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# Advances in analysis, quantification and modelling of N<sub>2</sub>O emission in SBRs under various DO set points

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**Abstract.** Nitrous oxide (N<sub>2</sub>O), considered a major greenhouse gas (GHG) in wastewater treatment plants (WWTPs), is produced during both nitrification and denitrification processes; hence, it needs to be controlled by internal and external strategies. Various factors, such as DO, temperature, and pH, could be incorporated into the mitigation of emissions in WWTPs. In this research, potential operational strategies were investigated in order to find the optimal range for DO and temperature for controlling the N<sub>2</sub>O production during the nitrification process. In parallel, the activity of nitrite oxidizing bacteria (NOB) could also be limited under optimal conditions to make the process more cost-effective and energy-saving. In this regard, under a lab-scale environment, DO = 0.7 mg/l was detected as the optimal range for inhibiting NOB activity and maintaining AOB activity. Moreover, the importance of developing mathematical modelling methods has gained significant attention in order to better understand the possibility of minimizing GHG in WWTPs. In this study, advanced mathematical modelling methods were used for simulating the kinetics of the nitrification process to determine the interaction among different operating factors compared to nitrification rates.

## 1. Introduction

Nitrification is a two-stage process performed by the ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) for oxidizing ammonium to nitrite and nitrite to nitrate, respectively. However, nitrous oxide (N<sub>2</sub>O) as a major greenhouse gas in wastewater treatment plants (WWTPs), is produced within the nitrification process, as a main by-product of nitrifiers' activity. Oxygen supply needs to be provided for ammonium oxidation, which makes the process complex and expensive. At the same time, oxygen limitation results in higher N<sub>2</sub>O production. Therefore, the complex control of operational parameters such as DO and temperature can be a useful and less complicated method to enhance the process efficiency and make the process more environmentally friendly.

Aeration strategy optimization is also essential for implementation of the new biotechnological processes based on nitrite metabolism considered, as a main intermediate product, such as anammox or nitrification/denitrification etc. The wastewater treatment process using these technologies is characterized by a lower oxygen and COD demand, as well as lower CO<sub>2</sub> emissions and biomass production compared to conventional processes (nitrification-denitrification). Nowadays, the use of alternative nitrification processes has become a high priority in the case of WWTP configurations that will be energy neutral. Partial nitrification has been thoroughly described by many authors, and a number of its advantages in comparison to full nitrification have been thoroughly documented [1–3].



The main advantages of the oxidation of ammonia to nitrite are: (1) 40% saving of the carbon source in the process of denitrification; (2) 25% lower oxygen demand in the nitrification process (by "suffocating" the nitrification); (3) reduction of CO<sub>2</sub> emissions by 20% in the denitrification process. Oxidation in a conventional oxygen reactor of a wastewater treatment plant with active sludge ammonia consists of two steps. In the first step, the ammonia is oxidized to nitrites by the nitrifying bacteria *Nitrosomonas*, followed by oxidation of nitrites to nitrates by microorganisms of the *Nitrobacter* group. Under favourable growth conditions for AOB, i.e., temperature above 20°C, it was proven that the growth rate was higher than NOB.

However, an issue that has to be solved is how the nitrification process is related to the production of this harmful gas can be emitted with AOB, NOB, and denitrifying micro-organisms [4–6]. Nitrous oxide (N<sub>2</sub>O) is a key greenhouse gas (GHG) with a global warming potential 300 times stronger than carbon dioxide. Nitric oxide (NO) is toxic to micro-organisms and has a negative impact on the environment, contributing to ozone layer depletion. Many researchers have investigated that emissions are affected dramatically when DO changes within nitrogen removal processes [7–11]. Zheng et al., (1994) [12] revealed that the maximum concentration of nitrous oxide occurred under very low DO concentration of around 0.2 mg/l. In the same study, Pijuan et al., (2014) [13] showed that N<sub>2</sub>O emission was inversely proportional to the DO changes, N<sub>2</sub>O production was decreased from 6% to 2.2% within the increase of DO concentration from 1.1 mg/l. In another research, the adverse effect of DO changes on emission was coupled with pH changes to enhance the oxidation rate of ammonium [14]. Harper et al., (2015) [15] revealed that the effective range of DO for predicting N<sub>2</sub>O production needs to be between 0.8 to 4.3 mg/l. In contrast, other studies have found the opposite effect of DO changes on N<sub>2</sub>O fluctuations, Peng et al., (2017) [16] reported that the increase of DO concentration together with granule size can increase N<sub>2</sub>O emission, the results also indicated a linear relation among N<sub>2</sub>O emission and ammonia oxidation rate under different DO and granule size conditions.

The aim of this study was to investigate the effect of different operational conditions (DO set point and temperature) on the efficiency of the nitrification process and N<sub>2</sub>O production. Mathematical modelling methods were also developed to better understand the behaviour of different parameters under various operational conditions. Furthermore, such models could increase productivity by finding the optimal range for design parameters in order to minimize costs and energy consumption.

