Adapting Partial Nitritation-Anammox Process to Mainstream Wastewater Treatment

By

Maciej S. Kowalski

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University of Manitoba

Winnipeg

ABSTRACT

The primary objective of the thesis was to adapt partial nitritation and anammox technology to the challenges of mainstream municipal wastewater treatment. Issues resulting from low temperature, low nitrogen concentration, slow growth and relatively low anammox activity as well as extreme difficulty in controlling partial nitritation under these conditions had to be resolved.

As a result, a pre-treatment technique was developed to reduce the start-up times of biofilm anammox reactors. Anammox activity was four times higher with the bioprimer before inoculation as compared to the control. Bioprimer acted as a scaffold that allowed for a very quick attachment of anammox bacteria. The start-up of anammox moving bed biofilm reactors (MBBR) was also quickly achieved by inoculating with waste sludge from granular systems like DEMON®. This study showed that granular biomass can be easily transitioned to a biofilm.

A novel control method for cold partial nitritation was developed. Mainstream partial nitritation was achieved by combining two control strategies. The target level of ammonium oxidation was achieved by controlling dissolved oxygen to total ammonium nitrogen (DO/TAN) ratio and nitritation was obtained by free ammonia (FA) inhibition of nitrite oxidizers at high pH. Long term stability was obtained at DO/TAN ratio of 0.06 and at FA concentration of 1.1±0.2 mg NH_3-N L^{-1}.

A sidestream partial nitritation-anammox (PNA) MBBR reactor treating anaerobically digested sludge dewatering centrate was operated in order to farm anammox biomass used for bio-augmentation purposes. Very fast start-up of PNA process could be achieved by incorporating the bioprimer technique. Nitrogen removal of 2.5 g-N m^{-2} d^{-1} was achieved in as little as 56 days.

Moreover, FA was found to be the predominant inhibitor of nitratation rather than selective wash-out of nitrifying biomass.

Finally, a mainstream PNA reactor incorporating all previous techniques was developed. The proposed configuration comprised of partial nitritation-MBBR and anammox-IFAS reactor has achieved nitrogen removal rates comparable with conventional mainstream nitrogen removal treatment. Partial nitritation-MBBR achieved partial nitritation at 2.0±0.3 g-N m^{-2} d^{-1} and nitrogen removal reached 0.45±0.1 g-N m^{-2} d^{-1} in the anammox-IFAS reactor at 19±3 °C with an average effluent nitrogen concentration of 11±4 mg-N L^{-1}.

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List of publications included in the thesis:

I. Kowalski, M.S., Devlin, T.R., Oleszkiewicz, J.A., 2017. Attachment of anaerobic ammonium-oxidizing bacteria to augmented carrier material. Environ. Technol. 40, 576–583.

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III. Kowalski, M.S., Devlin, T.R., di Biase, A., Oleszkiewicz, J.A., 2019. Controlling cold temperature partial nitritation in moving bed biofilm reactor. Chemosphere 227, 216-224.

IV. Kowalski, M.S., Devlin, T., di Biase, A., Basu, S., Oleszkiewicz, J.A., 2019. Accelerated start-up of a partial nitritation-anammox moving bed biofilm reactor. Biochem. Eng. J. 145, 83–89.

V. Kowalski, M.S., Devlin, T.R., di Biase, A., Oleszkiewicz, J.A., 2019. Effective nitrogen removal in a two-stage partial nitritation-anammox reactor treating municipal wastewater – Piloting PN-MBBR/AMX-IFAS configuration. Bioresour. Technol. 289, 121742.

My contributions to the publications:

I (Chapter 2)	I conceived the experiments with T. Devlin. I have designed the experiment, collected data, performed the analysis and wrote the paper. The paper was reviewed by the co-authors. The co-authors assisted with revisions.
II (Chapter 3)	I conceived the experiments, designed the experiment, performed the analysis and wrote the paper. Some data was collected and analysed by co-authors. The paper was reviewed by the co-authors. The co-authors assisted with revisions.
III (Chapter 4)	I conceived the experiments, designed the experiment, performed the analysis and wrote the paper. Some data was collected and analysed by co-authors. The paper was reviewed by the co-authors. The co-authors assisted with revisions.
IV (Chapter 5)	I conceived and designed the experiment. T. Devlin assisted in fabrication of the reactor set-up. Some data was collected and analysed by co-authors. I wrote the paper. The paper was reviewed by the co-authors. The co-authors assisted with revisions.
V (Chapter 6)	I conceived and designed the experiment. T. Devlin assisted in fabrication of the reactor set-up. Some data was collected and analysed by co-authors. I wrote the paper. The paper was reviewed by the co-authors. The co-authors assisted with revisions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AFR	air flow rate
AMO	ammonia monooxygenase
AMX	anammox
Anammox	anaerobic ammonium oxidation
AnAOB	anaerobic ammonium oxidising bacteria
AOB	ammonium oxidising bacteria
ATP	adenosine triphosphate
BNR	biological nutrient removal
BOD	biochemical oxygen demand
C	carbon
CEPT	chemically enhanced primary treatment
COD	chemical oxygen demand
DAF	dissolved air flotation
DEMON®	deammonification
DI	de-ionised water
DNOHO	denitrifying ordinary heterotrophs
DO	dissolved oxygen
EBPR	enhanced biological phosphorus removal
EPS	extracellular polymeric substances
FA	free ammonia
ffCOD	filtered-flocculated COD
FNA	free nitrous acid
GAC	granular activated carbon

HAO	hydroxylamine oxidoreductase
HDH	hydrazine/hydroxylamine oxidoreductase
HDPE	high density polyethylene
HRT	hydraulic retention time
HZS	hydrazine hydrolase
IFAS	integrated fixed film activated sludge
MBBR	moving bed biofilm reactor
MLSS	mixed liquor suspended solids
N	nitrogen
NAD	Nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide + hydrogen
Nir	nitrate reductase
NLR	nitrogen loading rate
NOB	nitrite oxidizing bacteria
NXR	nitrite oxidoreductase
OHO	ordinary heterotrophic organisms
P	phosphorus
PAO	phosphorus accumulating organism
PE	primary effluent
PN	partial nitritation
PNA	partial nitritation-anammox
RAS	returned activated sludge
RBC	rotary biological contractor
SBR	sequential batch reactor
sCOD	soluble COD

SEM	scanning electron microscopy
SNLR	surface nitrogen loading rate
SNRR	surface nitrogen removal rate
SOLR	surface organic loading rate
SORR	surface organic removal rate
SpAA	specific anammox activity
SRT	solids retention time
Stdev	standard deviation
SuAA	surface anammox activity
T	temperature
TAN	total ammonium nitrogen
TIN	total inorganic nitrogen
TN	total nitrogen
TSS	total suspended solids
UASB	up flow anaerobic sludge blanket reactor
UV	ultraviolet
VSS	volatile suspended solids
WWTP	wastewater treatment plant

1. Chapter 1: Introduction

1.1. The aim and scope of the thesis

The central point of this thesis was to adapt partial nitritation and anammox technology to the challenges of mainstream municipal wastewater treatment with an ultimate objective of developing novel mainstream PNA technology. This was achieved as result of a multistep process that required development of several new techniques and/or process control strategies as well as parallel operation of two pilot reactors in order two simulate anammox bioaugmentation schemes. In the end, a novel reactor configuration for cold PNA was developed and both, the potentials and limitations of the developed technology were evaluated. Effectively, this thesis covers several topics and references to a broad range of issues.

1.2. Structure of the thesis

The thesis consists of seven chapters followed by five publications divided into two parts, this structure is graphically represented in Figure 1-1. The first chapter is the Introduction where the basic concepts of nitrogen removal from wastewater are presented and explained. The Introduction is aimed to provide only the most important background information as each experimental chapter is preceded with a more detailed introduction specifically suited for the particular topics described in each of them.

Part I of the thesis includes Chapters 2-4 (Publication I-III) and describes the development of novel techniques used to aid in the integration of partial nitritation-annamox process into the mainstream treatment train.

Chapter 2 focuses on the problem of long formation times of anammox biofilm and therefore relatively long start-up times of deammonifying reactors. This chapter showcases a simple solution to that problem by pre-treating the surface of the carrier material, thereby increasing the attachment rate of anammox cells and creating a significant improvement in the adhesion

of cells to the carrier material. Rapid attachment of the anammox biomass was achieved in a reactor with media that had a pre-developed layer of biofilm. This biofilm acted as a scaffold with EPS matrix that allowed for a very quick attachment of anammox bacteria. In a way, this approach is analogical to a primer or an undercoat that is put on materials before painting to ensure better adhesion of paint to the surface, hence the suggested name – bioprimer.

Chapter 3 also deals with anammox reactor start-up problems. In this chapter the formation of anammox biofilm has been investigated after seeding the reactor with granular biomass. Availability of granular anammox sludge is much higher than biofilm seed carriers and the sludge is easier to transport. This paper shows that granular biomass can be transitioned to a biofilm relatively easily which opens a new window of opportunity for starting-up anammox MBBRs. Moreover, the anammox biomass grown in that reactor was used as a seed for the start-up of a pilot PNA reactor treating real centrate as described in Chapter 5.

Chapter 4 describes the development of a novel process control strategy for cold partial nitritation. Effective control of partial nitritation under mainstream conditions is the biggest obstacle in successful implementation of anammox process in the treatment of municipal wastewater. This paper proves that partial nitritation can be successfully controlled in a biofilm reactor treating wastewater with low nitrogen concentration, relatively high COD/N ratio and at low temperature. An algorithm for dynamic process control of partial nitritation has been also developed.

Part II of the thesis includes Chapters 5 and 6 (Publications IV-V) and describes the integration of the developed techniques into the pilot scale reactors.

Chapter 5 describes an accelerated start-up and long-term operation of the pilot PNA reactor treating real centrate. Methods developed in Chapter 2 were used to shorten the start-up time of the reactor and anammox biomass grown during the study described in Chapter 3 was used to seed the pilot reactor. Very fast start-up of PNA process was achieved by developing partial

nitritation biofilm first and then seeding with anammox biomass. Incorporating previously suggested bioprimer technique a nitrogen removal rate of 2.5 g-N m^{-2} d^{-1} could be achieved in as little as 56 days. This reactor was also a source of anammox biomass used to bio-augment the mainstream anammox reactor described in Chapter 6.

Chapter 6 contains the last experimental paper of the thesis which takes all the previous findings and the entirety of the gained experience whilst building upon them to develop a PNA technology suited for mainstream municipal wastewater treatment. The feasibility of PNA process was investigated in a novel reactor configuration. Primary effluent from full-scale municipal wastewater treatment plant was treated in a two-stage biofilm system incorporating innovative process control method for cold partial nitritation.

The last chapter of the thesis (Chapter 7) offers a summary of the whole thesis, presents the most significant findings and suggests future research perspectives.

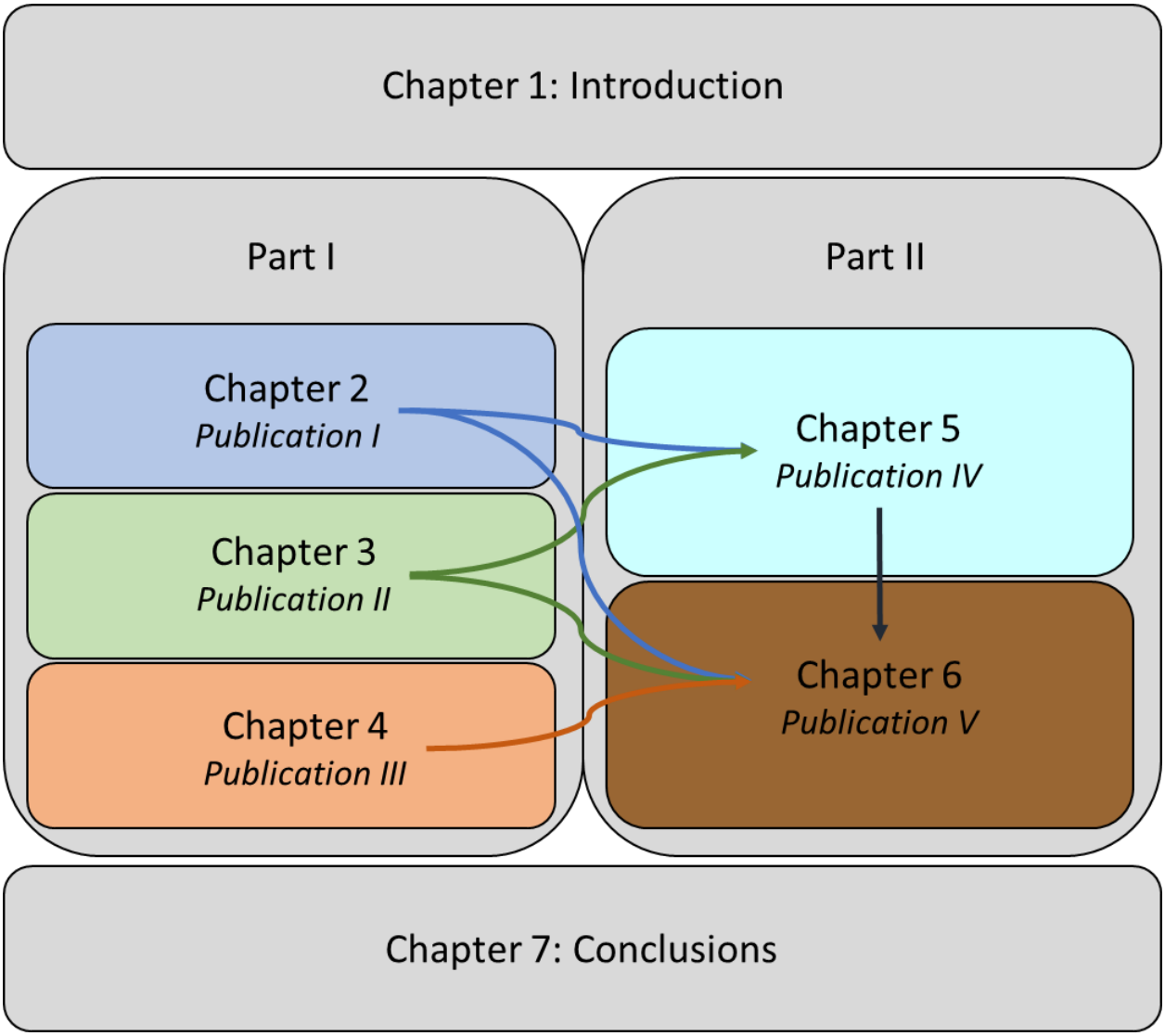

Figure 1-1 Graphical representation of the structure of the thesis. Arrows represent how different chapters relate to each other.

1.3. Background

Human activity has profoundly altered global nitrogen (N) and phosphorus (P) cycle with flowing waters acting as convenient wastewater disposal systems. The loadings of N into lakes, rivers and oceans are derived from a variety of sources including wastewater effluents. External loadings of N and P have profound effects on aquatic ecosystems and the environmental consequences of nutrient enrichment are far more serious and far-reaching then simple excessive production of biomass. The consequences of eutrophication can lead to deterioration

of aquatic ecosystems including loss of species and services that these systems provide. Secondary effects are usually deleterious to the users of the water resource e.g. eutrophic lakes tend to shift towards dominance of the phytoplankton by cyanobacteria, some of which produce substances that are highly toxic. Eutrophication is in fact the major water quality problem in many countries in the world. N removal is particularly important in brackish as sea water where it is considered the main driver of eutrophication. P, on the other hand, is considered to be the limiting factor to eutrophication in discharges to freshwater bodies. Nevertheless, N removal from wastewater is now considered a crucial and effective procedure to prevent eutrophication problems in receiving waters and most industrialized countries have implemented discharge limits on N from wastewater effluents. Moreover, the limits are becoming more stringent as the environmental awareness grows.

1.4. Conventional nitrogen removal

Nitrogen in wastewater is present in both organic and inorganic forms. The most common and important inorganic nitrogen forms in raw wastewater are free ammonia (NH_3) and ammonium (NH_4^+). Organic fraction consists of complex mixture of compounds including amino acids, proteins and amino sugars. Nitrogen in those compounds is easily hydrolysed to ammonium by microorganisms.

Nitrogen is conventionally removed from wastewater in a series of biological processes called nitrification and denitrification. In the first step (nitrification) the ammonium nitrogen is converted to nitrates. In the second step (denitrification) the previously produced nitrate is converted to gaseous nitrogen. Nitrification is in itself a two-step biological process in which ammonium oxidizing bacteria (AOB) oxidize ammonium to nitrite and subsequently nitrite oxidizing bacteria (NOB) oxidize nitrite to nitrate. The first step is termed nitritation and the second nitratation.

AOB and NOB belong to the most important groups of bacteria responsible for nitrification although some archea as well as a newly discovered commamox (complete ammonium oxidation) bacteria are also capable of nitrification. *Nitrosococcus mobilis* and *Nitrosomonas europaea* are found to be the dominant AOB in WWTPs. Whereas genus *Nitrospira* and *Nitrobacter* species are the most important NOB in WWTPs. These organisms are chemoautotrophs which means that they do not need organic carbon to obtain energy. Their only source of carbon is carbon dioxide dissolved in water, however, they do need oxygen to grow and metabolize (Soliman and Eldyasti, 2018).

1.4.1. Nitritation

There are few intermediate compounds in the process of nitritation in which nitrogen changes its oxidation state from -3 to +3. In the first step, ammonia is oxidized to hydroxylamine – NH_2OH, according to the following equation:

$$NH_4^+ + 0.5\,O_2 + 2\,e^- \rightarrow NH_2OH + H^+ \qquad \textbf{(Eq. 1-1)}$$

This reaction is endothermic and is catalysed by an enzyme containing copper – ammonia monooxygenase (AMO) which is located in cell membrane. At the beginning, AMO is reduced which allows for hydroxylamine to be produced. Then hydroxylamine is oxidized to nitrite by hydroxylamine oxidoreductase (HAO) located in periplasm:

$$NH_2OH + H_2O + 2\,e^- \rightarrow NO_2^- + 5\,H^+ + 4\,e^- \qquad \textbf{(Eq. 1-2)}$$

Water is the oxygen donor in this reaction. Four electrons released during the oxidation of hydroxylamine are introduced into the respiratory chain on cytochrome c. Two electrons are used to oxidize ammonia and two others are transported to NAD dehydrogenase in order to produce NADH that is necessary to synthesise biomass. Reduction of NAD undergoes with the

use of ATP energy. Oxidation of hydroxylamine is not a single step process and there are few intermediate compounds being produced including nitric oxide (NO) and nitrous oxide (N_2O).

Nitritation is a key process in technologies to reduce or remove carbon requirements for nitrogen removal.

1.4.2. Nitratation

Nitratation is a single step process and occurs with the presence of nitrite oxidoreductase. Electrons removed from nitrite during oxidation are introduced into the respiratory chain on cytochrome c. At the same time, no proton transport was observed during this process. Oxidation of nitrite first produces NADH and then its oxidation with oxygen as a final electron acceptor to synthesise ATP.

The process of ammonia oxidation is biochemically very complex and requires the presence of certain enzymes and energy transmitters which initiate the reactions in cytoplasm, cell membrane and periplasm.

Stoichiometrically both steps of nitrification can be written in the following way:

$$NH_4^+ + 1.5\,O_2 + 2\,HCO_3^- \rightarrow NO_2^- + 2\,H_2CO_3 + H_2O + (58-84)\ \text{kcal} \quad \textbf{(Eq. 1-3)}$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- + (15.4-20.9)\ \text{kcal} \quad \textbf{(Eq. 1-4)}$$

these reactions can be summarized as follows:

$$NH_4^+ + 2\,O_2 + 2\,HCO_3^- \rightarrow NO_3^- + 2\,H_2CO_3 + H_2O + (73.4 - 104.9)\ \text{kcal} \quad \textbf{(Eq. 1-5)}$$

The energy gained in the processes of ammonia and nitrite oxidation is used by bacteria for cell growth. Reactions describing growth of bacteria can be written as follows:

for AOB:

$$13\,NH_4^+ + 23\,HCO_3^- \rightarrow 10\,NO_2^- + 8\,H_2CO_3 + 3\,C_5H_7O_2N + 19\,H_2O \quad \textbf{(Eq. 1-6)}$$

for NOB:

$$NH_4^+ + 10\,NO_2^- + 4\,H_2CO_3 + HCO_3^- \rightarrow 10\,NO_3^- + C_5H_7O_2N + 3\,H_2O \quad \textbf{(Eq. 1-7)}$$

In the above equation biomass is denoted as $C_5H_7O_2N$.

During the first stage of nitrification as well as in the process of biomass production alkalinity is consumed. It is shown by the equations describing the processes. Alkalinity consumption is equal to 7.14 g $CaCO_3$/g N-NH_4^+. If alkalinity of the wastewater is too low, excessive pH drop can occur which can then cause disruption of the nitrification process. Optimum pH for the nitrification process falls between 7.2 and 9.0. It can be seen from the overall reaction of nitrification that oxygen demand equals to 4.57 g O_2/g N-NH_4^+. In practice, this value is a little lower because part of the ammonium nitrogen is used for the synthesis of new biomass and thus does not undergo oxidation to nitrates.

1.4.3. Denitrification

Denitrification is a process in which nitrate or nitrite is reduced to gaseous products. This process requires anoxic conditions. General scheme of denitrification is shown below:

$$2\,NO_3^- \rightarrow 2\,NO_2^- \rightarrow 2\,NO \rightarrow N_2O \rightarrow N_2 \qquad \textbf{(Eq. 1-8)}$$

Examples of true denitrifying bacteria include the following genera and species: *Bacillus, Pseudomonas stutzeri, Pseudomonas aereginosa, Pseudomonas celcis, Achromobacter denitrificans*. The demand for organic compound in denitrification is equal to 2.3-2.9 g BOD_5/g $N\text{-}NO_3$. It is assumed that for each gram of nitrate nitrogen falls a decrease of 2.86 g of BOD_5 (theoretically). Denitrification is accompanied by alkalinity rise of 3 g $CaCO_3$/g $N\text{-}NO_3$, considering the process with the use of organic compounds from the raw wastewater. The denitrification rate is not limited if $BOD_5/N\text{-}NO_3$ ratio is higher than 3.5. At lower $BOD_5/N\text{-}NO_3$ ratios it is necessary to introduce external organic compounds to wastewater, including external carbon source.

1.5. Completely autotrophic nitrogen removal

Anaerobic ammonium oxidation (anammox) is one of the novel biotechnologies for nitrogen removal. Anammox process is a significant component of the biogeochemical nitrogen cycle. It has been estimated that 30-70% of the annual release of N_2 to the atmosphere is contributed through anammox process (Song and Tobias, 2011). The process was discovered when it was noticed that ammonium was being converted to dinitrogen in a fluidized-bed reactor system at a yeast factory. In the process, two pollutants: ammonium and nitrite are removed simultaneously. This was first discovered in 1994 (Mulder et al., 1995).

The overall reaction for the anammox process is given below:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O\ (\Delta G^\circ = -357\ \text{kj mol}^{-1}) \quad \textbf{(Eq. 1-9)}$$

This reaction may be further broken down into the redox half-reactions that occur within the cell (Kartal et al., 2011):

$$2\ NO_2^- + 2\ H^+ + e^- \rightarrow NO + H_2O\ (E^\circ = +0.38V) \quad \textbf{(Eq. 1-10)}$$

$$NO + NH_4^+ + 2\ H^+ + 3\ e^- \rightarrow N_2H_4 + H_2O\ (E^\circ = +0.06V) \quad \textbf{(Eq. 1-11)}$$

$$N_2H_4 \rightarrow N_2 + 4\ H^+ + 4\ e^-\ (E^\circ = -0.75V) \quad \textbf{(Eq. 1-12)}$$

These catabolic reactions occur within the anammoxosome, a specialized pseudo-organelle within the bacterium, and create a proton gradient across the anammoxosome membrane. The first step involves the reduction of nitrite to nitric oxide by nitrate reductase (Nir). Ammonium is then combined with nitric oxide by hydrazine hydrolase (HZS) to form hydrazine (van Niftrik et al., 2008). Soluble cytochrome-c proteins in the anammoxosome lumen provide the electrons required for these two steps (van Niftrik and Jetten, 2012). The final step involves oxidation of hydrazine to dinitrogen gas via hydrazine/hydroxylamine oxidoreductase (HDH) (van Niftrik et al., 2008). The electrons liberated by hydrazine oxidation are transferred to another 4-electron cytochrome-c protein, which in turn delivers these electrons to ubiquinone present in the anammoxosome membrane. Ubiquinone then transfers these electrons to cytochrome–bc1 in the membrane, which then transfers them to the soluble cytochrome-c proteins that originally supplied Nir and HZS with electrons, restoring them as electron donors. Cytochrome–bc1 is a proton pump, translocating 6 protons into the anammoxosome lumen for every 4 electrons

transferred. This results in a buildup of protons inside the anammoxosome, which can be used to synthesize ATP (van Niftrik and Jetten, 2012).

Anammox bacteria are specialized into a group of deep-branching planctomycetes that were found in both wastewater treatment systems and natural waters. Anammox-capable bacteria are obligately anaerobic chemolithoautotrophs. Five "*Candidatus"* anammox genera have been identified so far: "*Ca*. Kuenenia", "*Ca*. Brocadia", "*Ca*. Anammoxoglobus", "*Ca*. Jettenia", and "*Ca*. Scalindua" (van Niftrik and Jetten, 2012).

General anammox reaction including cell mass synthesis that was derived from experimental observations follows Equation 1.13 (Strous et al., 1998):

$$NH_4^+ + 1.32\,NO_2^- + 0.066\,HCO_3^- + 0.13\,H^+ \rightarrow \quad \textbf{(Eq. 1-13)}$$

$$\rightarrow 1.02\,N_2 + 0.26\,NO_3^- + 0.066\,CH_2O_{0.5}N_{0.15} + 2.03\,H_2O$$

As illustrated in the above equation approximately 11% of the nitrogen is converted into NO_3^-. Nitrate is produced as a result of new biomass synthesis.

1.6. Advantages of Partial Nitritation Anammox

Figure 1-2 shows a comparison between nitrification denitrification and partial nitritation anammox (PNA) pathways. PNA presents a very beneficial shortcut to the extremely energy intensive process of full nitrification and denitrification. During partial nitritation only about 55% of ammonium needs to be oxidised to nitrite. That means that the oxygen requirements are 60% lower compared to full nitrification which translates to very substantial savings in energy consumption associated with aeration in wastewater treatment. Especially since aeration can account for approximately 60 to 90% of energy requirements of the conventional treatment plant (EPRI, 1994).

