Pilot scale partial denitratation coupled anammox: an evaluation

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by

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Picture on front page: K5 carriers from PDA pilot. Photo by the author.

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Preface

This masters thesis has been carried out on behalf of Sweden Water Research. Lab and office space was provided by the Water and Environmental Engineering group at the Department of Chemical Engineering, Lund University. Samples were gathered at Källby wastewater treatment plant where the PDA pilot under study was located.

I would like to thank everyone at the Department of Chemical Engineering for including me in their welcoming workplace. I would especially like to thank Ellen Edefell for the kind help troubleshooting IC issues and Juho Uzkurt Kaljunen for the many fun and productive conversations during our time sharing an office.

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Finally I would like to extend my gratitude to my supervisors Per Falås and David Gustavsson for their invaluable help and support during this project.

Tobias Ellingsen

Lund, January 2022

Summary

In wastewater treatment plants (WWTP) which implement nitrogen removal, the most common process is nitrification followed by denitrification. This requires extensive aeration which comes at a large energy cost while at the same time reducing biogas potential. Anaerobic ammonium oxidation (anammox) is an alternative treatment process that requires significantly less aeration by using nitrite as an electron acceptor in the oxidation of ammonium to diatomic nitrogen gas. It has been successfully and widely implemented in the treatment of ammonium rich sidestreams that have a high temperature, such as sludge liquor from dewatered digester sludge. Implementation of anammox in the mainstream at wastewater treatment plants has the potential to result in an energy-positive process, where more energy is recovered as biogas than is used in the wastewater treatment. Practical implementation however remains challenging and the focus of much research. One promising approach is anammox coupled to partial denitrification (PDA) where nitrite is provided to anammox through the partial denitrification of nitrate to nitrite.

This thesis evaluates the performance of a mainstream PDA pilot at Källby WWTP (Lund, Sweden) during a period of 11 weeks. The pilot consists of three moving bed biofilm reactors configured for COD removal, nitrification and PDA and was fed flow proportionately to the WWTP with mainstream wastewater taken after screening and primary filtration.

After inoculation with biofilm carriers taken from a sidestream process the anammox, denitratation and denitritation activity were followed over the course of 11 weeks using ex-situ batch activity tests. A cycle study was also conducted in which samples were taken from the pilot regularly over the course of a day in order to observe activity and activity variation in-situ.

A significant specific anammox activity remained in the biomass at the end of the experimental period with batch tests showing 0.68 ± 0.11 g N_2-N $\cdot$ $m^{-2} \cdot d^{-1}$. A low anammox activity of 0.07 g N_2-N $\cdot$ $m^{-2} \cdot d^{-1}$ was also calculated to be present in-situ during the cycle study performed towards the end of the 11 week period. This indicates that anammox bacteria were not completely outcompeted in the biomass by denitrifiers. Over the course of the experimental period ex-situ tests showed the development of denitrification activity from low levels with max denitratation and denitritation reaching 0.60 and 0.35 g N $\cdot$ $m^{-2} \cdot d^{-1}$ respectively using acetate as the carbon source. The difference in these rates indicates the biomass is capable of supplying nitrite to anammox. The in-situ nitrogen removal rates were calculated in three different ways, yielding rates 85 - 34 % lower than the denitrification rates at nearby WWTPs. These low values suggest that the pilot was not able to achieve sufficiently high removal rates to be practically implementable.

It was revealed in ex-situ tests that the pilot biofilm was very sensitive to nitrite inhibition. The anammox activity was investigated at two different initial nitrite concentrations, 25 and 75 mg N $\cdot$ L^{-1} and the activity at the higher initial concentration was 94 % lower than that at the lower initial concentration. This is a lower inhibitory concentration than commonly reported in the literature and underscores the importance of investigating nitrite inhibition concentrations of a specific biomass prior to testing anammox activity.

Sammanfattning

På avloppsreningsverk (ARV) som tillämpar kväverening är den vanligaste tekniken nitrifikation följt av denitrifikation. Denna process kräver omfattande luftning, vilket både är elintensivt och minskar mängden biogas som kan utvinnas. Anaerob ammoniumoxidation (anammox) är en alternativ reningsmetod som minskar luftningsbehovet genom att nitrit används som elektronacceptor istället för syre i oxidationen av ammonium direkt till kvävgas. Anammox har fått stort genomslag i rening av varma avloppsströmmar med hög ammoniumhalt, så som i behandlingen av rejektvattnet som uppstår efter biogasrötning. Om anammox skulle tillämpas i rening av huvudströmmen på ARV finns potential att göra hela processen energipositiv, dvs. att mer energi utvinns som biogas än som går åt i behandlingen. Detta har i praktiken visat sig vara svårt att genomföra och mycket forskning bedrivs idag på området. En lovande tillnärmning är anammox kopplat till partiell denitrifikation (PDA), dvs. att nitrit produceras till anammox genom den partiella denitrifikationen av nitrat till nitrit.

Detta examensarbete utvärderar prestationen till PDA-piloten vid Källby ARV i Lund under en 11 veckors period. Piloten består av tre MBBR reaktorer (moving bed biofilm reactor) som utför COD-rening, nitrifikation och PDA. Inflödet till piloten togs flödesproportionerligt från reningsverket efter gallring och förfiltration.

Piloten ympades med nya bärare från en befintlig rejektvattensbehandling och följdes sedan under 11 veckor. Under denna tid utfördes regelbundna aktivitetstest på bärare från piloten i labbet. En cykelstudie genomfördes även mot slutet av perioden för att undersöka aktiviteten i själva piloten samt dygnsvariationen i aktivitet. Detta gjordes genom att prover togs vid flera punkter i piloten under en dag.

En betydande anammoxaktivitet (SAA) fanns kvar i biomassan vid undersökningens slut, i labbförsök uppmätt till 0.68 ± 0.11 g N_2-N $\cdot$ m^{-2} $\cdot$ d^{-1} samt i piloten den lägre aktiviteten 0.07 g N_2-N $\cdot$ m^{-2} $\cdot$ d^{-1}. Detta visar att anammoxorganismerna inte helt utkonkurrerades från biomassan av denitrifierare. Från nivåer nära noll utvecklades en denitratations- och denitritations-aktivitet fram under undersökningsperioden med slutgiltiga värden på 0.60 and 0.35 g N $\cdot$ m^{-2} $\cdot$ d^{-1}, med acetat som kolkälla. Skillnaden mellan dessa värden indikerar att biomassan är förmögen att tillgodose anammox med nitrit. Kvävereningshastigheten beräknades på tre olika sätt till 85 - 34 % lägre än denitrifikaitonshastigheten vid närliggande ARV. Detta visar att piloten inte uppnådde tillräckliga reningshastigheter för att kunna implementeras i stor skala.

Labbtesten visade att biomassan var mycket nitritkänslig. Anammoxaktiviteten undersöktes vid initiala nitritkoncentrationer på 25 och 75 mg N $\cdot$ L^{-1}. Aktiviteten vid den högre initiala koncentrationen var 94 % mindre än vid den lägre initiala koncentrationen. Detta är en lägre inhibitionskoncentration än vad som generellt anges i litteraturen och visar på vikten att undersöka nitritinhiberingskoncentrationen hos en specifik biomassa innan anammoxaktivitet mäts.

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1 Introduction

Many municipal wastewater treatment plants (WWTP) in Sweden and around the world face the dual pressures of reducing nutrient effluents while at the same time reducing their costs and climate impact. This incentivises research into new treatment methods that reduce energy and chemical usage, maximise energy extraction from wastewater in the form of biogas while at the same time maintaining or improving treatment efficiency.

In a regional context the Baltic sea suffers from poor ecological status driven by anthropogenic nitrogen (N) and phosphorous (P) loading leading to eutrophication (HELCOM 2015). This has prompted contributing countries, including Sweden, to agree to nutrient reduction targets within the framework of the HELCOM Baltic Sea Action Plan (BSAP) (HELCOM 2013). According to the latest published pollution load compilation (HELCOM 2015) 4% of the total waterborne nitrogen input came from point sources discharging directly into the Baltic sea in 2010. The majority of this comes from municipal WWTPs with Sweden contributing the most point-source N per country. This shows the importance of continued work with improving wastewater treatment facilities.

Nitrogen presents in municipal wastewater mainly in the form of ammonium (NH_4^+). In conventional biological nitrogen removal (BNR) the ammonium is first fully oxidised via nitrite (NO_2^-) to nitrate (NO_3^-) in a process known as nitrification. After which nitrate is anoxically reduced via nitrite, nitric oxide (NO) and nitrous oxide (N_2O) to diatomic nitrogen gas (N_2) in a process called denitrification. Nitrification requires a high dissolved oxygen (DO) content leading to the process step being aerated. The aeration is negative for the overall energy balance of the WWTP in two ways; firstly the aeration pumps are a major electricity consumer. Secondly the high DO content leads to the proliferation of heterotrophic microorganisms that consume most of the chemical oxygen demand (COD) in the water. This in turn reduces the biogas potential.

Aeration constitutes 16-76% of the total electricity usage at Swedish WWTPs (Lingsten & Lundkvist 2008; Balmér 2018). In total biological treatment likely stands for more than 50% at most plants, with sludge and pre-denitrification recirculation being the other major contributors (Balmér 2018).

These factors have, in recent years, driven the search for treatment processes that reduce the energy usage and increase the biogas potential while maintaining high treatment efficiency. One such process is anaerobic ammonium oxidation (anammox).

Anammox is performed by autotrophic anaerobic ammonium oxidising bacteria (AnAOB) which are able to directly oxidise ammonium to dinitrogen gas using nitrite as an electron acceptor (Strous et al. 1998). Discovered in the late 1980's anammox has rapidly been implemented around the world in the treatment of ammonium rich wastewater streams at high temperatures (Lackner et al. 2014). If, as is the case in municipal wastewater, there isn't a significant amount of nitrite in the waste stream it can be produced in one of two ways. Either through partial nitrification (PN), where nitrite oxidation is inhibited or through full nitrification followed by partial denitrification (PD) where reduction of nitrite is inhibited, ie. denitratation. Coupled to anammox these two methods (PNA and PDA) both have the potential to significantly reduce the need for aeration (57 and 48% respectively) as well as the sludge production (84 and 66%) (Zhang et al. 2019). PNA being fully autotrophic and PDA being a hetertrophic/autotrophic hybrid also leads to the processes consuming significantly less COD

than BNR (Zhang et al. 2019). Taken together these factors lead to both methods having significant potential energy savings. PNA, when implemented in the mainstream, has been shown to theoretically be energy positive (Kartal et al. 2010) and while no such calculations were found for PDA it seems likely the same would apply, given the similar energy savings.

While application of PNA to ammonium rich sidestreams at high temperature such as sludge liquor from dewatered, anaerobically digested sludge is common today, this is not the case in the treatment of the mainstream at WWTPs. The main challenges facing mainstream PNA are competition from heterotrophic bacteria in the biomass, trouble inhibiting nitrite oxidising bacteria (NOB) due to low ammonium and nitrous acid concentrations and the low activity of the autotrophic AnAOB at low temperatures (Cao et al. 2017).

PDA has recently been the focus of much research. Many lab-scale experiments have been performed to investigate the effect of different process parameters, with several achieving total nitrogen removal efficiencies of 80-95% (Cao et al. 2021). While some larger scale studies have been conducted, (Li et al. 2019; Wang et al. 2021; Zhao et al. 2021), upscaling the PDA process remains understudied with challenges remaining such as maintaining AnAOB activity at low temperatures and maintaining nitrite competitiveness for AnAOB under mainstream conditions.

After PNA pilot experiments at the Sjölunda WWTP (Malmö, Sweden), (Stefansdottir 2014; Gustavsson et al. 2020), Sweden Water Research conducted a thesis project studying PDA at lab-scale (Holmin 2020). Building upon this a PDA pilot was constructed at Källby WWTP (Lund, Sweden), which is the subject of this thesis.

1.1 Aim

The overarching aim of this thesis is contributing to the development of a viable mainstream PDA treatment process for municipal wastewater through evaluating the performance of the PDA pilot at Källby WWTP.

Specifically this thesis set out to combine pilot measurements with ex-situ batch activity tests in order to assess whether:

- Anammox activity could be maintained in the PDA pilot.
- Partial denitratation could provide sufficient nitrite to anammox in the PDA pilot.
- Practically relevant nitrogen removal rates could be achieved in the PDA pilot.

2 Background

2.1 Nitrification and denitrification

Nitrogen is found in municipal wastewater mainly in the form of ammonium. Traditional WWTP removal process have mainly been in the form of BNR comprising of assimilation, nitrification and denitrification (Khunjar et al. 2014), see Figure 2.1.

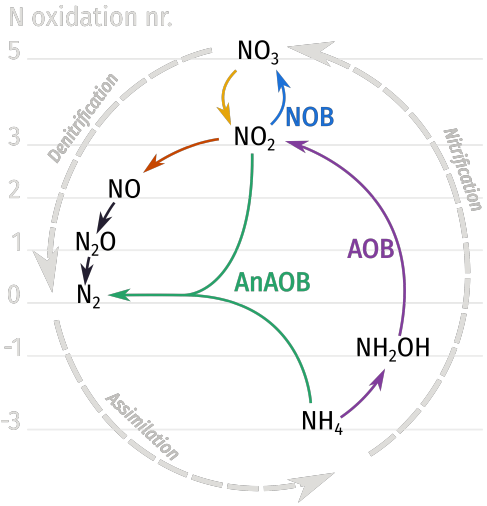


Figure 2.1.Overview of the nitrogen cycle and reaction pathways of aerobic ammonia oxidising bacteria (AOB), nitrite oxidising bacteria (NOB) and anaerobic ammonia oxidising bacteria (AnAOB).

2.1.1 Nitrification

Full nitrification is the oxidation of ammonium, first to nitrite and then to nitrate. The oxidation to nitrite is performed by (aerobic) ammonia oxidising bacteria (AOB) and archaea (AOA). NOB then complete the oxidation to nitrate. This simplified outline is being complicated by recent research showing additional pathways taken by AOB from hydroxylamine (NH_2OH) to nitric oxide and nitrous oxide (Khunjar et al. 2014; Caranto & Lancaster 2017).

AOB and NOB are primarily chemolithoautotrophs and as such have a comparatively slow growth rate compared to the hetertophic denitrifiers (Khunjar et al. 2014).

2.1.2 Denitrification

Denitrification is the reduction of nitrate or nitrite to diatomic nitrogen gas. Nitrate and nitrite are generally the most energetically favourable electron acceptors in anoxic environments and most denitrifiers, comprising a variety of bacterial genera but also archaea, are heterotrophic facultative anaerobes (Zumft 1997; Liu et al. 2013). Full denitrification comprises four reductions, catalysed by different enzymes; nitrate reduction (nitrate reductase), nitrite reduction (nitrite reductase), nitric oxide reduction (nitric oxide reductase), and nitrous oxide reduction (nitrous oxide reductase) (Liu et al. 2013).

Denitrifiers oxidise organics, in municipal wastewater often in the form of volatile fatty acids (VFA) such as acetate (CH_3COOH) or propionate ($CH_3CH_2CO_2$) in order to generate biomass which is often approximated by $C_5H_7NO_2$. Of particular interest in the context of PDA is nitrate and nitrite reduction, called denitratation and denitritation.

The exact stoichiometric relationships of denitratation and denitritation are dependent on the stoichiometry of the carbon source, the yield constant, that is to say biomass formation per mass carbon source consumed, of the specific biomass to the carbon source as well as the biomass composition. Given the biomass composition above, acetate as carbon source and assuming yield constants, the reactions can be described as (1) and (2) respectively (Strohm et al. 2007; Ma et al. 2017).

$$0.048\,NH_4^+ + NO_3^- + 0.37\,CH_3COO^- + 0.32\,H^+ \rightarrow NO_2^- + 0.048\,C_5H_7NO_2 + 0.64\,H_2O + 0.5\,CO_2 \quad (1)$$

$$0.12\,NH_4^+ + NO_2^- + 0.68\,CH_3COO^- + 1.57\,H^+ \rightarrow 0.5\,N_2 + 0.12\,C_5H_7NO_2 + 1.62\,H_2O + 0.75\,CO_2 \quad (2)$$

Martienssen & Schöps (1997) classify denitrifiers into three groups according to their ability to reduce nitrate and nitrite, see Table 2.1. Group A is only capable of reducing nitrate to nitrite. Group B can reduce both nitrate and nitrite with preferential and more rapid nitrite reduction leading to no nitrite accumulation. Group C can reduce both nitrate and nitrite with more rapid nitrate reduction, leading to nitrite accumulation.

Table 2.1. Classification system for denitrifying bacteria by Martienssen & Schöps (1997). Arrow thickness denoting relative reaction rate.

Group A	$NO_3^- \rightarrow NO_2^-$
Group B	$NO_3^- \rightarrow NO_2^- \rightarrow N_2$
Group C	$NO_3^- \rightarrow NO_2^- \rightarrow N_2$

2.2 Anammox

A history of the discovery, investigation and implementation of anammox can be found in Kuenen (2008). The process was first observed in the late 80's and patented (Mulder 1989). During the 90's studies were performed to determine the reaction pathways, stoichiometry and ultimately the responsible microorganisms. AnAOB were also discovered to already exist in wastewater treatment plants (Egli et al. 2001) as well as to play a significant role in the N_2 production in marine environments making it a major contributor to the global nitrogen cycle (Dalsgaard et al. 2005). The first full-scale treatment step was in full operation in 2006 in The Netherlands after a two year startup period treating a high ammonium side-stream at an existing WWTP. In 2014 over 100 full-scale anammox plants were reported, with many new plants likely entering operation since then (Lackner et al. 2014).

