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# Modelling AOB-NOB competition in shortcut nitrification compared with conventional nitrification-denitrification process

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**Abstract.** In particular, mainstream deammonification and/or shortened nitrification-denitrification via nitrite (so-called “nitrite shunt”) is a promising new treatment concept that has the potential to revolutionise how nitrogen removal is achieved at WWTPs. Understanding the role of the AOB/NOB competition in the nitrogen cycle in wastewater treatment systems will change operational strategies of the novel nitrogen removal processes. The key role in this process is inhibition of NOB activity undesirably affects AOB activity and leads to inefficient partial nitrification process and when used as pre-treatment for Anammox it can limit nitrite supply to Anammox bacteria. Successful NOB repression requires a combination of such factors as a low DO concentration, a rapid transition from aerobic to anoxic conditions, and tight control of Temperature and/or pH. The major driving force behind the successful NOB washout is the inhibition of those bacteria based on the difference in the growth rate between AOB and NOB. The obtained results from this study show the mechanisms and operating conditions (e.g. DO concentration, Temp.) leading to complete domination of AOB over NOB under aerobic conditions. This paper presents the perspectives on modelling AOB-NOB competition in shortcut nitrification. The combined deammonification, shortened nitrification-denitrification and/or nitrification-anammox process was compared with conventional nitrification-denitrification based on own experiments and literature data. Its successful application as shortcut nitrification technology and new control system will represent a paradigm shift for the wastewater industry, offering the opportunity for sustainable wastewater treatment, energy-neutral or even energy-positive facilities, and substantial reductions in treatment costs.

## 1. Introduction

Modelling and optimisation have become a key aspect of modern-day wastewater treatment and is highly topical in wastewater engineering and research. The rising cost of energy and increased concern about sustainability have caused many wastewater treatment facilities to implement energy cost reduction programs in terms of innovative operational strategies of the novel nitrogen removal processes. Emerging technologies in wastewater treatment plants (WWTPs) are expected to decrease the high costs of energy consumption within nitrite pathways. Several internal and external factors such as temperature, pH could be incorporated into the efficiency of innovative technologies which their influences could be optimised by the application of mathematical modelling. Therefore, the importance



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of interaction between different operational conditions and their recovery effects for each other could achieve the nitrogen removal process and maintain the process stability. Conventional methods require remarkable energy to apply oxygen for nitrification and organic matters for denitrification which makes such process expensive [1-4]. Nowadays, novel technologies have gained increasing attention to alleviate energy input used for nitrogen removal process and carbon needs for denitrification using operational factors especially dissolved oxygen, while low DO concentrations can successfully inhibit NOB activity as well as making the process much more cost-effective due to the reduction of oxygen demand.

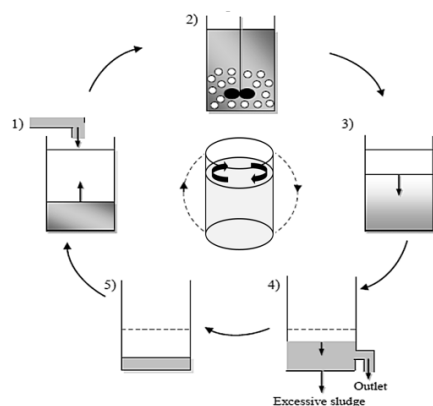
Mainstream deammonification and/or shortened nitrification-denitrification via nitrite (so-called “nitrite shunt”), compared with the conventional nitrification-denitrification process, is a promising new treatment concept that has the potential to revolutionise how nitrogen removal is achieved at WWTPs. The main challenge in implementing shortcut nitrogen removal processes for mainstream wastewater treatment is the out-selection of nitrite-oxidizing bacteria (NOB) to limit nitrate production. Understanding the role of the AOB/NOB competition in the nitrogen cycle in wastewater treatment systems can be useful in operational strategies of the novel nitrogen removal processes. The key role in this process is inhibition of NOB activity undesirably affects AOB activity and leads to inefficient partial nitrification process and when used as pre-treatment for Anammox it can limit nitrite supply to Anammox bacteria. Successful NOB repression requires a combination of such factors as a low DO concentration, a rapid transition from aerobic to anoxic conditions, and tight control of Temperature and/or pH. This assists in meeting a target effluent concentration leading to an increased capacity for denitrification (through simultaneous nitrification-denitrification and less oxygen transfer to anoxic zones) and potential supplemental carbon savings [5,6].