Moreover, anammox (unlike denitrification) does not require any biodegradable carbon source to remove nitrogen and sufficient amount of BOD might not be available in wastewater when enhanced biological phosphorus removal is incorporated into the treatment train, especially in relatively weak North American wastewater (Oleszkiewicz et al., 2015).

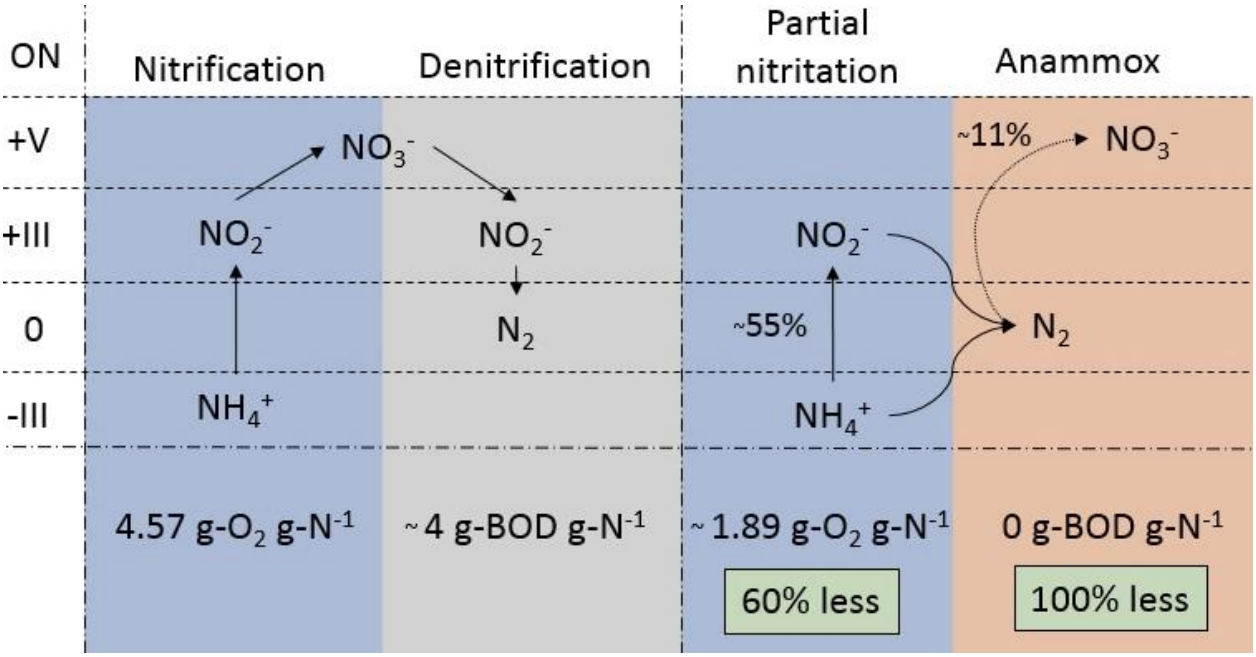

Figure 1-2 Simplified nitrogen conversion pathways during nitrification denitrification, and partial nitritation and anammox processes.

Both, partial nitritation and anammox are also processes that are fully autotrophic and use CO_2 as a sole source of carbon for biomass growth. As a result, biomass yields are extremely low compared to heterotrophic denitrification bacteria (DNOHO) (see Table 1-1). With sludge management and disposal costs being the costliest processes during wastewater treatment, any reduction in the volume of excess sludge produced is tremendously beneficial.

Table 1-1 Comparison of kinetic parameters between[1]

PARAMETER	UNITS	AOB AT 20°C	NOB AT 20°C	DNOHO AT 20°C	ANAMMOX AT 30-35°C
μ_{max}	g VSS/ g VSS•d	0.85-1.5	0.7-1.6	3.2	0.06-0.07
Yield	g VSS/ g NH_4-N	0.10-0.15	0.04-0.07	0.54	0.07-0.13
R_O	g O2/g N	3.43	1.14	0	0
BOD	g BOD/ g NO3-N	-	-	4	0

Apart from obvious operational savings, climate effects of implementation of mainstream PNA should also be considered. Replacing nitrification denitrification with autotrophic nitrogen removal could potentially increase eutrophication benefits without increasing or even reducing climate change effects (Hauck et al., 2016). Although, there is still no consensus in literature about the level of nitrous oxide (N_2O) emissions from PNA reactors (Kampschreur et al., 2009; Schaubroeck et al., 2015) as they are governed by many factors. Data from full-scale WWTPs is necessary to obtain trustworthy greenhouse gas emission profiles from mainstream PNA.

1.7. Mainstream Partial Nitritation Anammox

PNA has become an established technology in the treatment of high-temperature, high-strength reject waters with more than a 100 full scale installations worldwide (Lackner et al., 2014). Reject waters like centrate or filtrate from dewatering of anaerobically digested sludge are characterised by a temperature of 35-37°C and contain on average 500-1500 mg NH_4-N L^{-1}. In contrast the temperature of municipal wastewater varies seasonally from around 9°C to 20°C and the average TAN concertation ranges from 40-50 mg NH_4-N L^{-1}. Further application of anaerobic ammonium oxidation to mainstream wastewater treatment would significantly reduce energy requirements due to reduced aeration as well as eliminated need for external carbon

[1] Adopted from Metcalf and Eddy (2014), Pai et al. (2014) and ASM3

dosing and could bring wastewater treatment plants close to energy autarky. However, anammox process has not yet been successfully applied to mainstream wastewater treatment. There are several challenges that have to be overcome in order to achieve reliable mainstream PNA, they include low temperatures and low nitrogen loadings, stringent effluent quality requirements and long-term process stability (Laureni et al., 2016).

The research is now focused to explore the potential of mainstream PNA and to solve those problems. Table 1-2 presents a review of the recent research on the topic. Current trends are divided between one-stage SBR (granular) and MBBR (biofilm) systems with only a handful of two-stage configurations. Generally, there is much more research available for SBR reactors rather than biofilm systems, like MBBRs. This is mainly because of extreme difficulties in controlling cold partial nitritation.

One of the first pilot scale mainstream PNA studies was done in Europe at Strass WWTP in Austria. A performance of a granular reactor equipped with hydrocyclones was evaluated during 2011-2012. Operational performance of the pilot reactor showed effective nitrogen removal with bio-augmentation from a sidestream DEMON® reactor. The process was, however, unstable and experienced some nitrite accumulation. Inhibition of NOB was possible only due to high bio-augmentation rates from the sidestream reactor (Wett et al., 2015).

As part of the same trans-Atlantic R&D co-operation, a similar bench-scale study was conducted at Blue Plains WWTP (Washington, DC) in 2011. The goal was to asses the mechanisms and process kinetics of mainstream deammonification and to identify key process components. The study identified the importance of aggressive SRT management for effective NOB wash-out with simultaneous retention of anammox biomass. Intermittent aeration regime with high DO set-point was shown to effectively inhibit nitrite oxidation. However, bioaugmentation of anammox biomass from sidestream and hydrocyclones were necessary to maintain viable anammox population in the mainstream reactor (Wett et al., 2013).

Pilot study at Chesapeake Elizabeth WWTP (VA, USA) has tested an application of two-stage mainstream deammonification process without bioaugmentation for nitrogen polishing only. The first-stage activated sludge process was partially nitrifying effluent wastewater by controlling the ratio of ammonia over nitrite/nitrate (NO_x-N). This was followed by anammox MBBR in a second stage. Overall, the system was only partially successful in outcompeting NOB utilizing transient aeration with high DO set-point and short SRT but only at 20 °C. The process was very difficult to control. The performance of polishing anammox MBBR was stable, however, nitrogen removal was limited by the influent NO_2/NH_4 ratio (O'Shaughnessy, 2015).

Since then, research on mainstream deammonification utilizing anammox has grown rapidly due to the evident benefits. Further research is required to investigate the capacity of anammox bacteria to grow and perform under low temperatures (15°C or lower) and to develop the most efficient technology to improve poor effluent quality at mainstream conditions (Ali and Okabe, 2015).

Table 1-2 Literature overview of the research focused on mainstream PNA process.

Process	Wastewater type and strength	T[2]	NRR[3]	SpAA[4]	Comment	Reference
RBC						
PNA (one stage)	Pretreated primary effluent 30-60 mg N L^{-1}	15	435 g-N m^3 d^{-1}	NA	NO_2 and NO_3 accumulation	(De Clippeleir et al., 2013)
MBBRs						
PNA (one stage)	Synthetic 50 mg N L^{-1}	20	NA	0.14-1 g-N kg-TSS^{-1} d^{-1}	NO_2/NO_3 accumulation	(Gilbert et al., 2014)
		10	5-11 mg-N m^{-2} d^{-1} 8-16 g-N m^3 d^{-1}	0.14-1 g-N kg-TSS^{-1} d^{-1}		
	Pretreated primary effluent 40 mg N L^{-1}	25	0.04-0.13 g-N m^{-2} d^{-1} 18	NA	NO_3 overproduction	(Malovanyy et al., 2015b)

[2] Temperature, °C

[3] Nitrogen Removal Rate

[4] Specific Anammox Activity

			g-N m^{-3} d^{-1}			
PNA (two stage)	Pretreated primary effluent with centrate dosing	15	0.20 g-N L^{-1} d^{-1}	NA	Alternate centrate supply and thin biofilm controlled PN	(Piculell et al., 2016b, 2015)
PNA (one stage)	Pretreated municipal wastewater 21 mg N L^{-1}	15	30 g-N m^{-3} d^{-1}	103 ± 18 mg N L^{-1} d^{-1} (max anammox activity)	anammox activity suppression during operation at 11 °C.	(Laureni et al., 2016)
IFAS						
PNA (one stage)	Pretreated primary effluent 40 mg N L^{-1}	25	55 g-N m^3 d^{-1}	NA	NO_3 overproduction	(Malovanyy et al., 2015a)
SBRs						
PNA (one stage)	Synthetic 60 mg N L^{-1}	10	90 g-N m^3 d^{-1}	NA	Unstable operation at 10°C	(T Lotti et al., 2014)
PNA (one stage)	Synthetic	20	NA	0.1/0.8 g-N g-VSS^{-1} d^{-1}	Non-acclimated/acclimated	(Lotti et al., 2015)
		10		0/0.2 g-N g-VSS^{-1} d^{-1}		
PNA (one stage)	Pretreated primary effluent 15-30 mg N L^{-1}	23.2	223 ± 29 g-N m^3 d^{-1}	70 mg-N g-VSS^{-1} d^{-1} (20°C)	Observed unstable removal in situ	(Hoekstra et al., 2018)
		13.4	97 ± 16 g-N m^3 d^{-1}			
		15	400 g-N m^3 d^{-1}			

1.8. References

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Part I

Development of novel techniques for integration of partial nitritation-annamox into the mainstream treatment train

2. Chapter 2: Attachment of anaerobic ammonium oxidizing bacteria to augmented carrier material

2.1. Abstract

The formation of stable and highly active anammox biofilm is a lengthy process leading to long start-up times of deammonifying reactors of several months or more. This study aims to provide a quick and simple solution to the problem of long start-up periods for biofilm reactors by pre-treating the surface of carrier material, thereby increasing attachment rates of anammox cells and creating a significant improvement in the adhesion of cells to the carrier material. Two different techniques were investigated. The first one focused on growing a layer of heterotrophic biofilm on the surface of the plastic carriers prior to inoculation with anammox biomass. Specific anammox activity increased by almost 400% as compared to seed values and was equal to 250 mg NH_4-N g-VSS^{-1} d^{-1}. In the second technique, carrier material was coated with high porosity thin film of granular activated carbon to provide higher surface area. The anammox activity increased by approximately 50%. In comparison, control reactor with no media pretreatment did not develop any biofilm and no anammox activity was detected. Rapid attachment of the anammox biomass was achieved in a reactor with media that had a pre-developed layer of a biofilm. This biofilm acted as a scaffold with EPS matrix that allowed for a very quick attachment of anammox bacteria. In a way, this approach is analogous to a primer or an undercoat that is put on materials before painting to ensure better adhesion of paint to the surface, hence the suggested name – bioprimer.

2.2. Introduction

Excess discharge of nitrogen to receivers is a major contributing factor to eutrophication of natural water bodies and therefore poses a great environmental problem. Nitrogen is conventionally removed from wastewaters via nitrification and denitrification processes during

which ammonium is oxidized to nitrate and subsequently reduced to gaseous nitrogen. Nitrification is energy intensive and requires extensive aeration while denitrification necessitates substantial amounts of biodegradable carbon. With the discovery of the anaerobic ammonium oxidation (anammox) process, nitrogen removal can be achieved with much lower aeration and almost insignificant organic carbon source. Ammonium removal or deammonification using anammox is a fully autotrophic two-stage process combining partial nitritation and anammox process. First, approximately half of the ammonium is oxidized to nitrite and then the remaining ammonium together with nitrite are combined and transformed during the anammox process to gaseous nitrogen and some nitrate. The residual nitrate, constituting approximately 10% of the original nitrogen load, must be denitrified or re-introduced into the anammox metabolism.

Nitrogen removal from warm, high strength streams via anaerobic ammonium oxidation has become an established technology with over 100 installations worldwide. The available technologies range from suspended to attached growth systems in batch or continuous operation (Ali and Okabe, 2015). High anammox activity and relative abundance of anaerobic ammonium oxidizing bacteria (AnAOB) in biofilms together with the relative ease of controlling the population of nitrite oxidizing bacteria (NOBs) and retaining anammox biomass in biofilm systems (MBBR, IFAS) gives them a pronounced advantage over other technologies (Guo et al., 2016). The formation of stable and highly active anammox biofilm, however, is a lengthy process leading to long start-up times of deammonifying reactors of several months or more (Morales et al., 2015).

Even with anammox inoculum on the carriers the start-up periods can reach a few months. The start-up of a full-scale ANITAmox plant in Malmö took approximately 4 months to reach 90% ammonia removal. By incorporating flexible nitrogen loading rates the start-up period was reduced to 2 months at another ANITAmox plant in Växjö (Christensson et al., 2013). An

ANITAmox system installed in Durham, North Carolina was started within 3 months with subsequent problems with nitrate overproduction due to continuous aeration that exceeded minimum oxygen requirements. The reactor achieved only 80% ammonia removal and 70% TIN removal (Bilyk et al., 2016).

This start-up technique requires substantial amounts of seed carriers with already established anammox biofilm, usually 3-15% of the design media fill. This amount of seed material might not be always readily available (Araujo et al., 2011), especially in North America where there are only a few full-scale MBBR plants. To the best knowledge of the authors there are 4 full-scale ANITAmox installations in the United States (as of 2017) located in James River (VA), South Durham (NC), Chicago (IL) and Denver Metro (CO). In Canada, there is not a single one.

Recent studies have focused on inoculum-free start-ups of moving bed biofilm deammonification reactors. Inoculum-free development of a deammonifying biofilm in a pilot-scale reactor treating reject water took more than 10 months (Rikmann et al., 2017). Free ammonia inhibition of the microbial populations was suggested as the main reason behind such a long start-up period. After introducing automatic pH control the biofilm developed in 3-4 months. Kanders et al. (2016) reported similar findings while starting-up a deammonification process treating reject water with indigenous anammox bacteria in approximately 4 months.

One of the reasons for slow start-ups of full-scale deammonification plants is a very slow growth rate of AnAOBs that leads to a minimum doubling time of 14 days (Strous et al., 1997). The affinity of AnAOBs to carrier material is also of great importance in the speed of biofilm development. But while specific maximum growth rate of AnAOB is an inherent kinetic property of the specific species and cannot be directly enhanced, the rate of AnAOB attachment can be altered.

There has been also extensive research into reducing the start-up times of anammox reactors by enhancing the anammox activity. The techniques range from addition of chemicals to developing new control strategies. Addition of hydrazine (Xiao et al., 2015), reduced graphene oxides (Yin et al., 2016), ferrous iron (Bi et al., 2014) or acyl-homoserine lactones (Zhao et al., 2016) were shown to increase the anammox activity in lab-scale operation. Majority of the research, however, has focused on activity enhancements of granular anammox reactors and cannot be directly applied to biofilm systems. The limiting factor in fast start-up of biofilm reactors is not the activity but the ability to retain anammox biomass in the system and increase the attachment of anammox to the carrier material. Immobilizing anammox biomass in gel carriers has been recently studied (Ali et al., 2015; Isaka et al., 2017, 2013) and was shown to be successful in retaining the anammox biomass in the system as well reducing the start-up time of the reactor to approximately 2 months. Zhang et al. (2017) reported growth rates for immobilized anammox bacteria to be equal to 0.33 d^{-1} (doubling time of around 3 days), which is much faster than ever reported. These results point to the fact that anammox in a biofilm aggregate can grow much faster than in suspension as studied by Strous, given optimum conditions (Zhang et al., 2017). The gel immobilization technique, however, requires the production of the gel and its further processing.

This study aims to provide a quick and simple solution to the problem of long start-up periods for biofilm reactors by pre-treating the surface of plastic carrier material in a specific way, thereby increasing attachment rates of anammox cells and creating a significant improvement in the adhesion of cells to the carrier material. Two different techniques were investigated. The first one focused on growing a layer of heterotrophic biofilm on the surface of the plastic carriers prior to inoculation with anammox biomass. This biofilm acted as a scaffold with hydrophobic EPS matrix that allowed for a very quick attachment of AnAOBs. In a way, this approach is analogous to a primer or an undercoat that is put on materials before painting. This

technique was chosen because OHOs are characterized by very high growth rates and the ability to form biofilms very quickly. They also form complex EPS matrices, especially under high F/M ratios. Due to these reasons, heterotrophic biofilm is grown on the media prior to inoculation with anammox biomass. This creates a scaffold with high affinity to AnAOBs thus establishing the anammox biofilm faster and more easily. AnAOBs have been shown to produce highly hydrophobic and significantly different type of EPS matrix than ordinary heterotrophic organisms (OHOs) which is crucial in biofilm formation (Sheng et al., 2010; Hou et al., 2015).

In the second technique, carrier material was coated with high porosity thin film of granular activated carbon (GAC) in order to provide higher surface area with great potential cation exchange capacity making it a superior scaffold for anammox bacteria to attach to. GAC has been used as a carrier for anammox in several studies and has been recently proven as a feasible carrier material for anaerobic biomass which also improved the process. SEM results showed that more biomass was attached to GAC compared to zeolites (Dutta et al., 2014). Chen et al. (2012) investigated bamboo charcoal as a carrier material for anammox biomass and showed increased anammox performance in GAC augmented UASB reactor.

2.3. Materials and Methods

2.3.1. Analytical Procedures

The performance of the reactors was monitored using volumetric nitrogen removal rates and ratios of nitrate produced to nitrogen used and ratios of nitrite used to ammonia used. Nitrite, nitrate and ammonia were measured spectrophotometrically by QuickChem flow injector analyser (Lachat). Total Nitrogen (TN) was calculated as the sum of nitrite, nitrate and ammonia nitrogen since no organic source was provided in the medium. Chemical oxygen demand (COD) was measured spectrophotometrically using Hach vial tests. Volatile suspended solids (VSS)

and total suspended solids (TSS) were measured according to Standard Methods (APHA 2012). Pictures of the biofilm were taken using Zeiss stereoscopic microscope.

2.3.2. Media pre-treatment

AnoxKaldnes K3 media were used as a carrier material for the biofilm and were pre-treated using two methods.

Development of bioprimer

During the first treatment, heterotrophic biofilm was grown on the media in aerobic conditions. A Plexiglas reactor with a working volume of 5 L was seeded with activated sludge and inoculated with media to 40% volume (ca 200 carriers). The reactor was kept at 33 ± 1°C with constant mixing and aeration. The pH was monitored and controlled at 7.6 ± 0.1 with a 3M H_2SO_4 solution dosed by a peristaltic pump (Masterflex L/S, Cole-Parmer). The reactor was continuously fed with synthetic wastewater consisting of either acetate (1-14 d) or beef extract (15-22 d), ammonia chloride, potassium phosphate monobasic and microelements. Hydraulic retention time (HRT) of the reactor was controlled at 6 h. Biomass washing-out of the reactor was not returned. During the first 2 weeks, the reactor was operating at an organic loading rate of approximately 1.1 g-sCOD L^{-1} d^{-1}. Subsequently, the loading was increased to approximately 3.6 g-sCOD L^{-1} d^{-1} and rapid development of heterotrophic biofilm was observed. On day 22 the biofilm was clearly visible colouring the media with an orange tint (Figure 2-2A). At that point solids attached to the media were measured. 10 carriers were removed from the reactor and the biofilm was mechanically removed from them to a solution of DI water. On average 7.82 mg of VSS were attached to each media which translates to a biomass concentration of approximately 313 mg-VSS L^{-1} with a VSS/TSS ratio of 0.86. On average sCOD removal oscillated between 60 and 80% (Figure 2-1).

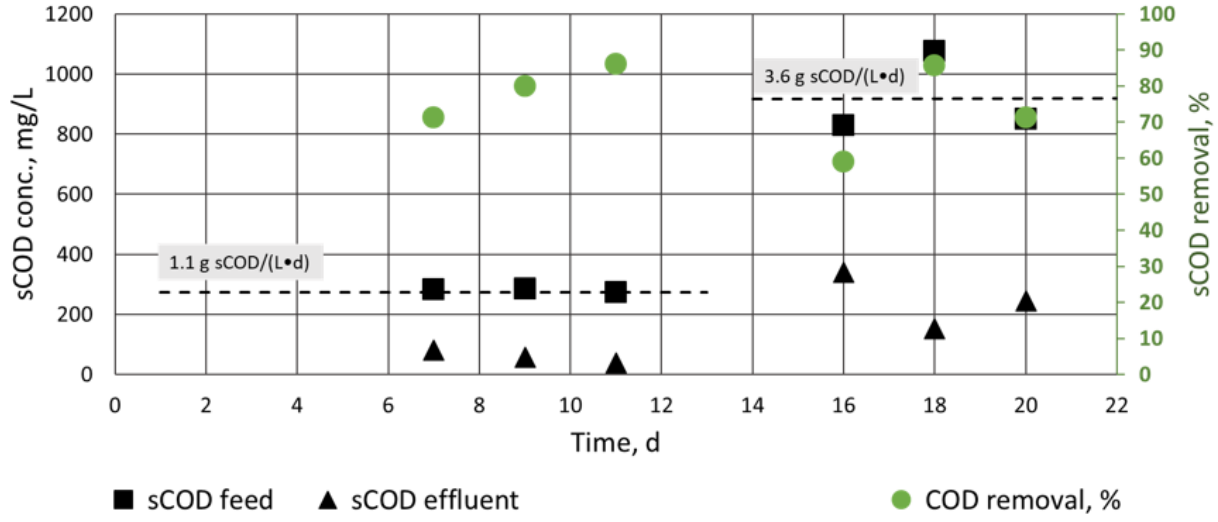

Figure 2-1 Organic loading rate and sCOD removal in a pre-treatment reactor.

On day 22 a rapid decrease in pH to values below 6 was observed pointing to the onset of nitrification and a consequent attachment of nitrifiers to the biofilm. Thus, the final biofilm (Figure 2-2A and B) is regarded to be a mixture of heterotrophs and autotrophs. On day 23 the media were introduced to the anammox reactors.

GAC Coating

In the second method, media were coated with a thin layer of GAC prior to transfer to the anammox reactor. In the first step of the procedure clean media were covered with a 3M contact adhesive diluted with acetone to create a thin film on the surfaces of the media. Immediately after that the media were put in the container with GAC and shaken so that the GAC could penetrate to the inside of the media and attach itself. The media were left to dry for 48h after which they were put in a water bath for 24h to remove excess carbon and wash the excess adhesive. The coated media is shown in Figure 2-2C and D. After that the media were introduced to the anammox reactor.

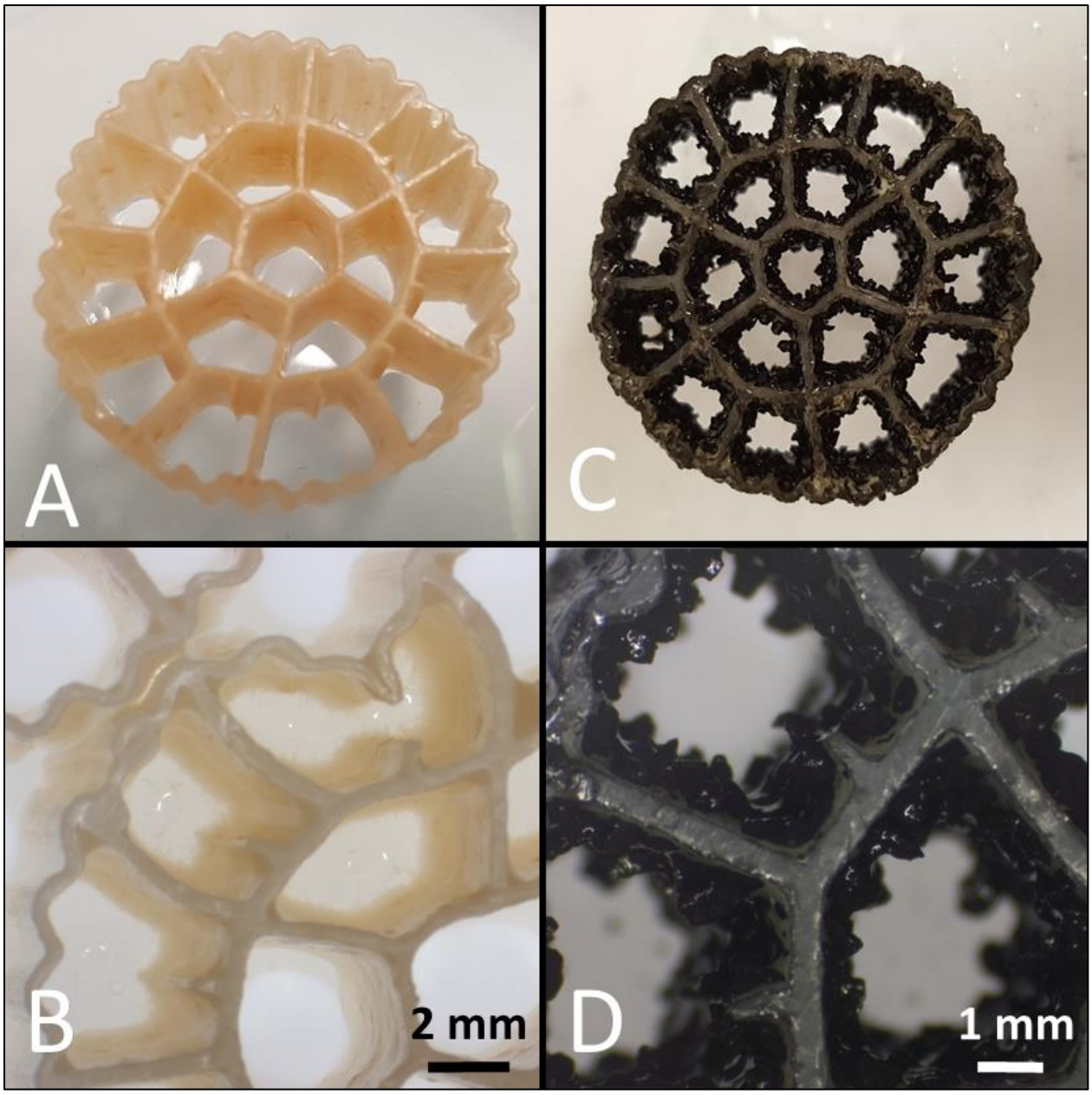

Figure 2-2 Aerobic biofilm formed during media pre-treatment (A and B) as well as a view of a media covered with a granular carbon coat (C and D).