Energy in anammox is generated from the partial reaction (3) which is then used for cell synthesis. The stoichiometry of cell synthesis appears to vary based on process configuration and several values can be found in the literature, e.g. in Lotti et al. (2014b) and Zhang et al. (2018). For the purposes of this thesis the stoichiometric relationship shown in (4) from Strous et al. (1998) will be assumed.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\,H_2O \quad (3)$$

$$NH_4^+ + 1.32\,NO_2^- + 0.066\,HCO_3^- + 0.13\,H^+ \rightarrow 1.02\,N_2 + 0.26\,NO_3^- + 0.066\,CH_2O_{0.5}N_{0.15} + 2.03\,H_2O \quad (4)$$

Typically AnAOB have been considered to be slow-growing organisms. Strous et al. (1998) reported a max specific growth rate (μ_{max}) at 30 °C of 0.065 d^{-1} translating to a doubling time (t_d) of 11 d. The growth rate also appears to be highly temperature dependent with values at 15 and 10 °C being reported as μ_{max} = 0.017 d^{-1}, t_d = 41 d and μ_{max} = 0.011 d^{-1}, t_d = 63 d respectively (Hendrickx et al. 2014; Lotti et al. 2014a). The situation has been complicated somewhat with the successful selection of a high growth-rate anammox strain with a μ_{max} = 0.33 d^{-1}, t_d = 2 d at 30 °C (Lotti et al. 2015a). This indicates that the growth rate also can be highly dependent on the specific anammox biomass.

Anammox activity is also generally considered to be highly temperature dependent with specific anammox activity at 10 °C reported to be well below 20 % of that at 30 °C (Stefansdottir 2014; Lotti et al. 2015b). However variation in low-temperature activity response appears to differ somewhat between different biomasses. Nitrogen removal rates (NRR) have however been shown to be maintainable at lower temperatures using strategies such as supplementing AnAOB biomass from a high-temperature sidestream (Gustavsson et al. 2020).

AnAOB can grow both in suspended culture as well as immobilised in biofilm or granules (Lotti et al. 2014b; Du et al. 2017a; Gustavsson et al. 2020).

2.3 Partial nitrification anammox

The most commonly implemented type of anammox process in wastewater treatment today is PNA. In municipal wastewater treatment the process is most commonly applied to treating the ammonium rich and high temperature sludge liquor from dewatered digester sludge. Kartal et al. (2010) calculated that applying anammox to this sidestream could decrease WWTP net energy consumption by 52% from 44 to 21 $Wh \cdot p^{-1} \cdot d^{-1}$ while treating all the wastewater in the main treatment stream with PNA could lead to a net energy production of 24 $Wh \cdot p^{-1} \cdot d^{-1}$. These calculations include aeration and mixing energy in biological treatment as well as energy production from biogas. The calculated BNR biostep electricity consumption is in-line with that reported for the largest WWTPs in Sweden according to Balmér (2018) but is an underestimation for smaller plants.

Anammox produces NO_3^- at a molar ratio of 0.26 / 2.32 ≃ 11% given the stoichiometry in (4). Given perfect NOB inhibition this limits the theoretical max nitrogen removal efficiency of PNA to around 89%.

According to Cao et al. (2017) some of the main process control aspects required for successful mainstream PNA implementation are efficient suppression of NOB, high AnAOB activity as well as preventing the AOB from being outcompeted by heterotrophic bacteria (HB). AnAOB activity can be highly temperature dependent (Lotti et al. 2015b) and thus the higher temperatures of digester effluent allow for high AnAOB activity. Similarly in such streams the high free ammonia and free nitrous acid concentrations have an inhibitory effect on NOB, which is not attainable in mainstream treatment (Cao et al. 2017).

Significant progress towards mainstream PNA has been made in recent years from lab to full scale (Cao et al. 2017). For example Cao et al. (2018) describes how PNA has been observed in treatment train 2 of the recently constructed, step-fed activated sludge, Changi water reclamation plant in Singapore. During a study period from 2011 to 2016 it was estimated that approximately 30% of the ammonium was removed in the anoxic zones, thus attributable to PNA. The total nitrogen (TN) removal efficiency (NRE) during this period was 86% with an average total inorganic nitrogen (TIN) effluent concentration of 4.6 mg $N \cdot L^{-1}$ (average of yearly averages). It should be noted that the wastewater in the treatment has a natural temperature of around 30 °C. Further Wett et al. (2015) describe how a high degree of NOB suppression and AnAOB enrichment were achieved in the Strass WWTP in Austria during winter months. However the amount of nitrogen removal attributable to anammox was not assessed.

While these results are promising, NOB suppression and maintaining high AnAOB activity under low temperatures often remain a challenge. One proposed strategy to address the latter concern is sidestream augmentation, where AnAOB biomass is transferred from high temperature sidestreams. This was done in a large scale pilot by Gustavsson (2020) achieving a relat-

ively temperature independent NRR of 0.42 g N_2-N · m^{-2} · d^{-1} at temperatures of 10-23 °C. However NOB suppression remained an issue with a relative nitrate production of 40%.

2.4 Partial denitritation anammox

PDA has been proposed as an alternative process in order to achieve mainstream anammox and has in the past decade been the focus of much research. In contrast to PNA, PDA systems fully nitrify around half of the incoming wastewater producing nitrate, then supply nitrite to AnAOB through partial denitrification, creating conditions where AnAOB are able to outcompete for nitrite the organisms performing denitritation.

Much of the research so far has been lab-scale, using artificial wastewater and not at low temperatures. While many of them have achieved nitrogen removal efficiencies > 90 % less is known about how PDA functions under the varying conditions of full scale treatment (Du et al. 2019b; Cao et al. 2021).

Unlike PNA, which has a theoretical maximum NRE of 89 %, PDA could achieve 100 %. In PNA insufficient NOB inhibition can lead to significant nitrate accumulation in the effluent (Gustavsson et al. 2020), while insufficient inhibition of denitritation in PDA results in full denitrification, at a cost of increased energy and COD consumption. It has been suggested that PDA can be configured such that during operation it can shift between partial and full denitrification, allowing flexibility to meet effluent demands during periods when temperature may inhibit anammox activity (Le et al. 2019a).

PDA was observed after addition of biofilm carriers to the anoxic zones of the mainstream A/A/O process at the Xi'an WWTP (China). Operating at temperatures of 11-25 °C the plant was able to maintain an effluent TIN concentration of 6.4-8 mgN/L. The NRE was not reported but the 16% of the nitrogen removal was likely due to PDA (Li et al. 2019; Wang et al. 2021). In a pilot-scale setup with wastewater from the ShaHe WWTP (China) Zhao et al. (2021) where able to achieve up to 45% contribution of PDA to nitrogen removal with an average NRE of 74% and effluent TIN of 9.8 mgN/L, albeit at an undisclosed temperature.

PDA appears mostly to have been studied in immobilised setups in a multitude of different reactor configurations (Cao et al. 2021). These include one-stage designs where denitrification and anammox take place in the same reactors as well as two-stage designs in which they take place in different reactors. Some examples of configurations are sequencing batch reactors (SBR), upflow anaerobic sludge blanket reactors (UASB) and moving bed biofilm reactors (MBBR) (Du et al. 2017b, 2019a; Holmin 2020; Han et al. 2021).

In two-stage reactor configurations as well as in suspended growth processes it is crucial to establish a nitrite residual, as the mass transfer takes place in the liquid phase. This is not necessary in attached growth systems however, as the mass transfer takes place within the biofilm. Here instead competition and diffusion dynamics within the biofilm are likely important in nitrite supply.

Several factors have been shown to affect either nitrite accumulation or the competitive balance between AnAOB and organisms performing denitritation. These include the carbon source, COD/NO_3^--N ratio and operation under famine or feast conditions, DO concentration, presence of a nitrate residual and pH (Gong et al. 2013; Le et al. 2019a, 2019b; Ma et al. 2020; Cao et al. 2021; Chen et al. 2021). These factors may both have a short term effect on the microorganisms in the process as well as exert a long-term selection pressure on the micro-ecology in the biomass as a whole (Ma et al. 2020).

The autotrophic AnAOB generally have a significantly slower growth rate than the heterotrophic denitrifiers, posing the risk that AnAOB can be outcompeted in the biomass (Cao et al. 2021). COD/NO_3^--N, DO and process type and operation are significant control parameters that can be used to establish and maintain the correct biomass composition (Le et al. 2019b; You et al. 2020; Cao et al. 2021).

A few main challenges remain in the successful establishment of mainstream PDA. These include achieving a high percentage of anammox contribution to nitrite reduction, i.e. in achieving a significant competitive advantage to AnAOB over organisms performing denitritation. A further challenge is maintaining a high anammox activity, especially in colder conditions and preventing the out-competition of AnAOB by denitrifiers in the biomass.

2.5 MBBR

A moving bed biofilm reactor (MBBR) is a type of attached growth process in which biofilm grows on small plastic carriers that circulate freely within the reactor. This retains active biofilm within the reactor, simplifying sludge separation and preventing wash-out. MBBR was developed in Norway in the 1980's and has been implemented for many different processes, including nitrification, denitrification, COD removal and anammox (Ødegaard 2006; Lackner et al. 2014; Cao et al. 2021).

MBBR reaction kinetics are highly dependent on transport phenomena within the biofilm. la Cour Jansen & Harremoës (1985) show that in cases when the substrate fully penetrates the biomass, zero-order rate kinetics are applicable and in cases of less than full penetration the rate is diffusion limited and half-order kinetics are applicable.

3 Method

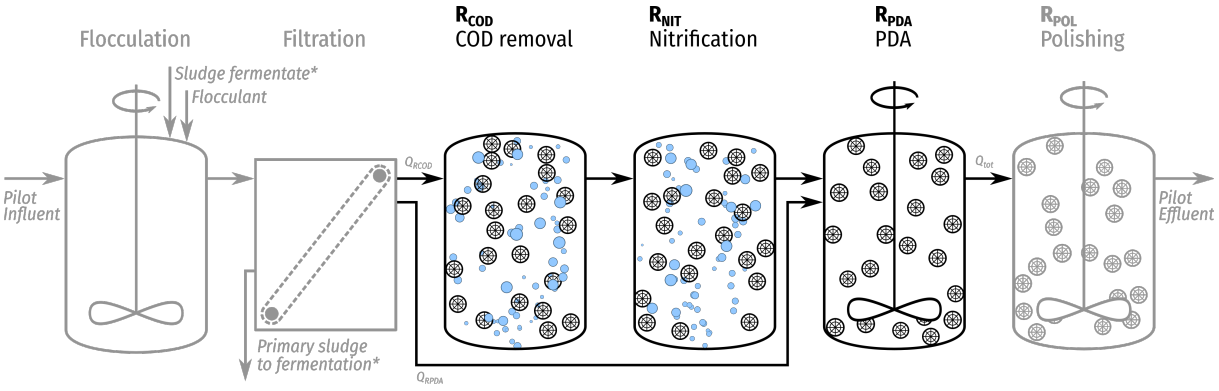
3.1 Pilot setup

The PDA pilot that is the topic of this thesis was a part of a larger pilot project at the Källby WWTP in Lund, Sweden. The pilot had flow-proportional (with upper bound; compared to the main plant) influent, pumped from the WWTP´s inlet after the screening. The influent was dosed with a cationic polymer in a stirred flocculation reactor followed by rotating belt filtration with a filter size of 350 µm. The pilot was designed such that filter-sludge could be hydrolysed and fermented to produce VFAs and that the fermentate could be returned to the flocculation reactor. However, during the experimental period of this thesis sludge fermentate addition was not in operation. The filter supernatant comprised the influent to the PDA portion of the pilot.

A process diagram of the PDA pilot can be seen in Figure 3.1. It consisted of three reactors, R_{COD}, R_{NIT}, and R_{PDA}. R_{COD} and R_{NIT} were aerated and designed to achieve COD reduction and complete nitrification respectively. R_{PDA} was anoxic and intended to achieve PDA. Following the PDA pilot was R_{POL}, which acted as a final polishing step. All reactors contained Anox^{TM} K5 biofilm carriers with a specific surface area of 800 $m^2 \cdot m^{-3}$. The reactor volumes, hydraulic retention times (HRT), carrier filling ratio and surface area can be seen in Figure 3.1.

The filter supernatant constituting the PDA pilot influent was split into two flows, Q_{RCOD} and Q_{RPDA}, entering R_{COD} and R_{PDA} respectively. Q_{RCOD} passes through R_{COD} and R_{NIT} before entering R_{PDA}, thus the total inflow to R_{PDA}, $Q_{tot} = Q_{RCOD} + Q_{RPDA}$. The bypass ratio is defined as

$$\text{bypass ratio} = \frac{Q_{RPDA}}{Q_{tot}} \tag{5}$$



Volume (L)	70	114	118	285
Mean HRT (h)	2.5	3.9	2.6	
Filling ratio (%)	25	40	40	40
Total carrier area (m^2)	15	34	38	93

*Figure 3.1.Pilot process diagram and reactor parameters. ⊛ denotes reactors with biomass carriers. ● denotes aeration. * Sludge fermentation not operational during exp. period.*

The pilot was equipped with automatic flow proportionate diurnal composite samplers, located to sample from the influent to R_{COD} and in R_{PDA}. Samples were analysed on a semi-regular basis, together with grab-samples from R_{COD}, R_{NIT} and R_{PDA} by WWTP staff. The pilot inflow was continuously logged with a resolution of 3 min.

3.1.1 Pilot operations

The experimental period of this thesis was from the 10th of September until the 24th of November 2021. The pilot was in operation for several months prior to this but due to declined anammox activity it was decided to introduce new carriers to the process. This was done on the 9th of September. The carriers were taken from the digester sludge liqour PNA process at Ryaverket WWTP in Gothenburg. A new reactor, R_{PDA}, was introduced to the pilot together with the new carriers and the reactor with the original carriers (R_{POL}) was kept as a final polishing step.

Issues with the primary filtration unit resulted in irregular inflow to the pilot for around a month from the 5th of October to the 5th of November. For 14 days from the 6th to the 20th of October the pilot was run without primary filtration.

The bypass ratio, Eq. (5), was adjusted approximately seven weeks prior, from 42 to 36 %, as well as immediately subsequent, 34 %, to the commencement of the experimental period. Daily average influent flow, Q_{tot}, and bypass ratio can be seen in Figure I.1, Appendix I. The temperature generally fell from 21.7 °C at the start of the experimental period to 15.6 °C at the end with a max and min temperature of 21.8 and 15.1 °C respectively. The daily average temperatures can be seen in Figure I.2, Appendix I. These temperatures can be seen in relation to the 10 °C design temperature for denitrification at nearby WWTP's (Mases et al. 2010).

3.2 Ex-situ batch activity tests

3.2.1 Setup and procedure

The development of the biomass activity was assessed through ex-situ batch tests. K5 carriers were extracted from the pilot's R_{PDA} reactor (Figure 3.1) the morning of the test. The tests were performed in 2 L, lidded glass reactors with stirring paddles, Figure 3.2. The entire experimental setup can be seen in Figure 3.3.

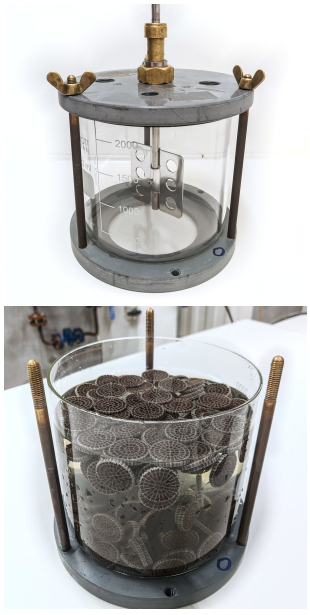


Figure 3.2. Empty reactor (top), reactor with carriers (bottom)

Each test contained 225 carriers and had a total liquid volume of 1700 mL. The reactor liquid contained tap-water, a carbon source and a combination of ammonium, nitrite and nitrite unique to each test and detailed in Table 3.1 below as well as a trace element solution.

Each test had a duration of 2 h and two tests were performed at once. During a test the reactors were stirred at 68 rpm with IDA EUROSTAR 20 stirrers and the temperature was controlled using a water-bath together with a heating (Grant GD100) and cooling (Grant C1G) unit. The pH and temperature were monitored using pH electrodes (Endress+Hauser Liquiline and WTW Multi 3620 IDS) and adjusted with 1 M HCl and NaOH solution. The pH and temperature set-points were 7.0 and 20.0 °C respectively.

An anoxic environment was established and maintained by bubbling N_2 gas into the reactor for a period of time before and during each test. The dissolved oxygen (DO) content of each reactor was monitored using a DO probe (Hach HQ40d with LDO probe).

10 mL samples were extracted from each reactor at 1, 20, 40, 60, 80, 100 and 120 min using a syringe. The samples were immediately filtered with a 0.45 µm syringe filter (GVS membrane filter) and refrigerated at 4 °C.

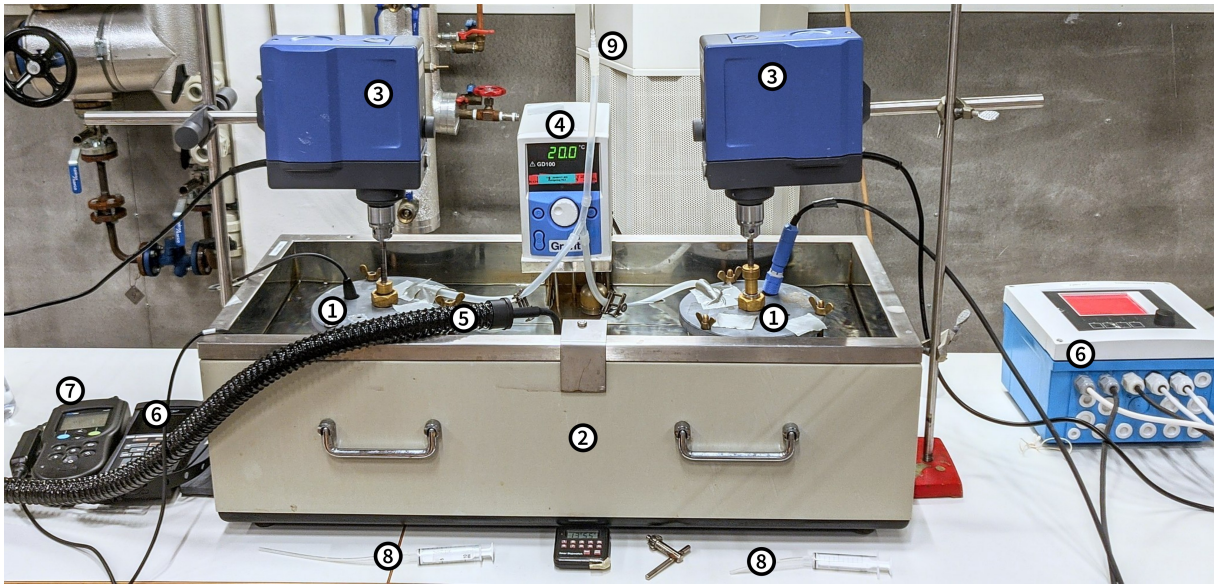


Figure 3.3: Experimental setup with the following components: reactors (1) submerged in a water-bath (2) and stirred with stirrers (3). Heating and cooling units (4, 5). Combined pH and temperature meters (6) and DO meter (7). Syringes (8) and N_2 supply (9).