In this research, a relationship between different operational factors has been developed under lab-scale experiments to better understand the concept of balance AOB/NOB within the nitrification process. Nitrogen shortcut removal processes were modelled to illustrate the contribution of NOB out-selection mechanisms depend on different DO and Temp. conditions. Finally, the modelling study was used in a scenario analysis, simulating hypothetical optimised performance based on the lab-scale process using a batch test: e.g. AUR-NPR. The study aimed at developing a mathematical model of two-step nitrification – IWA ASM2d [7], based on own experiments and literature data.

## 2. Materials and methods

### 2.1 Study area

The Swarzewo WWTP consists of three parts of wastewater treatment: mechanical, biological, and chemical. This paper concerns the second part and one SBR with a capacity of 6400 m<sup>3</sup>/day. Wastewater treatment is based on the activated sludge method. Figure 1 presents a single SBR cycle reflecting the following phases: feeding, biological reactions (aerobic/anaerobic), sedimentation, decantation, and idle state. Maximum acceptable pollutant concentrations of effluent, according to the water-law permit for Swarzewo WWTP, are chemical oxygen demand (COD) = 125 g O<sub>2</sub>/m<sup>3</sup>, total nitrogen (N<sub>tot</sub>) = 15 g N/m<sup>3</sup>, total phosphorus (P<sub>tot</sub>) = 2 g P/m<sup>3</sup>.



**Figure 1.** Phases of the SBR operating cycle: 1) feeding 2) biological reactions (e.g. aerobic/anaerobic) 3) sedimentation 4) decantation 5) idle state.

## 2.2 Lab set-up

Experimental set-up and measurements were based on a series of laboratory experiments carried out in a batch reactor with a working volume of 4 dm<sup>3</sup> reflecting different conditions in full-scale SBR. The reactor was equipped with the systems for continuous monitoring and control of pH, temperature, and DO concentration. Activated sludge used in the experiments originated from the local large biological nutrient removal (BNR) facility located in the city of Swarzewo. The concentration of the biomass ranged from 2.0 to 2.5 g MLVSS/m<sup>3</sup>. The nitrification tests were run at different DO setpoints: 0.5; 0.7; 1.0 and 1.5 g O<sub>2</sub>/m<sup>3</sup>. Ammonium constituted the sole nitrogen source. At the beginning of the tests, its concentration was increased to approximately 20 g N/m<sup>3</sup>. During each experiment, the process temperature set point was kept at 10°, 16°, and 30°C, pH remained in the range of 7.5 to 8.0 and the mixing intensity was set to approximately 200 rev/min. The adequate amount of alkalinity was ensured by the addition of 3 moles NaHCO<sub>3</sub> per each gram of nitrogen. In order to control the process performance, mixed liquor samples were withdrawn from the batch reactor with a set frequency and then filtered under vacuum pressure on the Whatman GF/C. Concentrations of NH<sub>4</sub>-N, NO<sub>3</sub>-N, NO<sub>2</sub>-N were determined using Xion 500 spectrophotometer (Dr. Lange GmbH, Germany). The total nitrogen concentration was determined in Total Nitrogen Measuring Unit TNM-1 (Shimadzu, Japan). Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the reactor were determined by the gravimetric method according to the Polish Standards (PN-72/C-04559).