2.3.3. Anammox Inoculum

Anammox inoculum was obtained from a membrane partial nitritation-anammox bioreactor operated in the same laboratory. The reactor was operated at 28°C with nitrogen loading rate of 0.2 kg m^{-3} d^{-1}. Waste sludge from the membrane bioreactor was used as a seed for the following experiments. The membrane reactor was originally seeded with waste sludge from a full-scale DEMON® reactor (HRSD York River, VA).

2.3.4. Experimental Set-up

The study was conducted in 3 Plexiglas circular reactors with 5 L working volume. Schematic diagram of the experimental configurations is shown in Figure 2-3. Reactor 1 acted as a control with virgin media. Reactor 2 was working with media coated with a bioprimer, Reactor 3 was working with GAC coated media. Each reactor was filled to 40% with media. The reactors were then seeded with WAS from an already operating suspended growth deammonification reactor and placed in an incubator at 33 ± 1°C. The initial biomass concentration in the reactors was equal to 0.7 g-VSS L^{-1}. The reactors were constantly mixed and fed with synthetically prepared medium. The medium contained ammonium chloride, sodium nitrite and sodium bicarbonate. Micronutrients solution was also added with minor changes according to Graff et al. (1996). HRT of the reactors was controlled with peristaltic pumps at 24h. The pH in the reactors was monitored and controlled at 7.6 ± 0.1 with a 3M H_2SO_4 solution dosed by a peristaltic pump (Masterflex L/S, Cole-Parmer). Biomass washing out of the reactors was trapped in settling tanks and returned to the reactors at specified time intervals. Volumetric nitrogen load to the reactors during the period of the study was equal to 116.5 ± 3.7 mg-N L^{-1} d^{-1}.

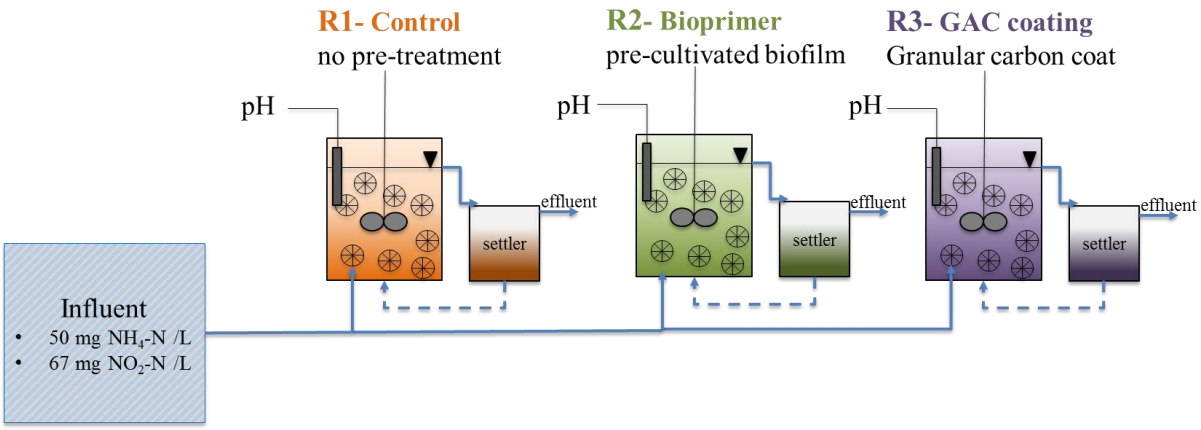

Figure 2-3 Schematic diagram of the experimental set-up.

2.3.5. Specific Anammox Activity Batch Tests

Specific anammox activity (SpAA) batch tests were conducted on day 28 in triplicates in 0.5 L glass bottles. Each bottle had 20 carriers from R1, R2 or R3 and was filled with 0.5 L of synthetically prepared medium with NH_4-N and NO_2-N concentrations of 31 and 41 mg L^{-1} respectively. The bottles were sealed and put in a water batch at 35°C and constantly mixed. Samples were taken every hour for 5 h and analysed for NH_4-N, NO_2-N and NO_3-N. SSA was reported as mg NH_4-N g-VSS^{-1} d^{-1}.

2.4. Results and Discussion

Performance of the reactors was monitored for 50 days. Figure 2-4 (top) shows TN removal expressed as percentage of nitrogen removed considering produced nitrate. The biomass washed out from the reactors was returned on days: 4, 8, 11, 13, 15 and 17. On day 36 the reactors were bioaugmented with fresh biomass from a lab-scale deammonifying bioreactor.

2.4.1. Ammonium and nitrite removal

During first 2 weeks of intensive wash-out and bioaugmentation the removal trends were similar for all reactors. However, from day 18 on, three unique trends could be observed. Performance of R1 dropped rapidly after last bioaugmentation on day 17 and the performance was lost completely on day 28. Total loss of performance in the control reactor was associated with lack of attachment of AnAOBs to the carrier material and complete washout of the suspended biomass from the reactor. The reactors were bioaugmented on day 36 with a fresh biomass and some recovery of the removal was observed in R1. On day 44 ammonium and nitrite removal reached 50% and stabilized on this level for the reminder of the study.

A significantly different trend was observed in R2 with a layer of bioprimer on the media. Rapid increase in the removal of ammonia and nitrite was observed from day 18 and 94% removal was reached on day 25, only a week after bioaugmentation was stopped. The performance

increased even further in the next 2 weeks and reached a maximum of 97%. These results show great potential of the bioprimer method in increasing the attachment rate of AnAOBs to carrier material and significantly shortening the start-up times of anammox biofilm reactors.

Reactor 3 was operating with GAC coated media. The performance of the reactor oscillated between 57-81% with a relatively steady trend and average ammonium and nitrite removal of 65±7 % throughout the period of the study. Reactor 3 did not reach full ammonium and nitrite removal like R2 and operated at a quasi-steady state from day 17. The lack of improvement in nitrogen removal suggested the existence of a certain limiting factor or an inhibition effect preventing additional performance improvement. This hypothesis was further explored by conducting a series of specific anammox activity batch tests with original blank media and original GAC coated media inoculated with suspended deammonifying biomass. The results showed that SpAA was approximately 15.6 % lower during the test with GAC coated media ($P<0.05$). This difference strongly implies partial inhibition of anammox bacteria by the carbon coating. The authors believe that this inhibition originated from the contact adhesive that was used to fix the granular carbon to carrier material.

2.4.2. TN removal

During first 4 days of operation total nitrogen removal followed similar pattern in all 3 reactors. Nitrogen removal oscillated between 55 and 67% on day 1 and decreased as the biomass was washed out from the reactors to 39-53% on day 4. The biomass was then returned, and the nitrogen removal recovered and then gradually decreased to 9 and 8% in R1 and R3 respectively. The performance of R2, however, did not drop so dramatically and was equal to 41% on day 8. From day 11 to 17 the biomass was returned to the reactors more frequently to avoid extensive wash-out and to accelerate attachment. During this period nitrogen removal trends in R1 and R2 were virtually the same and oscillated between 30-46% but nitrogen removal in R3 was much more stable and slightly higher – 46%. Since day 18 the biomass was

no longer returned and was let to completely wash-out from the reactors. From this point, a unique trend for each of the reactors could be observed.

Performance in R1 decreased dramatically after day 17 and dropped to 0% on day 26. Nitrogen removal was almost completely lost after the wash-out implying minimal attachment. There was also no visible biofilm on the media. The reactors were bioaugmented with fresh biomass on day 36 and nitrogen removal in R1 recovered slightly and reached 23% on day 41, however, it decreased again afterwards and dropped below 10% during the last week of the study.

Performance in R2 was highest and most stable. Nitrogen removal increased from 45% to 53% on day 30 but then decreased to 37% on day 35. After last bioaugmentation on day 38 nitrogen removal in R2 increased significantly and reached 71% on day 43. Afterwards, however, the performance dropped slightly again and stabilized at 63%.

Between days 18 and 50 nitrogen removal in R3 decreased steadily with a linear trend to 15%. No improvement in performance of R3 was observed after bioaugmentation on day 36.

2.4.3. $Nitrate_{produced}/Nitrogen_{used}$

The cause behind relatively low total nitrogen removal values was mainly due to overproduction of nitrate. The ratios of nitrate produced to nitrogen used are shown on Figure 2-4 (bottom). Ideally, the ratio should be oscillating around 0.11 as calculated from the general anammox reaction (Strous et al., 1997). It is particularly useful as it shows the fraction of nitrogen oxidized to nitrate. Values higher than 0.11 would indicate prevalence of nitrite oxidizing bacteria (NOB). On day 17 the ratios were equal to 0.51, 0.42 and 0.35 for R1, R2 and R3, respectively. Since then the ratios started to gradually increase in all reactors. The ratio increased up to 0.62 in R2 on day 37 but after last bioaugmentation it started to decrease rapidly and dropped to 0.26 on day 44 and then stabilized at 0.34 at the end of the study. In R3, the

ratio climbed all the way to 0.57 on day 35 and after bioaugmentation increased even more and stabilized at approximately 0.75.

The fact that at one point as much as 60% of nitrogen load was oxidized to nitrate in a non-aerated and lid-covered reactor was very irregular. This amount of nitrate production cannot be explained by oxidation due to oxygen mass transfer into the reactor. It was estimated that only approximately 5-10% of the nitrogen load could have been oxidized by that route, which means that the rest of the nitrate was being produced by a different type of metabolism. It is, therefore, hypothesised that the remaining nitrate was produced by anoxic rather than aerobic metabolism of AOBs and NOBs. Based on a proteome overview of anammox reactions (de Almeida et al., 2016) nitrite oxidation to nitrate is catalysed by nitrite:nitrate oxidoreductase (NXR) which replenishes the electrons that are withdrawn from the cyclic electron flow for CO_2 fixation. Under steady state conditions 11% of nitrogen load is transformed to nitrate by periplasmically oriented NXR. However, in this study this fraction was much higher which might be explained by a shift of the anammox metabolism towards NO_3. The electrons produced from this process are used by acetyl-CoA synthase to fix carbon and produce new biomass. It is reasonable to assume that under studied conditions the anammox consortium would have been in a prolonged log phase favouring faster reproduction, and hence the higher ratio.

Moreover, according to the study by Lücker et al. (2010) anammox bacteria share the periplasmically bound NXR complex with *Nitrospira* and *Nitrospina* which forms a distinct phylogenetic lineage with AnAOBs and implies common ancestry (Lücker et al., 2010). The constitutive expression of NXR in these organisms enables them to use NO_2^- immediately after this energy source becomes available. That would agree with the observations in this study where NO_2-N, present in R2 even in very low concentrations, would turn this metabolic pathway on and explain the increased production of nitrate under anoxic conditions. In contrast, *Nitrobacter* and *Nitrococcus* possess a different type of the NXR enzyme which is a membrane-

bound complex that is competitive only in higher nitrite concentrations. Nitrite concentration in R3 was significantly higher compared to R2 which could point to the prevalence of this type of NOBs in the system and high nitrate production.

Additionally, newest proteomic overview of anammox metabolism may suggest that anammox bacteria could possess both types of NXR complexes. The role of the additional NXR is yet unknown (de Almeida et al., 2016).

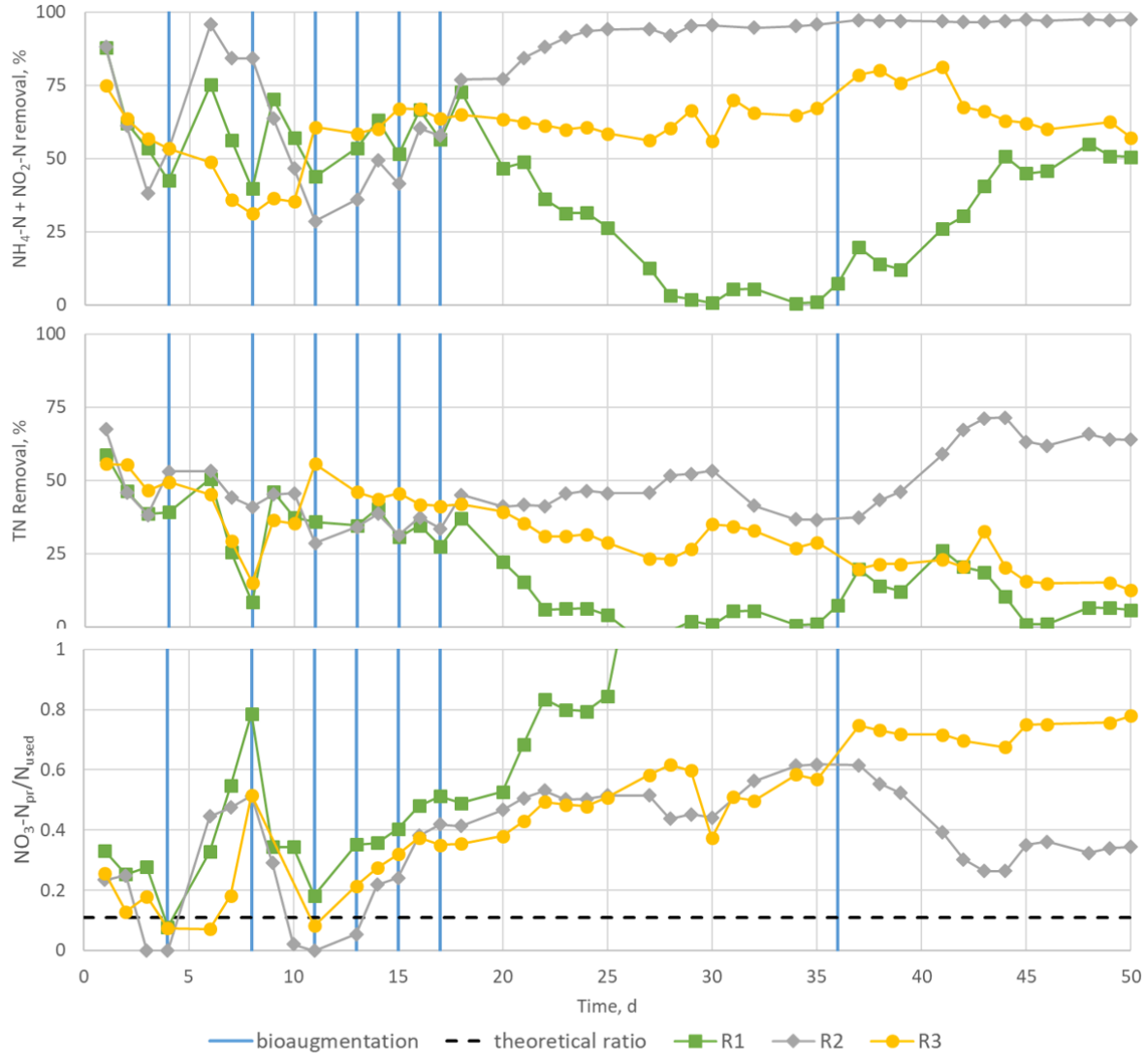

Figure 2-4 TN removal (top), ratio of nitrite to ammonia used (middle) and ratio of nitrate produced to nitrogen used.

2.4.4. Ecological competition in the biofilm

Very interesting trend was observed in TN removal in R2 which is presented on Figure 2-5. TN removal increased after bioaugmentation on day 17, reached a certain maximum and then started decreasing. The same pattern was observed after bioaugmentation on day 36. TN removal would significantly increase, reach a higher maximum value than previously but then decrease slightly. This behaviour is most probably related to the competition between the bioprimer's original microbiome and the anammox culture. While AnAOBs attached to the bioprimer very rapidly they had to compete with the organisms in a mixed biofilm and due to their slow growth rate started to be outcompeted and the TN removal decreased subsequently.

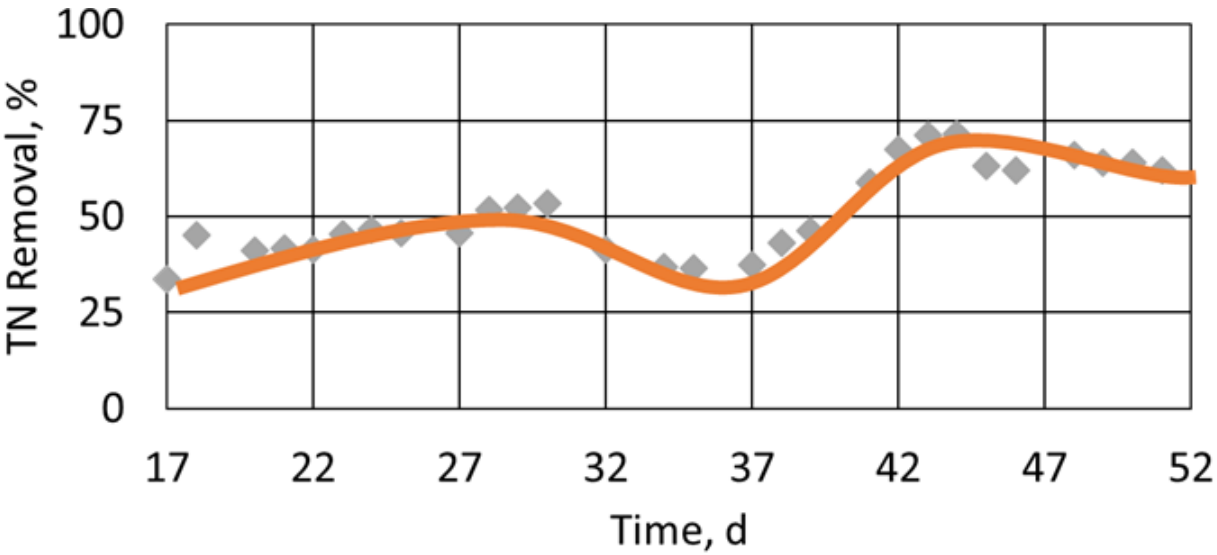

Figure 2-5 Trend of TN removal in R2.

The problem with the competition effect could be easily solved by chemically or physically treating the bioprimer to inhibit the original microbiome prior to inoculation with anammox biomass but without destroying the EPS matrix that allows for quick attachment of AnAOBs.

On the other hand, biological diversity reliably increases biofilm formation compared to more homogenous conditions, as such biofilm formation is a response to ecological competition. This

effect was studied on a collection of *Pseudomonas aureginosa* isolates and has been described by Oliveira et al. (2015).

2.4.5. SpAA Batch Tests

Batch tests to measure specific anammox activity of the biofilm were conducted on day 28 and the results are shown on Figure 2-6. Specific anammox activity in R2 increased by almost 400% as compared to seed values and was equal to 250 mg NH_4-N g-VSS^{-1} d^{-1}. This shows great potential of the pretreatment method using media with pre-developed biofilm to increase the attachement of anammox biomass. In R3 the anammox activity increased by approximately 50%. In comparison, R1 (control reactor with no media pretreatment) did not develop any biofilm and no anammox activity was detected during batch tests.

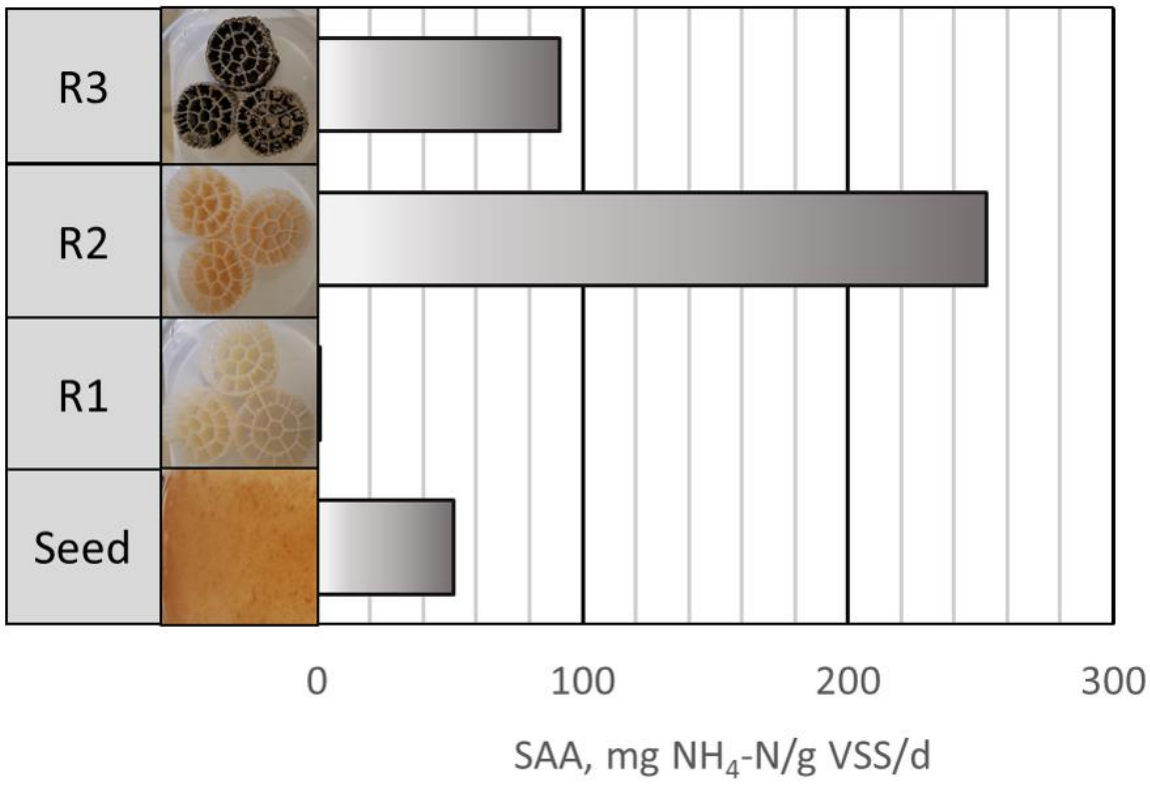

Figure 2-6 Specific anammox activity of the biofilm measured on day 28 as compared to seed.

2.5. Conclusions

Results obtained from the study show great potential of the developed pre-treatment methods in significantly reducing start-up times of biofilm anammox reactors. Rapid attachment of the anammox biomass was achieved in a reactor with media that had pre-developed layer of a biofilm undercoat. This biofilm acted as a scaffold with EPS matrix that allowed for a very quick attachment of AnAOBs. In a way, this approach is analogous to a primer or an undercoat that is put on materials before painting to ensure better adhesion of paint to the surface, hence the suggested name – bioprimer.

This study demonstrated for the first time the ground-breaking advantages of the bioprimer for rapid development of anammox biofilms.

2.6. Acknowledgements

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3. Chapter 3: Start-up and long-term performance of anammox moving bed biofilm reactor seeded with granular biomass

3.1. Abstract

Availability of granular anammox sludge is much higher than biofilm seed carriers and the sludge is easier to transport. This paper describes and investigates a formation of mature anammox biofilm originated from granular sludge and proves that an anammox moving bed biofilm reactors (MBBR) can be easily and quickly started-up by seeding with granular sludge. The reactor was fed with synthetic wastewater containing ammonium and nitrite. Successful start-up was completed in as little as 50 days when TN removal increased to more than 80%. Surface nitrogen loading rate during start-up was equal to 0.75 g m^{-2} d and was stepwise increased up to 5.3 g m^{-2} d. Biofilm thickness reached 1269±444 µm at the end of the study with specific anammox activity of 22.0±2.1 mg N g^{-1} VSS h. This study shows that granular biomass can be transitioned to a biofilm relatively easily which opens a new window of opportunity for starting-up anammox MBBRs.

3.2. Introduction

Nitrification is energy intensive and requires extensive aeration while denitrification necessitates substantial amounts of biodegradable carbon. With the discovery of the anaerobic ammonium oxidation (anammox) process (Mulder et al., 1995), nitrogen removal can be achieved with much lower aeration and almost insignificant organic carbon source. Ammonium removal using anammox is a fully autotrophic process where ammonium together with nitrite are combined and transformed to gaseous nitrogen and some nitrate (Strous et al., 1997).

Nitrogen removal from warm, high strength streams of wastewater via anaerobic ammonium oxidation has become an established technology with over 100 installations worldwide. The

available technologies range from suspended to attached growth systems in batch or continuous operation (Ali and Okabe, 2015; Lackner et al., 2014). High anammox activity and relative abundance of anaerobic ammonium oxidizing bacteria (AnAOB) in biofilms together with the relative ease of controlling the population of nitrite oxidizing bacteria (NOBs) and retaining anammox biomass in biofilm systems (MBBR – moving bed biofilm reactor, IFAS – integrated fixed film activated sludge) gives them a pronounced advantage over other technologies (Guo et al., 2016). The advantages of biofilm systems include higher resistance to temperature variations and nitrite accumulation (Eva M. Gilbert et al., 2015). Biofilms tend to be more resilient to inhibitors like free ammonia or other toxic compounds due to diffusion gradients protecting deeper layers of biofilm. Biofilms are more effective at removal of recalcitrant or toxic pollutants (Venkata Mohan et al., 2013). Several micropollutants were also shown to have higher removal rates in biofilm compared to suspended sludge (Falås et al., 2013). The formation of stable and highly active anammox biofilm, however, is a lengthy process leading to long start-up times of deammonifying reactors of several months or more (Morales et al., 2015).