Seven different types of activity test were performed; denitratation with acetate as carbon source (NA-A), denitritation with acetate as carbon source (NI-A), denitratation with propionate as carbon source (NA-P), anammox with hydrogen carbonate as carbon source (AMX) and partial denitratation coupled to anammox with acetate and hydrogen carbonate as carbon source (PDA). Additionally, the anammox with hydrogen carbonate and denitritation with acetate as carbon source tests were performed with lower nitrite concentrations (AMX-Lo and NI-A-Lo).

Nitrate, nitrite and ammonium were added to the test reactors in the form of 5 g N · L^{-1} KNO_3, $NaNO_2$ and NH_4Cl solution in order to reach the target concentrations shown in Table 3.1. Likewise, with acetate and propionate in the form of 20 g COD · L^{-1} $NaCH_3COO$ and propionic acid ($CH_3CH_2COO^-$) solution as well as hydrogen carbonate in the form of 3 g C · L^{-1} $NaHCO_3$ solution. The target concentrations were based on Stefansdottir (2014) with the AMX-Lo and NI-A-Lo tests performed with lower nitrite concentrations. Additionally, a trace element solution was added to the test reactors according to Holmin (2020).

Table 3.1.Target concentrations of nutrients and carbon source in ex-situ batch activity tests.

	NA-A [mg·L^{-1}]	**NI-A** [mg·L^{-1}]	**NI-A-Lo** [mg·L^{-1}]	**AMX** [mg·L^{-1}]	**AMX-Lo** [mg·L^{-1}]	**NA-P** [mg·L^{-1}]	**PDA** [mg·L^{-1}]
NO_2-N		75	30	75	30		
NO_3-N	75					75	75
NH_4-N				75	75		75
Acetate-COD	300	300	300				300
Propionate-COD						300	
HCO_3^-				45	45		45

3.2.2 Analysis

All samples collected from the test reactors were diluted and analysed for nitrate-, nitrite- and ammonium- concentration using an ion chromatograph (Metrohm Eco IC). Additionally, the 1 and 120 min samples from the NA-A, NI-A, NA-P and PDA test reactors were analysed for COD using LCK314 and LCK114 HACH-Lange cuvettes and a DR2800 spectrophotometer.

3.2.3 Activity calculation

In this context the biomass activity, r_x, is characterised by the mass in grams of nitrogen of species x consumed per square meter of carrier surface area and day [g N · m^{-2} · d^{-1}].

This was calculated by first plotting the change in x N concentration over time and calculating the slope of the linear regression, which has the unit of [g N · L^{-1} · d^{-1}]. Multiplying with the reactor volume, V_{avr}, and dividing the total carrier area, A_c, yields r_x as can be seen in (6). Since the reactor volume changes over the course of a test as samples are extracted the time-weighted average was used.

$$r_x = \frac{\text{slope}}{A_c} \cdot V_{avr} \tag{6}$$

In a one-stage reactor PDA implementation the denitratation, denitritation and anammox reactions are coupled though production, consumption and assimilation of ammonium, nitrate and nitrite. In the NA-A, NA-P, NI-A and AMX tests, including the low nitrite variants, the reactor conditions are configured in such a way that each process can be studied separately. This is done in the AMX tests by limiting the availability of a carbon source required by the heterotrophic denitrifiers, in the NI-A tests by not adding the nitrate or ammonium required for denitratation or anammox and in the NA-A tests by not adding the ammonium required for anammox and studying the change in nitrate concentration, which is unaffected by denitritation. Following from the assumption that only one of the three reactions are affecting the target species concentration it can be assumed that the nitrate activity in the NA tests is equal to the denitratation activity (dna), the nitrite activity in the NI-A tests is equal to the denitritation activity (dni) and that the ammonium activity in the AMX tests is equal to the anammox activity (amx).

However, in the PDA tests all three reactions are taking place at once and must be taken into consideration when determining dna, dni and amx. This is done through solving the system of linear equations that describe the contribution of the three reactions to the observed species removal activity. Here the coefficients are taken from the stoichiometric relationships described in Eq. (1),(2) and (4), which is specific to the carbon source used in denitrification.

$$\begin{aligned} r_{NO3} &= \text{dna} - 0.26\,\text{amx} \\ r_{NO2} &= -\text{dna} + \text{dni} + 1.32\,\text{amx} \\ r_{NH4} &= 0.048\text{dna} + 0.12\,\text{dni} + \text{amx} \end{aligned} \tag{7}$$

3.2.4 Specific anammox activity and anammox contribution

The specific anammox activity (SAA) is here defined in relation to N_2-N formation instead of in relation to NH_4^+-N removal. Here the stoichiometric relationship between N_2-N and NH_4^+-N, seen in Eq. (4) is used. The SAA value is useful for comparison with other publications.

$$\text{SAA} = 2.04 \cdot \text{amx} \quad [\text{g}\,N_2\text{–N} \cdot \text{m}^{-2} \cdot \text{d}^{-1}] \tag{8}$$

The fractional contribution of anammox to N_2 formation, given acetate as carbon source and the stoichiometry in Eq. (2), can be calculated as

$$\text{amx cont.} = \frac{1.02 \cdot \text{amx}}{1.02 \cdot \text{amx} + 0.5 \cdot \text{dni}} \tag{9}$$

3.3 Pilot cycle study

3.3.1 Sampling and sample analysis

On the 10th of November sampling was conducted at the pilot over the course of 8 h in order to study the pilot activity across different inflow conditions. The samples were taken from 07:30 until 15:30 in order to capture the entirety of a typical morning flow and concentration peak. The type and frequency of samples can be seen in Table 3.2. Samples were collected from the PDA pilot inflow (filter effluent) as well as in the R_{COD}, R_{NIT} and R_{PDA} reactors. The filtered samples were immediately filtered using 0.45 µm syringe filters. All filtered samples were analysed using ion chromatography (IC) for ammonium-, nitrate- and nitrite- concentration as well as using Hach-Lange colorimetry for COD. The unfiltered samples were analysed using Hach-Lange colorimetry for total nitrogen concentration (totN) and COD.

Table 3.2. Sample type, frequency and analysis method conducted during the pilot daily cycle study.

Sample type	Frequency	Analysis
Filtered grab samples	Every 30 min from 07:30 until 15:30.	IC (NH_4^+, NO_3^-, NO_2^-) COD spectrophotometry
Unfiltered grab samples	07:30, 09:00 and every subsequent 2 h until 15:00.	Total nitrogen spectrophotometry COD spectrophotometry

Online flow measurements for Q_{RCOD} and Q_{RPDA} with a resolution of 3 min were logged and dissolved oxygen concentration (DO) and temperature sampled six times across the day.

3.3.2 Data interpolation

The filtered 30 min sample analysis results were linearly interpolated to 3 min data points and used in the subsequent data analysis. The unfiltered samples were interpolated non-linearly to 30 min data points using the function `inpaints_nans` method 0 (D'Errico 2006) and then linearly interpolated to 3 min data points.

3.3.3 Data analysis

Mass balance and reaction rate constant

R_{COD}, R_{NIT} and R_{PDA} can be analysed as continuously stirred tank reactors (CSTRs). In order to calculate dna, dni and amx as well as COD removal, the nitrate, nitrite, ammonium and COD removal rates must be determined. This can be done by studying the mass balances across the reactors. In reality the flow into/out of a reactor as well as the inflow concentration varies continuously, however here they are assumed to be constant across the 3 min time intervals.

Assuming 0-order reactions and with *M(t)* denoting the mass of a species in a control volume at the time *t*, a mass balance can be expressed as

$$M'(t) = C_{\text{in}} \cdot Q - M(t) \cdot \frac{Q}{V} - k \Leftrightarrow M(t) = C_{\text{in}} \cdot V - k \cdot \frac{V}{Q} + \frac{\xi_1}{e^{Q \cdot t/V}} \quad (10)$$

$$\xi_1 = M(0) - C_{\text{in}} \cdot V + k \cdot \frac{V}{Q} \quad (11)$$

where C_{in} is the inflow concentration, Q is the volumetric flow, V is the volume, k is the reaction rate constant in mass per unit time, eg. [mg · h^{-1}], and ξ_1 is an integration constant.

Eq. (10) is however not directly solvable for k given both k and are ξ_1 unknown.

The inert case $M_i(t)$ where no reaction takes place however is calculable as

$$M_i'(t) = C_{\text{in}} \cdot Q - M_i(t) \cdot \frac{Q}{V} \Leftrightarrow M_i(t) = C_{\text{in}} \cdot V + \frac{\xi_2}{e^{Q \cdot t/V}} \quad (12)$$

$$\xi_2 = M(0) - C_{\text{in}} \cdot V \quad (13)$$

Assuming the same starting conditions ($M_i(0) = M(0)$), V, Q and C_{in}, Eq. (10)-(13) can then be combined to yield

$$k = \frac{M_i - M}{1 - e^{-Q \cdot t/V}} \cdot \frac{Q}{V} \quad (14)$$

with $M_i(t)$ and $M(t)$ written as M_i and M. By definition $M = C \cdot V$ and M_i can be calculated according to (12).

Mass consumption and activity calculation

The mass consumed through reaction m_r after t time can then be calculated as

$$m_r = k \cdot t \quad (15)$$

The nitrate, nitrite and ammonium k and m_r in R_{PDA} were calculated step-wise for each 3 min time interval in the sampling period. Likewise for filtered COD in R_{COD} and R_{NIT}. The total mass consumed though reaction $m_{r,t}$ was calculated as the sum m_r over all time steps and the overall reaction rate constant as $m_{r,t}$ / 8 h.

The species activity r_x was calculated as, adjusting for units,

$$r_x = \frac{k}{A_c} \quad (16)$$

where A_c is the total carrier area. The dna, dni and amx activities were then calculated for each time-step and for the entire period using (7).

Mass balance control calculations

The method described above was checked by calculating and comparing the left- and right-hand side of the mass balance

$$m_{in,t} = m_{out,t} + m_{r,t} + m_{acc,t} \quad (17)$$

where, given a timestep length of *ts* the total masses are defined as; the mass entering through inflow $m_{in,t} = \sum(C_{in} \cdot Q \cdot ts)$, exiting through outflow $m_{out,t} = \sum(C \cdot Q \cdot ts)$ and accumulated in the reactor $m_{acc,t} = C(0) \cdot V - C(end) \cdot V$.

Nitrogen removal efficiency

The nitrogen removal efficiency (NRE) was calculated as

$$\text{NRE} = \frac{\text{Removed N}}{\text{Influent N}} \tag{18}$$

The calculations were performed across R_{PDA} using totN and TIN (NO_3^- + NO_2^- + NH_4^+) measurements and calculated reduction.

COD calculations

The COD value of main interest in this thesis is the readily biodegradable soluble COD (rbsCOD). In this context it is chosen to define the rbsCOD as the part of the filtered COD that is reduced in R_{COD} and R_{NIT}. The rbsCOD / sCOD ratio is defined as

$$\text{rbsCOD / sCOD} = \frac{\text{sCOD removed in } R_{COD} \text{ and } R_{NIT}}{\text{Influent sCOD to } R_{COD}} \tag{19}$$

where sCOD is the soluble COD, measured as the filtered COD.

4 Results and discussion

4.1 Ex-situ batch activity tests

Each batch test resulted in a concentration profile. Two such plots can be seen in Figure 4.1 and the rest in Appendix II. In general the concentration decline is linear after the first 20 min, indicating that the reduction rate is not concentration dependent and validating the assumption of 0-order kinetics. This also holds for the tests with reduced nitrite concentration. The difference in reduction rate between the first 20 min and the rest of the trials is not explored in this thesis. The subsequent results are based on the reduction rate after the first 20 min, i.e. for the period 20 - 120 min.

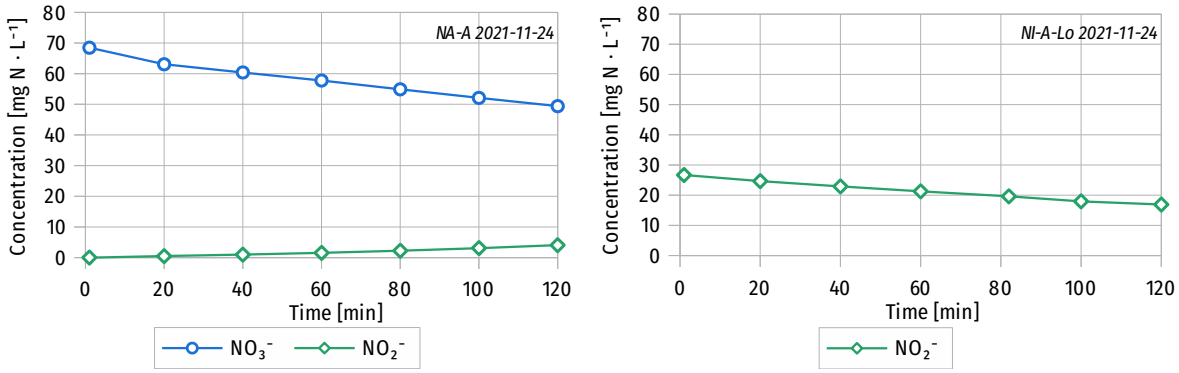


Figure 4.1. NA-A (left) and NI-A-Lo (left) example ex-situ batch test concentration curves from 2021-11-24.

4.1.1 Denitrification

The denitratation rates with acetate (NA-A) and propionate (NA-P) and denitritation rates with acetate (NI-A and NI-A-Lo) across the experimental period can be seen in Figure 4.2 and Table 4.2. It can be observed that all initial denitrification rates are very low, indicating the original biomass contained little or no denitrifiers and then developed across the experimental period. Given this low initial value, mean denitratation and denitritation values are calculated excluding this first data point and are marked with †.

The denitratation rate appeared to be significantly affected by the carbon source. Propionate yielded consistently and significantly lower rates ($0.24^{\dagger} \pm 0.02$ g N · m^{-2} · d^{-1}) than acetate ($0.52^{\dagger} \pm 0.09$ g N · m^{-2} · d^{-1}). This is consistent with the findings of Holmin (2020).

Over the course of the experimental period a differential in the acetate denitratation (NA-A) and denitritation (NI-A) rates developed with the denitratation rates being consistently and significantly higher than the denitritation rates, mean values of $0.52^{\dagger} \pm 0.09$ and $0.26^{\dagger} \pm 0.04$ g N · m^{-2} · d^{-1} respectively. Furthermore, as observed e.g. in Figure 4.1, there is some nitrite accumulation in the NA-A tests, but none in the NA-P tests (Figure II.4, Appendix II). The nitrite accumulation shows that the biomass is not comprised solely of group B denitrifiers, which are incapable of nitrite accumulation, and the fact that the nitrite accumulation is less than the nitrate reduction shows that it also does not solely comprise group A denitrifiers, which are incapable of nitrite reduction. The differential NA-A and NI-A rates along with the biomass not solely comprising of group B denitrifiers suggests that the biomass is capable of partial denitratation, and thus has the potential to function as a nitrite source for anammox.

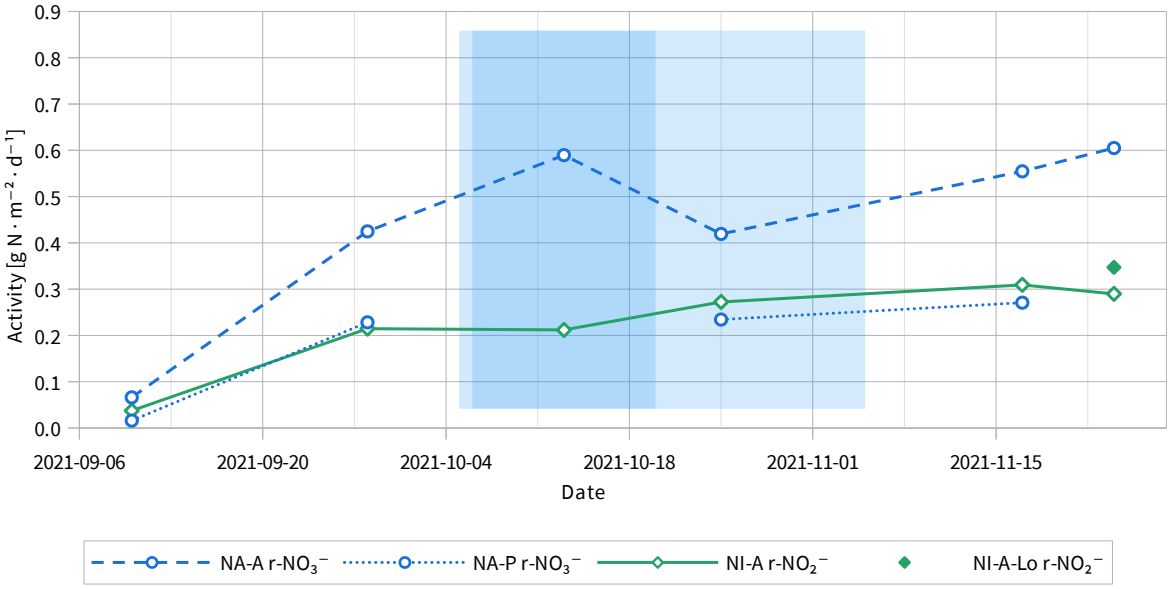


Figure 4.2. Denitratation NO_3^- and denitritation NO_2^- rates with acetate and propionate over the experimental period. Period of irregular flow and no primary filtration marked with blue and darker blue area respectively.

The denitritation test with lower nitrite concentration (NI-A-Lo) showed a slightly higher activity than the NI-A test the same day (0.35 versus 0.29 g N · m^{-2} · d^{-1}). This may indicate some mild nitrite inhibition in the denitritation tests. However, the small difference in value and number of data points (one), mean that the difference may well be attributable to experimental error and more tests would need to be conducted in order to determine nitrite inhibition.