## 2.3 Model concept

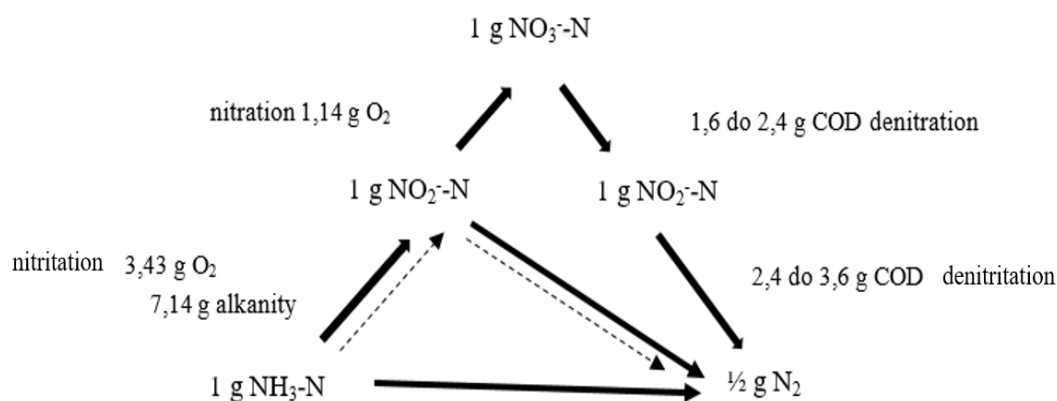
The effective operation of deammonification and/or shortened nitrification-denitrification via nitrite (so-called “nitrite shunt”), compared with the conventional nitrification-denitrification process in WWTP requires the use of modern control and optimisation techniques. The costs of implementing effective control and optimisation algorithms in a WWTP are much lower than its modernisation. The biological nitrification-denitrification processes were modelled by applying ASM2d. It is a popular mathematical description of biological processes at WWTPs. The overall model was calibrated/validated according to the data records from batch tests and Swarzewo WWTP. The simulator used in this study was Gps-x v7.1 (Hydromantis, Canada). The model was implemented in MATLAB and/or a simulation package, GPS-x, in order to obtain information data from the controlled plant. Simulations of the AOB/NOB competition in the nitrogen cycle in wastewater treatment systems such as deammonification and/or shortened nitrification-denitrification via nitrite (so-called “nitrite shunt”), were conducted using ASM2d within a two-step nitrogen removal model from the literature (table 3).

## 3. Result and discussion

Traditional methods have disadvantages due to the operational characteristics including requirements of remarkable energy to apply oxygen for nitrification and organic matters for denitrification [1,2,8]. The aeration in conventional nitrification and denitrification processes may consume from about 50% to 90% of the electricity used by a treatment plant (depending on its size and the employed technological solutions), while the cost of consumed power may constitute as much as 15–49% of total costs within a whole WWTP [9].

Several studies have proved that partial nitrification/anammox (deammonification) can be successfully applied for lab and full-scale designs for side-stream lines in plants due to the elimination of carbon demand for denitrification and having cost-effective benefits [10,11]. Besides, different process configurations have been implemented for the anammox process, including, single reactor system for high activity ammonium removal over nitrite (SHARON), partial nitrification/anammox (deammonification) under pH-controlled one-stage or two-stage configuration, completely autotrophic nitrogen removal over nitrite (CANON), SNAP (single-stage nitrogen removal using anammox and partial nitrification), simultaneous partial nitrification, anammox and denitrification (SNAD) [12-14]. Currently, novel technologies (such as deammonification and/or shortened nitrification-denitrification via nitrite (so-called “nitrite shunt”) have been investigated in order to alleviate energy input used for the nitrogen removal process and carbon needs for denitrification. An estimated potential saving of 60%

in carbon addition for nitrogen removal by implementing full-scale mainstream deammonification was predicted by Al-Omari et al. [12]. These technologies are assumed to suppress the nitrification/denitrification processes and improve the capacity of nitrification combined with an anammox-based process.



**Figure 2.** The process of shortened nitrification-denitrification and anammox and its advantages over conventional nitrification/denitrification [14].