Recent studies have focused on inoculum-free start-ups of moving bed biofilm deammonification reactors. Inoculum-free development of a deammonifying biofilm in a pilot-scale reactor treating reject water took more than 10 months (Rikmann et al., 2017). Free ammonia inhibition of the microbial populations was suggested as the main reason behind such a long start-up period. After introducing automatic pH control the biofilm developed in 3-4 months. Kanders et al. (2016) reported similar findings while starting-up a deammonification process treating reject water with indigenous anammox bacteria in approximately 4 months.

Even with anammox inoculum on the carriers the start-up periods can reach a few months. The start-up of a full-scale ANITA™mox (single-stage MBBR) plant in Malmö took approximately 4 months to reach 90% ammonia removal. By incorporating flexible nitrogen loading rates the

start-up period was reduced to 2 months at another ANITA™mox plant in Växjö (Christensson et al., 2013). An ANITA™mox system installed in Durham, North Carolina was started within 3 months with subsequent problems with nitrate overproduction due to continuous aeration that exceeded minimum oxygen requirements. The reactor achieved only 80% ammonia removal and 70% TIN removal (Bilyk et al., 2016).

This start-up technique, however, requires substantial amounts of seed carriers with already established anammox biofilm, usually 3-15% of the design media fill. This amount of seed material might not be always readily available (Araujo et al., 2011), especially in North America where there are only few full-scale MBBR plants. To the best knowledge of the authors there are only 4 full-scale ANITA™mox installations in the United States (as of 2017) located in James River (VA), South Durham (NC), Chicago (IL) and Denver Metro (CO). There are none in Canada. Anammox process has been, however, recently recognized by the Canadian Water Network as a one of the most important technologies to decrease energy consumption and improve nutrient removal (Oleszkiewicz et al., 2015).

On the other hand, availability of suspended granular sludge from DEMON® (deammonifying sequential batch reactor with granular sludge) plants is much higher and the sludge is easier to transport. The objective of this research was to investigate the formation of mature anammox biofilm from granular sludge and to assess the ease and rate of acclimation of an anammox MBBR. Subsequently, long-term performance of the acclimated reactor and rate of its recovery after pH upsets will be presented.

3.3. Material and Methods

3.3.1. Experimental set-up and seeding strategy

The study was conducted in a plastic circular reactor with 16 L working volume. The reactor was filled to 40% (app. 6 L) with AnoxKaldnes K3 media with a specific surface area of 500

m^2 m^{-3}. The reactor was then seeded with waste sludge from a full-scale DEMON® reactor (York River) and placed in an environmental chamber (SR-Chamber, CONVIRON, USA) at 33 ± 1°C.

The initial biomass concentration in the reactor was equal to approximately 1.25 g VSS/L. The reactor was constantly mixed and fed with synthetically prepared medium. The medium contained ammonium chloride (NH_4Cl), sodium nitrite ($NaNO_2$) and sodium bicarbonate ($NaHCO_3$). Micro- and macro-nutrients solution (Table 3-1) was also added with minor changes according to Graff et al. (1996). Hydraulic retention time (HRT) of the reactor was controlled with a peristaltic pump (Masterflex L/S, Cole-Parmer) at 48h during the start-up period. The pH in the reactor was monitored (Alpha pH200 meter, Eutech Instruments, USA) and controlled at 7.6 ± 0.2 with a 3M H_2SO_4 solution dosed by a peristaltic pump (Masterflex L/S, Cole-Parmer). Biomass washing out of the reactors was trapped in a settling tank and returned to the reactors manually during first two weeks of operation. Volumetric nitrogen load to the reactor during the period of the start-up was equal to approximately 140 mg N L^{-1} d.

Table 3-1 Macro- and micro-elements provided with the feed.

Component	Concentration in feed, mg/l
KH_2PO_4	27.2
EDTA	15
$ZnSO_4•7H_2O$	0.43
$MnSO_4•H_2O$	0.99
$CoCl_2•6H_2O$	0.24
$CuSO_4•5H_2O$	0.25
$Na_2MoO_4•2H_2O$	0.22
$NiCl_2•6H_2O$	0.19

$NaSeO_4 \cdot 10H_2O$	0.21
H_3BO_4	0.014
$MgSO_4$	147
$FeSO_4 \cdot 7H_2O$	15
$CaCl_2 \cdot 2H_2O$	180

3.3.2. Operation strategy

Following the start-up, operation of the reactor was divided into several periods (referred to as Phases 1, 2, 3 and 4) during which the volumetric and surface nitrogen loading rates (NLRs) to the reactor were increased stepwise as indicated in Table 3-2. Increase of the NLRs was achieved by increasing nitrogen concentration in the feeding medium, shortening HRT and/or increasing the working volume of the reactor, all while maintaining original number of carriers. On days 155 and 241 the reactor had experienced a sudden and prolonged drop in pH to values of approximately 5.5 due to the malfunction of the acid dosing pump. The effect of the pH shocks on a performance of MBBR reactor and its subsequent recovery was observed.

Table 3-2 Operational parameters of the reactor during start-up and consequent phases of operation.

Period of the study		**Start up**	**Phase 1**	**Phase 2**	**Phase 3**	**Phase 4**
Days		**11-106**	**106-136**	**136-209**	**209-240**	**240-320**
N concentration in feed, mg L^{-1}	Average	290	430	550	580	560
	Stdev	20	30	30	20	40
Volumetric NLR, mg L^{-1} d	Average	140	210	270	520	530
	Stdev	10	20	10	50	40
Surface NLR, mg m^{-2} d	Average	750	1110	1440	2570	5330
	Stdev	60	90	70	190	400

3.3.3. Analytical methods

The performance of the reactor was monitored using volumetric and surface nitrogen removal rates, as well as stoichiometric nitrogen ratios. Nitrite, nitrate and ammonium concentrations in the influent and effluent were measured three times a week by QuickChem flow injection analyser (Lachat QuikChem 8500, HACH, CA). Total Nitrogen (TN) was calculated as the sum of nitrite, nitrate and ammonium nitrogen since no organic source was provided in the medium. Volatile suspended solids (VSS) and total suspended solids (TSS) were measured according to Standard Methods (APHA, 2005). Pictures of the biofilm were taken using a stereoscopic microscope (Zeiss StereoDiscovery.V6, USA) with magnification that varied from 0.315x to 1.5x. All samples that required filtration were run through medium porosity Q5 filter paper (Fisher Scientific, CA).

3.3.4. Specific anammox activity batch tests

Specific anammox activity (SpAA) batch tests were conducted in triplicates in 0.5 L glass bottles. Each bottle had 10 carriers and was filled with 0.5 L of synthetically prepared medium with NH_4-N and NO_2-N concentrations of 32±1 and 40±1 mg/L respectively. The bottles were sealed and put in a water bath at 35°C and constantly mixed. Samples were taken every hour for 5 h and analysed for NH_4-N, NO_2-N and NO_3-N. SpAA was reported as mg N removed per g VSS in an hour. Surface anammox activity (SuAA) was reported as mg N removed per square meter in an hour. N removed was calculated as the sum of NH_4-N and NO_2-N removed.

3.4. Results and Discussion

3.4.1. Reactor start-up

The MBBR reactor was seeded with granular deammonifying sludge from full-scale DEMON reactor. The seed sludge was shipped from HRSD's York River reactor in an insulated cooler and then stored at 4°C for approximately a week prior to seeding. The sludge was characteristic

in appearance with dark reddish-brown colour and micro-granules (Figure 3-1A). The average size of the granules was equal to 170±75 µm (n=21). Successful start-up of the reactor was completed in as little as 50 days when TN removal increased to more than 80% and subsequently stabilized as shown in Figure 3-1B. During first two weeks of operation NLR was optimized to match the removal and accelerate start-up. The reactor was successfully started-up at NLR of approximately 140 mg L^{-1} d. During that time, the biomass washed out from the bioreactor was periodically returned in order to accelerate attachment to the media. From the third week forward the suspended biomass was let to naturally wash out.

The authors believe that the start-up time could be further decreased with the implementation of an attachment enhancing strategy like a recently described bioprimer that showed great potential to increase the attachment of anammox bacteria to the carrier material (Maciej S Kowalski et al., 2019).

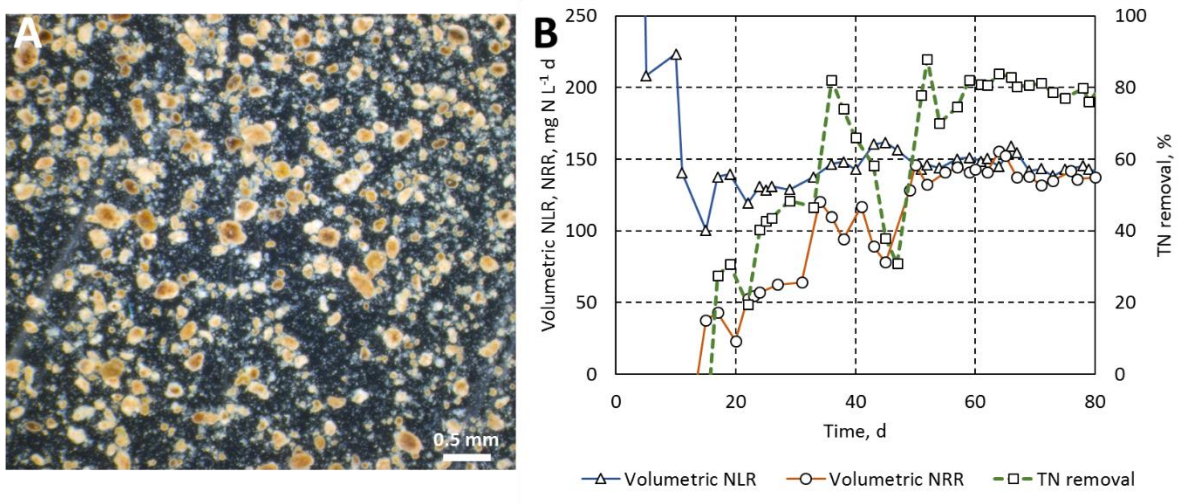

Figure 3-1 A – Stereoscopic photograph of granular DEMON sludge used as a seed, B – volumetric NLR vs NRR during start-up and TN removal.

3.4.2. Reactor operation

Following successful start-up, nitrogen concentration in the feed and consequently NLR were increased stepwise in Phases 1 to 4. Performance of the reactor was tracked over a period of 320 days and the results are shown in Figure 3-2A, B and C.

During Phase1 the concentration of nitrogen in the feed was increased to approximately 430 mg N L^{-1}, volumetric and surface NLR consequently increased to 200 mg N L^{-1} d and 1.1 g N m^{-2} d. The biomass adapted to the new load very quickly without any loss in performance. The reactor was reliably removing 86.2% TN.

On day 136 the nitrogen concentration in the feed was increased again to approximately 550 mg N L^{-1} and the volumetric NLR increased to 270 mg N L^{-1} d. The surface NLR during Phase 2 was equal to approximately 1.4 g N m^{-2} d. On day 155 the pH in the reactor dropped to 5.5 due to pH control system failure and as a result the performance of the reactor was affected. TN removal dropped to as low as 50.1% immediately following the pH upset. Interestingly, however, neither ammonium nor nitrite accumulated in the reactor. Rather a significant overproduction of nitrate was observed with NO_3-N:N_{rem} ratio reaching 0.48 (instead of a theoretical 0.11). The reactor recovered during following weeks and removed >80% TN at the end of Phase 2 but the average TN removal during Phase 2 decreased to 75.9%.

Phase 3 was initiated on day 209 when the HRT was decreased from 48h to 24h. The nitrogen concentration in the feed was not changed. Volumetric and surface NLR was therefore increased to approximately 500 mg N L^{-1} d and 2.5 g N m^{-2} d, respectively. The performance of the reactor was very stable during the whole period and the average TN removal was equal to 81.6%.

On day 240 the working volume of the reactor was increased to 30 L, but the HRT was kept at 24 h and the feed concentration remained the same. By doing so, the volumetric NLR was

approximately the same as in Phase 3 but the surface NLR was increased significantly to 5.3 g N m^{-2} d. At the beginning of Phase 4 the reactor experienced another pH upset and observations very like those from Phase 2 were made. The TN removal dropped to approximately 50% and NO_3-N:N_{rem} ratio increased to 0.47. The reactor recovered during the next 4 weeks and TN removal increased to 75%. Nitrogen removal stabilized at this level and did not increase further mainly because the NO_3-N:N_{rem} ratio did not decrease below 0.2 after the pH upset.

Additionally, during Phase 4 a rapid growth of biofilm was observed due to a very high surface loading rate. Such a high NLR might have negatively affected performance and create an added effect with the pH perturbance.

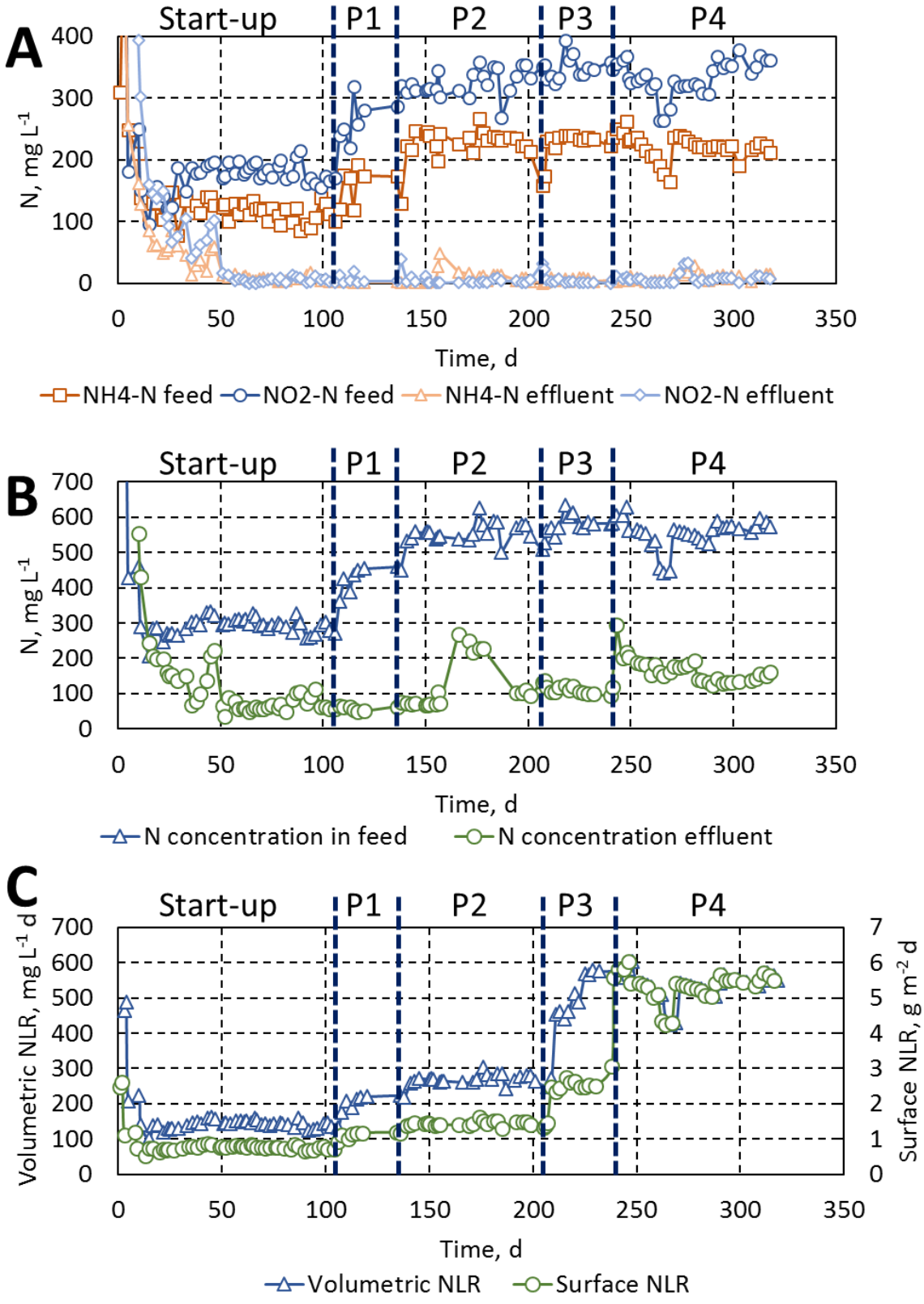

Figure 3-2 Reactor performance during long-term reactor operation; A – NH_4-N and NO_2-N concentrations in the feed vs effluent; B – TN concentrations in the feed vs effluent; C – volumetric and surface nitrogen lading rates.

The two mentioned pH shocks had a significant impact on the long-term performance of the MBBR reactor. Table 3-3 summarizes and compares the process parameters at steady states before and after the pH upsets. Most notable was the decrease in TN removal which dropped from 87.1% to 81.6% and 75.5% after first and second upset respectively. The drop in the performance was accompanied by an increase in the observed stoichiometric nitrogen ratios which are discussed further in section 3.4.

Table 3-3 Comparison of NLR and TN removal as well as nitrogen ratios during steady state before and after the two pH upsets.

Parameter	115-155		211-240		283-320	
	steady state		steady state after 1st upset		steady state after 2nd upset	
	average	SD[a]	average	SD	average	SD
NLR, g m^{-2} d	1.3	0.1	2.6	0.2	5.4	0.2
TN removal, %	87.1	1.5	81.6	1.3	75.5	1.6
NO_2-N:NH_4-N [b]	1.40	0.13	1.53	0.08	1.65	0.15
NO_3-N:NH_4-N [c]	0.28	0.03	0.43	0.03	0.58	0.05

[a] standard deviation

[b] ratio of nitrite nitrogen consumed to ammonium nitrogen consumed

[c] ratio of nitrate nitrogen produced to ammonium nitrogen used

3.4.3. Biofilm development and activity

On day 44 stereoscopic pictures of the media were taken (Figure 3-3). The biomass was attached to the media in uneven, small patches that were characterized by the same dark brown colour as the seed. At that point, the biofilm was attached to the carrier material quite loosely and tended to detach easily when mixing was increased. The mixing was therefore kept at relatively low intensity at around 60 rpm to prevent the biomass wash-out. Development of the biofilm

progressed significantly over a period of the next several months and at the end of Phase 2 a bright red uniform layer of the biofilm had formed on the surface of the carriers that was firmly attached to the carrier. On day 210 the thickness of the biofilm was on average 105±37 µm. At the end of Phase 3 (on day 250) biofilm thickness was on average 450±157 µm. During Phase 4 surface lading rate was increased significantly to more than 5 g N m^{-2} d and rapid growth of the biofilm was observed. On day 320 the thickness of the biofilm was as high as 1269±444 µm. At this point the media were overgrown with biomass and the spaces inside the media started to clog. With such a high surface loading rate, a significant biofilm growth on the bottom and the walls of the reactor was also observed.

Figure 3-3 Progression of biofilm development during the study.

Specific anammox activity assay was conducted at the end of Phase 4. The amount of biomass attached to the carriers averaged 12.8±0.3 g VSS m^{-2} with VSS/TSS ratio of 0.92±0.02. That translates to a total of approximately 41 g VSS in the reactor. The activity assay showed excellent anammox performance with SuAA of 276.9±2.1 mg N m^{-2} h and SpAA of 22.0±2.1 mg N g^{-1} VSS h (0.53 g N g^{-1} VSS d). The reported activity compares very well to other studies. Zhang et al. (2016), reported SpAA values between 0.3 and 0.45 g N g^{-1} VSS d. Similarly, Fernández et al. (2008) reported specific anammox activity between 0.35 and 0.5 g N g^{-1} VSS d.

On day 241 the reactor had experienced a pH upset and during the following weeks unusual growth on the surface of the anammox biofilm had appeared. It had grown significantly while the performance of the reactor was recovering and on day 285 was clearly visible with a naked eye appearing as brown flocs attached to the red biofilm (Figure 3-4). During following weeks,

the brown biomass disappeared naturally without outside intervention. The authors hypothesize that these brown flocs were somehow connected to the recovery of the bioreactor after the pH upset.

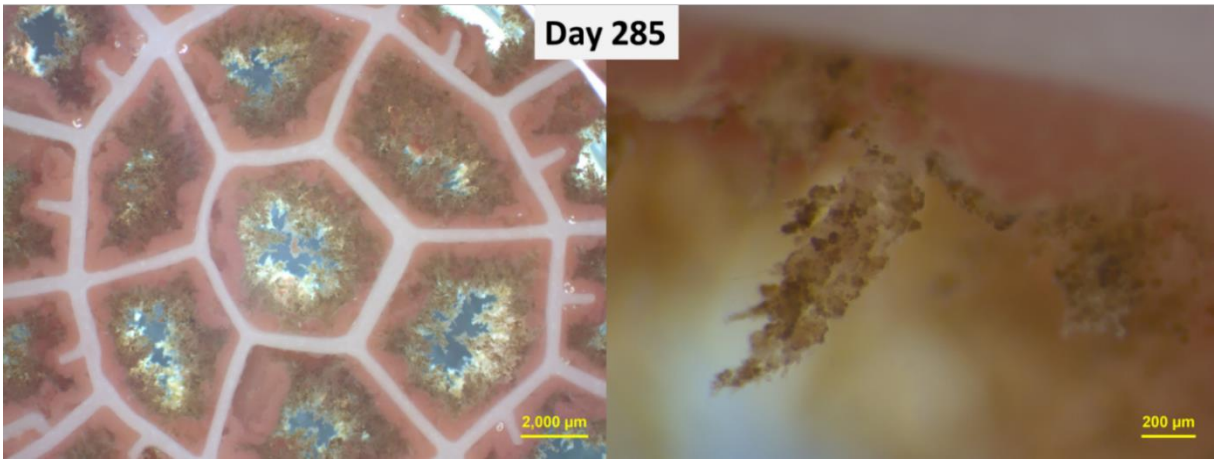

Figure 3-4 Stereoscopic photographs of anammox biofilm (red) and the unidentified growth (brown).

3.4.4. Process stoichiometry and pH upsets

Anammox process stoichiometry has been reported in a few studies over the years with values stated by Strous et al., (1997) being the most widely cited and generally accepted. The nitrogen ratios have been shown to vary slightly depending on the type of reactor and/or the type of biomass agglomerate as shown in Table 3-4.

Table 3-4 Stoichiometric nitrogen ratios reported in literature for different types of biomass compared to the values from this study.

Nitrogen ratio			Doubling time	T, °C	Biomass	Reactor type	Reference
NH_4	NO_2	NO_3					
1	1.32	0.26	11 d	32±3 °C	Suspended (granules)	SBR	(Strous et al., 1998)
1	1.1–1.3	0.10–0.25	3.6-5.4 d	38 °C	Suspended (free cells)	MBR	(Van Der Star et al., 2008)
1	1.278	0.353	7 d	30±0.1 °C	Enriched flocculent Brocadia spp/model	Batch experiments	(Puyol et al., 2013)
1	1.219	0.211	3.3 d	30 °C	Suspended (mix of flocs and granules)	MBR	(Lotti et al., 2014)
1	1.40	0.28	NA	33±1 °C	Biofilm	MBBR	This study

NO_2-N:NH_4-N ratio (nitrite consumed vs ammonium consumed) during Phase 1 was equal to 1.40 which is slightly higher than previously reported (Strous et al., 1997). This ratio increased with time and averaged to 1.51 over the whole study. NO_2-N:NH_4-N ratios in the feed to the consumed values are compared in Figure 3-5A.

The results from this study suggests that the observed anammox stoichiometry can be quite significantly affected by environmental perturbances such as pH upsets and high surface NLR as well. During the period of the study the reactor experienced two pH upsets. The first on day 155 and the second on day 241. On both occasions, it took approximately 40 days for the reactor to recover and reach a steady state. The nitrogen ratios after the pH upsets have been compared to the values before and are shown in Figure 3-5B. NO_3-N:NH_4-N ratio (nitrate produced vs

ammonium used) increased from 0.28 to 0.43 after first upset and to 0.58 after the second upset. Simultaneously, the TN removal dropped from 87.1% to 81.6% and 75.5%, respectively.

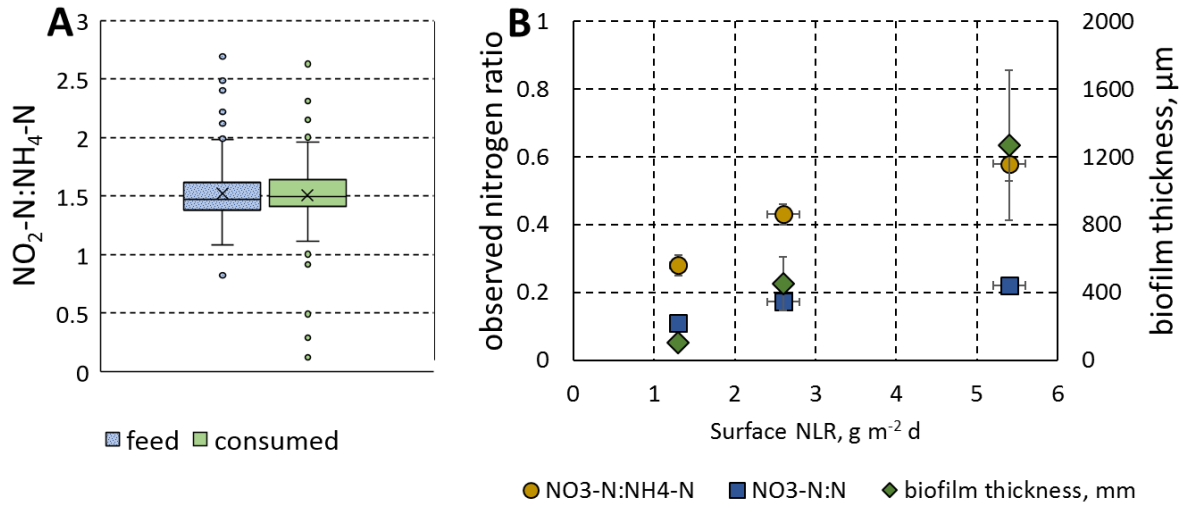

Figure 3-5 A – NO_2-N:NH_4-N ratio in the feed vs NO_2-N:NH_4-N consumed in the reactor; B – observed stoichiometric nitrogen ratios and biofilm thickness as a function of surface NLR.

pH is one of the most important parameters in an anammox reactor as it governs the equilibriums between ammonium and free ammonia (FA) and between nitrite and free nitrous acid (FNA) (Jaroszynski et al., 2011). FA and FNA are known to easily inhibit the process when their concentration rises above certain threshold. The threshold concentrations, however, have been long a subject of a debate in literature with many different values being reported (Tomaszewski et al., 2017). Lower pH increases FNA concentration significantly while FA concentration increases with higher pH (Anthonisen et al., 1977). The physiological pH range for anammox process has been shown to be between 6.7 and 8.3 in a granular system (Strous et al., 1999). In a biofilm system of a rotating biological contractor an optimum pH value of 8 was shown for anammox (Egli et al., 2001). Anammox process also increases the pH in the system and so the pH control must be applied.

