in terms of low N_2O emissions relating to this observation. However, also the contrary case of low DO concentrations resulting in decreased

N₂O emissions has been reported (Kampschreur et al., 2009a; Peng et al., 2014), which is in agreement with the findings of this study and could be explained by the combined consideration of DO and nitrite concentrations. Nitrite itself was observed to generally trigger N₂O formation, but Peng et al. (2014, 2015) claimed that low nitrite concentrations potentially are responsible for a reduced stimulative effect on the AOB denitrification pathway, even though this N₂O production pathway is encouraged at lower DO levels. These explanations apply for the investigated system here as well, as continuous aeration causing a low DO concentration enables an immediate consumption of produced nitrite by the anammox process in deeper layers of microbial aggregates and thus, prohibits an accumulation of nitrite. Additionally, a lower aeration intensity not only decreases the stripping effect, but could have also been hold responsible for a lower N₂O formation rate by Castro-Barros et al. (2015). Furthermore, continuous aeration also prohibits sudden changes in the DO concentration (especially the recovery from anoxic conditions), which were demonstrated to be favorable for the N₂O production (Castro-Barros et al., 2015; Yu et al., 2010). For all these reasons, continuous aeration seemed to be advantageous over (highly) intermittent aeration for mitigating N₂O emissions.

Furthermore, less frequent feeding causing a higher gradient of ammonium concentration was observed to result in lower N₂O emissions. Decreasing ammonium concentrations have been demonstrated before to decrease N₂O peaks as well as N₂O production rates (Domingo-Félez et al., 2014). Therefore, also potential N₂O emissions could be expected to be lower, which would favor the approach of a (highly) intermittent feeding strategy. However, others could not find such an indication (Kampschreur et al., 2009a). Nevertheless, ammonia shock loads were observed to influence the N₂O production rate, yet having reached its maximum at ammonia loads exceeding 1.6 mg NH₃-N/g TSS (Burgess et al., 2002). Considering all possible conditions during the DoE, nearly all settings fulfilled this load threshold (ammonia concentrations were calculated according to Anthonisen et al. (1976)). Thus, N₂O formation

was most likely only induced once by single feeding instead of multiple times by (highly) intermittent feeding. Therefore, the amount of produced dissolved N_2O , which is potentially emitted, could be reduced. Furthermore, an infrequent feeding strategy for mitigated N_2O emissions is most likely also related to a further increase of the pH value at the beginning of the cycle if feeding takes place rarely.

With respect to the pH value as the most influencing factor, the lowest N_2O emissions were observed with the highest pH value of pH 7.8 whereby a change from 'level 0' to 'level +1' caused a considerable higher decrease of N_2O emissions than a change to 'level -1' (Figure 2). These observations are in good agreement with Rathnayake et al. (2015) who found the N_2O emissions to peak at pH 7.5 and to decrease with lower and higher values. However, this study only investigated the partial nitrification system, whereas N_2O emissions of the entire deammonification system were investigated in this study providing more general conclusions. Inhibited AOBs and with it decreased activity of AOB denitrification and reduced production of intermediates being available for N_2O formation due to elevated FA concentrations at higher pH values causing also a decreased nitrogen removal rate could be one explanation for the observations. FNA has been demonstrated to inhibit strongly the N_2O reduction during denitrification (Zhou et al., 2008). FNA built-up was avoided by both an elevated pH value and a continuous aeration strategy enabling for an immediate consumption of produced nitrite. Thus, these conditions are favorable for a simultaneous reduction of dissolved N_2O leading to a decrease of potentially emitted N_2O and are in line with the predictions of the model.