## 2. Materials and Methods

### 2.1. Biomass origin

In this study, the biomass from the Swarzewo WWTP was applied. Swarzewo WWTP contains three different parts of wastewater treatment: mechanical, biological, and chemical. This WWTP has 180 000 PE, located in northern Poland, provided fresh inoculum biomass samples for each experiment. Six batch reactors are used in parallel to undertake biological nutrient removal in the biological component of the plant (SBRs). The activated sludge technique of wastewater treatment employs a single SBR cycle that reflects the phases of feeding, biological reactions (aerobic/anaerobic), sedimentation, decantation, and idle-state. According to the European Union Urban Wastewater Directive (91/21/EEC), the effluent criteria are as follows: Total N (TN) = 10 mg N/L and total P (TP) = 1 mg P/L. These regulations applied to the Swarzewo WWTP as well.

### 2.2. Lab set-up

The tests were carried out on a laboratory scale with the use of two batch test reactors (R1, R2) with a capacity of 4 dm<sup>3</sup>, equipped with a dissolved oxygen measurement system, temperature and pH. Two Clark-type microsensors (Unisense AS, Denmark) were used to measure the N<sub>2</sub>O concentration once in the liquid phase and in the gas phase. The N<sub>2</sub>O formation rate (N<sub>2</sub>O<sub>FR</sub>) was determined from the slope of the N<sub>2</sub>O concentration (linear regression), plus the concentration of the consumed NO<sub>2</sub>-N. All parameters were recorded by the computer online. Additionally, the reactors were equipped with a water jacket in order to maintain a constant temperature during the test. The pH was corrected by an

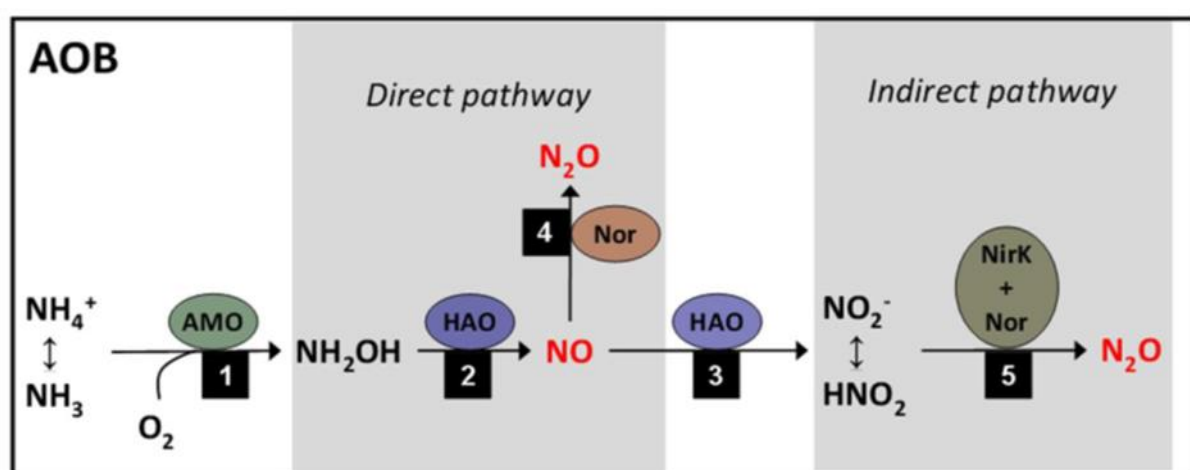
automatic 1M solution dosing system of NaOH. The DO value was kept constant with the oxygen probe and system control combined with an aerating pump.

The biomass had a 2.0 to 2.5 g MLVSS/m<sup>3</sup> concentration. Different DO set points were used for the nitrification tests, including 0.5, 0.7, 1.0, and 1.5 g O<sub>2</sub>/m<sup>3</sup>. The only supply of nitrogen was ammonium. Its concentration was increased to roughly 20 g N/m<sup>3</sup> before the testing began. The process temperature set points of each experiment were maintained at 10, 16, and 30 degrees Celsius, the pH remained between 7.5 and 8.0, and the mixing rate was set at roughly 200 revolutions per minute. Three moles of NaHCO<sub>3</sub> were added for every gram of nitrogen to provide an acceptable level of alkalinity. The concentrations of NH<sub>4</sub>-N, NO<sub>3</sub>-N, and NO<sub>2</sub>-N were determined using Xion 500 spectrophotometer (Hach Lange GmbH, Germany). Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the reactor were determined by using the gravimetric method according to the Polish Standards (PN-72/C-04559).

