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The author of the PhD dissertation: Marcin J. Marcinkowski
Scientific discipline: Environmental Engineering, Mining and Energy

DOCTORAL DISSERTATION

Title of the PhD dissertation: Mathematical modelling of two-step nitrification in activated sludge systems treating ammonia-rich wastewater

Title of the PhD dissertation (in Polish): Modelowanie matematyczne dwustopniowej nitryfikacji w układach osadu czynnego w procesie oczyszczania ścieków o wysokiej zawartości azotu amonowego

Supervisor *signature*
Prof. dr hab. inż. Jacek Mąkinia

Gdańsk, September 2024

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DESCRIPTION OF DOCTORAL DISSERTATION

Author of the PhD dissertation: Marcin J. Marcinkowski

Title of PhD dissertation: Mathematical modelling of two-step nitrification in activated sludge systems treating ammonia-rich wastewater.

Language of PhD dissertation: English

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Keywords of PhD dissertation in English: mathematical modelling; activated sludge; wastewater treatment; comammox; reject water; two-step nitrification.

Keywords of PhD dissertation in Polish: modelowanie matematyczne; osad czynny; oczyszczanie ścieków; comammox; wody poosadowe; dwustopniowa nitryfikacja.

Summary of PhD dissertation in English:

This dissertation presents the development and application of a mechanistic mathematical model in an activated sludge BNR system, where the assessment of a conventional two-step nitrification model was extended. The primary goal was to identify the bottlenecks in modelling full-scale wastewater treatment technologies, focusing on nitrite shunt and comammox. Major challenges (kinetic parametrization and calibration of models) were examined. The research utilized kinetic-based competition mechanisms between AOB and NOB, with the nitrogen removal process controlled by DO concentration and aerobic SRT. A conceptual two-step nitrification model, incorporating comammox activity, was evaluated during the start-up and operation of SBR, operated at elevated temperatures, under limited DO, and fed with ammonia-rich influent wastewater. Local sensitivity analysis (LSA) was applied to identify the most critical kinetic parameters of the newly developed model. Correlation matrix was used to identify pairs of the most sensitive parameters. The model performance was comprehensively evaluated using statistical measures, including RSME, MAE, and NSE. The study highlighted the significant impact of DO on the accuracy of two-step nitrification model predictions. It emphasized the importance of accounting for the distinct comammox pathways. Special attention was given to substrate limitation mechanisms, inhibition processes, the adaptive properties of unacclimatized biomass, and biomass washout.

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Summary of PhD dissertation in Polish:

W niniejszej rozprawie przedstawiono opracowanie i zastosowanie mechanistycznego modelu matematycznego w systemie osadu czynnego z biologicznym usuwaniem biogenów, do opisu którego zastosowano rozszerzony model dwustopniowej nitryfikacji. Głównym celem badań było zidentyfikowanie ograniczeń w modelowaniu pełnoskalowych technologii oczyszczania ścieków, ze szczególnym uwzględnieniem procesu nitrytacji oraz comammox. Główne wyzwania w tym obszarze to określenie parametrów kinetycznych oraz kalibracja modeli. W badaniu wykorzystano mechanizmy kinetycznej konkurencyjności pomiędzy mikroorganizmami AOB i NOB, przy czym proces był kontrolowany poprzez stężenie tlenu rozpuszczonego i wiek osadu czynnego. Dokonano oceny koncepcyjnego dwustopniowego modelu nitryfikacji, uwzględniającego aktywność comammox podczas rozruchu i eksploatacji SBR, pracującego w podwyższonej temperaturze, w ograniczonych warunkach tlenowych i zasilanego ściekami zawierającymi wysokie stężenia amoniaku. Przeprowadzono lokalną analizę wrażliwości LSA w celu zidentyfikowania najważniejszych parametrów kinetycznych nowo opracowanego modelu. Do identyfikacji par najbardziej wrażliwych parametrów wykorzystano macierz współczynników korelacji. Wydajność modelu oceniono przy użyciu wskaźników statystycznych RSME, MAE oraz NSE. Wyniki badań wykazały istotny wpływ stężenia DO na dokładność wyników symulacji modelu dwustopniowej nitryfikacji. Podkreślono konieczność uwzględnienia odrębnych ścieżek utleniania związków azotu w procesie comammox. Szczególną uwagę zwrócono na mechanizmy ograniczające dostępność substratu, procesy inhibicji, właściwości adaptacyjne nieaklimatyzowanej biomasy oraz wypłukiwanie biomasy w fazie rozruchu.

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"Without data you're just another person with an opinion."

W. Edwards Deming

GDAŃSK UNIVERSITY
OF TECHNOLOGY
FACULTY OF CIVIL AND ENVIRONMENTAL
ENGINEERING

mgr inż. Marcin J. Marcinkowski

Mathematical modelling of two-step nitrification in activated sludge systems treating ammonia-rich wastewater

DOCTORAL DISSERTATION

Supervisor:

Prof. dr hab. inż. Jacek Mąkinia

Gdańsk, September 2024

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Acknowledgments

I want to thank two professors whose influence on my academic development was invaluable. I am grateful to the late Professor Jens A. Hansen from Aalborg University for guiding me through the early stages of my scientific journey and stimulating my research interests in the field of activated sludge wastewater treatment during my master's studies. Also, Professor Jacek Mąkinia from Gdańsk University of Technology, who inspired me and made me curious about wastewater engineering during my bachelor studies and became my mentor and supervisor during this PhD journey, for his continuous guidance and support.

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List of abbreviations

AMO	ammonia monooxygenase
AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
ASM	Activated Sludge Model
AUR	ammonia uptake rate
BNR	biological nutrient removal
BOD	Biological Oxygen Demand
CMX	comammox bacteria
COD	Chemical Oxygen Demand
CSTR	continuously stirred tank reactor
DO	dissolved oxygen
FA	free ammonia
FNA	free nitrous acid
HAO	hydroxylamine dehydrogenase
HAOB	heterotrophic ammonia oxidizing bacteria
HET	heterotrophic bacteria
MAE	mean absolute error
NOB	nitrite-oxidizing bacteria
NUR	nitrite uptake rate
PN/A	partial nitritation – anammox
RMSE	root mean squared error
SBR	sequencing batch reactor
SRT	solids retention time
TSS	total suspended solids
VSS	volatile suspended solids
WWTP	wastewater treatment plant

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I. Introduction

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1. General introduction

Nitrogen (N) is a crucial macronutrient whose speciation and accessibility are known to influence terrestrial, freshwater, and marine environments (Vitousek et al., 2013). Before the Industrial Revolution, the conversion of nitrogen gas (non-reactive nitrogen form) to reactive forms – directly or indirectly supporting biological growth – was mainly accomplished by biological nitrogen fixation mediated by bacteria and archaea (Galloway et al., 2004; Gruber and Galloway, 2008; Galloway et al., 2014). While human metabolism processes are responsible for approximately 5% of the global reactive nitrogen pool, a large fraction of metabolized nitrogen is associated with the discharge to sensitive water reservoirs and coastal waters (Larsen et al., 2007; Larsen, 2015).

Local accumulations of reactive nitrogen species have been linked to water pollution issues, including eutrophication and algal blooms, which can lead directly to hypoxia and aquatic fauna death (Gruber and Galloway, 2008). Wastewater treatment (i.e., biological nutrient removal) before discharge to these water bodies remains an effective strategy for directly removing the reactive nitrogen fractions associated with human metabolism. The following text addresses nitrogen removal's importance as related to wastewater treatment and discusses existing and developing activated sludge model concepts where novel processes for nitrogen removal are implemented.

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1.1. Nitrogen cycle in nature

Nitrogen content is approximately 80% of the atmosphere (N_2 gas) and, in parallel, is an essential component of organics, e.g., proteins' structure that are integral materials for living organisms. However, the gaseous N form is generally unavailable for most organisms to support their growth. Nitrogen gas can be converted to ammonia by nitrogenase enzymes in N_2-fixing bacteria, by photochemical reactions, and through industrial processes, as Galloway et al. (2004) described. In contrast to nitrogen gas, ammonia N consists of ammonium ions and free ammonia and is readily available for supporting growth since it does not need to be reduced to form organic compounds containing nitrogen. Upon cell death and lysis, organic N can be released and re-converted to ammonia in the ammonification process.

In addition to being incorporated into biomass, NH_4-N oxidation can also be used as an electron donor. In the autotrophic nitrification process, the energy generated from the ammonia oxidation can be used to fix the CO_2 from the environment to synthesize ammonia oxidizers and nitrite oxidizers (Liu and Wang, 2012). In the nitrification process, NH_4-N is oxidized by aerobic ammonia oxidizing bacteria (aerobic AOB) or archaea (aerobic AOA) to nitrite (+III). Nitrite oxidizing bacteria (NOB) can then convert nitrite to nitrate (+V) also under aerobic conditions. Both nitrite N and nitrate N can be used as electron acceptors during the denitrification process. Further during denitrification, nitrite and/or nitrate are sequentially converted to N_2 gas, mainly by heterotrophic bacteria. These heterotrophs use organic carbon compounds for biomass synthesis. Anaerobic ammonia oxidation (anammox) is another major process in the nitrogen cycle, which enables the conversion of ammonia and nitrite to N_2 gas and nitrate by autotrophic organisms. Figure 1.1 presents an overview of the major pathways of the conventional nitrogen cycle.

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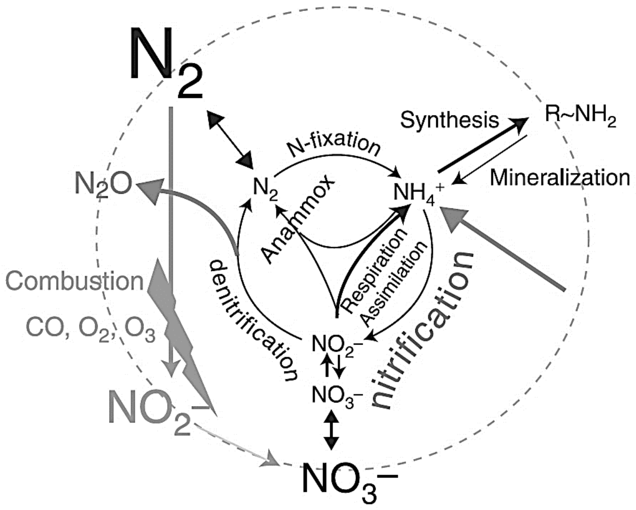

Figure 1.1. Key pathways in the conventional nitrogen cycle
(from Klotz and Stein, 2008).

Since there are five outermost electrons in the nitrogen atom, therefore it is possible to form different oxidation states from -III to +V. In wastewater, ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) are the three common stable soluble N forms among many other nitrogen compounds. Nitrogen is being changed from one form to another continuously by microbial activity. In the biological wastewater treatment processes, organic N and ammonia are considered as the influent nitrogen source and enter the nitrogen removal cycle, while ammonia is considered the main component of dissolved inorganic N in the influent. In some cases, other nitrogen forms (e.g. nitrite and nitrate) may also be present in the influent.

Excessive amounts of inorganic nitrogen and phosphorus provide nutrient sources for algae blooms and eutrophication in receiving waters (Anderson et al., 2002). Algae can cause taste and odor problems in any water body. Because algae cover the surface of the water body and hinder oxygen transfer into the water from the air, the dissolved oxygen (DO) concentration may become low, resulting in the death of fish and other aquatic organisms. Additionally, a high concentration of ammonia is toxic to many aquatic species, even killing fish and aquatic organisms in the receiving stream. Nitrate can act as a nutrient material in receiving streams and poses a health risk by contaminating drinking water supplies when they are beyond a certain level.

If the water with high nitrate concentration was supplied for drinking purposes, this problem could be significant and harmful to human health. The study of Fan (2011)

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has shown that when water contains elevated levels of nitrate (> 20 mg/L), an illness known as methemoglobinemia, hypertension and stomach cancer can occur, according to the World Health Organization (WHO). Methemoglobinemia, known also as “blue baby” syndrome, is caused by high nitrite concentrations in the bloodstream (Fan, 2011; Ward et al., 2018).

For all the above reasons and factors, controlling nitrogenous compounds and removing excess nitrogen in water bodies became mandatory over 50 years ago (Stensel et al., 1973; Stensel and Shell, 1974).

1.2. Nitrogen cycle in wastewater treatment plants

Nitrification is a commonly known key process of nitrogen removal in municipal wastewater treatment plants (WWTPs), consisting usually of two steps: ammonia oxidation to nitrite (nitritation) followed by nitrite oxidation to nitrate (nitratation). While nitrification has been known since the end of the 19th century, the process insights have changed considerably in the last 30 years. The idea that the oxidation of ammonia to nitrate is, in fact, a biological process was first given by Louis Pasteur in 1862 (Pasteur, 1862). The evolution of nitrification understanding was reflected by several expanding descriptions in engineering manuals and academic books (e.g., the well-known series of Metcalf and Eddy's authoritative book on wastewater engineering, 1991 - 2014). The improved understanding of the microbial processes of nitrification resulted in growing attention to nitrite as a key nutrient component in novel autotrophic N removal processes, e.g. deammonification referred to as a shortcut nitrogen removal process via nitrite (“nitrite shunt”), where various steps of traditional nitrification/denitrification N removal process are bypassed or eliminated. Therefore, the role of nitrite-oxidizing bacteria (NOB) has received growing attention; however, due to limited knowledge of their metabolism, the NOB group remains a “big unknown of the nitrogen cycle” (Daims et al., 2016). Particularly, the latest discovery of complete ammonia oxidation (comammox) by a single *Nitrospira* microorganism (Daims et al., 2015; van Kessel et al., 2015) has overturned “a century-old dogma of nitrification research”. However, the actual role of comammox *Nitrospira* in full-scale WWTPs is unclear (Koch et al., 2019; H. Chen et al., 2023).

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Biological wastewater treatment

The activated sludge treatment process was originally developed as an alternative to the anaerobic treatment process for removing bulk organic carbon from wastewater. During the early years of the process development, researchers found that extended aeration of sewage transformed ammonium to nitrate as described by Ardern and Lockett (1914). However, apart from delivering a ground-breaking aerobic process of wastewater treatment, the process of nitrification was mostly unexplored until the middle of the 20th century when nitrogen and phosphorus species from wastewater were linked with the cause of eutrophication (Henze et al., 2008; Rahimi et al., 2020). At this point, research into the biological N removal focused on the combined process of nitrification-denitrification to convert reactive nitrogen (primarily ammonia, nitrite and nitrate) to nitrogen gas.

Further research by several pioneers led to the development of a two-step nitrogen removal process that combined nitrification and denitrification (Sawyer and Bradney, 1945; Ludzack and Ettinger, 1962; Wuhrmann, 1964; Downing and Hopwood, 1964; Johnson, 1966; Young and McCarty, 1969). These findings were further developed by Balakrishnan and Eckenfelder (1970) and Barnard (1975) to create multi-stage processes (e.g. modified Balakrishnan and Eckenfelder, modified Ludzak & Ettinger, Bardenpho) that utilized varying redox conditions to enable carbon and nutrient removal from the mainstream flow (i.e. different configurations of aerobic, anoxic and anaerobic processes).

Sidestream N removal has extensively been implemented in both pilot- and full-scale installations. These flows can account for 5 to 30% of the total nitrogen load to the mainstream process (Khunjar et al., 2014; Mehta et al., 2015). Removal of this side-stream nitrogen using biological processes can help facilities meet strict effluent discharge limits. The side-stream treatment systems can utilize conventional nitrification/denitrification, shortcut N removal (nitrite shunt) or deammonification.

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Nitrogen removal in wastewater treatment plants

Mainstream N removal in WWTPs has traditionally relied on nitrification and denitrification processes (Figure 1.2). In this strategy, ammonia is oxidized to nitrate which is then denitrified to N_2 gas. Complete nitrification requires 4.57g O_2/g NH_4-N and 7.14 g alkalinity/g NH_4-N, while denitrification requires approximately 4 to 6 g COD/g NO_4-N. When coupled in a multi-stage process, such as the Modified Ludzak & Ettinger (MLE) process, denitrification can recover 2.86 g O_2/g NO_3-N denitrified and 3.6 g alkalinity/g NO_3-N denitrified.

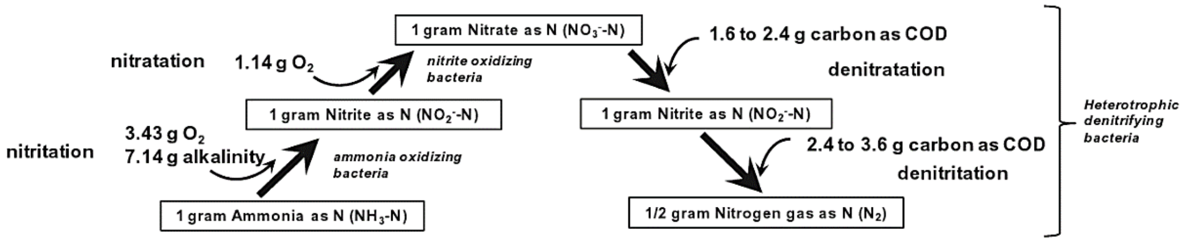

Figure 1.2. Conventional nitrification and denitrification overview
(adapted from Khunjar et al., 2014).

Today, “engineered” nitrification is considered a two-step aerobic process. The first step, nitritation, is catalyzed by aerobic ammonia oxidizing bacteria (AOB) or ammonia oxidizing archaea (AOA). The second step (nitratation) is catalyzed by the nitrite oxidizing bacteria (NOB) group. These organisms primarily display chemolithoautotrophic metabolism, deriving their energy from inorganic chemical sources (lithotrophy) and utilize CO_2 as their primary carbon source (autotrophy). Because of the need to fix CO_2, nitrifiers' growth rates and biomass yields are low compared to chemoorganoheterotrophic organisms. Equation 1.1 shows the combined processes of ammonia and nitrite oxidation.

$$NH_4^+ + 1.9O_2 + 0.069CO_2 + 0.017HCO_3^- \rightarrow 0.017C_5H_7O_2N + 0.98NO_2^- + 0.97H_2O + 1.8H^+ \quad (1.1)$$

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Downing and Hopwood (1964) demonstrated that nitrification is limited by the maximum specific growth rate of the nitrifiers and that this rate is highly liable to environmental conditions, such as changes in temperature, dissolved oxygen (DO) concentration, and inhibition by toxicants. A consequence of the low growth rate and biomass yield is the need to provide sufficient aerobic solids retention time (SRT) for nitrifiers to ensure the biomass washout does not occur.

The aerobic SRT requirements vary with temperature, but typical values range from 10 to 20 d at 10°C and 4 to 7 d at 20°C (Tchobanoglous and Burton, 1991). Expending upon this work, Nowak et al. (1995), Chandran and Smets, (2000, 2005) and Chandran et al. (2008) developed two-step approaches for mathematically describing the process of nitrification that considered the growth dynamics of aerobic AOB and NOB separately and allowed for a more realistic representation of the nitrifier microbial groups.

Denitrification is the reduction of nitrogen oxides to N_2 gas. Heterotrophic organisms (HET) catalyze these reactions in the absence of oxygen. During the reactions, nitrogen oxides are utilized as electron acceptors, and the reduced organic carbon compounds in wastewater are used as electron donors. If needed the wastewater's reduced carbon content can be supplemented with such compounds as methanol, glycerol and fermentation products to achieve low effluent nitrogen concentrations (<5 mg N/L). Equation 1.2 describes denitrification, assuming that the organic carbon present in wastewater (generalized as $C_{10}H_{19}O_2N$) is used as the electron donor.

$$C_{10}H_{19}O_2N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \qquad (1.2)$$

1.3. Novel nitrogen removal processes in wastewater treatment plants

Shortcut nitrogen removal is another option that can be used to remove nitrogen from wastewater. In this process, ammonia is oxidized to nitrite (nitritation), which is then denitrified to N_2 gas (denitritation), as presented in Figure 1.3. Nitritation requires 3.43 gO_2/g NH_4-N and 7.14 g alkalinity/g NH_4-N, while denitritation requires approximately 3 to 4 gCOD/g NO_4-N. By avoiding the conversion of nitrite to nitrate, this process can theoretically result in 25% oxygen savings and 40% electron donor savings compared

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to the nitrification/denitrification (Hellinga et al., 1998; Makinia et al., 2011; Torresi et al., 2017).

The promotion of nitrite accumulation to provide operational savings contrasts with the historical (conventional) approaches in which nitrite accumulation was viewed as an indicator of poor nitrification performance and a source of operating problems, such as bulking sludge or excessive chlorine demand during effluent disinfection (Khunjar et al., 2014).

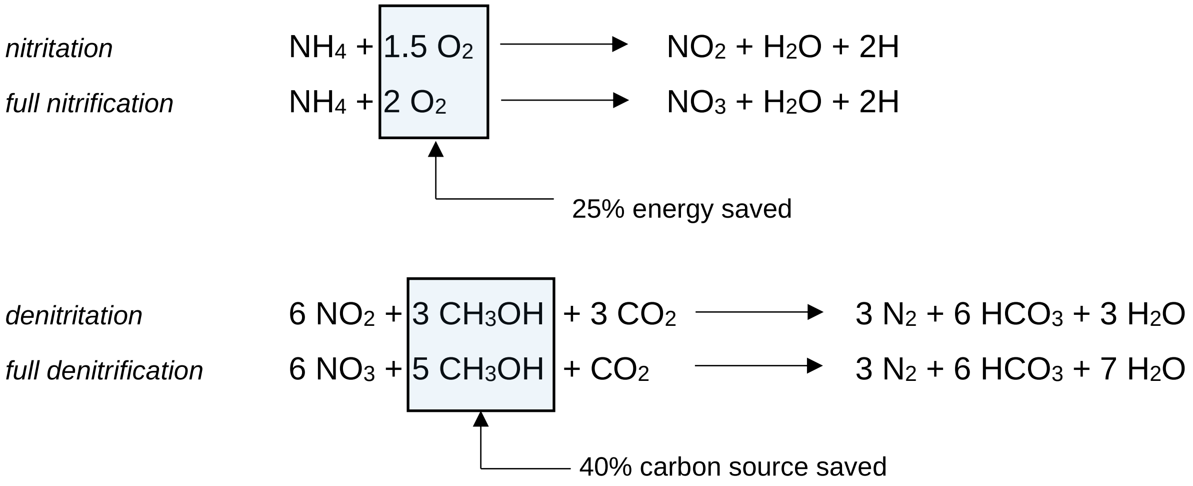

Figure 1.3. Shortcut nitrogen removal overview.

Nitrite shunt

One of the first examples of recognizing the potential for savings by utilizing the shortcut approach were researchers at TU Delft and Water Board ZHEW together with the City of Utrecht and Rotterdam, the Netherlands (Hellinga et al., 1998). They have tested pilot and full-scale nitritation/denitritation processes for treating side-stream flows with high nitrogen loads. This process, known as Single reactor system for High activity Ammonium Removal Over Nitrite (SHARON®) has been implemented in various configurations at nearly one hundred full-scale treatment facilities worldwide (Lackner et al., 2014). In this process, NOB repression is achieved using a combination of free ammonia (FA), DO, and short aerobic SRT, which enable washout of the NOBs.

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Following this process, a supplemental carbon source, such as methanol, is usually added to drive denitrification (van Dongen et al., 2001; Choi et al., 2019).

Nowadays, extensive research is carried out on using mainstream nitritation/denitritation processes for nitrogen removal. In these systems, low ammonia concentrations and low temperatures limit the ability to use FA and low aerobic SRT to control NOB growth. Recent studies (Regmi et al., 2013; Roots et al., 2020; Duan et al., 2022) have shown that successful NOB repression in the mainstream flow will require a combination of the factors that include DO concentrations, a rapid transition from aerobic to anoxic conditions and a tight control of SRT.

Deammonification (partial nitritation and anammox)

Another recent approach for removing nitrogen is the deammonification process that consists of partial nitritation and anammox (PN/A), where approximately 50% of the influent ammonia is converted into nitrite (Wett, 2007; Lackner et al., 2014; Cao et al., 2017). Nitrite and ammonia nitrogen are then consumed through anaerobic ammonia oxidation (anammox) to produce N_2 gas (Equation 1.3) and a small amount of nitrate. Compared to conventional nitrification/denitrification, this process can theoretically save 62.5% of the oxygen and all the electron donors (Figure 1.4).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 0.26NO_3^- + 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1.3)$$

Initially, the deammonification process was developed as a two-step side-stream process (treatment of anaerobically digested sludge liquor) in which nitritation was performed in a separate reactor (e.g. SHARON®) (Loosdrecht and Salem, 2006). The partially nitrified wastewater was later combined with non-nitrified digested liquor (ammonia source) to enable the nitrogen removal process. Since then, single reactor approaches have been developed in which, partial nitrification and anammox process can be carried out in a compact footprint (Sliekers, 2002; Christensson et al., 2013; Cao et al., 2017).

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Deammonification has been used for the treatment of side-stream flows containing high nutrient loads, e.g. ANAMMOX/CANON (Sliekers, 2002); DEMON (Wett, 2007); AnitaMOX (Christensson et al., 2013); CLEARGREEN (Khunjar et al., 2013). In these approaches, control of nitrite oxidation is accomplished by controlling a combination of several factors, including FA concentration, free nitrous acid (FNA) concentration, temperature, pH, DO and aerobic SRT (Gabarró et al., 2012; Zhao et al., 2023). Many process setups, such as a sequencing batch reactor (SBR), granular up-flow reactors and moving bed biofilm reactor (MBBR), have been used for this purpose.

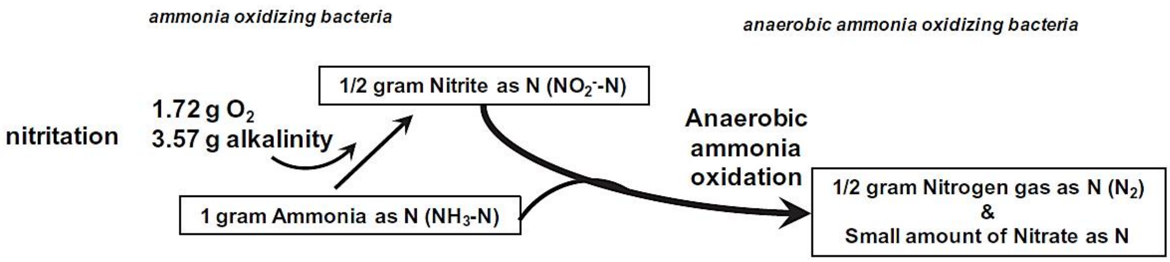

Figure 1.4. PNA process overview
(Khunjar et al., 2014).

Additional extensive research has been performed to determine the procedure for performing deammonification in the mainstream flow (Wett et al., 2013, 2015). In order to achieve the nitrite diversion pathway in mainstream systems, NOB repression depends on maintaining residual ammonia concentrations, occasionally high DO concentrations, rapid transition to anoxic conditions and tight SRT control (Regmi et al., 2013; Izadi et al., 2021). However, mainstream deammonification requires maximum retention of the anammox biomass besides suppressing the NOB activity. This can be achieved using fixed film media and SRT decoupling devices such as settlers or hydrocyclones (Wett et al., 2013; Podmirseg et al., 2022).

Partial denitrification-anammox (PdN/A)

One of the alternatives to the mainstream deammonification process explored widely is partial denitrification-anammox (PdN/A). Stable operation of the PdN/A process relies on the interaction and competition between anammox bacteria and denitrifiers. However, the mechanism of metabolic between the functional bacteria in the PdN/A

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system remains unclear, especially in the treatment of high-strength wastewater (G. Chen et al., 2023).

In the PdN/A process, a part of the influent NH_4-N is aerobically oxidized to NO_3-N. Next, the nitrate N is reduced to nitrite N by heterotrophic activity (denitratation), and the resulting mix of remaining ammonia and nitrite acts as a substrate source for the anammox process (Le et al., 2019; Lu et al., 2021; Al-Hazmi et al., 2022), as presented in Figure 1.5. This process does not involve NOB washout and requires a carbon donor to achieve partial denitrification (M. Zhang et al., 2019). Compared to the PN/A path, the PdN/A process consumes slightly more resources (energy for aeration and carbon). However, the nitrite-generating pathway is better documented than the NOB washout pathway (e.g. You et al., 2020; Izadi et al., 2021).

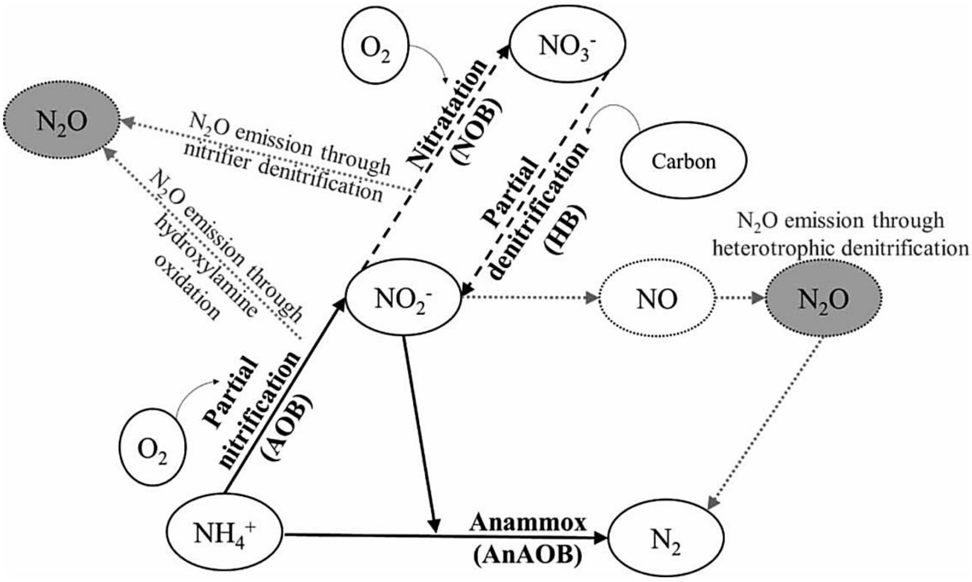

Figure 1.5. Deammonification pathways PN/A and PdN/A process including nitrous oxide emission pathways (adapted from Kirim et al., 2022).

Theoretically, 50% of aeration demand and 80% of carbon demand can be saved, and sludge production can be decreased by 60% compared to conventional N removal process (Z. Zhang et al., 2019; McCullough et al., 2022). Recent research attempts to focus on reducing the external carbon need (M. Zhang et al., 2019; Justin et al., 2022) and aim to take advantage of alternative solutions, e.g. slowly biodegradable organics in wastewater or production of soluble microbial products through fermentation (Ladipo-Obasa et al., 2022).

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1.4. Problem definition and research objectives

Activated sludge modelling aims to understand and control the biological processes and interactions in activated sludge systems. This requires a dynamic process model that describes the physiology of the individual microorganisms and the ecological interactions in activated sludge processes. Improved process knowledge should, finally, result in the development of more efficient wastewater treatment processes.

This work is intended to develop a complete biokinetic model that could describe the dynamics of the two-step nitrification, especially in bioreactors fed with high ammonia-concentrated influent. The thesis presents research results in an SBR-type process operated at elevated temperatures and limited DO concentrations. Special attention was given to substrate limitation mechanisms, the inhibition processes, the biomass adaptive properties and biomass washout during the start-up phase.

The mechanism of two-step partial nitrification and competition between AOB and NOB microorganisms at elevated ammonia nitrogen concentrations was studied. The model calibration and validation were performed with experimental data from laboratory batch tests and long-term bench scale operations, using biomass and sludge digester liquor from the Municipal Wastewater Treatment Plant “Wschod” in Gdansk, Poland.

This research aims to identify the bottlenecks in modelling full-scale wastewater treatment technologies focused on new processes, such as nitrite shunt and comammox. One of the major known bottlenecks is kinetic parametrisation and model calibration. Several approaches have been proposed, which rely on a kinetic-based AOB and NOB competition mechanism, where AOB and other microorganisms (i.e. comammox *Nitrospira*) compete for substrates. The background of controlling the nitrogen removal process towards outcompeting of NOB included (1) low DO operation, (2) high DO operation, and (3) control of the aerobic SRT. This research extends the assessment of a conventional two-step nitrification model, where the model will be used to simulate a series of lab-scale experiments.

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Besides the main goal of this research, several objectives were identified and studied, including theoretical aspects of the partial nitrification process and its practical control strategies. These key objectives are:

a. influence of nitrite on two-step nitrification process kinetics concerning high and low initial nitrite concentration.
b. study of two-step nitrification process kinetics under variable aerobic conditions (oxygen as an inhibiting factor) at a high initial concentration of nitrites.
c. determination of the most critical biokinetic model parameters for calibration and validation of the partial nitrification process.
d. application of the biokinetic model to simulate nitritation and nitratation in side stream activated sludge treatment systems of sludge digester liquors.
e. use of the biokinetic model of two-step nitrification to simulate and evaluate the impact of specific process control strategies to achieve nitrite shunt via NOB out-selection.
f. extension of the established two-step nitrification model with comammox type bacterial activity performing complete oxidation of ammonia to nitrate.

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II. Theoretical background

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2.1. Nitrification background

Nitrification is the oxidation of reduced forms of nitrogen to nitrite and nitrate. The process is an essential link in the nitrogen cycle of natural, industrial, and agricultural systems. In wastewater treatment, nitrification is a key step in nitrogen removal, linked to denitrification and fixed nitrogen loss (Ward et al., 2010).

Understanding the critical environmental factors that influence the nitrifying activity of autotrophic microorganisms leading to the nitrification process is discussed below. The most influential factors are:

- Temperature,
- pH & alkalinity,
- nitrogen substrate concentration,
- dissolved oxygen (DO).

It is important to note that the oxidation of ammonia is usually the rate-limiting step in the overall nitrification process. This means that nitrite accumulation will only appear in large amounts when the considered process is non-stationary, e.g. due to varying loads, start-up of the treatment process and washout of biomass, or other operational problems in the treatment unit (Henze et al., 2001). Many other factors, such as metals can inhibit the nitrification process in activated sludge treatment systems. As conventional nitrifying treatment plants are designed to allow the nitrification process to occur at the design temperature, even a limited inhibition could cause the nitrification to stop completely. However, this will not take place instantaneously but after several weeks (e.g. over a washout period). Such a stop in nitrification is, therefore, not a result of the nitrifying population being completely inhibited by a toxic substance or environmental factor concerned, but it is a result of the washing out of the nitrifying biomass (Henze et al., 2001; Tchobanoglous et al., 2003; Carrera et al., 2004).

Temperature

The influence of temperature on wastewater treatment efficiency is commonly known. Temperature is an important factor influencing nitrifying biomass activity, which is crucial to maintaining effective biological wastewater treatment and good-quality effluent. It also affects other physiochemical properties, such as DO concentration. The

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exact influence of temperature on biological wastewater treatment remains difficult to determine because of its interaction with mass transfer, chemical equilibria, and growth rates.

Recent studies (Gustavsson et al., 2020; Liu et al., 2021; Regmi et al., 2014) focus on temperature effect on the competition between AOB and NOB groups (difference in growth rates) and substrate (free ammonia) availability. The growth and decay rates are temperature dependent, and thus, a change in temperature can result in treatment process performance variations. Temperature rise results in two opposite effects, including increased free ammonia inhibition and increased activity of microorganisms according to the general Arrhenius relationship (Equation 2.1.). This change in microbial activity is maintained only up to a specific critical temperature, above which biological activity decreases again (not described by this equation).

$$\frac{d(\ln k)}{dT} = \frac{E_a}{R \cdot T^2} \qquad (2.1)$$

where
k – reaction rate (or equilibrium) constant, T^{-1}
E_a – activation energy, J/mol
T – temperature, K (273.15 + C°)
R – ideal gas constant, 8.314 J(mol·K)

Equation 2.1. may be integrated between the temperature limits (T and T_0), yielding:

$$ln\frac{k_T}{k_{T_0}} = \frac{E_a(T-T_0)}{R \cdot T \cdot T_0} \qquad (2.2)$$

Because most of the activated sludge systems are operated in a relatively narrow temperature range, the term $E_a/(R \cdot T \cdot T_0)$ may be constant, and thus:

$$k_T = k_{T_0} e^{C_T(T-T_0)} \qquad (2.3)$$

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The term e^{C_T} in Equation 2.3 is expressed as temperature coefficient (temperature correction factor or Arrhenius coefficient), θ, which gives:

$$k_T = k_{T_0}\theta^{(T-T_0)} \tag{2.4}$$

The accuracy of k_T is strongly dependent on the accuracy of the Arrhenius coefficient θ. To avoid an error in k_T above 10%, the temperature must be accurate to within 1°C to 1.5°C for values of θ between 1.07 and 1.10 (Argaman and Adams, 1977). A list of θ values for various biochemical reactions in the activated sludge process is presented in Table 2.1.

Table 2.1. Selected values of temperature correction factor θ for various kinetic coefficients.

Parameter	Dold et al. (1980)	Grady et al. (1999)	Salem et al. (2003)	Hwang and Oleszkiewicz (2007)	Guo et al. (2010)	Mannucci et al. (2015)
μ_{AOB}	1.123	1.098 – 1.118	1.088	1.116	1.051	1.062
μ_{NOB}		1.068 – 1.112	-	-	-	-
$K_{NH,AOB}$	1.029	1.125	-	-	-	-
$K_{NO2,NOB}$		1.157	-	-	-	-

Some authors reported that the modified Arrhenius equation (2.4) is not accurate when applied to evaluate temperature effects on treating domestic wastewater in a completely stirred tank reactor (CSTR) activated sludge process in the range of 4 – 31 °C (Sayigh and Malina, 1978; Painter and Loveless, 1983). These authors found and confirmed that the coefficient θ depends on variability in substrate characteristics and biomass adaptation (including shifts in the biomass population and the acclimation of specific bacterial groups). However, many other studies revealed that the biochemical reactions in mixed microbial cultures, e.g. activated sludge, would follow the modified Arrhenius relationship in the temperature range from 5 °C to 25 °C, with gradual temperature variation of 0.1 °C·d^{-1} (Guo et al., 2010; Mannucci et al., 2015).