The maximum denitratation rate, measured on the 24th of November of 0.60 g N · m^{-2} · d^{-1} can be compared with observed MBBR denitrification rates in nearby WWTPs. According to Mases et al. (2010) the average observed denitrification rates in the Sjölunda and Klagshamn WWTPs (Malmö, Sweden) for the period 2004-2009 were 0.9 and 1.3 g N · m^{-2} · d^{-1} using methanol and ethanol as carbon source respectively. It must however be noted that the average temperature over this period at Sjölunda and Klagshamn likely was under the 20 °C used in the ex-situ batch experiments in this thesis, and that different carbon sources were used. Both these factors make directly comparing the rates unuseful, however it is likely that the pilot carrier biomass had a significantly less denitrifying capacity than that at WWTPs.

4.1.2 Anammox

The mean specific anammox activity (SAA) for the three ex-situ reactor configurations can be seen in Figure 4.3. The initial value for the AMX reactor is excluded from the average, as it was measured at the time of carrier introduction and doesn't reflect the conditions in the pilot.

It can be seen that the SAA in the AMX tests, with an initial nitrite concentration of 75 mg N · L^{-1} was significantly lower than that in the PDA and AMX-Lo tests with 0 and 25 mg N · L^{-1} initial nitrite concentration respectively. This strongly indicates that a nitrite concentration of 75 mg N · L^{-1} had a significant inhibitory effect on the AnAOB in the biomass.

Previous studies investigating the inhibitory effects of nitrite on anammox have found a wide range of inhibitory concentrations, with IC_{50} values ranging from 80 to over 430 mg N · L^{-1} (Bettazzi et al. 2010; Kimura et al. 2010; Lotti et al. 2012; Raudkivi et al. 2016). Stefansdottir (2014), on who's method the 75 mg N · L^{-1} concentration is based, investigated initial nitrite concentrations from 25 to 125 mg N · L^{-1}. No nitrite inhibition was observed within this range. At concentrations < 50 - 75 mg N · L^{-1} the maximum SAA appeared to be concentration dependent while in the range 75 - 125 mg N · L^{-1} this was not the case, indicating a shift from half to zero order kinetics.

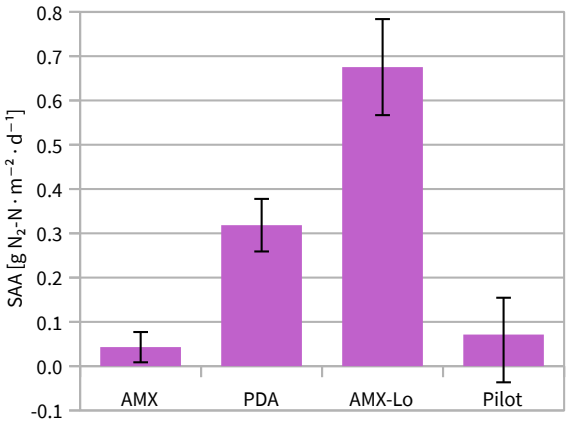


Figure 4.3. Mean ex-situ SAA for the different test configurations as well as SAA during cycle study (Pilot). AMX value excluding the initial measurement. Error bars for ex-situ results denote standard deviation and for Pilot denote a simulated max and min value.

In summary, initial nitrite concentration appears to be a significant affecting factor when determining SAA though both the mechanisms of nitrite inhibition at high concentrations and substrate limitation at low concentrations. Nitrite inhibition concentrations appear to be highly variable between different anammox biomasses and future studies would be well advised to investigate inhibitory, as well as substrate limiting concentrations of the biomass in question, determining the optimum concentration in which to measure SAA.

The AMX-Lo tests, performed at the end of the experimental period, show that the biofilm did retain anammox activity. Few studies on mainstream anammox using MBBR were found that report SAA. Table 4.1 compares the AMX-Lo SAA to the literature values found. It can be seen that the mean AMX-Lo SAA of 0.68 ± 0.1 g N_2-N · m^{-2} · d^{-1} is relatively low, however in line with some values reported towards the end of the experimental period of Holmin (2020).

Table 4.1. Comparison of ex-situ batch SAA determined in this thesis with literature values.

SAA [g N_2-N · m^{-2} · d^{-1}]	**T** [°C]	**Process**	**Source**	**Note**
0.75	20	PDA	*this thesis*	AMX-Lo max
0.68 ± 0.11	20	"	"	AMX-Lo mean
2.7	20	PNA	(Stefansdottir 2014)	
0.75	15	"	"	
2.04	20	PDA	(Holmin 2020)	Max, week 1
1.56	"	"	"	Week 6
0.55	"	"	"	Final, week 10
4-9	28	PNA	(Gustavsson et al. 2020)	

The SAA determinations over the entire experimental period can be seen in Figure 4.4 as well as Table 4.2. It can be seen that after the first AMX SSA determination the activity dropped and remained close to zero for the entire experimental period (mean $0.04^{\dagger} \pm 0.03$ g N_2-N · m^{-2} · d^{-1}). As was explored above, the AMX activity was likely highly nitrite inhibited and the AMX-Lo tests show that some anammox activity did remain at the end of the experimental period with the mean AMX-Lo being 0.68 ± 0.11 g N_2-N · m^{-2} · d^{-1}. The drop in AMX activity does however indicate a change in biomass composition, either through loss of AnAOB and/or a shift in biomass composition to one which is more easily nitrite inhibited.

The (ex-situ) PDA SAA (mean 0.32 ± 0.06 g N_2-N · m^{-2} · d^{-1}) determinations were consistently in between the AMX and AMX-Lo values. As no nitrite was added to the PDA tests they were not nitrite inhibited. Two possible explanations as to why the PDA SAA is less than the AMX-Lo are as follows. Firstly AnAOB PDA tests may be nitrite limited due to the nitrate reduction rate being to low. From Eq. (4) it can be seen that for anammox the ratio of NO_3^- to NH_4^+ removal is 1.32. Comparison of the removal rates (Table 4.2) shows that this is unlikely the reason. Secondly AnAOB PDA tests may be nitrite limited due to competition for nitrite with denitrifiers. The biomass contains organisms capable of performing denitritation. Given a limited nitrite supply a situation of competition will arise within the biomass which may lead to anammox activity being rate limited. The anammox contribution to nitrite reduction of 39-53 % is inline with that reported by Le et al. (2019b) for their high AnAOB, acetate tests (39-48 %).

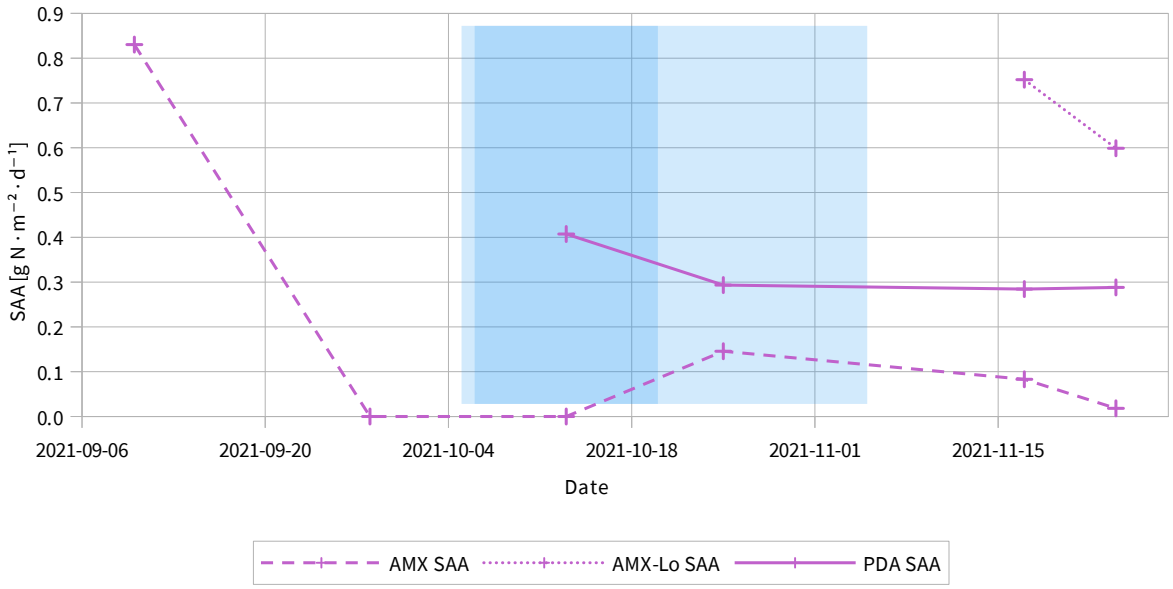


Figure 4.4. SAA determinations across the experimental period for ex-situ batch activity tests with nitrite (AMX), lower nitrite concentration (AMX-Lo) and nitrate + carbon source (PDA). Period of irregular flow and no primary filtration marked with blue and darker blue area respectively.

Table 4.2. Ex-situ batch activity test results. dna, dni and amx denote the denitratation, denitritation and anammox rate calculated according to Eq 7. Values marked with ⊢ calculated excluding the results of 2021-09-10.

Reactor	Type	Unit	2021-09-10	2021-09-28	2021-10-13	2021-10-25	2021-11-17	2021-11-24	Mean	Stdev
NA-A	r_{NO3}	$g\ N \cdot m^{-2} \cdot d^{-1}$	0.07	0.42	0.59	0.42	0.55	0.60	$0.52^{\vdash}$	$0.09^{\vdash}$
NA-P	r_{NO3}	"	0.02	0.23	-	0.23	0.27	-	$0.25^{\vdash}$	$0.02^{\vdash}$
NI-A	r_{NO2}	"	0.04	0.21	0.21	0.27	0.31	0.29	$0.26^{\vdash}$	$0.04^{\vdash}$
NI-A Lo	r_{NO2}	"	-	-	-	-	-	0.35	-	-
AMX	r_{NO2}	"	0.62	0.13	0.17	0.27	0.27	0.23	-	-
	r_{NH4}	"	0.41	0.00	0.00	0.07	0.04	0.01	-	-
	SAA	$g\ N_2\text{-}N \cdot m^{-2} \cdot d^{-1}$	0.83	0.00	0.00	0.15	0.08	0.02	$0.04^{\vdash}$	$0.03^{\vdash}$
AMX-Lo	r_{NO2}	$g\ N \cdot m^{-2} \cdot d^{-1}$	-	-	-	-	0.66	0.56	-	-
	r_{NH4}	"	-	-	-	-	0.37	0.29	-	-
	SAA	$g\ N_2\text{-}N \cdot m^{-2} \cdot d^{-1}$	-	-	-	-	0.75	0.60	0.68	0.11
PDA	r_{NO3}	$g\ N \cdot m^{-2} \cdot d^{-1}$	-	-	0.57	0.46	0.60	0.58	-	-
	r_{NO2}	"	-	-	0.00	0.00	0.00	0.00	-	-
	r_{NH4}	"	-	-	0.27	0.20	0.22	0.22	-	-
	dna	$g\ NO_3\text{-}N \cdot m^{-2} \cdot d^{-1}$	-	-	0.62	0.50	0.63	0.62	-	-
	dni	$g\ NO_2\text{-}N \cdot m^{-2} \cdot d^{-1}$	-	-	0.36	0.31	0.45	0.43	-	-
	amx	$g\ NH_4\text{-}N \cdot m^{-2} \cdot d^{-1}$	-	-	0.20	0.14	0.14	0.14	-	-
	SAA	$g\ N_2\text{-}N \cdot m^{-2} \cdot d^{-1}$	-	-	0.41	0.29	0.28	0.29	0.32	0.06
	Amx cont.	%	-	-	53	49	39	40	-	-

4.2 Pilot

4.2.1 Pilot performance

Flow proportionate diurnal composite samples were taken at the PDA pilot inflow (n = 8) and in R_{PDA} (n = 7) during the experimental period and analysed by WWTP staff. Table 4.3 shows mean analysis results, excluding one sample point taken during the period of irregular flow (2021-10-05 to 2021-11-05). There can be seen a measurable reduction of total nitrogen concentrations across the pilot, though mean effluent concentrations are still high at 28.1 ± 4.6 mg $N \cdot L^{-1}$. There appears to be a significant effluent nitrate residual of 11.9 ± 1.3 mg $N \cdot L^{-1}$, indicating that the denitratation rate may be a limiting factor in total nitrogen removal. The mean inflow and R_{PDA} pH of 7.7 ± 0.0 and 7.5 ± 0.1, are both close to / within the optimum pH range for anammox according to a recent review (Tomaszewski et al. 2017). Significant alkalinity remains in R_{PDA} (mean 147 ± 32 mg $HCO_3^- \cdot L^{-1}$), indicating this likely is not a limiting factor.

The pilot sampling strategy is not such that the PDA reactor, R_{PDA}, inflow concentrations can be calculated. However, given the flow proportionate sampling the NRE for the pilot as a whole can be calculated based on same day pilot inflow and R_{PDA} totN concentration samples (n = 6). The resulting mean NRE during the experimental period is 40 ± 4 %, calculated across the entire PDA pilot. Sample analysis results can be seen in Appendix I Figure I.3 and Table I.1.

The mean pilot inflow (Q_{tot}), only counting days when composite samples were analysed for R_{PDA}, was 51 ± 9.6 $L \cdot h^{-1}$. Combining the mean diurnal flow and totN concentration reduction across the entire pilot allows for the calculation of the diurnal totN nitrogen removal. The mean nitrogen removal rate (NRR) can then be calculated across R_{PDA} (carrier area 38 m^2) as 0.59 ± 0.11 g $N \cdot m^{-2} \cdot d^{-1}$. The NRR calculated in this way does not include the carrier area required for nitrification and does therefore not represent the process as a whole, however it does serve as a comparison with the denitrification step of BNR.

The MBBR denitrification reactors at Sjölunda and Klagshamn WWTPs (Malmö, Sweden) had during the period 2004-2009 a mean annual average NRR of 0.9 and 1.3 g $N \cdot m^{-2} \cdot d^{-1}$ using methanol and ethanol as carbon source respectively (Mases et al. 2010). This is higher than the NRR at the pilot calculated across R_{PDA} of 0.59 g $N \cdot m^{-2} \cdot d^{-1}$. However as mentioned above, the pilot NRR does not include nitrification. The full Sjölunda and Klagshamn processes involve nitrification of all wastewater while the PDA process theoretically only involves nitrifying 50 % (in the pilot during the experimental period around 66 %). After accounting for nitrification, the pilot NRR will be slightly better off, however this is not expected to be sufficient to make the pilot reach similar removal rates. Complicating comparison is the unknown temperature response of the anammox in the pilot as well as the unknown distribution of nitrite removal between denitritation and anammox.

There is a significant decrease in both sCOD and total COD across the pilot, which is to be expected. It is possible to calculate the rbsCOD concentration drop across R_{PDA} to 34 mg $O_2 \cdot L^{-1}$, however, the limited data set and relatively large variation in the underlying data makes the accuracy of this value highly uncertain. The mean (n = 2) effluent filtered 7-day biological oxygen demand (BOD_7) concentration of 9 ± 1.4 mg $O_2 \cdot L^{-1}$ can be seen as promising in relation to the monthly average effluent requirement of Källby WWTP of 10 mg $O_2 \cdot L^{-1}$.

Table 4.3. Mean composite sample data for pilot inflow and outflow calculated based on diurnal composite samples taken within the experimental period but excluding samples in the period of irregular flow (2021-10-05 to 2021-11-05).

	Inflow		Outflow		
	Mean	**Stdev**	**Mean**	**Stdev**	**Unit**
totN	46.8	8.2	28.1	4.6	[mg N · L^{-1}]
NH_4^+	37.1	5.9	9.5	2.2	"
NO_3^-	-	-	11.9	1.3	"
NO_2^-	-	-	0.4	0.1	"
sCOD	159	26	49	6	[mg O_2 · L^{-1}]
total COD	318	71	206	37	"
pH	7.7	0.0	7.5	0.1	-
Alkalinity	296	55	147	31	[mg HCO_3^- · L^{-1}]
VFA	16.7	9.8	3.1	1.3	[mg O_2 · L^{-1}]

4.2.2 Pilot cycle study

The purpose of the pilot cycle study was to analyse the performance of the pilot from a typical night-time flow across a morning/mid day peak flow event. The mean temperature in R_{PDA} during the collection period was 16.1 ± 0.1 °C and the mean DO concentration was 0.21 ± 0.02 mg O_2 · L^{-1} (both measured with a Hach DO probe). The inorganic N-species and totN inflow and outflow concentrations to the R_{PDA} reactor, as well as the pilot flow during the cycle study can be seen in Figure 4.5. The R_{PDA} sCOD inflow and outflow concentrations are shown in Figure III.1, Appendix III.

The calculated total values across the observation period for NRE, NRR, SAA, anammox contribution to nitrite removal as well as COD characterisation and COD/N ratio can be seen in Table 4.4. During the cycle study the NRE was low at 27 or 19 % calculated for total nitrogen and total inorganic nitrogen respectively. The relatively large difference between these two values may be a reflection of the difference in sampling frequency and hence interpolation error. Similarly there is a large difference between the NRR across R_{PDA} based either on calculated totN removal (0.43 g N · m^{-2} · d^{-1}) or the value defined as 0.5 · dni + 2.04 · amx (0.19 g N · m^{-2} · d^{-1}).

The continuous denitratation rate (dna), denitritation rate (dni) and anammox rate (amx) in units of [g N_x-N · m^{-2} · d^{-1}] can be seen in Figure 4.6 where Nx is NO_3^-, NO_2^- and NH_4^+ respectively. Observe that SAA = 2.04 · amx. It should be noted that the interpolation of the 30 min samples to 3 min samples likely is a significant source of error and may explain much of the sudden rate-changes displayed in the figure. It is deemed highly unlikely that the rates undergo such rapid and frequent changes as shown and Figure 4.6 should only be interpreted as showing the general trend and development of the rates across the observation period.