### 2.3. Methodology of N<sub>2</sub>O model development

In the quest for mitigation techniques based on optimal design and control, mathematical dynamic modelling of N<sub>2</sub>O emission is crucial. Two pathways are presently thought to be the main processes in charge of the emissions during nitrification among the various potential mechanisms for N<sub>2</sub>O production by AOB [17, 18]. The autotrophic denitrification of nitrite (noted "ND") [19], which involves the reduction of nitrite to NO by the NirK enzyme and the subsequent reduction of NO to N<sub>2</sub>O by the Nor enzyme, is the first main pathway. The second pathway (noted "NN") involves the HAO enzyme performing incomplete hydroxylamine oxidation [20–22], which results in the build-up of NO, which is then reduced to N<sub>2</sub>O by the Nor enzyme.

A minimum of four distinct models, each based on a single route, have been put forth and tested against various lab-scale or full-scale N<sub>2</sub>O data [23,24], yielding appropriate explanations in a variety of situations. It was not feasible to create a single model structure based on a single route that could adequately characterize all the data published in the literature, according to the conclusions of these calibration exercises, which either used batch results [23] or continuous long-term data [24]. The primary presumption used was that both NN and ND routes may exist concurrently, with each pathway's contribution depending on the operational environment. According to the batch test data records and Swarzewo WWTP data records, the overall N<sub>2</sub>O model was calibrated and verified. The study was simulated by GPS-x ver. 7.1. (Hydromantis, Canada). In order to collect data from the controlled plant, the model was constructed in MATLAB and/or a simulation tool called GPS-x. Previously, switching function was used to describe the concurrent consumption of various electron acceptors for the same donor (as well as the rivalry between them). In light of the fact that oxygen is involved in the control of the ND pathway, it was recently recommended [25] to use a new expression to characterize the inhibition of nitrite reduction by oxygen.



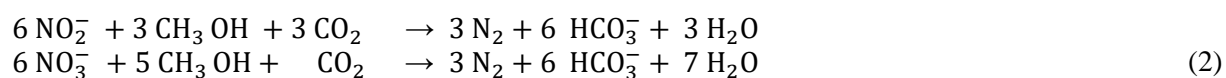
**Figure 1.** Schematic representation of the five enzymatic reactions considered in the model [25].

Finally, there is not one single way to consider two AOB pathways simultaneously in a unified approach, which still needs to be confronted with experimental observations. However, emissions were measured in aerobic batch tests and a sequencing batch reactor (SBR), in which full nitrification and denitrification were achieved. Therefore, single or multiple pathway models have been used simultaneously confronted with  $N_2O$  and  $NO$  emissions for their calibration or validation in many studies. According to a POCQUET [17] methodology and model development (Table 1 see below). Quantification, Analyses and Modelling of  $N_2O$  production in the SBRs were used in this study as an example of concept model in order to compare other modelling approaches. They were conducted using a two-pathway model from the literature.

According to Equation (1), 25% energy safe from low DO:



According to Equation (2), 40% carbon source safe:



**Table 1.** Stoichiometry and kinetics of two-paths model according to a POCQUET [17]

Model Components- 2-P model							
Process	$S_{NH}$	$S_{NH_2OH}$	$S_{NO}$	$S_{NO_2^-}$	$S_{N_2O}$	$S_{O_2}$	$X_{AOB}$
1	-1	1	-	-	-	-8/7	-
2	$-i_{N,BM}$	$\frac{-1}{Y_{AOB}}$	$\frac{1}{Y_{AOB}}$	-	-	$-\left(\frac{12}{7} - Y_{AOB}\right)$	1
3	-	-	-1	1	-	$\frac{-4}{7}$	-
4	-	-1	-4	1	4	-	-
5	-	-1	-	-1	2	-	-

Process	Kinetic rate expressions- 2-P model	
1	$q_{AOB,AMO}$	$\frac{S_{O_2}}{S_{O_2} + K_{O_2,AOB,1}} \frac{S_{NH_3}}{S_{NH_3} + K_{NH_3,AOB}} X_{AOB}$
2	$\mu_{AOB,HAO}$	$\frac{S_{O_2}}{S_{O_2} + K_{O_2,AOB,2}} \frac{S_{NH_2OH}}{S_{NH_2OH} + K_{NH_2OH,AOB}} X_{AOB}^*$
3	$q_{AOB,HAO}$	$\frac{S_{O_2}}{S_{O_2} + K_{O_2,AOB,2}} \frac{S_{NO}}{S_{NO} + K_{NO,AOB,HAO}} X_{AOB}$
4	$q_{AOB,N_2O,NN}$	$\frac{S_{NH_2OH}}{S_{NH_2OH} + K_{NH_2OH,AOB}} \frac{S_{NO}}{S_{NO} + K_{NO,AOB,Nor}} X_{AOB}$
5	$q_{AOB,N_2O,ND}$	$\frac{S_{NH_2OH}}{S_{NH_2OH} + K_{NH_2OH,AOB}} \frac{S_{HNO_2}}{S_{HNO_2} + K_{HNO_2,AOB}} X_{AOB}$

\*In the rate of process 2, an ammonium limitation for growth is mathematically imposed with

$$\frac{S_{NH}}{(S_{NH} + 10^{-12})}$$

#### 2.4. Correlation of process parameters using Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a technique for examining the connection between a number of variables and responses. RSM is helpful when data are statistically important for assessing the impact of particular individual variables and their combined interaction on each response [18]. In the present study, a standard RSM model was implemented in “OriginPro” to determine the interactions of two process variables including DO concentration and temperature influencing on the N<sub>2</sub>O production (response) in nitrogen removal system.