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Like any other bacteria, nitrifying microorganisms are especially sensitive to sudden variations in temperature. The maximum specific growth rate (μ_{max}) of AOB often exceeds that of NOB at higher temperatures (Dongen et al., 2001; Van Hulle et al., 2010). When the temperature rise is fast (measured in hours), the increase in the growth rate is lower than expected. In contrast, a sudden temperature drop gives a much higher activity decline than expected. Investigations conducted in the temperature range between 35 and 45°C (Van Hulle et al., 2007; Wett et al., 2010) showed an optimal range for partial nitrification for short-term effects. The long-term exposure of nitrifying cultures to temperatures above 40°C is expected to inhibit and finally disable the nitritation process (Hellinga et al., 1999). Moreover, nitrification cannot take place at thermophilic temperatures (50 - 60°C) (Henze et al., 2008). Application and operation of the SHARON process (Hellinga et al., 1998) is based on the conclusion that temperatures above 25°C lead to the increased specific growth rate of AOB.

The traditional nitrification theory states that the rate of nitrification is controlled by the conversion of ammonia to nitrite by *Nitrosomonas* species (Ward et al., 2010). It is widely accepted that the oxidation of nitrite to nitrate by *Nitrobacter* species commonly occurs much more rapidly, and consequently, nitrite concentration typically remains very low (< 1 mg N/L). This postulate has been repeatedly confirmed by investigations within the typical range of nitrification process temperatures 15° to 25°C in WWTPs and has been accepted as a fundamental principle (Tomlinson et al., 1966; Henze et al., 2001; Guo et al., 2010).

pH & alkalinity

Literature shows a wide disagreement in the reported optimum range and pH effects on nitrification in general. Still, in overall agreement the optimum pH for both AOB and NOB is between 7 and 8. A very first explanation is the actual influence on the NH_4/NH_3 and HNO_2/NO_2 balance:

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$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O$$

$$NO_2^- + OH^- \leftrightarrow HNO_2$$

The preference of AOB for slightly alkaline environments possibly explains the fact that these organisms can use NH_3 as substrate (Suzuki, I. et al., 1974), while at certain pH values, ammonia and nitrous acid can explain the inhibitory effects. Besides the influence of pH on chemical equilibrium where substrate/inhibitors are involved, other studies show the direct pH effects on the nitrifiers' activity (Van Hulle et al., 2007). The first studies on the nitritation process (Hellinga et al., 1998) showed a decrease in the growth rate of the NOB population at pH = 7 compared with pH = 8, while changes in the growth rate of AOB at these pH values were insignificant. A drop in the pH value below 7 results in a nitrification rate decrease since a carbon limitation due to CO_2 stripping occurs (Wett and Rauch, 2003; Guisasola et al., 2007). Nevertheless, high nitrification rates at low pH were detected in reactors with a buffer system, e.g., a fluidized bed reactor with chalk as a biofilm carrier (Tarre and Green, 2004). Figure 2.1 presents an example of the pH effect on *Nitrobacter* specific growth rate at 32°C (W. C. Hiatt and Grady, 2008).

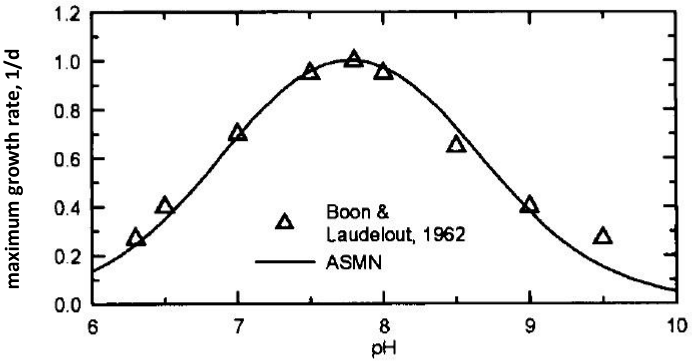

Figure 2.1. Effect of pH on the *Nitrobacter* growth rate
(Hiatt and Grady, 2008).

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Nitrogen substrate concentration

Free ammonia (FA) and free nitrous acid (FNA) concentrations have a significant influence on the nitrification process as these uncharged nitrogen forms are the actual substrate/inhibitor for AOB and NOB instead of ammonium and nitrite (Suzuki, I. et al., 1974; Anthonisen et al., 1976). This was confirmed for AOB active in the SHARON process application (Van Hulle et al., 2005, 2007). Based on the standard total ammonium nitrogen (TNH_4) and total nitrite nitrogen (TNO_2) analysis, which gives the sum of ionized and unionized compounds, the free ammonia and nitrous acid concentration can be calculated incorporating pH and temperature according to Anthonisen et al. (1976):

$$[FA] = \frac{[TNH_4]10^{pH}}{e^{6344/(T+273)} + 10^{pH}} \qquad (2.1)$$

$$[FNA] = \frac{[TNO_2]10^{-pH}}{e^{-2300/(T+273)} + 10^{-pH}} \qquad (2.2)$$

The ratio between the ionized (NH_4^+ and NO_2^-) and the unionized forms (NH_3 and HNO_2) of the substrate is determined by the pH and temperature values in the reactor and can be calculated based on acid-base equilibrium. The amount of FA increases with increasing pH, while the amount of HNO_2 decreases. This causes the promotion of ammonium oxidizers but suppresses nitrite oxidizers. Thus, NOB can be outcompeted in a weak alkaline environment (7.5–8) to produce a nitrite-rich (Anammox-suited) effluent in the nitritation reactor (Van Hulle et al., 2005, 2007, 2010). However, the potential of using this engineering approach seems somewhat limited since the adaptation of the NOB to increased FA concentrations has also been reported (Turk and Mavinic, 1989; Joss et al., 2009). Therefore, it was stated that stable partial nitritation only occurs when factors other than FA and FNA are regulated (Peng and Zhu, 2006; Ma et al., 2016).

Regarding inhibition, it can be concluded that free ammonia is the main inhibitor of nitrification at high pH (above 8), whereas HNO_2 is the main inhibitor at low pH (below 7.5). In literature, different threshold values were proposed for nitrification inhibition,

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but these are very sensitive to microorganism adaptation (Prakasam and Loehr, 1972; Anthonisen et al., 1976) and require an individual approach.

Anthonisen et al. (1976) stated that AOB are inhibited at NH_3 concentrations of 8–120 mgN/L and HNO_2 concentrations of 0.2–2.8 mgN/L, while inhibition of nitrite oxidation is observed at an NH_3 concentration of 0.08–0.82 mgN/L and HNO_2 concentration of 0.06–0.83 mgN/L. Further studies have confirmed that free ammonia only partially affects the inhibition of nitrite oxidation (Hawkins et al., 2010; Joss et al., 2011; Cao et al., 2017). They found that pH variations had a greater influence on the NOB activity than AOB activity.

Dissolved oxygen (DO)

The requirement, tolerance or sensitivity to molecular oxygen varies widely among microorganisms. Aerobes use oxygen and may need it (or require it), can function in its absence (facultative requirement) or require it in low levels (microaerophilic). Anaerobes do not use oxygen but may tolerate it (aerotolerant) or not (obligate). During the nitrification process, the DO concentration is highly important for both AOB and NOB populations (Philips et al., 2002; Park and Noguera, 2004). AOB are more robust against low DO concentration than NOB. Accumulation of nitrite at low DO concentrations is usually explained by the difference in the DO half saturation constant (K_O) for AOB and NOB (Hanaki et al., 1990; Park and Noguera, 2004; Liu *et al.*, 2020). In other words, oxygen deficiency due to low DO concentration influences the activity of NOB more significantly compared to AOB (Hanaki et al., 1990; Laanbroek and Gerards, 1993; Park and Noguera, 2004). This difference could be explained by the higher energy released per amount of oxygen consumed by AOB compared to NOB. However, values reported for the half-saturation constant for activated sludge systems vary in the range of 0.25 – 0.5 mgO_2/L for AOB and0.34 – 2.5 mgO_2/L for NOB (Van Hulle et al., 2010; Kits et al., 2017). The selection of values of the KO parameter for AOB and NOB found in the literature are presented in Table 2.2.

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Table 2.2. Oxygen affinity constant (K_O) values for AOB and NOB (adapted in part from Sin et al., 2008)

Type of wastewater →		**SSW**	**SSW**	**WW**	**WW**	**SSW**	**SSW**	**WW**	**SSW**
Reference →		Hellinga et al., 1999 (35°C)	Volcke, 2006 (35°C)	Moussa et. al., 2005 (30°C)	Kampschreur et al., 2008 (20°C)	Wett &Rauch, 2003 (26°C)	Jones et al., 2007 (35°C)	Sin et al., 2008 (15°C)	Mehrani et al, 2021 (20°C)
Parameter	Definition								
$K_{O,AOB}$	AOB affinity for oxygen (mgO$_2$/L)	1.45	0.9	1.0	0.5	0.4	0.25	0.1	0.3
$K_{O,NOB}$	NOB affinity for oxygen (mgO$_2$/L)	1.1	1.1	1.0	1.0	1.0	0.5	0.3	0.2

SSW – sidestream wastewater; WW – mainstream wastewater

This variation may be due to the reactors' different oxygen mass transfer efficiencies. The oxygen concentration inside a sludge floc or biofilm does not necessarily equal that of the water phase. The half saturation constant is, therefore, dependent on the biomass density, the floc size, the mixing intensity, and the rate of oxygen diffusion in the floc (Blackburne et al., 2008; Lackner et al., 2014).

Significant oxygen-limiting conditions can be considered another way to outcompete NOB. Nevertheless, it is also suggested that free hydroxylamine inhibition of NOB rather than a difference in oxygen affinity constants causes nitrite build-up in nitrifying systems at low DO concentrations (Yang and Alleman, 1992; Jianlong and Ning, 2004). Table 2.3. presents a summary of the effects of DO concentration on nitrification in activated sludge systems with a focus on nitrite accumulation.

Table 2.3. Effects of DO concentration on nitrification in activated sludge systems (adapted in part from Paredes et al., 2007)

DO [mg/L]	Effect	Reference
<0.5	nitrite and ammonium accumulation	(Ruiz et al., 2003; Ciudad et al., 2005)
0.7	nitrite accumulation up to 67% of the applied NH_4^+	(Ruiz et al., 2003; Ciudad et al., 2005)
1.0	80% oxidation of NH_4^+, 80% as NO_2^-	(Ciudad et al., 2005)
1.4	99% oxidation of NH_4^+, 70% as NO_2^-	(Ciudad et al., 2005)
>1.7	full nitrification	(Ruiz et al., 2003)
2.4	99% oxidation of the applied NH_4^+, 10% as NO_2^-	(Ciudad et al., 2005)

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2.2. Nitrification in mainstream systems

Biological nitrogen removal (BNR) in most modern wastewater treatment plants (WWTP), usually present in the form of ammonium or organic nitrogen, is carried out by conventional nitrification/denitrification processes in various configurations (Figure 2.2). This conventional nitrogen removal approach is an energy-demanding and resource-intensive process. Nitrification requires oxygen and alkalinity, and denitrification requires carbon sources as either influent or supplemental carbon (Fu et al., 2022; Kirim et al., 2022). The benefits of the conventional process are the high potential removal efficiency, high process stability and reliability, relatively easy process control, low area requirement and moderate cost (Tchobanoglous et al., 2003). The overall disadvantage of the conventional activated sludge BNR processes is a requirement for significant tank volumes with associated capital costs, particularly in cold climates where the reliability of nitrification is a concern in terms of system sludge age (Hu et al., 2012). Other limitations of the activated sludge BNR process include filamentous sludge bulking, complex aeration and mixing setups, and more complicated process design and operation (Tchobanoglous et al., 2013). Generally, the conventional biological nitrogen removal process is applied in the treatment of low-strength wastewater (low nitrogen concentrations with total nitrogen concentration less than 100 mg N/L).

Fundamentally, all aerobic biological treatment systems operate with the same principles, i.e., trickling filters, aerated lagoons, contact-stabilization, extended aeration, etc. They only vary in the conditions under which the biological reactions are limited to run. A typical activated sludge system comprises the flow regime in the reactor, its size and shape, the number and configuration of the reactors, recycle flows, influent flow and other features combined either intentionally or present in the treatment system unintentionally or unavoidably (Henze et al., 2008). Although the reaction of the nitrifying microorganisms follows their nature, i.e. biological process behaviour, the response of the system is controlled by a combination of the microorganisms' performance and physical characteristics of the treatment system, i.e. environmental conditions or system limitations under which the biological processes are constrained to operate (e.g. mixing regime, sludge retention time, hydraulic retention time, etc.) (Henze et al., 2001, 2008).

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It can be concluded that nitrogen removal is important for large mainstream treatment systems not only for fulfilling legal regulations and standards for effluent but also as a key factor for the design and extension of WWTP, as the reaction volume design is based on biological nitrogen elimination process rates (Rosenwinkel et al., 2009).

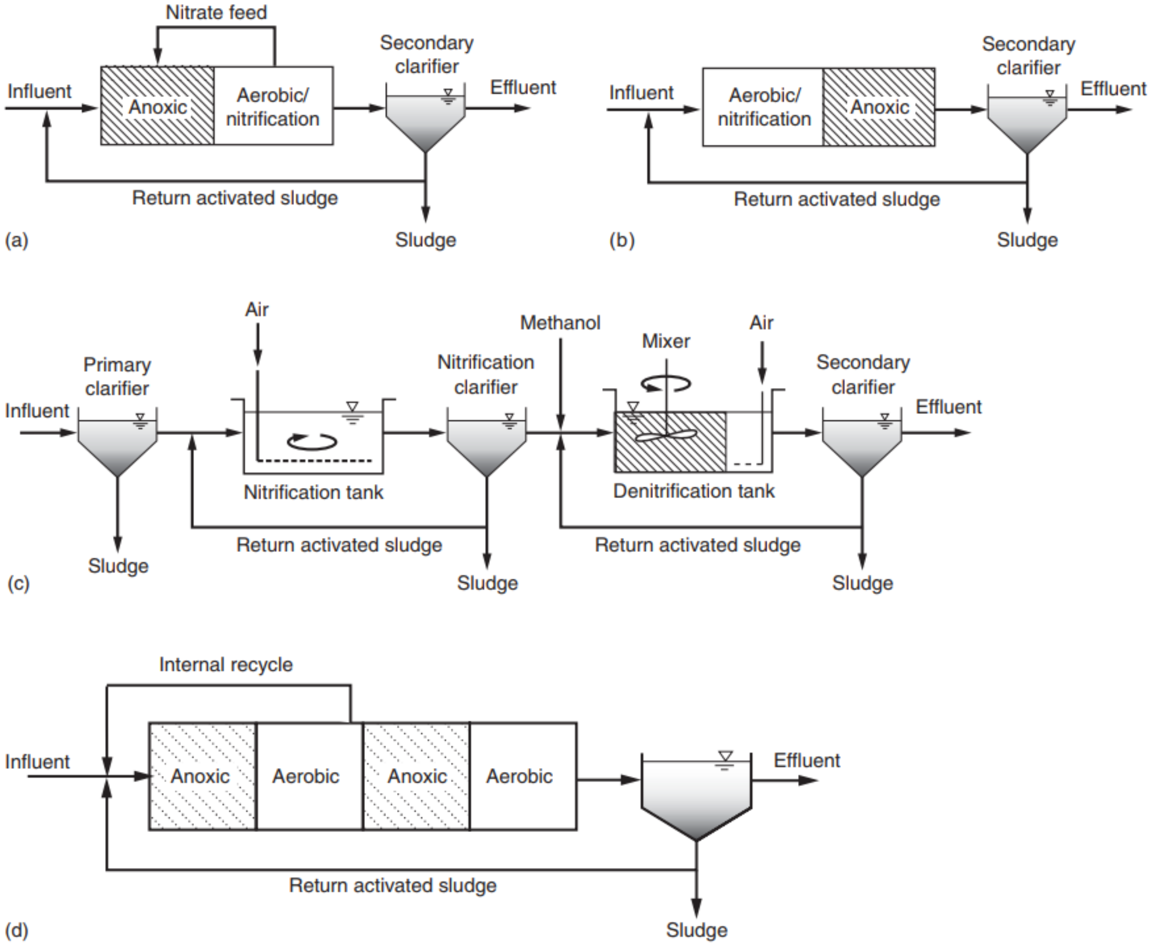

Figure 2.2. Schematic diagram of four basic biological nitrogen removal process configurations (a) preanoxic, (b) postanoxic, (c) two-sludge nitrification-denitrification, and (d) Bardenpho process (Tchobanoglous et al., 2013).

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2.3. Nitrification in side-stream systems

Compared with municipal wastewater, some wastewater streams, such as anaerobic digester effluents, landfill leachate and industrial wastewater, contain high nitrogen concentrations. Side streams originating from sludge and biosolids processing are generally treated in the mainstream plant. A typical solution is that the side streams are returned to the WWTP inlet, the inlet of primary sedimentation tanks or directly to the secondary treatment process (e.g. channels carrying primary effluent or return sludge to the activated sludge reactors). These high-strength effluents return points are dependent on the nutrient, BOD and suspended solids loadings associated with particular side-stream together with WWTP's physical limitations, such as the plant piping configuration and the location of the dewatering process relative to the main treatment process (Henze et al., 2001; Tchobanoglous et al., 2003).

The conventional BNR process, as mentioned previously, is carried out by nitrification and denitrification processes in a controlled environment. While these are effective treatment technologies, both steps produce nitrous oxide (greenhouse gas), besides nitrification being an energy-intensive process and denitrification being potentially costly in operation where organic carbon is required for wastewater with a low COD/N ratio. However, the nitritation process coupled with denitritation or anaerobic ammonium oxidation was identified as a promising alternative by many research projects and applications (Van Hulle et al., 2010; Winkler and Straka, 2019; Liu et al., 2020).

Nitritation/denitritation

Denitrifying microorganisms can use nitrite or nitrate as their electron acceptor, and while full nitrification requires 4.57 mgO_2/mgN, nitritation only requires 3.43 mgO_2/mgN. Therefore, suppressing nitratation can save approximately 25% of aeration costs (Peng and Zhu, 2006; Joss et al., 2009). Furthermore, denitritation rates appear 1.5 to 2 times more rapid than denitrification, organic carbon requirement is up to 40% smaller (decrease in total COD), and sludge production is theoretically reduced by approximately 33% for nitrification and 55% for denitrification (Regmi et al., 2013). Different strategies have been established and implemented to control NOB activity and washout while enriching AOB activity. These include alternating anoxic and oxic

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conditions, SRT control, step feeding, and intermittent aeration (Blackburne et al., 2008; Liu et al., 2020).

Partial nitritation-anammox (PN/A)

Extended energy-saving improvement to the nitritation/denitritation process is connecting partial nitritation with anaerobic ammonia oxidation (*Anammox*) (PN/A). In existing applications, approximately 50% of the ammonia is oxidized by AOB to nitrite, and the remaining 50% is anaerobically oxidized by *Anammox*. Key advantages of PN/A process over the conventional BNR process are: 1) no organic carbon required (fully autotrophic process), 2) about 60% smaller energy demand for aeration, 3) about 75% less sludge production, and 4) decreased emission of CO_2 and potentially N_2O since both gases are not produced in *Anammox* metabolism (Lackner et al., 2014; Ma et al., 2016; Cao et al., 2017). However, it is important to note that the AOB activity is reported to yield more nitrous oxide under DO limited conditions (Massara et al., 2017).

Complete ammonia oxidation (comammox)

NOB catalysing the second step of nitrification (nitrite oxidation to nitrate) were recently identified as nitrifying microorganisms capable of ammonia oxidation directly to nitrate (complete ammonia oxidation; comammox) (Daims et al., 2015). This finding of NOB genus *Nitrospira* already has broad implications for present and future research on nitrification and the nitrogen cycle, where comammox links two steps of a known process into one rather than representing a novel link in the nitrogen cycle (Costa et al., 2006). Comammox organisms show an extremely high affinity for ammonia (Kits et al., 2017), therefore, their presence may be challenging in PN/A systems if comammox bacteria can produce nitrate. However, early results indicate that comammox *Nitrospira* is not very effective at nitrite oxidation and may function as an ammonia oxidizer (Daims et al., 2016; Kits et al., 2017; Lawson and Lücker, 2018). Furthermore, it has been suggested by Kits et al. (2017) that comammox *Nitrospira* can perform dissimilatory nitrate reduction to nitrite, which could be beneficial to Anammox systems, but so far, it is the theoretical approach. Since their first discovery, comammox *Nitrospira* has been identified in several engineered systems, including aquaculture biofiltration units, drinking water treatment and distribution systems, and wastewater treatment plants (Lawson and Lücker, 2018).

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2.4. Factors affecting shortcut nitrification process

The key step for controlling partial nitrification is to obtain a nitrifying reactor with a stable nitrite accumulation. The main parameters reported to allow nitrite accumulation are oxygen limitation (Garrido et al., 1997; Pollice, 2002), temperature effect (Hellinga et al., 1998), and free ammonia and nitrite inhibition (Anthonisen et al., 1976; Surmacz-Gorska et al., 1997; Yoo et al., 1999). One of the examples that follow the” nitrite path” is a single stirred bioreactor with no biomass retention (i.e. chemostat type) that has been proposed for treating nitrogen-rich wastewater via partial nitrification: the SHARON® technology (Hellinga et al., 1998; van Dongen et al., 2001).

An advantage of this system is that the hydraulic retention time controls the sludge retention time. Therefore, in a system stabilized at 35°C and with a hydraulic retention time of about 1 day, it is possible to maintain only ammonia-oxidizing bacteria (AOB) in the reactor (Hellinga et al., 1998; Fux et al., 2002). As the chemostat reactor is operated at a given hydraulic retention time, the designed volume of this process depends only on the wastewater flow. In contrast, in a system with biomass retention, like the sequencing batch reactor (SBR), the maximum volumetric load depends mainly on the maximum oxygen transfer capacities.

Partial nitrification can be carried out with different types of reactors, such as attached-growth or suspended-growth processes, SBR, or CSTR (Hellinga et al., 1998; Pambrun et al., 2006). Regardless of the configuration, maintaining a long-term stable nitrite accumulation is one of the most stringent issues.

DO concentration

Adjusting DO concentration in a biological reactor is one of the possible ways to enhance nitrite accumulation (see Table 2.3). It is based on the differences between the oxygen saturation coefficients of the Monod kinetics for ammonium oxidation and nitrite oxidation (Arnaldos et al., 2015). A possible mechanism for inhibiting NOB activity (and nitrite oxidation) by lower DO concentrations is based on the accumulation of hydroxylamine, an intermediate product of ammonia oxidation. Under low DO conditions and high ammonia concentration, hydroxylamine accumulates and causes an inhibitory effect for NOB at values as low as 0.2 mg (Jofra et al., 2020). Hydroxylamine concentrations above 2000 mg/L were also reported to inhibit the AOB activity (Jofra et al., 2020; Yang and Alleman, 1992). However, in wastewater treatment engineering, hydroxylamine presence is typically ignored in nitrification

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processes due to an indirect hypothesis that it will be available at significant levels (Zhao et al., 2022).

For suspended growth biomass systems (i.e. activated sludge) under limited DO supply, complete and stable conversion of ammonia into nitrite was obtained, independent of the solids retention time (SRT). However, when there was no DO limitation, the SRT became the critical parameter for partial nitrification. Yang and Allemann (1992) concluded that a combination of free ammonia inhibition, low DO concentration and hydroxylamine accumulation were the main factors of nitrite build-up within an enriched nitrifying culture under batch conditions.

pH

Interpreting the optimum pH range for nitrification (see section 2.1 pH & alkalinity), three different effects of pH on the nitrifying microorganisms have been identified and reported: activation and deactivation of nitrifying biomass (reversible process); nutritional effects associated with the alkalinity and the species of inorganic carbon available, and inhibition through free ammonia and free nitrous acid (Antoniou et al., 1990). Activation-deactivation of nitrifying bacteria is linked to binding H^+ or OH^- ions to the weak basic groups of the enzymes (Grunditz and Dalhammar, 2001). Nutritional effects are mainly associated with the availability of the mineral carbon, which is required as a carbon source for the nitrifying autotrophic microorganisms. The CO_2 species can be easily removed from water by stripping associated with the reactor's aeration at low pH. A high pH value favours mineral carbon presence in the form of carbonate species, which is barely assimilated in most cases (Paredes et al., 2007). At higher pH values, free ammonia increases, while nitrous acid concentration increases at low pH.

Substrate inhibition

Both AOB and NOB can inhibited by either free ammonia or nitrous acid, but NOB are more sensitive than AOB to free ammonia (Anthonisen et al., 1976). However, the threshold concentration above 150 mg/L of free ammonia, at which the nitrite oxidation is inhibited, increases with time. Wong-Chong and Loehr (1975) observed that pure cultures of *Nitrobacter* adapted to free ammonia could tolerate concentrations above

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40 mg N/L, while a concentration of 3.5 mg N/L could inhibit non-adapted NOB. Further studies on biofilm and suspended biomass treatment systems showed that NOB can be adapted to higher free ammonia concentrations (Villaverde, 2000), and after a long exposure time (i.e. 6 to 12 months), the nitrite accumulation decreases in the system and the nitrate concentration increases (Fux et al., 2004), making it difficult to maintain long-term partial nitrification in a nitrifying reactor.

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2.5. Nitrification microbiology

Microorganisms known to be involved in the nitrification process include autotrophic ammonia-oxidizing bacteria (AOB), heterotrophic ammonia-oxidizing bacteria (HAOB), ammonia-oxidizing archaea (AOA), nitrite-oxidizing bacteria (NOB), and complete ammonia oxidizers (Comammox). The following section describes the three most important groups, AOB, NOB, and Comammox, with a general overview of heterotrophic activity during nitrification.

Autotrophic ammonia oxidizing bacteria

There are three identified species of autotrophic ammonia-oxidizing bacteria (Ward et al., 2010): two Betaproteobacteria (*Nitrosomonas* and *Nitrosospira*) and Gammaproteobacteria (*Nitrosococcus*). Ammonia oxidation by autotrophic AOB involves two main enzymes: ammonia monooxygenase (AMO) and hydroxylamine dehydrogenase (HAO). AMO from autotrophic ammonia oxidizing bacteria catalyses the oxidation pathway's first step by oxidizing ammonia to hydroxylamine (reaction 2.1). Next, HAO catalyses the oxidation of hydroxylamine to nitrite (reaction 2.2):

$$O_2 + NH_3 + 2e^- \xrightarrow{AMO} NH_2OH + H_2O \tag{2.1}$$

$$NH_2OH + H_2O \xrightarrow{HAO} HNO_2 + 4H^+ + 4e^- \tag{2.2}$$

In the first reaction, the actual substrate (Suzuki, I. et al., 1974), ammonia (NH_3) rather than ammonium (NH_4^+) is oxidized to hydroxylamine (NH_2OH) via catalysis by ammonia monooxygenase (AMO). In the second step, NH_2OH is oxidized to NO_2^- via hydroxylamine oxidoreductase (HAO) catalysis.

AOB was reported to be present not only in most natural aerobic environments, including soils, freshwater, and marine ecosystems but also in some low-oxygen environments like brackish waters and subsurface sediments (Holmes et al., 2018). AOB are also dominant in many ammonium-rich environments that have been impacted by anthropomorphic nitrogen sources such as fertilizers, wastewater, and industrial by-products (Ward et al., 2010).

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Heterotrophic ammonia oxidizing bacteria

Several differences must be understood to distinguish between chemolithoautotrophic ammonia-oxidizing and heterotrophic ammonia-oxidizing prokaryotes. Most importantly, heterotrophic ammonia oxidation does not generate energy (Stein, 2011). Consequently, many heterotrophic nitrifiers couple ammonia oxidation to aerobic denitrification to dispose of surplus reducing power generated during ammonia oxidation (Richardson et al., 1998; Ward et al., 2010). The heterotrophic nitrification rate is considerably lower than the autotrophic rate. However, large heterotrophic populations in various ecosystems allow them to make major contributions to the global nitrogen cycle. A wide diversity of bacteria from various phyla are capable of heterotrophic ammonia oxidation, and they can make substantial contributions to nitrogen cycling in such wastewater treatment systems as activated sludge (Stein, 2011), landfill leachate treatment systems (Chen et al., 2012) and ammonium-rich industrial wastewater treatment system (Yang et al., 2011).

The ammonia oxidation pathway utilized by heterotrophic nitrifiers is not clear, but it has been proposed that ammonia oxidation occurs like autotrophic nitrification. First, ammonia is oxidized by AMO to hydroxylamine, which is then oxidized to nitrite (Holmes et al., 2018).

Autotrophic nitrite oxidizing bacteria

Chemolithoautotrophic nitrite-oxidizing bacteria (NOB) include the second group of microorganisms involved in nitrification. NOB obtain energy from the oxidation process of nitrite to nitrate according to the following reaction (2.3):

$$NO_2^- + H_2O \longleftrightarrow NO_3^- + 2H^+ + e^- \qquad (2.3)$$

$$2H^+ + 2e^- + 0.5\ O_2 \longrightarrow H_2O$$

Nitrite-oxidizing microorganisms are abundant in a wide range of terrestrial, marine and freshwater ecosystems and play a major role in nitrogen cycling (Ward *et al.*, 2010; Holmes et al., 2018). In fact, it has been estimated that NOB contributes to the formation of approximately 88% of all nitrate in the oceans (Gruber, 2004). Nitrate

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formation is also essential for most life on the planet, as it is the only form of inorganic nitrogen that many primary producers (i.e. plants) can assimilate into biomass (Gruber and Galloway, 2008). Furthermore, NOB activity is a critical mechanism of nitrogen removal treatment processes in many wastewater treatment systems. NOB is generally considered metabolically restricted and dependent on ammonia oxidizers.

Koch et al. (2015) reported that the key NOB of many ecosystems (*Nitrospira*) convert urea, an essential ammonia source in nature, to NH_3 and carbon dioxide. Therefore, *Nitrospira* delivers urease-negative AOB with ammonia and receives nitrite produced by ammonia oxidation in return, leading to a reciprocal feeding interaction of nitrifiers. Previously, *Nitrobacter* was regarded as the most important NOB. Recently, *Nitrospira* was a more influential and specialized NOB in most WWTPs (Ge et al., 2015) and drinking water systems (Ward *et al.*, 2010; Dumont et al., 2016). Fukushima et al. (2013) found that *Nitrospira* was dominant at high inorganic carbon conditions, while *Nitrobacter* was in a low inorganic carbon environment. Moreover, *Nitrospira* was regarded as K-strategists (with high substrate affinities and low maximum activity) for nitrite and DO. At the same time, *Nitrobacter* represented r-strategists under limited substrate conditions and could utilize organic energy sources as a part of facultative autotrophs and anaerobes (Schmidt et al., 2003; Huang et al., 2010).

Complete ammonia oxidizing bacteria (comammox)

Until recently aerobic microbial ammonia oxidation to nitrate was regarded as a process catalysed by two different groups of microorganisms that both take care of a separate part of the process: ammonia oxidizing bacteria (or archaea) oxidize ammonia to nitrite, and nitrite oxidizing bacteria oxidize nitrite to nitrate. Both conversions were considered aerobic conversions (Henze et al., 2001).

The basis why this pathway is segregated in two different types of microorganisms has been intensively questioned and discussed, but so far no convincing assumption has been suggested to justify the advantage of pathway segregation. It has been proposed that longer catabolic pathways of nitrifying microorganisms provide a competitive advantage over short pathways in case of severe substrate limitation (e.g. ammonia and nitrite), because it allows for boosting of the amount of energy that can be gained per unit of the substrate (Kleerebezem and Lücker, 2021). This approach for biomass

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yield maximum values search was suggested to support the development of comammox microorganisms in the case of slow growing substrate-limited systems such as attached biofilm wastewater treatment reactors (Costa et al., 2006).

Recently, convincing proof was presented that specific *Nitrospira* species have the capability to oxidize ammonia to nitrate (Daims et al., 2015; van Kessel et al., 2015), and that ammonia oxidation to nitrate is not necessarily catalysed by AOB and NOB alone. *Nitrospira* are primarily known as aerobic chemolithoautotrophic nitrite oxidizing bacteria, with the capacity to conduct a number of other functions such as respiration of simple organic carbon molecules or hydrogen (Kits et al., 2017). The first described *Nitrospira* species was *Nitrospira marina*, isolated and named in late 1980's (Watson et al., 1986) from water collected in the Gulf of Maine (USA). Meanwhile, a great variety of members of the genus *Nitrospira* have been found such environments as freshwaters, soils, groundwaters, geothermal springs, and WWTPs (Daims and Wagner, 2018).

Nitrospira uses the enzyme nitrite oxidoreductase for nitrite oxidation, and the comammox *Nitrospira* members use ammonia monooxygenase (AMO) and hydroxylamine dehydrogenase (HAO) for oxidation of ammonia via hydroxylamine to nitrite. Carbon dioxide is fixed by the reductive citric acid cycle. One of the features of *Nitrospira* is adaptation to oligotrophic conditions, and therefore ability to successfully compete with canonical nitrifiers. The very high affinity for ammonia of comammox strain *Nitrospira inopinata* exceeds that of many other AOB (Daims et al., 2016; Kits *et al.*, 2017; Daims and Wagner, 2018; Roots *et al.*, 2019).

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2.6. Modelling nitrogen removal in wastewater treatment plants

2.6.1. Historical background

The well-known series of activated sludge models (ASMs): ASM1, ASM2, ASM2d, ASM3 recognized the most important processes in activated sludge environment, but all of the traditional models are built to model single-step nitrification and denitrification processes (Henze et al., 2001). Recent developments in modelling activated sludge treatment systems tend to focus on expansion of nitrification-denitrification via nitrite. This approach promotes reduction in the oxygen demand for nitrification and organic carbon demand for denitrification. In this way, it is possible to save 25% of the oxygen uptakes for nitrification and 40% of the carbon needs for denitrification (particularly interesting for low COD/N ratio effluents), as described in Chapter 1.2. Figure 2.3 presents most important nitrogen conversions compared to ASM1, the two-step nitrification model concept, and multi-step nitrification model concept. It is important to note that most of the studies found in literature consider nitrite as a model state variable and describe nitrite by assuming two-step nitrification and two-step denitrification.

A possible way of model optimization is to carry out partial nitrification, involving blocking of ammonia oxidation at the stage of nitrite, and to treat nitrite by denitrification. Consequently, this technique can either be combined with a traditional denitrification of nitrite or with an anoxic conversion of ammonia and nitrite by *anammoxidans* bacteria found in Anammox systems (Jetten et al., 2001; Fux et al., 2002).

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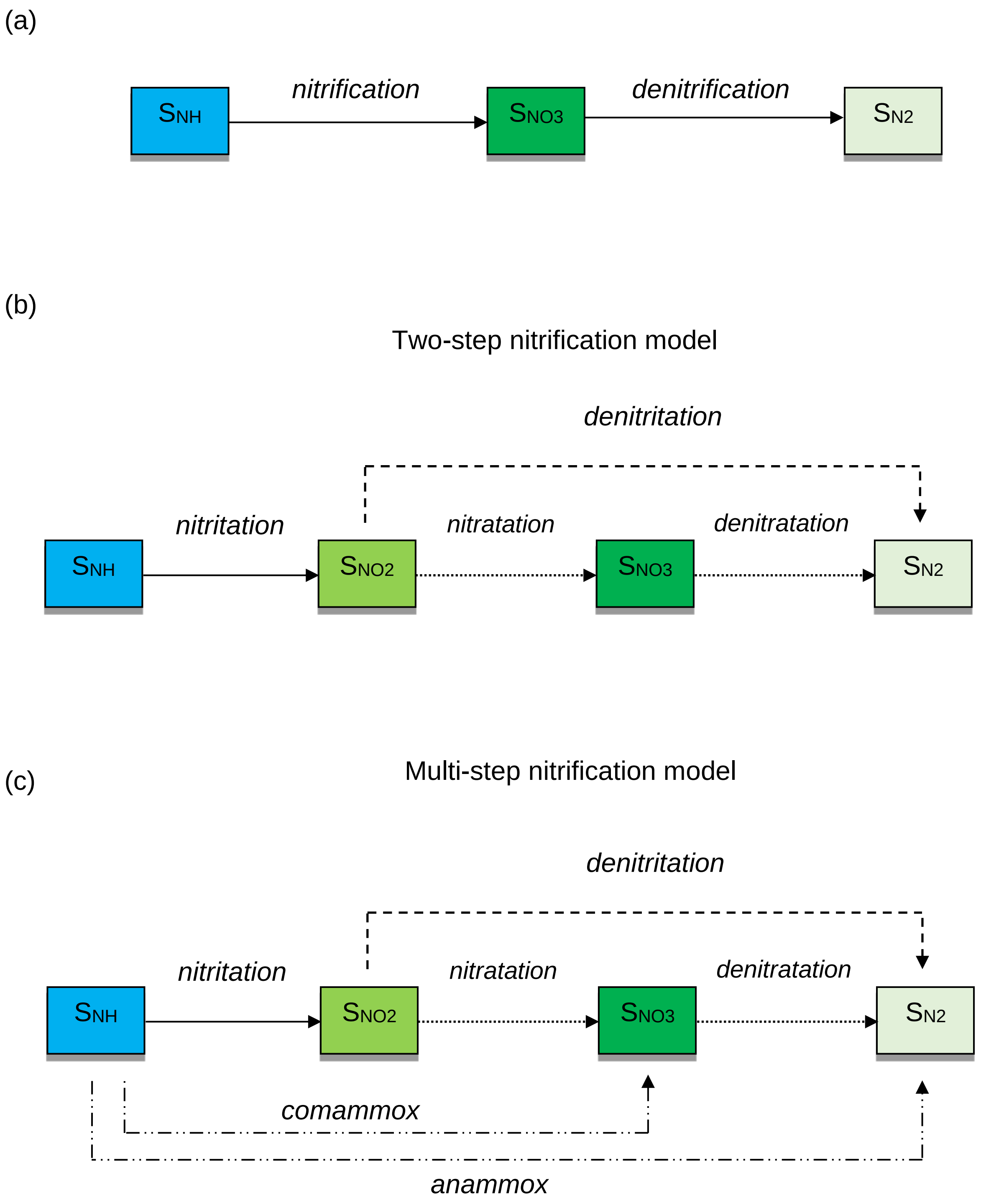

Figure 2.3. Schematic illustration of nitrogen transformations as readily biodegradable substrate (Ss) according to the ASM1/ASM3 principle (a), two-step nitrification model concept (b), and multi-step nitrification model concept (c).