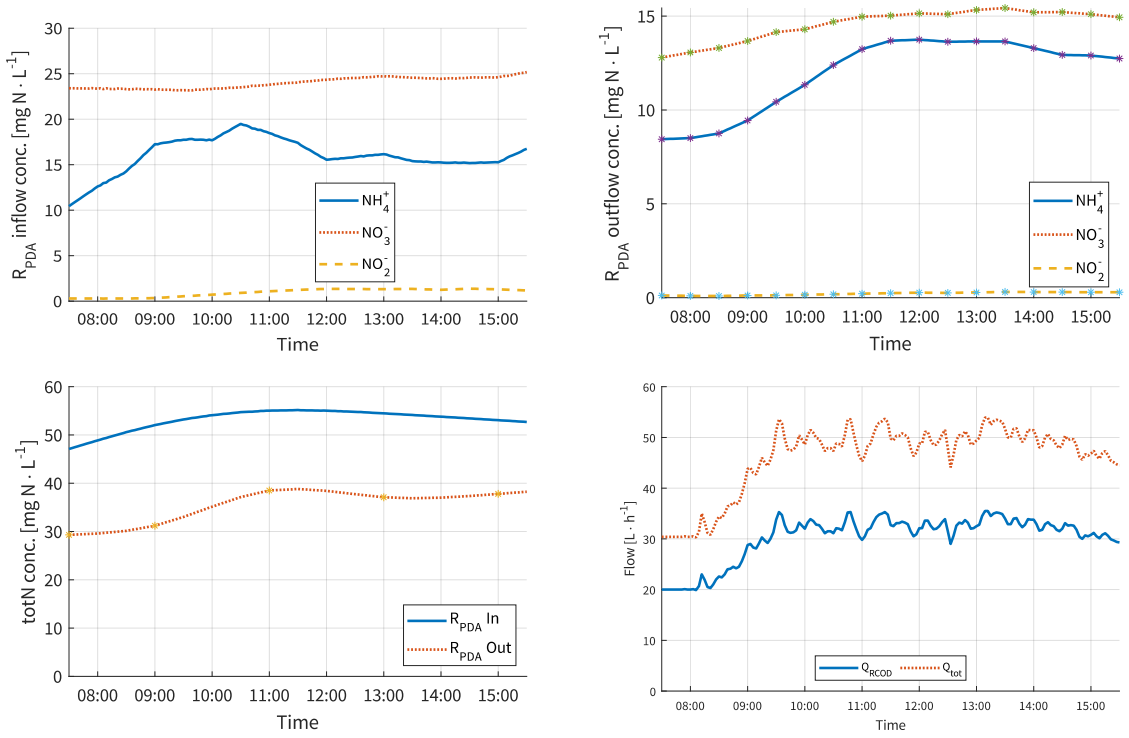


Figure 4.5. R_{PDA} inorganic nitrogen infow (top left) outflow (top right) as well as total nitrogen inflow and outflow (bottom left) and pilot flows (bottom right) during the cycle study. Stars (*) mark measured values.

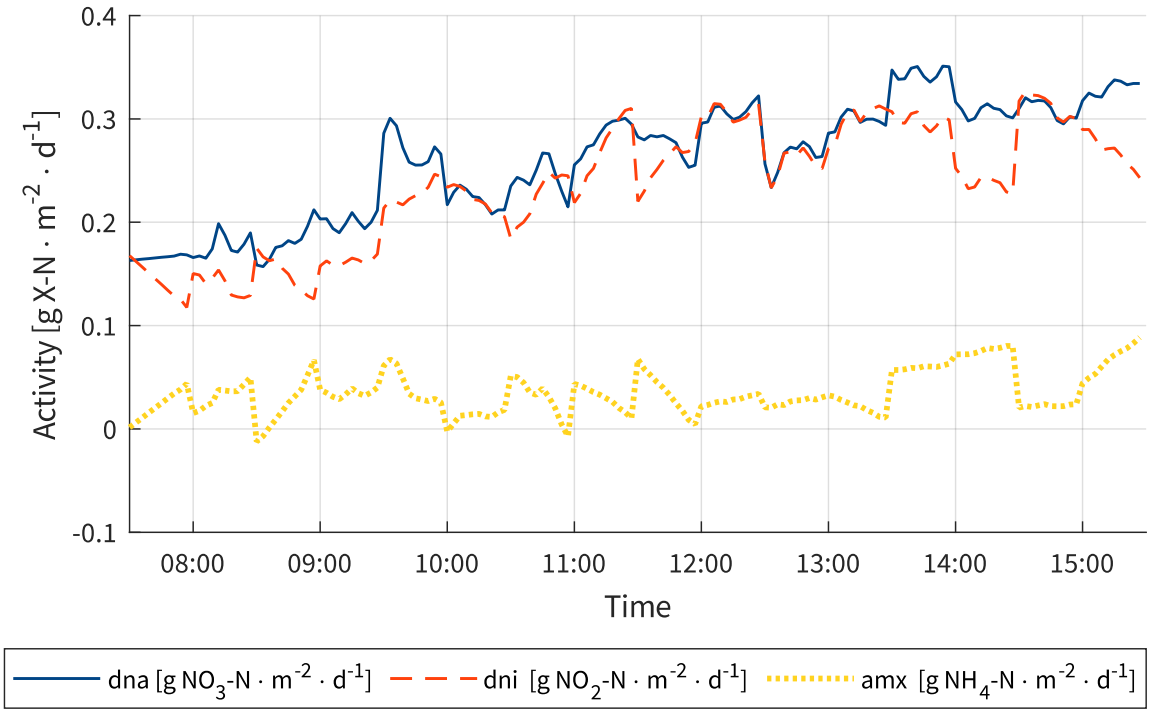


Figure 4.6. Calculated dna, dni and amx across the observation period.

The calculation method described in 3.3.3 can be checked by looking at the mass balances. As can be seen Figure 4.7 the mass present in the inflow very closely equals the sum of mass present in the outflow, calculated reduction and accumulation. This also holds true both for N-species in R_{PDA} as well as for sCOD in the three pilot reactors, indicating that the model developed in section 3.3.3 well describes the reduction in the pilot.

The HRT and rbsCOD / NO_3^--N ratio across the observation period can be seen in Figure 4.8. It can be seen that the HRT shifts across the observation period, as a response to changing inflows. The rbsCOD / NO_3^--N ratio shows a generally increasing trend, but remains relatively low.

According to a recent review, many different optimum COD/N ratios have been reported. The value of 1.52 during the cycle study falls within the range of reported optimums (Cao et al. 2021). Le et al. (2019b) suggest that the COD/N ratio response may be complex and dependent on carbon source and biomass community. As such the COD/N ratio during the cycle study may be seen as reasonable, but not enough data exists to determine if it is optimal.

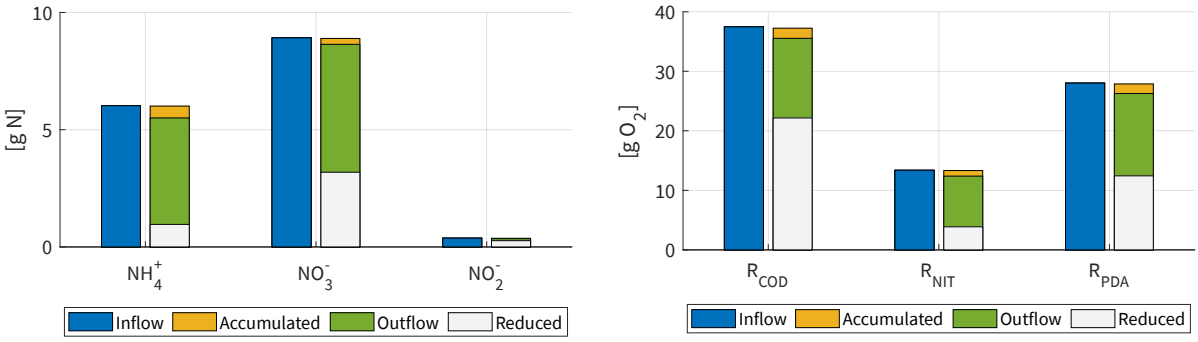


Figure 4.7. Total mass comparison of inflow to the sum of outflow, accumulation and reduction for NH_4^+, NO_3^- and NO_2^- in R_{PDA} (left) and sCOD in R_{COD}, R_{NIT} and R_{PDA} (right).

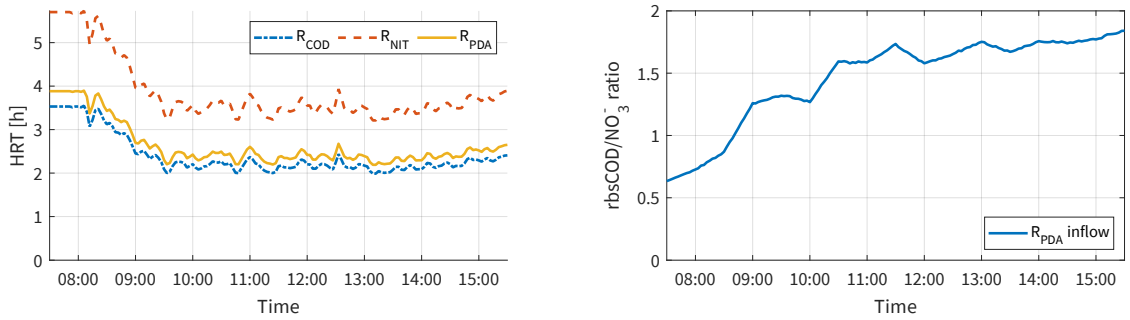


Figure 4.8. The HRT in the three pilot reactors (left) and the rbsCOD/NO_3^- ratio in RPDA across the observation period.

Table 4.4. Pilot cycle study results. Values calculated as totals for the entire observation period. Values marked with ‡: negative values not possible, assumed to be zero.

	Value	Unit
NRE totN	27	[%]
NRE TIN	19	"
NRR totN	0.43	[g N · m^{-2} · d^{-1}]
NRR - activity based	0.19	"
dna	0.26	[g NO_3-N · m^{-2} · d^{-1}]
dni	0.24	[g NO_2-N · m^{-2} · d^{-1}]
amx	0.04	[g NH_4-N · m^{-2} · d^{-1}]
SAA	0.07	[g N_2-N · m^{-2} · d^{-1}]
min, max	-0.04‡, 0.15	"
Anammox contribution	23	[%]
min, max	-14‡, 45	"
rbsCOD / sCOD ratio	0.70	-
rbsCOD / NO_3 ratio	1.52	-

The SAA, both in total over the observation period (Table 4.4), and the continuous calculation (Figure 4.6) are low, with the total value being 0.07 g N_2-N · m^{-2} · d^{-1}. This can be compared to the mean ex-situ batch AMX-Lo SAA of 0.67 g N_2-N · m^{-2} · d^{-1} and PDA SAA of 0.32, performed at roughly the same time, see Figure 4.3 above. It should be noted that the ex-situ tests were performed at 20 °C while R_{PDA} was at 16.1 °C during the observation period. According to data from Stefansdottir (2014), who compared PNA SSA at 15 and 20 °C, the SAA at 16.1 °C is 44 % that at 20 °C. Assuming the same temperature response in the biomass in the pilot, the temperature adjusted AMX-Lo SAA is 0.30 and PDA SAA is 0.14 g N_2-N · m^{-2} · d^{-1}.

The calculation of SAA according to Eq. (7) and (8) above is relatively sensitive to the stoichiometric coefficients in the reaction equations for denitratation and denitritation; Eq. (1) and (2), specifically the ammonium assimilation coefficients. These in turn are based both on the stoichiometry of the carbon source and the biomass yield coefficient of the specific biomass to the carbon source in question. The calculations above assume the stoichiometric relationships using acetate as carbon source from Ma et al. (2017). However, this is likely to be a significant source of error when calculating the SAA in the pilot as pure acetate is not the sole carbon source.

A Monte Carlo simulation was employed to attempt to quantify the order of magnitude of the error in calculated SAA due to incorrect ammonium assimilation coefficients. It was assumed that the denitritation and denitratation assimilation coefficients were between 0 and two times their acetate value. 10^6 random pairings of assimilation coefficients were selected, linearly distributed within this range and the total SAA calculation, according to Eq. (7) and (8), repeated for each pairing. The min and max SAA values, as can be seen in Table 4.4 and as er-

ror bars in Figure 4.3, were -0.04 and 0.15 g N_2-N · m^{-2} · d^{-1} respectively with the corresponding min and max anammox contribution to nitrite removal being -14 and 45 %. The max value was achieved when the assimilation coefficients both approached zero. As this is unlikely to happen in a functioning PDA process where denitratation is required to take place, the calculated max value is unlikely to be exceeded. As negative SAA values and anammox contributions are definitionally not possible the negative min values are interpreted as 0. It therefore seems likely that the SAA in the pilot calculated over the whole observation period was between 0 and 0.15 g N_2-N · m^{-2} · d^{-1}. The maximum continuous amx, dna and dni, equivalent to Figure 4.6 but calculated using zero values for denitritaion and denitratation ammonium assimilation coefficients can be seen in Figure III.2, Appendix III.

4.2.3 Pilot summary and discussion

The NRR in R_{PDA} is calculated in three ways in this study. Firstly, from flow proportionate diurnal composite totN concentrations, secondly during the cycle study from totN mass reduction and thirdly during the cycle study from dni and amx. The obtained values display a large spread, 0.59, 0.43 and 0.19 g N · m^{-2} · d^{-1} respectively.

The high value of 0.59 g N · m^{-2} · d^{-1} is not solely the result of it being calculated based on data points over the entire experimental period and the rate falling towards the end of the period when the cycle study was conducted. NRR values calculated according to the first method from data taken three days before and 11 days after the cycle study are 0.4 and 0.5 respectively. An unexplained N sink, apart from anammox and denitritation would explain the low third value, however this is deemed highly unlikely. The more probable cause is that the difference is explained by the combination of multiple source of error. Specifically major sources of error arising from using the composite sample concentration values, interpolation error and model error from incorrect stoichiometric relationship assumptions.

All the NRR values are significantly lower compared to the 2004-2009 mean annual average denitrification rates at the Sjölunda and Klagshamn WWTPs of 0.9 and 1.3 g N · m^{-2} · d^{-1} using methanol and ethanol as carbon source respectively. Coupled with the low anammox contribution to N_2 formation of 23 % it can be concluded, despite the uncertainty in the exact values, that the pilot as operated is not practically up-scalable.

The mean, temperature adjusted ex-situ AMX-Lo and PDA SSA values of 0.30 and 0.14 g N_2-N · m^{-2} · d^{-1} are significantly higher than the cycle study SAA of 0.07 g N_2-N · m^{-2} · d^{-1}. However, the temperature adjusted PDA SAA is close to the maximum cycle study SAA (obtained assuming zero ammonium assimilation) of 0.15 g N_2-N · m^{-2} · d^{-1}. While zero ammonium assimilation is not feasible in a working PDA reactor it does indicate that temperature and stoichiometric error may be significant factors explaining the difference. Another potential explaining factor is the carbon source. It has been suggested that the carbon source may influence the competitive balance for nitrite between AnAOB and denitritation bacteria with Le et al. (2019b) showing a large difference in anammox activity in PDA setups with different carbon sources, where acetate yielded the highest activity.

The evidence, both ex-situ and in-situ, indicate that the AnAOB were not completely outcompeted in the biomass. However the SAA was low, especially in-situ, compared with e.g. Stefansdottir (2014) and Gustavsson (2020). The PNA pilot in those studies continuously transferred carriers from a sidestream PNA process and little temperature affect was observed on NRR. As AnAOB are temperature sensitive the competitive balance in the biomass during the WWTP's coldest operational period is important to the viability of the PDA process. This was not investigated in this thesis and may be of interest for future study.

5 Conclusions

The mainstream partial denitritation coupled anammox pilot at Källby WWTP was evaluated using ex-situ batch activity tests over the course of 11 weeks, composite samples from the pilot analysed by WWTP staff as well as a cycle study performed at the pilot. The conclusions are as follows:

- A low anammox activity was maintained in the pilot and AnAOB were not completely outcompeted in the biomass. Ex-situ tests showed a mean SAA of 0.68 ± 0.11 g N_2-N · m^{-2} · d^{-1} during the last weeks of the experimental period and the cycle study indicated an in-situ SAA of 0.07 g N_2-N · m^{-2} · d^{-1}.

- Partial denitratation did provide nitrite to anammox. Over the experimental period a biomass developed that in ex-situ tests had a 69 % higher denitratation than denitritation rate when using acetate as the carbon source. Further, nitrite accumulation was observed in denitratation tests and significant SAA was observed in ex-situ PDA batch tests.

- The in-situ nitrogen removal rates were calculated in three different ways, yielding rates 85 - 34 % lower than the denitrification rates at nearby WWTPs. These low values suggest that the pilot was not able to achieve sufficiently high removal rates to be practically implementable.

Additionally:

- It was revealed in ex-situ tests that the pilot biofilm was very sensitive to nitrite inhibition. The anammox activity was investigated at two different initial nitrite concentrations, 25 and 75 mg N · L^{-1} and the activity at the higher initial concentration was 94 % lower than that at the lower initial concentration. This is a lower inhibitory concentration than commonly reported in the literature and underscores the importance of investigating nitrite inhibition concentrations of a specific biomass prior to testing anammox activity.

6 Future work

As has been mentioned at several points in this thesis many AnAOB properties appear to be biomass specific. As such it would be of interest to study the biomass species composition and development over the experimental period. This may yield more insight into PNA biomass adaptation to PDA environments, allow for analysis of biomass response to process disturbances as experienced during and after the filter malfunction as well as contribute to future meta-analysis of anammox species behaviour. Carrier samples were frozen over the course of the experimental period to allow for future work in this area.

Analysis of ex-situ batch tests during and immediately after the period of coldest annual inflow temperatures would be of interest in order to investigate the potential long-term competitive balance between AnAOB and denitrifiers.

Sludge fermentate return flow was not active during the experimental period. Future studies could use this VFA-rich stream to investigate the effect of carbon source and COD/N ratio on PDA performance.

Strategies for establishing higher SAA and NRR could also be explored, such as sidestream carrier augmentation as well as introduction of PNA carriers from multiple sources in order to increase biomass diversity and potentially select for a more optimal biomass composition.

7 Reference list

Balmér, P. (2018). *Resultatrapport för VASS Reningsverk 2016*. Stockholm: Svenskt Vatten Utveckling. No. 2018–05.

Bettazzi, E., Caffaz, S., Vannini, C. & Lubello, C. (2010). Nitrite inhibition and intermediates effects on Anammox bacteria: A batch-scale experimental study. *Process Biochemistry*, 45(4), pp. 573–580, doi:10.1016/j.procbio.2009.12.003.

Cao, S., Du, R. & Zhou, Y. (2021). Coupling anammox with heterotrophic denitrification for enhanced nitrogen removal: A review. *Critical Reviews in Environmental Science and Technology*, 51(19), pp. 2260–2293, doi:10.1080/10643389.2020.1778394.