The observed significant overproduction of nitrate might have been a response to a higher nitrite (FNA) toxicity at a lower pH (Puyol et al., 2014) and a consequence of a detoxification

mechanism deployed as a reaction to an environmental stressor. To the best knowledge of the authors such behaviour was not previously reported in literature and warrants further study. It is also possible that higher NO_3-N concentrations after the second pH upset might have been related to the appearance of the afore-mentioned brown biomass on the surface of anammox biofilm.

Finally, elevated NO_3-N:NH_4-N ratio might have been a side effect of a very high growth rate of anammox bacteria at peak surface nitrogen loading rate at the end of Phase 4. Metabolically this phenomenon could be explained by increased nitrite consumption during carbon fixation process in the anammox cell, as a result increased nitrate production ratio would be observed (Kartal et al., 2012). And indeed, a significant correlation between nitrogen ratios and surface NLR was observed as depicted in Figure 3-5B. The ratios increased linearly (R^2=0.9247 for NO_3-N:N_{rem} ratio and R^2 = 0.9573 for NO_3-N:NH_4-N ratio) with increased surface NLR as compared between the three steady states defined previously. Somewhat similar observations were made by Jaroszynski et al. (2011) where the NO_3-N:N_{rem} increased from 0.12 at low load conditions to 0.21 at high load. Additionally, low pH shifts the chemical equilibrium of inorganic carbon forms to CO_2 which provides more substrate for carbon fixation potentially increasing the growth rate. Not surprisingly, biofilm thickness also increased significantly with higher loads. The observed correlation was even stronger with R^2 of 0.9995.

3.5. Conclusions

Results obtained from this study show for the first time that the start-up of anammox MBBR reactors can be easily achieved by seeding with waste sludge from granular systems like DEMON® reactors. More than 80% TN removal was achieved in approximately 50 days. This study shows that granular biomass can be transitioned to a biofilm relatively easily which opens a new window of opportunity for starting-up anammox MBBRs. Most importantly, granular

sludge from DEMON® reactors is a lot more available than seed carriers and the start-up of biofilm reactors does not necessitate the use of proprietary seed carriers from biofarms.

3.6. Acknowledgement

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4. Chapter 4: Controlling cold temperature partial nitritation in moving bed biofilm reactor

4.1. Abstract

Mainstream partial nitritation was studied at 10°C in a moving bed biofilm reactor treating synthetic wastewater containing both nitrogen (≈40 mg L^{-1}) and organic carbon at COD/N ratio ranging from 1.3 to 2.2. Three different control strategies were investigated to achieve partial nitritation. Initially, biofilm age was controlled by incorporating a media replacement strategy. Next, separately from the media replacement, oxygen limited conditions were investigated and finally pH control was incorporated together with oxygen limitation. Successful partial nitritation was achieved only by combining oxygen limitation with pH control. The average NH_4-N concentration was equal to 16.0±1.6 mg L^{-1} and average NO_2-N concentration was equal to 15.7±2.4 mg L^{-1} during steady state partial nitritation. The average residual NO_3-N concentration was equal to 2.6±2.2 mg L^{-1}. The results obtained from this study prove for the first time that partial nitritation can be successfully controlled in a biofilm reactor treating wastewater with low nitrogen concentration, relatively high COD/N ratio and at low temperature. An algorithm for dynamic process control of partial nitritation has been also developed.

4.2. Introduction

Conventional nitrification/denitrification process in which ammonium is oxidized to nitrate and subsequently reduced to gaseous nitrogen is very energy intensive. Nitrification requires extensive aeration which can account for approximately 60 to 90% of energy requirements of the treatment plant (EPRI, 1994)). Denitrification in turn necessitates substantial amounts of biodegradable carbon which might not be available in wastewater in sufficient amounts when enhanced biological phosphorus removal is incorporated into the treatment train, especially in

relatively weak North American wastewater (Oleszkiewicz et al., 2015). Although it has been shown that denitrifying PAOs could reduce the organic carbon requirements for denitrification (Mandel et al., 2019).

There are, however, few emerging technologies that have a potential to significantly minimize energy consumption, improve nutrient removal and even minimize greenhouse gas emissions. One such technology is aerobic granular sludge that has been intensively studied recently (Corsino et al., 2017; Derlon et al., 2016; Devlin et al., 2017). Aerobic granular sludge has the capability to provide nutrient removal within more compact footprints and higher energy efficiencies than conventional technologies and is commercially available as Nereda® (Adav et al., 2008). One of the problems with this technology, however, is that it is only available in sequencing batch reactors and the nitrogen removal is still based on conventional nitrification/denitrification (Morgenroth et al., 1997; Pronk et al., 2015).

A more promising technology that is getting a significant attention is the combination of mainstream partial nitritation (PN) with anaerobic ammonium oxidation (anammox). With the discovery of the anammox process (Mulder et al., 1995), nitrogen removal can be achieved with much lower aeration costs and almost insignificant organic carbon requirements . Nitrogen removal using anammox is a fully autotrophic process where ammonium together with nitrite is combined and transformed to gaseous nitrogen and some nitrate (Strous et al., 1997). But since there is no nitrite in the wastewater partial nitritation is a necessary pre-treatment step in which approximately half of the ammonium in the wastewater is oxidized to nitrite. Nitrogen removal from warm, high strength waste streams via partial nitritation and anammox has become an established technology with over 100 installations worldwide (Ali and Okabe, 2015; Lackner et al., 2014) and has been also proven effective in treatment of industrial wastewater (Zekker et al., 2018).

The application of the anammox process technology to mainstream conditions is, however, proving extremely difficult. Firstly, municipal wastewater in places with moderate climate is subjected to seasonal temperature variations between approximately 20°C and 10°C and anammox efficiency has been shown to decrease rapidly below 20°C with removal rates falling down to 0.03 kg N m^{-3} d^{-1} (Hendrickx et al., 2014; Sánchez Guillén et al., 2016). Nevertheless, the growth and enrichment of anammox bacteria at low temperatures is feasible as shown by Lotti et al. (2014) or Hu et al. (2013).

Secondly, even more problematic is the partial nitritation (De Clippeleir et al., 2013). At lower temperatures (<20°C), nitrite oxidizing bacteria (NOB) have a kinetic advantage over ammonium oxidizing bacteria (AOB) with maximum specific growth rates higher than AOB (Kaelin et al., 2009; Sin et al., 2008; Wett and Rauch, 2003). For that reason, NOB must be constantly and selectively washed-out over AOB (and/or anaerobic ammonium oxidizing bacteria - AnAOB) in partial nitritation-anammox systems operating at low temperatures. Else, NOB activity should be supressed such that they cannot propagate within the system. Over the last several years, there has been some research into simultaneous PN/anammox with granular sludge. Lotti et al. (2014) reported very good TN removal of 86% at 20°C but the removal decreased below 40% at 10°C mainly due to nitrate production. Controlling residual ammonium concentration has been recently shown to partially supress NOB activity (Poot et al., 2016). Isanta et al. (2015) achieved stable partial nitritation in a granular sludge system at 12.5 °C and Reino et al. (2016) at 10 °C by maintaining an adequate ratio between dissolved oxygen (DO) and ammonium concentrations in the bulk liquid. The synthetic wastewater used in those studies, however, did not contain any or very little organic carbon and nitrogen concentration was still relatively high compared to a typically very weak (40 - 50 mg TN L^{-1}) North American wastewater (Metcalf & Eddy et al., 2013).

More recently the research has become focused on partial nitritation using biofilm rather than granular systems. Moving bed biofilm reactors (MBBRs) have been shown to perform better and retain more anammox biomass at low temperatures (Eva M Gilbert et al., 2015; Gilbert et al., 2014). Piculell et al. (2016) reported successful partial nitritation at 15°C in an MBBR reactor treating synthetic municipal wastewater by maintaining thin biofilm and alternating feeding of synthetic centrate. Laureni et al. (2016) reported successful NOB suppression at micro-aerophilic conditions (0.15 - 0.18 mg-O_2 L^{-1}) at 15°C. Bian et al. (2017) achieved stable partial nitritation through maintaining a constant ratio of 0.17 between DO and total ammonium nitrogen (TAN) concentration at 6 - 16°C, similar to Isanta et al. (2015).

This study has examined the feasibility of mainstream partial nitritation treating carbon-rich synthetic wastewater with low nitrogen concentrations (≈40 mg N L^{-1}) and at a temperature of 10 °C in a biofilm reactor. Three different control strategies were investigated to achieve partial nitritation. Initially, biofilm age was controlled by incorporating a media replacement strategy. Next, separately from the media replacement, oxygen limited conditions were investigated and finally pH control was incorporated together with oxygen limitation. Stable partial nitritation was only achieved by combining high pH and oxygen limitation.

4.3. Materials and Methods

4.3.1. Experimental Set-up

Partial nitritation was conducted in a plexiglas circular reactor with 5 L working volume. The reactor was filled to 40% with HeadworksBio 450 type media (protected surface area of 402 m^2/m^3). The reactor was then seeded with return activated sludge (RAS) from a full-scale biological nutrient removal (BNR) plant in Winnipeg (Manitoba, Canada). The reactor was constantly aerated at the air flowrate of 0.1-5 L/min and fed with synthetically prepared medium. The medium contained ammonium chloride (NH_4Cl), yeast extract (as source of COD)

and sodium bicarbonate ($NaHCO_3$). Micronutrients solution was also added according to (Lashkarizadeh et al., 2015). The reactor was kept in an environmental chamber (Conviron, USA) at 10±1 °C throughout the experimental period. HRT of the reactor was controlled with a peristaltic pump (Masterflex L/S, Cole-Parmer) at 6-12h. pH was monitored with a general-purpose pH probe (Oakton®, USA) but online measurements were not recorded. Prior to the installation of pH control (Oakton pH/ORP controller, USA), the pH oscillated between 6.5 and 7.5 with a single event when the pH dropped to 5.5. On day 202 pH control was installed and the pH was maintained at 8.6±0.1. DO was measured with an optical probe (Orion RDO, Thermo Scientific, USA) and portable DO meter (Orion 3 Star, Thermo Scientific, USA). Biomass washing out of the reactor was not returned to the reactor after seeding. Table 4-1 summarizes experimental conditions throughout the different periods of the study.

Table 4-1 Summary of operational parameters during different periods of the study. St.dev. – standard deviation

Period of the study	**Start-up and full nitrification**			**Media replacement**	**Aeration Control**	**pH Control**
Days	**0-17**	**18-33**	**34-115**	**116-146**	**147-199**	**200-250**
NH_4-N in feed, mg L^{-1}	**38**	**41**	**37**	**38**	**39**	**38**
St.dev.	3	2	2	5	3	4
Surface NLR, g N m^{-2} d^{-1}	**0.94**	**0.51**	**0.46**	**0.5**	**0.49**	**0.46**
St.dev.	0.08	0.02	0.03	0.6	0.05	0.03
COD in feed, mg L^{-1}	**79**		**52**	**58**	**86**	**82**
St.dev.	2		25	28	33	23
Surface OLR, g COD m^{-2} d^{-1}	**1.95**	**1**	**0.6**	**0.6**	**1.1**	**0.8**
St.dev.	0.04	-	0.3	0.2	0.4	0.4

HRT, h	**6**	**12**		
AFR, L min^{-1}	**5**	**5-0.1**	**0.1**	

4.3.2. Analytical methods

The performance of the MBBR reactor was monitored by observing the fractionation of inorganic nitrogen species in the effluent. The ammonium fractions (f_{NH4}) were expressed with the following equation (1):

$$f_{NH4} = \frac{(NH_4 - N_R)}{(\mathrm{TIN})}. \quad (1)$$

Nitrite and nitrate fractions (f_{NO2}, f_{NO3}) which describe how much of the oxidized ammonium was converted to either of the two were calculated according to equation (2) and (3):

$$f_{NO2} = \frac{(NO_2 - N_R)}{(TIN)} \quad (2)$$

$$f_{NO3} = \frac{(NO_3 - N_R)}{(\mathrm{TIN})}, \quad (3)$$

where: NH_4-N_R – ammonium nitrogen concentration in the reactor, NO_2-N_R – nitrite nitrogen concentration in the reactor, NO_3-N_R – nitrate nitrogen concentration in the reactor, TIN – total inorganic nitrogen concentration in the reactor.

It was assumed that total inorganic nitrogen (TIN) in the effluent was made up of only NH_4, NO_2 and NO_3 and so the sum of f_{NH4}, f_{NO2} and f_{NO3} equals to 1. Organic nitrogen was not analyzed in the study due to negligible concentration.

Average surface nitrogen loading rate (SNLR), as well as surface organic loading rate (SOLR) were also calculated for each period of the study. Nitrite, nitrate and ammonium concentrations in the influent and effluent were measured three times a week by a flow injection analyser

(Lachat QuikChem 8500, HACH, CA). TIN was calculated as the sum of nitrite, nitrate and ammonium nitrogen.

Free ammonia (FA) concentration was calculated using the following equation according to (Anthonisen et al., 1977):

$$FA\ (mg\ NH_3 - N\ L^{-1}) = \frac{TAN_R * 10^{pH}}{e^{6344^{(273+T)}} + 10^{pH}}, \quad (4)$$

where: TAN_R - total ammonium nitrogen concentration in the reactor, T - temperature.

Chemical oxygen demand (COD), volatile suspended solids (VSS) and total suspended solids (TSS) were measured according to Standard Methods (APHA, 2005). Biofilm solids on the plastic carriers were measured after scarping the biomass off with a cotton swab and de-ionized water. Pictures of the biofilm were taken using a stereoscopic microscope (Zeiss Stereo Discovery.V6, USA) with magnification that varied from 0.315x to 1.5x. Biofilm thickness was measured using CMEIAS©, an open-source image analysis software. All samples that required filtration were run through medium porosity Q5 filter paper (Fisher Scientific, CA).

4.4. Results and Discussion

4.4.1. Reactor start-up

The MBBR was seeded with BNR sludge. The initial concentration of biomass in the reactor of approximately 1.7 g VSS L^{-1}. After seeding, the biomass was left to naturally wash out from the reactor. The initial SNLR and SOLR were equal to 0.94 ±0.08 g N m^{-2} d^{-1} and 1.95±0.04 g COD m^{-2} d^{-1}, however, no nitrification was observed during the first 17 days. The media were microscopically inspected, and a layer of biofilm had already been formed on the surface of the media. The thickness was measured to be equal to 166±61 µm. Since no nitrification was observed, the loading rates were lowered to 0.51±0.02 g N m^{-2} d^{-1} and 1 g COD m^{-2} d^{-1} by increasing the HRT of the reactor from 6 to 12h. On day 34, the biofilm thickness was equal to

159±38 µm. To accelerate nitrification the SOLR was further decreased to 0.65±0.31 g COD m^{-2} d^{-1} during the rest of the start-up period. After decreasing the SOLR, a quick start-up of nitrification was observed. On day 64, the pH in the reactor dropped to 5.5 due to alkalinity deficiency and ammonium oxidation dropped from 62% to 32% on day 71. Nitrification recovered quickly (10 days) and 96% ammonium removal was reached on day 83 with subsequent stable operation throughout the rest of this phase. The biofilm thickness was measured on day 113 and was equal to 147±44 µm. Figure 4-1 shows the performance of the reactor during the whole start-up period.

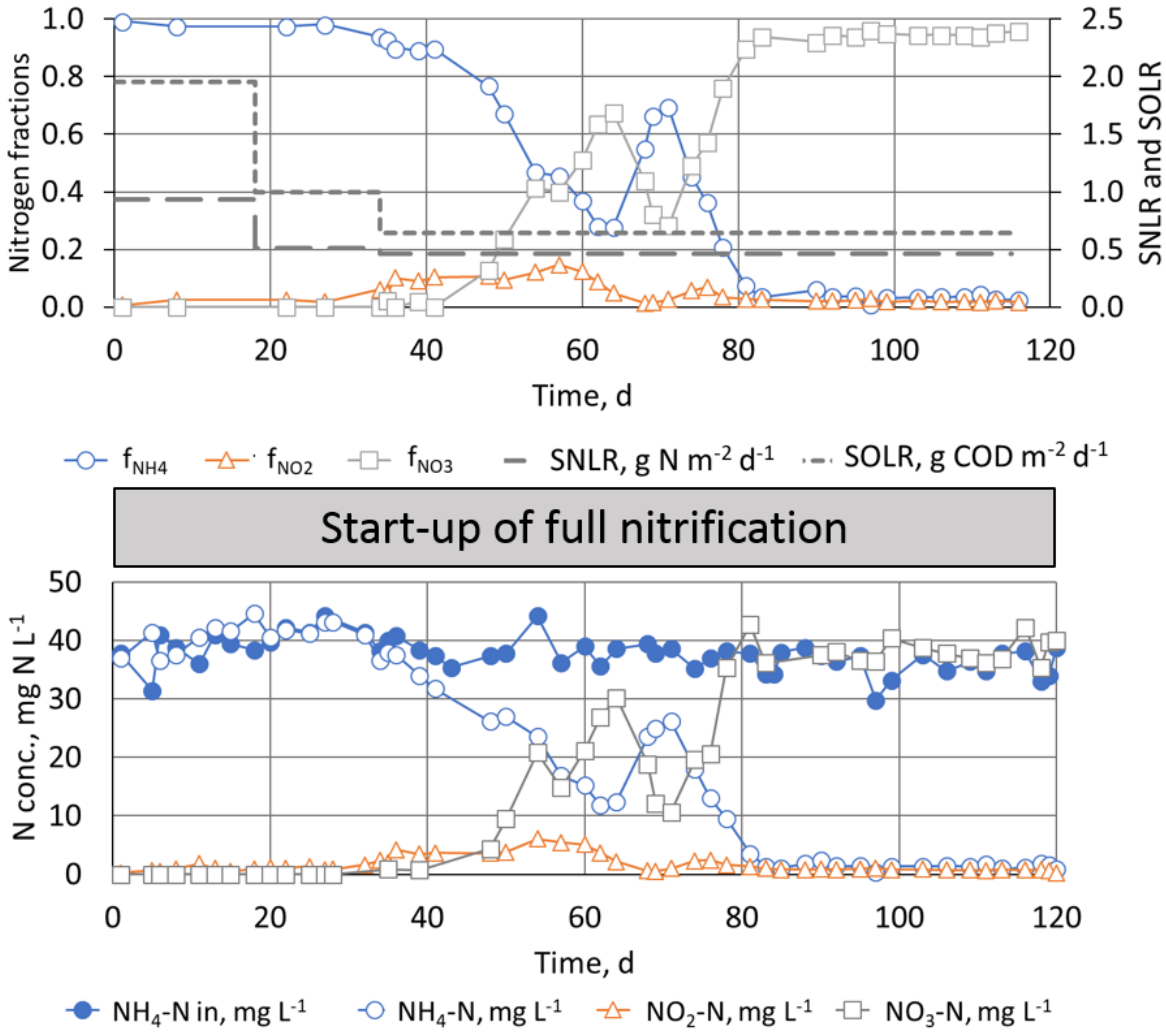


Figure 4-1 Top – Effluent nitrogen species fractionation during start-up period: f_{NH4} – ammonium fraction, f_{NO2} – nitrite fraction and f_{NO3} – nitrate fraction as well as surface nitrogen loading rate (SNLR; g N m^{-2} d^{-1}) and surface organic loading rate (SOLR; g COD m^{-2} d^{-1}). Bottom – Effluent ammonium, nitrite and nitrate concentration as well as influent ammonium concentration during start-up period.

The aim of the start-up phase was to establish full nitrification and consequently focus on controlling partial nitritation. Some build-up of nitrite was observed during start-up. Maximum nitrite concentration reached 6 mg L^{-1} on day 54 but then quickly subsided to lower than 1 mg L^{-1}. No nitrite accumulation was also observed during the pH upset.

4.4.2. Media replacement

The first strategy to control partial nitritation was based on a concept of sludge retention time (SRT). NOB have higher maximum growth rates than AOB at temperatures lower than 27°C

which gives them a significant kinetic advantage and consequently nitrite is consumed by NOB as soon as it becomes available (Wett et al., 2011). This makes it necessary to selectively control the population of NOB over AOB. However, under transient conditions (e.g. during nitrification start up), nitrite can accumulate to significant concentrations since NOB growth cannot occur until AOB produce nitrite. To simulate these conditions a strategy based on replacing media at a certain rate was implemented. AOB would grow on a surface of a new media before NOB and produce nitrite before getting replaced with a virgin media hence maintaining a form of a constant start-up in the reactor. As a side-effect of this control strategy an average thickness of the biofilm was expected to decrease, and thin biofilms were previously shown to contribute to more efficient nitritation (Piculell et al., 2016).

Starting from day 116 (Figure 4-3), 10 media/day were replaced (10 carriers were manually withdrawn from the reactor and 10 virgin carriers were put back in) producing a theoretical SRT of about 17 d. From day 113 to 132 ammonium oxidation dropped only slightly from 0.95 to 0.90 and no nitrite production was observed. Rapid decrease in nitrification was observed after day 136 when ammonium oxidation dropped to 0.53 but still no nitrite production was observed with f_{NO2} and f_{NO3} of 0.04 and 0.43, respectively. The average biofilm thickness in the reactor decreased significantly and was equal to 111±29 µm on day 146. These results stand contrary to the observations made by Piculell et al. (2016, 2015) who reported increased nitrite production in thin biofilms. Under conditions studied in this research, however, thickness of the biofilm had no impact on nitrite production.

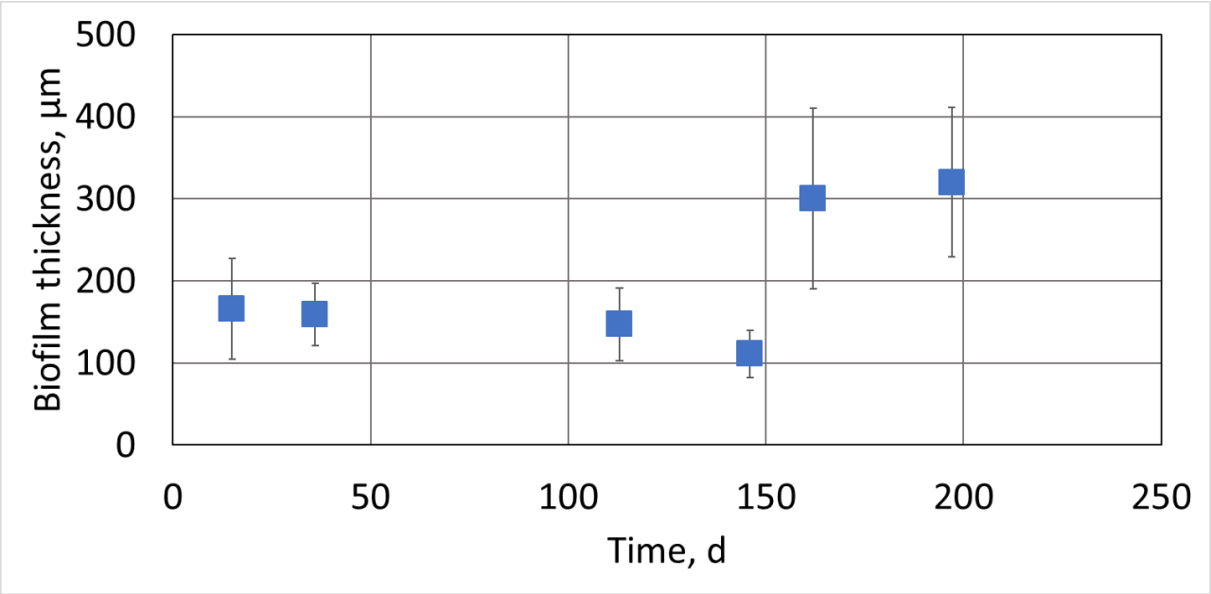


Figure 4-2 Changes in biofilm thickness throughout the study.

4.4.3. Oxygen limitation

On day 146 media replacement was stopped as the approach was deemed unsuccessful and in order to prevent a total loss of nitrification. Immediate recovery of nitrification occurred and on day 160 the ammonium oxidation reached 87%. Biofilm thickness was measured on day 162 and was equal to 300±110 µm. From that point air flow rate to the reactor was adjusted stepwise in order to control ammonium oxidation at about 0.6 which is required for anammox. As the air flow rate was decreased to 0.1 L min^{-1} the mixing in the reactor became insufficient and an additional nitrogen sparging was installed to mix the reactor. Nitrogen flow rate to the reactor was set to 5.5 L min^{-1}.

During this period of the study the reactor was submitted to significant oxygen limitation. Oxygen limitation was assessed based on R value, which represents a ratio of DO to TAN concentration in the reactor. When the R value is lower than 3.94 the conditions are assumed to be oxygen limited for full nitrification. Application of this ratio as a control strategy for partial nitritation was used by Bian et al. (2017) who reported successful partial nitritation at R values lower than 0.17. Contrary to the observations made by Bian et al. (2017), even though the

reactor operated at oxygen-limited conditions while nitrifying approximately 60% of influent ammonium still no nitrite accumulation was observed. At these conditions R value in the reactor oscillated at around 0.1 but still no nitritation was observed. The authors believe that the apparent discrepancy between the observations may arise from the difference in influent characteristics. While Bian el al. (2017) used synthetic wastewater with no organic carbon, this study treated carbon-rich wastewater with COD/N ratio of approximately 2.2 (average from the period between days 146 and 199). On day 197 the media were microscopically inspected, and the biofilm thickness was equal to 320±91 µm.