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Several attempts have been made in the past to model the accumulation of these intermediate products (Schulthess and Gujer, 1996). Also, the two-step nitrification has been modelled previously (Volcke et al. 2006), but several aspects governing the ammonia and nitrite degradation, still remained unclear. First, the mechanisms and biokinetic models for NH_3 and HNO_2 inhibition need to be clarified. Secondly, the discussion on true substrates of AOB and NOB is ongoing.

However, nitric oxide (NO) and nitrous oxide (N_2O) were not integrated into the most common ASMs for several reasons. First, these products do not contribute significantly to the total nitrogen mass-balance in the system and activated sludge model series has not been used to predict NO/N_2O in the treatment plants so far. Moreover, municipal treatment systems do not consider nitrite accumulation as a significant process. The partial nitrification (nitritation) process in municipal WWTPs is usually not recognized due to the lack of NO_2 measurements in standard effluent tests or combined NO_2+NO_3 measurements (NO_x tests).

According to (Sin et al., 2008a), nitrite can play an important role in some specific situations, e.g. unstable operation of WWTP due to insufficient DO concentration, operation of the plant at high temperatures, sidestream processes and industrial wastewater treatment plants (e.g. food processing facilities). These cases present clearly that a single-step process assumption is no longer applicable and, consequently, nitrite needs to be included in the modelling process. The guidelines given by Schulthess and Gujer (1996) stated 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. Such an approach would generate false model predictions. Some of the state variables used in different activated sludge models are presented in Table 2.4. An example of the stoichiometric matrix for growth of AOB and NOB in the two-step nitrification process is presented in Table 2.5.

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Table 2.4. Selection of state variables and their notation used in different biokinetic models (from Pambrun et al., 2006; Hiatt and Grady, 2008)

Description	ASM 1	ASM2d	ASM3	***Two-step model***
Dissolved oxygen	S_O	S_{O2}	S_{O2}	S_O
Ammonia nitrogen	S_{NH}	S_{NH4}	S_{NH4}	S_{NH}
Free ammonia nitrogen	-	-	-	S_{FA}
Total nitrite + nitrate nitrogen	S_{NO}	S_{NO3}	S_{NOX}	-
Nitrite nitrogen	-	-	-	$\mathbf{S_{NO2}}$
Nitrate nitrogen	-	-	-	$\mathbf{S_{NO3}}$
Nitrogen gas	-	-	S_{N2}	-
Ammonia oxidizing bacteria (AOB)	$X_{B,A}$	X_{AUT}	X_A	X_{AOB}
Nitrite oxidizing bacteria (NOB)	-	-	-	X_{NOB}

Table 2.5. Stoichiometric matrix and process rate equations for growth of AOB and NOB in two-step nitrification process (modified from Iacopozzi et al., 2006, Hiatt and Grady, 2008)

Process	$\mathbf{S_O}$	$\mathbf{S_{NH}}$	$\mathbf{S_{NO2}}$	$\mathbf{S_{NO3}}$	$\mathbf{X_{AOB}}$	$\mathbf{X_{NOB}}$	$\mathbf{S_{ALK}}$
	gO_2/m^3	gN/m^3	gN/m^3	gN/m^3	$gCOD/m^3$	$gCOD/m^3$	$mole/m^3$
Growth of X_{AOB}	$-\frac{(3.43-Y_{AOB})}{Y_{AOB}}$	$-\frac{1}{Y_{AOB}}-i_{N,BM}$	$\frac{1}{Y_{AOB}}$		1		$-\frac{1}{7Y_{AOB}}-\frac{i_{N,BM}}{14}$
Growth of X_{NOB}	$-\frac{(1.14-Y_{NOB})}{Y_{NOB}}$	$-i_{N,BM}$	$-\frac{1}{Y_{NOB}}$	$\frac{1}{Y_{NOB}}$		1	

where: $i_{N,BM}$ – mass fraction of nitrogen in active biomass gN/gCOD

It must be noted that a misunderstanding occurs when considering the real substrate for AOB (ammonium or free ammonia) and NOB (nitrite or free nitrous acid). The assumption behind use of ionized or non-ionized forms of nitrogen still undergoes investigations when it is considered for industrial wastewaters i.e. ammonia-rich effluents. Studies concluded by several authors (e.g. Painter and Loveless, 1983; Antoniou *et al.*, 1990) estimated – at different pH values – a unique value of half-saturation constant for free ammonia (NH_3) but not for free nitrous acid (HNO_2) (Ward et al., 2010). Hence, the authors concluded that ammonia and nitrite should be considered as the true substrate of nitritation and nitratation, respectively.

Nitritation and denitritation naturally occur in all WWTPs, if only to a limited extent and it takes special process conditions to turn them into dominant nitrogen pathway.

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Although the extensive research and rich literature on the nitrite shortcut modelling can be found (Al-Omari et al., 2015; Mehrani et al., 2022b; Park and Bae, 2009; Pérez et al., 2014; Sharif Shourjeh et al., 2021a; Volcke, 2006; Yu et al., 2020), the complexity of the processes and conditions leading to nitrite accumulation has not yet been fully agreed (Giusti et al., 2011). In fact, the operating conditions favouring the nitrite shortcut induce radical changes in the microbial community, which normally reflect in a change of the kinetic parameters. Although a few examples exist where the specific saturation constants were estimated with *ad hoc* experiments (Chandran and Smets, 2000; Giusti et al, 2011; Pérez *et al.*, 2014), the problem of approximating the coefficients of the nitritation kinetics is still open (Cao et al., 2017). The two-step nitrification model concept presented further in this work considers the approach proposed by several studies on the subject of nitrite-pathway modelling (Chandran and Smets, 2000; Mehrani et al., 2021; Wu et al., 2014).

One-step nitrification models

The conversion of ammonia to nitrate is a multi-step, biologically mediated process with several intermediates (where nitrite is the most important). However, from the perspective of the oxygen demand and alkalinity consumption, the first models considered nitrification as a one-step aerobic process in which ammonia was oxidized to nitrate mediated by autotrophic nitrifying organisms. This simplification provided a more manageable mathematical structure and, in typical activated sludge systems where the SRT was above a critical value, the results obtained were almost identical (Dold et al., 1980; Vanhaandel et al. 1981). The maximum specific growth rate (typically assumed to be 0.9 d^{-1}), the decay rate (0.17 d^{-1}) and temperature sensitivity in the range of 12 to 26°C (double every 10°C; Arrhenius coefficient = 1.072) are the key design parameters. If the aerobic SRT falls below the critical SRT for nitrification, the nitrifying microorganisms activity stops and may lead to wash out of the system, and finally nitrification stops (Dold et al., 1980).

Based on Marais and Ekama's steady-state model (Marais and Ekama, 1976), other pioneers published a dynamic model which included all the elements of the Marais-Ekama model, extended by heterotrophic organisms denitrification (Dold et al., 1980; Vanhaandel et al., 1981), one-step nitrification, and alkalinity use and production calculations. One of the new elements was the separation of influent biodegradable

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substrate into readily (S) and slowly (X) biodegradable substrate, both being used directly but with different kinetics (dual substrate model). In 1982, IWA (at that time IAWPRC) formed a Task Group which in 1987 published a modified, simplified and updated form of the Dold/van Haandel model converted to single substrate use known as Activated Sludge Model No. 1 (Henze et al., 1987).

While the original models consisted of only a few equations, ASM1 (applied to a complex nitrifying/denitrifying plant configuration) consists of numerous lines of calculations and dynamically changing input parameters. Other models and their extensions are often many times larger than ASM1. In order to use in practice these models, they have to accept parameters that can be measured in practice and produce relevant results quickly (Ekama and Takacs, 2014).

In the past decade, nitrification pathways specifically and N pathways in general have been investigated in detail, and two-, four- and five-step nitrification and autotrophic denitrification models have been proposed to describe the relationships between total ammonia (NH_3 and NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), hydroxylamine (NH_2OH), nitric oxide (NO) and nitrous oxide (N_2O) (Chandran et al., 2011).

Two-step nitrification models

A detailed review of available two-step model structures was given in detail by several authors (e.g. Sin et al., 2008; Makinia and Zaborowska, 2020) with respect to main and side-stream processes. These models are based on and similar to the ASM1 structure. The most common aspect in all these models is that the growth rate is assumed as the rate-limiting step and used to describe the rate of other substrate conversions via stoichiometric yields — similar to the ASM1 convention. Overall, it becomes clear from Table 2.6 and 2.7 that some diversity exists in the mathematical structures developed so far. This diversity originates from differences either (a) in the underlying assumptions or (b) in the choice of the kinetics of substrate limitation and inhibition.

Table 2.6 summarizes AOB model structure for main- and sidestream treatment processes (Sin et al., 2008a). The following most significant differences for the ammonia oxidation models were observed:

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1. Ammonium versus ammonia as the substrate: Except for Hellinga et al. (1999), Volcke (2006) and Van Hulle et al. (2007), the other models assumed ammonium to be the substrate type for the growth of AOB. Notice that ammonia as substrate source was mainly considered for the models intended for the side-stream processes. Results of Anthonisen et al. (1976) support the claim that ammonia is the true substrate source as well as molecular simulation studies (Yang et al. 2007). In either case, the Monod equation is used to describe the substrate limitation on growth (Equation 2.4).

$$r_{N,AOB} = \mu_{NH} X_{NH} \cdot \frac{S_O}{K_{O,NH}+S_O} \cdot \frac{S_{NH_3}}{K_{NH_3,NH}+S_{NH_4}} \cdot \frac{K_{IHNO_2,NH}}{K_{IHNO_2,NH}+S_{HNO_2}} \tag{2.4}$$

(growth rate of AOB in side-stream wastewater treatment process, Hellinga et al., 1999)

2. Ammonia inhibition: It was considered by Wett & Rauch (2003) and Van Hulle et al. (2007) using a simple switch function and included in the model of Pambrun et al. (2006) using Haldane kinetics (Equation 2.5). Notice that the respective inhibition coefficients were set high, which means that ammonia will probably not be inhibitory in the model in practice, except maybe for some side-stream treatment processes (Sin et al., 2008a).

$$r_{N,AOB} = \mu_{NH} X_{NH} \cdot \frac{S_O}{K_{O,NH}+S_O} \cdot \frac{S_{NH_3}}{K_{NH_3,NH}+S_{NH_3}+\frac{S_{NH_3}^2}{K_{INH_3,NH}}} \cdot \frac{K_{IHNO_2,NH}}{K_{IHNO_2,NH}+S_{HNO_2}} \tag{2.5}$$

(growth rate of AOB in side-stream wastewater treatment process, Pambrun et al., 2006)

3. Nitrous acid inhibition: It was considered only in the models developed for the side-stream processes using a switching function (Hellinga et al. 1999; Pambrun et al. 2006; Volcke 2006; Van Hulle et al. 2007). This is probably because nitrite is not present in high levels in the main-stream processes, and hence the nitrous acid concentration remains at low levels justifying its exclusion.

4. pH effect on the growth rate: The main-stream process models ignore the pH effect on the growth rate since typically pH remains constant in municipal WWTPs. In the side-stream, three approaches are employed to describe the pH effect: (a) direct approach considering a Gaussian like inhibition function of pH (Van Hulle et al.

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2007), (b) indirect approach in which the pH effect is represented by considering pH dependent equilibrium of weak acid/base reactions (Hellinga et al. 1999; Wett & Rauch 2003), and (iii) combination of both approaches (Jones et al., 2007).

5. Inorganic carbon limitation: This term has been considered in the main-stream models in the form of bicarbonate alkalinity using a Monod approach (Sin & Vanrolleghem 2006), which is inspired by the ASMs. Notice that in the main-stream process models influent alkalinity is assumed to be sufficiently high, which practically means hardly any limitation. It could be limiting, however, in crystalline regions (geologically speaking) with low alkalinity water or in deep aeration tanks with high oxygen uptake efficiency (creating CO_2 profile along depth). Concerning sidestream models, it was included in the model of Wett & Rauch (2003) using an exponential term. Some experimental studies (Tijhuis et al. 1995; Guisasola et al. 2007; Vadivelu et al. 2007) also confirmed the importance of inorganic carbon limitation on the growth of nitrifiers. In practice, therefore, this becomes important to model especially when the wastewater is relatively low in bicarbonate.

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Table 2.6. Mathematical model structure for the growth rate of AOB
(adapted from Sin G. et al., 2008)

Process type →	SSW	SSW; WW	SSW	SSW	SSW	WW	WW
Reference → Mathematical form ↓	Hellinga et al. (1999) and Volcke (2006)	Hao et al. (2002); Kampschreur et al. (2007, 2008)	Wett and Rauch (2003)	Van Hulle et al. (2007)	Pambrun et al. (2006)	Sin and Vanrolleghem (2006)	Kaelin et al. (2008)
$\mu_{NH} X_{NH}$	1	1	1	1	1	1	1
$\frac{S_O}{K_{O,NH} + S_O}$	1	1	1	1	1	1	1
$\frac{S_{NH4}}{K_{NH4,NH} + S_{NH4}}$		1	1			1	1
$\frac{S_{NH3}}{K_{NH3,NH} + S_{NH3}}$	1			1			
$\frac{S_{INH3}}{K_{INH3,NH} + S_{NH3}}$			1	1			
$\frac{S_{NH3}}{K_{NH3,NH} + S_{NH3} + \frac{S_{NH3}^2}{K_{INH3,NH}}}$					1		
$\frac{S_{IHNO2,NH}}{K_{IHNO2,NH} + S_{HNO2}}$	1		1	1	1		
$\frac{S_{PH}}{K_{PH} - 1 + 10^{pHop-pH}}$				1			
$\frac{S_{ALK}}{K_{N,ALK} + S_{ALK}}$						1	1

SSW – side-stream wastewater; WW – main-stream wastewater.

Table 2.7 summarizes the NOB model structure for main- and side-stream treatment processes (Sin et al., 2008a). The following most significant differences for the nitrite oxidation models were observed:

1. Nitrite versus nitrous acid as substrate source: Hellinga et al. (1999), Pambrun et al. (2006) and Volcke (2006) considered the nitrous acid as the substrate source and used different kinetic terms to describe it. Others assumed the nitrite as substrate source and described the substrate limitation using the Monod equation (Equation 2.5).

$$r_{N,NOB} = \mu_{NO} X_{NO} \cdot \frac{S_O}{K_{O,NO} + S_O} \cdot \frac{S_{HNO_2}}{K_{HNO_2,NO} + S_{HNO_2}} \qquad (2.5)$$

(growth rate of NOB in side-stream wastewater treatment process, Volcke et al., 2006)

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2. Nitrous acid inhibition: Mostly the reasoning to include this term (Equation 2.6) depends on the extent of nitrite concentration that prevails in the system being modelled. From that it appears that it is deemed necessary by the following studies: Hellinga et al. (1999), Wett and Rauch (2003) and Pambrun et al. (2006). The experimental results from early works, such as Boon & Laudeloot (1962) and Anthonisen et al. (1976), also demonstrate a clear inhibition by nitrous acid. Each study, on the other hand, assumed a different inhibition kinetics ranging from a simple switch function to Haldane, non-competitive inhibition versus mixed inhibition kinetics. It is desirable to statistically compare these kinetics on experimental observations and reach a consensus.

$$r_{N,NOB} = \mu_{NO} X_{NO} \cdot \frac{S_O}{K_{O,NO}+S_O} \cdot \frac{S_{NO_2}}{K_{NO_2,NO}+S_{NO_2}} + \frac{K_{IHNO_2,NO}}{K_{IHNO_2,NO}+S_{HNO_2}} \qquad (2.6)$$

(growth rate of NOB in side-stream wastewater treatment process, Wett and Rauch, 2003)

3. Ammonia inhibition: This term was considered by Wett and Rauch (2003) and Pambrun et al. (2006) using a simple switch function, while not considered by the rest of the models (Equation 2.7). It should be noted that the respective inhibition coefficients were much lower compared to the coefficients reported for ammonia inhibition of the AOB, meaning that the ammonia inhibition is considered more severe on NOB. Especially at low SRTs when biomass is not adapted to high ammonia operation levels ammonia inhibition is one of the driving factors to outcompete NOBs.

$$r_{N,NOB} = \mu_{NO} X_{NO} \cdot \frac{S_O}{K_{O,NO}+S_O} \cdot \frac{S_{NO_2}}{K_{NO_2,NO}+S_{NO_2}} + \frac{S_{NO_2}}{K_{NO_2,NO}\left(\frac{1+\frac{S_{NH3}}{K^{I}_{INH_3,NO}}}{1+\frac{S_{NH3}}{K^{i}_{INH_3,NO}}}\right)} + \frac{K_{INH_3,NO}}{K_{INH_3,NO}+S_{NH_3}} \qquad (2.7)$$

(growth rate of NOB in side-stream wastewater treatment process, Pambrun et al., 2006)

4. pH effect on the growth rate: It was considered implicitly by some of the models through weak/acid base equilibrium reactions between nitrite-nitrous acid and ammonium–ammonia. However, the model of Jones et al. (2007) considered it both implicitly and explicitly. From a microbiological point of view, Boon and Laudeloot (1962) argued that while the acid region of the pH-activity curve can

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be explained by nitrous acid inhibition (see point 2), the alkaline inhibition of pH can be explained by OH^- ion activity. The latter was described by competitive inhibition kinetics. Such results may indeed support the assumption to adopt direct inhibition of the growth rate by pH - particularly for the alkaline pH spectrum.

5. Inorganic carbon limitation: This term has been considered in the main-stream models in the form of bicarbonate alkalinity using a Monod approach (Sin et al. 2008), which results from the ASMs that only consider the low pH inhibition and not alkaline situations. As the alkalinity is assumed to be high in the main-stream models, inorganic carbon is practically not growth-limiting in these models. On the other hand, only Wett and Rauch (2003) considered it growth-limiting in the side-stream models using an exponential term. Their results indicated that inorganic carbon could become an important limiting factor especially in high-rate nitrification systems with low influent bicarbonate to ammonium ratio.

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Table 2.7. Mathematical model structure for the growth rate of NOB
(adapted from Sin G. et al., 2008)

Type →	SSW		SSW; WW	SSW	SSW	SSW	WW	WW
Reference → Mathematical form ↓	Hellinga et al. (1999)	Volcke (2006)	Hao et al. (2002); Kampschreur et al. (2007, 2008)	Wett and Rauch (2003)	Pambrun et al. (2006)	Jones et al. (2007)	Sin et al. (2008)	Kaelin et al. (2008)
$\mu_{NO} X_{NO}$	1	1	1	1	1	1	1	1
$\frac{S_O}{K_{O,NO} + S_O}$	1	1	1	1	1	1	1	1
$\frac{S_{HNO2}}{K_{HNO2,NO} + S_{HNO2}}$		1						
$\frac{S_{NO2}}{K_{NO2,NO} + S_{NO2}}$			1	1		1	1	1
$\frac{S_{IHNO2,NO}}{K_{IHNO2,NO} + S_{HNO2}}$				1		1		
$\frac{S_{HNO2}}{K_{HNO2,NO} + S_{HNO2} + \frac{S_{HNO2}^2}{K_{IHNO2,NO}}}$	1							
$\frac{S_{NO2}}{K_{NO2,NO}\left(\frac{1+\frac{S_{NH3}}{K_{INH3,NO}^{I}}}{1+\frac{S_{NH3}}{K_{INH3,NO}^{i}}}\right) + S_{NO2}}$					1	(similar nitrite preference)		
$\frac{K_{INH3,NO}}{K_{INH3,NO} + S_{NH3}}$				1	1	1 (nutrient)		
$\frac{e^{((S_{HCO3}-K_{HCO3})/a)}}{e^{((S_{HCO3}-K_{HCO3})/a)} + 1}$				1		1		
$\frac{S_{ALK}}{K_{N,ALK} + S_{ALK}}$							1	1

SSW – side-stream wastewater; WW – main-stream wastewater.

2.6.2. Model parameter estimation methodology

Model calibration is known as the estimation of the model parameters to fit a selected data obtained from the full-scale WWTP being studied. The requirements for a model calibration depend on the model use. In the case of model use for education (e.g. to increase basic understanding of the treatment processes), for assessment of design alternatives of newly engineered municipal WWTPs or in other situations where qualitative comparisons are sufficient, default parameter values can be applied (Petersen et al., 2002).

Parameter estimation is an important step in the development of mathematical process models. ASMs contain parameters with physical significance that may vary drastically

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from plant to plant. In order to develop process models that can be used for predictive purposes, it is important to estimate the unknown process parameters using measured process data (Henze, 1992). Applying literature values for the unknown parameters will often result in a model that is not very useful for predicting actual plant behaviour (Jeppsson et al., 2013).

Modelling of the shortcut nitrification processes requires application of robust calibration methodologies and model developments to find the optimal range of influential model parameters (e.g. Mehrani *et al.*, 2021). Process models for shortcut nitrogen removal found in literature are usually applied to a specific case scenario analysis and assessment of treatment process performance under variable conditions (Mehrani et al., 2021; Mozumder et al., 2014; Pérez et al., 2014).

The information needed for the description of stoichiometric and kinetic parameters in biokinetic models can fundamentally be gathered from three sources (Petersen et al., 2002):

- Default values found in literature.
- Full-scale WWTP data, e.g. average or dynamic data from grab or time/flow proportional samples, mass balances of the full-scale data, online data collection, and measurements in reactors to characterise process dynamics.
- Data sets from different lab-scale experiments with wastewater and biomass from the WWTP under study.

Manual calibration

Simple curve fitting is a manual calibration method where parameters are adjusted by trial and error to achieve the best fit between model predictions and observed data (Sin et al., 2008b). This method relies heavily on the expertise and judgment of the engineer (model user) and can be divided into three steps:

1. Selection of Parameters: identification of critical parameters that significantly influence model outputs. Typical parameters include kinetic rates (e.g., maximum specific growth rate μ_{max}) and stoichiometric coefficients (e.g., yield coefficient Y) (Henze et al., 2000).

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2. Data visualization: plotting observed data against model predictions. This visualization helps in understanding the trends and inconsistencies.
3. Manual adjustment: iterative adjustment of parameters and comparing the model outputs with observed data until a satisfactory fit is achieved (Rittmann and McCarty, 2020).

The advantage of simple curve fitting is its intuitive approach, allowing user to apply their experience to make learned modifications. However, it can be time-consuming and may not always yield the most accurate results due to the potential for subjective parameter preference during the manual calibration. In most applications, no attempts are made to estimate all parameters simultaneously from the data collected from a single system (Brun et al., 2002). Most parameters remain fixed at the default values and only small subsets of parameters are adjusted, either by “ad hoc” tuning or a parameter estimation algorithm (Mehrani et al., 2022a). It was demonstrated by a number of authors (Brdjanovic, 2000; Meijer et al., 2001; Gernaey et al., 2004; Trojanowicz et al., 2017; Lu et al., 2018) that even complex biokinetic models can be calibrated with small sets of parameters.

Automated parameter estimation

The Nelder-Mead (NM) method (Nelder and Mead, 1965; Olsson and Nelson, 1975) is a widely used optimization algorithm for automated parameter estimation in complex environmental models like ASM series. The method is a simplex-based optimization technique that does not require the calculation of gradients, making it advantageous for calibrating ASMs where the objective function landscape may be irregular (Lagarias et al., 1998). The NM method is particularly effective for ASM calibration due to its robustness in handling irregular objective functions. In ASM calibration, the method iteratively adjusts parameters such as biomass yield, decay rates, and substrate affinity constants to minimize the difference between observed data (e.g., effluent concentrations) and model predictions (Makinia and Zaborowska, 2020; Szeląg et al., 2022).

The approach presented by Pryce et al. (2022) concluded that parameter estimation by the NM algorithm identified only minor adjustments were required to influential

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parameters for the model to predict actual system outputs with sufficient accuracy. Finally, parameter uncertainty was observed to be minimal for the BOD and TSS models, however, the TN model demonstrated greater uncertainty that may warrant further work to support the drawn conclusions.

Computer simulation software GPS-x by Hydromantis includes the optimizer module, where a computational optimization can be performed to estimate the kinetic and stoichiometric parameters of greatest influence using the maximum likelihood function. Further details of the maximum likelihood function utilized can be found in the GPS-x Technical Reference Guide (Hydromantis, 2017).

2.6.3. Modelling AOB and NOB competition

It was emphasized in Section 2.3 that nitrite is no longer viewed as a toxic and undesirable intermediate product in WWTPs, but it has been receiving growing attention as the critical component in a variety of new shortcut nitrogen removal processes. It was stated by Cao et al. (2017) that the activated sludge nitrification models should be revised to accurately reflect the competition between AOB and NOB in order to understand factors influencing the competition between autotrophic nitrifying microorganisms. Furthermore, the approach of modelling nitrification as a one-step conversion is not usually acceptable for ammonia-rich wastewaters, elevated temperatures or inhibiting conditions (Hiatt and Grady, 2008b). Representative examples of such conditions include anaerobic digester supernatant effluents, food processing industry, and industrial facilities that manufacture fertilizers, chemicals, pharmaceuticals, and other nutrient-rich products (Hiatt and Grady, 2008; Van Hulle et al., 2010).

Based on the kinetic differences, the nitrifiers were classified into two categories: ‘‘r-strategists” and ‘‘K-strategists” (Schramm et al., 1999). The r-strategists nitrifiers growth is rapid at high substrate concentration. The Monod-type equation used for describing nitrifying r-strategists growth is identified by high maximum specific growth rate (μ_{max}, d^{-1}) and high value of substrate half-saturation (K_S, mg/L). In contrast, the values of μ_{max} and K_S for nitrifying K-strategists are low. K-strategists nitrifiers grow slowly but can develop a competitive behaviour under low substrate concentration due to their high substrate affinities (Yuan and Blackall, 2002; Vannecke and Volcke, 2015).

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Nitrosomonas (AOB) and *Nitrobacter* (NOB) were usually assumed to be r-strategists (r-AOB and r-NOB), while the *Nitrosospira* (AOB) and *Nitrospira* (NOB) were classified as K-strategists (K-AOB and K-NOB). NOB suppression is critical for successful application and operation of the recently developed autotrophic nitrogen removal (Pérez et al., 2014; Mehrani et al., 2021). However, the ongoing discussion on the competitions among different species of nitrifiers, i.e. r/K AOB and NOB will affect the r/K NOB suppression is still unresolved (Sharif Shourjeh et al., 2021b; Mehrani et al., 2022b). The selection of r-AOB and r-NOB cannot be explained by the measured nitrifier kinetic parameters alone (Yu et al., 2020), as their measurements are complex and controlled by several factors, e.g. nitrifier species, floc morphology, and substrate feeding condition, which increases complexity and uncertainty in using the kinetic selection of r/K AOB and NOB.

Functional differences between the species of NOB population were confirmed by Cao et al. (2017). The half-saturation constants for *Nitrospira* (K_{NO2} of 0.16-1.2 mgN/L) were lower in comparison with those for *Nitrobacter* (K_{NO2} of 1.7-13 mgN/L), which suggests that these bacteria could indeed be regarded as typical K-strategists and r-strategists, respectively. This difference may also explain why *Nitrobacter* is a well-functioning competitor in sidestream treatment systems (high substrate concentration), while *Nitrospira* can grow in mainstream systems under conditions of substrate shortage.

The Monod equation (Monod, 1949), used as the basis of ASM (Henze et al., 2000) for modelling of the majority of biological wastewater treatment processes, is a fundamental mathematical model that describes the growth rate of the nitrifying microorganisms as a function of substrate concentration. While increasing number of studies are being published presenting and discussing measured and calibrated values of K_S, it has become obvious that these values have a high degree of inconsistency (the values found are not always consistent between publications) (Arnaldos et al., 2015). A good example of these inconsistencies is the case of the partial nitrification process, where AOB must outperform NOB. The overall process understanding is that AOB have higher DO “affinities” (lower K_O values) than NOB (Ward et al., 2010), and therefore low DO conditions can be used consistently as a one of methods to washout NOB from the process. Table 2.8 summarizes different K_O values reported for AOB and NOB for a selected number of studies.

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It can be observed that the values reported for $K_{O,AOB}$ and $K_{O,NOB}$ change significantly from one study to the other (0.03 - 1.16 mgO_2/L for AOB and 0.13 - 3 mgO_2/L for NOB). Furthermore, some studies have reported lower values of half-saturation constants for NOB than AOB, denying fundamental process understanding (Regmi et al., 2014). Using this basic knowledge it was stated that applying a low DO concentration to washout NOB biomass cannot be comprehensive, and the actual performance of nitritation has to be assessed in a case-to-case basis (Arnaldos et al., 2015).

Table 2.8. Selection of affinity constants for oxygen (K_O) of AOB and NOB (adapted in part from Arnaldos et al, 2015)

$K_{O,AOB}$ (mgO_2/L)	$K_{O,NOB}$ (mgO_2/L)	Biological process / system setup	Reference
0.03	0.43	Suspended growth / lab scale	Blackburne et al., 2008
1.16	0.16	Suspended growth / pilot scale	Regmi et al., 2014
1.45	1.1	Suspended growth / full-scale	Hellinga et al., 1999
0.28	0.39	Suspended growth / lab scale	Yu et al., 2020
0.3	0.2	Suspended growth / lab scale	Mehrani et al., 2021
0.29	0.09	Suspended growth / lab scale	Wang et al., 2021
0.17	0.13	Suspended growth / lab scale	Mehrani et al., 2023

The “r/K strategist” explanation is generally used for the AOB and NOB substrate competition to account for the inconsistencies found in published data. Some modelling studies (e.g. Volcke et al., 2008) have connected the unexpected lower K_O values for NOB (as compared to AOB) to the fact that low DO conditions might benefit NOB species that are K-strategists (*Nitrospira sp.*), as compared to the more commonly encountered *Nitrobacter sp.* (identified as r-strategists) (Dytczak et al., 2008). Hence, NOB are outcompeted by AOB group (Regmi et al., 2014; Al-Omari et al., 2015).

2.6.4. Estimation of half-saturation constants

The Monod-type equations are commonly used to present the nitrifying activity of AOB and NOB (Henze et al., 2008). However, these equations are used based on the measured ammonia utilization rate (AUR) and nitrite utilization rate (NiUR), under the unlimited NO_2-N conditions, as follows:

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$$r_{AOB} = r_{max,AOB} \frac{S_O}{S_O + K_{O,AOB}} \tag{2.8}$$

$$r_{AOB} = r_{max,AOB} \frac{S_O}{S_O + K_{O,AOB}} \tag{2.9}$$

where r_{AOB} and r_{NOB} are the observed specific process rates for AOB (AUR) and NOB (NiUR) (mg N/(g VSS·h), $r_{max,AOB}$ and $r_{max,NOB}$ are the maximum specific process rates for AOB and NOB (mgN/(gMLVSS·h), $K_{O,AOB}$ and $K_{O,NOB}$ are DO affinity constants for AOB and NOB (mgO_2/L), respectively, and S_O is the DO concentration (mgO_2/L).

Three linearized forms of Equations (2.8 and 2.9) can be used for the estimation of r_{max} and K_O, based on the following three linearization methods:

Lineweaver-Burk

$$\frac{S_O}{r} = \frac{1}{r_{max}} S_O + \frac{K_O}{r_{max}} \tag{2.10}$$

Hanes

$$\frac{1}{r} = \frac{K_O}{r_{max}} \frac{1}{S_O} + \frac{1}{r_{max}} \tag{2.11}$$

Hofstee

$$r = r_{max} - K_O \frac{r}{S_O} \tag{2.12}$$

In addition to the linearized forms of the Monod equation, the least-square method is also used for parameter estimation. The Least-Squares method is widely used to estimate the numerical values of the parameters by fitting a function to a set of measured data.

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III. Materials and methods

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3.1. Biomass origin

Samples of mixed liquor's suspended solids (inoculum biomass) were collected for each batch test trial and long-term washout experiments from a recycled sludge stream at the municipal WWTP "Wschod" in Gdansk, Poland (Figure 3.1). The "Wschod" WWTP is the largest treatment facility in the Northern Poland region and one of the largest facilities located on the Baltic Sea coast, serving a population of Gdansk and neighbouring smaller towns (total population approx. 570,000). The treated effluent is directly discharged to the Bay of Gdansk, which is directly connected to the Baltic Sea. The total pollutant load of the treatment plant corresponds to approximately 700,000 PE, where industrial (non-domestic) and hospital (undisinfected) wastewater is approximately 10% and 0.2% of the daily average flow rate, respectively. The industrial inflow originates from the food processing industry, chemical industry, and shipyards. The WWTP "Wschod" suspended growth basic process configuration for biological nutrients removal is The Modified University of Cape Town (MUCT) setup designed for $Q_{d,max}$= 96.000 m^3/d. The treatment plant has operated since 1976 (mechanical treatment only). Later, from 1996 to 1999, the plant was modified for biological treatment process configuration. Finally, from 2009 to 2012, it was modified and extended for process configuration of full BNR process to meet stringent EU nutrients effluent standards. The effluent standards were set according to the EU Urban Wastewater Directive (91/21/EEC), i.e., total N (TN) = 10 mgN/L and total P (TP) = 1 mgP/L.

Figure 3.1: WWTP "Wschod" in Gdansk, Poland.
(Courtesy of GIWK, Gdansk. Photographed by aeromedia.pl)

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3.2. Feed characterization

The laboratory batch tests were carried out with the inoculum biomass (samples from recycled sludge tank) diluted with treated effluent from the WWTP “Wschod” to reach MLSS concentration of approximately 4000 mg/L. Each batch test was processed with two types of wastewater feed: diluted sludge digester liquor and a synthetic substrate (ammonia and nitrite).

The sludge digester liquor was collected weekly from the sludge management facility at WTTP “Wschod” in Gdansk. Table 3.1 presents the characteristics of the decanter sludge liquor. Each batch test was operated with wastewater feed diluted with treated effluent from the WWTP and supplied directly to the reactors, where the initial ammonia concentration was controlled at approximately 45 mgN/L.

Table 3.1. WWTP “Wschod” digester liquor characteristics.

Parameter	**Symbol**	**Unit**	**Value**
Total nitrogen	N_{tot}	mg/L	975 ±50
Ammonia nitrogen	NH_4-N	mg/L	920 ±120
Nitrate nitrogen	NO_3-N	mg/L	1 ±0.50
Nitrite nitrogen	NO_2-N	mg/L	0.5 ±0.20
COD	COD	mg/L	990 ±200
Temperature	T	°C	15 ÷ 35 ±1

The synthetic substrate was a liquid medium containing either an ammonium source (NH_4Cl) or an ammonium and nitrite source ($NaNO_2$). Since the experiments were focused on autotrophic nitrogen removal and competition between three groups of nitrifiers, the heterotrophic activity was ignored. Thus, supplemental inorganic carbon source was not included (Yu et al., 2020). In batch tests without adding a nitrite source, the initial concentration of ammonia substrate was approximately 45 mg N/L. In contrast, tests with additional nitrite substrate had a controlled initial concentration of NO_2-N equal to 10 mg N/L.

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3.3. Laboratory setups

A series of batch tests was carried out to validate the model and evaluate experimental data regarding the predicted nitrogen concentrations. Each batch test included a mix of MLSS, digester liquor, and treated effluent (according to the description given in section 3.2). The mixture was placed in two parallel, fully automated plexiglass sequencing batch reactors (SBR1 and SBR2) with a working volume of 4 L each.

A typical SBR is a fill-and-draw biological reactor system involving a single complete mix reactor where all steps of the activated sludge process occur (Tchobanoglous et al., 2003). The mixed liquor remains in the reactor during all cycles, eliminating the need for separate sedimentation tanks. However, for laboratory-scale short-term batch tests, SBRs were operated without sludge settling and retention, as the tests were focused on the single cycle of complete nitrification (ammonia reduction to a concentration equal to 0 mgN/L). The operational cycle of each reactor was limited to aeration and mixing. Each SBR was equipped with high-quality measurement electrodes and probes (WTW) for continuous monitoring of pH and temperature (SenTix 21), oxidation-reduction potential (SenTix ORP), and DO (CellOx 325). The SBR double wall construction acted as a water jacket to circulate a heat transfer media. The water jacket was coupled with a thermostatic water bath (F12-ME Refrigerated/Heating Circulator, Julabo), and this automated control system helped regulate and sustain the selected temperature parameters. Automated control for the aeration system was based on DO probe monitoring and air compressor operation. Mixing was applied by a mechanical stirrer (Hei-Torque 100, Heidolph Instruments), and the mixing intensity was set to approximately 180 rpm.

Long-term washout experiments were carried out on a similar setup of two parallel fully automated SBRs with V_a=10L each. The automatic pump setup supplied wastewater feed automatically at room temperature, connected to a 20L plastic container (diluted digester liquor) or a 5L glass container (synthetic medium).

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3.4. Reactor configuration and operation

Batch tests

Aerobic batch tests were carried out at three different DO setpoints equal to 0.5, 2.0, and 6.0 mgO₂/L at two different temperatures of 20˚C and 25˚C with continuous aeration. Table 3.2 gives a detailed description of the batch tests' parameters.