Cao, Y., Kwok, B. H., van Loosdrecht, M. C. M., Daigger, G., Png, H. Y., Long, W. Y. & Eng, O. K. (2018). The influence of dissolved oxygen on partial nitritation/anammox performance and microbial community of the 200,000 m3/d activated sludge process at the Changi water reclamation plant (2011 to 2016). *Water Science and Technology*, 78(3), pp. 634–643, doi:10.2166/wst.2018.333.

Cao, Y., van Loosdrecht, M. C. M. & Daigger, G. T. (2017). Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies. *Applied Microbiology and Biotechnology*, 101(4), pp. 1365–1383, doi:10.1007/s00253-016-8058-7.

Caranto, J. D. & Lancaster, K. M. (2017). Nitric oxide is an obligate bacterial nitrification intermediate produced by hydroxylamine oxidoreductase. *Proceedings of the National Academy of Sciences*, 114(31), pp. 8217–8222, doi:10.1073/pnas.1704504114.

Chen, H., Tu, Z., Wu, S., Yu, G., Du, C., Wang, H., Yang, E., Zhou, L., Deng, B., Wang, D. & Li, H. (2021). Recent advances in partial denitrification-anaerobic ammonium oxidation process for mainstream municipal wastewater treatment. *Chemosphere*, 278, p. 130436, doi:10.1016/j.chemosphere.2021.130436.

la Cour Jansen, J. & Harremoës, P. (1985). Removal of Soluble Substrates in Fixed Films. *Water Science and Technology*, 17(2–3), pp. 1–14, doi:10.2166/wst.1985.0115.

Dalsgaard, T., Thamdrup, B. & Canfield, D. E. (2005). Anaerobic ammonium oxidation (anammox) in the marine environment. *Research in Microbiology*, 156(4), pp. 457–464, doi:10.1016/j.resmic.2005.01.011.

D'Errico, J. (2006). *inpaint_nans*. https://se.mathworks.com/matlabcentral/fileexchange/4551-inpaint_nans [2021-12-20].

Du, R., Cao, S., Li, B., Niu, M., Wang, S. & Peng, Y. (2017a). Performance and microbial community analysis of a novel DEAMOX based on partial-denitrification and anammox treating ammonia and nitrate wastewaters. *Water Research*, 108, pp. 46–56, doi:10.1016/j.watres.2016.10.051.

Du, R., Cao, S., Li, B., Wang, S. & Peng, Y. (2017b). Simultaneous domestic wastewater and nitrate sewage treatment by DEnitrifying AMmonium OXidation (DEAMOX) in sequencing batch reactor. *Chemosphere*, 174, pp. 399–407, doi:10.1016/j.chemosphere.2017.02.013.

Du, R., Cao, S., Peng, Y., Zhang, H. & Wang, S. (2019a). Combined Partial Denitrification (PD)-Anammox: A method for high nitrate wastewater treatment. *Environment International*, 126, pp. 707–716, doi:10.1016/j.envint.2019.03.007.

Du, R., Peng, Y., Ji, J., Shi, L., Gao, R. & Li, X. (2019b). Partial denitrification providing nitrite: Opportunities of extending application for anammox. *Environment International*, 131, p. 105001, doi:10.1016/j.envint.2019.105001.

Egli, K., Fanger, U., Alvarez, P. J. J., Siegrist, H., van der Meer, J. R. & Zehnder, A. J. B. (2001). Enrichment and characterization of an anammox bacterium from a rotating

biological contactor treating ammonium-rich leachate. *Archives of Microbiology*, 175(3), pp. 198–207, doi:10.1007/s002030100255.

Gong, L., Huo, M., Yang, Q., Li, J., Ma, B., Zhu, R., Wang, S. & Peng, Y. (2013). Performance of heterotrophic partial denitrification under feast-famine condition of electron donor: A case study using acetate as external carbon source. *Bioresource Technology*, 133, pp. 263–269, doi:10.1016/j.biortech.2012.12.108.

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, p. 136342, doi:10.1016/j.scitotenv.2019.136342.

Han, Y., Qian, J., Guo, J., Song, Y., Lu, C., Li, H., Hou, Y. & Chen, Z. (2021). Feasibility of partial denitrification and anammox for removing nitrate and ammonia simultaneously in situ through synergetic interactions. *Bioresource Technology*, 320, p. 124390, doi:10.1016/j.biortech.2020.124390.

HELCOM (2013). *Summary report on the development of revised Maximum Allowable Inputs (MAI) and updated Country Allocated Reduction Targets (CART) of the Baltic Sea Action Plan*. HELCOM.

HELCOM (2015). *Updated Fifth Baltic Sea Pollution Load Compilation (PLC-5.5). Baltic Sea Environment Proceedings No. 145*. HELCOM.

Hendrickx, T. L. G., Kampman, C., Zeeman, G., Temmink, H., Hu, Z., Kartal, B. & Buisman, C. J. N. (2014). High specific activity for anammox bacteria enriched from activated sludge at 10°C. *Bioresource Technology*, 163, pp. 214–221, doi:10.1016/j.biortech.2014.04.025.

Holmin, F. W. (2020). *Partial denitrification as nitrite provider for mainstream anammox*. Masters thesis. Lund University, Lund.

Kartal, B., Kuenen, J. G. & van Loosdrecht, M. C. M. (2010). Sewage Treatment with Anammox. *Science*, 328(5979), pp. 702–703, doi:10.1126/science.1185941.

Khunjar, W. O., Pitt, P. A., Bott, C. B. & Chandran, K. (2014). Nitrogen. In: Jenkins, D., Wanner, J., & International Water Association (eds.) *Activated sludge - 100 years and counting*. Presented at the IWA Conference Activated Sludge - 100 Years and Counting, London: IWA Publishing.

Kimura, Y., Isaka, K., Kazama, F. & Sumino, T. (2010). Effects of nitrite inhibition on anaerobic ammonium oxidation. *Applied Microbiology and Biotechnology*, 86(1), pp. 359–365, doi:10.1007/s00253-009-2359-z.

Kuenen, J. G. (2008). Anammox bacteria: from discovery to application. *Nature Reviews Microbiology*, 6(4), pp. 320–326, doi:10.1038/nrmicro1857.

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, pp. 292–303, doi:10.1016/j.watres.2014.02.032.

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. (2019a). Nitrate residual as a key parameter to efficiently control partial denitrification coupling with anammox. *Water Environment Research*, 91(11), pp. 1455–1465, doi:10.1002/wer.1140.

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. (2019b). Impact of carbon source and COD/N on the concurrent operation of partial denitrification and anammox. *Water Environment Research*, 91(3), pp. 185–197, doi:10.1002/wer.1016.

Li, J., Peng, Y., Zhang, L., Liu, J., Wang, X., Gao, R., Pang, L. & Zhou, Y. (2019). Quantify the contribution of anammox for enhanced nitrogen removal through metagenomic

analysis and mass balance in an anoxic moving bed biofilm reactor. *Water Research*, 160, pp. 178–187, doi:10.1016/j.watres.2019.05.070.

Lingsten, A. & Lundkvist, M. (2008). *Nulägesbeskrivning av VA- verkens energianvändning*. Stockholm: Svenskt Vatten Utveckling. No. 2008–01.

Liu, B., Mao, Y., Bergaust, L., Bakken, L. R. & Frostegård, Å. (2013). Strains in the genus Thauera exhibit remarkably different denitrification regulatory phenotypes. *Environmental Microbiology*, 15(10), pp. 2816–2828, doi:10.1111/1462-2920.12142.

Lotti, T., Kleerebezem, R., Abelleira-Pereira, J. M., Abbas, B. & van Loosdrecht, M. C. M. (2015a). Faster through training: The anammox case. *Water Research*, 81, pp. 261–268, doi:10.1016/j.watres.2015.06.001.

Lotti, T., Kleerebezem, R., Hu, Z., Kartal, B., Jetten, M. S. M. & van Loosdrecht, M. C. M. (2014a). Simultaneous partial nitritation and anammox at low temperature with granular sludge. *Water Research*, 66, pp. 111–121, doi:10.1016/j.watres.2014.07.047.

Lotti, T., Kleerebezem, R. & van Loosdrecht, M. c. m. (2015b). Effect of temperature change on anammox activity. *Biotechnology and Bioengineering*, 112(1), pp. 98–103, doi:10.1002/bit.25333.

Lotti, T., Kleerebezem, R., Lubello, C. & van Loosdrecht, M. C. M. (2014b). Physiological and kinetic characterization of a suspended cell anammox culture. *Water Research*, 60, pp. 1–14, doi:10.1016/j.watres.2014.04.017.

Lotti, T., van der Star, W. R. L., Kleerebezem, R., Lubello, C. & van Loosdrecht, M. C. M. (2012). The effect of nitrite inhibition on the anammox process. *Water Research*, 46(8), pp. 2559–2569, doi:10.1016/j.watres.2012.02.011.

Ma, B., Qian, W., Yuan, C., Yuan, Z. & Peng, Y. (2017). Achieving Mainstream Nitrogen Removal through Coupling Anammox with Denitratation. *Environmental Science & Technology*, 51(15), pp. 8405–8413, doi:10.1021/acs.est.7b01866.

Ma, B., Xu, X., Wei, Y., Ge, C. & Peng, Y. (2020). Recent advances in controlling denitritation for achieving denitratation/anammox in mainstream wastewater treatment plants. *Bioresource Technology*, 299, p. 122697, doi:10.1016/j.biortech.2019.122697.

Martienssen, M. & Schöps, R. (1997). Biological treatment of leachate from solid waste landfill sites—Alterations in the bacterial community during the denitrification process. *Water Research*, 31(5), pp. 1164–1170, doi:10.1016/S0043-1354(96)00364-8.

Mases, M., Dimitrova, I., Nyberg, U., Gruvberger, C. & Andersson, B. (2010). Experiences from MBBR Post-Denitrification Process in Long-term Operation at two WWTPs. In: *Water Environment Federation and International Water Association Biofilm Reactor Technology Conference*. Portland, Oregon: Water Environment Federation.

Mulder, A. (1989). *Anoxic ammonia oxidation*. World Intellectual Property Organization: WO1989007089A1, 10/08/1989.

Ødegaard, H. (2006). Innovations in wastewater treatment: –the moving bed biofilm process. *Water Science and Technology*, 53(9), pp. 17–33, doi:10.2166/wst.2006.284.

Raudkivi, M., Zekker, I., Rikmann, E., Vabamäe, P., Kroon, K. & Tenno, T. (2016). Nitrite inhibition and limitation – the effect of nitrite spiking on anammox biofilm, suspended and granular biomass. *Water Science and Technology*, 75(2), pp. 313–321, doi:10.2166/wst.2016.456.

Stefansdottir, D. (2014). *Manometric method for evaluation of anammox activity in mainstream anammox at Sjölunda WWTP*. Masters thesis. Lund University, Lund.

Strohm, T. O., Griffin, B., Zumft, W. G. & Schink, B. (2007). Growth Yields in Bacterial Denitrification and Nitrate Ammonification. *Applied and Environmental Microbiology*, 73(5), pp. 1420–1424, doi:10.1128/AEM.02508-06.

Strous, M., Heijnen, J. J., Kuenen, J. G. & Jetten, M. S. M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidiz-

ing microorganisms. *Applied Microbiology and Biotechnology*, 50(5), pp. 589–596, doi:10.1007/s002530051340.

Tomaszewski, M., Cema, G. & Ziembińska-Buczyńska, A. (2017). Influence of temperature and pH on the anammox process: A review and meta-analysis. *Chemosphere*, 182, pp. 203–214, doi:10.1016/j.chemosphere.2017.05.003.

Wang, Z.-B., Liu, X.-L., Bu, C.-N., Ni, S.-Q. & Sung, S. (2021). Microbial diversity reveals the partial denitrification-anammox process serves as a new pathway in the first mainstream anammox plant. *Science of The Total Environment*, 764, p. 142917, doi:10.1016/j.scitotenv.2020.142917.

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. *Water Environment Research*, 87(12), pp. 2084–2089, doi:10.2175/106143015X14362865227319.

You, Q.-G., Wang, J.-H., Qi, G.-X., Zhou, Y.-M., Guo, Z.-W., Shen, Y. & Gao, X. (2020). Anammox and partial denitrification coupling: a review. *RSC Advances*, 10(21), pp. 12554–12572, doi:10.1039/D0RA00001A.

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, pp. 393–401, doi:10.1016/j.scitotenv.2019.07.293.

Zhang, Y., Ma, H., Chen, R., Niu, Q. & Li, Y.-Y. (2018). Stoichiometric variation and loading capacity of a high-loading anammox attached film expanded bed (AAEEB) reactor. *Bioresource Technology*, 253, pp. 130–140, doi:10.1016/j.biortech.2018.01.043.

Zhao, Q., Chen, K., Li, J., Sun, S., Jia, T., Huang, Y., Peng, Y. & Zhang, L. (2021). Pilot-scale evaluation of partial denitrification/anammox on nitrogen removal from low COD/N real sewage based on a modified process. *Bioresource Technology*, 338, p. 125580, doi:10.1016/j.biortech.2021.125580.

Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiology and molecular biology reviews : MMBR*, 61(4), pp. 533–616, doi:10.1128/.61.4.533-616.1997.

Appendix I - Pilot sample data

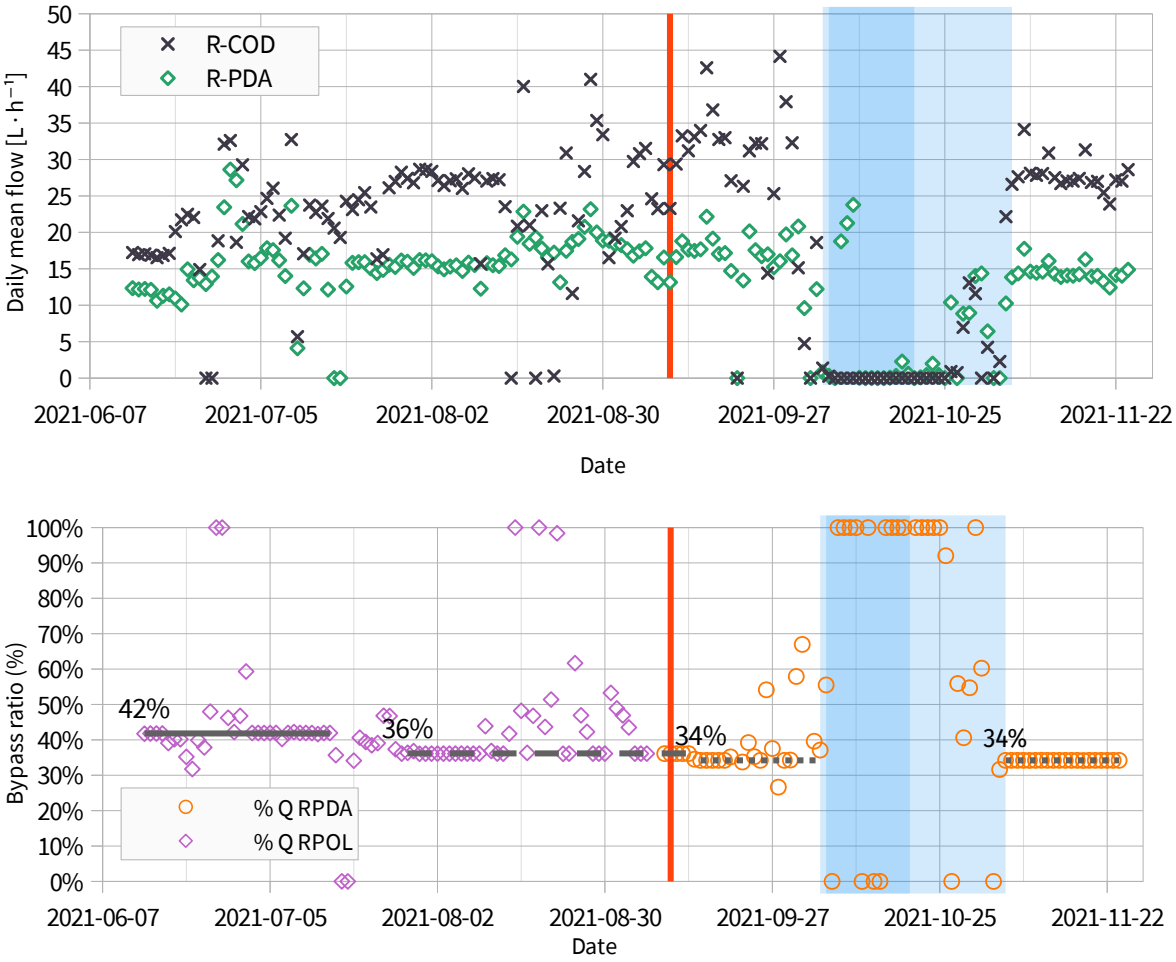


Figure I.1. Inflow to R_{COD} and R_{PDA} (upper) during the experimental period and bypass ratio to R_{POL} and R_{PDA} respectively (lower), start of experimental period marked with vertical bar. Period of irregular flow and no primary filtration marked with blue and darker blue area respectively.

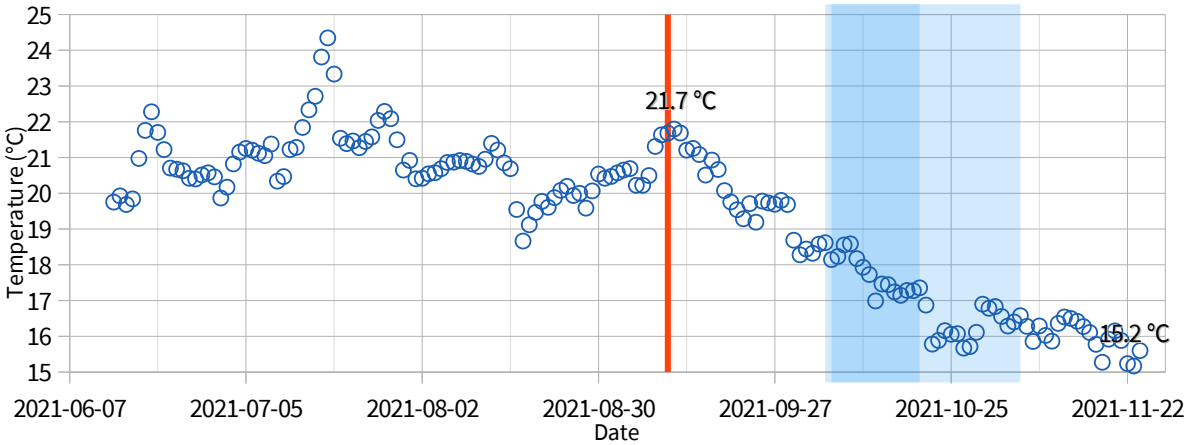


Figure I.2.Temperature in R_{POL} (before experimental period) and R_{PDA} (during experimental period). Start of experimental period marked with vertical bar. Period of irregular flow and no primary filtration marked with blue and darker blue area respectively.