To reduce the N_2O emissions to a minimum, an optimum set-point was calculated to be at the edges of the operational settings investigated, namely at pH 7.8, a single feeding strategy, and a continuous aeration. Whether a further reduction of the N_2O emissions beyond the edges of the influencing factors investigated would be possible, should be evaluated in future studies, whereby a further increase of the pH value is the only possibility since the feeding and

aeration strategy are already at the lowest edge. However, the performance of the deammonification process would most likely further decrease with an additional increase of the pH value (see Figure 1), which would already drop by 46% to a nitrogen removal rate of $0.24 \pm 0.03 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$ in comparison to the mean value at the proposed optimized operational settings for N_2O reduction.

3.2.3 Practical operation strategies to achieve high nitrogen removal rates and reduced N_2O emissions

Ideally, a maximization of the nitrogen removal rate would come along with a minimization of the N_2O emissions for an economic and an environmentally friendly operation of the deammonification process. However, the results of the DoE tests revealed that such a negative correlation did not exist between those two response variables for single-stage deammonification (Figure 3). More specifically, only a weak positive correlation was identifiable, which is supported by a low R^2 , that is yet in a comparable range for the measured values by the DoE with $R^2=0.086$ and the predicted values by the models with $R^2=0.107$. These findings are supported by a comparison of predicted values for all possible combinations of settings (see Table SI-3) proving that a single optimized operational setting did not exist. Consequently, a compromise between the two aims of reducing the N_2O emissions and improving the nitrogen removal rate is needed for an environmentally friendly or efficient operation of deammonification, e.g. at pH 7.7, a single feeding, and a moderate intermittent aeration (here represented by 22 times/cycle). With these settings, the nitrogen removal rate is predicted to be $0.41 \pm 0.03 \text{ kg}_\text{N}/(\text{m}^3 \cdot \text{d})$, thus exceeding the mean by 17%. In contrast, the N_2O emissions were estimated to be decreased by 55% in comparison to the mean to $49.6 \pm 32 \text{ mg}_\text{N}/\text{d}$. For an even more environmentally friendly operation, settings at pH 7.8, with single feeding, and fewer aeration intervals of 11 times/cycle were calculated to lead to a more than fivefold decrease of the N_2O emissions if a nitrogen removal reduction of

approximately 10% is acceptable. If the focus is on a high nitrogen removal rate, operational settings for instance at pH 7.55, single feeding, and a higher aeration interval of 28 times/cycle would allow for an increase of the mean performance by over 31% while N₂O emissions would still be below the mean value.

Figure 3

3.3 Validation and reliability of the mathematical models

Both models for the prediction of the nitrogen removal rate and the N₂O emission were validated repeatedly and simultaneously at three different set-points for the purpose of low N₂O emissions, of a moderate nitrogen removal rate at moderate N₂O emissions, and of a high nitrogen removal rate. Details regarding the operational settings with their specific model predictions and experimental results for both response variables are listed in Table 3.

Table 3

Results demonstrated a good agreement between the predicted and experimental values for both response variables at settings for low N₂O emissions. The experimentally evaluated values had a low deviation from the predicted mean, thus were all securely within the prediction range and therefore able to validate both models at this set-point. In contrast, experiments for the validation of the models at settings leading to a high nitrogen removal rate did not meet the prediction criterion with one exception for the N₂O emissions. At settings for a moderate nitrogen removal rate and N₂O emissions, the validation experiments could confirm the predictions for the nitrogen removal rate while results of the N₂O emissions exceeded the prediction range. Thus, two out of three cases could be validated for the nitrogen removal rate model, while only one was confirmed by the N₂O emission model.