Table 3.2. Operational parameters of batch tests.

Batch series	T (˚C)	pH	DO (mgO₂/L)	Initial MLSS/MLVSS average values and SD (mg/L)	Nitrogen substrate
1	20	7.5 ±0.2	2.0 ±0.1	2800 ±94 / 2200 ±82	NH_4-N
2	20	7.5 ±0.2	2.0 ±0.1		NH_4-N + NO_2-N
3	25	7.5 ±0.2	0.5 ±0.1	3500 ±190 / 2835 ±150	NH_4-N
4	25	7.5 ±0.2	0.5 ±0.1		NH_4-N + NO_2-N
5	25	7.5 ±0.2	6.0 ±0.1		NH_4-N
6	25	7.5 ±0.2	6.0 ±0.1		NH_4-N + NO_2-N

The batch series 1 and 2 operational cycle included a 4h (240 min) aeration phase. During the test, MLSS samples were collected at intervals of 30 minutes and filtered before analytical measurements. Batch series 3 to 6 had an extended aeration phase to 5h (300 min). The concentration of suspended and volatile solids (MLSS and MLVSS) was measured at the beginning of each batch cycle. The final concentration of MLSS was calculated based on the solids concentration of inoculum biomass from the recycled stream (MLSS 5500 ÷ 7000 mg/L, SD 200 mg/L) to reach test values presented in Table 3.2.

Long-term experiments

The experimental lab-scale SBR operated two cycles a day. Each cycle consisted of three phases: aeration, mix and fill (0.5 h), aeration and mix (11 h), and aeration and discharge (0.5 h). The settling phase was excluded to create a chemostat environment

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for easy SRT control. In this way, the resulting HRT (hydraulic retention time) was equal to SRT. The SRT setpoint was maintained by wasting a calculated volume of MLSS at the end of each cycle. Operational parameters and conditions in the reactor during the long-term washout experiments are presented in Table 3.3.

Table 3.3. Operational parameters and conditions of long-term SBR experiments

Experiment	T (˚C)	pH	DO setpoint (mgO_2/L)	Initial MLSS / MLVSS (mg/L)	Substrate source
1	30	7.5 ±0.2	2.0 ±0.1	2260 ±200 / 1870 ±151	*digester liquor*
2	30	7.5 ±0.2	0.5 ±0.1	2260 ±200 / 1870 ±151	*digester liquor*

Initial biomass concentration and composition

ASM setup for dynamic simulations requires setting the initial concentration of MLSS for each biomass component (Henze et al., 2008). Since autotrophic biomass was defined in ASM1 as $X_{B,A}$ was replaced in the two-step nitrification model by X_{AOB} and X_{NOB} notation and further extended with comammox biomass component X_{CMX} (see Table 2.4 and Table S3.2), initial biomass calculations must include each nitrifying group of microorganisms (AOB, NOB, comammox). The calculations for this study followed a modified protocol proposed by (Mehrani et al., 2022c), who proposed mass balance calculations combined with the results of microbiological analyses. First, nitrifier concentrations were calculated using the mass balance equations (Tchobanoglous et al., 2003; Henze et al., 2008). For the long-term SBR experiments, the initial MLSS and MLVSS concentrations were approximately 2260 and 1870 mg/L, respectively (Table 3.3). Since the microbial analysis of inoculum biomass was not conducted to support this study, the overall nitrifying microorganisms' concentrations were assumed based on the results of studies by Mehrani et al. (2021) and Kowal et al. (2022) for similar WWTPs in Poland. Seasonal changes in the nitrogen-transforming bacterial communities in activated sludge systems were considered, as described by Stratton et al. (2024). The calculated initial concentrations of the individual microbial groups (AOB, NOB, comammox) are presented in Appendix A (Table S.4). For dynamic simulations, the averaged initial ratios of AOB:NOB:comammox bacteria were set at 3:9:1 for long-term experiments and batch tests. The total nitrifier abundance

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and AOB:NOB proportions (including *Nitrospira* abundance as the representative of the nitrite-oxidizing microbial group and comammox group) were within the ranges reported in the literature for similar studies (Griffin and Wells, 2017; Roots et al., 2019; S. Shourjeh et al., 2020).

3.5. Model development and implementation

One of the key objectives of this study is the evaluation of ammonia and nitrite substrate influence on two-step nitrification process kinetics and the application of the developed biokinetic model to simulate nitritation and nitratation in the treatment of ammonia-rich wastewater streams. The model presented in this study (denoted by ASM1 structure) separates the autotrophic biomass into AOB and NOB, where ammonia N and nitrite N are oxidized by nitrifying autotrophic biomass (AOB and NOB) according to the concept of two-step and multi-step nitrification model presented on Figure 2.3 and AOB growth rate equations given in Table 2.5 (see: Chapter 2 Theoretical background).

3.5.1. Conceptual two-step model

The aerobic growth rates of the individual nitrifying groups in the two-step model are:

$$\mu_{AOB} \cdot \frac{\mathrm{S_{NH4}}}{\mathrm{S_{NH4}}+\mathrm{K_{NH4}}} \cdot \frac{\mathrm{S_O}}{\mathrm{S_O}+\mathrm{K_{O,AOB}}} \cdot \mathrm{X}_{AOB} \qquad 3.1$$

$$\mu_{NOB} \cdot \frac{\mathrm{S}_{NO2}}{\mathrm{S_{NO2}}+\mathrm{K}_{NO2}} \cdot \frac{\mathrm{S_O}}{\mathrm{S_O}+\mathrm{K_{O,NOB}}} \cdot \mathrm{X}_{NOB} \qquad 3.2$$

Modelling the two-step ammonia oxidation described by Equations 3.1 and 3.2 requires the introduction of two new variables (Henze et al., 2008; Sin et al., 2008a), representing the concentration of ammonia oxidizers (X_{AOB}) and nitrite oxidizers (X_{NOB}). Equation 3.1 describes the first step of ammonia N oxidation into nitrite N, where both ammonia (S_{NH4}) and DO (S_{O2}) are limiting factors. In Equation 3.2, DO (S_{O2}) has the same function for further oxidation of nitrite N (S_{NO2}) to nitrate N (S_{NO3}).

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3.5.2. Incorporation of comammox process in two-step nitrification model

Extension of the above model concept with the activity of comammox *Nitrospira* (Kits et al., 2017; Koch et al., 2019) was presented and verified by Mehrani et al. (2021) with three conceptual models. Even though it was established that comammox is a two-step process and comammox *Nitrospira* possesses key enzymes for both ammonia N and nitrite N oxidation, there is still no compromise in the source literature whether nitrite is released outside of the cells during the process or not (Roots et al., 2019; van Kessel et al., 2015). However, while some model studies focused on initial biomass concentrations and comammox (Mehrani et al., 2022b), extension of ASMs with potential nitrification pathways, including the comammox process (Mehrani et al., 2022b; Mei et al., 2023), and introduction of comammox process together with two parallel pathways of nitrite oxidation to nitrate (Kowal et al., 2022) is considered acceptable, the model concept of simple modification and extension of ASM1 with one-step ammonia N oxidation to nitrate N (comammox process) remains the most accurate and efficient under high ammonia concentrations and specific operational conditions (Mehrani et al., 2021). The newly introduced process aerobic growth rate of comammox (CMX) following ASM1 notation is:

$$\mu_{CMX} \cdot \frac{\mathrm{S_{NH4}}}{\mathrm{S_{NH4}+K_{NH4}}} \cdot \frac{\mathrm{S_O}}{\mathrm{S_O+K_{O,CMX}}} \cdot \mathrm{X}_{CMX} \tag{3.3}$$

where X_{CMX} represents the concentration of CMX ammonia oxidizer, and $K_{O,CMX}$ is the CMX oxygen half-saturation constant.

The present model study attempted to identify the possible activity of the comammox process during the treatment of ammonia-rich effluent and oxidation of ammonia N to nitrate N, under unlimited substrate conditions. Different two-step nitrification model concepts, including comammox, were considered for this study and are presented in Figure 3.2. A mathematical notation of these models, including their stoichiometric matrices and vectors of kinetic expressions, is presented in Table S2.2 and S3.2 in Appendix A.

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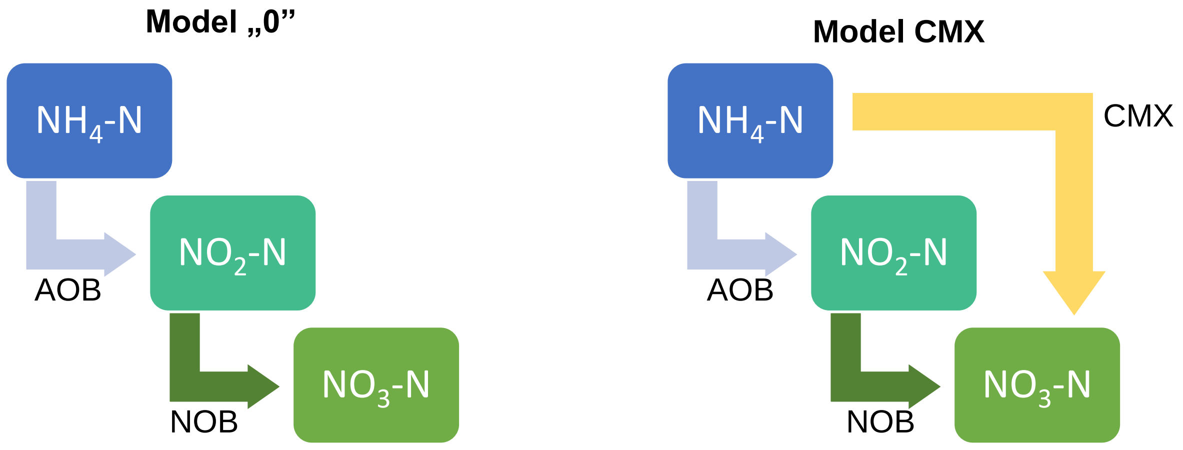

Figure 3.2. ASM1-based conceptual model of two-step nitrification (Model “0”) and extended two-step nitrification with one-step comammox (Model CMX).

The conceptual comammox model (Model CMX) includes the complete NH_4–N oxidation via comammox, and is assumed to be a one-step process (NH_4–N→NO_3–N) without the release of extracellular NO_2–N, i.e. comammox bacteria are unable to utilize extracellular NO_2–N as electron donors. Next, model CMX was compared to Model “0” (two-step nitrification) and extension of CMX model, where heterotrophic (HET) denitrification was included to incorporate the reduction of nitrate N to nitrite N and reduction of nitrite N to nitrous oxide (N_2O). See Table 3.4 for the summary of conceptual models’ processes. In this way, the traditional two-step nitrification model considers the potential two additional nitrogen oxidation processes (CMX and HET). However, due to very limited organic carbon source in the waster feed (C:N ratio ≥ 1), the HET activity was ignored in CMX model, where extensive biomass washout was estimated. The reasoning behind this generalisation is the fact that the main objective of the model study was to investigate the N removal by AOB and NOB and competition among the nitrifiers. Therefore, the CMXH Model was used for benchmarking purposes only (see Appendix A.).

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Table 3.4. Conceptual models and bacterial groups' activities.

	Microbial activity						
Model concept	HET** denitrification ($NO_3 \rightarrow NO_2$)	HET** denitrification ($NO_2 \rightarrow N_2O$)	AOB NH_2OH oxidation	AOB denitrification ($NO_2 \rightarrow N_2O$)	AOB ($NH_4 \rightarrow NO_2$)	NOB ($NO_2 \rightarrow NO_3$)	CMX* ($NH_4 \rightarrow NO_3$)
Model „0"	n/a	n/a	n/a	n/a	+	+	n/a
Model CMX	**n/a**	**n/a**	**+**	**+**	**+**	**+**	**+**
Model CMXH**	+	+	+	+	+	+	+

* one-step comammox activity without nitrite release; ** HET denitrification included only for benchmarking.

3.5.3. Modelling of partial nitrification reactor start-up

Modelling the start-up of a partial nitrification reactor is a complex process involving integrating biological kinetics, environmental factors, and engineering principles. The goal is to optimize the reactor conditions to achieve efficient ammonia oxidation to nitrite while suppressing the activity of NOB. Guidelines and remarks given by some authors include several aspects related to reactor start-up modelling, e.g., kinetic and stoichiometric considerations (Hellinga et al., 1998; Sliekers, 2002) and dynamic simulations setup (Joss *et al.*, 2009; Giusti *et al.*, 2011). The presented study combines the knowledge on competition and coexistence of AOB, NOB and Comammox microorganisms. It presents modelling of two-step nitrification biochemical processes (two-step N conversion) occurring in activated sludge system start-up. Special attention is given to the growth of microorganisms, biomass washout and nitrogen substrate accumulation. Configuration and limitations of the laboratory SBR indicated that the process is controlled by re-occurring 12-hour influent dynamics and active biomass loss (washout) while the activated sludge composition changes. It was proven that model kinetics become sensitive when a shift of the microbial population is induced by means of operational parameters (e.g., DO, and SRT) under start-up conditions, i.e., the "shock" effect of inoculum biomass. To evaluate the model kinetics during the start-up of the laboratory SBR, the system was evaluated in a simulation study.

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3.5.4. Model stoichiometry and kinetics

The stoichiometric matrix and kinetic equations of the evaluated Model “0” extension with comammox Nitrospira activity are presented in Table 3.5.

Table 3.5. Stoichiometric matrix and kinetic equations of comammox activity

	Stoichiometric matrix									**Kinetic equations**
	X_{CMX}	X_S	X_U	X_{nd}	S_O	S_{NH4}	S_{NO2}	S_{NO3}	Salk	
Growth of comammox *Nitrospira*	1				$\frac{4.57 - Y_{CMX}}{Y_{CMX}}$	$-\frac{1}{Y_{CMX}} - ixbn$		$\frac{1}{Y_{CMX}}$	$-\frac{1}{7 \cdot Y_{CMX}} - \frac{ixbn}{14}$	$\mu_{CMX} \cdot \frac{S_{NH4}}{S_{NH4}+K_{NH4}} \cdot \frac{S_O}{S_O+K_{O,CMX}} \cdot X_{CMX}$
Decay of comammox *Nitrospira*	-1	$1 - f_u$	f_u	$ixbn - f_u \cdot ixun$						$b_{CMX} \cdot X_{CMX}$

3.5.5. Simulation software

The GPS-x version 7.0 software (Hydromantis ESS, Inc.) was used for modelling, optimization, and simulations. The Carbon-Nitrogen-Industrial Component Library (CNIPLIB) was used for basic wastewater components and state variables. The ASM1 suspended growth biological model with a one-step nitrification process was expanded with a two-step nitrification process and comammox.

3.5.6. Model implementation

The GPS-x built-in Model Developer (MD) module was used to create and edit new models. This model development uses the model matrix format for model specification, the standard format in the wastewater modelling literature (Henze et al., 2008). MD is an application that organizes model content into pages containing information on model structure, parameters, and GPS-X model variables (Hydromantis, 2017).

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3.6. Data collection and evaluation

3.6.1. Data collection for model calibration and validation

MLSS samples were collected daily during weekdays at the start and end of the reaction phase (SBR cycle). Samples were filtered to separate particulates from the aqueous sample and analysed for the nitrogen forms of NH_4-N, NO_3−N, and NO_2−N. Spectrophotometric cuvette tests were used to determine nitrogen concentrations. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentrations were analysed at the start-up of the reactor operation. The analytical methods used were based on the gravimetric technique according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

3.6.2. Initial simulations

For this study, a series of dynamic simulations of the long-term washout experiments and batch test experiments were performed with default kinetic parameters to evaluate the model behaviour under limited and unlimited substrate concentrations and DO. Initial simulations were run with the literature-based set of model parameters. At this point, it was of primary interest to look at the simulation results of the models in terms of trends and to which extent these trends reflect observed data. At this stage, the focus was on trends only, and good calibration, exact fitting and accurate knowledge of parameter values was unnecessary.

3.6.3. Evaluation of key parameters

A simple manual calibration method was used to identify the key parameters influencing the model outputs. Maximum specific growth rates were determined for AOB ($\mu_{max,AOB}$) and NOB ($\mu_{max,NOB}$) in a trial-and-error manual adjustment of the parameters. Next, the simulation software build-in mathematical optimizer tool (NM method) was used to adjust $\mu_{max,AOB}$ and $\mu_{max,NOB}$ together with oxygen ($K_{O,AOB}$ and $K_{O,NOB}$) and substrate half-saturation constants ($K_{NH4,AOB}$ and $K_{NO2,NOB}$). In this way, the estimated set of kinetic parameters at DO concentrations of 0.5, 2.0 and 6.0 mg O_2/L was determined for the two-step nitrification model (Model “0”). The same procedure was repeated in the extended model (Model “CMX”) to include CMX specific growth

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rate ($\mu_{max,CMX}$) and oxygen half-saturation constant ($K_{O,CMX}$). Ammonia half-saturation constant for CMX ($K_{NH4,CMX}$) was adapted from literature. The extended model was used to evaluate the effect of different microbial groups (i.e. AOB, NOB and CMX) on the predicted nitrogen concentrations.

3.6.4. Mass balance

Mass balance is fundamental in environmental engineering, especially in the activated sludge process used for wastewater treatment. In the context of two-step nitrification involving AOB, NOB, and comammox *Nitrospira* (CMX), maintaining an accurate mass balance is critical for several reasons, e.g. accurate prediction of biological processes and nutrient transformations. Prediction of biological processes includes microbial dynamics, where a mass balance is essential for accurately tracking the growth, decay, and interactions of AOB, NOB, and CMX. By accounting for these processes, the model can predict the rates of ammonia oxidation to nitrite (by AOB), nitrite oxidation to nitrate (by NOB), and complete oxidation of ammonia to nitrate (by comammox) (Daims *et al.*, 2006).

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3.7. Organization of the modelling study

The following section describes a process of evaluating the examined model against the experimental data set, including model calibration and a procedure to determine values of the model parameters that allow for minimum differences between the model predictions and measured values from the studied activated sludge systems. Figure 3.3 presents a general sequence diagram of the procedure with all steps, and a detailed procedure is given in the supplementary material S.1 (Appendix A.).

3.7.1. Sensitivity analysis and correlation matrix

A one-variable-at-a-time approach for the parameters' local sensitivity analysis (LSA) was applied to evaluate the model uncertainty. The LSA method determines the influence of one or more variables on results or quantities of importance in mathematical models (Hong et al., 2019; Makinia, 2010). The sensitivity was evaluated using the normalized sensitivity coefficient ($S_{i,j}$), defined as the ratio of the percentage change in the *j-th* model parameter (x_j) with ±10% uncertainty of the adjusted values (Lu et al., 2018; Mehrani et al., 2021):

$$S_{i,j} = \left|\frac{\Delta y_{i,j}}{y_i} \cdot \frac{x_j}{\Delta x_j}\right| \tag{3.4}$$

where the $S_{i,j}$ coefficient is defined as a ratio of the percentage change ($\Delta y_{i,j}/y_i$) in the *i-th* output variable (y_i) to the percentage change ($\Delta x_j/x_j$) in the *j-th* model parameter (x_j).

The influence of each adjusted parameter on the specific model output was defined using the following classification (Cao et al., 2020; Lu et al., 2018): insignificantly influential ($S_{i,j} < 0.25$), influential ($0.25 \leq S_{i,j} < 1$), very influential ($1 \leq S_{i,j} < 2$), and highly influential ($S_{i,j} \geq 2$). The analysis was carried out based on the results from the batch test series. Two trials, including AOB and NOB activity, and AOB, NOB and CMX activity, were conducted for the sensitivity analysis of ammonia N, nitrite N and nitrate N model performance.

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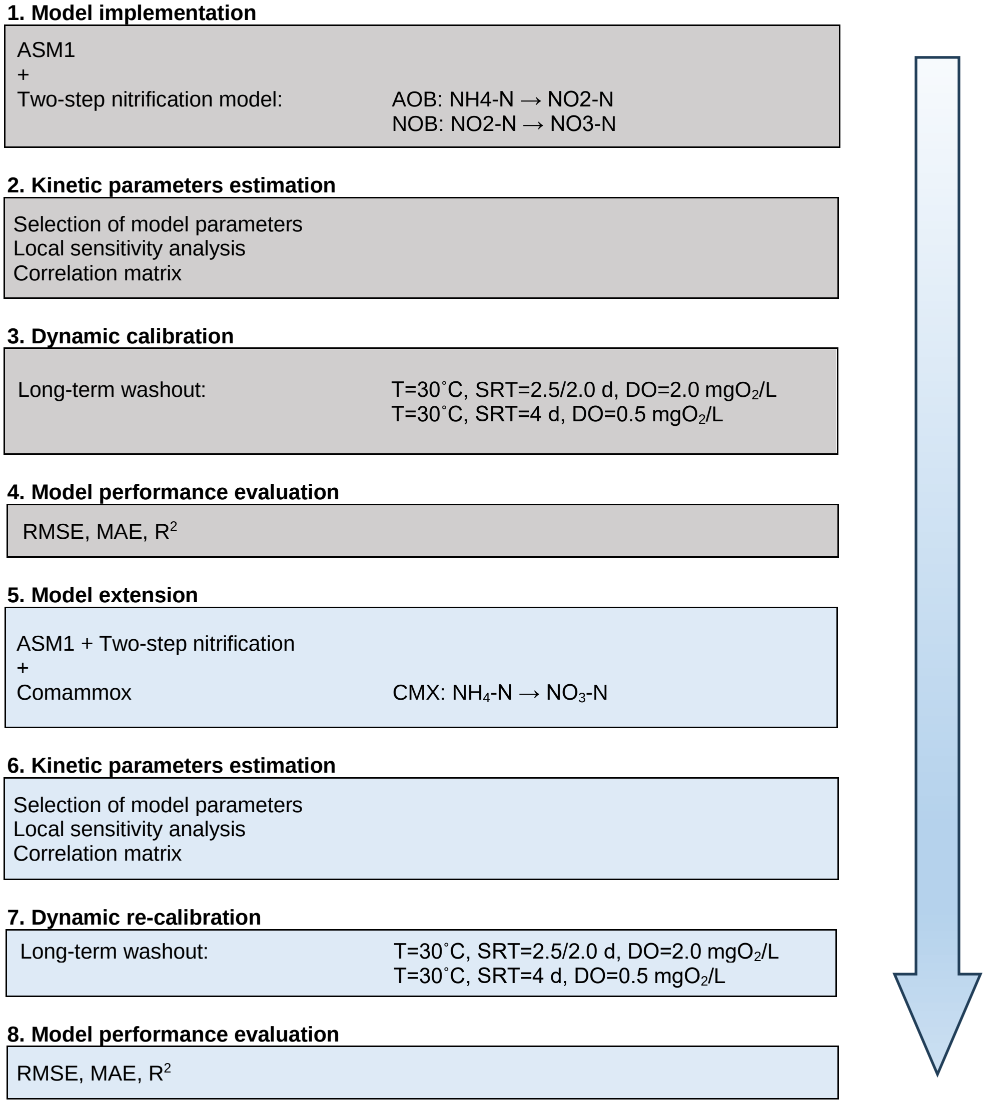

Figure 3.3. Model implementation and calibration sequence diagram.

3.7.2. Parameter correlation

As a result of the sensitivity analysis, pairs of the most sensitive kinetic parameters were evaluated using the correlation matrix. If the correlation coefficient for any pair is high enough, the calibration procedure can be simplified by adjusting only one of these parameters. The correlation matrix (C_θ) was calculated from the Equation 3.5:

$$(C_\theta)_{k,l} = \frac{(V_\theta)_{k,l}}{\sqrt{(V_\theta)_{l,l}(V_\theta)_{k,k}}} \tag{3.5}$$

where (V_θ) is the variance-covariance matrix of the individual parameter estimates (*k,k*) and (*l,l*), and the different parameter estimates (*k,l*).

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The correlation matrix evaluates a linear relationship, strength, and direction (positive vs. negative) of the pairs of influential model parameters. If the determined correlation coefficient is high enough for any pair, the calibration process can be simplified by adjusting only one of the two parameters (Cao et al., 2020; Zhu et al., 2015). Standard classification for the correlation values of pair parameters is classified as strong (>0.68), moderate (0.36–0.68), and weak (<0.36), respectively. This classification was adopted in the present study.

3.7.3. Parameter estimation

The model kinetic parameters' values were selected using three methods: data adapted from literature, manual calibration of the experimental data, and mathematical optimization (GPS-x Optimizer Module, NM method). Based on the local sensitivity analysis, values for the least influential parameters were adapted from literature. SBR long-term washout experiments were used for the estimation of specific growth rates of AOB ($\mu_{max,AOB}$) and NOB ($\mu_{max,NOB}$) in Model "0" and additionally CMX ($\mu_{max,CMX}$) in Model "CMX". It was recommended by Henze *et al.* (2008) that the growth rates and saturation coefficients for autotrophic microorganisms can be estimated and adjusted using non-dynamic data. A combination of sensitivity analysis data and process knowledge was applied as appropriate method for the selection of parameters to be adjusted during the calibration step of the model implementation (Petersen *et al.*, 2002; Makinia *et al.*, 2005; Henze *et al.*, 2008) to verify model's sensitivity of the adjusted parameters.

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3.8. Model performance measures

Evaluation of the performance of environmental models like ASM should be completed using appropriate statistical measures to assess how well the model predictions match observed data. Three commonly used performance metrics in this context are Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and the Coefficient of Determination (R^2), often interpreted as the Nash-Sutcliffe Efficiency (NSE). Each metric provides different insights into model performance, and their combined use is recommended for a comprehensive evaluation.

RMSE is a widely used measure to evaluate the differences between predicted and observed values. It is defined as:

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(O_i - P_i)^2} \tag{3.6}$$

where:

O_i = the observed (measured) value
P_i = the predicted (simulated) value
n = number of data points
i = the *i-th* observation

RMSE offers a measure of the magnitude of prediction errors. Since it squares the differences between predicted and observed values, larger errors have a disproportionately higher impact, making RMSE sensitive to outliers. A lower RMSE indicates better model accuracy, and it is particularly valuable in models predicting concentrations of pollutants like ammonia and nitrite in wastewater treatment processes. In ASM applications and simulations, RMSE is useful for determining the overall error magnitude in predicting key parameters. However, RMSE does not differentiate between over- and under-predictions, which can be important in certain contexts.

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MAE is the average of the absolute differences between predicted and observed values. It is calculated as:

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|O_i - P_i| \tag{3.7}$$

where:

O_i = the observed (measured) value
P_i = the predicted (simulated) value
n = number of data points
i = the *i-th* observation

MAE provides a linear measure of average prediction error, meaning all errors are treated equally, without giving extra weight to larger errors. This makes MAE a robust indicator of model performance when extreme errors are less significant to the analysis. In multi-step nitrification simulations, MAE is particularly useful for understanding the typical prediction error, offering an intuitive measure of how close model predictions are to observed values on average. This metric is beneficial when modelling concerns the average deviation rather than the impact of extreme values.

The Nash-Sutcliffe Efficiency (NSE), also known as the Coefficient of Determination (R^2), is a normalized statistic that measures the relative magnitude of the residual variance ("noise") compared to the variance of the observed data ("signal"). It is defined as:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(O_i - P_i)^2}{\sum_{i=1}^{n}(O_i - O_m)^2} \tag{3.8}$$

where:

O_i = the observed (measured) value
O_m = mean of the observed (measured)

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P_i = the predicted (simulated) value
n = number of data points
i = the *i-th* observation

R^2 values range from - ∞ to 1. Coefficient value of 1 indicates a perfect match between model predictions and observations, while an R^2 of 0 indicates that the model predictions are as accurate as the mean of the observed data. Negative R^2 values suggest that the model predictions are worse than using the mean of the observed values as a predictor. NSE is widely used in environmental modelling, including ASM, to assess overall predictive power. However, R^2 can be sensitive to extreme values and might not adequately represent model performance if outliers dominate the error structure.

A comprehensive evaluation of ASM multi-step nitrification simulations employs all three metrics – RMSE, MAE, and R^2 – to provide a robust assessment of the examined model's predictive accuracy, average error magnitude, and ability to capture the variability in observed data. When used together, these metrics will offer a detailed picture of the model's strengths and weaknesses, guiding improvements and ensuring reliable simulations in this study.

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3.9. Model application

The practical applications of complex biokinetic models, as documented in the literature, can be broadly categorized into four main areas: optimizing the performance of existing plants, upgrading current facilities, designing new facilities, and developing innovative treatment concepts (Makinia and Zaborowska, 2020). Among these, optimizing existing facilities is considered the most beneficial for employing mathematical modeling approaches. This is primarily due to the ability of these models to be calibrated and validated using actual data from the facilities in question. According to the literature (Al-Omari et al., 2015; Zaborowska et al., 2016; Lu et al., 2018; Maktabifard et al., 2022; Mehrani et al., 2022a), model-based optimization is defined as the evaluation of various scenarios involving alternative operational strategies. This includes adjustments, i.e., in wastewater or recycle flow rates, changes in pollutant loads, and modifications to the physical configuration of the plant.

Model-based optimization also serves as a reliable tool for the energy optimization of WWTP operations in terms of power consumption for aeration, greenhouse gas emissions, and carbon footprint. Within this framework, mathematical modeling and computer simulation have primarily been employed as tools to achieve optimal process performance.

In this study, the model was calibrated and validated to optimize process performance. The model was used to determine the maximum tank volume (V_{max}) and the optimal DO concentration. The tank reactor under study was the first stage of the PN/A process, a nitritation tank reactor (1st stage of PN/A) that produces effluent with maximum NO_2-N concentration for the subsequent anammox tank reactor (2nd stage of PN/A). Two scenarios were considered, including constant vs. variable influent NH_4-N concentrations.

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IV. Results

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4.1. Simulations results

Long-term SBR simulation results are presented for experiments conducted at the two DO concentrations (0.5 and 2.0 mgO_2/L). The initial and adjusted values of the most influential model parameters are provided and discussed in relation to the operational conditions and model concepts outlined in Chapter 3, “Materials and Methods”. This includes both the two-step nitrification model (Model “0”) and the two-step nitrification model with comammox (Model “CMX”). Validation results are presented separately for batch tests at different DO concentrations (i.e., DO=0.5, 2.0, and 6.0 mgO_2/L) and with different initial substrate sources i.e., NH_4-N vs. a mixture of NH_4-N and NO_2-N, as specified in Table 3.2.

4.2. Basic two-step nitrification model

4.2.1. Initial simulations

The kinetic parameters for the evaluated two-step nitrification Model “0” at the DO concentrations of 0.5 and 2.0 mgO_2/L are presented in Table 4.1. Initial simulations were conducted with AOB and NOB kinetic parameter values from the literature, and the model outputs were compared with the experimental data. Subsequently, the model was automatically calibrated using the GPS-x built-in Optimizer tool. An optimization algorithm (NM simplex method) with a maximum likelihood objective function was selected for parameter adjustment, as described in Section 3.6.3. The calibration process began with the direct estimation of the specific growth rate of AOB (μ_{AOB}) and NOB (μ_{NOB}) was conducted. This was followed by approximations of the nitrite- and DO half-saturation constants for NOB ($K_{NO2,NOB}$ and $K_{O,NOB}$, respectively). Finally, $K_{NH4,AOB}$ and $K_{O,AOB}$ were adjusted to better fit the model predictions to the long-term test data.

4.2.2. Sensitivity analysis and correlation matrix

Figure 4.1 presents calculated sensitivity coefficients ($S_{i,j}$) for evaluated AOB and NOB kinetic parameters based on the long-term washout test data at DO = 2.0 mgO_2/L. A total of 6 kinetic parameters were analyzed. The highly influential ($1 \leq S_{i,j} \leq 2$) parameter was μ_{AOB} with respect to changes in nitrite N concentration (Si,j = 1.06), while the sensitivity of $K_{NH4,AOB}$ for NO_2-N was moderate ($S_{i,j}$ = 0.75). For all three

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nitrogen compounds, the maximum specific growth rate of AOB (μ_{AOB}) was the dominant parameter for nitrite N.

The sensitivity coefficient of 1.06 for μ_{AOB} indicates that the system's response to changes in that parameter is significant when considering NO_2-N concentrations. This suggests that even small changes in μ_{AOB} can substantially impact NO_2-N levels. Moreover, a previous study (Sepehri and Sarrafzadeh, 2019) has indicated that elevated nitrite N levels significantly influence AOB activity, potentially leading to the build-up of toxic intermediates like nitrous oxide. For example, Dytczak *et al.* (2008) found that elevated nitrite N concentrations can either enhance or inhibit ammonium utilization rate. This is crucial for processes like partial nitritation, where NO_2-N levels are intentionally manipulated (Peng and Zhu, 2006; Fang et al., 2009; Cua and Stein, 2011). Additionally, studies by Stein and Arp (1998) and Daims *et al.* (2011) showed that elevated nitrite levels can be toxic to AOB, as nitrite can penetrate bacterial cells and disrupt metabolic processes, reducing growth rates and potentially causing cell death.

Moderately influential parameters for NO_2-N were μ_{NOB} and $K_{O,NOB}$. The sensitivity coefficient $S_{i,j}$ of 0.46 for μ_{NOB} indicates a reasonable sensitivity to changes in the maximum specific growth rate of NOB, impacting NO_2-N levels. The DO half-saturation constant for NOB, $K_{O,NOB}$, with a sensitivity coefficient of 0.39, also plays a critical role in influencing NO_2-N concentrations.

Apart from the moderately low sensitivity of NH_4-N to μ_{NOB} ($S_{i,j}$ = 0.27), the sensitivity to ammonia N was generally low ($S_{i,j} \leq 0.01$) across other parameters and microbial groups. This suggests that NH_4-N concentration changes do not drastically affect the microbial activities involved in nitrification, and relative stability of these processes concerning ammonia fluctuations (Peng and Zhu, 2006).

A correlation matrix was developed from the initial simulation results to reduce the number of adjusted kinetic parameters during calibration. The overall correlation matrix for the six kinetic parameters evaluated is presented in Figure 4.2. The highest positive correlation (0.99) was between μ_{NOB} and $K_{O,NOB}$. Other strongly correlated parameters (0.95) included $K_{NH4,AOB}$ and $K_{O,AOB}$. The negative correlation between the growth rates of AOB and NOB (μ_{AOB} and μ_{NOB}, -0.34) suggests significant competition for resources. This is further supported by the negative correlation between μ_{AOB} and $K_{O,NOB}$ (- 0.34). These results indicate that as AOB growth increases, NOB growth is inhibited, likely

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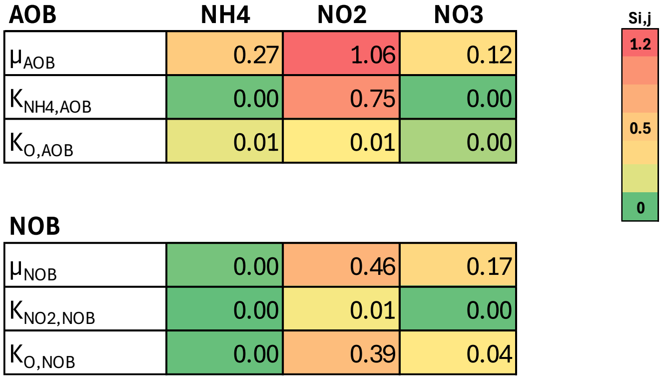
due to competition for shared resources, such as dissolved oxygen (S. Shourjeh et al., 2020). The strong positive correlation between the growth rate and the half-saturation constant for DO within NOB (μ_{NOB} and $K_{O,NOB}$, 0.99) suggests that the metabolic processes regulating growth and oxygen uptake are highly interdependent and closely linked within each nitrifying group (Martens-Habbena et al., 2009).

Table 4.1. Model "0" long-term experiment estimated kinetic parameters.

Bacterial group	**Kinetic parameter**	**Unit**	**Initial value**	**Model 0 DO=0.5* mgO$_2$/L**	**Model 0 DO=2** mgO$_2$/L**	**References**
AOB	μ_{AOB}	d^{-1}	1.01	1.19	1.19	washout test
	$K_{NH4,AOB}$	mgN/L	0.67	0.99	0.99	washout test
	$K_{O,AOB}$	mgO$_2$/L	0.30	0.61	0.61	washout test
	b_{AOB}	d^{-1}	0.15	0.15	0.15	Yu et al. (2020)
NOB	μ_{NOB}	d^{-1}	0.31	0.64	0.64	washout test
	$K_{NO2,NOB}$	mgN/L	0.08	0.34	0.34	washout test
	$K_{O,NOB}$	mgO$_2$/L	0.45	1.12	1.12	washout test
	b_{NOB}	d^{-1}	0.05	0.05	0.05	Yu et al. (2020)
	$K_{NH4\ INH,NOB}$	mgN/L	25	25	25	Sin et al. (2008)

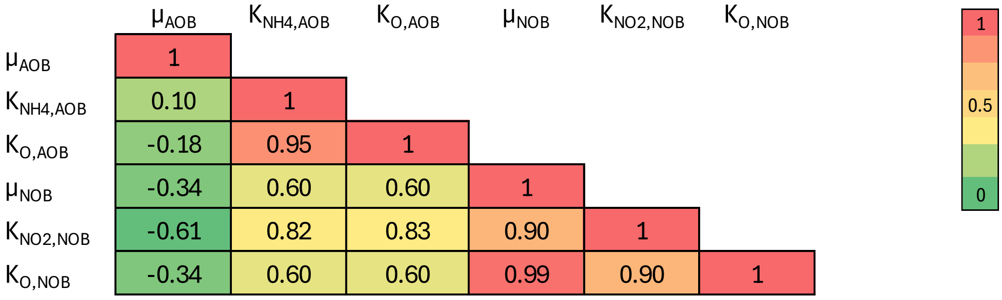
* dynamic calibration: DO=0.5 mgO2/L, T=30˚C, variable influent NH$_4$-N concentration
** dynamic calibration: DO=2.0 mgO2/L, T=30˚C, constant influent NH$_4$-N concentration



AOB	**NH4**	**NO2**	**NO3**
μ_{AOB}	0.27	1.06	0.12
$K_{NH4,AOB}$	0.00	0.75	0.00
$K_{O,AOB}$	0.01	0.01	0.00

NOB			
μ_{NOB}	0.00	0.46	0.17
$K_{NO2,NOB}$	0.00	0.01	0.00
$K_{O,NOB}$	0.00	0.39	0.04

Figure 4.1. Heat map of the sensitivity coefficients for the Model "0" kinetic parameters.