A mathematical model between the response (Y) and eight independent inputs ( $x_i$ ,  $x_j$ ) was described by a second-order polynomial equation [19] :

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{i < j} \beta_{ij} x_i x_j + \epsilon \quad (3)$$

where  $\beta_0$  is a constant coefficient,  $\beta_i$  are the linear coefficients,  $\beta_{ii}$  are the quadratic coefficients,  $\beta_{ij}$  are the interplay coefficients,  $x_i$  and  $x_j$  are inputs, and  $\epsilon$  is the residual error.

Moreover, the analysis of variance was used to determine which model inputs were statistically significant ( $p=0.05$ ), and then the importance level was used to determine the level of significance on the response for each input parameter and their interaction Equation (4):

$$P_i = \left( \frac{b_i^2}{\sum b_i^2} \right) \quad (i \neq 0) \quad (4)$$

where  $P_i$  is the importance level of each single model input or their interaction ( $b_i$ ).

### 3. Results and Discussion

In the literature, different influencing random conditions such as pH, O<sub>2</sub> level, or temperature are given to favour N<sub>2</sub>O accumulation and emission [20]. The N<sub>2</sub>O emissions from wastewater treatment have been reported to constitute 0.22% of the total anthropogenic N<sub>2</sub>O emissions in 2010 and have increased for almost 25% in the last 20 years. It is thus important to understand the biological mechanisms involved in these emissions in order to control and reduce the environmental impacts of wastewater treatment systems. N<sub>2</sub>O is formed as a by-product during nitrification and as an intermediate during denitrification [26, 30]. In relation to the denitrification process, N<sub>2</sub>O gas is emitted into the atmosphere if the N<sub>2</sub>O concentration in the liquid phase accumulates and exceeds the saturation concentration. Moreover, high NO<sub>2</sub> concentrations have an influence on the N<sub>2</sub>O accumulation. However, the influence of NO<sub>2</sub> is not constant but is attenuated over time. This phenomenon has already been noted by [21] and is was also confirmed by own investigations [22,23].

Table 2 illustrates different DOs and temperatures, maximum value of N<sub>2</sub>O, Ammonium utilization rate (AUR) and nitrate production rate (NPR) measured. The results show that an increase in N<sub>2</sub>O emission is not only dependent on the height of temperature, but also on the DO concentration. The same applies to temporarily elevated NO<sub>3</sub> concentrations but with a lower effect on the N<sub>2</sub>O accumulation. Nitrous oxide at a temperature of 10°C was elevated from 0.038 mg/l to 0.163 mg/l when DO varied from 0.5 mg/l to 1.5 mg/l. This increasing trend was repeated for other temperatures and the maximum amount of N<sub>2</sub>O was 0.187 mg/l at a temperature of 30°C and DO=1.5 mg/l. AUR and NPR also had same behaviour during temperature and DO fluctuation, while the minimum value of both AUR=0.59 mg NH<sub>4</sub>-N/(gVSS·h) and NPR=0.51 mg NO<sub>3</sub>-N/(gVSS·h) was observed at temperature 10 °C and DO=0.5 mg/l, the upward trend of AUR and NPR changes occurred during the increase of both temperature and DO, where the peak of AUR and NPR were 2.89 mg NH<sub>4</sub>-N/(gVSS·h) and 2.75 mg NH<sub>4</sub>-N/(gVSS·h) at temperature 30 °C and DO=1.5 mg/l.



**Table 2.** Nitrification components changes under various DO and temperature conditions.

Phase	Temp 10°C				Temp 16°C				Temp 30°C			
DO (mg O <sub>2</sub> /l)	0.5	0.7	1.0	1.5	0.5	0.7	1.0	1.5	0.5	0.7	1.0	1.5
Nitrous oxide (mg/l)	0.038	0.096	0.089	0.163	0.127	0.144	0.185	0.187	0.096	0.104	0.167	0.187
AUR (mg NH <sub>4</sub> - N/(g.VSS.h))	0.59	0.79	1.01	1.07	0.78	0.89	1.21	1.58	1.61	1.89	1.97	2.89
NPR (mg NO <sub>3</sub> - N/(g.VSS.h))	0.51	0.56	0.55	0.57	0.71	0.65	1.19	1.57	1.59	1.83	1.93	2.75

Figure 2 illustrates the N<sub>2</sub>O changes under different DO and temperature conditions. This increasing trend was repeated for other temperatures and the maximum amount of N<sub>2</sub>O was 0.187 mg/l at a temperature of 30°C and DO=1.5 mg/l. AUR and NPR also had same behaviour during temperature and DO fluctuation, while the minimum value of both AUR=0.59 mg NH<sub>4</sub>-N/(gVSS·h) and NPR=0.51 mg NH<sub>4</sub>-N/(gVSS·h) was observed at a temperature of 10 °C and DO=0.5 mg/l, the upward trend of AUR and NPR changes occurred during the increase of both temperature and DO, where the peak of AUR and NPR were 2.89 mg NH<sub>4</sub>-N/(gVSS·h) and 2.75 mg NH<sub>4</sub>-N/(gVSS·h) at a temperature of 30°C and DO=1.5 mg/l.