From a statistical point of view, this fact can be explained by the quality of the models. The prediction accuracy of both models was limited to some extent, which is reflected by Figure 4 depicting the correlation between observed and predicted values. The model for the nitrogen removal rate prediction had a coefficient of determination of $R^2=0.82$, whereas R^2 of the N_2O emission model was well below with $R^2=0.50$. Even though a wide range of emitted N_2O concentrations have been reported before for different WWTPs (Foley et al., 2010; Kampschreur et al., 2009b), N_2O emissions were expected to be comparable within the repetitions of each run of the DoE as the settings of influencing factors were the same and all other conditions were kept stable as far as possible. However, the N_2O emission exhibited rather high fluctuations within the repetitions (Table 1 and Figure 4), which influenced directly the quality of the model. Thus, the N_2O emission model exhibited a low Q^2 value and therefore can be more characterized as a trend model. Consequently, the model for nitrogen removal rate was expected to have a higher prediction accuracy with 75% of the experimental data being expected to meet the predictions within one standard deviation ($Q^2_{N\text{-removal rate}}=0.75$), and therefore being more reliable than the model of N_2O emission with only 34% ($Q^2_{N_2O\text{ emission}}=0.34$), which could be reproduced by the validation experiments (Table 3).

Figure 4

From a practical point of view, different error sources are conceivable, which were attempted conscientiously to be minimized, however, could not be completely excluded. These might have negatively influenced the results of the DoE and thus, the quality and the validation probability of the models. The accuracy of photochemical testing kits and the feeding pumps as well as the precision of the pH value adjustment and of the initial ammonium concentration at the beginning of each cycle are generally limited. Additionally, rapidly changing process

conditions have been reported to increase N₂O emissions (Kampschreur et al., 2009b). Even though a fast change in the pH value has not been described in that context, the adjustment of the pH value at the beginning of the cycle might have stressed the microorganisms and therefore, increased the N₂O production potentially dependent on the induced pH gradient. Although conditions apart from the pH value, the aeration strategy, and the feeding strategy were tried to being kept constant, it cannot be excluded that also other unidentified factors could have influenced the response variables, such as a possible alternation of the sludge retention time (SRT). Due to a mediocre settling behavior of the sludge during the DoE experiments, no surplus sludge removal was necessary to keep the TSS concentration at a stable level of 2.0 ± 0.2 g/L, whereas the TSS concentration increased up to 3.6 g/L until the validation experiments, consequently prolonging the SRT. Preliminary validation results demonstrated that N₂O emissions were well below the predicted N₂O emission (results not shown), which is supported by the negative correlation of N₂O emissions and SRT described in a previous study (Noda et al., 2003). Nitrogen removal rates were also below the model predictions. This can be explained by a higher endogenous respiration at higher TSS concentrations, while the aeration volume over one cycle was kept constant as one fundamental condition of this experimental setup. Thus, the available dissolved oxygen for the purpose of the single-stage deammonification process was likely reduced causing the nitrogen removal rate to drop. Consequently, the TSS concentration was adjusted for the validation experiments to match those during the DoE experiments and therefore enable again for comparable conditions, which could improve the nitrogen removal rate, however also shortened the SRT. In contrast to the preliminary validation experiments, the N₂O emissions exceeded the predictions in two out of three validation cases (Table 3). A possible explanation for the deviating results could potentially also be found in the changed composition of the process water between the calibration experiments and the validation experiments. Even though the process water had been stored at -20 °C to minimize differences in quality over the

entire experimental period, an alteration was observed which was especially apparent for the COD concentration. Thereby, the COD concentrations during the validation experiments were in average decreased by 38 % in comparison to those during the model development and calibration. This circumstance is supposed not to have negatively influenced the validation at settings for low N_2O emissions with continuous aeration, because potential denitrification for the reduction of N_2O is known to be inhibited by oxygen (Oh and Silverstein, 1999).

However, decreased COD concentrations causing a hypothesized decreased denitrification activity could be an explanation, why the N_2O emissions exceeded the predicted values for the other two cases during which anoxic phases were supplied. For these reasons, it is more likely that not all predicted values were reproducible. Consequently, changing COD concentrations of the process water as well as the SRT would be interesting additional influencing factors to be investigated jointly in further studies.