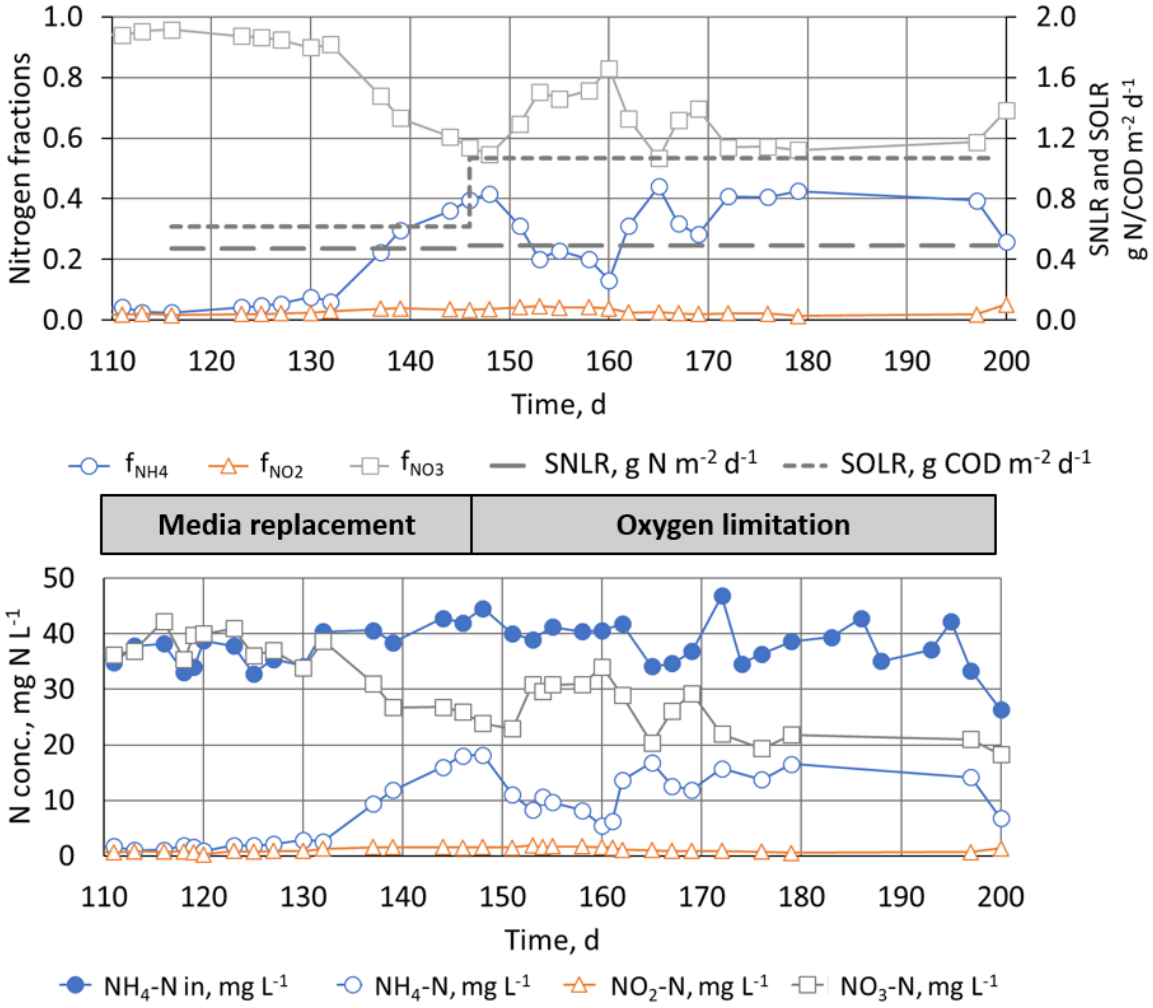


Figure 4-3 Top – Effluent nitrogen species fractionation during media replacement and aeration control period: f_{NH4} – ammonium fraction, f_{NO2} – nitrite fraction and f_{NO3} – nitrate fraction as well as surface nitrogen loading rate (SNLR; g N m^{-2} d^{-1}) and surface organic loading rate (SOLR; g COD m^{-2} d^{-1}). Bottom – Effluent ammonium, nitrite and nitrate concentration as well as influent ammonium concentration.

4.4.4. pH control strategy

Starting from day 200, the pH in the reactor was controlled at 8.6±0.1 by dosing 0.125 M aqueous solution of sodium hydroxide (NaOH). The air flow rate to the reactor was kept constant at 0.1 L min^{-1} and none of the other operational parameters were changed. Following the increase in pH, a significant increase in f_{NO2} and simultaneous decrease in f_{NO3} was observed during the next two weeks (Figure 4-4 - top). On day 214 the fractions of NH_4-N, NO_2-N and NO_3-N in the reactor were equal to 0.40, 0.48 and 0.12, respectively with NO_2-N/NH_4-N ratio of 1.2. The average SNLR during this period was equal to 0.46±0.02 g N m^{-2} d^{-1} and average SOLR was equal to 0.83±0.42 g COD m^{-2} d^{-1}. Figure 4-4 (bottom) shows the respective nitrogen species concentrations. The average NH_4-N concentration was equal to 16.0±1.6 mg L^{-1} and average nitrite concentration equaled to 15.7±2.4 mg L^{-1} during the period of stable partial nitritation with the NO_2-N/NH_4-N ratio of 0.98. The average residual nitrate concentration during steady state partial nitritation was equal to 2.6±2.2 mg L^{-1}. Biofilm thickness was equal to 248±29 µm on day 250.

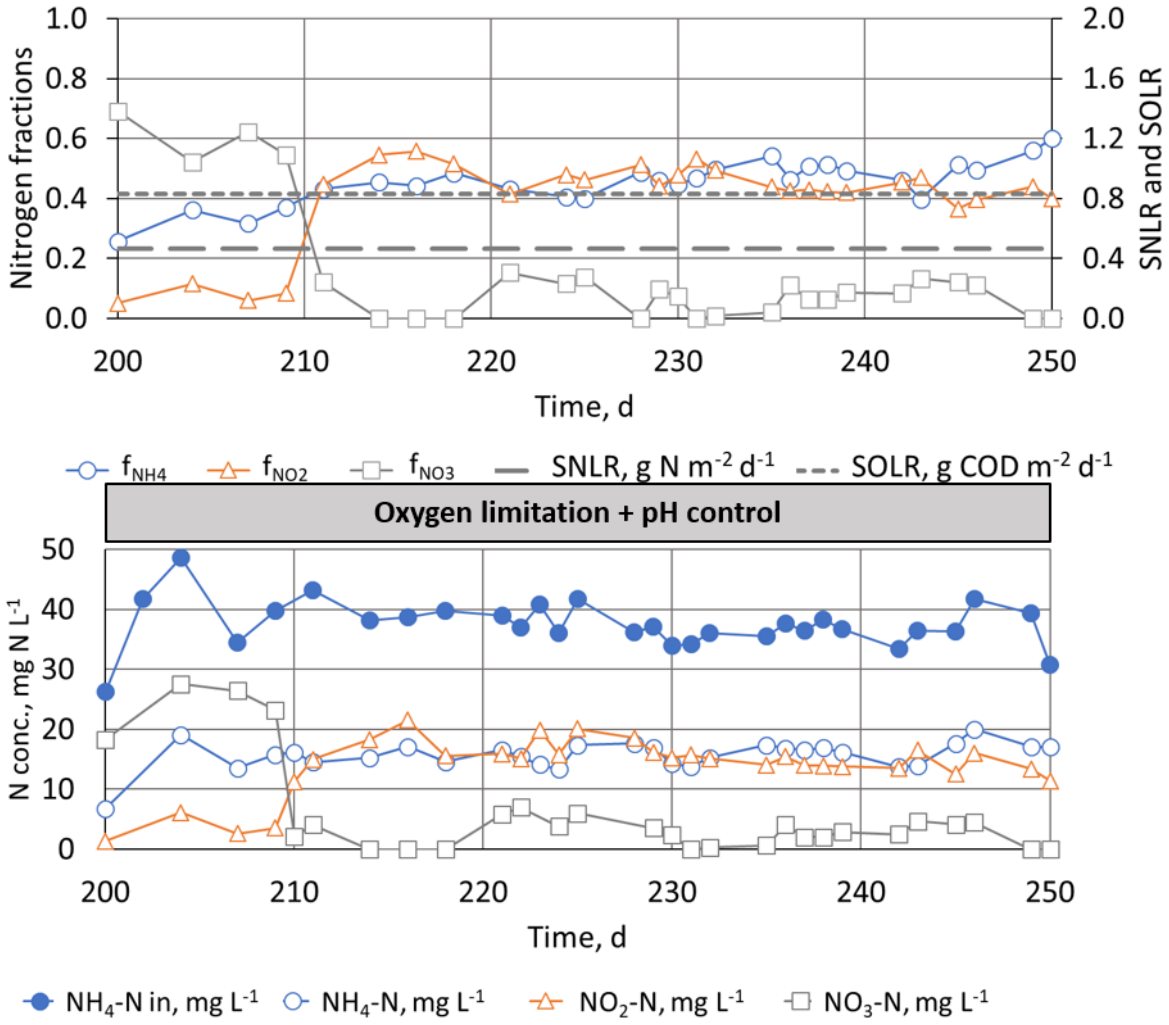


Figure 4-4 Top – Effluent nitrogen species fractionation during pH control period: f_{NH4} – ammonium fraction, f_{NO2} – nitrite fraction and f_{NO3} – nitrate fraction as well as surface nitrogen loading rate (SNLR; g N m^{-2} d^{-1}) and surface organic loading rate (SOLR; g COD m^{-2} d^{-1}). Bottom – Effluent ammonium, nitrite and nitrate concentration as well as influent ammonium concentration during pH control period.

4.4.5. Free ammonia inhibition of NOB activity

The concentration of free ammonia is directly affected by pH of the solution and increases at higher pH (Tenno et al., 2018). Free ammonia concentration had significantly increased after the pH set point was changed to 8.6 with average concentration of 1.1±0.2 mg NH_3-N L^{-1}. This concentration of FA did not negatively affect ammonia oxidation which is in agreement with Anthonisen (1977) who reported AOB inhibition at 7 mg NH_3-N L^{-1}. NOB activity was, however, successfully supressed at these conditions. These results agree with the observations

that nitrite oxidizers are more susceptible to FA toxicity than AOB with inhibition reported at concentrations as low as 0.1 to 1.0 mg NH_3-N L^{-1} (Turk and Mavinic, 1986). Other studies reported, however, NOB sensitivity comparable with AOB at 6.6 to 8.9 mg NH_3-N L^{-1} (Mauret et al., 1996). Wong-Chong and Loehr (1975) reported, for a system of high ammonia of 100 to 1000 mg N/L, that acclimated culture of NOB could tolerate FA concentrations as high as 40 mg NH_3-N L^{-1}. In this study, however, NOB did not acclimate even to relatively low FA concentrations and during the 40 d of steady state operation effective inhibition was maintained.

In regard to Piculell et al. (2016), the authors believe that better nitritation was observed in thinner biofilms because of FA diffusion through the biofilm. The depth of FA penetration into the biofilm is directly proportional to the concentration in bulk and in thin biofilms FA can penetrate the whole depth of the biofilm hence successfully inhibiting NOB metabolism. In thicker biofilms, NOB communities in inner layers would be protected from exposition to FA.

Figure 4-5 shows an overview of inorganic nitrogen fractionation obtained in the reactor with no process control, oxygen limitation (DO/NH4-N ratio control) and a combination of oxygen limitation and pH control (all at 10°C). Employing DO/NH_4-N ratio control resulted in successful limitation of ammonium oxidation to approximately 60% (partial nitrification), however ammonium was still oxidized to nitrate, and no nitrite production was observed. Mainstream partial nitritation was only achieved by combining DO/NH_4-N ratio control and pH control at 8.6. Nitritation was obtained by the inhibition of NOB metabolism by elevated concertation of FA at high pH.

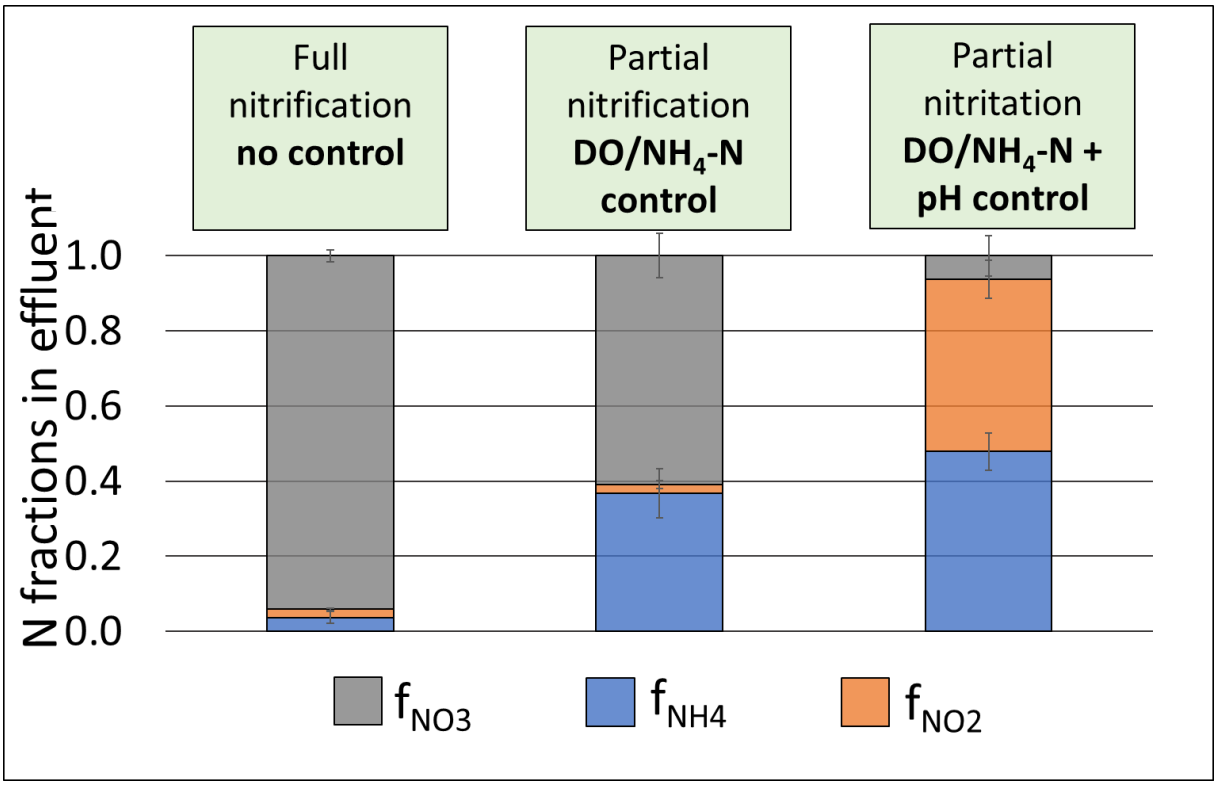


Figure 4-5 Inorganic nitrogen fractions in the effluent from the MBBR during different control strategies at 10°C.

4.4.6. Integration to mainstream and process control

The results obtained from this study prove that two-stage MBBR approach for incorporating anammox process into mainstream is a viable option. Figure 4-6 shows a schematic diagram of the proposed mainstream process configuration. Chemically enhanced primary treatment (CEPT) is proposed to remove phosphorus and capture a significant portion of organic carbon. Arguably, CEPT could be replaced with conventional primary clarification and subsequent enhanced biological phosphorus removal (EBPR) in anaerobic/oxic process (A/O). CEPT or EBPR is followed by a two-stage partial nitritation-anammox process and the treatment is finished with solids separation (DAF) and disinfection (UV).

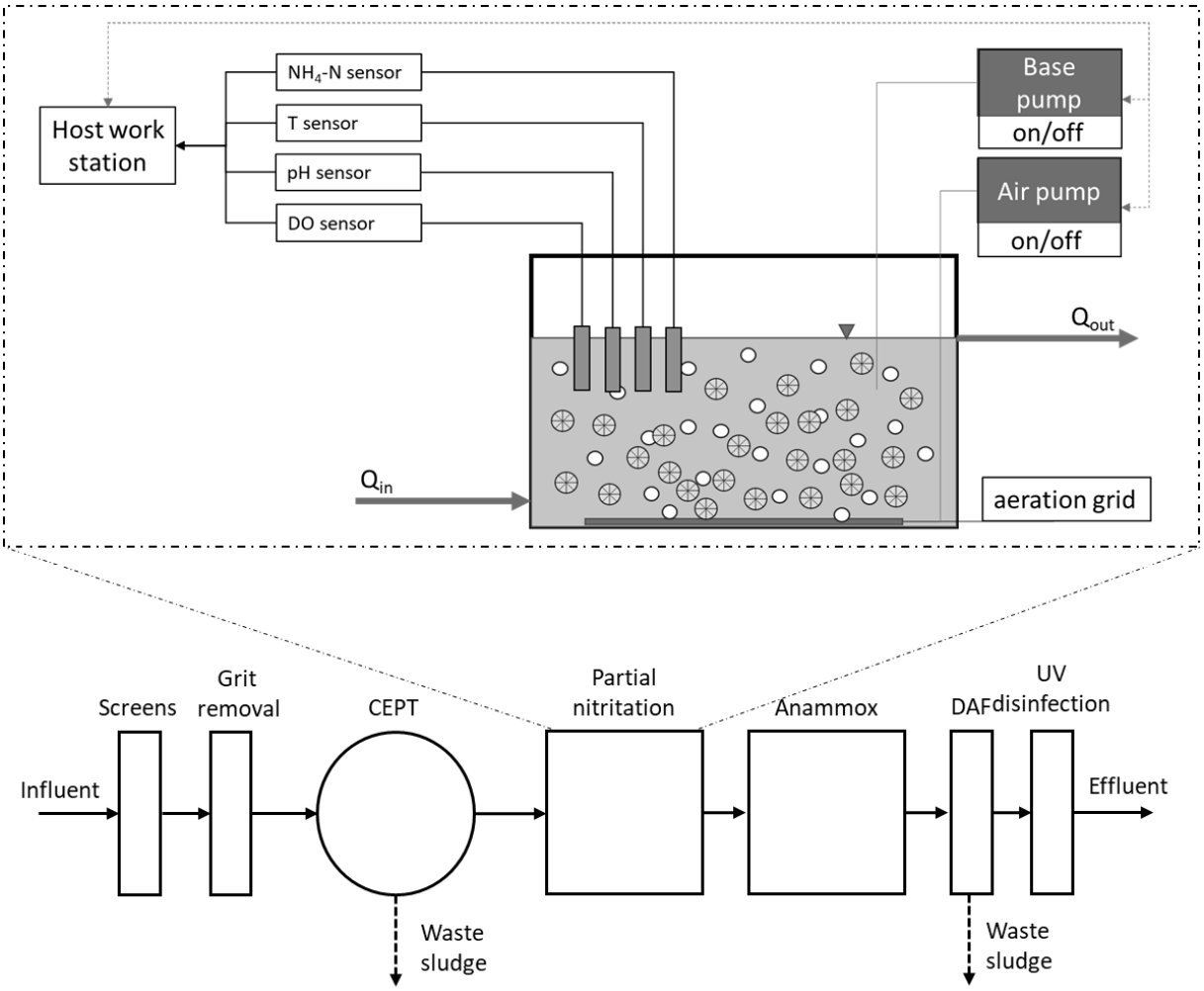
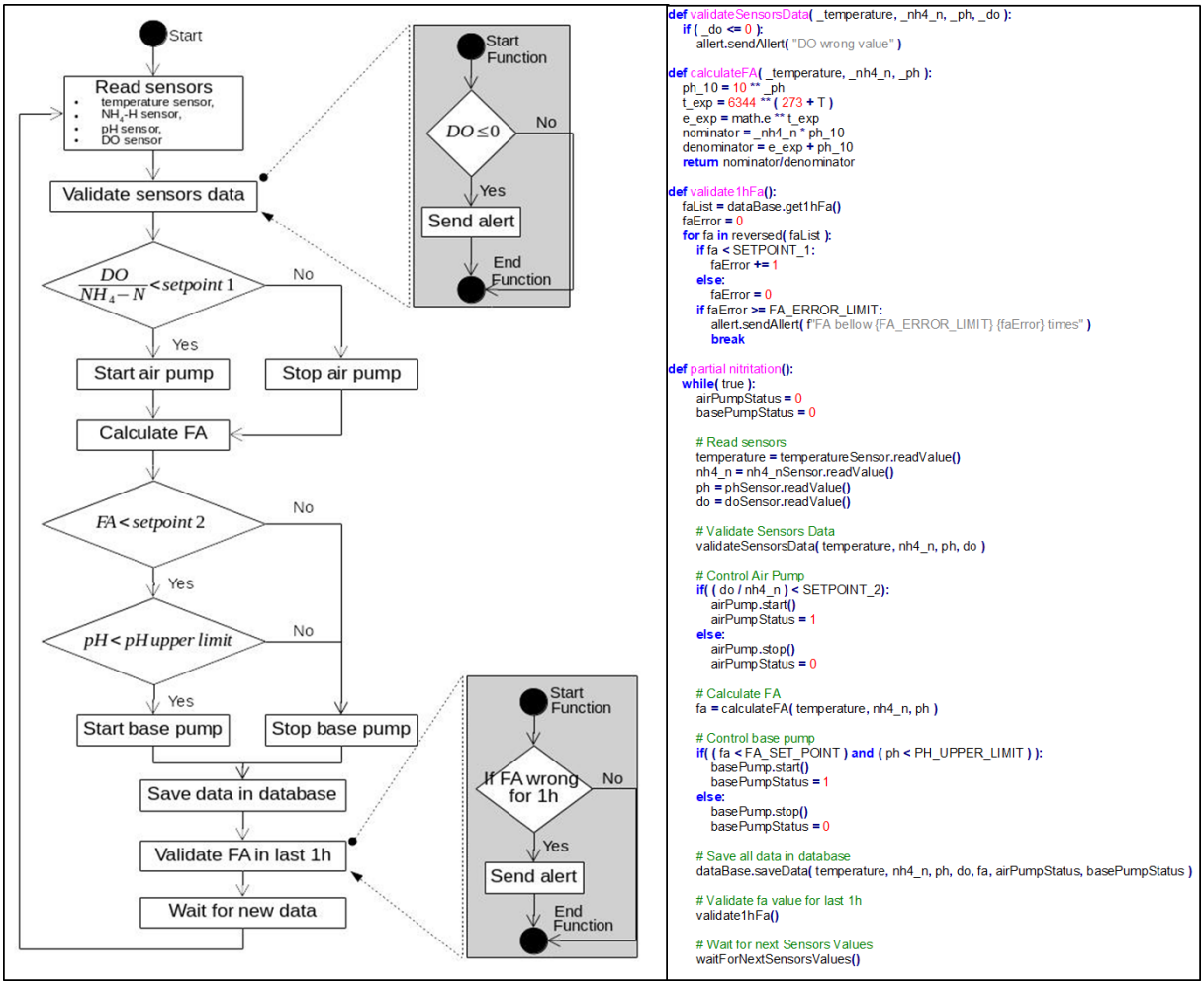


Figure 4-6 Schematic diagram of the proposed mainstream process configuration with two-stage partial nitritation-anammox integrated into the treatment train.

The proposed configuration could arguably be more efficient than one-stage approach where partial nitritation and anammox occur in one reactor. The main reason for this is that with the presented control strategy it is relatively easy to control partial nitritation and solve the biggest problem in one-stage systems which is nitrate overproduction due to proliferation of NOB. This is especially true for temperatures below 15 °C and very low nitrogen concentrations. While one-stage systems are advantageous in side-stream applications (Lackner et al., 2014), the authors believe that separation of partial nitritation and anammox is a better solution for mainstream processes.

Dynamic process control algorithm for mainstream partial nitritation is presented in Figure 4-7. The process is controlled by simultaneously maintaining two user-specified control setpoints. Firstly, DO/NH_4-N ratio is controlled at a certain setpoint (setpoint 1 in Fig. 6) to obtain TAN oxidation at 50% by monitoring NH_4-N concentration in the reactor and controlling DO concentration. And secondly, FA concentration is controlled at a certain setpoint (setpoint 2 in Fig. 6) to obtain nitritation by inhibiting NOB activity. FA concentration is governed by temperature, pH and TAN concentration in bulk but only pH can be externally controlled. Hence, a base dosing system is used to increase the pH to obtain the specified FA concentration.

The exact values for both setpoints are dictated by the wastewater characteristics and temperature. Primarily by the concentration of biodegradable COD since both setpoints must be increased the higher the organic loading rate (OLR) gets. The biofilm will grow thicker at higher OLRs hence the FA setpoint has to be increased in order to enhance the FA penetration depth into the biofilm and successfully inhibit NOB. The setpoint for DO/NH_4-N ratio also must be increased at higher OLRs as more oxygen is needed to oxidize the additional organic carbon. For the conditions and synthetic wastewater studied in this paper the setpoints were defined through the course of the experiment to be 0.06 for DO/NH_4-N ratio and 1.1 mg NH_3-N L^{-1} for FA.




```python
def validateSensorsData( _temperature, _nh4_n, _ph, _do ):
    if ( _do <= 0 ):
        allert.sendAllert( "DO wrong value" )

def calculateFA( _temperature, _nh4_n, _ph ):
    ph_10 = 10 ** _ph
    t_exp = 6344 ** ( 273 + T )
    e_exp = math.e ** t_exp
    nominator = _nh4_n * ph_10
    denominator = e_exp + ph_10
    return nominator/denominator

def validate1hFa():
    faList = dataBase.get1hFa()
    faError = 0
    for fa in reversed( faList ):
        if fa < SETPOINT_1:
            faError += 1
        else:
            faError = 0
        if faError >= FA_ERROR_LIMIT:
            allert.sendAllert( f"FA bellow {FA_ERROR_LIMIT} {faError} times" )
            break

def partial nitritation():
    while( true ):
        airPumpStatus = 0
        basePumpStatus = 0

        # Read sensors
        temperature = temperatureSensor.readValue()
        nh4_n = nh4_nSensor.readValue()
        ph = phSensor.readValue()
        do = doSensor.readValue()

        # Validate Sensors Data
        validateSensorsData( temperature, nh4_n, ph, do )

        # Control Air Pump
        if( ( do / nh4_n ) < SETPOINT_2):
            airPump.start()
            airPumpStatus = 1
        else:
            airPump.stop()
            airPumpStatus = 0

        # Calculate FA
        fa = calculateFA( temperature, nh4_n, ph )

        # Control base pump
        if( ( fa < FA_SET_POINT ) and ( ph < PH_UPPER_LIMIT ) ):
            basePump.start()
            basePumpStatus = 1
        else:
            basePump.stop()
            basePumpStatus = 0

        # Save all data in database
        dataBase.saveData( temperature, nh4_n, ph, do, fa, airPumpStatus, basePumpStatus )

        # Validate fa value for last 1h
        validate1hFa()

        # Wait for next Sensors Values
        waitForNextSensorsValues()
```

Figure 4-7 Process control algorithm for partial-nitritation (left) and Python pseudocode (right) describing the algorithm.