	μ_{AOB}	$K_{NH4,AOB}$	$K_{O,AOB}$	μ_{NOB}	$K_{NO2,NOB}$	$K_{O,NOB}$
μ_{AOB}	1					
$K_{NH4,AOB}$	0.10	1				
$K_{O,AOB}$	-0.18	0.95	1			
μ_{NOB}	-0.34	0.60	0.60	1		
$K_{NO2,NOB}$	-0.61	0.82	0.83	0.90	1	
$K_{O,NOB}$	-0.34	0.60	0.60	0.99	0.90	1

Figure 4.2. Correlation matrix of the adjusted kinetic parameters for Model "0".

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4.2.3. Model calibration with SBR long-term test data

DO unlimited conditions

The experimental reactor operating at DO=2.0 mgO_2/L and SRT=2 d was fed with diluted sludge digester liquor solution with a stable NH_4-N concentration at 282 ±10 mgN/L. During the startup phase (0 ÷ 5d), an intense increase in the concentration of nitrate N was observed (197 mgN/L at t = 4.5 d), together with a partial NH_4-N accumulation at 40÷60 mgN/L, and some amount of nitrite N (14 mgN/L). After 4.5 days of operation (9 cycles), the SRT was reduced to 2 days, followed by an intense reactor response with an increasing accumulation of NO_2-N and a decrease in NO_3-N concentration. After 16 days of operation, ammonia and nitrate N concentrations stabilized at approximately 41 and 53 mgN/L, respectively. Nitrite N accumulation in the system increased throughout the operational period, reaching a concentration of 212 mgN/L on day 31 (completed 63 cycles).

Figure 4.3 presents long-term SBR simulation results for NH_4-N, NO_2-N, and NO_3-N with Model “0” adjusted parameters used at the DO concentration of 2.0 mgO_2/L and initial SRT = 2.5d. The model predictions of NH_4-N with the R^2 value equal to 0.74 suggest that the model adequately explains the variance in the observed data, indicating a strong predictive capability. However, comparing R^2 values of NO_2-N and NO_3-N indicates that the model performs best in predicting NO_2-N (R^2=0.82), with a moderate model performance of NO_3-N (R^2=0.52) and explains a large portion of the variance in the observed NO_3-N data.

The Model “0” predicted concentrations of NH_4-N have relatively low errors as indicated by the RMSE (3.99) and MAE (3.47) values. Still, the R^2 value suggests that the model does not explain much of the variance in the observed data, indicating a limited fit despite low error metrics. The model estimates for NO_2-N have higher error values (RMSE and MAE). A high MAE (734.42) shows potential extreme values or variability issues. Still, the high R^2 value indicates a good fit to the observed data, meaning the model explains most of the variance despite the more significant errors compared to NH_4-N predictions. Similar to NO_2-N, the model predictions for nitrate N show high

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values of RMSE and MAE (31.30 and 979.67, respectively). However, a relatively high R^2 value indicates an average fit to the observed data, explaining a significant portion of the variance. After 20 days of simulation, it can be observed that Model "0" cannot predict the accumulation of NO_3-N in the system. This indicates a need for further refinement or extension of the model to improve accuracy and data fit across all nitrogen species (see Table 4.2).

Table 4.2. Model "0" performance metrics during long-term SBR operation.

SBR operational conditions	*Variable*	*RMSE*	*MAE*	R^2
DO=0.5 mgO₂/L *SRT=4.0 d* *T=30˚C*	NH_4-N	5.81	3.76	0.94
	NO_2-N	43.33	1877.38	0.76
	NO_3-N	38.00	1443.90	0.85
DO=2.0 mgO₂/L *SRT=2.5/2.0 d* *T=30˚C*	NH_4-N	3.99	3.47	0.74
	NO_2-N	27.10	734.42	0.82
	NO_3-N	31.30	979.67	0.52

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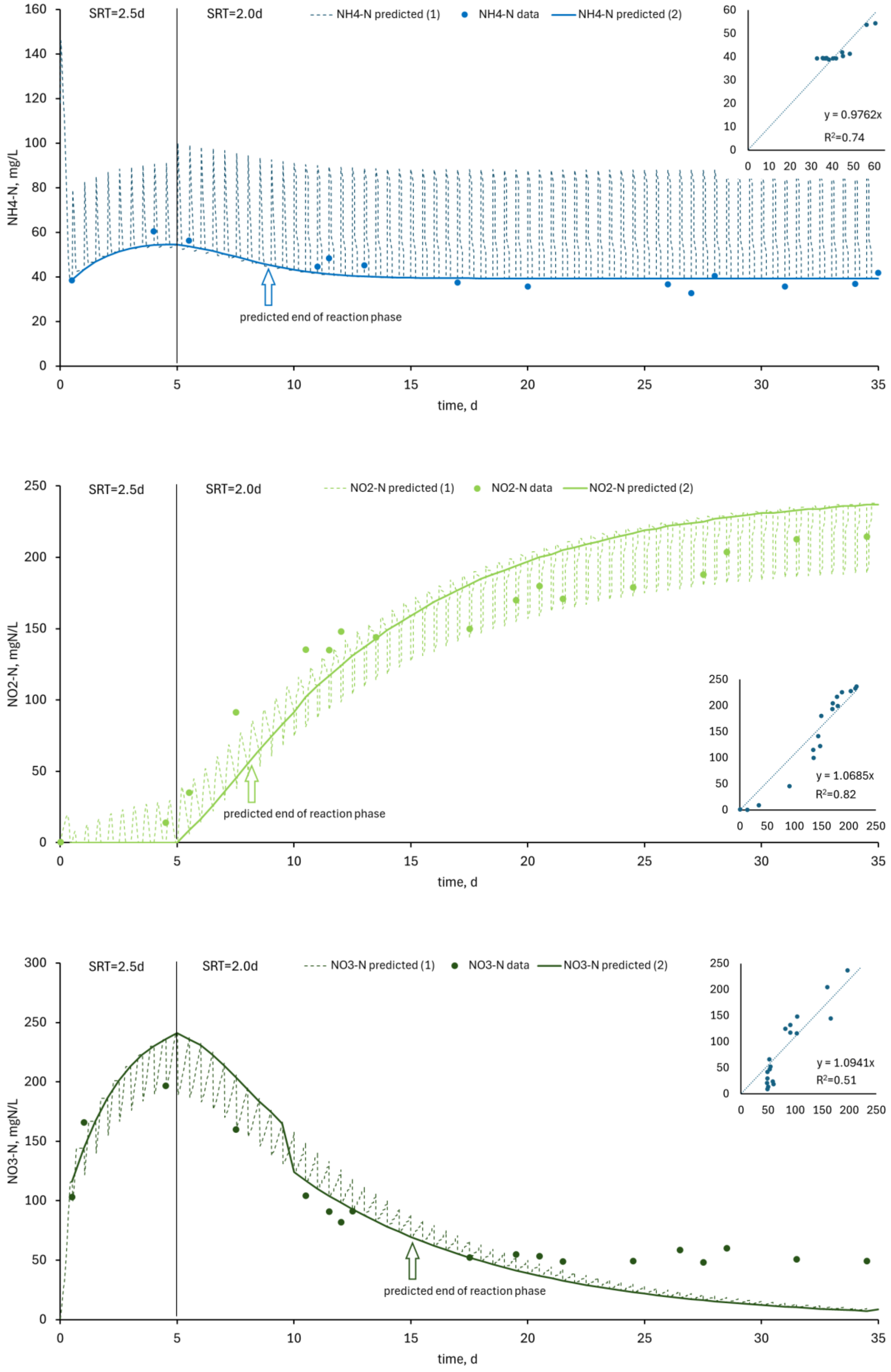

Figure 4.3. Measured and predicted nitrogen concentrations in the long-term washout experiment. DO=2.0 mgO$_2$/L; influent NH$_4$-N concentration 282 mgN/L; initial SRT=2.5d.

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DO limited conditions

Long-term SBR washout experiment simulation results at the limited DO conditions are presented in Figure 4.4. The experimental reactor operating at DO=0.5 mgO_2/L and SRT=4 d was fed with diluted sludge digester liquor solution, with variable NH_4-N concentration 410÷550 ±10 mgN/L. The influent NH_4-N concentration was controlled based on AUR and adjusted between SBR cycles. During the startup phase (0 ÷ 7d), the controlled ammonia concentration in the feed was 450 ÷ 550 mgN/. The observed concentration of nitrate N reached 430 mgN/L after 15 cycles (t = 7.5d), with a partial nitrite N accumulation at 28 mgN/L, and some residual NH_4-N (5 mgN/L). After 28 days of operation, nitrate N concentration stabilised at approximately 104 mgN/L. Nitrite N accumulation in the system constantly increased throughout the experiment, reaching a concentration of 287 mgN/L on day 36 (completed 73 cycles).

The model predictions for NH_4-N have a relatively low RMSE (5.81), indicating that the model's predictions are close to the observed values. MAE is also low (3.76), showing that, on average, the predicted concentrations are only 3.76 away from the observed data. The R^2 is very high (0.94), demonstrating that the model explains 94% of the variance in the actual NH_4-N concentrations and implies that AOB activity is well predicted. It also indicates the model captures the effect of AOB behaviour on NH_4-N concentrations effectively, even under high and variable influent conditions. In the case of nitrite and nitrate N, the R^2 values are relatively high (0.76 and 0.85, respectively), suggesting that the model does not explain much of the variance in the observed data. The model predictions for NO_2-N have a relatively high RMSE (43.33) and very high MAE (1877.38), indicating significant prediction errors and average accuracy. The model predictions for NO_3-N have high RMSE (38.00), similar to NO_2-N, indicating the model's considerable deviations from the actual values. MAE for nitrite N is very high (1443.90) and shows that, on average, the predictions are off by a significant margin, indicating substantial prediction errors.

The high error metrics (Table 4.2) and relatively high R^2 values for NO_2-N and NO_3-N suggest that the model may not adequately account for inhibitory effects on NOB, resulting in less accurate predictions. While the performance for NO_2-N and NO_3-N is reasonable regarding R^2, it needs improvement in reducing prediction errors, as indicated by the high RMSE and MAE values.

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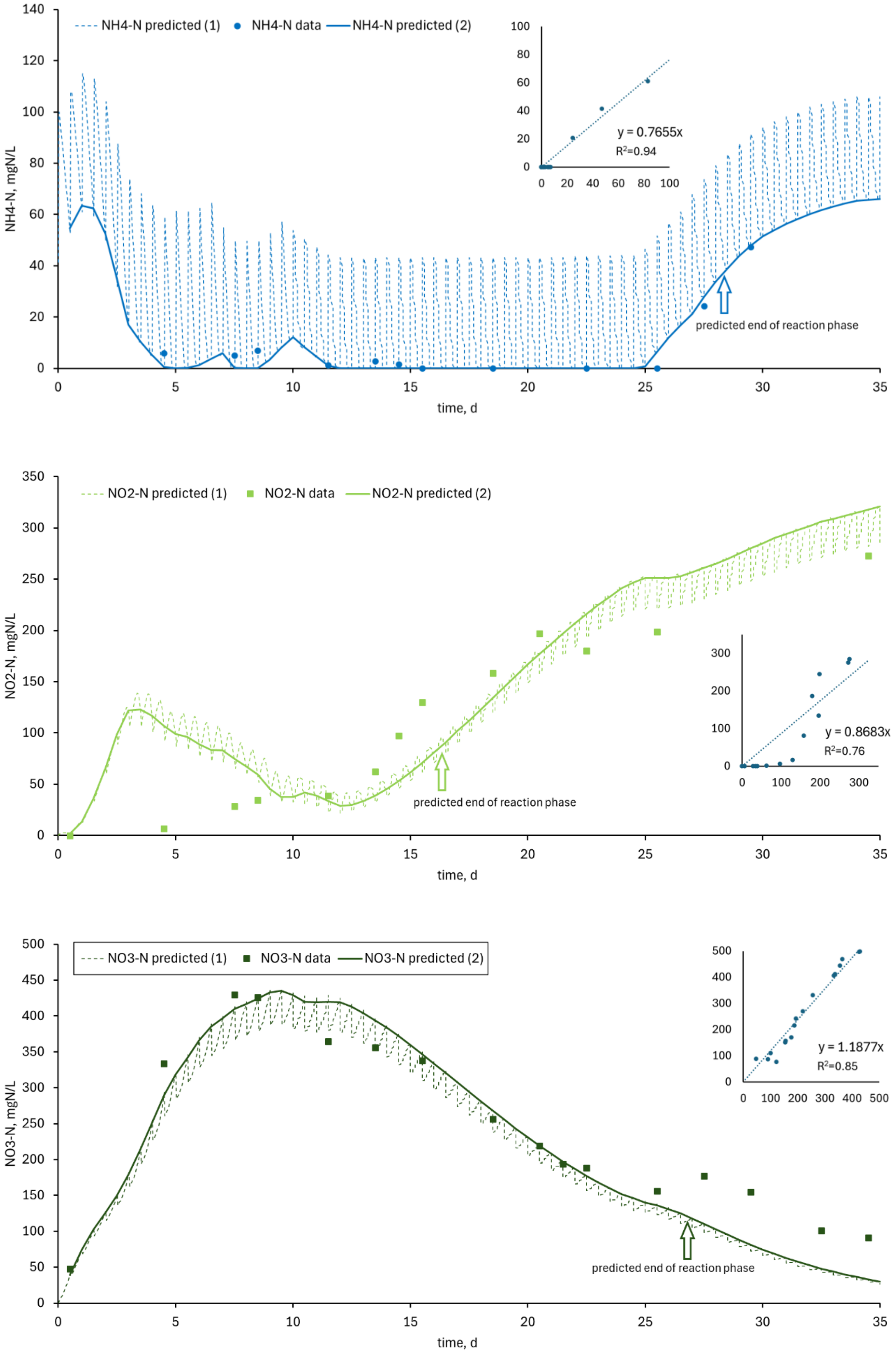

Figure 4.4. Measured and predicted nitrogen concentrations in the long-term washout experiment. DO=0.5 mgO$_2$/L; influent NH$_4$-N concentration 410÷550 mgN/L; SRT=4d.

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4.2.4. Model validation with batch tests data

Figure 4.5 presents a selection of batch tests measured and predicted nitrogen concentrations during the validation phase at DO concentrations 0.5, 2.0, and 6.0 mgO_2/L with various initial nitrite N concentrations. The overall correlation between each nitrogen component's observed and model-predicted data shows very good model performance with $R^2 > 0.8$ (Table 4.3). The batch test at DO concentration of 6.0 mgO_2/L had the lowest R^2 (0.76) when compared with ammonia and nitrate N compounds, but still reasonably high, and nitrite N compound has the lowest RMSE and MAE, suggesting that the model performs best in predicting NO_2-N concentrations at DO concentration of 6.0 mgO_2/L. However, its R^2 value is the lowest among the three nitrogen compounds but still indicates a good fit.

Table 4.3. Model “0” performance metrics during validation phase.

Batch reactor operational conditions	*Variable*	*RMSE*	*MAE*	R^2
DO=0.5 mgO₂/L *T=25˚C*	NH_4-N	2.19	1.56	0.89
	NO_2-N	0.76	0.58	0.95
	NO_3-N	1.25	1.56	0.98
DO=2.0 mgO₂/L *T=20˚C*	NH_4-N	0.83	0.51	0.99
	NO_2-N	2.02	4.08	0.97
	NO_3-N	1.88	3.55	0.98
DO=6.0 mgO₂/L *T=25˚C*	NH_4-N	5.02	4.04	0.88
	NO_2-N	1.90	3.60	0.76
	NO_3-N	6.91	47.87	0.86

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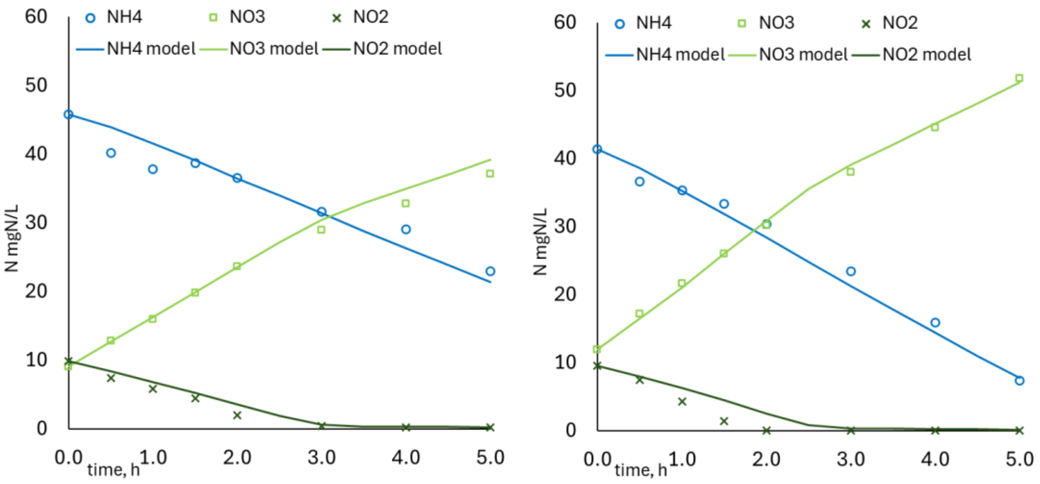
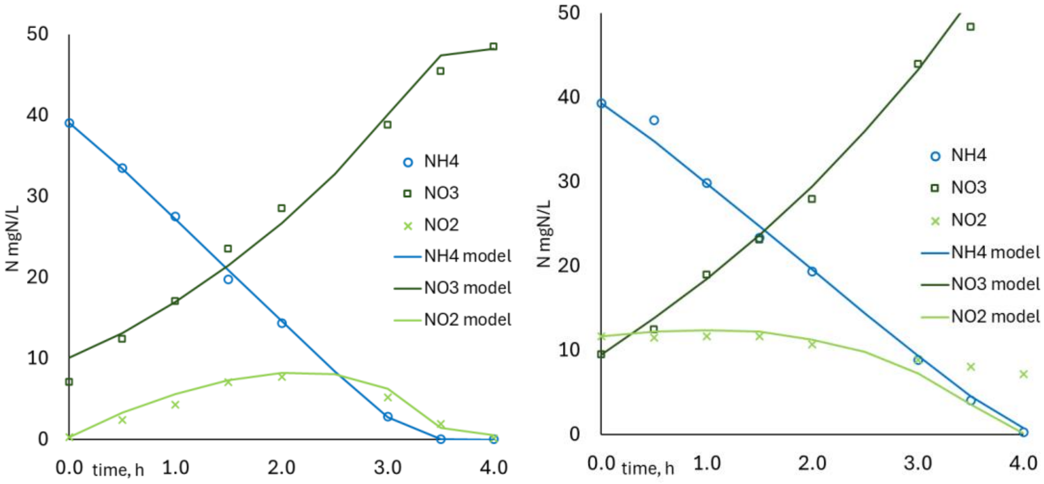
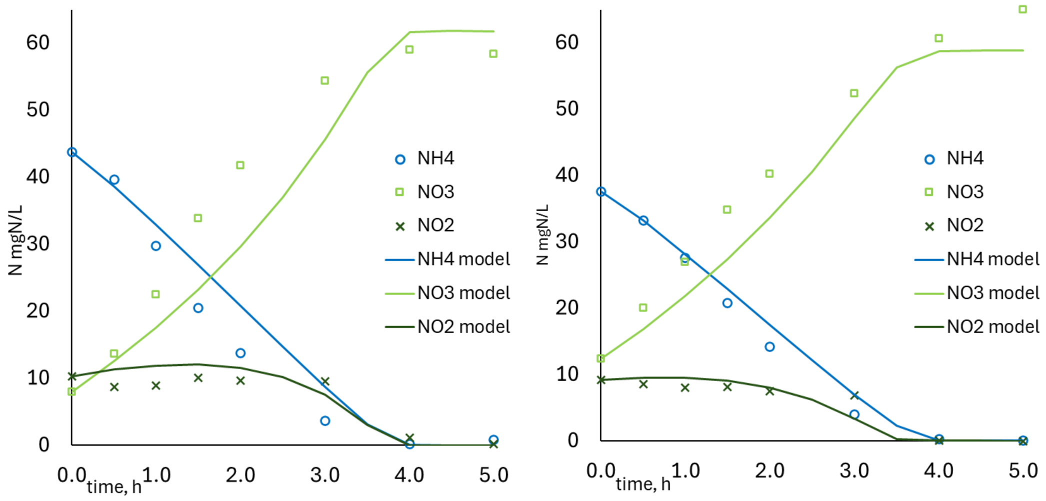


a) DO=0.5 mg$_2$/L , T=25˚C b) DO=0.5 mg$_2$/L, T=25˚C



c) DO=2 mgO$_2$/L, T=20˚C d) DO=2 mgO$_2$/L, T=20˚C



e) DO=6 mgO$_2$/L, T=25˚C f) DO=6 mgO$_2$/L, T=25˚C

Figure 4.5. Measured and predicted nitrogen concentrations in batch tests experiments validation phase at various DO and initial NO$_2$-N concentrations.

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4.3. Model extension with Comammox

Based on the long-term simulation results, where Model “0” had average quality performance metrics, the extended Model “CMX” with one-step comammox oxidation of ammonia nitrogen (NH_4-N → NO_3-N) was applied. Initial simulations were run with values of AOB, NOB, and comammox kinetic parameters adopted from the literature study, and model outputs were compared with the experimental data. Model calibration and validation procedure was the same as in the case of Model “0”, described in supplementary material S1 (Appendix A.).

4.3.1. Sensitivity analysis and correlation matrix

Local sensitivity analysis based on NH_4-N, NO_2-N, and NO_3-N variations for the kinetic parameters of AOB, NOB and Comammox microorganisms is presented in Figure 4.6. The maximum specific growth rate is consistently the most sensitive parameter across all species, particularly for AOB influencing NO_2-N and NO_3-N concentrations. Nitrite N is highly sensitive to μ_{AOB} with $S_{i,j}$ coefficient of 1.95, indicating a very significant influence. Figure 4.7 presents changes in predicted NO_2-N concentrations at the base value of μ_{AOB}, and ±10% model response. NO_3-N also shows considerably high sensitivity to μ_{AOB} with $S_{i,j}$ coefficient equal to 1.10. However, the sensitivity of NH_4-N to μ_{AOB} is moderate ($S_{i,j} = 0.31$). NOB maximum specific growth rate (μ_{NOB}) with $S_{i,j}$ coefficient of 0.60 for NH_4-N shows influential sensitivity, low sensitivity for NO_2-N with $S_{i,j}$ of 0.21, and very low (insignificant) sensitivity to NO_3-N (0.07). Nitrite N model output has noticeable sensitivity to oxygen half-saturation constant ($K_{O,NOB}$) with a coefficient of 0.39. Comammox kinetic parameters show the lowest sensitivity for NH_4-N of μ_{CMX} ($S_{i,j} = 0.07$) compared to AOB and NOB and no sensitivity to $K_{NH4,CMX}$ and $K_{O,CMX}$ (0.00 each). NO_2-N shows significant sensitivity to μ_{CMX} ($S_{i,j} = 0.74$), though less than AOB, and low sensitivity for oxygen half-saturation constant $K_{O,CMX}$ with $S_{i,j}$ of 0.19. Within the low range of moderate sensitivity was μ_{CMX} ($S_{i,j} = 0.31$) for NO_3-N. Overall analysis shows that AOB exhibits the highest overall sensitivity, particularly to its maximum specific growth rate (μ_{AOB}), which remains critical for controlling NO_2-N and NO_3-N concentrations. NOB kinetic parameters have reasonably high sensitivity, with the most impact seen in NH_4-N concentrations. CMX kinetic parameters show average sensitivity, particularly affecting NO_2-N but generally less influential than AOB.

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Consequently, a correlation matrix was developed based on the initial simulation results of the extended model to reduce the number of adjusted kinetic parameters during the calibration phase, focusing on Comammox kinetics. The correlation matrix for the most sensitive kinetic parameters evaluated is presented in Figure 4.8. The highest positive correlation (0.99) was between μ_{NOB} and $K_{O,NOB}$. A very strong positive correlation shows that the growth rate of NOB is closely tied to its oxygen affinity. As the oxygen half-saturation constant increases, so does the growth rate of NOB. This indicates that NOB requires higher oxygen levels to maintain its growth, which is crucial for converting nitrite to nitrate in the conventional nitrification process but not in the partial nitrification systems. This finding highlights the importance of maintaining adequate oxygen levels to support NOB activity, particularly in systems where NOB is vital in completing the nitrification. Another highest positive correlation was between μ_{CMX} and $K_{O,CMX}$ (0.99), which indicates oxygen availability is crucial for CMX, directly affecting its ability to perform complete nitrification. As oxygen availability increases, CMX becomes more efficient in converting NH_4-N to NO_3-N, making it a key player in environments where oxygen is plentiful. Comammox strong dependence on oxygen mirrors that of NOB, but because CMX is capable of bypassing the nitrite stage, it might compete directly with NOB under high oxygen conditions, potentially reducing the need for NOB in the system. This confirms that the metabolic processes regulating growth and oxygen uptake are closely linked within each nitrifying microorganism (Martens-Habbena et al., 2009) and highly interdependent. Then again, the very weak correlation between the oxygen affinities of AOB and CMX (-0.10) implies that these groups can coexist without significantly competing for oxygen. This allows for simultaneous operation in partial nitrification systems without compromising efficiency (Sharif Shourjeh et al., 2021b).

Next, the model was automatically calibrated with the GPS-x built-in Optimizer Tool. First, a direct estimation of the specific growth rate of AOB (μ_{AOB}), NOB (μ_{NOB}), and Comammox (μ_{CMX}) was conducted, followed by an approximation of the nitrite- and DO half-saturation constants for NOB ($K_{NO2,NOB}$ and $K_{O,NOB}$, respectively). Next, the oxygen half-saturation constant for Comammox ($K_{O,CMX}$) was estimated. Finally, $K_{NH4,AOB}$ and $K_{O,AOB}$ were estimated to fit the predicted long-term test data better. The Model “CMX” estimated kinetic parameters for AOB, NOB, and Comammox at unlimited DO

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conditions (DO=2.0 mgO$_2$/L) and limited DO conditions (DO=0.5 mgO$_2$/L) are presented in Table 4.4.

Table 4.4. Model “CMX” long-term experiment estimated kinetic parameters.

Bacterial group	**Kinetic parameter**	**Unit**	**Initial value**	**Model CMX DO=0.5* mgO$_2$/L**	**Model CMX DO=2** mgO$_2$/L**	**References**
AOB	μ_{AOB}	d^{-1}	1.01	0.94	0.94	washout test
	$K_{NH4,AOB}$	mgN/l	0.67	0.71	0.71	washout test
	$K_{O,AOB}$	mgO$_2$/l	0.30	0.24	0.24	washout test
	b_{AOB}	d^{-1}	0.15	0.15	0.15	Yu et al. (2020)
NOB	μ_{NOB}	d^{-1}	0.31	0.26	0.26	washout test
	$K_{NO2,NOB}$	mgN/l	0.08	0.06	0.06	washout test
	$K_{O,NOB}$	mgO$_2$/l	0.45	0.1	0.1	washout test
	b_{NOB}	d^{-1}	0.05	0.05	0.05	Yu et al. (2020)
	$K_{NH4\ INH,NOB}$	mgN/l	25	25	25	Sin et al. (2008)
CMX	μ_{CMX}	d^{-1}	0.20	0.82	0.82	washout test
	$K_{NH4,CMX}$	mgN/l	0.01	0.01	0.01	Koch et al. (2019)
	$K_{O,CMX}$	mgO$_2$/l	0.30	0.51	0.61	washout test
	b_{CMX}	d^{-1}	0.05	0.05	0.05	Yu et al. (2020)

* dynamic calibration: DO=0.5 mgO2/L, T=30˚C, variable influent NH_4-N concentration
** dynamic calibration: DO=2.0 mgO2/L, T=30˚C, constant influent NH_4-N concentration

Calibrated AOB oxygen half-saturation constant ($K_{O,AOB}$) remains constant at 0.24 mgO$_2$/L across both DO conditions. This suggests that AOBs have a stable, moderate oxygen efficiency, and their activity does not significantly vary with changes in oxygen levels. This aligns with findings by Blackburne et al. (2007) that show AOBs can sustain activity in low-oxygen environments by enhancing their enzyme activity. Estimated $K_{O,NOB}$ remains steady at 0.1 mgO$_2$/L in both scenarios, indicating that NOBs are efficient oxygen users with high oxygen affinity. However, their overall performance is limited by their strict dependence on oxygen for nitrite oxidation, and their overall sensitivity to oxygen limitation reduces their competitive advantage in low-oxygen environments (Koch *et al.*, 2019). Comammox bacteria exhibit a slightly higher $K_{O,CMX}$ under low DO (0.51 mgO$_2$/L) than high DO (0.61 mgO$_2$/L). This slight change suggests that comammox bacteria are adaptable, maintaining high efficiency in oxygen utilization across different conditions (Koch *et al.*, 2019). The finding aligns with studies by Mei et al. (2023), who found that oxygen half-saturation constant changes when ammonia-oxidizers and nitrite-oxidizers are cultured at low (0.5 mgO$_2$/L) and high (4.0 mgO$_2$/L) DO concentrations. The phenomenon was also observed by Wang et al.

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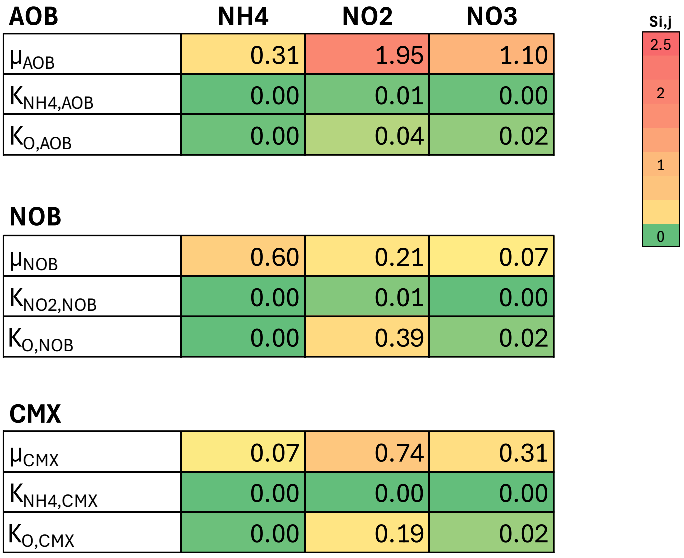
(2021), who found that K_O decreased when DO concentration decreased and showed that the adaptation of NOB to low oxygen conditions was much stronger than that of AOB. Summarizing the calibration results, both AOB and NOB show stable oxygen utilization across different DO levels. Still, comammox bacteria demonstrate higher adaptability, which may give them an advantage in fluctuating oxygen environments.



AOB	**NH4**	**NO2**	**NO3**
μ_{AOB}	0.31	1.95	1.10
$K_{NH4,AOB}$	0.00	0.01	0.00
$K_{O,AOB}$	0.00	0.04	0.02

NOB			
μ_{NOB}	0.60	0.21	0.07
$K_{NO2,NOB}$	0.00	0.01	0.00
$K_{O,NOB}$	0.00	0.39	0.02

CMX			
μ_{CMX}	0.07	0.74	0.31
$K_{NH4,CMX}$	0.00	0.00	0.00
$K_{O,CMX}$	0.00	0.19	0.02

Figure 4.6. Heat map of the sensitivity coefficients for the Model “CMX” kinetic parameters.

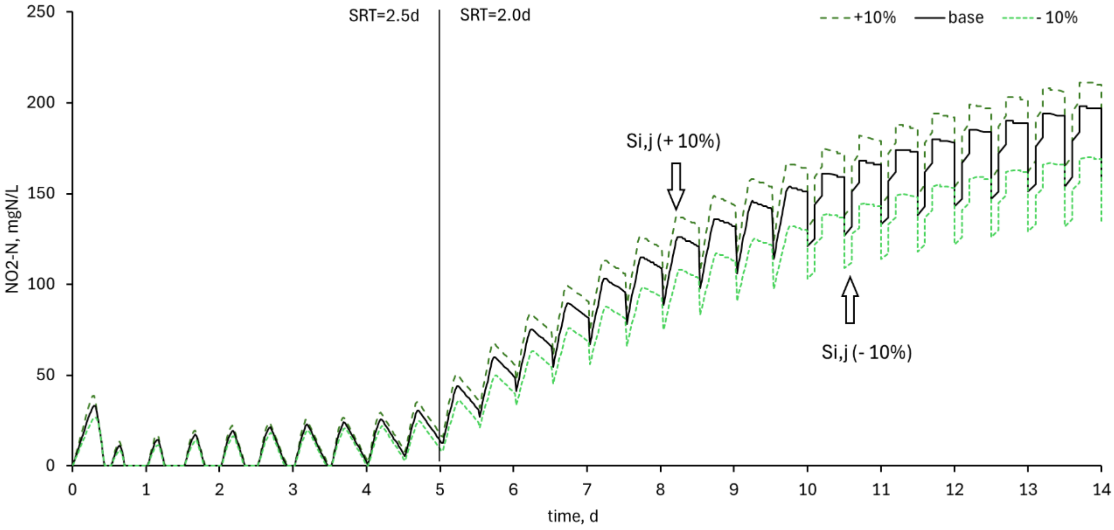


Figure 4.7. AOB growth rate (μ_{AOB}) sensitivity response and changes in predicted NO_2-N concentration.

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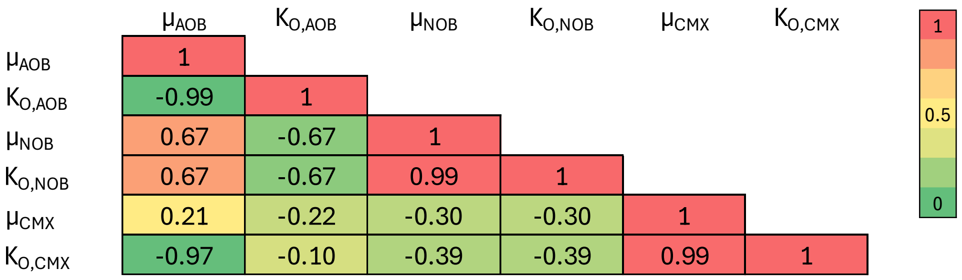

	μ_{AOB}	$K_{O,AOB}$	μ_{NOB}	$K_{O,NOB}$	μ_{CMX}	$K_{O,CMX}$
μ_{AOB}	1					
$K_{O,AOB}$	-0.99	1				
μ_{NOB}	0.67	-0.67	1			
$K_{O,NOB}$	0.67	-0.67	0.99	1		
μ_{CMX}	0.21	-0.22	-0.30	-0.30	1	
$K_{O,CMX}$	-0.97	-0.10	-0.39	-0.39	0.99	1

Figure 4.8. Correlation matrix of the adjusted kinetic parameters for Model “CMX”.

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4.3.2. Model calibration with SBR long-term test data

DO unlimited conditions

Figure 4.9 presents long-term SBR simulation results with Model CMX estimated parameters. Table 4.5 summarizes the Model CMX efficiency (RMSE, MAE, R^2) during the simulations using the extended model with estimated AOB, NOB, and CMX parameters. The extended Model "CMX" consistently outperforms the base model (Model "0") across all metrics and variables. It demonstrates lower RMSE and MAE values, indicating higher accuracy and fewer average prediction errors. The significant improvements in these metrics suggest that Model "CMX" is a more efficient and reliable model for predicting the concentrations of all three nitrogen species. Given the substantial improvements shown by Model "CMX", it is a more reliable tool for predicting concentrations of nitrogen compounds in the studied system.

Table 4.5. Model "CMX" performance metrics during long-term SBR operation.

SBR operational conditions	*Variable*	*RMSE*	*MAE*	R^2
DO=0.5 mgO₂/L *SRT=4.0 d* *T=30˚C*	NH_4-N	11.23	6.58	0.78
	NO_2-N	24.03	577.68	0.92
	NO_3-N	33.06	1092.65	0.92
DO=2.0 mgO₂/L *SRT=2.5/2.0 d* *T=30˚C*	NH_4-N	2.65	2.07	0.89
	NO_2-N	15.49	240.01	0.93
	NO_3-N	12.25	150.07	0.94

The performance metrics suggest that the extended model better predicts nitrogen species concentrations in the SBR under higher DO levels (2.0 mgO_2/L) and shorter SRT. Both RMSE and MAE values are lower under these conditions, while the R^2 values are higher, indicating more accurate and reliable predictions.