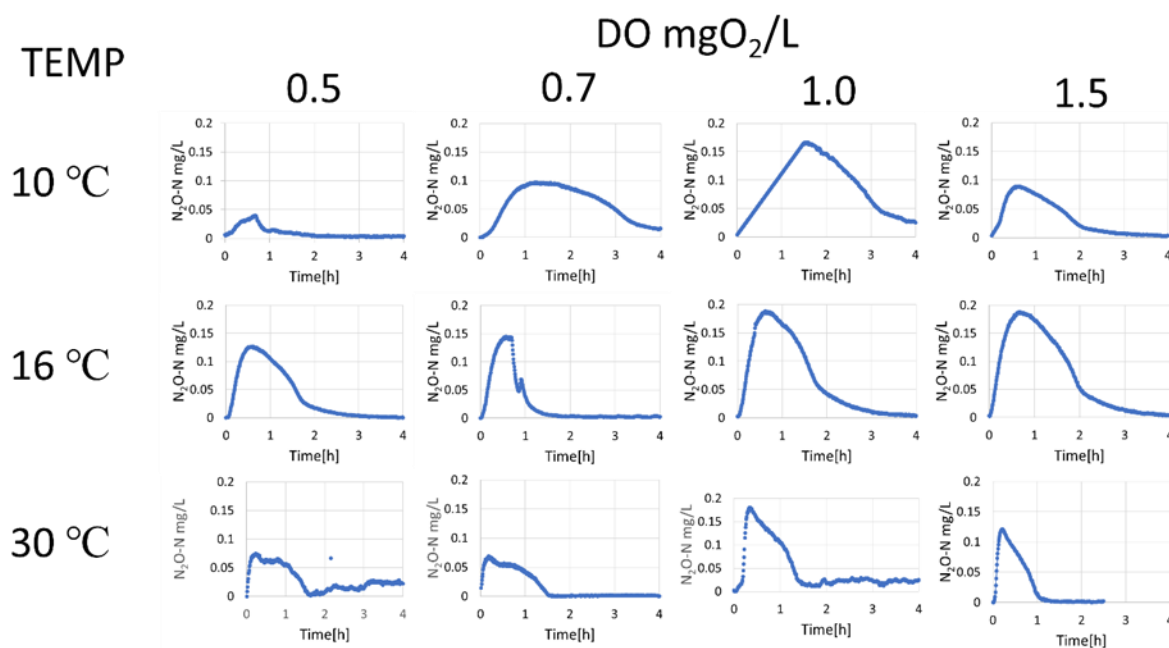
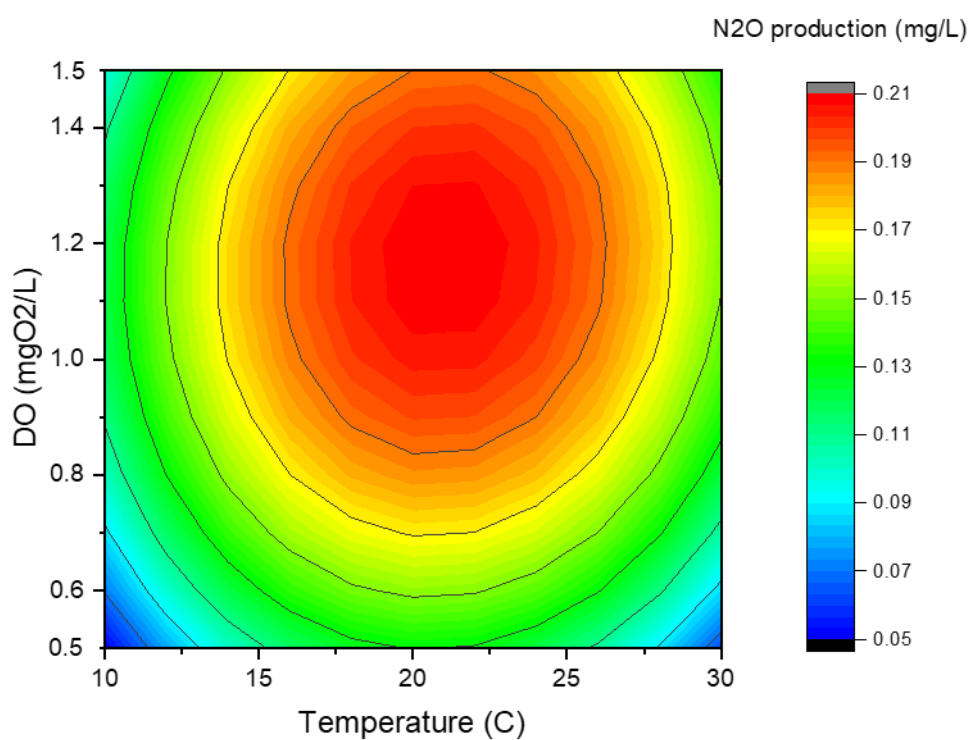
**Figure 2.** N<sub>2</sub>O changes under different DO and temperature conditions.