Generally, the models were not expected to predict the exact values for every deammonification plant as the influencing factors apart of the pH value are hardly directly transferable. However, to prove that the models were rather qualitatively than quantitatively reliable, the most significant effects for both models were tested in additional trend validation experiments. Thus, the aeration strategy has been changed on all three levels for the investigation of the nitrogen removal rate, while the pH value and the feeding strategy were kept stable (pH 7.5; feed=13 times/cycle). For the trend validation of the N_2O emissions, the effect of the pH value was examined with the feeding and aeration strategy being unchanged (feed=25 times/cycle; aeration=30 times/cycle). In Figure 5, the changes of the nitrogen removal rate and N_2O emissions related to the lowest values at changing conditions are depicted. Results demonstrated a good agreement between the development of the trend validation values and the predicted values of both response variables and therefore, could confirm the qualitative applicability of both models. Only the experiment for the trend validation of the N_2O emission at pH 7.2 diverged clearly, however, it still could mimic the

predicted trend of N_2O emissions at changing pH values. Therefore, it could be proved that the developed mathematic models are suitable to give qualitatively recommendations for improving the nitrogen removal rate and reducing the N_2O emissions.

Figure 5

Conclusions

Two models were developed via a systematic DoE approach for the prediction of the nitrogen removal rate (Y_1) and N_2O emissions (Y_2) of a single-stage deammonification process influenced by three adjustable operation factors (pH value, aeration and feeding strategy). The highest degree of influence on Y_1 and Y_2 had the squared effect of the aeration regime and the pH value, respectively. $Y_{1,\max}$ was predicated at a pH value of 7.46, intermittent feeding and aeration, while $Y_{2,\min}$ was predicted at a pH value of 7.80, single feeding, and continuous aeration. Furthermore, results suggested a weak positive correlation between Y_1 and Y_2 .

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Figure captions

Figure 6. 2D-response contour plots for the influencing factors pH value, feeding strategy, and aeration strategy on the response variable of nitrogen removal rate (Y_1)

Figure 7. 2D-response contour plots for the influencing factors pH value, feeding strategy, and aeration strategy on the response variable of N_2O emission (Y_2)

Figure 8. Correlation between the nitrogen removal rate (Y_1) and the N_2O emission (Y_2) for both the model predictions and the DoE experiments

Figure 9. Observed vs. predicted values for the model of the nitrogen removal rate (left) and the N_2O emission (right)

Figure 10. Trend validation for the model of the nitrogen removal rate (left) and the N_2O emissions (right)

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Table 1. Box-Behnken design matrix with experimentally observed results regarding the response variables (Y_i) for the nitrogen removal rate and the N_2O emission as well as the respective emission factors (three influencing factors (X_j) were varied on three levels including the cycle's initial pH value: (-1) = pH 7.2, (0) = pH 7.5, (+1) = pH 7.8; Feeding strategy: (-1) = 1 time/cycle, (0) = 13 times/cycle, (+1) = 25 times/cycle; Aeration strategy: (-1) = 1 time/cycle, (0) = 25 times/cycle, (+1) = 49 times/cycle))

Box- Behnken design	Influencing factor (X_j)			Response variable (Y_i)				Emission factor
	pH value (X_1)	Feeding strategy (X_2)	Aeration strategy (X_3)	Nitrogen removal rate (Y_1)	n	N_2O emission (Y_2)	n	
Run order	[-]	[times/ cycle]	[times/ cycle]	[$kg_N/(m^3 \cdot d)$]	[-]	[mg_N/d]	[-]	[%]
1	+1	0	+1	0.34±0.04	2	107.9±24.1	2	3.3±1.1
2	+1	0	-1	0.24±0.04	4	33.4±2.1	3	1.4±0.2
3	0	+1	-1	0.28±0.02	5	93.9±21.4	6	3.5±0.7
4	-1	-1	0	0.42±0.04	6	66.2±26.1	7	1.5±0.7
5*	0	-1	-1	0.36±0.01	3	50.4±12.6	5	1.6±0.4
6	0	0	0	0.48±0.01	3	125.7±4.4	3	2.6±0.2
7	+1	-1	0	0.34±0.01	3	27.3±15.7	4	0.7±0.5
8	-1	0	+1	0.38±0.02	3	92.8±13.0	3	2.5±0.5
9*	0	-1	+1	0.38±0.01	3	44.6±17.7	3	0.9±0.0
10	-1	0	-1	0.27±0.04	3	64.9±43.2	4	2.7±1.8
11*	0	+1	+1	0.38±0.03	3	108.3±57.8	4	2.5±1.5
12	-1	+1	0	0.37±0.07	3	139.8±59.7	4	4.1±1.2
13	+1	+1	0	0.34±0.03	3	62.5±8.5	3	1.9±0.2
Total				0.35±0.07	44	77.0±42.8	51	2.3±1.3