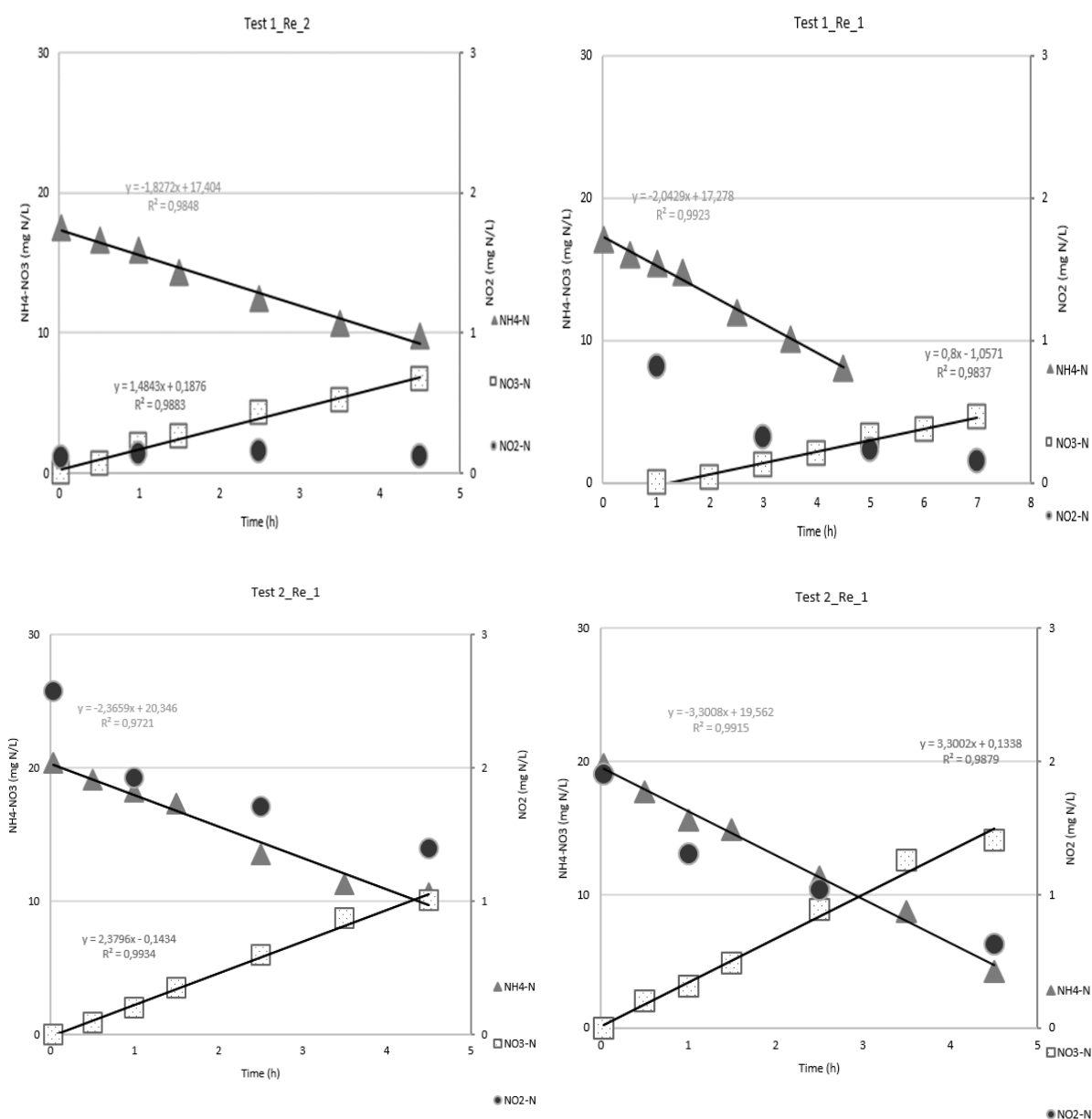
The model concept of AOB-NOB competition in shortcut nitrification compared with conventional nitrification-denitrification process started with conducting lab-scale batch tests and analysing data from Swarzewo WWTP. During a batch test, the ammonium utilisation and nitrate production rates (AUR-NPR) had an upward trend even under low-temperature conditions which confirmed the mutual influence of increasing DO concentration on process efficiency while ammonium successfully converted around 65% even under low temperature (table 1). On the other hand, increasing temperature from 10 to 30 °C could play an essential role even under low DO concentration 0.5 mg/L to maintain AOB activity and the process stability by applying high temperature 30 °C. In figure 1 under DO=0.5 mg/L condition, when the temperature changed to 30 °C, ammonium conversion rate had a faster slope and at the end of the test, the ammonium concentration decreased down to around 3.6 mg N/L, and efficiency close to 81%, demonstrating the strong relationship between operating environment which could remain the process stable.