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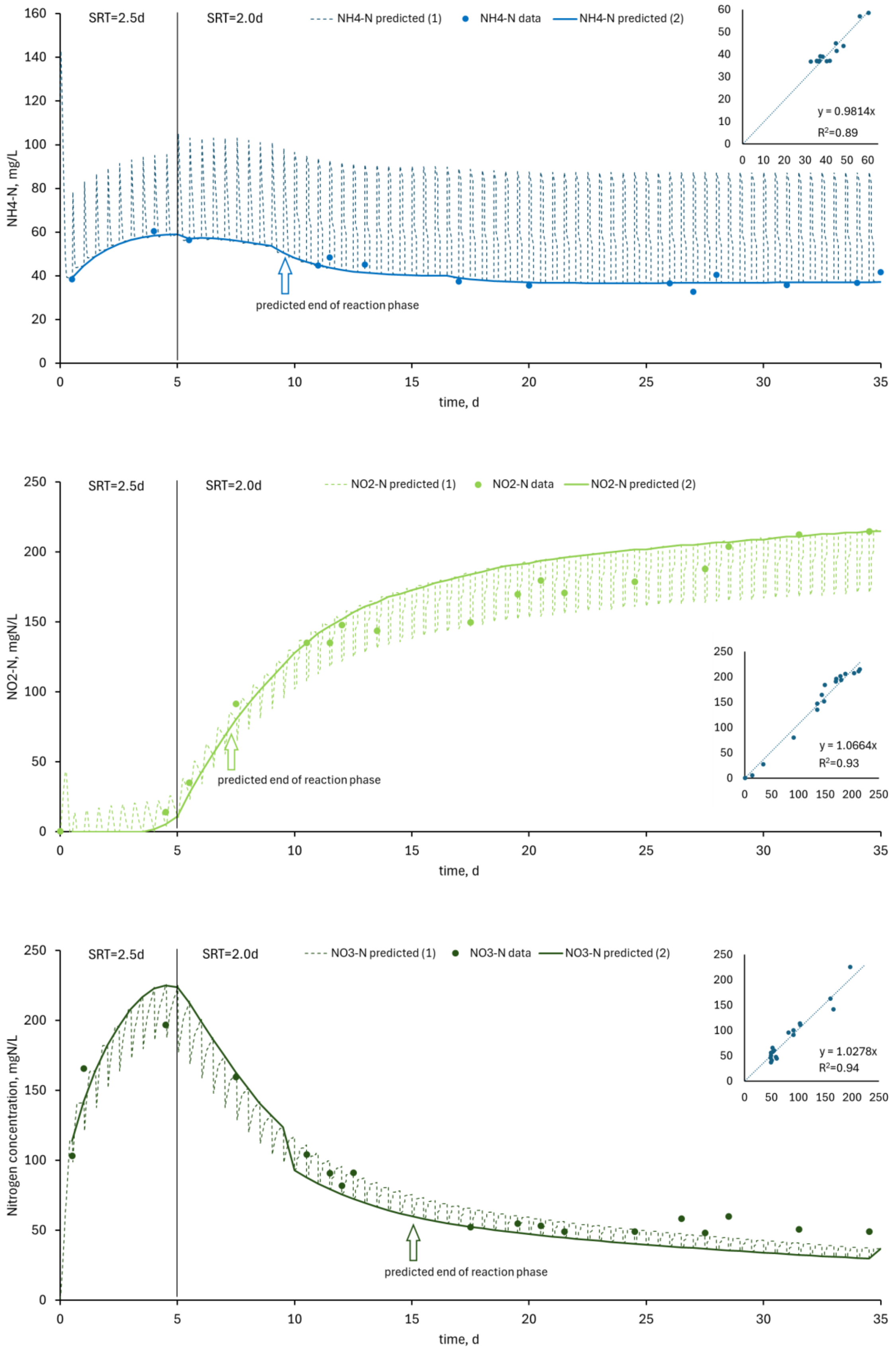

Figure 4.9. Measured and predicted nitrogen concentrations in the long-term washout experiment

DO=2.0 mgO_2/L; influent NH_4-N concentration 282 mgN/L; initial SRT=2.5d.

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DO limited conditions

Figure 4.10 presents Model CMX predicted nitrogen concentrations at limited DO conditions (0.5 mgO_2/L) during a long-term SBR washout experiment. The coefficient of determination (R^2) values for Model "CMX" are higher in all cases, except NH_4-N at DO concentration 0.5 mgO_2/L (R^2 = 0.78), showing that the extended model better captures the variability in the data at higher DO concentrations compared to the base model. Initial fluctuations of NH_4-N concentrations and the sharp increase reflect changes in influent nitrogen loading and indicate a phase where AOB activity is primed, leading to a subsequent decrease in NH_4-N as AOB converts it to NO_2-N. The eventual stabilization in NH_4-N (4 – 26 days of reactor operation) suggests that most of the available ammonium is processed by AOB and Comammox bacteria. The delayed but steady increase in NO_2-N concentration reflects AOB activity reaching a point where significant amounts of nitrite are produced and retained in the system. This rise in NO_2-N is closely followed by an increase in NO_3-N, indicating that NOB is effectively converting NO_2-N to NO_3-N. Nitrate N concentrations rise steadily as both NOB and Comammox bacteria work on oxidizing NO_2-N and NH_4-N (respectively). The peak in NO_3-N concentration around 12 days and the gradual decline thereafter suggest the system is reaching a point where nitrite N oxidation is slowing due to NOB biomass washout, and the only contributing microbial group producing nitrate N is Comammox.

The observed trends in NH_4-N, NO_2-N, and NO_3-N clearly demonstrate the extended model's good performance, with AOB initially increasing NO_2-N concentrations, followed by NOB converting nitrite N to nitrate N. The extended model also predicts the potential for NO_2-N accumulation, where NOB activity is limited, and their biomass is eventually washed out. The concentrations of all three nitrogen species in certain overlapping time frames of the washout test hint at the activity of Comammox bacteria, which can directly convert NH_4-N to NO_3-N, potentially explaining some of the rapid changes in their concentrations. The shift from NH_4-N to NO_2-N and finally to NO_3-N as the dominant nitrogen species over time indicates the temporal shift in dominance of AOB and Comammox. It shows the nature of nitrification, with AOB and NOB sequentially playing their roles, and Comammox bacteria contributing to the overall efficiency of the process. Figure 4.11 shows predicted changes in biomass concentrations (MLSS and MLVSS) and individual nitrifier groups.

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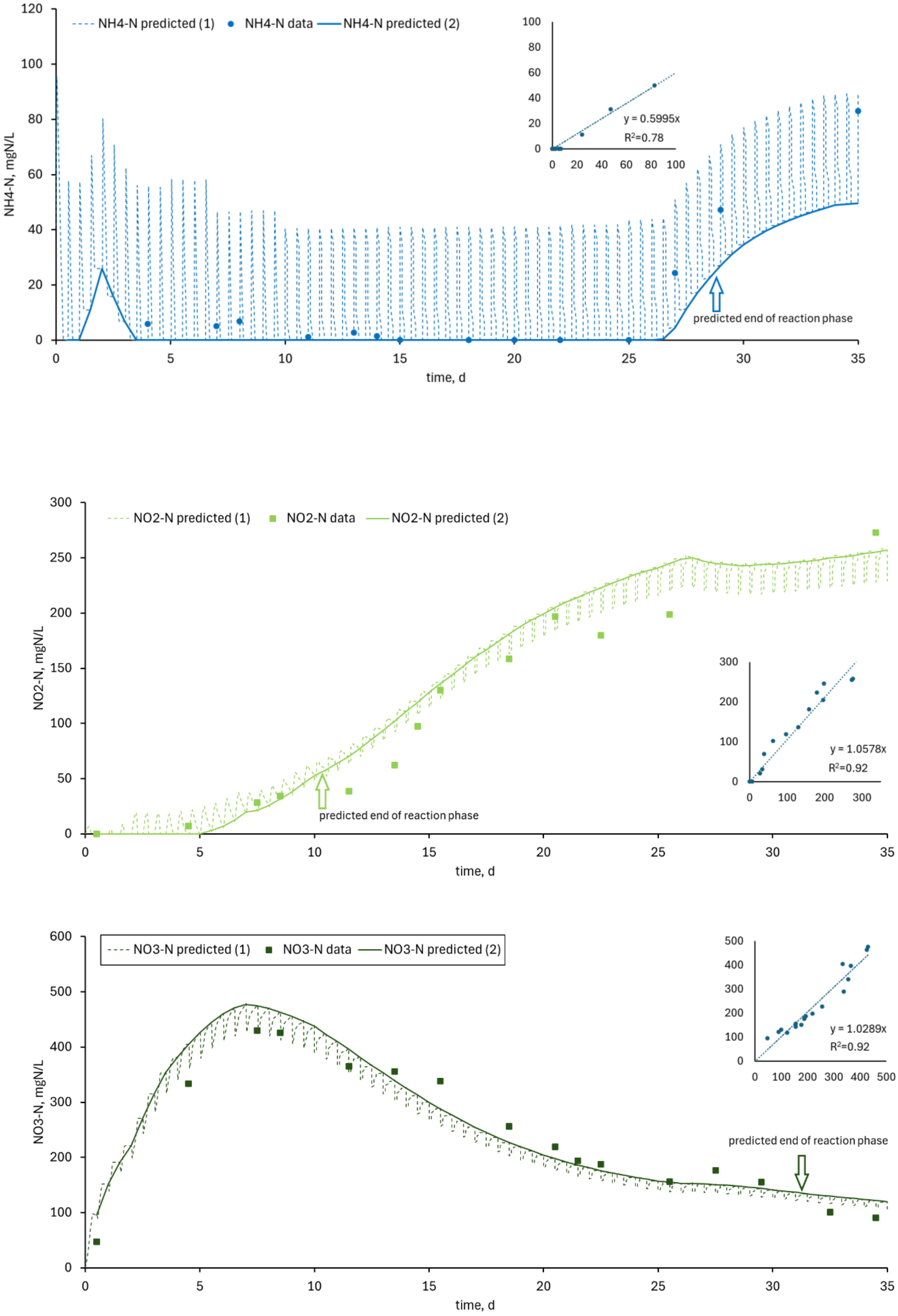

Figure 4.10. Measured and predicted nitrogen concentrations in the long-term washout experiment DO=0.5 mgO$_2$/L; influent NH$_4$-N concentration 410÷550 mgN/L; SRT=4d.

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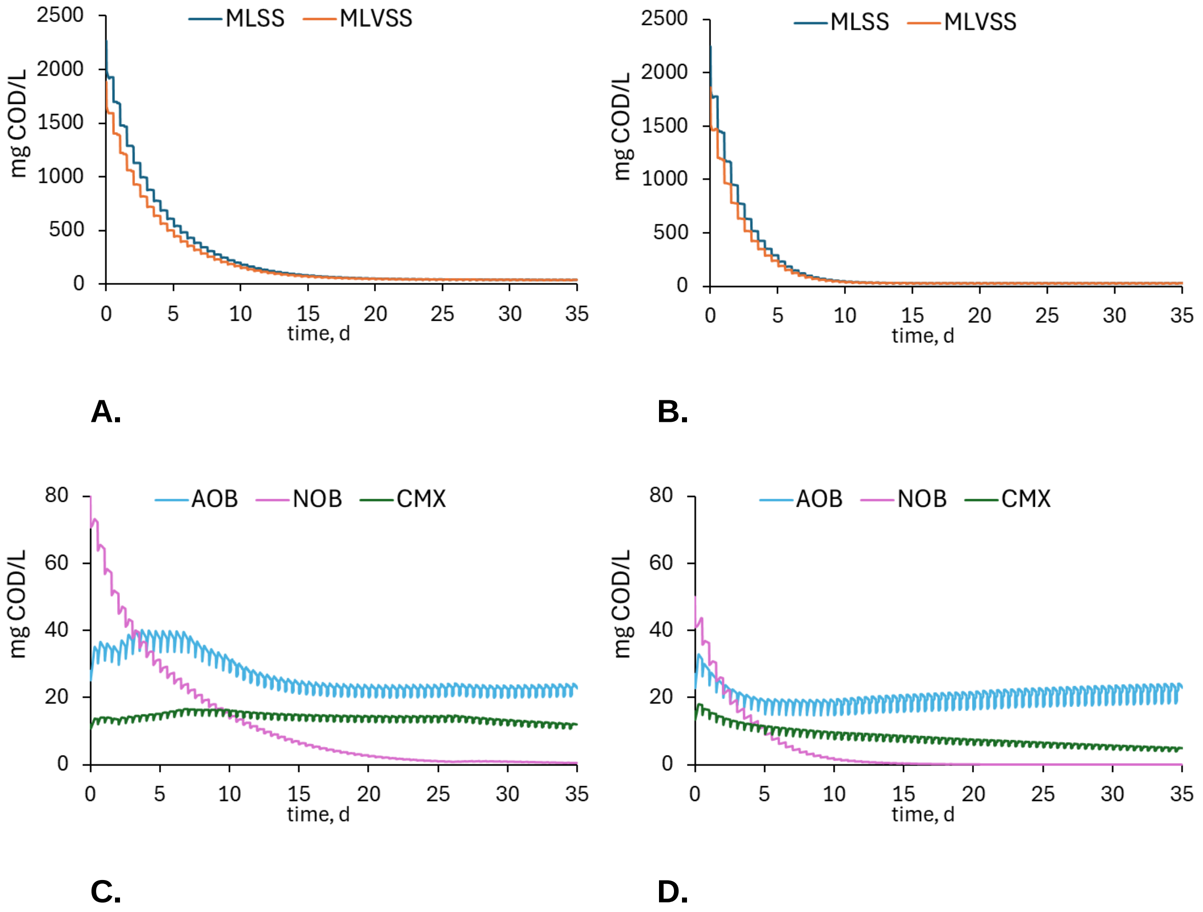

Figure 4.11. Predicted changes in biomass concentrations and nitrifiers groups

A. and C. dynamic simulations at DO=0.5 mg O_2/L, B. and D. dynamic simulations at DO=2.0 mg O_2/L.

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4.3.3. Model validation with batch tests data

Figure 4.12 presents a selection of batch tests measured and predicted nitrogen concentrations during the validation phase at DO concentrations 0.5, 2.0, and 6.0 mgO_2/L with various initial nitrite N concentrations. The overall correlation between each nitrogen component's observed and model-predicted data shows very good model performance with $R^2 > 0.8$ (Table 4.6). The batch test at DO concentration of 6.0 mgO_2/L had the lowest R^2 (0.73) for NO_2-N when compared with ammonia and nitrate N compounds, but still reasonably high, and nitrite N compound has low RMSE and MAE, suggesting that the model performs best in predicting NO_2-N concentrations at DO concentration of 6.0 mgO_2/L. However, its R^2 value is the lowest among the three nitrogen compounds but still indicates a good fit.

Table 4.6. Model “CMX” performance metrics during validation phase.

Batch reactor operational conditions	*Variable*	*RMSE*	*MAE*	R^2
DO=0.5 mgO_2/L *T=25˚C*	NH_4-N	3.88	3.28	0.87
	NO_2-N	1.35	1.84	0.85
	NO_3-N	3.20	10.26	0.93
DO=2.0 mgO_2/L *T=20˚C*	NH_4-N	2.30	1.74	0.97
	NO_2-N	0.64	0.40	0.80
	NO_3-N	1.80	3.25	0.98
DO=6.0 mgO_2/L *T=25˚C*	NH_4-N	3.07	2.29	0.95
	NO_2-N	1.98	3.93	0.73
	NO_3-N	4.28	18.33	0.94

At low DO concentration (0.5 mgO_2/L), the model shows moderate predictive accuracy for NH_4-N, with relatively low RMSE (3.88) and MAE (3.28) and a good R^2 (0.87). This indicates that the model explains approximately 87% of the variance in NH_4-N concentrations. The model performs well for NO_2-N with low error values and a high R^2 (0.85), although slightly less than for NH_4-N, reflecting good predictive capability. However, the model struggles with NO_3-N prediction, as indicated by the high MAE (10.26), though the R^2 (0.93) suggests the model still explains 93% of the variance, highlighting a discrepancy between explained variance and prediction accuracy.

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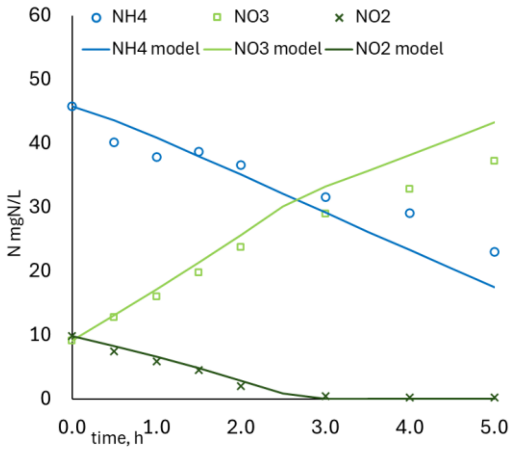
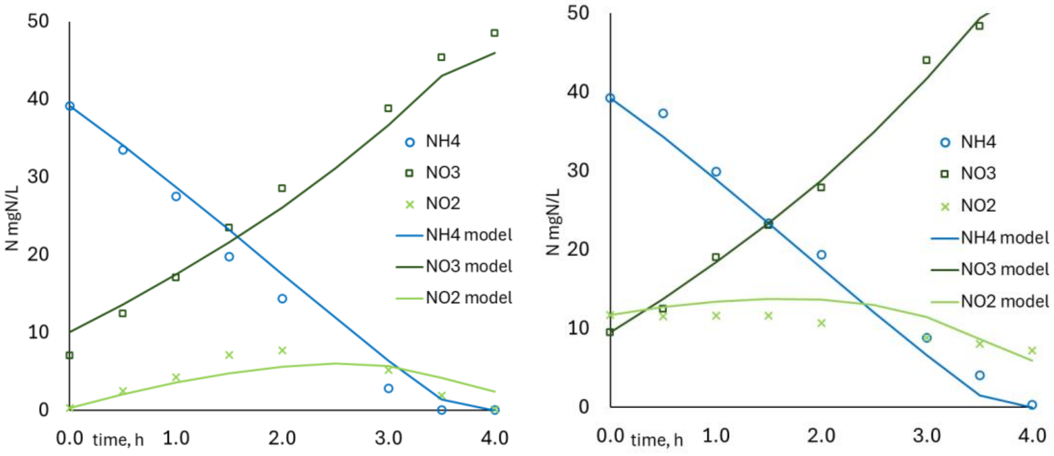


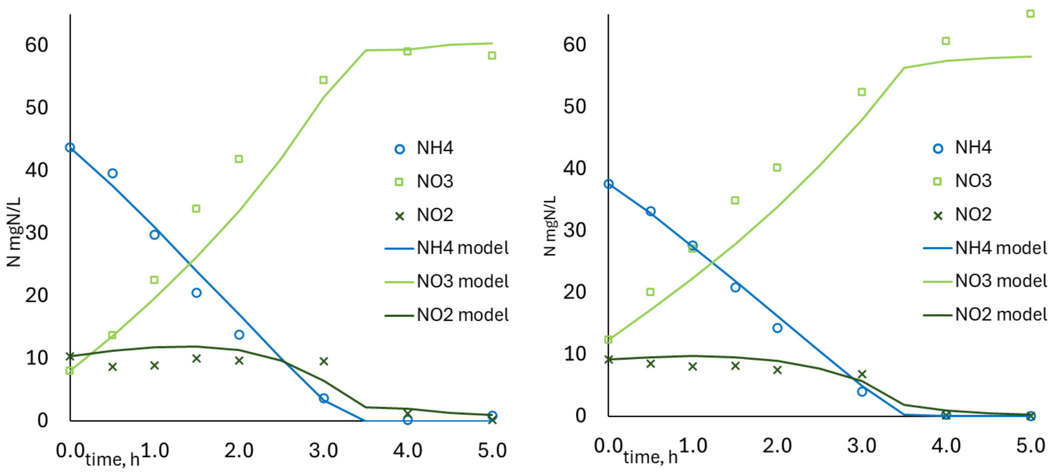
a) DO=0.5 mgO$_2$/L, T=25˚C

b) DO=0.5 mgO$_2$/L, T=25˚C



c) DO=2.0 mgO$_2$/L, T=20˚C

d) DO=2.0 mgO$_2$/L, T=20˚C



e) DO=6 mgO$_2$/L, T=25˚C

f) DO=6 mgO$_2$/L, T=25˚C

Figure 4.12. Measured and predicted nitrogen concentrations in batch tests experiments validation phase at various DO and initial NO_2-N concentrations.

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The model validation under unlimited DO conditions (2.0 mgO_2/L) shows excellent performance for NH_4-N with low RMSE and MAE, and an exceptionally high R^2 (0.97), indicating near-perfect predictive accuracy under these conditions. The extended model demonstrates very low prediction errors for NO_2-N but with a lower R^2 (0.80). It suggests that while the model accurately predicts, on average, it may not fully capture the variability in NO_2-N concentrations. The model's performance for NO_3-N is strong, with very high R^2 (0.98) and relatively low error metrics, indicating a reliable prediction model under these oxygen conditions.

Another validation under unlimited DO conditions (6.0 mgO_2/L) has shown that the extended model maintains high predictive accuracy for NH_4-N, with a slightly higher error than at DO = 2.0 mgO_2/L but still strong overall, as indicated by the high R^2 (0.95). The model's predictive power decreases for NO_2-N, with the lowest R^2 (0.73) observed across all DO conditions. The higher RMSE (1.98) and MAE 3.93) indicate that the model struggles to predict NO2-N concentrations at this DO level accurately. Moreover, the model exhibits the highest error metrics for NO_3-N under evaluated high DO conditions, particularly regarding MAE (18.33). Despite this, the high R^2 suggests that the model still explains a significant proportion of the variance, though the high errors indicate potential issues with prediction accuracy.

The overall validation of Model “CMX” verified that the model’s performance varies under the influence of the SBR’s operational conditions. Intermediate DO concentrations (2.0 mgO_2/L) and lower temperatures (20°C) generally improve predictive accuracy for most nitrogen species, especially NH_4-N and NO_3-N. Higher DO concentrations (6.0 mgO_2/L) at 25°C show an apparent decrease in predictive performance, particularly for NO_2-N and NO_3-N.

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4.4. Model application

The primary goal was to maximize the effluent NO_2-N concentration by optimizing both physical and operational parameters. This study considered two scenarios for model application: constant influent NH_4-N concentration (SBR 1) and variable influent NH_4-N concentration (SBR 2). The optimization variables were the maximum tank volume (V_{max}) and DO concentration. Table 4.7. presents the results of the model-based optimized parameters.

Table 4.7. Physical and operational parameters of the model-based optimized tank reactors.

	T (˚C)	NH_4-N influent concentration	*Initial values*			*Optimized values*		
			DO setpoint (mgO_2/L)	Max. volume V_{max} (m^3)	Effluent NO_2-N (mg N/L)	DO setpoint (mgO_2/L)	Max. volume V_{max} (m^3)	Effluent NO_2-N (mg N/L)
SBR 1	30	*constant*	2.0	10	215	0.68	7.36	253
SBR 2	30	*variable*	0.5	10	256	0.22	3.29	344

Process optimization was performed for a two-stage PN/A process setup, with the first stage being the nitritation reactor and the second stage - the anammox reactor. For SBR1, with constant influent NH_4-N concentration, the optimized V_{max} was 7.36 m^3, 26% lower than the initial volume of 10 m^3. The DO setpoint was reduced from 2.0 mgO_2/L to 0.68 mgO_2/L. These optimized settings resulted in a 15% increase in the effluent NO_2-N concentration, reaching 253 mgN/L.

For SBR2, with variable influent NH_4-N concentration, the optimization of physical (V_{max}) and operational (DO) parameters yielded a higher NO_2-N effluent concentration, where NO_2-N increasing by 25%, reaching 344 mgN/L. The maximum operational volume was reduced significantly to 3.29 m^3, with the DO setpoint reduction to 0.22 mgO_2/L.

In both scenarios, lowering the DO setpoint was crucial for optimizing NO_2-N production. Lower DO concentrations favor the partial nitritation process, enhancing NO_2-N production. Reducing the maximum volume (V_{max}) in both SBRs indicates that a smaller reactor volume can be more effective for nitritation when coupled with optimized DO levels. This suggests a more efficient process with a reduced footprint

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and lower energy requirements for aeration. SBR 2, with variable influent NH_4-N concentrations, achieved a higher effluent NO_2-N concentration than SBR 1. This suggests that the system can adapt more effectively to changes in influent conditions when physical and operational parameters are optimized.

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V. Discussion

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5.1. Influence of DO concentration

The developed CMX Model accurately predicted NH_4-N and NO_2-N concentrations under unlimited DO conditions (2.0 mgO_2/L), although NO_3-N predictions were less accurate. These results align with those of Pérez et al. (2020), who noted that high DO enhances AOB and NOB activities but complicates NO_3-N predictions due to potential shifts in microbial community dynamics. The model's validation through batch tests showed high R^2 values, particularly at higher DO concentrations. This supports the general understanding that higher DO levels favor more consistent nitrification, as reported by Liu et al. (2018), who highlighted the role of sufficient DO (above 2.0 mgO_2/L) in maintaining stable nitrification rates and NO_2-N accumulation. Therefore, achieving successful operation of a partial nitritation treatment system (i.e. the first stage of a two-stage PN/A system configuration) should not only focus on the suppression and washout of the NOB biomass but also supporting the AOB activity. Recent studies of mainstream nitritation in the activated sludge process (Liu et al., 2017) involved high DO concentrations (DO > 1.0 mgO_2/L) and sufficient residual NH_4-N in the bulk liquid. Further studies by Liu *et al.* (2018) under limited and unlimited DO conditions showed that NO_2-N accumulation was not possible without residual NH_4-N present together with sufficient influent ammonium concentrations and was based on the low SRT. The study confirms that selecting fast-growing AOB against NOB is a key strategy for achieving high-rate nitritation, underscoring the practical implications of this research.

The differences observed in NO_2-N and NO_3-N predicted concentrations (Model CMX) under limited DO conditions are consistent with existing literature. Yu et al. (2020) highlighting the challenges of modeling nitrification under low DO conditions. Several studies have shown that traditional nitrification models often underestimate the effects of low DO, leading to inaccurate predictions of intermediate (NO_2-N) and end-product (NO_3-N) concentrations (Liu et al., 2017; Regmi et al., 2014). While the DO limitations and relatively short SRT, i.e. 2.5 – 6.0 d (Regmi *et al.*, 2014; Cao *et al.*, 2017), are the most common methods for the effective NOB suppression, studies carried out under intermittent aeration in SBR at DO concentrations as low as 0.2 mgO_2/L (Roots et al., 2019) showed high NOB activity and extensive NO_2-N oxidation to NO_3-N. Thus, these

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two operational factors may not always be sufficient to suppress NOB activity. Kowal *et al.* (2022) demonstrated that expending two-step nitrification models to simulate novel processes, i.e. partial nitrification in the presence of comammox microorganisms, must include the resistance of comammox bacteria for the biomass washout conditions. They suggested that the expended two-step nitrification model should incorporate two parallel pathways of nitrite oxidation to nitrate, catalyzed by two groups of NOB (e.g. *Nitrospira* or *Ca. Nitrotoga*), and a one-step pathway of the direct ammonia oxidation to nitrate by comammox *Nitrospira*.

A study by Shourjeh *et al.* (2020) provided insights into the interaction between temperature and DO concentration, showing that even a lower abundance of NOB (compared to AOB) in the total autotrophic bacterial community can lead to predominant NOB activity at increased temperatures (~30˚C), making the DO limitation more suitable for the NOB suppression. These key findings confirm the Model CMX accuracy of predicted NO_2-N and NO_3-N concentrations at limited oxygen conditions (DO=0.5 mgO_2/L) and good model fit to observed data, compared to Model "0" predicted nitrite and nitrate concentrations. Limited DO availability resulted in a lower accuracy (Model CMX) of the predicted NH_4-N concentrations compared to unlimited oxygen conditions (DO=2.0 mgO_2/L), where long-term experimental data R^2 value was 0.78 and 0.89, respectively.

5.2. Influential model parameters

The application of local sensitivity analysis (LSA) and the calibration methodology enabled a comparison between the nitrogen concentrations simulated by Model "0" and Model CMX. Most importantly, the correlation matrix identified the most influential kinetic parameters (μ_{AOB}, μ_{CMX}, and $K_{O,NOB}$) critical for model calibration and comparison with the observed data. This identification revealed which observed data are more beneficial for model calibration, thus guiding modeling efforts. For example, the higher sensitivity of AOB and CMX growth rates over NOB growth rates for NO_2-N is significant. In microbial competition modeling, growth kinetics and substrate affinities are crucial for understanding substrate competition and population shift (Kirim et al., 2022; Kits et al., 2017). The growth kinetics of nitrifying microorganisms are substrate-limited based on Monod's formulation used in the ASMs, which states a fixed relation between growth rate and bulk substrate concentration (Henze et al., 2008). Thus, the

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substrate half-saturation (affinity) constant is crucial for process performance. However, according to Arnaldos *et al.* (2015), model calibration may lump the effects of different phenomena in the system (e.g. mixing, advection, and diffusion limitations), potentially leading to variation in the apparent values of the half-saturation constants and affecting the prediction power of the calibrated model.

Calibration results of Model CMX under unlimited DO conditions (2.0 mgO_2/L) for the maximum specific growth rates for AOB (μ_{AOB}) and NOB (μ_{NOB}) were within the reported literature range for two-step nitrification models (Mehrani et al., 2022b, 2021; Mei et al., 2023). The adjusted values of $K_{O,AOB}$ (0.24 mgO_2/L) and $K_{O,NOB}$ (0.1 mgO_2/L) were within the literature ranges of 0.16–1.22 mgO_2/L for canonical r-AOB *Nitrosomonas* (Mehrani et al., 2022b; D. Zhang et al., 2019) and 0.17–4.32 mgO_2/L for canonical r-NOB *Nitrospira* (Park *et al.*, 2017; Mei *et al.*, 2023). Although the DO half-saturation constant for NOB was slightly lower than the reported range, indicating a very high oxygen affinity of this bacterial group throughout the long-term experiment, it also suggested reduced capabilities of NO_2-N oxidation at low DO concentrations.

For comammox, calibrated kinetic parameters (Table 4.4) showed that the maximum specific growth rate (μ_{CMX}) and oxygen half-saturation constant ($K_{O,CMX}$) were above the reported literature values for temperatures ranging from 17-22˚C. The estimated μ_{CMX} (0.82 d^{-1}) was higher than the value reported by Park et al. (2017) for enriched *Nitrospira* culture and above the reported range of 0.15–0.22 reported by Mehrani *et al*. (2021). It is important to note that only the study by Mehrani *et al*. (2021) separated different NH_4-N oxidation pathways, distinguishing between one-step NH_4-N oxidation to NO_3-N (without NO_2-N released), two-step NH_4-N and NO_2-N oxidation, and two-step parallel pathway of NH_4-N and NO_2-N oxidation catalyzed by AOB, NOB, and comammox. The estimated $K_{O,CMX}$ (0.61 mgO_2/L) was above the literature range for enriched *Nitrospira* culture (0.33 mgO_2/L, T=22˚C), and above the adjusted value (0.3 mgO_2/L) from the study by Mehrani et al. (2022) at T=20˚C and DO=0.6 mgO_2/L.

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5.3. Extension of two-step nitrification model with comammox

The new CMX Model developed and validated in this study was successfully applied to simulate the start-up phase and long-term SBR operation under both limited and unlimited DO conditions with varying influent NH_4-N concentrations. The model was used to simulate competition between AOB, NOB and comammox bacteria found in unacclimatized inoculum biomass from a municipal WWTP. Extending the two-step nitrification model to include complete ammonia oxidizer (one-step oxidation of NH_4-N to NO_3-N) increased the prediction accuracy of the studied partial nitrification system. The simulation results of the present study support the hypothesis that comammox bacteria tend to grow on NH_4-N as it appears to be a preferable substrate (Kowal et al., 2022; Mehrani et al., 2022b). However, since the discovery of comammox, it is still ongoing debate if comammox *Nitrospira* possess the ability to grow on NO_2-N in the absence of NH_4-N (Koch et al., 2019).

Additionally, simulation results indicate that the two-step nitrification model, extended with the comammox pathway of direct NH_4-N oxidation to NO_3-N, can better predict residual NO_3-N concentrations measured in long-term SBR experiments. It should be noted that during the start-up phase of both reactors, i.e. 4.5 days for DO=2.0 mgO_2/L and 7 days for DO=0.5 mgO_2/L, complete nitrification was performed by AOB, NOB and comammox together. The SRT played a crucial role in influencing the competition between AOB and NOB. During the start-up phase, the SBR operated with DO=2.0 mgO_2/L and a longer SRT (2.5 d). Subsequently, the SBR continued its operation with a reduced SRT of 2.0 days, resulting in a rapid increase in NO_2-N concentration and a parallel reduction in NO_3-N concentration.

The simulation results by the extended model were more accurate and stable in explaining the nitrification process in the presence of three nitrifying microorganism groups. Despite the variability in reported kinetic parameters (both measured and calibrated) and different concepts of the comammox process found in the literature (Kowal et al., 2022; Mehrani et al., 2021; Mei et al., 2023), the consistency and robustness of the ASM1, extended with comammox oxidation of NH_4-N to NO_3-N, proved its strength in terms of predicting nitrogen conversions.

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5.4. Innovation and impact of the research on wastewater engineering

Since the discovery of comammox by Daims et al. (2015), most of the research focused on the biological aspects of this process, with particular attention given to ecosystems, such as soil (Kits et al., 2017; Pjevac et al., 2017; Koch et al., 2019) and freshwater systems (Bartelme et al., 2017), rather than engineered systems, such as WWTPs. In WWTP studies, efforts have focused on metagenomics and novel PCR assays to identify comammox *Nitrospira* (Roots et al., 2019; Kowal et al., 2022) and explore their biological mechanisms, rather than developing kinetic models based on mechanistic, data-driven, or hybrid approaches.

The key novelty of this study lies in incorporating comammox into wastewater treatment modeling and using such a model to explain the influence of comammox on the nitritation process, particularly its impact on NO_2-N accumulation in nitritation tank reactors. The major innovation presented is the adaptation of the ASM framework to develop a process modelling strategy that addresses the limitations of current mechanistic models for side-stream wastewater treatment and NO_2-N prediction. As these models become increasingly complex, challenges in model validation and full-scale application become evident, highlighting the need for advanced approaches like the one proposed in this study.

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VI. Conclusions

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6. Conclusions

This study provides several key insights into the complex dynamics of nitrification processes in wastewater treatment systems, offering important conclusions and final remarks:

1. The study demonstrated the critical role of DO concentration in determining the accuracy of two-step nitrification model predictions for NH_4-N, NO_2-N, and NO_3-N concentrations, especially in the presence of comammox bacteria. Higher DO levels facilitated more consistent nitritation by enhancing AOB activity. On the contrary, low DO conditions present challenges in accurately modelling the nitritation process, underscoring the complexity of nitrification under varying DO conditions.

2. The presence of comammox *Nitrospira* in the nitrification process was shown to play a significant role in nitrification, requiring the incorporation of several process variables for successful model validation and application. This finding emphasizes the need for accounting the distinct pathways of comammox bacteria, particularly under low DO conditions, to improve the accuracy of model predictions.

3. The study underscored the challenges in modelling two-step nitrification under low DO conditions and with variable influent NH_4-N concentrations, particularly during the start-up phase of nitritation reactors. Traditional nitrification models often underestimate the effects of low DO, leading to inaccurate predictions of intermediate (NO_2-N) and final product (NO_3-N) concentrations. This highlights the urgent need for improved models to better predict nitrification dynamics, especially in treating ammonia-rich wastewater, e.g., anaerobic sludge digester liquors.

4. The study emphasized the importance of local sensitivity analysis and model calibration for identifying the most influential kinetic parameters to achieve accurate predictions of nitrogen concentrations. The AOB group was the most critical contributor to nitrification, even in the presence of comammox bacteria.

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However, the initial biomass concentration of individual nitrifiers, as confirmed by microbiological analysis, proved to be a crucial factor that should not be overlooked in successful model calibration and validation. This study provides valuable new insights into modelling the complex dynamics of nitrification processes under varying substrate concentrations, with significant implications for optimizing the operation of wastewater treatment systems and improving modeling approaches. The findings underscore the practical relevance and applicability of incorporating comammox pathways.

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VII. Future perspectives

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7. Future perspectives

Future research should explore novel approaches for integrating the influence of DO on microbial competition into two-step nitrification modeling, including developing advanced models that accurately capture the effects of comammox pathways under low DO conditions. Additionally, there is a need for continued investigations into practical strategies for enhancing AOB activity and suppressing NOB in wastewater treatment systems, aiming to improve the efficiency and reliability of nitrification processes under diverse operational conditions.

Further exploration of microbial community dynamics and substrate competition in nitrification processes will provide valuable insights for advancing modeling and operational practices in environmental engineering. Extending the two-step nitrification model with comammox should also include pathways for N_2O production and emission. Incorporating heterotrophic denitrification and NH_2OH oxidation into the model may help to identify the dominant N_2O emission pathway during wastewater treatment in the nitritation process under different DO conditions. This will help develop and design the two-stage PN/A treatment system for ammonia-rich wastewater with minimized GHG emissions and optimized energy requirements for aeration.

However, before incorporating new model concepts, such as comammox nitrogen oxidation pathways in nitrification modelling, a comprehensive microbial structure analysis and evaluation should be conducted under various environmental conditions (e.g. DO and SRT). Most recently, it was emphasized by Wang et al. (2021) and Mei et al. (2023) that the microbial relative abundance of *Nitrospira* should be included in kinetics and modelling studies of the wastewater treatment process in the presence of comammox. Quantitative polymerase chain reaction (qPCR) can be effectively applied to quantify the nitrifiers, such as *Nitrosomonas* AOB, *Nitrospira* NOB, *Nitrobacter* NOB, and comammox *Nitrospira* in the nitrifying activated sludge. This can be followed by a high-throughput sequencing to investigate bacterial community structures.

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As highlighted by Kowal et al. (2022), NO_2-N production pathways, driven by canonical AOB, are distinct from the more diverse NO_3-N production pathways, which may include the metabolism of two different canonical NOBs (*Nitrospira* and *Ca. Nitrotoga*). While mathematical modelling is an essential tool for kinetic studies in wastewater treatment systems, it should be led by the microbiological analysis of the nitrifying microbial population.