* Biological removal of trace organic compounds (TO_{RC}) was analyzed for these runs as a side project with results being presented in the SI.

Table 2. Statistical analysis for the response variables nitrogen removal rate (Y_1) and N_2O emission (Y_2) (Aer: Aeration)

		Nitrogen conversion rate (Y_1)			N_2O emission (Y_2)		
		p-Value	Effect	β	p-Value	Effect	β
C_0		$7.9 \cdot 10^{-25}$	-	-50.8659	$2.4 \cdot 10^{-08}$	-	-17363.60
X_1 :	pH	$1.5 \cdot 10^{-03}$	-0.04	13.7265	$3.3 \cdot 10^{-03}$	-37.32	4700.12
X_2 :	Feed	$1.1 \cdot 10^{-02}$	-0.03	0.0034	$1.1 \cdot 10^{-05}$	54.08	6.27
X_3 :	Aer	$4.3 \cdot 10^{-07}$	0.08	0.0091	$7.3 \cdot 10^{-02}$	22.03	2.83
X_1X_1 :	pH·pH	$5.8 \cdot 10^{-08}$	-0.17	-0.9200	$1.9 \cdot 10^{-02}$	-57.15	-317.48
X_2X_2 :	Feed·Feed	$5.2 \cdot 10^{-03}$	-0.07	-0.0003	$6.3 \cdot 10^{-02}$	-44.45	-0.15
X_3X_3 :	Aer·Aer	$2.2 \cdot 10^{-09}$	-0.19	-0.0002	$2.5 \cdot 10^{-02}$	-54.54	-0.05
X_2X_3 :	Feed·Aer	$3.0 \cdot 10^{-02}$	0.04	0.0001	-	-	-
ε		-	-	± 0.0324	-	-	± 32.12
		$R^2=0.82$		$Q^2= 0.75$	$R^2=0.50$		$Q^2= 0.34$

Table 3. Validation of both models for the estimation of the nitrogen removal rate and the N₂O emission by comparing predicted vs. experimental results

Nitrogen removal rate [kg _N /(m ³ ·d)]			N ₂ O emission [mg _N /d]		
Prediction	Experiment	Validated* (Y/N)	Prediction	Experiment	Validated* (Y/N)
Low N ₂ O emission (pH 7.80, four-fold feeding, continuous aeration)					
	0.24	Y		6.9	Y
0.25±0.03	0.25	Y	7.5±32.1	4.6	Y
	0.27	Y		6.7	Y
Moderate nitrogen removal rate and N ₂ O emission (pH 7.32, single feeding, highly intermittent aeration)					
	0.37	Y		162.1	N
0.37±0.03	0.38	Y	61.1±32.1	139.1	N
	0.36	Y		148.1	N
High nitrogen removal rate (pH 7.46, eleven-fold feeding, intermittent aeration (30 times/cycle))					
	0.41	N		222.2	N
0.49±0.03	0.38	N	123.7±32.1	155.8	Y
	0.42	N		258.9	N

* Test, if experiments for validation met the prediction criterion.

Exemplary at pH 7.5:

56.