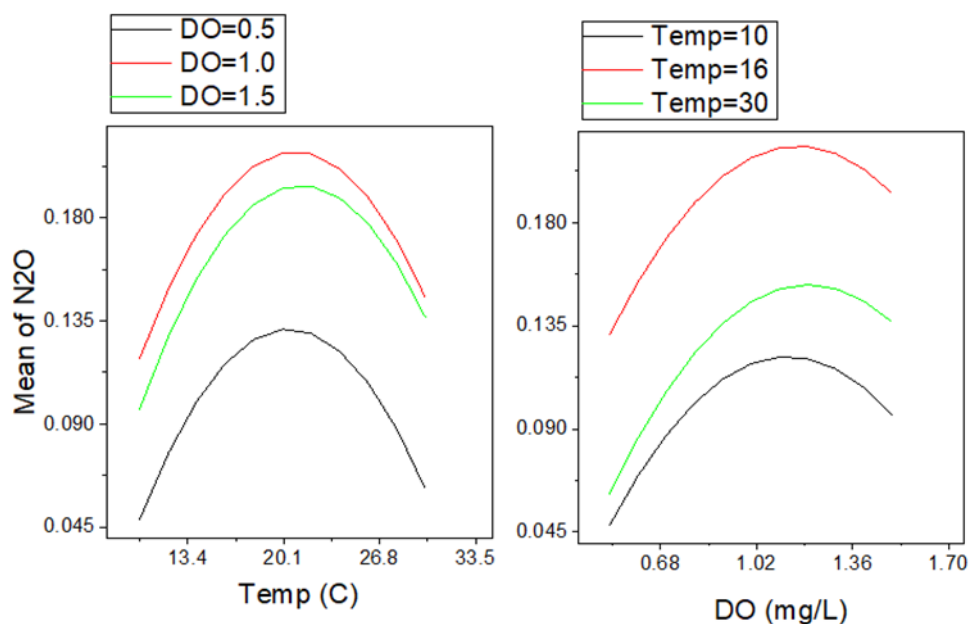
Table 3 represents the 12 possible combinations of different process parameters (temperature and DO) on the response (N<sub>2</sub>O production). The range of tested temperature was between 10 and 30°C whereas the range of DO was 0.5 to 1.5 mg O<sub>2</sub>/l.

**Table 3.** Possible combination of different process parameters (temperature and DO) on the response ( $N_2O$  production)

Iteration number	Temperature ( $^{\circ}C$ )	DO ( $mgO_2/l$ )	$N_2O$ production ( $mg/l$ )
1	10	0.5	0.04
2	16	0.5	0.12
3	30	0.5	0.08
4	10	0.7	0.1
5	16	0.7	0.15
6	30	0.7	0.07
7	10	1.0	0.17
8	16	1.0	0.19
9	30	1.0	0.17
10	10	1.5	0.09
11	16	1.5	0.18
12	30	1.5	0.13

**Figure 3.** Contour plot of the interaction effect of the process parameters (temperature and DO concentration) on the response ( $N_2O$  production).





**Figure 4.** The importance level of process parameters on the response.

For the most crucial input pairs (DO and temperature) on the response, a counter plot was made to show the coinciding effect. Figure 1 shows the counter plot of RSM study for the analysis the combined influence of temperature and DO concentrations on the  $N_2O$  production in liquid phase of nitrification-denitrification process. As can be seen in Figure 3, the highest  $N_2O$  production among all temperatures was observed for the DO range of 1.0-1.5 mg  $O_2/l$ . At low DO concentrations (<1.0 mg  $O_2/l$ ), the oxygen was lower than required for efficiently running the nitrification process; thus, low  $N_2O$  activity was achieved. The highest  $N_2O$  production can be achieved on interaction of DO and temperature at 1.2 mg  $O_2/l$  and 20°C, respectively, and the lowest  $N_2O$  production were achieved at low DO of 0.5 mg  $O_2/l$  at high and low temperature of 10°C and 30°C.