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VIII. References

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Al-Hazmi, H., Derwis, D., Majtacz, J., Ziembińska-Buczyńska, A., Zhai, J., Makinia, J., 2022. Combined Partial Denitrification/Anammox Process for Nitrogen Removal in Wastewater Treatment. Journal of Environmental Chemical Engineering. https://doi.org/10.1016/j.jece.2022.108978

Al-Omari, A., Wett, B., Nopens, I., Clippeleir, H.D., Han, M., Regmi, P., Bott, C., Murthy, S., 2015. Model-based evaluation of mechanisms and benefits of mainstream shortcut nitrogen removal processes. Water Science and Technology 71, 840–847. https://doi.org/10.2166/wst.2015.022

Anthonisen, A.C., Loehr, R.C., Prakasam, T.B.S., Srinath, E.G., 1976. Inhibition of Nitrification by Ammonia and Nitrous Acid. Journal (Water Pollution Control Federation) 48, 835–852.

Antoniou, P., Hamilton, J., Koopman, B., Jain, R., Holloway, B., Lyberatos, G., Svoronos, S., 1990. Effect of temperature and pH on the effective maximum specific growth rate of nitrifying bacteria. Water Research 24, 97–101. https://doi.org/10.1016/0043-1354(90)90070-M

APHA (Ed.), 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association.

Ardern, E., Lockett, W.T., 1914. Experiments on the oxidation of sewage without the aid of filters. Journal of the society of chemical industry 33, 523–539.

Argaman, Y., Adams, C., 1977. COMPREHENSIVE TEMPERATURE MODEL FOR AERATED BIOLOGICAL-SYSTEMS. Progress in Water Technology 9, 397.

Arnaldos, M., Amerlinck, Y., Rehman, U., Maere, T., Hoey, S., Naessens, W., Nopens, I., 2015. From the affinity constant to the half-saturation index: Understanding conventional modeling concepts in novel wastewater treatment processes. Water Research 70, 458–470. https://doi.org/10.1016/j.watres.2014.11.046

Balakrishnan, S., Eckenfelder, W.W., 1970. Nitrogen Removal by Modified Activated Sludge Process. J. Sanit. Engrg. Div. 96, 501–512. https://doi.org/10.1061/JSEDAI.0001090

Barnard, J.L., 1975. Biological nutrient removal without the addition of chemicals. Water Research 9, 485–490.

Bartelme, R., Mclellan, S., Newton, R., 2017. Freshwater Recirculating Aquaculture System Operations Drive Biofilter Bacterial Community Shifts around a Stable Nitrifying Consortium of Ammonia-Oxidizing Archaea and Comammox Nitrospira. Frontiers in Microbiology 8. https://doi.org/10.3389/fmicb.2017.00101

Blackburne, R., Yuan, Z., Keller, J., 2008. Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. Biodegradation 19, 303–312. https://doi.org/10.1007/s10532-007-9136-4

Brdjanovic, D., 2000. Modeling COD, N and P removal in a full-scale wwtp Haarlem Waarderpolder. Water Research 34, 846–858. https://doi.org/10.1016/S0043-1354(99)00219-5

Brun, R., Kühni, M., Siegrist, H., Gujer, W., Reichert, P., 2002. Practical Identifiability of ASM2d Parameters—Systematic Selection and Tuning of Parameter Subsets. Water research 36, 4113–27. https://doi.org/10.1016/S0043-1354(02)00104-5

Cao, J., Zhang, T., Wu, Y., Sun, Y., Zhang, Y., Huang, B., Fu, B., Yang, E., Zhang, Q., Luo, J., 2020. Correlations of nitrogen removal and core functional genera in full-scale wastewater treatment plants: Influences of different treatment processes and influent characteristics. Bioresource Technology 297, 122455. https://doi.org/10.1016/j.biortech.2019.122455

Cao, Y., van Loosdrecht, M.C.M., Daigger, G.T., 2017. Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies. Appl Microbiol Biotechnol 101, 1365–1383. https://doi.org/10.1007/s00253-016-8058-7

Carrera, J., Jubany, I., Carvallo, L., Chamy, R., Lafuente, J., 2004. Kinetic models for nitrification inhibition by ammonium and nitrite in a suspended and an immobilised biomass systems. Process Biochemistry 39, 1159–1165. https://doi.org/10.1016/S0032-9592(03)00214-0

Chandran, K., Hu, Z., Smets, B.F., 2008. A critical comparison of extant batch respirometric and substrate depletion assays for estimation of nitrification biokinetics. Biotechnology and Bioengineering 101, 62–72. https://doi.org/10.1002/bit.21871

Chandran, K., Smets, B.F., 2005. Optimizing experimental design to estimate ammonia and nitrite oxidation biokinetic parameters from batch respirograms. Water Research 39, 4969–4978. https://doi.org/10.1016/j.watres.2005.10.001

Chandran, K., Smets, B.F., 2000. Applicability of two-step models in estimating nitrification kinetics from batch respirograms under different relative dynamics of ammonia and nitrite oxidation. Biotechnology and Bioengineering 70, 54–64. https://doi.org/10.1002/1097-0290(20001005)70:1<54::AID-BIT7>3.3.CO;2-8

Chandran, K., Stein, L.Y., Klotz, M.G., Loosdrecht, M.C.M. van, 2011. Nitrous oxide production by lithotrophic ammonia-oxidizing bacteria and implications for engineered nitrogen-removal systems. Biochemical Society Transactions 39, 1832–1837. https://doi.org/10.1042/BST20110717

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Chen, G., Lin, L., Wang, Y., Zhang, Z., Cao, W., Zhang, Y., 2023. Unveiling the interaction mechanisms of key functional microorganisms in the partial denitrification-anammox process induced by COD. Front. Environ. Sci. Eng. 17, 103. https://doi.org/10.1007/s11783-023-1703-3

Chen, H., Liu, K., Yang, E., Chen, J., Gu, Y., Wu, S., Yang, M., Wang, H., Wang, D., Li, H., 2023. A critical review on microbial ecology in the novel biological nitrogen removal process: Dynamic balance of complex functional microbes for nitrogen removal. Science of The Total Environment 857, 159462. https://doi.org/10.1016/j.scitotenv.2022.159462

Chen, P., Li, J., Li, Q.X., Wang, Y., Li, S., Ren, T., Wang, L., 2012. Simultaneous heterotrophic nitrification and aerobic denitrification by bacterium *Rhodococcus* sp. CPZ24. Bioresource Technology 116, 266–270. https://doi.org/10.1016/j.biortech.2012.02.050

Choi, D., Cho, K., Jung, J., 2019. Optimization of nitrogen removal performance in a single-stage SBR based on partial nitritation and ANAMMOX. Water Research 162, 105–114. https://doi.org/10.1016/j.watres.2019.06.044

Christensson, M., Ekström, S., Chan, A.A., Vaillant, E.L., Lemaire, R., 2013. Experience from start-ups of the first ANITA Mox Plants. Water Science and Technology 67, 2677–2684. https://doi.org/10.2166/wst.2013.156

Ciudad, G., Rubilar, O., Muñoz, P., Ruiz, G., Chamy, R., Vergara, C., Jeison, D., 2005. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. Process Biochemistry 40, 1715–1719. https://doi.org/10.1016/j.procbio.2004.06.058

Costa, E., Pérez, J., Kreft, J.-U., 2006. Why is metabolic labour divided in nitrification? Trends in Microbiology 14, 213–219. https://doi.org/10.1016/j.tim.2006.03.006

Cua, L.S., Stein, L.Y., 2011. Effects of nitrite on ammonia-oxidizing activity and gene regulation in three ammonia-oxidizing bacteria. FEMS Microbiology Letters 319, 169–175. https://doi.org/10.1111/j.1574-6968.2011.02277.x

Daims, H., Lebedeva, E.V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R.H., Bergen, M. von, Rattei, T., Bendinger, B., Nielsen, P.H., Wagner, M., 2015. Complete nitrification by *Nitrospira* bacteria. Nature 528, nature16461. https://doi.org/10.1038/nature16461

Daims, H., Lücker, S., Le Paslier, D., Wagner, M., 2011. Diversity, Environmental Genomics, and Ecophysiology of Nitrite-Oxidizing Bacteria. https://doi.org/10.2166/9781789064742_ch12

Daims, H., Lucker, S., Wagner, M., 2016. A New Perspective on Microbes Formerly Known as Nitrite-Oxidizing Bacteria. Trends in Microbiology 24, 699–712. https://doi.org/10.1016/j.tim.2016.05.004

Daims, H., Taylor, M., Wagner, M., 2006. Wastewater treatment: a model system for microbial ecology. Trends in Biotechnology 24, 483–489. https://doi.org/10.1016/j.tibtech.2006.09.002

Daims, H., Wagner, M., 2018. Nitrospira. Trends in Microbiology 26. https://doi.org/10.1016/j.tim.2018.02.001

Dold, P., Ekama, G., Marais, G., 1980. The Activated Sludge Process 1. A General Model For Activated Sludge Process. Progress in Water Technology 12, 47–77.

Dongen, U., Jetten, M., Loosdrecht, M., 2001. The SHARON((R))-Anammox((R)) process for treatment of ammonium rich wastewater. Water science and technology : a journal of the International Association on Water Pollution Research 44, 153–60. https://doi.org/10.2166/wst.2001.0037

Downing, A.L., Hopwood, A.P., 1964. Some observations on the kinetics of nitrifying activated-sludge plants. Schweizerische Zeitschrift für Hydrologie 26, 271–288.

Duan, H., Watts, S., Zheng, M., Wang, Z., Zhao, J., Li, H., Liu, P., Dwyer, J., McPhee, P., Rattier, M., Larsen, E., Yuan, Z., Hu, S., 2022. Achieving robust mainstream nitrite shunt at pilot-scale with integrated sidestream sludge treatment and step-feed. Water Research 223, 119034. https://doi.org/10.1016/j.watres.2022.119034

Dumont, M., Godon, J.-J., Harmand, J., 2016. Species Coexistence in Nitrifying Chemostats: A Model of Microbial Interactions. Processes 4, 51. https://doi.org/10.3390/pr4040051

Dytczak, M.A., Londry, K.L., Oleszkiewicz, J.A., 2008. Activated sludge operational regime has significant impact on the type of nitrifying community and its nitrification rates. Water Research 42, 2320–2328. https://doi.org/10.1016/j.watres.2007.12.018

Ekama, G.A., Takacs, I., 2014. Modeling. Iwa Publishing, London.

Fan, A.M., 2011. Nitrate and Nitrite in Drinking Water: A Toxicological Review, in: Nriagu, J.O. (Ed.), Encyclopedia of Environmental Health. Elsevier, Burlington, pp. 137–145. https://doi.org/10.1016/B978-0-444-52272-6.00563-8

Fang, F., Ni, B.-J., Li, X.-Y., Sheng, G.-P., Yu, H.-Q., 2009. Kinetic analysis on the two-step processes of AOB and NOB in aerobic nitrifying granules. Appl Microbiol Biotechnol 83, 1159–1169. https://doi.org/10.1007/s00253-009-2011-y

MOST WIEDZY Downloaded from mostwiedzy.pl

Fu, X., Hou, R., Yang, P., Qian, S., Feng, Z., Chen, Z., Wang, F., Yuan, R., Chen, H., Zhou, B., 2022. Application of external carbon source in heterotrophic denitrification of domestic sewage: A review. Science of The Total Environment 817, 153061. https://doi.org/10.1016/j.scitotenv.2022.153061

Fux, C., Boehler, M., Huber, P., Brunner, I., Siegrist, H., 2002. Biological treatment of ammonium-rich wastewater by partial nitritation and subsequent anaerobic ammonium oxidation (anammox) in a pilot plant. Journal of Biotechnology 99, 295–306. https://doi.org/10.1016/S0168-1656(02)00220-1

Fux, C., Huang, D., Monti, A., Siegrist, H., 2004. Difficulties in maintaining long-term partial nitritation of ammonium-rich sludge digester liquids in a moving-bed biofilm reactor (MBBR). Water Sci. Technol. 49, 53–60.

Gabarró, J., Ganigué, R., Gich, F., Ruscalleda, M., Balaguer, M.D., Colprim, J., 2012. Effect of temperature on AOB activity of a partial nitritation SBR treating landfill leachate with extremely high nitrogen concentration. Bioresource Technology 126, 283–289. https://doi.org/10.1016/j.biortech.2012.09.011

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vöosmarty, C.J., 2004. Nitrogen Cycles: Past, Present, and Future. Biogeochemistry 70, 153–226. https://doi.org/10.1007/s10533-004-0370-0

Galloway, J.N., Winiwarter, W., Leip, A., Leach, A.M., Bleeker, A., Erisman, J.W., 2014. Nitrogen footprints: past, present and future. Environmental Research Letters 9, 115003. https://doi.org/10.1088/1748-9326/9/11/115003

Garrido, J.M., van Benthum, W.A.J., van Loosdrecht, M.C.M., Heijnen, J.J., 1997. Influence of dissolved oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. Biotechnology and Bioengineering 53, 168–178. https://doi.org/10.1002/(SICI)1097-0290(19970120)53:2<168::AID-BIT6>3.0.CO;2-M

Ge, S., Wang, S., Yang, X., Qiu, S., Li, B., Peng, Y., 2015. Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: A review. Chemosphere, Wastewater-Energy Nexus: Towards Sustainable Wastewater Reclamation 140, 85–98. https://doi.org/10.1016/j.chemosphere.2015.02.004

Gernaey, K.V., van Loosdrecht, M.C.M., Henze, M., Lind, M., Jørgensen, S.B., 2004. Activated sludge wastewater treatment plant modelling and simulation: state of the art. Environmental Modelling & Software 19, 763–783. https://doi.org/10.1016/j.envsoft.2003.03.005

Giusti, E., Marsili-Libelli, S., Spagni, A., 2011. Modelling microbial population dynamics in nitritation processes. Environmental Modelling & Software 26, 938–949. https://doi.org/10.1016/j.envsoft.2011.02.001

Griffin, J.S., Wells, G.F., 2017. Regional synchrony in full-scale activated sludge bioreactors due to deterministic microbial community assembly. The ISME Journal 11, 500–511. https://doi.org/10.1038/ismej.2016.121

Gruber, N., 2004. The Dynamics of the Marine Nitrogen Cycle and its Influence on Atmospheric CO2 Variations. https://doi.org/10.1007/978-1-4020-2087-2_4

Gruber, N., Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature 451, 293–296. https://doi.org/10.1038/nature06592

Grunditz, C., Dalhammar, G., 2001. Development of nitrification inhibition assays using pure cultures of Nitrosomonas and Nitrobacter. Water research 35, 433–40. https://doi.org/10.1016/S0043-1354(00)00312-2

Guisasola, A., Petzet, S., Baeza, J.A., Carrera, J., Lafuente, J., 2007. Inorganic carbon limitations on nitrification: Experimental assessment and modelling. Water Research 41, 277–286. https://doi.org/10.1016/j.watres.2006.10.030

Guo, J., Peng, Y., Huang, H., 2010. Short- and long-term effects of temperature on partial nitrification in a sequencing batch reactor treating domestic wastewater. Journal of Hazardous Materials 179, 471–479. https://doi.org/10.1016/j.jhazmat.2010.03.027

Gustavsson, D.J.I., Suarez, C., Wilén, B.-M., Hermansson, M., Persson, F., 2020. Long-term stability of partial nitritation-anammox for treatment of municipal wastewater in a moving bed biofilm reactor pilot system. Science of The Total Environment 714, 136342. https://doi.org/10.1016/j.scitotenv.2019.136342

Hanaki, K., Wantawin, C., Ohgaki, S., 1990. Nitrification at low levels of dissolved oxygen with and without organic loading in a suspended-growth reactor. Water Research 24, 297–302. https://doi.org/10.1016/0043-1354(90)90004-P

MOST WIEDZY Downloaded from mostwiedzy.pl

Hawkins, S., Robinson, K., Layton, A., Sayler, G., 2010. Limited impact of free ammonia on Nitrobacter spp. inhibition assessed by chemical and molecular techniques. Bioresource Technology 101, 4513–4519. https://doi.org/10.1016/j.biortech.2010.01.090

Hellinga, C., Loosdrecht, M.C.M. van, Heijnen, J.J., 1999. Model Based Design of a Novel Process for Nitrogen Removal from Concentrated Flows. Mathematical and Computer Modelling of Dynamical Systems 5, 351–371. https://doi.org/10.1076/mcmd.5.4.351.3678

Hellinga, C., Schellen, A., Mulder, J., Vanloosdrecht, M., Heijnen, J., 1998. The sharon process: An innovative method for nitrogen removal from ammonium-rich waste water. Water Science and Technology 37, 135–142. https://doi.org/10.1016/S0273-1223(98)00281-9

Henze, M., 1992. Characterization of Wastewater for Modelling of Activated Sludge Processes. Water Science and Technology 25, 1–15.

Henze, M., Grady, C.P.L., Gujer, W., Marais, G.V.R., Matsuo, T., 1987. A general model for single-sludge wastewater treatment systems. Water Research 21, 505–515. https://doi.org/10.1016/0043-1354(87)90058-3

Henze, M., Gujer, W., Mino, T., Loosdrecht, M., 2000. Activated Sludge Models ASM1, ASM2, ASM2D, ASM3, Water Intelligence Online. https://doi.org/10.2166/9781780402369

Henze, M., Harremoes, P., Jansen, J. la C., Arvin, E., 2001. Wastewater Treatment: Biological and Chemical Processes. Springer Science & Business Media.

Henze, M., Loosdrecht, M.C.M. van, Ekama, G.A., Brdjanovic, D., 2008. Biological Wastewater Treatment: Principles, Modeling and Design. IWA Publishing.

Hiatt, W., Grady, L., 2008. Application of the Activated Sludge Model for Nitrogen to Elevated Nitrogen Conditions. Water environment research : a research publication of the Water Environment Federation 80, 2134–44. https://doi.org/10.2175/106143008X304767

Hiatt, W.C., Grady, C.P.L., 2008. An Updated Process Model for Carbon Oxidation, Nitrification, and Denitrification. Water Environment Research 80, 2145–2156. https://doi.org/info:doi/10.2175/106143008X304776

Holmes, D., Dang, Y., Smith, J., 2018. Nitrogen cycling during wastewater treatment, in: Advances in Applied Microbiology. https://doi.org/10.1016/bs.aambs.2018.10.003

Hong, Y., Liao, Q., Bonhomme, C., Chebbo, G., 2019. Physically-based urban stormwater quality modelling: An efficient approach for calibration and sensitivity analysis. Journal of Environmental Management 246, 462–471. https://doi.org/10.1016/j.jenvman.2019.06.003

Hu, Z., Houweling, D., Dold, P., 2012. Biological Nutrient Removal in Municipal Wastewater Treatment: New Directions in Sustainability. Journal of Environmental Engineering 138, 307–317. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000462

Huang, Z., Gedalanga, P.B., Asvapathanagul, P., Olson, B.H., 2010. Influence of physicochemical and operational parameters on *Nitrobacter* and *Nitrospira* communities in an aerobic activated sludge bioreactor. Water Research 44, 4351–4358. https://doi.org/10.1016/j.watres.2010.05.037

Hydromantis (Ed.), 2017. GPS-x Technical Reference. Hydromantis Environmental Software Solutions.

Izadi, Parin, Izadi, Parnian, Eldyasti, A., 2021. Towards mainstream deammonification: Comprehensive review on potential mainstream applications and developed sidestream technologies. Journal of Environmental Management 279, 111615. https://doi.org/10.1016/j.jenvman.2020.111615

Jeppsson, U., Alex, J., Batstone, D.J., Benedetti, L., Comas, J., Copp, J.B., Corominas, L., Flores-Alsina, X., Gernaey, K.V., Nopens, I., Pons, M.-N., Rodríguez-Roda, I., Rosen, C., Steyer, J.-P., Vanrolleghem, P.A., Volcke, E.I.P., Vrecko, D., 2013. Benchmark simulation models, quo vadis? Water Science and Technology 68, 1–15. https://doi.org/10.2166/wst.2013.246

Jetten, M.S.M., Wagner, M., Fuerst, J., van Loosdrecht, M., Kuenen, G., Strous, M., 2001. Microbiology and application of the anaerobic ammonium oxidation (‘anammox’) process. Current Opinion in Biotechnology 12, 283–288. https://doi.org/10.1016/S0958-1669(00)00211-1

Jianlong, W., Ning, Y., 2004. Partial nitrification under limited dissolved oxygen conditions. Process Biochemistry 39, 1223–1229. https://doi.org/10.1016/S0032-9592(03)00249-8

Jofra, A., Perez, J., Loosdrecht, M., 2020. Hydroxylamine and the nitrogen cycle: A review. Water Research 190, 116723. https://doi.org/10.1016/j.watres.2020.116723

Johnson, G.E., 1966. The use of coal and modified coals as adsorbents for removing organic contaminants from waste waters. US Department of the Interior, Bureau of Mines.

Jones, R.M., Dold, P.L., Takács, I., Chapman, K., Wett, B., Murthy, S., Shaughnessy, M., 2007. Simulation for operation and control of reject water treatment processes. Proceedings of the Water Environment Federation 2007, 4357–4372.

Joss, A., Derlon, N., Cyprien, C., Burger, S., Szivak, I., Traber, J., Siegrist, H., Morgenroth, E., 2011. Combined Nitritation–Anammox: Advances in Understanding Process Stability. Environmental Science & Technology 45, 9735–9742. https://doi.org/10.1021/es202181v

MOST WIEDZY Downloaded from mostwiedzy.pl

Joss, A., Salzgeber, D., Eugster, J., König, R., Rottermann, K., Burger, S., Fabijan, P., Leumann, S., Mohn, J., Siegrist, H., 2009. Full-Scale Nitrogen Removal from Digester Liquid with Partial Nitritation and Anammox in One SBR. Environmental Science & Technology 43, 5301–5306. https://doi.org/10.1021/es900107w

Justin, M., Long, C., Klaus, S., Parsons, M., Chandran, K., De Clippeleir, H., Bott, C., 2022. Nitrogen Removal Capacity and Carbon Demand Requirements of Partial Denitrification/Anammox MBBR and IFAS Processes. Water Environment Research 94. https://doi.org/10.1002/wer.10766

Khunjar, W.O., Bilyk, K., Bruton, T., Yi, P., Latimer, R., Pitt, P., Mehta, S., Desmottes, C., Graveleau, L., Grandstaff, J., 2013. Use of Free Ammonia and Dissolved Oxygen for Process Control of a Sidestream Deammonification Sequential Batch Reactor. Proceedings of the Water Environment Federation 2013, 1995–2000. https://doi.org/10.2175/193864713813673613

Khunjar, W.O., Pitt, P.A., Bott, C.B., Chandran, K., 2014. Nitrogen, in: Activated Sludge - 100 Years and Counting. IWA Publishing, pp. 77–91.

Kirim, G., McCullough, K., Bressani Ribeiro, T., Domingo-Félez, C., Duan, H., Al-Omari, A., De Clippeleir, H., Jimenez, J., Klaus, S., Ladipo-Obasa, M., Mehrani, M., Regmi, P., Torfs, E., Volcke, E., Vanrolleghem, P., 2022. Mainstream short-cut N removal modelling: current status and perspectives. Water Science and Technology 85. https://doi.org/10.2166/wst.2022.131

Kits, K.D., Sedlacek, C.J., Lebedeva, E.V., Bulaev, A., Daebeler, A., Daims, H., Han, P., Stein, L.Y., Albertsen, M., Wagner, M., Pjevac, P., Romano, S., 2017. Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. Nature 549, 269. https://doi.org/10.1038/nature23679

Kleerebezem, R., Lücker, S., 2021. Cyclic Conversions in the Nitrogen Cycle. Frontiers in Microbiology 12, 622504. https://doi.org/10.3389/fmicb.2021.622504

Koch, H., van Kessel, M.A.H.J., Lücker, S., 2019. Complete nitrification: insights into the ecophysiology of comammox Nitrospira. Appl Microbiol Biotechnol 103, 177–189. https://doi.org/10.1007/s00253-018-9486-3

Kowal, P., Mehrani, M., Sobotka, D., Ciesielski, S., Makinia, J., 2022. Rearrangements of the nitrifiers population in an activated sludge system under decreasing solids retention times. Environmental Research. https://doi.org/10.1016/j.envres.2022.113753

Laanbroek, H.J., Gerards, S., 1993. Competition for limiting amounts of oxygen between Nitrosomonas europaea and Nitrobacter winogradskyi grown in mixed continuous cultures. Archives of Microbiology 159, 453–459. https://doi.org/10.1007/BF00288593

Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H., van Loosdrecht, M.C.M., 2014. Full-scale partial nitritation/anammox experiences – An application survey. Water Research 55, 292–303. https://doi.org/10.1016/j.watres.2014.02.032

Ladipo-Obasa, M., Forney, N., Riffat, R., Bott, C., deBarbadillo, C., De Clippeleir, H., 2022. Partial denitrification-anammox (PdNA) application in mainstream IFAS configuration using raw fermentate as carbon source. Water Environ Res 94, e10711. https://doi.org/10.1002/wer.10711

Lagarias, J., Reeds, J., Wright, M., Wright, P., 1998. Convergence Properties of the Nelder--Mead Simplex Method in Low Dimensions. SIAM Journal on Optimization 9, 112–147. https://doi.org/10.1137/S1052623496303470

Larsen, T.A., 2015. CO2-neutral wastewater treatment plants or robust, climate-friendly wastewater management? A systems perspective. Water Research 87, 513–521. https://doi.org/10.1016/j.watres.2015.06.006

Larsen, T.A., Maurer, M., Udert, K.M., Lienert, J., 2007. Nutrient cycles and resource management: implications for the choice of wastewater treatment technology. Water Science and Technology 56, 229–237. https://doi.org/10.2166/wst.2007.576

Lawson, C.E., Lücker, S., 2018. Complete ammonia oxidation: an important control on nitrification in engineered ecosystems? Current Opinion in Biotechnology, Energy biotechnology • Environmental biotechnology 50, 158–165. https://doi.org/10.1016/j.copbio.2018.01.015

Le, T., Peng, B., Su, C., Massoudieh, A., Torrents, A., Al-Omari, A., Murthy, S., Wett, B., Chandran, K., deBarbadillo, C., Bott, C., De Clippeleir, H., 2019. Nitrate residual as a key parameter to efficiently control partial denitrification coupling with anammox. Water Environment Research 91, 1455–1465. https://doi.org/10.1002/wer.1140

Liu, G., Wang, J., 2012. Probing the stoichiometry of the nitrification process using the respirometric approach. Water Research 46, 5954–5962. https://doi.org/10.1016/j.watres.2012.08.006

Liu, W., Chen, W., Yang, D., Shen, Y., 2018. Functional and compositional characteristics of nitrifiers reveal the failure of achieving mainstream nitritation under limited oxygen or ammonia conditions. Bioresource Technology 275. https://doi.org/10.1016/j.biortech.2018.12.065

MOST WIEDZY Downloaded from mostwiedzy.pl

Liu, W., Yang, Q., Ma, B., Li, J., Ma, L., Wang, S., Peng, Y., 2017. Rapid Achievement of Nitritation Using Aerobic Starvation. Environ. Sci. Technol. 51, 4001–4008. https://doi.org/10.1021/acs.est.6b04598

Liu, X., Kim, M., Nakhla, G., Andalib, M., Yuan, F., 2020. Partial nitrification-reactor configurations, and operational conditions: Performance analysis. Journal of Environmental Chemical Engineering 8. https://doi.org/10.1016/j.jece.2020.103984

Liu, Y., Li, S., Ni, G., Duan, H., Huang, X., Yuan, Z., Zheng, M., 2021. Temperature Variations Shape Niche Occupation of Nitrotoga-like Bacteria in Activated Sludge. ACS EST Water 1, 167–174. https://doi.org/10.1021/acsestwater.0c00060

Loosdrecht, M.C.M. van, Salem, S., 2006. Biological treatment of sludge digester liquids. Water Science and Technology 53, 11–20. https://doi.org/10.2166/wst.2006.401

Lu, W., Zhang, Y., Wang, Q., Wei, Y., Bu, Y., Ma, B., 2021. Achieving advanced nitrogen removal in a novel partial denitrification/anammox-nitrifying (PDA-N) biofilter process treating low C/N ratio municipal wastewater. Bioresource Technology 340, 125661. https://doi.org/10.1016/j.biortech.2021.125661

Lu, X., D. S. Pereira, T., Al-Hazmi, H.E., Majtacz, J., Zhou, Q., Xie, L., Makinia, J., 2018. Model-Based Evaluation of N2O Production Pathways in the Anammox-Enriched Granular Sludge Cultivated in a Sequencing Batch Reactor. Environ. Sci. Technol. 52, 2800–2809. https://doi.org/10.1021/acs.est.7b05611

Ludzack, F.J., Ettinger, M.B., 1962. Controlling operation to minimize activated sludge effluent nitrogen. Journal (Water Pollution Control Federation) 920–931.

Ma, B., Wang, S., Cao, S., Miao, Y., Jia, F., Du, R., Peng, Y., 2016. Biological nitrogen removal from sewage via anammox: Recent advances. Bioresource Technology 200, 981–990. https://doi.org/10.1016/j.biortech.2015.10.074

Makinia, J., 2010. Mathematical Modelling and Computer Simulation of Activated Sludge Systems. IWA Publishing.

Makinia, J., Czerwionka, K., Oleszkiewicz, J., Kulbat, E., Fudala-Ksiazek, S., 2011. A distillery by-product as an external carbon source for enhancing denitrification in mainstream and sidestream treatment processes. Water Science and Technology 64, 2072–2079. https://doi.org/10.2166/wst.2011.624

Makinia, J., Wells, S.A., Zima, P., 2005. Temperature Modeling in Activated Sludge Systems: A Case Study. Water Environment Research 77, 525–532. https://doi.org/info:doi/10.2175/106143005X67449

Makinia, J., Zaborowska, E., 2020. Mathematical Modelling and Computer Simulation of Activated Sludge Systems - Second Edition. https://doi.org/10.2166/9781780409528

Maktabifard, M., Blomberg, K., Zaborowska, E., Mikola, A., Makinia, J., 2022. Model-based identification of the dominant N2O emission pathway in a full-scale activated sludge system. Journal of Cleaner Production 336, 130347. https://doi.org/10.1016/j.jclepro.2021.130347

Mannucci, A., Munz, G., Mori, G., Lubello, C., Oleszkiewicz, J.A., 2015. Applicability of the Arrhenius model for ammonia oxidizing bacteria subjected to temperature time gradients. Front. Environ. Sci. Eng. 9, 988–994. https://doi.org/10.1007/s11783-014-0751-0

Marais, G., Ekama, G., 1976. Activated Sludge Process Part 1: Steady State Behaviour. Water 2, 164–200.

Massara, T.M., Malamis, S., Guisasola, A., Baeza, J.A., Noutsopoulos, C., Katsou, E., 2017. A review on nitrous oxide (N2O) emissions during biological nutrient removal from municipal wastewater and sludge reject water. Science of The Total Environment 596–597, 106–123. https://doi.org/10.1016/j.scitotenv.2017.03.191

McCullough, K., Klaus, S., Parsons, M., Wilson, C., Bott, C., 2022. Advancing the Understanding of Mainstream Shortcut Nitrogen Removal: Resource Efficiency, Carbon Redirection, and Plant Capacity. Environmental Science: Water Research & Technology 8. https://doi.org/10.1039/D2EW00247G

Mehrani, M., Azari, M., Teichgräber, B., Jagemann, P., Schoth, J., Denecke, M., Makinia, J., 2022a. Performance evaluation and model-based optimization of the mainstream deammonification in an integrated fixed-film activated sludge reactor. Bioresource Technology 351, 126942. https://doi.org/10.1016/j.biortech.2022.126942

Mehrani, M., Kowal, P., Sobotka, D., Godzieba, M., Ciesielski, S., Guo, J., Makinia, J., 2022b. The coexistence and competition of canonical and comammox nitrite oxidizing bacteria in a nitrifying activated sludge system – Experimental observations and simulation studies. Science of The Total Environment 161084. https://doi.org/10.1016/j.scitotenv.2022.161084

Mehrani, M., Lu, X., Kowal, P., Sobotka, D., Makinia, J., 2021. Incorporation of the complete ammonia oxidation (comammox) process for modeling nitrification in suspended growth wastewater

MOST WIEDZY Downloaded from mostwiedzy.pl

treatment systems. Journal of Environmental Management 297, 113223. https://doi.org/10.1016/j.jenvman.2021.113223

Mehrani, M., Sobotka, D., Kowal, P., Guo, J., Makinia, J., 2022c. New insights into modeling two-step nitrification in activated sludge systems – The effects of initial biomass concentrations, comammox and heterotrophic activities. Science of The Total Environment 848, 157628. https://doi.org/10.1016/j.scitotenv.2022.157628

Mehta, C.M., Khunjar, W.O., Nguyen, V., Tait, S., Batstone, D.J., 2015. Technologies to Recover Nutrients from Waste Streams: A Critical Review. Critical Reviews in Environmental Science and Technology 45, 385–427. https://doi.org/10.1080/10643389.2013.866621

Mei, P., Wang, Z., Guo, W., Gao, Y., A. Vanrolleghem, P., Li, Y., 2023. The ASM2d model with two-step nitrification can better simulate biological nutrient removal systems enriched with complete ammonia oxidizing bacteria (comammox *Nitrospira*). Chemosphere 335, 139169. https://doi.org/10.1016/j.chemosphere.2023.139169

Meijer, S.C.F., van Loosdrecht, M.C.M., Heijnen, J.J., 2001. Metabolic modelling of full-scale biological nitrogen and phosphorus removing wwtp's. Water Research 35, 2711–2723. https://doi.org/10.1016/S0043-1354(00)00567-4

Monod, J., 1949. The Growth of Bacterial Cultures. Annual Review of Microbiology 3, 371–394. https://doi.org/10.1146/annurev.mi.03.100149.002103

Mozumder, M.S.I., Picioreanu, C., Loosdrecht, M.C.M. van, Volcke, E.I.P., 2014. Effect of heterotrophic growth on autotrophic nitrogen removal in a granular sludge reactor. Environmental Technology.

Nelder, J.A., Mead, R., 1965. A Simplex Method for Function Minimization Comput. The Computer Journal 7. https://doi.org/10.1093/comjnl/7.4.308

Olsson, D., Nelson, L., 1975. The Nelder-Mead Simplex Procedure for Function Minimization. Technometrics 17, 45–51. https://doi.org/10.1080/00401706.1975.10489269

Painter, H.A., Loveless, J.E., 1983. Effect of temperature and pH value on the growth-rate constants of nitrifying bacteria in the activated-sludge process. Water Research 17, 237–248. https://doi.org/10.1016/0043-1354(83)90176-8

Paredes, D., Kuschk, P., Mbwette, T.S.A., Stange, F., Müller, R.A., Köser, H., 2007. New Aspects of Microbial Nitrogen Transformations in the Context of Wastewater Treatment – A Review. Engineering in Life Sciences 7, 13–25. https://doi.org/10.1002/elsc.200620170

Park, H.-D., Noguera, D.R., 2004. Evaluating the effect of dissolved oxygen on ammonia-oxidizing bacterial communities in activated sludge. Water Research 38, 3275–3286. https://doi.org/10.1016/j.watres.2004.04.047

Park, M.-R., Park, H., Chandran, K., 2017. Molecular and Kinetic Characterization of Planktonic Nitrospira spp. Selectively Enriched from Activated Sludge. Environ. Sci. Technol. 51, 2720–2728. https://doi.org/10.1021/acs.est.6b05184

Park, S., Bae, W., 2009. Modeling kinetics of ammonium oxidation and nitrite oxidation under simultaneous inhibition by free ammonia and free nitrous acid. Process Biochemistry 44, 631–640. https://doi.org/10.1016/j.procbio.2009.02.002

Pasteur, L., 1862. Etudes sur les micodermes. Rôle des plantes dans la fermentation acetique. C. r. Acad. Sci. Vol. 54. 1862.

Peng, Y., Zhu, G., 2006. Biological nitrogen removal with nitrification and denitrification via nitrite pathway. Applied Microbiology and Biotechnology 73, 15–26. https://doi.org/10.1007/s00253-006-0534-z

Pérez, J., Lotti, T., Kleerebezem, R., Picioreanu, C., van Loosdrecht, M.C.M., 2014. Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: A model-based study. Water Research 66, 208–218. https://doi.org/10.1016/j.watres.2014.08.028

Petersen, B., Vanrolleghem, P.A., Gernaey, K., Henze, M., 2002. Evaluation of an ASM1 model calibration procedure on a municipal–industrial wastewater treatment plant. Journal of Hydroinformatics 4, 15–38.