**Table 1.** The influence of DO variations under low temperature 16°C on changing rates of ammonium, nitrite, and nitrate.

Parameters	Units	Dissolved oxygen (mg/L)			
		0.5	0.7	1	1.5
AUR	-	0.87	0.97	1.13	1.57
NPR	-	0.71	0.50	1.13	1.57
Maximum NO <sub>2</sub> <sup>-</sup>	(mg N/L)	0.16	0.81	2.58	1.91
Ammonium conversion efficiency	(%)	47	53	47	65

Ammonium removal rate and nitrite-nitrate accumulation rate were measured under different temperatures 16, 30, and 10 °C and DO concentrations 0.5, 0.7, 1, and 1.5 mg/L within reactors 1 and 2 (figure 1). The temperature decrease from 30 to 10 °C caused a sharp reduction in the ammonium conversion by approximately half but still, nitrate accumulation happened during temperature changes. The peak of nitrite accumulation was around 1.21, 2.58 and 0.68 mg N/L for temperatures 30, 16, and

10 °C, respectively. The ammonium uptake rate (AUR) and nitrate production rate (NPR) started to increase by applying higher DO to the system, at temperature 30°C, AUR and NPR at DO=1.5 mg/L were almost 2 and 1.5 times more than DO=0.5 mg/L (Table 1). The results confirmed that the increasing rate of DO and temperature improved ammonium removal efficiency up to 93%. Under low temperatures down to 10 °C, NPR and nitrite accumulation rates were interestingly affected, which caused unstable conditions for the nitrification process in comparison with higher temperatures. AUR showed a suitable trend even under low temperature based on ammonium removal efficiency as well as and nitrite accumulation, which may indicate that temperature reduction can influence NOB activities more than other bacteria within the nitrification process.

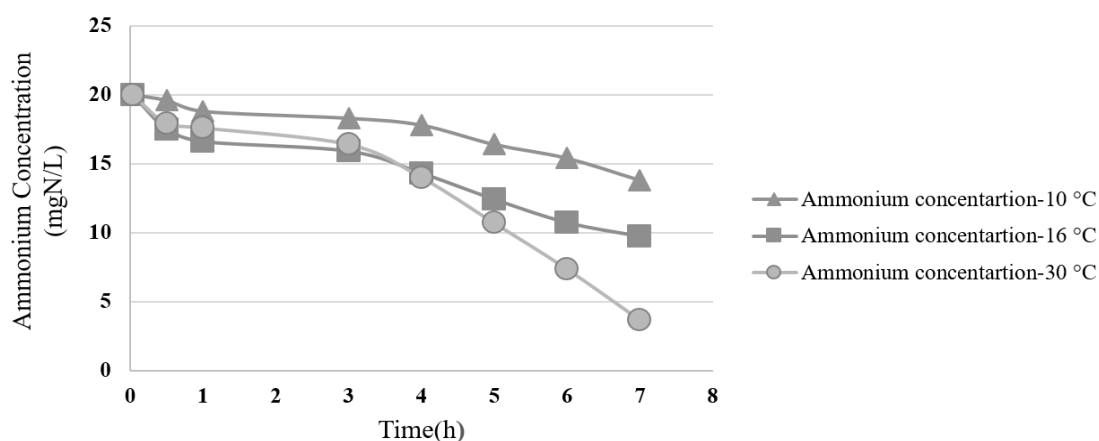


**Figure 3.** The DO influences under temperature 16 °C on the AOB-NOB competition during nitrification test in reactors 1 and 2.