4.4.7. Cost of nitrogen removal from wastewater

The chemical requirement needed to artificially increase pH is an additional operating cost associated with the proposed control strategy. Sodium hydroxide was chosen as the source of basicity due to it's ease of handling, ability to make 50% solutions and it has been a proven chemical of choice used in full-scale applications for sidestream phosphorus recovery or sludge treatment. The average consumption of NaOH was estimated at 0.2 kg/m^3 of wastewater. The cost of industrial grade NaOH varies greatly depending on the purity, however, assuming the average price of 200 \$/tonne gives approximately 0.04 \$/m^3 of additional chemical cost. It is not easy to precisely estimate the operational costs of BNR plants as they vary greatly

depending on process design, scale, energy costs, location etc. According to (Gratziou and Chrisochoidou, 2011) the total annual operating cost of an activated sludge process with pre-denitrification zone was 1.54[5] $/m^3. Molinos-Senante et al. (2010) reported much lower operating costs estimated at 0.265[1] $/m^3. Similarly, Zessner et al. (2010)who showed that the cost of operating BNR plants in Austria ranges from 0.154 to 0.225[1] $/m^3. Rodriguez-Garcia et al. (2011) reported moderately higher range of 0.271-0.320[1] $/m^3.

Integration of anammox into mainstream process is associated with potentially significant savings in operating costs. Energy requirement for aeration could be reduced by 50% compared to conventional nitrification-denitrification. External carbon requirement could be completely removed, and sludge production would be reduced at least by 50%. Assuming that energy and sludge disposal (including chemical costs and maintenance) make up around 60% of total operational costs of the treatment plant (Molinos-Senante et al., 2010), the authors believe that the additional chemical costs could be easily offset by the gained savings.

4.5. Conclusions

Partial nitritation at 10°C, low nitrogen concentration and high COD/N ratio (>2) has been shown to be feasible. Mainstream partial nitritation was achieved by combining two control strategies. The target level of ammonium oxidation was achieved by controlling DO/TAN ratio and nitritation was obtained by FA inhibition at high pH. Long term stability was successfully obtained at DO/TAN ratio of 0.06 and at FA concentration of 1.1±0.2 mg NH_3-N L^{-1}. NOB did not acclimate to FA during 40 days of steady state operation and were effectively inhibited. An algorithm for dynamic process control of partial nitritation has been also developed.

[5] The price was converted to USD and the inflation from the year of original publication was taken into account.

4.6. Acknowledgements

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Part II

Integration of the developed techniques in pilot scale reactors

5. Chapter 5: Accelerated start-up of a partial nitritation-anammox moving bed biofilm reactor

5.1. Abstract

The formation of anammox biofilm is a lengthy process, leading to reactor start-up times of several months even with addition of anammox inoculum. To demonstrate the effectiveness of combining bio-primer technique with seed carriers in further accelerating the start-up time of anammox reactors a pilot moving bed biofilm reactor was set-up at a dewatering facility of a municipal wastewater treatment plant. The reactor was filled to 40% with plastic media with a specific surface area of 402 m^2 m^{-3} and was operated at 34 ± 1 °C. Using bio-primer coated media it was shown that a surface nitrogen removal rate of 2.5 g-N m^{-2} d^{-1} could be achieved in as little as 56 days. Free ammonia (FA) was thought to be the predominant inhibitor of nitrite oxidizing bacteria activity rather than the selective wash-out of nitrifying biomass. A minimum FA concentration of 2 mg NH_3-N L^{-1} was suggested for optimal performance of partial nitritation and to prevent the build-up of nitrates. Biomass from the pilot reactor had developed a specific anammox activity of 19 ± 2 mg-N g-VSS^{-1} h^{-1} which was 75% higher than the seed biomass from an anammox-only reactor fed with synthetic wastewater.

5.2. Introduction

Nitrogen removal from warm, high strength waste streams via anaerobic ammonium oxidation (anammox) has become an established technology with over 100 installations worldwide (Ali and Okabe, 2015; Lackner et al., 2014). Even though the anammox process has been proven effective and reliable, most of the installations are located in Europe and China with only a handful in North America. In Canada, there is only one (as of 2018) installation of DEMON® (granular sludge) in Guelph. Anammox technology is therefore still relatively new and uncommon in North American perspective (Oleszkiewicz et al., 2015) and municipalities

require proof of performance through a pilot demonstration prior to commencing a full-scale application.

Available technologies range from attached (biofilm) to suspended (granules) growth systems, however, biofilm reactors like MBBR (moving bed biofilm reactor) or IFAS (integrated fixed film activated sludge) posses few major advantages over suspended growth technologies (Guo et al., 2016; Lotti et al., 2015). Biofilms are characterized by high anammox activity and relative abundance of anaerobic ammonium oxidizing bacteria (AnAOB). Moreover, a control over the population of nitrite oxidizing bacteria (NOBs) is significantly easier in MBBRs. They are also relatively immune to the wash out of anammox biomass. The development of anammox biofilm, however, is a slow process leading to lengthy start-up times of anammox reactors reaching several months or more (Morales et al., 2015).

Seeding the reactor with high quantities of inoculum can shorten the start-up time significantly, however, even then the start-up periods can reach several months. The start-up of a full-scale MBBR plant in Malmö took approximately 4 months to reach 90% ammonia removal. An MBBR in Durham, North Carolina was started within 3 months with subsequent problems with nitrate overproduction. The reactor achieved only 80% ammonia removal and 70% total inorganic nitrogen (TIN) removal (Bilyk et al., 2016).

The start-up techniques used require seed carriers with already established anammox biofilm, usually 3-15% of the design media fill. This amount of seed material might not be always readily available (Araujo et al., 2011), especially in North America where there are currently (as of 2018) only 4 full-scale biofilm installations located in James River (VA), South Durham (NC), Chicago (IL) and Denver Metro (CO).

AnAOB are characterised by a very slow growth rate with a minimum doubling time of 14 days (Strous et al., 1997) which is one of the reasons for slow formations of anammox biofilms and long start-up of full-scale anammox plants. The affinity of AnAOB to carrier material is also of

great importance in the course of biofilm development. Maximum growth rate of AnAOB is an intrinsic kinetic property of the specific anammox species and cannot be increased, the attachment rate of AnAOB can be influenced. It was shown that by growing a layer of heterotrophic biofilm on the surface of the plastic carriers prior to inoculation with anammox biomass, the attachment rate of AnAOB was significantly increased. The heterotrophic biofilm acted as a bio-primer that allowed for a very quick attachment of AnAOB. After 4 weeks from seeding the specific anammox activity on the media covered with a bio-primer reached 250 mg NH_4-N_{rem} g VSS^{-1} d^{-1} while the control showed no activity (Maciej S Kowalski et al., 2019).

In order to demonstrate the effectiveness of combining the bio-primer technique with seed carriers in further accelerating the start-up time of anammox reactors a pilot MBBR was set-up at a dewatering facility of a municipal wastewater treatment plant. Additionally, the piloted configuration was used to prove the feasibility and reliability of a biofilm-based anammox process in cost-effectively treating anaerobically digested sludge dewatering centrate.

5.3. Materials and Methods

5.3.1. Reactor configuration and operation

The MBBR reactor was constructed out of HDPE tank with a working volume of 100 L. Centrate was pumped through plastic tubing from a centrate equalization tank. The reactor was mixed and aerated with a fine-bubble diffuser. Air flow rate was controlled with an air flow meter. pH was monitored (Alpha pH200m, Eutech Instruments, USA) but not controlled. The reactor was filled to 40% (app. 40 L) with HeadworksBIO 450 type media with a specific surface area (protected[6]) of 402 m^2 m^{-3}. Effluent was pumped out of the reactor through a sieve that allowed biomass to wash out but retained the media in the reactor. External micro- or

[6] protected surface area is the surface area on the inside of the plastic carriers that is not exposed to external shear

macro-nutrients were not supplemented during the operation of the reactor. Alkalinity in the form of sodium bicarbonate ($NaHCO_3$) was added to centrate starting from day 131 at a dose of 0.75 g L^{-1}. On day 144 the dose was increased to 1.25 g L^{-1} and finally from day 172 to 2.0 g L^{-1}.

The pilot operation of the reactor was divided into 3 distinctive phases (Table 5-1). During Phase 1 partial nitritation was established in the reactor. During Phase 2 anammox biomass was introduced to the reactor and simultaneous partial nitritation and anammox process was established. During Phase 1 and 2 the reactor was fed with centrate diluted with process water at a ratio of approximately 1:1 (v/v). During Phase 3 full strength centrate was treated in the pilot reactor.

The extent of aeration, hydraulic retention time (HRT) and loading rates varied throughout the experimental period. Detailed values for these parameters are specified in sections 3.1 and 3.2.

Table 5-1 Operational parameters of the reactor during different phases of the experiment.

Period of the study	Phase 1	Phase 2	Phase 3
Process	Partial nitritation	Partial nitritation-anammox	Partial nitritation-anammox
Goal	Development of bio-primer and establishment of partial nitritation.	Anammox inoculation, process start-up and stabilization.	Investigation of process performance under high loading rates and the effect of full strength centrate on the process.
Feed	Diluted centrate	Diluted centrate	Non-diluted centrate
SNLR	1.5±0.4 g-N m^{-2} d^{-1}	1.5±0.4 g-N m^{-2} d^{-1}	2.8±0.3 g-N m^{-2} d^{-1}
SNRR	NA	gradual increase	2.2±0.5 g-N m^{-2} d^{-1}
TIN removal	NA	gradual increase	81.2±7.7%
TAN removal	NA	gradual increase	90.6±6.6%

Temperature	34 °C	33.8±1.2 °C	34.8±0.9 °C
pH	7.5	7.2±0.6	7.3±0.6
DO	NA	NA	2.1 ±0.1 mg L^{-1}
Length	90 days	200 days	275 days

5.3.2. Source of inoculum

For Phase 1 of the study the MBBR was inoculated with conventional activated sludge from a full-scale biological nutrient removal domestic wastewater treatment plant located in Winnipeg, Canada. The full-scale process operates a Westbank-type configuration with carbon removal, nitrification, and denitrification for total nitrogen (TN) removal, and enhanced biological phosphorus removal achieving effluent quality of <15 mg-TN L^{-1} and <1 mg-TP L^{-1} (i.e., TP is total phosphorus). The inoculum was introduced such that the original solids concentration in the reactor reached >2 g-TSS L^{-1} (TSS is total suspended solids).

For Phase 2 of the study, the MBBR was inoculated with anammox biomass from a lab scale MBBR operated for approximately 1 year (Kowalski et al., 2018). 6 L (approximately 15% of design media fill) of the carriers with fully developed biofilm were removed from the lab-scale MBBR and put in the pilot reactor. Additionally, on days 43, 57, 64, 71, 78 and 85, 1 L of seed carriers was added. The reactor was augmented due to a process upset at the beginning of the study (see section 3.4). It is important to note that each time seed carriers were added a respective volume of original carriers was removed from the MBBR to maintain a filling fraction of 40 %.

Specific anammox activity of the seed was measured in batch tests prior to seeding (procedure described in section 2.5). Specific surface anammox activity equaled to 138 ± 1 mg-N m^{-2} h^{-1}.

While specific anammox activity in respect to volatile solids concentration was equal to 11.0 ± 0.2 mg-N g-VSS^{-1} h^{-1}.

5.3.3. Centrate characteristics

Centrate from the full-scale plant's dewatering centrifuges was supplied to the MBBR. The centrate was collected 3 times a week in a 250 L HDPE barrel and from there continuously fed to the MBBR. Characteristics of the centrate were quantified on the samples from the dewatering facility's centrate sampling port (Figure 5-1). During the period when the reactor was fed with diluted centrate the characteristics of the feeding medium were quantified from the inlet to the reactor. The average COD, TAN, and TSS were 400 ± 100 mg-COD L^{-1}, 700 ± 100 mg-NH_4-N L^{-1}, and 120 ± 90 mg-TSS L^{-1}, respectively. Temperature and pH of the fresh centrate was also measured and the average was 34.5 ± 0.8 °C and 7.5 ± 0.1, respectively. COD/N ratio was relatively low on average and equaled to approximately 0.6. It is noteworthy that the TAN concentration in the centrate was relatively low. The centrate had low average solids concentration, with significant variability and peak concentration of almost 400 mg-TSS L^{-1}.

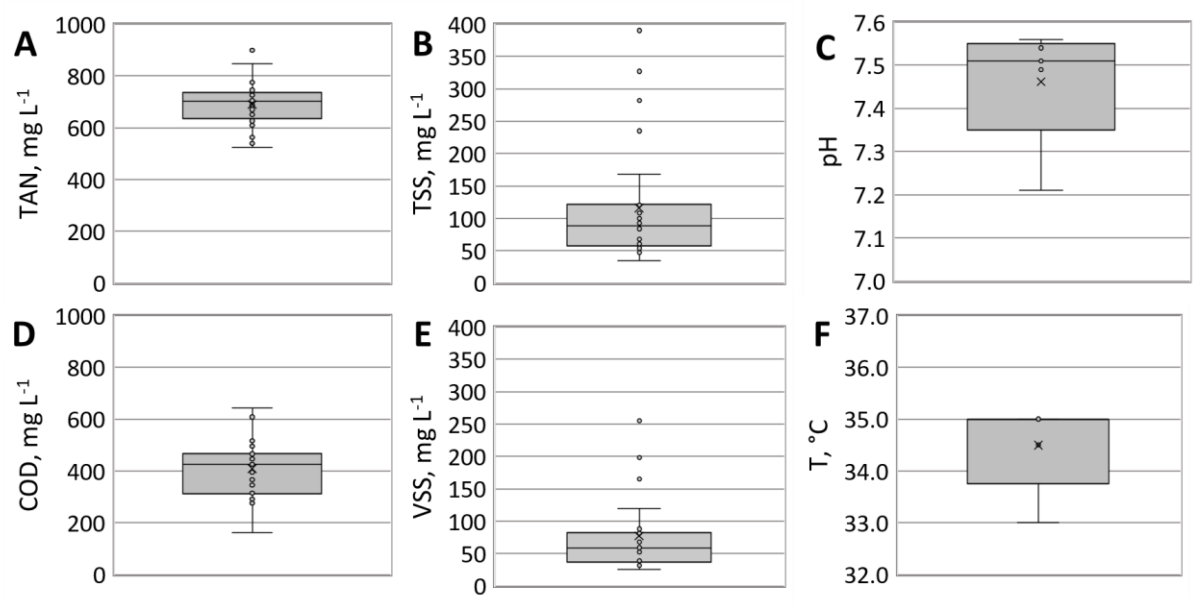


Figure 5-1 Full-strength centrate characteristics; A – total ammonium nitrogen (TAN; mg-N L-1), B – total suspended solids (TSS; mg-TSS L-1), C – pH, D – chemical oxygen demand (COD; mg-O2 L-1), E – volatile suspended solids (VSS; mg-VSS L-1), F – temperature (T; °C). Error bars represent one standard deviation.

5.3.4. Analytical methods

The performance of the reactor was monitored using surface nitrogen loading and removal rates, as well as nitrogen removal percentage. Nitrite, nitrate and ammonium concentrations in the influent and effluent were measured three times a week by QuickChem flow injection analyser (Lachat QuikChem 8500, HACH, CA). Total Inorganic Nitrogen (TIN) was calculated as the sum of nitrite, nitrate and ammonium nitrogen. Chemical oxygen demand (COD), volatile suspended solids (VSS) and total suspended solids (TSS) were measured according to Standard Methods (APHA, 2005). Pictures of the carriers were taken using a stereoscopic microscope (Zeiss Stereo Discovery.V6, USA) with magnification that varied from 0.315x to 1.5x. Biofilm thickness was measured as a distance from the surface of the carrier to the top of the biofilm using CMEIAS©, an open-source image analysis software. Biofilm solids on the plastic carriers were measured after physically removing the biofilm with a cotton swab and de-ionized water. The biomass sample was then measured for VSS and TSS using Standard Methods. All samples that required filtration were run through medium porosity Q5 filter paper (Fisher Scientific,

CA). One-way analysis of variance (VassarStats, USA) was used to determine if steady-state data was significantly different (i.e., $\alpha = 0.05$).

Free, un-ionized ammonia (FA) was calculated according to Anthonisen et al. (1977):

$$FA\ (mg\ NH_3 - N\ L^{-1}) = \frac{TAN_R * 10^{pH}}{e^{6344^{(273+T)}} + 10^{pH}}\ , \quad (1)$$

where: TAN_R - total ammonium nitrogen in the reactor, T - temperature.

Surface nitrogen lading rate (SNLR) was calculated based on the following equation:

$$SNLR\ (g\ N\ m^{-2}d^{-1}) = \frac{TAN_{in} * Q}{SA}\ , \quad (2)$$

and surface nitrogen removal rate (SNRR):

$$SNRR\ (g\ N\ m^{-2}d^{-1}) = \frac{(TAN_{in} - TIN_{out}) * Q}{SA}\ ; \quad (3)$$

where: TAN_{in} – total ammonium nitrogen in the centrate; TIN_{out} – total inorganic nitrogen in the effluent; Q – flow, SA – total protected surface area of the moving bed.

5.3.5. Batch tests

Anammox activity batch tests were conducted (in triplicates) in 0.5 L glass bottles. The tests were run separately on old seed carriers and original non-seed carriers (partial nitritation biofilm present prior to seeding). Each bottle was filled with 10-15 carriers and with 0.4 L of synthetically prepared medium with NH_4-N and NO_2-N concentrations of approximately 50 and 70 mg-N L^{-1}, respectively. The bottles were sealed and put in a water bath at 35°C and constantly mixed. Samples were taken every hour for 4 to 5 h and analysed for NH_4-N, NO_2-N and NO_3-N. Surface anammox activity (SuAA) was reported in mg-$N_{removed}$ m^{-2} h^{-1} and specific anammox activity (SpAA) in mg-$N_{removed}$ g-VSS^{-1} h^{-1}.

5.4. Results and Discussion

5.4.1. Partial nitritation – Phase 1

The MBBR was seeded with BNR sludge and continuously aerated at an average air flow rate (AFR) of 2 L min^{-1}. The HRT during this period was approximately 48 h with surface nitrogen loading rate (SNLR) of 1.5 g-N m^{-2} d^{-1}. Successful start-up of partial nitritation was completed in 2 weeks when significant production of NO_2^- was observed. Suspended biomass was let to naturally wash out from the reactor during this period. NO_2-N and NO_3-N was produced immediately after seeding and reached 120 and 50 mg-N L^{-1}, respectively on day 4. As the inoculum was washed out from the reactor the NO_2-N and NO_3-N production dropped to a minimum of 27 and 12 mg-N L^{-1}, respectively on day 8. From that point on, however, the biofilm had started developing and the nitritation had fully established on day 14 when the NO_2-N concentration reached 300 mg-N L^{-1}. At that point no more NO_3-N production was observed.

Following successful start-up, the performance of partial nitritation was tracked over a period of 2.5 months to gain steady state data. NH_4-N, NO_2-N and NO_3-N profiles during start-up and steady state partial nitritation are shown in Figure 5-2 (top). During 3 months of operation significant NO_3-N production was observed only once on day 25 when the concentration reached almost 200 mg NO_3-N L^{-1}. This was due to the malfunction of the feeding pump. The pump was subsequently replaced, and the operation of the reactor returned to normal. The average NO_2-N/NH_4-N ratio during steady state was 0.57 ± 0.06. The ratio was kept lower than the optimal for anammox to minimize free nitrous acid (FNA) toxicity after seeding with AnAOB biomass.

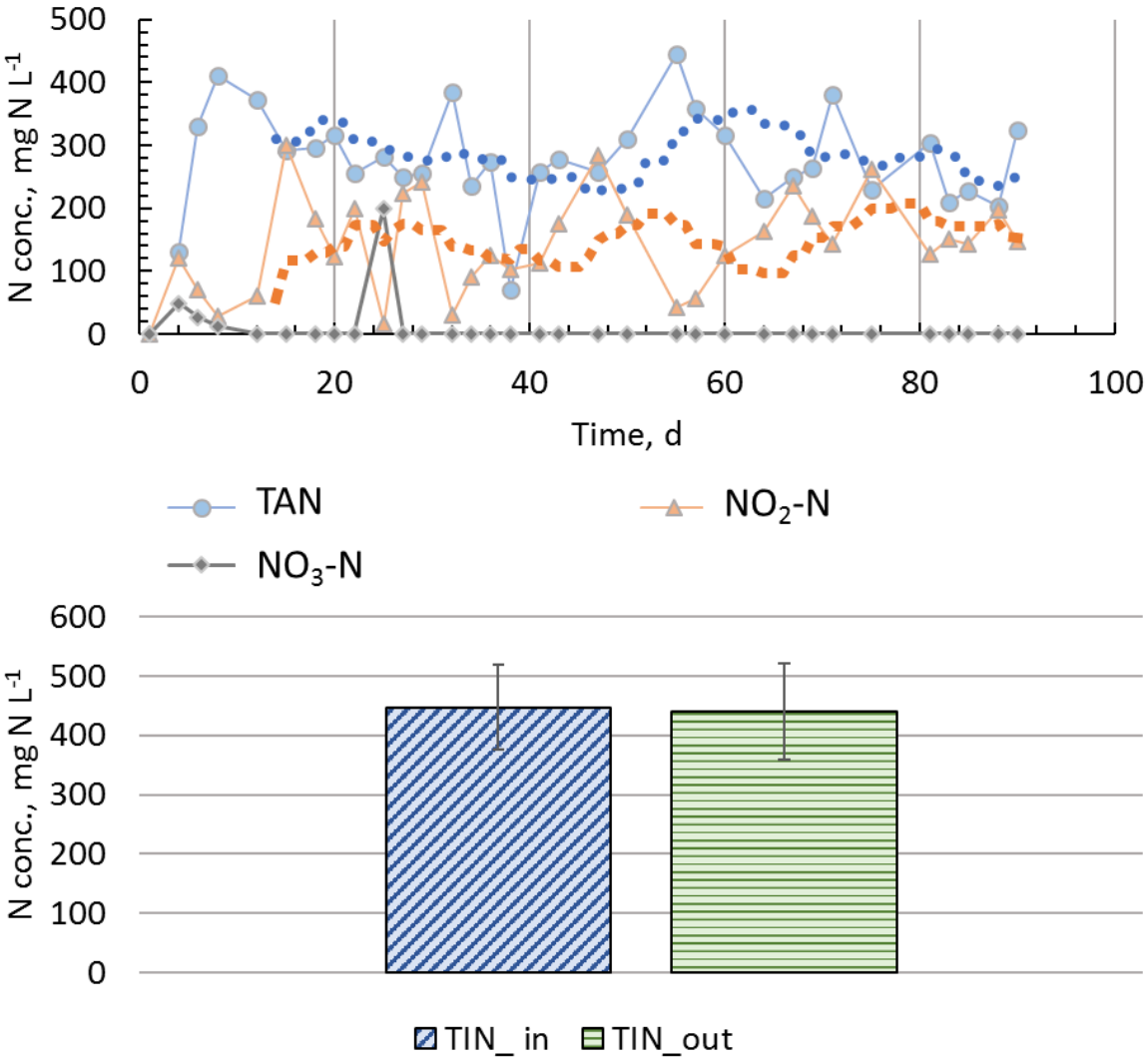


Figure 5-2 Top - NH_4-N, NO_2-N and NO_3-N concentration profiles during start-up and steady state partial nitritation; trend lines reflect a 14-day rolling average. Bottom - comparison of total inorganic nitrogen (TIN) concentration in the centrate and in the effluent of the MBBR. Error bars represent one standard deviation.

Nitrogen concentration in and out of the reactor was also monitored during the operation of the MBBR in order to assess possible nitrogen losses due to ammonia stripping or inoculum free development of anammox activity. Figure 5-2 (bottom) shows average TIN concentration in the centrate and in the effluent of the MBBR. Nitrogen concentration in the centrate was on average 450 ± 70 mg-TIN L^{-1} and the average nitrogen concentration in the effluent was 440 ± 80 mg-TIN L^{-1}. Analysis of variance (non-directional t-test) showed that the difference is not statistically significant with p-value of 0.73. No significant stripping of ammonia was therefore

observed. Anammox activity did not develop either although few recent studies (Kanders et al., 2016; Rikmann et al., 2017) have focused on inoculum-free start-ups of deammonifying biofilm reactors in a pilot-scale treating reject water. Rikmann et al. (2017) reported anammox biofilm development after more than 10 months. Inhibitory concentration of free ammonia (FA) was suggested as the main reason behind such a long start-up period. After introducing automatic pH control the biofilm developed in 3–4 months. In case of this study, the authors believe that the anammox activity did not readily develop on its own due to high DO concentration in the reactor.

5.4.2. Anammox – Phase 2 & 3

The MBBR was subsequently inoculated with anammox seed carriers (see section 2.2). Performance of the reactor was tracked over a period of 280 days divided into Phase 2 during which the centrate was diluted approximately 1:1 (v/v) with process water and Phase 3 during which full-strength centrate was used. The results are shown in Figure 5-3. Average SNLR during Phase 2 was equal to 1.5 ± 0.4 g-N m^{-2} d^{-1}. The SNRR increased steadily and the reactor reached 80% TIN removal on day 78. However, due to several process upsets (see section 3.4) in the following weeks the removal dropped significantly. The reactor recovered relatively quickly after each upset and TAN removal reached >90% (and TIN >80%) each time. Operational issues were ultimately solved and from day 175 the process stabilized. Starting from day 197 the reactor was fed with full-strength centrate (Phase 3) with an average SNLR of 2.8 ± 0.3 g-N m^{-2} d^{-1}. The nitrogen removal dropped to around 60% after the loading was increased but reached full removal again in as little as 10 days. The average TAN removal was 91 ± 7 % and average TIN removal was 81 ± 8 % during the last steady state period. The SNRR averaged to 2.2 ± 0.5 g-N m^{-2} d^{-1}.