Philips, S., Wyffels, S., Sprengers, R., WV, V., 2002. Oxygen-limited autotrophic nitrification/denitrification by ammonia oxidisers enables upward motion towards more favourable conditions. Applied microbiology and biotechnology 59, 557–66. https://doi.org/10.1007/s00253-002-1059-8

Pjevac, P., Schauberger, C., Poghosyan, L., Herbold, C., Kessel, M., Daebeler, A., Steinberger, M., Jetten, M., Lücker, S., Wagner, M., Daims, H., 2017. AmoA-Targeted Polymerase Chain Reaction Primers for the Specific Detection and Quantification of Comammox Nitrospira in the Environment. Frontiers in Microbiology 8. https://doi.org/10.3389/fmicb.2017.01508

Podmirseg, S.M., Gómez-Brandón, M., Muik, M., Stres, B., Hell, M., Pümpel, T., Murthy, S., Chandran, K., Park, H., Insam, H., Wett, B., 2022. Microbial response on the first full-scale DEMON®

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biomass transfer for mainstream deammonification. Water Research 218, 118517. https://doi.org/10.1016/j.watres.2022.118517

Pollice, A., 2002. Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. Water Research 36, 2541–2546. https://doi.org/10.1016/S0043-1354(01)00468-7

Prakasam, T., Loehr, R., 1972. Microbial nitrification and denitrification in concentrated wastes. Water Research 6, 859–869. https://doi.org/10.1016/0043-1354(72)90038-3

Pryce, D., Kapelan, Z., Memon, F.A., 2022. Modelling the performance of an integrated fixed-film activated sludge (IFAS) system: a systematic approach to automated calibration. Sci Rep 12, 9416. https://doi.org/10.1038/s41598-022-13779-w

Rahimi, S., Modin, O., Mijakovic, I., 2020. Technologies for biological removal and recovery of nitrogen from wastewater. Biotechnology Advances 43, 107570. https://doi.org/10.1016/j.biotechadv.2020.107570

Regmi, P., Holgate, B., Miller, M.W., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., 2013. NOB Repression for Mainstream Nitrite-Shunt and Deammonification: A Pilot Study. Proceedings of the Water Environment Federation 2013, 1959–1981. https://doi.org/10.2175/193864713813673677

Regmi, P., Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B., 2014. Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation. Water Research 57, 162–171. https://doi.org/10.1016/j.watres.2014.03.035

Richardson, D.J., Wehrfritz, J.-M., Keech, A., Crossman, L.C., Roldan, M.D., Sears, H.J., Butler, C.S., Reilly, A., Moir, J.W.B., Berks, B.C., Ferguson, S.J., Thomson, A.J., Spiro, S., 1998. The diversity of redox proteins involved in bacterial heterotrophic nitrification and aerobic denitrification. Biochemical Society Transactions 26, 401–408. https://doi.org/10.1042/bst0260401

Rittmann, B., McCarty, P.L., 2020. Environmental Biotechnology: Principles and Applications, Second Edition, 2nd ed. McGraw-Hill.

Roots, P., Sabba, F., Rosenthal, A.F., Wang, Y., Yuan, Q., Rieger, L., Yang, F., Kozak, J.A., Zhang, H., Wells, G.F., 2020. Integrated shortcut nitrogen and biological phosphorus removal from mainstream wastewater: process operation and modeling. Environ. Sci.: Water Res. Technol. 6, 566–580. https://doi.org/10.1039/C9EW00550A

Roots, P., Yubo, W., Rosenthal, A., Griffin, J., Sabba, F., Petrovich, M., Yang, F., Kozak, J., Zhang, H., Wells, G., 2019. Comammox Nitrospira are the dominant ammonia oxidizers in a mainstream low dissolved oxygen nitrification reactor. Water Research 157. https://doi.org/10.1016/j.watres.2019.03.060

Rosenwinkel, K.-H., Beier, M., Phan, L.-C., Hartwig, P., 2009. Conventional and Advanced Technologies for Biological Nitrogen Removal in Europe. Water Practice and Technology 4. https://doi.org/10.2166/wpt.2009.014

Ruiz, G., Jeison, D., Chamy, R., 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. Water Research 37, 1371–1377. https://doi.org/10.1016/S0043-1354(02)00475-X

S. Shourjeh, M., Kowal, P., Drewnowski, J., Szeląg, B., Szaja, A., Lagod, G., 2020. Mutual Interaction between Temperature and DO Set Point on AOB and NOB Activity during Shortcut Nitrification in a Sequencing Batch Reactor in Terms of Energy Consumption Optimization. Energies 13, 1–21. https://doi.org/10.3390/en13215808

Sawyer, C.N., Bradney, L., 1945. Rising of activated sludge in final settling tanks. sewage works journal 17, 1191–1209.

Sayigh, B.A., Malina, J.F., 1978. Temperature Effects on the Activated Sludge Process. Journal (Water Pollution Control Federation) 50, 678–687. https://doi.org/10.2307/25039610

Schmidt, I., Sliekers, O., Schmid, M., Bock, E., Fuerst, J., Kuenen, J.G., Jetten, M.S.M., Strous, M., 2003. New concepts of microbial treatment processes for the nitrogen removal in wastewater. FEMS Microbiology Reviews 27, 481–492. https://doi.org/10.1016/S0168-6445(03)00039-1

Schulthess, R.V., Gujer, W., 1996. Release of nitrous oxide (N2O) from denitrifying activated sludge: Verification and application of a mathematical model. Water Research 30, 521–530. https://doi.org/10.1016/0043-1354(95)00204-9

Sepehri, A., Sarrafzadeh, M.-H., 2019. Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: metabolite reduction and CO2 mitigation intensification process. Appl Water Sci 9, 131. https://doi.org/10.1007/s13201-019-1017-6

Sharif Shourjeh, M., Kowal, P., Lu, X., Xie, L., Drewnowski, J., 2021a. Development of Strategies for AOB and NOB Competition Supported by Mathematical Modeling in Terms of Successful Deammonification Implementation for Energy-Efficient WWTPs. Processes 9, 562. https://doi.org/10.3390/pr9030562

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Sharif Shourjeh, M., Kowal, P., Lu, X., Xie, L., Drewnowski, J., 2021b. Development of Strategies for AOB and NOB Competition Supported by Mathematical Modeling in Terms of Successful Deammonification Implementation for Energy-Efficient WWTPs. Processes 9, 562. https://doi.org/10.3390/pr9030562

Sin, G., Kaelin, D., Kampschreur, M.J., Takács, I., Wett, B., Gernaey, K.V., Rieger, L., Siegrist, H., Loosdrecht, M.C.M. van, 2008a. Modelling nitrite in wastewater treatment systems: a discussion of different modelling concepts. Water Science and Technology 58, 1155–1171. https://doi.org/10.2166/wst.2008.485

Sin, G., Pauw, D., Weijers, S., Vanrolleghem, P., 2008b. An efficient approach to automate the manual trial and error calibration of activated sludge models. Biotechnology and bioengineering 100, 516–28. https://doi.org/10.1002/bit.21769

Sliekers, A., 2002. Completely autotrophic nitrogen removal over nitrite in one single reactor. Water Research 36, 2475–2482. https://doi.org/10.1016/S0043-1354(01)00476-6

Stein, L., 2011. Heterotrophic Nitrification and Nitrifier Denitrification. Nitrification 95–114. https://doi.org/10.1128/9781555817145.ch5

Stein, L., Arp, D., 1998. Loss of Ammonia Monooxygenase Activity in Nitrosomonas europaea upon Exposure to Nitrite. Applied and Environmental Microbiology 64. https://doi.org/10.1128/AEM.64.10.4098-4102.1998

Stensel, H.D., Loehr, R.C., Lawrence, A.W., 1973. Biological Kinetics of Suspended-Growth Denitrification. Journal (Water Pollution Control Federation) 45, 249–261.

Stensel, H.D., Shell, G.L., 1974. Two Methods of Biological Treatment Design. Journal (Water Pollution Control Federation) 46, 271–283.

Stratton, H.M., Nittami, T., Esteban, N.H., Petrovski, S., Seviour, R.J., 2024. Influence of season on the microbial population dynamics of activated sludge. Journal of Applied Microbiology 135, lxae014. https://doi.org/10.1093/jambio/lxae014

Surmacz-Gorska, J., Cichon, A., Miksch, K., 1997. Nitrogen removal from wastewater with high ammonia nitrogen concentration via shorter nitrification and denitrification. Water Science and Technology 36, 73–78. https://doi.org/10.1016/S0273-1223(97)00000-0

Suzuki, I., Dular, U., Kwok, SC, 1974. Ammonia or ammonium ion as substrate for oxidation by Nitrosomonas-Europaea cells and extracts. Journal of Bacteriology 120, 556–558.

Szeląg, B., Kiczko, A., Zaborowska, E., Mannina, G., Mąkinia, J., 2022. Modeling nutrient removal and energy consumption in an advanced activated sludge system under uncertainty. Journal of Environmental Management 323, 116040. https://doi.org/10.1016/j.jenvman.2022.116040

Tarre, S., Green, M., 2004. High-Rate Nitrification at Low pH in Suspended- and Attached-Biomass Reactors. Appl. Environ. Microbiol. 70, 6481–6487. https://doi.org/10.1128/AEM.70.11.6481-6487.2004

Tchobanoglous, G., Burton, F.L., 1991. Wastewater Engineering: Treatment, Disposal, and Reuse. McGraw-Hill.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., Inc, M.& E., 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill Education.

Tchobanoglous, G., Stensel, H.D., Tsuchihashi, R., Burton, F.L., 2013. Wastewater Engineering: Treatment and Resource Recovery.

Tomlinson, T.G., Boon, A.G., Trotman, C.N.A., 1966. Inhibition of Nitrification in the Activated Sludge Process of Sewage Disposal. Journal of Applied Bacteriology 29, 266–291. https://doi.org/10.1111/j.1365-2672.1966.tb03477.x

Torresi, E., Casas, M., Polesel, F., Christensson, M., Bester, K., 2017. Impact of external carbon dose on the removal of micropollutants using methanol and ethanol in post-denitrifying Moving Bed Biofilm Reactors. Water Research 108, 95–105. https://doi.org/10.1016/j.watres.2016.10.068

Trojanowicz, K., Plaza, E., Trela, J., 2017. Model Extension, Calibration and Validation of Partial Nitritation-Anammox Process in Moving Bed Biofilm Reactor (MBBR) for Reject and Mainstream Wastewater. Environmental Technology 40, 1–49. https://doi.org/10.1080/09593330.2017.1397765

Turk, O., Mavinic, D.S., 1989. Maintaining nitrite build-up in a system acclimated to free ammonia. Water Research 23, 1383–1388. https://doi.org/10.1016/0043-1354(89)90077-8

van Dongen, U., Jetten, M.S.M., van Loosdrecht, M.C.M., 2001. The SHARON((R))-Anammox((R)) process for treatment of ammonium rich wastewater. Water Sci. Technol. 44, 153–160.

Van Hulle, S.W., Volcke, E.I., Teruel, J.L., Donckels, B., van Loosdrecht, M.C., Vanrolleghem, P.A., 2007. Influence of temperature and pH on the kinetics of the Sharon nitritation process. Journal of Chemical Technology & Biotechnology 82, 471–480. https://doi.org/10.1002/jctb.1692

Van Hulle, S.W.H., Van den Broeck, S., Maertens, J., Villez, K., Donckels, B.M.R., Schelstraete, G., Volcke, E.I.P., Vanrolleghem, P.A., 2005. Construction, start-up and operation of a continuously

MOST WIEDZY Downloaded from mostwiedzy.pl

aerated laboratory- scale SHARON reactor in view of coupling with an Anammox reactor. Water SA 31, 327–334.

Van Hulle, S.W.H., Vandeweyer, H.J.P., Meesschaert, B.D., Vanrolleghem, P.A., Dejans, P., Dumoulin, A., 2010. Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. Chemical Engineering Journal 162, 1–20. https://doi.org/10.1016/j.cej.2010.05.037

van Kessel, M.A.H.J., Speth, D.R., Albertsen, M., Nielsen, P.H., Op den Camp, H.J.M., Kartal, B., Jetten, M.S.M., Lücker, S., 2015. Complete nitrification by a single microorganism. Nature 528, 555–559. https://doi.org/10.1038/nature16459

Vanhaandel, A., Ekama, G., Marais, G., 1981. The activated sludge process?3 single sludge denitrification. Water Research 15, 1135–1152. https://doi.org/10.1016/0043-1354(81)90089-0

Vannecke, T., Volcke, E., 2015. Modelling microbial competition in nitrifying biofilm reactors. Biotechnology and bioengineering 112. https://doi.org/10.1002/bit.25680

Villaverde, S., 2000. Nitrifying biofilm acclimation to free ammonia in submerged biofilters. Start-up influence. Water Research 34, 602–610. https://doi.org/10.1016/S0043-1354(99)00175-X

Vitousek, P.M., Menge, D.N.L., Reed, S.C., Cleveland, C.C., 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philosophical Transactions of the Royal Society of London B: Biological Sciences 368, 20130119. https://doi.org/10.1098/rstb.2013.0119

Volcke, E., 2006. Modelling, analysis and control of partial nitritation in a SHARON reactor.

Volcke, E.I.P., Sanchez, O., Steyer, J.-P., Dabert, P., Bernet, N., 2008. Microbial population dynamics in nitrifying reactors: Experimental evidence explained by a simple model including interspecies competition. Process Biochemistry, Bioenergy Production by Process Engineering Approach 43, 1398–1406. https://doi.org/10.1016/j.procbio.2008.08.013

Volcke, E.I.P., van Loosdrecht, M.C.M., Vanrolleghem, P.A., 2006. Controlling the nitrite : ammonium ratio in a SHARON reactor in view of its coupling with an Anammox process. Water Sci. Technol. 53, 45–54. https://doi.org/10.2166/wst.2006.109

Wang, L., Li, B., Li, Y., Wang, J., 2021. Enhanced biological nitrogen removal under low dissolved oxygen in an anaerobic-anoxic-oxic system: Kinetics, stoichiometry and microbial community. Chemosphere 263, 128184. https://doi.org/10.1016/j.chemosphere.2020.128184

Ward, B.B., Arp, D.J., Klotz, M.G., Editors, 2010. Nitrification, 1st ed. ASM Press.

Ward, M.H., Jones, R.R., Brender, J.D., de Kok, T.M., Weyer, P.J., Nolan, B.T., Villanueva, C.M., van Breda, S.G., 2018. Drinking Water Nitrate and Human Health: An Updated Review. Int J Environ Res Public Health 15, 1557. https://doi.org/10.3390/ijerph15071557

Watson, S., Bock, E., Valois, F., Waterbury, J., Schlosser, U., 1986. Watson SW, Bock E, Valois FW, Waterbury JB, Schlosser U.. Nitrospira marina gen. nov. sp. nov.: a chemolithotrophic nitrite-oxidizing acterium. Arch Microbiol 144: 1-7. Archives of Microbiology 144, 1–7. https://doi.org/10.1007/BF00454947

Wett, B., 2007. Development and implementation of a robust deammonification process. Water Science and Technology 56, 81–88. https://doi.org/10.2166/wst.2007.611

Wett, B., Hell, M., Nyhuis, G., Puempel, T., Takacs, I., Murthy, S., 2010. Syntrophy of aerobic and anaerobic ammonia oxidisers. Water Science and Technology 61, 1915–1922. https://doi.org/10.2166/wst.2010.969

Wett, B., Omari, A., Podmirseg, S.M., Han, M., Akintayo, O., Brandón, M.G., Murthy, S., Bott, C., Hell, M., Takács, I., Nyhuis, G., O’Shaughnessy, M., 2013. Going for mainstream deammonification from bench to full scale for maximized resource efficiency. Water Science and Technology 68, 283–289. https://doi.org/10.2166/wst.2013.150

Wett, B., Podmirseg, S.M., Gómez-Brandón, M., Hell, M., Nyhuis, G., Bott, C., Murthy, S., 2015. Expanding DEMON Sidestream Deammonification Technology Towards Mainstream Application [WWW Document]. URL (accessed 11.20.16).

Wett, B., Rauch, W., 2003. The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. Water Research 37, 1100–1110. https://doi.org/10.1016/S0043-1354(02)00440-2

Winkler, M.K., Straka, L., 2019. New directions in biological nitrogen removal and recovery from wastewater. Current Opinion in Biotechnology, Energy Biotechnology • Environmental Biotechnology 57, 50–55. https://doi.org/10.1016/j.copbio.2018.12.007

Wu, J., Yan, G., Zhou, G., Xu, T., 2014. Model predictive control of biological nitrogen removal via partial nitrification at low carbon/nitrogen (C/N) ratio. Journal of Environmental Chemical Engineering 2, 1899–1906. https://doi.org/10.1016/j.jece.2014.08.007

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Wuhrmann, K., 1964. Nitrogen removal in sewage treatment processes: With 9 figures in the text and on 2 folders. SIL Proceedings, 1922-2010 15, 580–596. https://doi.org/10.1080/03680770.1962.11895576

Yang, L., Alleman, J., 1992. INVESTIGATION OF BATCHWISE NITRITE BUILDUP BY AN ENRICHED NITRIFICATION CULTURE. Water Sci. Technol. 26, 997–1005.

Yang, X.-P., Wang, S.-M., Zhang, D.-W., Zhou, L.-X., 2011. Isolation and nitrogen removal characteristics of an aerobic heterotrophic nitrifying–denitrifying bacterium, *Bacillus subtilis* A1. Bioresource Technology 102, 854–862. https://doi.org/10.1016/j.biortech.2010.09.007

Yoo, H., Ahn, K.-H., Lee, H.-J., Lee, K.-H., Kwak, Y.-J., Song, K.-G., 1999. Nitrogen removal from synthetic wastewater by simultaneous nitrification and denitrification (SND) via nitrite in an intermittently-aerated reactor. Water Research 33, 145–154. https://doi.org/10.1016/S0043-1354(98)00159-6

You, Q.-G., Wang, J.-H., Qi, G.-X., Zhou, Y.-M., Guo, Z., Shen, Y., Gao, X., 2020. Anammox and partial denitrification coupling: A review. RSC Advances 10, 12554–12572. https://doi.org/10.1039/D0RA00001A

Young, J.C., McCarty, P.L., 1969. The anaerobic filter for waste treatment. Journal (Water Pollution Control Federation) R160–R173.

Yu, L., Chen, S., Chen, W., Wu, J., 2020. Experimental investigation and mathematical modeling of the competition among the fast-growing "*r-strategists*" and the slow-growing "*K-strategists*" ammonium-oxidizing bacteria and nitrite-oxidizing bacteria in nitrification. Science of The Total Environment 702, 135049. https://doi.org/10.1016/j.scitotenv.2019.135049

Yuan, Z., Blackall, L.L., 2002. Sludge population optimisation: a new dimension for the control of biological wastewater treatment systems. Water Research, Modern Scientific Tools in Bioprocessing 36, 482–490. https://doi.org/10.1016/S0043-1354(01)00230-5

Zaborowska, E., Czerwionka, K., Makinia, J., 2016. Strategies for achieving energy neutrality in biological nutrient removal systems – a case study of the Slupsk WWTP (northern Poland). Water Science and Technology 75, 727–740. https://doi.org/10.2166/wst.2016.564

Zhang, D., Su, H., Antwi, P., Xiao, L., Liu, Z., Li, J., 2019. High-rate partial-nitritation and efficient nitrifying bacteria enrichment/out-selection via pH-DO controls: Efficiency, kinetics, and microbial community dynamics. Science of The Total Environment 692, 741–755. https://doi.org/10.1016/j.scitotenv.2019.07.308

Zhang, M., Wang, S., Ji, B., Liu, Y., 2019. Towards mainstream deammonification of municipal wastewater: Partial nitrification-anammox versus partial denitrification-anammox. Science of The Total Environment 692, 393–401. https://doi.org/10.1016/j.scitotenv.2019.07.293

Zhang, Z., Zhang, Y., Chen, Y., 2019. Recent advances in partial denitrification in biological nitrogen removal: From enrichment to application. Bioresource Technology 298, 122444. https://doi.org/10.1016/j.biortech.2019.122444

Zhao, H., Guo, Y., Wang, Q., Zhang, Z., Wu, C., Gao, M., Liu, F., 2022. The Summary of Nitritation Process in Mainstream Wastewater Treatment. Sustainability 14, 16453. https://doi.org/10.3390/su142416453

Zhao, W., Bi, X., Bai, M., Wang, Y., 2023. Research advances of ammonia oxidation microorganisms in wastewater: metabolic characteristics, microbial community, influencing factors and process applications. Bioprocess and Biosystems Engineering 46, 1–13. https://doi.org/10.1007/s00449-023-02866-5

Zhu, A., Guo, J., Ni, B.-J., Wang, S., Yang, Q., Peng, Y., 2015. A Novel Protocol for Model Calibration in Biological Wastewater Treatment. Sci Rep 5, 8493. https://doi.org/10.1038/srep08493

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Appendix A. Supplementary data

The author of the PhD dissertation:

Marcin Marcinkowski

Title of the PhD dissertation:

Mathematical modelling of two-step nitrification in activated sludge systems treating ammonia-rich wastewater

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S1 Model development and calibration procedure

I. Model development and implementation

Model “0”: two-step nitrification model (AOB+NOB)

II. Kinetic parameters assumption

1. Literature references & variables: μ_{AOB}, $K_{NH4,AOB}$, $K_{O,AOB}$, μ_{NOB}, $K_{NO2,NOB}$, $K_{O,NOB}$
2. Local sensitivity analysis and correlation matrix development

III. Dynamic calibration (GPSx Optimizer)

1. *Unlimited DO conditions* long-term washout (T=30˚C, SRT=2.5/2.0 d, DO=2.0 mgO$_2$/L)

Adjusted variables: μ_{AOB}, $K_{NH4,AOB}$, $K_{O,AOB}$, μ_{NOB}, $K_{NO2,NOB}$, $K_{O,NOB}$,

2. *Limited DO conditions* long-term washout (T=30˚C, SRT=4 d, DO=0.5 mgO$_2$/L)

Adjusted variables: $K_{O,AOB}$, $K_{O,NOB}$,

3. Model performance metrics: RMSE, MAE, R^2

IV. Model “0” validation

1. Batch tests data: DO 0.5/2.0/6.0 mgO$_2$/L
2. Model performance metrics: RMSE, MAE, R^2

V. Model extension

Model “CMX”: two-step nitrification (AOB+NOB) and comammox (NH$_4$ → NO$_3$)

VI. Kinetic parameters assumption

1. Literature references & variables: a. μ_{CMX}, $K_{NH4,CMX}$, $K_{O,CMX}$
   b. AOB and NOB kinetic parameters from Model “0”
2. Local sensitivity analysis and correlation matrix development

VII. Dynamic calibration (GPSx Optimizer)

1. *Unlimited DO conditions* long-term washout (T=30˚C, SRT=2.5/2.0 d, DO=2.0 mgO$_2$/L)

Adjusted variables: μ_{AOB}, $K_{NH4,AOB}$, $K_{O,AOB}$, μ_{NOB}, $K_{NO2,NOB}$, $K_{O,NOB}$, μ_{CMX}, $K_{NH4,CMX}$, $K_{O,CMX}$

2. *Limited DO conditions* long-term washout (T=30˚C, SRT=4 d, DO=0.5 mgO$_2$/L)

Adjusted variables: $K_{O,AOB}$, $K_{O,NOB}$, $K_{O,CMX}$

3. Model performance metrics: RMSE, MAE, R^2

VIII. Model “CMX” validation

1. Batch tests data: DO 0.5/2.0/6.0 mgO$_2$/L
2. Model performance metrics: RMSE, MAE, R^2

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S2 Model “0” stoichiometric matrix and kinetic rate equations

Table S2.1 Stoichiometric matrix related to AOB and NOB nitrogen conversions.

	Stoichiometric matrix									
	S_{NH4}	S_{NO2}	S_{NO3}	S_O	X_{AOB}	X_{NOB}	X_S	X_U	X_{nd}	Salk
R1	$-\frac{1}{Y_{AOB}} - ixbn$	$\frac{1}{Y_{AOB}}$		$-\frac{3.43 - Y_{AOB}}{Y_{AOB}}$	1					$-\frac{1}{7 \cdot Y_{AOB}} - \frac{ixbn}{14}$
R2					-1		$1 - f_u$	f_u	$ixbn - f_u \cdot ixun$	
R3		$-\frac{1}{Y_{NOB}} - ixbn$	$\frac{1}{Y_{NOB}}$	$-\frac{1.14 - Y_{NOB}}{Y_{NOB}}$		1				
R4						-1		f_u	$ixbn - f_u \cdot ixun$	

Table S2.2 Kinetic rate equations related to AOB and NOB.

Process	**Reaction rate**	**Kinetic equation**
Aerobic growth of AOB	*R1*	$\mu_{AOB} \cdot \frac{S_{NH4}}{S_{NH4} + K_{NH4,AOB}} \cdot \frac{S_O}{S_O + K_O} \cdot X_{AOB}$
Decay of AOB	*R2*	$b_{AOB} \cdot X_{AOB}$
Aerobic growth of NOB	*R3*	$\mu_{NOB} \cdot \frac{S_{NO2}}{S_{NO2} + K_{NO2,NOB}} \cdot \frac{S_O}{S_O + K_O} \cdot \frac{K_{INH4,NOB}}{K_{INH4,NOB} + S_{NH4}} \cdot X_{NOB}$
Decay of NOB	*R4*	$b_{NOB} \cdot X_{NOB}$

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S3 Model “CMX” stoichiometric matrix and kinetic rate equations

Table S3.1 Stoichiometric matrix related to AOB, NOB and comammox nitrogen conversions.

	Stoichiometric matrix											
	S_{NH4}	S_{NO2}	S_{NO3}	S_{N2O}	S_O	X_{AOB}	X_{NOB}	X_{CMX}	X_S	X_U	X_{nd}	Salk
R1	$-\frac{1}{Y_{AOB}} - ixbn$	$\frac{1}{Y_{AOB}}$			$-\frac{3.43 - Y_{AOB}}{Y_{AOB}}$	1						$-\frac{1}{7 \cdot Y_{AOB}} - \frac{ixbn}{14}$
R2						-1			$1 - f_u$	f_u	$ixbn - f_u \cdot ixun$	
R3		$-\frac{1}{Y_{NOB}} - ixbn$	$\frac{1}{Y_{NOB}}$		$-\frac{1.14 - Y_{NOB}}{Y_{NOB}}$		1					
R4							-1			f_u	$ixbn - f_u \cdot ixun$	
R5	$-\frac{1}{Y_{CMX}} - ixbn$		$\frac{1}{Y_{CMX}}$		$-\frac{4.57 - Y_{CMX}}{Y_{CMX}}$			1				$-\frac{1}{7 \cdot Y_{CMX}} - \frac{ixbn}{14}$
R6								-1	$1 - f_u$	f_u	$ixbn - f_u \cdot ixun$	
R7	1	-2		3								-1
R8	-1			1	-1							-1

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Table S3.2 Model “CMX” kinetic rate equations related to AOB, NOB and comammox.

Process	**Reaction rate**	**Kinetic equation**
Aerobic growth of AOB	*R1*	$\mu_{AOB} \cdot \frac{S_{NH4}}{S_{NH4} + K_{NH4,AOB}} \cdot \frac{S_O}{S_O + K_{O,AOB}} \cdot \frac{S_{ALK}}{K_{ALK,AOB} + S_{ALK}} \cdot X_{AOB}$
Decay of AOB	*R2*	$b_{AOB} \cdot X_{AOB}$
Aerobic growth of NOB	*R3*	$\mu_{NOB} \cdot \frac{S_{NO2}}{S_{NO2} + K_{NO2,NOB}} \cdot \frac{S_O}{S_O + K_{O,NOB}} \cdot \frac{K_{INH4,NOB}}{K_{INH4,NOB} + S_{NH4}} \cdot X_{NOB}$
Decay of NOB	*R4*	$b_{NOB} \cdot X_{NOB}$
Aerobic growth of comammox ($S_{NH4} \rightarrow S_{NO3}$)	*R5*	$\mu_{CMX} \cdot \frac{S_{NH4}}{S_{NH4} + K_{NH4,CMX}} \cdot \frac{S_O}{S_O + K_{O,CMX}} \cdot \frac{S_{ALK}}{K_{ALK,CMX} + S_{ALK}} \cdot X_{CMX}$
Decay of comammox	*R6*	$b_{CMX} \cdot X_{CMX}$
AOB denitrification (S_{NH4},$S_{NO2} \rightarrow S_{N2O}$)	*R7*	$\mu_{AOB} \cdot \eta_{2,AOB} \cdot \frac{K_{O,AOB}}{K_{O,AOB} + S_O} \cdot \frac{S_{NH4}}{K_{NH4,AOB} + S_{NH4}} \cdot \frac{S_{NO2}}{K_{NO2,AOB} + S_{NO2}} \cdot \frac{S_{ALK}}{K_{ALK,AOB} + S_{ALK}} \cdot X_{AOB}$
Hydroxylamine oxidation ($S_{NH4} \rightarrow S_{N2O}$)	*R8*	$\mu_{AOB} \cdot \eta_{1,AOB} \cdot \frac{S_O}{K_{O,AOB} + S_O} \cdot \frac{S_{NH4}}{K_{NH4,AOB} + S_{NH4}} \cdot \frac{S_{ALK}}{K_{ALK,AOB} + S_{ALK}} \cdot X_{AOB}$

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S4 Model “CMXH” stoichiometric matrix and kinetic rate equations

Table S4.1 Stoichiometric matrix related to HET, AOB, NOB and comammox nitrogen conversions

	Stoichiometric matrix												
	S_S	S_{NH4}	S_{NO2}	S_{NO3}	S_{N2O}	S_O	X_{HET}	X_{AOB}	X_{NOB}	X_{CMX}	X_S	X_U	X_{nd}	S_{alk}
R1		$-\frac{1}{Y_{AOB}}-\mathrm{ixbn}$	$\frac{1}{Y_{AOB}}$			$-\frac{3.43-Y_{AOB}}{Y_{AOB}}$		1						$-\frac{1}{7\cdot Y_{AOB}}-\frac{\mathrm{ixbn}}{14}$
R2								-1			$1-f_u$	f_u	$\mathrm{ixbn}-f_u\cdot\mathrm{ixun}$	
R3			$-\frac{1}{Y_{NOB}}-\mathrm{ixbn}$	$\frac{1}{Y_{NOB}}$		$-\frac{1.14-Y_{NOB}}{Y_{NOB}}$			1					
R4									-1			f_u	$\mathrm{ixbn}-f_u\cdot\mathrm{ixun}$	
R5		$-\frac{1}{Y_{CMX}}-\mathrm{ixbn}$		$\frac{1}{Y_{CMX}}$		$-\frac{4.57-Y_{CMX}}{Y_{CMX}}$				1				$-\frac{1}{7\cdot Y_{CMX}}-\frac{\mathrm{ixbn}}{14}$
R6										-1	$1-f_u$	f_u	$\mathrm{ixbn}-f_u\cdot\mathrm{ixun}$	
R7		1	-2		3									-1
R8		-1			1	-1								-1
R9	$-\frac{1}{Y_H}$	$-\mathrm{ixbn}$	$\frac{1-Y_{HET}}{1.143\cdot Y_{HET}}$	$-\frac{1-Y_{HET}}{1.143\cdot Y_{HET}}$			1							$-\frac{\mathrm{ixbn}}{14}+\frac{1-Y_{HET}}{14\cdot 1.143\cdot Y_{HET}}$
R10	$-\frac{1}{Y_H}$	$-\mathrm{ixbn}$	$-\frac{1-Y_{HET}}{1.143\cdot Y_{HET}}$		$\frac{1-Y_{HET}}{1.143\cdot Y_{HET}}$		1							$-\frac{\mathrm{ixbn}}{14}$

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Table S4.1 Model “CMXH” kinetic rate equations related to HET nitrate and nitrite reduction

Process	**Reaction rate**	**Kinetic equation**
Anoxic growth of HET (S_{NO3}→S_{NO2})	*R9*	$\mu_{HET} \cdot \eta_{1,HET} \cdot \frac{K_{O,HET}}{K_{O,HET} + S_O} \cdot \frac{S_S}{S_S + K_{S,HET}} \cdot \frac{S_{NO3}}{S_{NO3} + K_{NO3,HET}} \cdot \frac{S_{NH4}}{S_{NH4} + K_{NH4,HET}} \cdot X_{HET}$
Anoxic growth of HET (S_{NO2}→S_{N2O})	*R10*	$\mu_{HET} \cdot \eta_{2,HET} \cdot \frac{K_{O,HET}}{K_{O,HET} + S_O} \cdot \frac{S_S}{S_S + K_{S,HET}} \cdot \frac{S_{NO2}}{S_{NO2} + K_{NO2,HET}} \cdot \frac{S_{NH4}}{S_{NH4} + K_{NH4,HET}} \cdot X_{HET}$

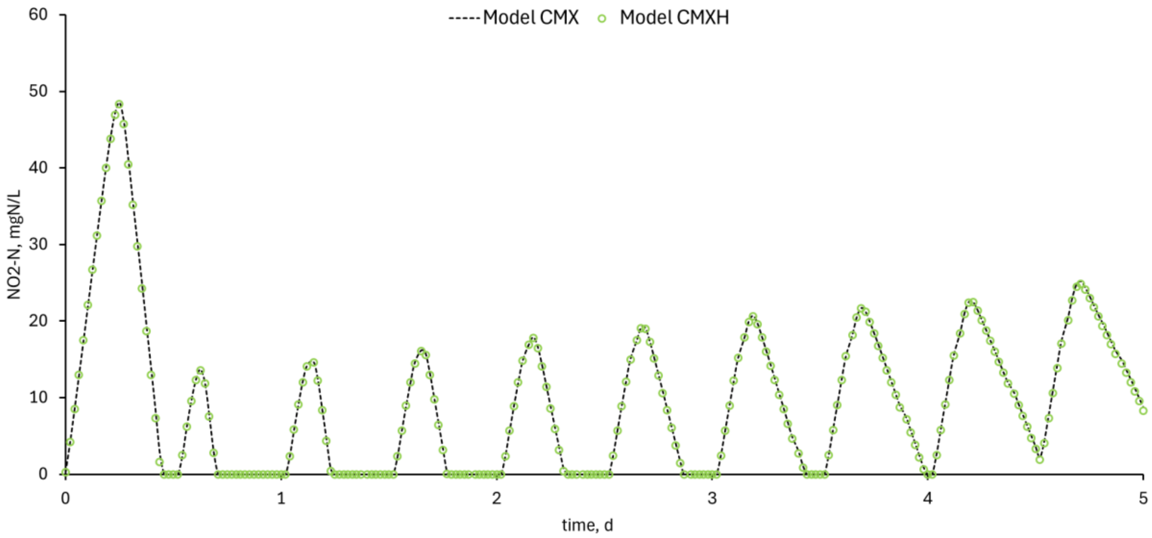

Figure S4.1 Model “CMX” and “CMXH” predicted NO_2-N concentrations in the long-term washout experiment during start-up phase (5 days) of SBR (DO=2.0mg O2/L; influent NH4-N 282 mgN/L; initial SRT=2.5d).

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S5 Initial biomass concentrations and composition

Table S.5 Initial biomass concentrations estimated for dynamic simulations

Nitrifying biomass	*Units*	*Long-term washout experiment*	
		DO=0.5 mgO_2/L	DO=2.0 mgO_2/L
X_{AOB}	mg COD/L	28	27
X_{NOB}	mg COD/L	80	54
X_{CMX}	mg COD/L	12	16

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S6 Model parameters related to HET, AOB, NOB, and CMX activity in the developed Model “CMX” and Model “CMXH”

Table S6.1 Kinetic parameters of Model “CMX” and Model “CMXH”.

Parameter	Description	Unit
	Heterotrophic bacteria, X_{HET}	
μ_{HET}	Maximum growth rate on substrate	d^{-1}
b_{HET}	Decay rate	d^{-1}
K_O	Half-saturation constant for oxygen	mg O_2/L
K_S	Half-saturation constant for readily biodegradable substrate	mg COD/L
K_{NO3}	Half-saturation constant for nitrate	mg N/L
K_{NO2}	Half-saturation constant for nitrite	mg N/L
K_{ALK}	Half-saturation constant for alkalinity	mole/L
$\eta_{1,HET}$	Nitrate reduction factor	–
$\eta_{2,HET}$	Nitrite reduction factor	–
	Ammonia oxidizing bacteria, X_{AOB}	
μ_{AOB}	Maximum growth rate of AOB	d^{-1}
b_{AOB}	Decay rate of AOB	d^{-1}
$K_{O,AOB}$	Half-saturation constant for oxygen	mg N/L
$K_{NH4,AOB}$	Half-saturation constant for free and ionized ammonia	mg N/L
$\eta_{1,AOB}$	Hydroxylamine oxidation pathway factor	–
$\eta_{2,AOB}$	AOB denitrification pathway factor	–
$K_{ALK,AOB}$	Half-saturation constant for alkalinity	mole/L
	Nitrite oxidizing bacteria, X_{NOB}	
μ_{NOB}	Maximum growth rate of NOB	d^{-1}
b_{NOB}	Decay rate of NOB	d^{-1}
$K_{O,NOB}$	Half-saturation constants for oxygen of NOB	mg N/L
$K_{NO2,NOB}$	Half-saturation constant for nitrite	mg N/L
$K_{INH\ NH4,NOB}$	Half-saturation constant for ammonia inhibition	mg N/L
	Comammox bacteria, X_{CMX}	
μ_{CMX}	Maximum growth rate of comammox	d^{-1}
b_{CMX}	Decay rate of comammox	d^{-1}
$K_{O,CMX}$	Half-saturation constant for oxygen	mg N/L
$K_{NH4,CMX}$	Half-saturation constant for free and ionized ammonia	mg N/L
$K_{ALK,CMX}$	Half-saturation constant for alkalinity	mole/L

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Table S6.2 State variables of Model “CMX” and Model “CMXH”.

Cryptic name	Variable name	Unit
X_{HET}	Active heterotrophic biomass	mg COD/L
X_{AOB}	Active AOB biomass	mg COD/L
X_{NOB}	Active NOB biomass	mg COD/L
X_{CMX}	Active CMX biomass	mg COD/L
S_{O}	Dissolved oxygen	mg O_2/L
S_{NH4}	Free and ionized ammonia	mg N/L
S_{NO2}	Nitrite nitrogen	mg N/L
S_{NO3}	Nitrate nitrogen	mg N/L
S_{ALK}	Alkalinity	mole/L

Table S6.3 Stoichiometric parameters of Model “CMX” and Model “CMXH”.

Cryptic name	Variable name	Unit
ixbn	Nitrogen content of active biomass	mg N / mg COD
ixun	Nitrogen content of inert mass	mg N / mg COD
fu	Fraction of biomass leading to particulate products	mg COD / mg COD
yh	HET yield	mg COD / mg COD
ya	AOB yield	mg COD / mg N
yb	NOB yield	mg COD / mg N
yc	CMX yield	mg COD / mg N

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