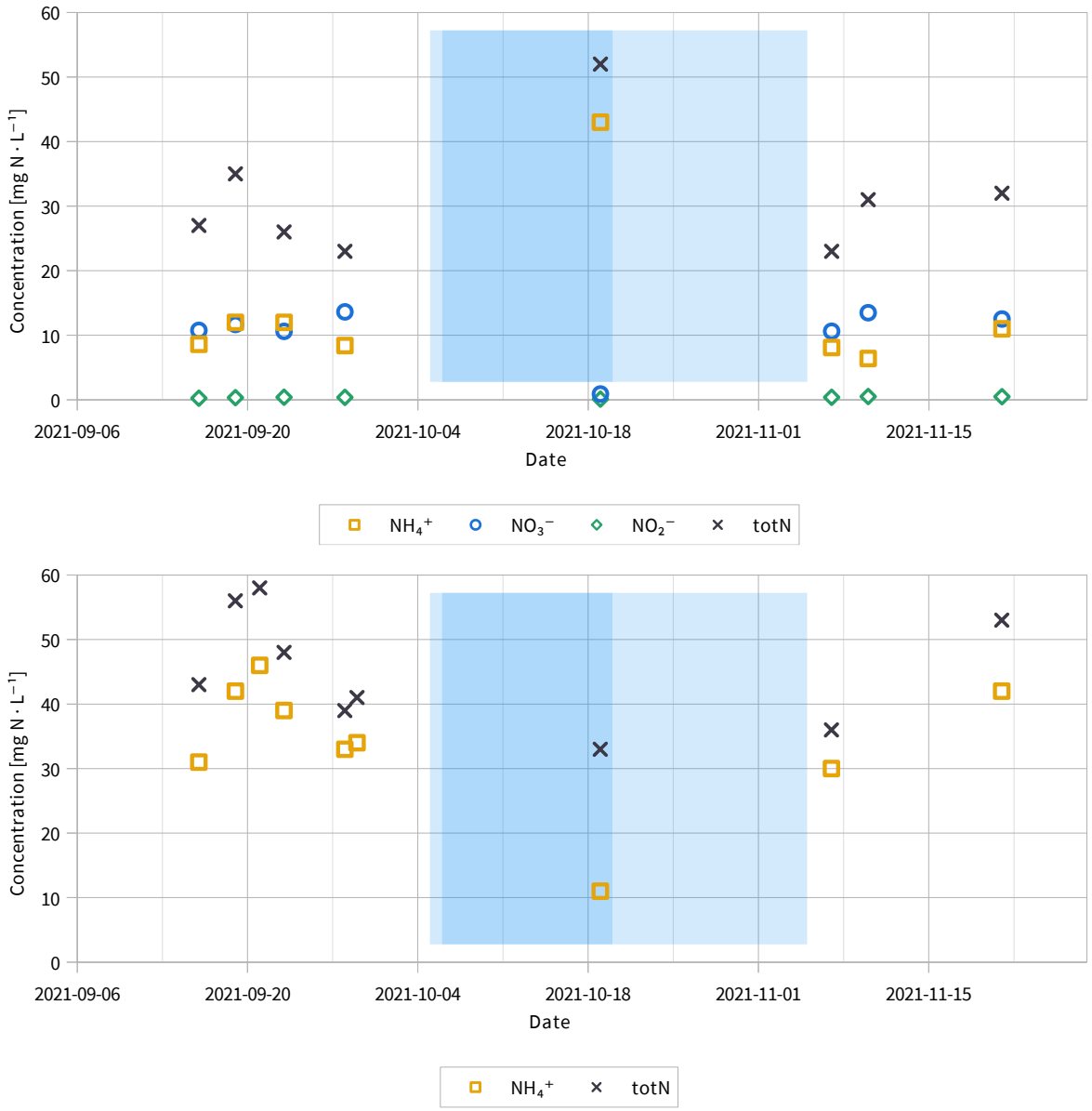


Figure I.3. R_{PDA} (upper) and pilot inflow (lower) flow proportionate diurnal composite sample concentrations. Period of irregular flow and no primary filtration marked with blue and darker blue area respectively.

Table I.1. Flow proportionate diurnal composite sample results from R_{PDA} and pilot inflow. Values from period of irregular flow striked through. Values marked with ∽ calculated excluding data point during irregular flow period.

R_{PDA}

Date	sCOD [mg $O_2 \cdot L^{-1}$]	total COD [mg $O_2 \cdot L^{-1}$]	NH_4^+ [mg $N \cdot L^{-1}$]	NO_3^- [mg $N \cdot L^{-1}$]	NO_2^- [mg $N \cdot L^{-1}$]	totN [mg $N \cdot L^{-1}$]	pH	Alkalinity [mg $HCO_3^- \cdot L^{-1}$]	VFA [mg $O_2 \cdot L^{-1}$]
2021-09-16	47	160	8.6	10.75	0.25	27	7.4	110	1.1
2021-09-19	61	220	12	11.67	0.33	35	7.5	170	2.2
2021-09-23	43	190	12	10.6	0.4	26	7.5	160	2.8
2021-09-28	43	210	8.4	13.62	0.38	23	7.3	100	3.2
~~*2021-10-19*~~	~~*160*~~	~~*540*~~	~~*43*~~	~~*0.9*~~	~~*0.1*~~	~~*52*~~	~~*7.7*~~	~~*330*~~	~~*21*~~
2021-11-07	48	170	8.1	10.61	0.39	23	7.5	140	3.6
2021-11-10	52	220	6.4	13.49	0.51	31	7.6	170	3
2021-11-21	47	270	11	12.51	0.49	32	7.5	180	5.5
Mean∽	48.7	205.7	9.5	11.9	0.4	28.1	7.5	147.1	3.1
Stdev∽	6.2	36.9	2.2	1.3	0.1	4.6	0.1	31.5	1.3

Pilot Inflow

2021-09-16	150	270	31	-	-	43	7.7	260	16
2021-09-19	190	360	42	-	-	56	-	-	-
2021-09-21	200	410	46	-	-	58	7.7	360	28
2021-09-23	150	330	39	-	-	48	-	-	-
2021-09-28	130	280	33	-	-	39	7.7	240	8.7
2021-09-29	130	250	34	-	-	41	-	-	-
~~*2021-10-19*~~	~~*59*~~	~~*510*~~	~~*11*~~	-	-	~~*33*~~	~~*7.4*~~	~~*160*~~	~~*4.6*~~
2021-11-07	150	230	30	-	-	36	7.7	270	5.6
2021-11-21	170	410	42	-	-	53	7.7	350	25
Mean∽	158.8	317.5	37.1	-	-	46.8	7.7	296.0	16.7
Stdev∽	25.9	70.7	5.9	-	-	8.2	0.0	55.0	9.8

Appendix II - Batch test concentrations

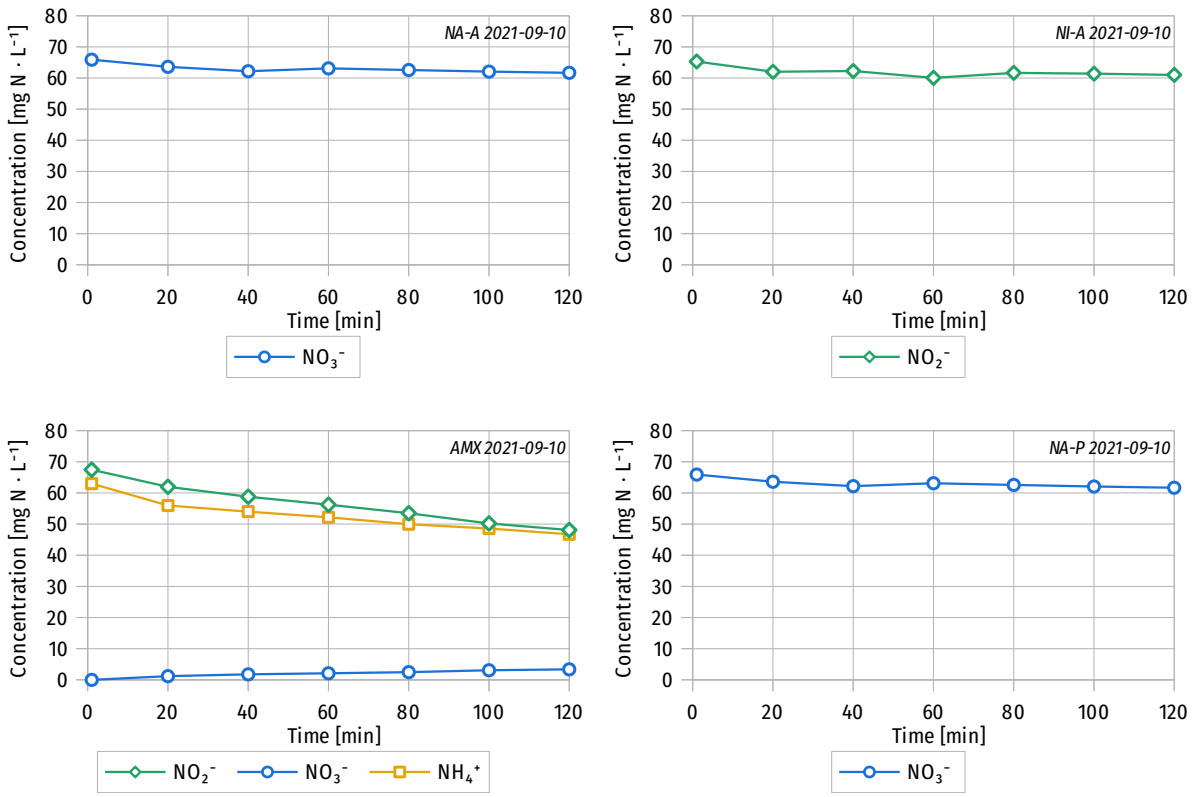


Figure II.1. Ex-situ batch test concentration plots for tests conducted 2021-09-10.

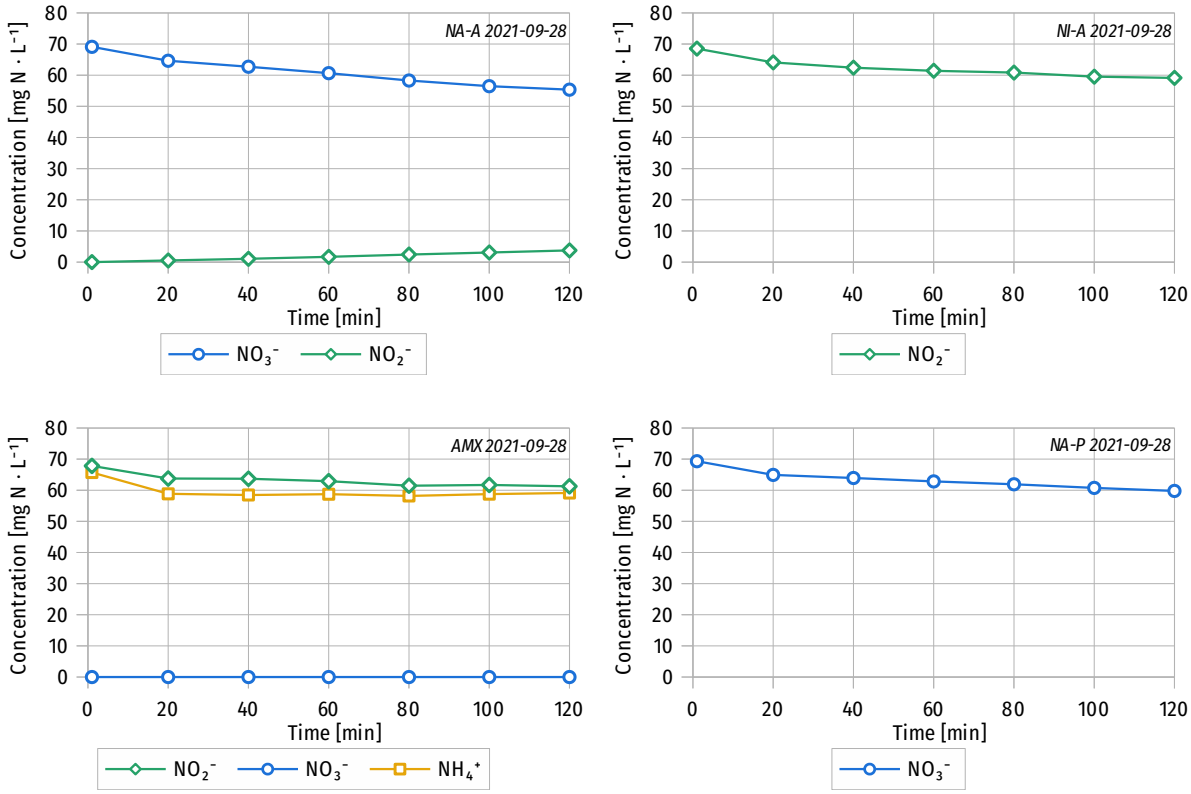


Figure II.2. Ex-situ batch test concentration plots for tests conducted 2021-09-28.

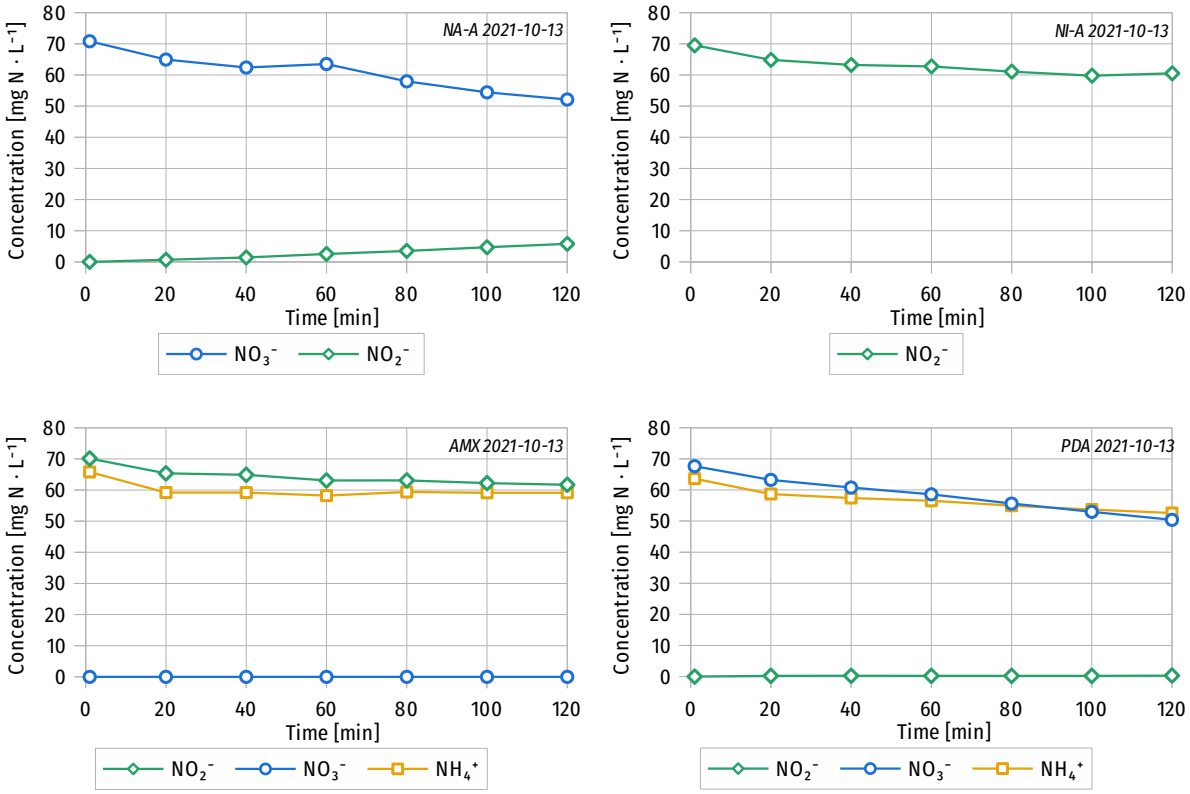


Figure II.3. Ex-situ batch test concentration plots for tests conducted 2021-10-13.