**Table 2.** The change of maximum nitrite and nitrous oxide in temperature 30 °C under DO variations.

Parameters		Dissolved oxygen (mg/L)			
		0.5	0.7	1	1.5
AUR	-	1.67	1.89	1.990	2.89
NPR	-	1.67	1.88	2.040	2.10
Maximum NO <sub>2</sub> <sup>-</sup>	(mg N/L)	0.28	0.38	0.037	1.21
Ammonium removal	(%)	82	84	91	93

The performance of deammonification as a complete autotrophic method depends on the inhibition of NOBs activities, the enrichment of AOB, and the start-up of the process based on the slow growth rate of anammox bacteria (AnAOB) [15]. The main challenge in performing nitrite pathway methods such as deammonification in the mainstream is related to controlling the key factors affecting the competition between AOB and NOB for using oxygen substrate and between NOB and AnAOB for using nitrite as substrate.

**Figure 4.** The influence of increasing temperature on ammonium conversion rates under low DO concentration 0.5 mg/L.

A misunderstanding occurs concerning the real substrate for AOB (NH<sub>4</sub><sup>+</sup> or free ammonia) and NOB (NO<sub>2</sub><sup>-</sup> or free nitrous acid). The assumption behind the use of ionised or non-ionised forms still needs to be investigated. Studies concluded by Pambrun et al. [16] estimated – at different pH values – a unique value of half-saturation constant for free ammonia (NH<sub>3</sub>) but not for free nitrous acid (HNO<sub>2</sub>). Hence, the authors concluded that NH<sub>3</sub> and NO<sub>2</sub><sup>-</sup> – should be considered as the true substrate of nitrification and nitritation, respectively.

Guidelines given by Sin et al. [17] and von Schultess et al. [18] state that modelling the intermediate nitrite production and consumption would be relatively easy in the context of nitrification. However, nitrite is also produced and consumed in the denitrification process, and thus considering nitrite as a state variable in the nitrification model (but neglecting in the denitrification model) would be inconsistent according to Sobotka et al. [19]. Such an approach could generate false model predictions according to a previous study conducted by Drewnowski and Makinia [20], as well as Drewnowski et al. [21]. In this circumstances, the concept model based on ASM2d presented in this work considers the approach proposed by different authors [22-25] in order to evaluate some of the state variables for various solutions of modelling. According to the model used for the simulations was a general IWA model ASM2d that was modified in order to include a two-step nitrogen model reflected AOB-NOB

competition and/or eventually compare with the conventional denitrification/nitrification process. To simulate the impact of inorganic carbon on AOB in a such model, separate half-saturation values for inorganic carbon limited growth need to be specified. Multiple Monod terms for AOB and NOB process rates are presented in table 3.

**Table 3.** Stoichiometric matrix and process rate equations for the growth of AOB and NOB.

Process	S <sub>O</sub>	S <sub>NH</sub>	S <sub>NO2</sub>	S <sub>NO3</sub>	X <sub>AOB</sub>	A <sub>NOB</sub>	S <sub>ALK</sub>
	gO <sub>2</sub> /m <sup>3</sup>	gN/m <sup>3</sup>	gN/m <sup>3</sup>	gN/m <sup>3</sup>	gCOD/m <sup>3</sup>	gCOD/m <sup>3</sup>	mole/m <sup>3</sup>
Growth of X <sub>AOB</sub>	$\frac{(3.43 - Y_{AOB})}{Y_{AOB}}$	$-\frac{1}{Y_{AOB}} - i_{N,BM}$	$\frac{1}{Y_{AOB}}$		1		$v_{ALK,NOB}^*$
Growth of X <sub>NOB</sub>	$\frac{(1.14 - Y_{NOB})}{Y_{NOB}}$	$-i_{N,BM}$	$-\frac{1}{Y_{NOB}}$	$\frac{1}{Y_{NOB}}$		1	