On the other hand, denitrification process contributed in experiments with DO of 0.5 and 0.7 mg  $O_2/l$ . Moreover, in the experiment with DO=1.5 mg  $O_2/l$ , the sub-optimal condition for nitrification, and excessive condition for denitrification occurred followed by more intensive stripping and lower  $N_2O$  accumulation in the liquid phase. In the experiment with DO = 1.0 mg  $O_2/l$ , based on combined flavour conditions, the highest  $N_2O$  concentrations and accumulation rates were obtained. Moreover, the importance level (Figure 4) reveals the average amount of  $N_2O$  production. As it can be seen, the highest average  $N_2O$  production during the 4 hours were correlated by DO=1.0 mg  $O_2/l$  and T=16°C (red lines).

The concentration of free ammonia, which was thought to be the actual substrate of AOB, was correlated with the ammonia oxidation rate [33], while the concentration of free nitrous acid was correlated with the nitrite reduction rate. This investigation did not take into account how  $NH_3$  and  $HNO_2$  can inhibit the development of AOB (concentrations were relatively low). Consideration of the inhibitions seen at high concentrations is a logical extension [34]. An inhibitory term as described by [25] was taken into consideration in reaction 5 to account for the impact of dissolved oxygen on the ND route.

This phrase depicts how a certain  $N_2O$  production rate rises as DO falls, up to a maximum production rate, after which the rate falls as DO approaches 0 [35]. The model was first evaluated without DO inhibition (option 1), then with a traditional uncompetitive inhibition term  $\frac{K_i}{K_i+S}$  (option 2), and finally with the modified inhibition term (option 3). With the third alternative, a significant improvement was seen, particularly for the prediction of long-term data (SBR) at relatively low DO

(1-2 mg/l). The experimental design in this study was primarily concentrated on the nitrite impact, thus it should be emphasized that this is not a general finding. With future research focused on the DO impact, this assumption would require more convincing proof.

The models also incorporated oxygen,  $N_2O$ , and NO gas liquid transfers. Utilizing the observed oxygen transfer coefficient and the corresponding diffusivity ratio, the transfer coefficients (KLa) for  $N_2O$  and NO were computed [36]. Both  $N_2O$  and NO are created in the WWTP during the nitrogen removal process, which is mostly carried out through nitrification by ammonium oxidizing bacteria (AOB). It is known that two production pathways—the NN pathway, which corresponds to the synthesis of  $N_2O$  and NO during the oxidation of ammonia to nitrite, and the ND pathway, which corresponds to the reduction of nitrite to  $N_2O$  and NO, are responsible for these emissions by AOB.

It is actually not quite clear how operational circumstances affect both NN and ND routes. Additionally, a number of  $N_2O$  models based on the NN or ND route have been created, but a generic  $N_2O$  model has yet to be developed. The main goal of this research is to use mechanistic models in conjunction with planned studies to better understand the biological mechanisms underlying the  $N_2O$  generation by AOB. The AOB  $N_2O$  production routes responsible for  $N_2O$  and NO emissions in response to environmental circumstances have been identified by comparing experimental results to the  $N_2O$  models based on a single pathway. On earlier single route models [23,37], the assumptions for the description of NN and ND pathways were predicated. Five processes were included in the  $N_2O$  model, which correspond to the following five enzymatic reactions. These five processes are as follows: (1)  $NH_3$  oxidation to hydroxylamine ( $NH_2OH$ ) with oxygen consumption; (2)  $NH_2OH$  oxidation to nitric oxide (NO) coupled with oxygen reduction; (3) NO oxidation to nitrite ( $N_2O-N$ ) coupled with oxygen reduction; and (4) NO reduction to  $N_2O$  by the enzyme "Nor" coupled with the ( $N_2O$  from ND pathway).

The original ND model [37,39] took into account two sequential processes: nitrite reduction to NO (NirK enzyme), then NO reduction to  $N_2O$ . (Nor enzyme). In the current study, it was decided to combine these two reactions into a single process (5) that would represent the direct reduction of nitrite to  $N_2O$  in a single step. This adjustment was required to prevent the NO loop, in which the nitrification process (3), which has a considerably faster rate [40], would quickly oxidize the NO generated by the ND route to nitrite. In such situation, the NO released by NirK may be seen to as (as a different state variable to avoid the NO loop). This was not necessary in the current investigation, though, since the predicted NO emissions matched the experimental findings. This is likely because at high nitrite levels (the case in this study), the Nor enzymes are extensively synthesized and quickly degrade the intermediate NO from the ND route. Thus, the impact of operating parameters on NO and  $N_2O$  emissions during nitrification by AOB has been highlighted by the study of batch tests and SBR cycles.

The simulations using the 2-P model demonstrate that  $HNO_2$  and DO have an additive influence on the routes for  $N_2O$  emission. An increase in the  $HNO_2$  concentration causes the  $N_2O$  emission factor, ND contribution, and the NO-EF/ $N_2O$ -EF ratio to drop at a constant DO (2 mgO<sub>2</sub>/l). The rise in DO causes a decrease in the  $N_2O$ -EF for a comparable  $HNO_2$  concentration (0.7 gN- $HNO_2$ /L), a little drop in the ND contribution along with a tiny increase in the NN contribution, and a slight increase in the NO-EF/ $N_2O$ -EF.