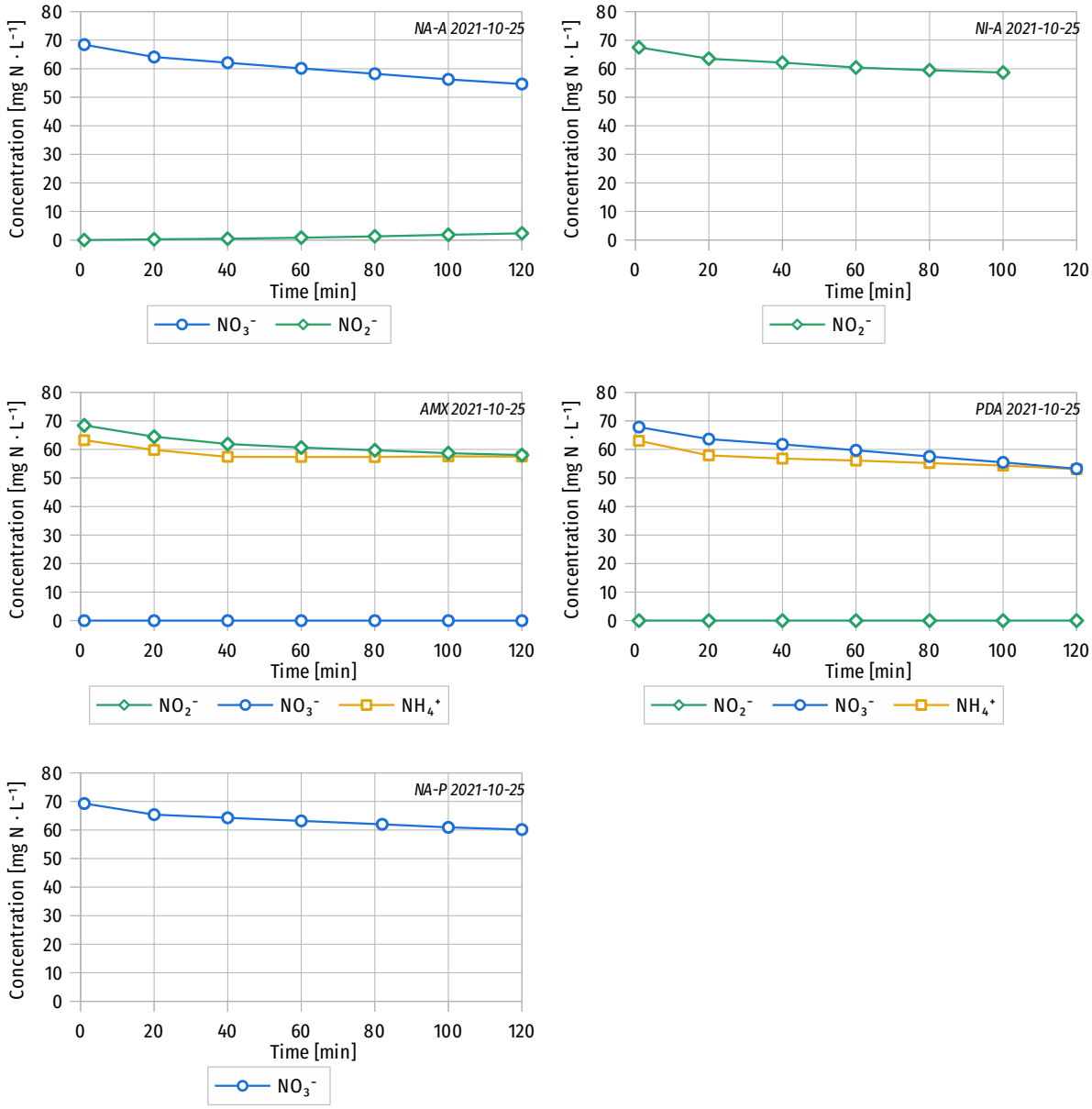


Figure II.4.Ex-situ batch test concentration plots for tests conducted 2021-10-25.

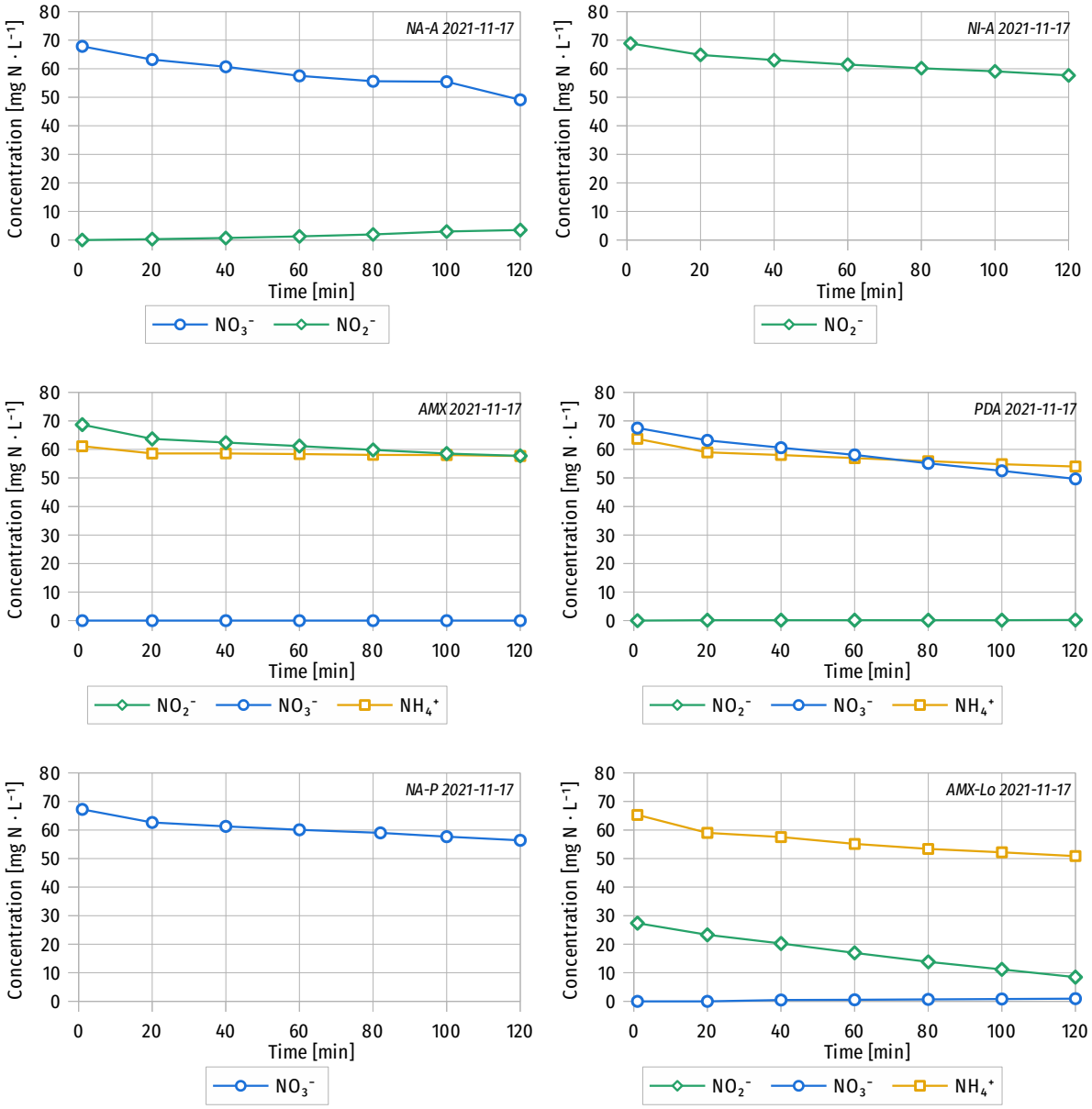


Figure II.5.Ex-situ batch test concentration plots for tests conducted 2021-11-17.

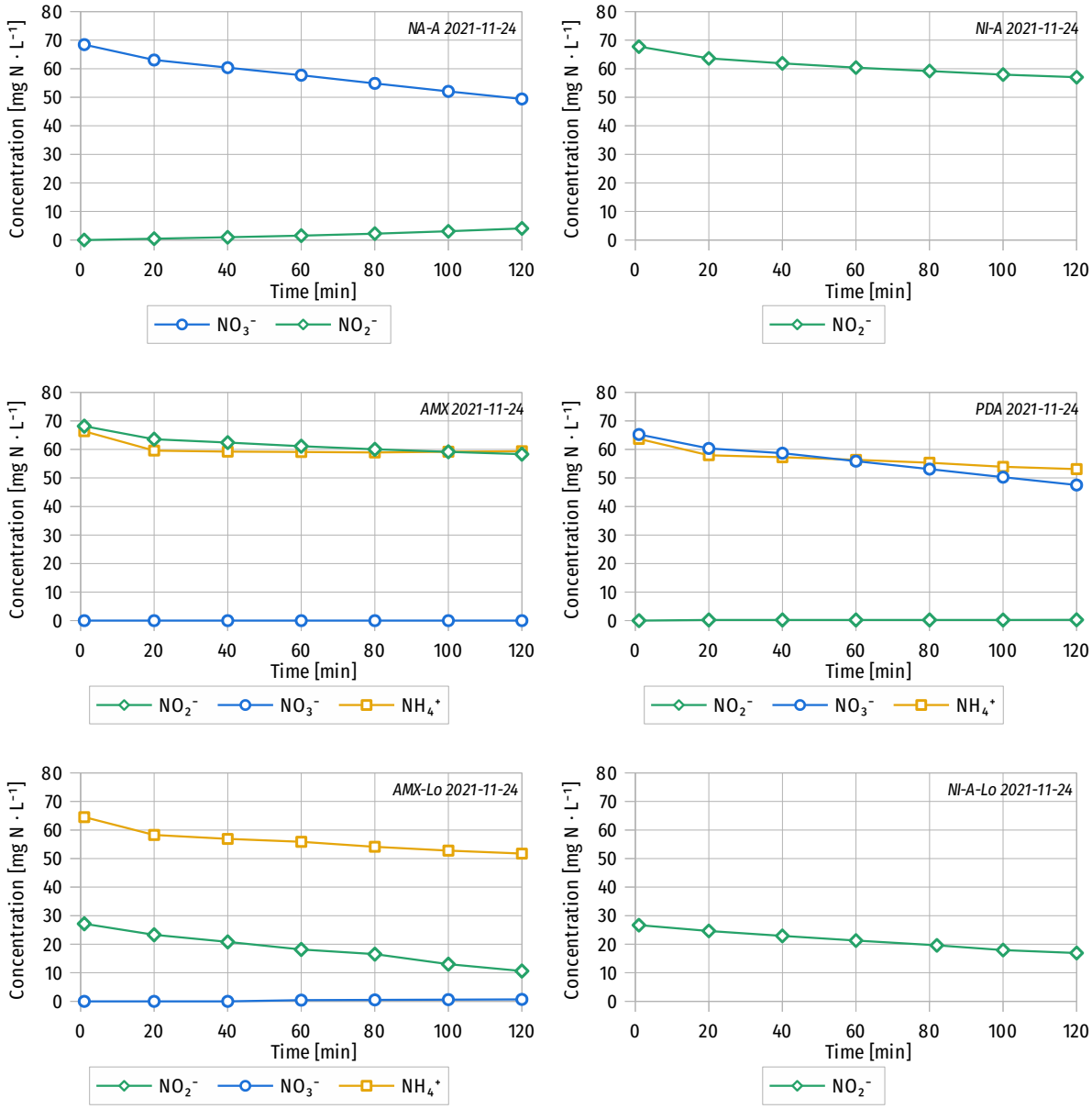


Figure II.6 Ex-situ batch test concentration plots for tests conducted 2021-11-24.

Appendix III - Pilot cycle study data

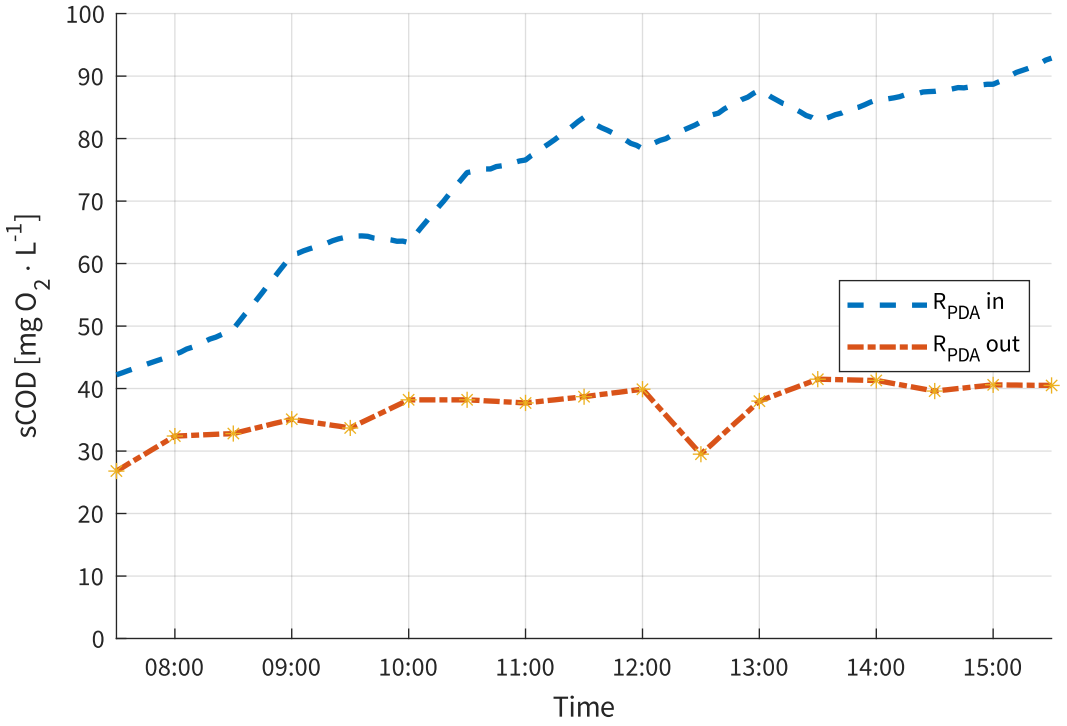


Figure III.1. R_{PDA} sCOD inflow and outflow concentrations during the cycle study. Stars (*) denote measured values.

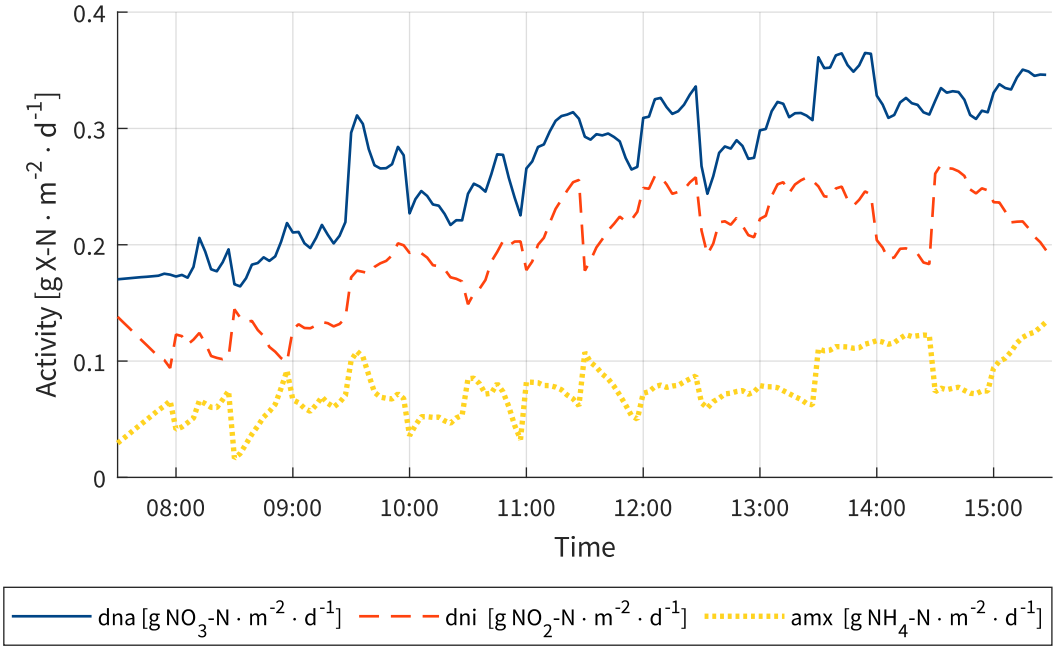


Figure III.2. dna, dni and amx during the pilot cycle study calculated using zero values for denitritation and denitratation ammonium assimilation. Representing a maximum SAA.

Appendix IV - Populärvetenskaplig sammanfattning

Ett steg närmare framtidens reningsverk?

Värme till våra hem, bränsle till fordonen, näring till våra åkrar, levande floder och hav - framtidens reningsverk kan bli en stor tillgång i klimatomställningen. Men för att nå dit krävs nya reningstekniker som bättre tar vara på de resurser som finns i avloppsvattnet. Vägen från idé, via labbet och till full skala i reningsverken är lång och stundtals krokig. I ett spännande samarbete mellan Lunds Tekniska Högskola och forskning och utvecklingsbolaget Sweden Water Research har ett examensarbete utvärderat en sådan process - är vi ett steg närmare framtiden?

En av avloppsreningsverkens funktioner är att minimera vår miljöpåverkan. Det innebär att avlägsna ämnen som kväve, fosfor och kol från avloppsvattnet. Om dessa släpps ut i naturen kan de leda till övergödning och allvarliga miljöproblem som t.ex. algblomning och syrebrist i Östersjön. Samtidigt utgör dessa ämnen en potentiell resurs - kväve och fosfor är viktiga näringsämnen för jordbruket. Kol kan omvandlas till biogas och därmed användas till att producera fjärrvärme, el eller användas som fordonsbränsle.

Idag är det vanligt att kväve avlägsnas i två biologiska reningssteg. Kväve finns löst i avloppsvatten i form av ammonium och i första steget omvandlar mikroorganismer det till nitrat. Detta kräver syre och därför syresätts det första reningssteget genom att pumpa in luft. I andra steget omvandlar andra mikroorganismer i en syrefri miljö nitratet först till nitrit och sedan till kvävgas som släpps ut i atmosfären. Atmosfären består redan av ca. 78 % kvävgas så där gör den ingen skada. Ett problem med denna reningsteknik är att det kräver stora mängder el att pumpa in den luft som behövs. Ett annat problem är att den syrerika miljön leder till att det frodas bakterier som äter upp det mesta av kolet och omvandlar det till koldioxid. Då finns det mindre kol kvar att göra biogas av.

På 90-talet upptäcktes ett nytt sätt att omvandla ammonium till kvävgas, det fick heta anammox. Mikroorganismerna som utför anammox behöver matas med hälften ammonium och hälften nitrit och kan då i en syrefri miljö omvandla dessa till kvävgas direkt. Endast hälften av avloppsvattnet behöver då luftas och därmed går det endast åt ca. hälften så mycket el, samtidigt som mer kol blir över för att göra biogas på. Men anammox är en mycket känslig process och än så länge har det mest fått genomslag i det lilla hörn på reningsverken som behandlar det varma, ammoniumrika rejektvattnet som blir över efter biogasrötning. De stora besparingarna finns i att lyckas få till anammox i behandlingen av allt vatten på reningsverket.

Under 2021 byggde Sweden Water Research en pilotanläggning på Källby ARV i Lund för att undersöka möjligheterna till just detta. I ett examensarbete vid LTH studerade den blivande civilingenjören Tobias Ellingsen pilotanläggningen. Resultaten visade att anammox förekom i piloten och bidrog med ca. 23 % av kvävereningen. "Det är lovande att anammox organismerna överlevde i piloten" säger Tobias, men tillägger att både högre reningsgrad och högre bidrag från anammox kommer krävas innan processen kan tas i bruk i större skala. Piloten finns kvar för vidare forskning och kan ses som ett litet steg mot vad som kan komma att bli en viktig del av framtidens reningsverk.