$$*v_{ALK,NOB} = -\frac{1}{7Y_{AOB}} - \frac{i_{N,BM}}{14}$$

Process rate equations:

$$r_{N,NOB} = \mu_{AOB} \frac{S_O}{K_{O,AOB} + S_O} \frac{S_{NH}}{K_{NH,AOB} + S_{NH}} \frac{S_{I,FA,AOB}}{K_{I,FA,AOB} + S_{FA}} \frac{S_{ALK}}{K_{ALK,AOB} + S_{ALK}} X_{AOB} \quad (1)$$

$$r_{N,NOB} = \mu_{NOB} \frac{S_O}{K_{O,NOB} + S_O} \frac{S_{NO_2}}{K_{NO_2,NOB} + S_{NO_2}} \frac{S_{NH}}{K_{NH,NOB} + S_{NH}} \frac{S_{I,FA,NOB}}{K_{I,FA,NOB} + S_{FA}} \frac{S_{ALK}}{K_{ALK,NOB} + S_{ALK}} X_{NOB} \quad (2)$$

The concept model of modified ASM2d as an initial study was implemented in MATLAB and/or GPS-x ver. 7.1 to compare features of process control strategy used for AOB-NOB competition and/or NOB out-selection by simulating SBR. While the control strategy and parameters were selected (DO, Temp., SRT, etc.) based on the goal of the treatment required, the objective here was to demonstrate some of the features according to a lab and full-scale data that NOB wash-out is possible to achieve based on literature data and own research on activated sludge process from SBR at municipal WWTP in Swarzewo (Poland). The model was also prepared to evaluate the impact of seeding AOB (AOB bioaugmentation) from the side-stream process on NOB out-selection. Besides, the model was created to anticipate the potential improvement of SBR technology in the future such as reactor depth and retention efficiency of NOB. Finally, the optimised model in GPS-x could be used to determine the potential savings in energy consumption and carbon addition between a conventional nitrification-denitrification system and a new technology based on nitrogen shortcut (i.e. repressed NOB). According to the latest study presented by Al-Omari et al. [12], such modelling study showed that for nitrogen removal efficiency was approximately 90% and effective about 70% NOB out the selection. The acetate saving as a carbon source due to nitrogen shortcut technology was simulated up to 60% compared to conventional nitrification-denitrification.

In this study model calibration was carried out with data obtained from own batch-test experiments by Nelder-Mead's simplex method. The literature data [19, 23, 24, 25] delivered set of default parameters for AOB and NOB maximum growth rates (e.g.  $\mu_{AOB} = 0.9$  1/d and  $\mu_{NOB} = 0.7$  1/d), half-saturation coefficients for AOB and NOB (e.g.  $K_{NH_4, AOB} = 0.7$  g N/m<sup>3</sup> and  $K_{NO_2, NOB} = 0.05$  g N/m<sup>3</sup>). According to Al-Omari et. [24, 25] DO half saturations values should previously be calibrated for AOB-NOB and total inorganic carbon half-saturation for AOB. The oxygen as DO half-saturation concentrations for AOB/NOB were verified using the previously calibrated values reported by Al-Omari et al. [24, 25] and presented in table 3 and table 4. It was observed that ammonia removals in the model were lower than measured when using the default total inorganic carbon half-saturation concentration ( $K_{CO_2}$  of 0.1 mmol/L) for AOB/NOB. To match the measured removal rates of ammonia removal profiles during the batch tests at temperature 30°C from Swarzewo WWTP,  $K_{CO_2}$  of 1.5 mmol/L was used for AOB according to literature data proposed by Jones et al. [23]. Supporting data were found in the literature where e.g. the use of 4 mmol/L for AOB was recommended by Wett et al. [26] in side



stream processes, and on the other hand Guisasola et al. [27] suggested that AOB were limited by inorganic carbon availability at concentrations as low as 3 mmol/L while the NOB were not limited even at concentrations below 0.1 mmol/L. The aerobic vs. anoxic/anaerobic decay rate were used 0.17 vs. 0.08 1/d as default model parameters for AOB/NOB according to previous study Jones et al. [23].