This effect of DO is in good agreement with a recent work by [42], who found that an increase in dissolved oxygen (DO) concentration from 0.2 to 3 mg O<sub>2</sub>/l led to an increase in the NN pathway contribution from 5% to 27% and that the ND pathway was the main contributor to  $N_2O$  production during ammonia oxidation (95% to 73% of  $N_2O$  from the ND pathway). Last but not least, substantial  $HNO_2$  concentrations and relatively modest DO (0.5-1 mgO<sub>2</sub>/l) emission factors are seen. This is also in line with the practical observations made, which show that the highest peak in the SBR process was seen when high  $HNO_2$  (0.9 gN/l) and low DO (1.0 mg/l) occurred simultaneously.

The conclusions concerning the oxygen impact should be treated with care because the independent effect of DO was not thoroughly examined in this experiment (greater attention was paid to the nitrite effect). Future research will focus on comparing 2-P models using the data that have both the combined DO and nitrite effect. None of the AOB models with a single route were able to account for the empirically found fluctuation in the NO/ $N_2O$  ratio. Furthermore, different pathways might have

varying effects on an operational parameter (like DO), depending on how much of a contribution they made.

Therefore, only a certain range of experimental circumstances should be used with single route models. The potential of a single route model to predictably represent the  $N_2O$  data produced by a multiple pathway model was examined by Peng et al. in 2015 [35]. The AOB denitrification model may be used at low DO (0.5 mg/l) or at high DO with considerable nitrite build up (DO > 0.5 and  $NO_2-N > 1$  mg N/l), according to the study's findings. The latter circumstance is consistent with the current work, and the conducted research supports that the ND model was the most accurate single route model to account for  $N_2O$  emission. This makes sense, given that the settings of this investigation have shown that this route significantly contributes to  $N_2O$  emission.

Nevertheless, it was also demonstrated that the 2-P model was far more effective for forecasting  $N_2O$  emissions as well as NO tendencies. In a system with low nitrite level (high NOB activity), which results in the  $N_2O$  emissions through the NN route, it was anticipated that the ND model would be unable to characterize the  $N_2O$  emissions [23]. As was previously shown, ND models [25,26,39] could represent experimental data in many systems, but they required a large and occasionally irrational change of critical parameters (AOB) to characterize a system with low nitrite levels [25]. This was presumably required to make up for the hydroxylamine route not being taken into account. The use of the hydroxylamine oxidation model was suggested by Peng et al. (2015) [35]. The hydroxylamine oxidation model may be used under the conditions of high DO (DO > 1.5 mg/l) and nitrite content between 0 and 5.0 mg N/l, according to Peng et al. (2015) [35]. The current research showed that this model was unable to forecast the findings across a wider range of nitrite values. Finally, the many route model recently provided by [40] was contrasted with the model proposed in this work. To compare the prediction capabilities of these two methods, more research would be needed. The first analysis conducted by the authors shows that while similar forecasts of  $N_2O$  emissions could be made, the 2-P model suggested in this work provided more accurate predictions of NO emissions that were in line with trials and other studies [41,43,44].

#### 4. Conclusions

In the face of a global climate change, the calculation of  $CO_2$ -footprints to quantify greenhouse gas (GHG) emissions has become an issue also for WWTPs. One of the most relevant gases, which is produced during nitrogen removal processes, is nitrous oxide ( $N_2O$ ) with a high global warming potential (GWP) of 298. In the conducted study, the effects of DO and temperature changes on the nitrification process and  $N_2O$  production were studied for batch test experiments in a lab-scale environment. Increasing DO concentration could raise the emission of  $N_2O$  and autotrophic activities during the nitrification process. When DO concentration was elevated to 1.5 mg/l for all temperatures,  $N_2O$  was significantly increased to 0.16 and 0.18 mg/l, resulting in a direct relation between nitrous oxide emissions and DO changes during the nitrification process. The results also showed that the activity of AOB and NOB was directly affected by the increase in DO and temperature, while both AUR and NPR were approximately doubled. The measurements of the  $N_2O$  production followed the same trend within different operational conditions, while the peak of  $N_2O$  production needs to be evaluated to better understand the level of its sensitivity to DO and temperature changes, which has been summarized in Table 2.

Mathematical modelling, as a reliable tool, could be beneficial to better interpret the relation between autotrophic activity and  $N_2O$  production within nitrification process. In this way, the inhibitory effect of different strategies and optimization of the nitrification process could be investigated simultaneously; however, alternatively, a variety of generalization tests can be used. Additional work will be necessary to determine which of these different  $N_2O$  model concepts would be recommended for use in each specific situation. The NO measurements from different experimental systems could be useful in order to test the proposed concept models and evaluate their ability to predict  $N_2O$  emission more thoroughly.

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