**Table 4.** The preliminary set of AOB/NOB default or calibrated parameters based on own experimental results vs. model predictions [24, 25].

Parameter	Arrhenius on maximum spec. growth rate	Nitrous acid inhibition constant (1/d)	Yield (gCOD/gN)	DO half-saturation assumed to modelling* (gO <sub>2</sub> /m <sup>3</sup> )	
				<i>Default</i>	<i>Calibrated</i>
AOB	1.072	0.005	0.15	0.25	0.4
NOB	1.06	0.075	0.09	0.5	0.14
Temperature [°C]	-	-	-	-	30

\*values of default and/or calibrated parameters at T = 30°C, which were further evaluated within data obtained during a batch test in order to validated model at T = 16/10°C in this study

The study identified similarities between model predictions as well as measured data during all batch tests and revealed that higher concentrations of NO<sub>2</sub>-N affect both: one- and two-steps of the nitrification process. Model validation aimed at a comparison between the calibrated model within data obtained during a batch test at temperature 30°C vs. 16 and/or 10°C. The verification of the calibrated/validated model and/or optimisation results was satisfactory when differences between experimental data and simulations were below 10%. However, a calibration/validation of the model will be required either with full or a pilot scale reactor to confirm the hypotheses introduced, especially that as initial research of concept model, only some of the state variables generally used in activated sludge models were evaluated in this paper in order to preliminary check and interpret experimental results vs. model predictions from SBR Swarzewo WWTP as a first step of modelling AOB/NOB competition.

#### 4. Conclusions

The main challenge in implementing shortcut nitrogen removal processes for wastewater treatment is a deep knowledge of AOB-NOB competition and especially the out-selection of nitrite-oxidizing bacteria (NOB) to limit nitrate production. In this paper, based on own experimental research from Swarzewo WWTP and data from the literature, a model-based approach of the key mechanisms for shortcut nitrogen removal as an AOB-NOB competition was identified. Simulations were conducted on GPS-x software using a two-step nitrogen removal ASM2d model based on own experimental results and data from the literature. Nitrogen shortcut removal processes from case study – Swarzewo WWTP were modelled to illustrate the contribution of AOB-NOB mechanisms and to simulate the impact of individual features of process control strategies (as DO, Temp., SRT, etc.) in order to achieve NO<sub>2</sub>-N shunt via NOB out-selection. Therefore, satisfactory control performance for all operating conditions in WWTP cannot be achieved without use of modern control and optimization techniques. The model could be recommended as a useful tool to separate the impact of individual mechanisms for AOB and NOB competition and/or out-selection of NOB to identify artifacts associated with lab-scale reactors. The concept model based on modified ASM2d with two-step nitrogen could be used in the future as a tool for a hypothetical scenario demonstrating potential external carbon and energy savings that would be done by converting a conventional nitrification-denitrification system to a new technology based on nitrogen shortcut (i.e. repressed NOB) such as combined deammonification, shortened nitrification-denitrification and/or nitrification-anammox process. The costs of implementing effective control and optimization algorithms in order to compare such innovative biochemical processes based on AOB-NOB competition in shortcut nitrification vs. conventional denitrification/nitrification in WWTP are much lower than modernization of this facilities in real conditions. However, a calibration/validation of

the model should be required either with full scale and/or pilot/lab study using modified ASM2d in order to confirm the hypotheses introduced in this paper.

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