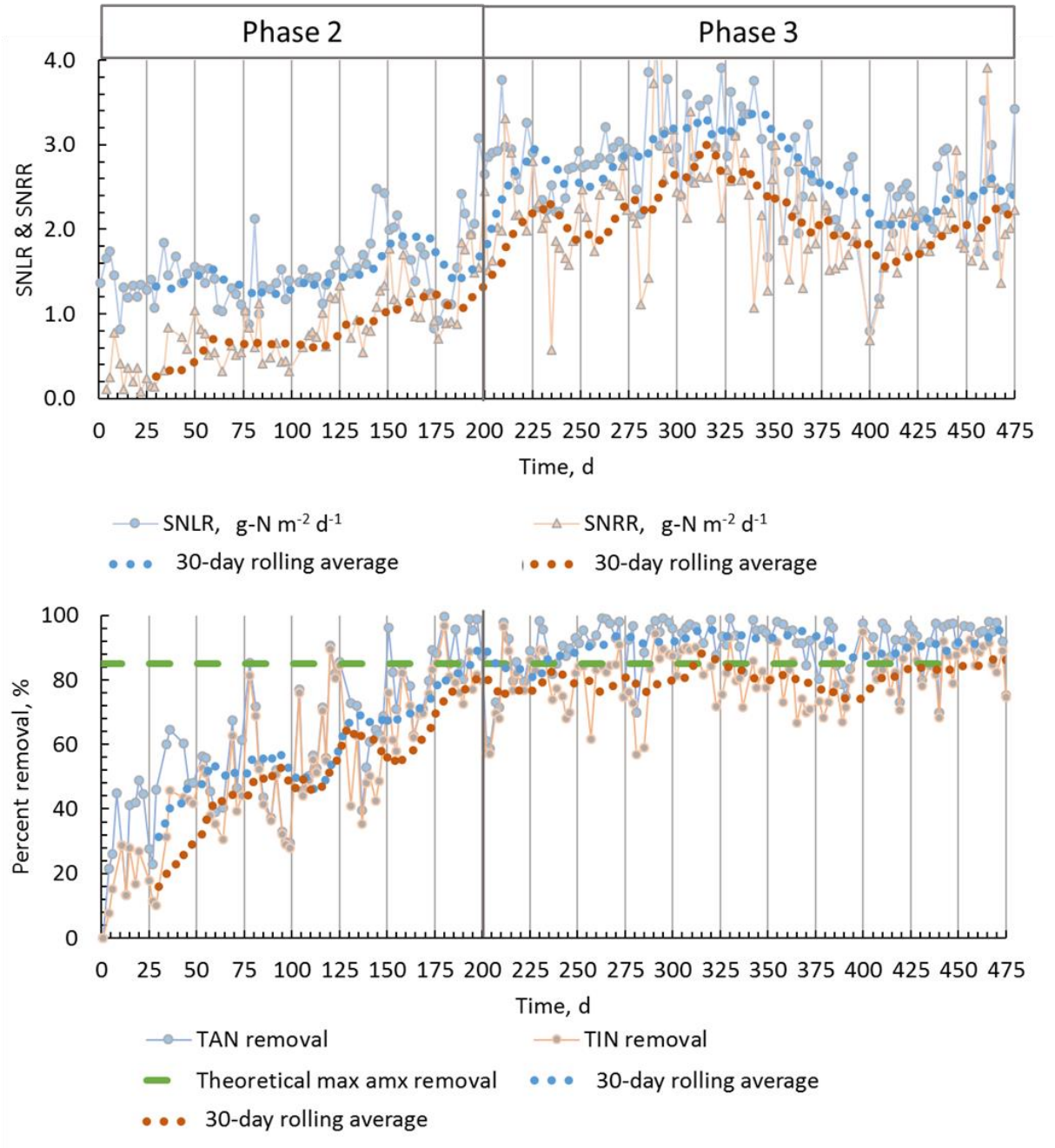
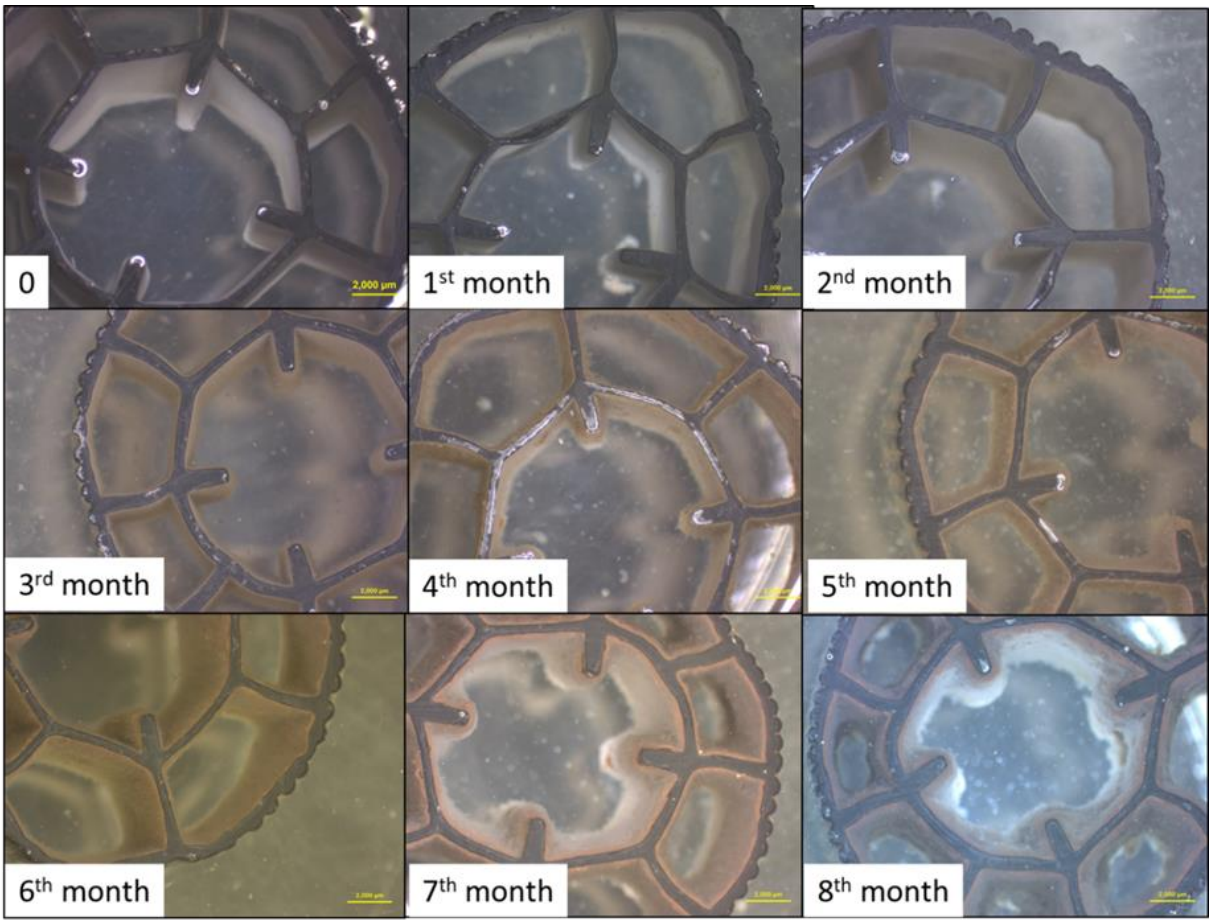


Figure 5-3 Top - Surface nitrogen loading and removal rates (SNLR & SNRR; g-N m^{-2} d^{-1}) during phase 2 and 3. Trend lines reflect a 30-day rolling average. Bottom - Percent total ammonium nitrogen (TAN) and total inorganic nitrogen (TIN) removal during Phase 2 and 3. Trendlines reflect a 30-day rolling average. Horizontal line reflects 88% removal.

Biofilm development was monitored throughout the study and microscopic pictures were taken monthly, biofilm thickness was also measured (Figure 5-4). The thickness of the bio-primer (partially nitritating biofilm) prior to inoculation with anammox was 96 ± 18 µm. During first two months after inoculation the thickness and appearance of the biofilm did not change

significantly. Third month after inoculation the biofilm was noticeably changing colour to reddish brown and the thickness increased to 190 ± 30 µm. During the following three months the thickness of the biofilm did not increase drastically, however, the biofilm became visibly red. After that the SNLR was increased in Phase 3 and during the next two months the biofilm thickness increased significantly and averaged to 420 ± 80 µm. The appearance of the biofilm also changed drastically, and two distinct layers of the biofilm developed and were easily discernible. The inner layer of the biofilm was red and the outer layer greyish-brown, suggesting a heterotrophic/nitrifying biofilm on top of anammox biofilm in the inner layers. These observations confirm the mechanism behind higher resistance of anammox to inhibition in biofilm systems reported previously (Jin et al., 2012). The anammox biomass was effectively protected against high FA concentration or pH variations by an extra layer of biofilm. The authors believe that a similar protective mechanism is not observed in granular systems like DEMON® because the average size of the granules in a full scale systems can be relatively small with a reported average diameter of 170 ± 75 µm (York River DEMON®, Kowalski et al., 2018). This means that the diffusion depth to the centre of the granule is only 85 µm. In case of biofilm systems, the diffusion depth to the bottom of the biofilm can be much higher. Biofilms tend to grow easier than granules and can be effectively thicker (Zhang et al., 2015). The biofilm is also protected by an impenetrable barrier of the plastic carrier substrate. The diffusion depth is equal to the biofilm thickness and in this study, it was almost 5 times higher (420 µm vs 85 µm) and most likely very effective at protecting inner anammox layers. HeadworksBIO 450 type media are relatively deep which allows biofilms to grow thicker and develop additional biofilm layers. This advantage is, however, effectively lost in very shallow "chip-like" carriers.



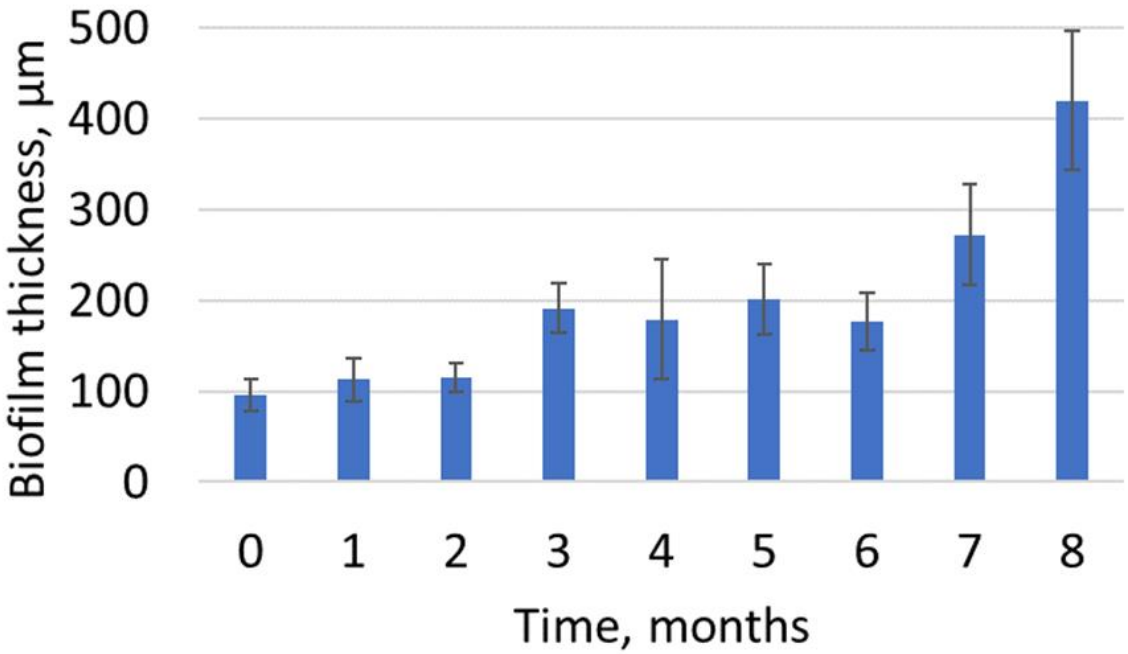


Figure 5-4 Top – development of anammox biofilm during 8 months of operation. Bottom – increase in biofilm thickness over the period of the study

5.4.3. Anammox activity assays

The activity of the seed was assessed prior to inoculation and during Phase 2 – Figure 5-6 (biofilm solids on the media are reported in Figure 5-5). The assay of the seed biomass showed good anammox performance with SuAA of 138 ± 1mg-N m^{-2} h^{-1} and SpAA of 11.0 ± 0.2 mg-N g-VSS^{-1} h^{-1} (0.26 g-N g-VSS^{-1} d^{-1}).

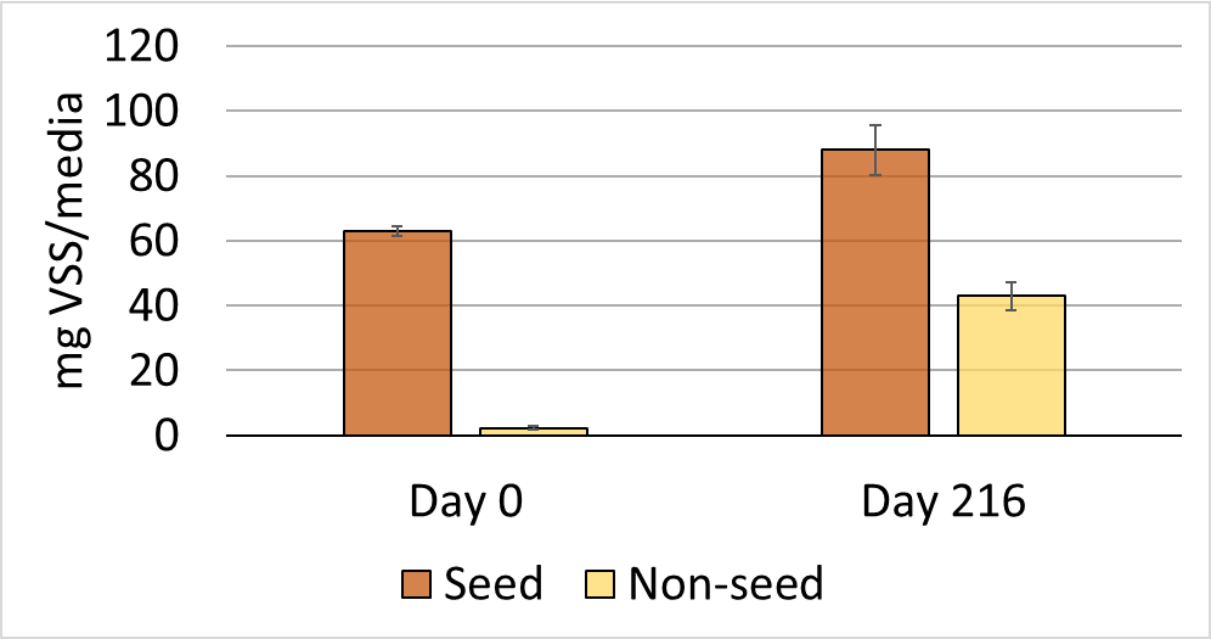


Figure 5-5 Volatile suspended solids on seed carriers and non-seed carriers on day 0 and 216.

After 216 days, anammox activity batch tests were conducted again. Anammox activity was measured separately on old seed carriers and original non-seed carriers (it was possible to differentiate between the two since seed carriers were white and non-seed carriers - black). The anammox activity on the non-seed carriers had reached 179 ± 9 mg-N m^{-2} h^{-1} which was more than twice as high as the old seed. The SpAA had reached 19 ± 2 mg-N g-VSS^{-1} h^{-1} which was almost 75% more than the activity of the original seed biomass prior to inoculation. On the other hand, the activity of the seed has decreased significantly (by approximately 60%) compared to the original values and was 4.2 ± 0.5 mg-N g-VSS^{-1} h^{-1} after 216 days in the reactor. The results obtained from the kinetic tests point to an interesting observation.

Anammox biomass that has developed on the non-seed media (covered originally with a bio-primer) had significantly better kinetic properties than the seed biomass (seed biomass has been grown in an anammox-only reactor on a synthetic wastewater). Furthermore, anammox has been shown to be easily inhibited which was reported in literature many times (Lackner et al., 2014; Li et al., 2011) and has been an important issue whether the inhibition was due to high concentrations of sulfide (Wu et al., 2016), organic carbon (Dapena-Mora et al., 2007; Molinuevo et al., 2009) or FA (Jaroszynski et al., 2011). However, the data obtained from this study shows that anammox biomass that was developed in a deammonifying reactor treating centrate was much more active and resilient than the biomass originating from the anammox-only reactor. The results point to the fact that microbial diversity in a partial nitritation-anammox reactor treating centrate has a significant positive effect on the anammox biomass activity and nitrogen removal performance.

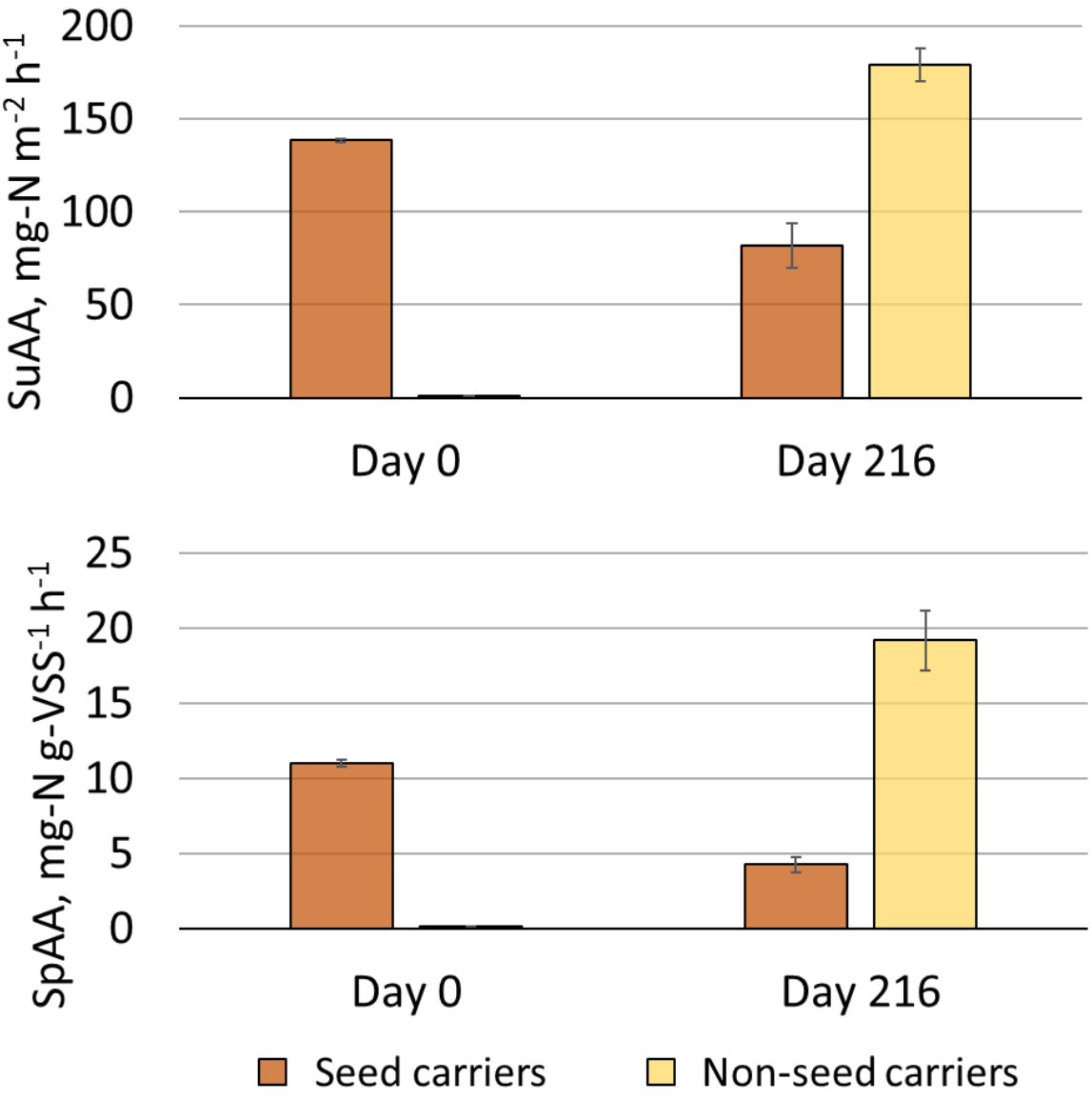


Figure 5-6 Top – Surface anammox activity (SuAA) of seed carriers and non-seed carriers on day 0 and 216. Bottom – Specific anammox activity (SpAA) of the seed biomass and non-seed biomass on day 0 and 216. Error bars represent one standard deviation.

It is also important to note that the difference in specific anammox activity was not due to acclimation as the seed was present in the pilot reactor for more than 200 days, i.e. long enough for the biomass to acclimate.

In fact, to confirm these observations, specific anammox activity has been simultaneously tracked in an anammox-only reactor treating synthetic wastewater. Figure 5-7 shows the

development of SuAA and SpAA in a lab anammox-only MBBR over a period of 3 months. The reactor was seeded with the same anammox biomass as the pilot reactor.

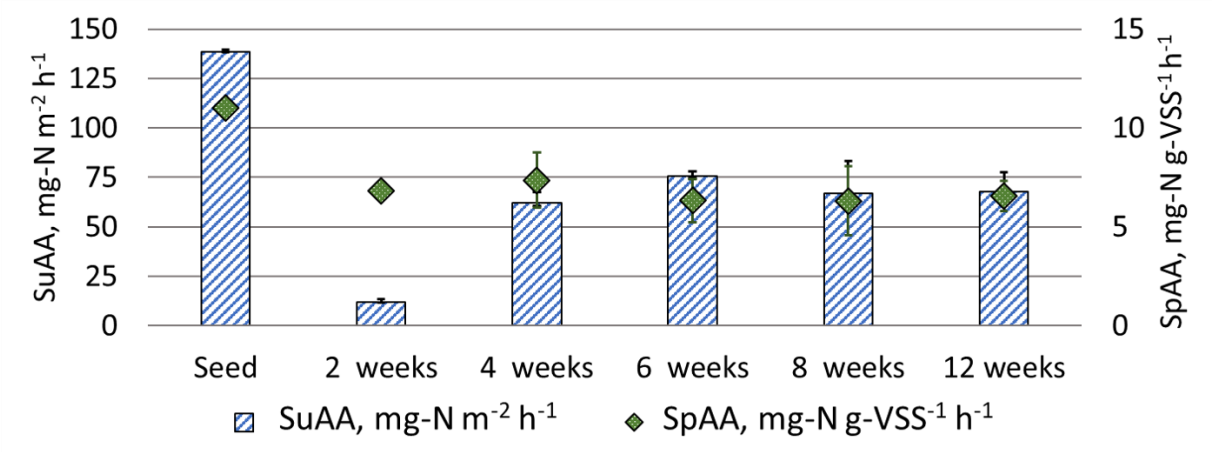


Figure 5-7 Surface anammox activity (SuAA; columns) and specific anammox activity (SpAA; diamonds) of the seed anammox biomass and new anammox biomass grown in anammox-only MBBR on synthetic wastewater. Error bars represent one standard deviation.

After seeding, the SuAA of the virgin media increased rapidly over a period of 6 weeks and subsequently stabilized at approximately 70 mg-N m^{-2} h^{-1}. The SpAA was very stable over the 12-week period and ranged from 6 to 7 mg-N g-VSS^{-1} h^{-1} which was approximately 3 times lower than 19 ± 2 mg-N g-VSS^{-1} h^{-1} observed in the pilot reactor.

The reported anammox activity in the pilot reactor (0.46 ± 0.05 g-N g-VSS^{-1} d^{-1}) compares very well to other studies. Zhang et al. (2016), reported SpAA values between 0.3 and 0.5 g-N g-VSS^{-1} d^{-1}. Similarly, Fernández et al. (2008) reported SpAA between 0.4 and 0.5 g-N g-VSS^{-1} d. Wu et al. (2016) reported average values of 0.39 ± 0.07 g-N g-VSS^{-1} d^{-1}.

5.4.4. Process upsets and FA concentration

pH is a very important parameter in a partial nitritation-anammox reactor as it governs the concentrations of FA and FNA. Both are known to easily inhibit the process when their concentration rises above a certain threshold. The inhibitory concentrations have been widely discussed in literature with many different values being reported (Jin et al., 2012; Tomaszewski et al., 2017). FA concentration increases with higher pH (Anthonisen et al., 1977) but because

of the pH dependent fluctuations of FA, it is difficult to distinguish pH effects from FA inhibition. The physiological pH range for the anammox process has been shown to be between 6.7 and 8.3 in a granular system (Strous et al., 1999) with optimal values of around 8 reported for a biofilm system (Egli et al., 2001).

During the period of the study the pilot reactor had experienced several process upsets, mainly due to low pH. In total, there were 9 incidents when pH dropped below 6 – showed as dots in Figure 5-8. The drops in pH were due to mechanical failure of the feeding pump/clog in the feeding tube (days 67, 76, 151, 235, 257), or alkalinity deficiency (days 131, 137, 144, 172). After each upset a significant decrease in performance was observed (Figure 5-3) caused by a drop in ammonium oxidation and high production of nitrate. The performance of the reactor usually recovered within a week after each upset and no long-term inhibition of either partial nitritation or anammox was observed.

Figure 5-8 also shows the concentration of FA during the operation of the pilot reactor. The FA concentration averaged 4 ± 5 mg NH_3-N L^{-1} with a maximum concentration of 25.5 mg NH_3-N L^{-1}. The FA concentration varied greatly, however, even the highest reported concentration did not cause any long lasting toxic effects which is in agreement with previous observation. Fernández et al. (2012) reported 50% inhibition in SpAA at FA concentration of 38 mg NH_3-N L^{-1}. The optimal concentration of FA to maintain stable operation of a granular reactor was found to be less than 20-25 mg NH_3-N L^{-1}. (Tang et al., 2014) studied inhibitory effects of FA in biofilm reactors and reported that only concentrations as high as 57-187 mg NH_3-N L^{-1} caused inhibitory effects suggesting that biofilm reactors are more resilient to FA inhibition. Jaroszynski et al. (2011), however, reported much higher anammox performance at a pH of 6.5 with average FA concentration of 0.4 ± 0.3 mg NH_3-N L^{-1} than at a pH of 7.8 ± 0.2 with the bulk FA averaging 4 ± 3 mg-N L^{-1}. This relation was observed, however, only at a very high nitrogen removal rates (around 6 g-N L^{-1} d^{-1}) that were never observed in full-scale or pilot

installations (Lackner et al., 2014). At loading rates between 1 and 2 g-N L^{-1} d^{-1} there was no significant difference. It is also important to note that Jaroszynski et al. (2011) had a two-stage system where the anammox reactor was separate from partial nitritation. The authors believe that anammox was shown to be more susceptible to FA inhibition because in a two-stage configuration anammox biofilm is directly exposed to bulk FA concentrations and is not protected by a layer of nitrifying biofilm.

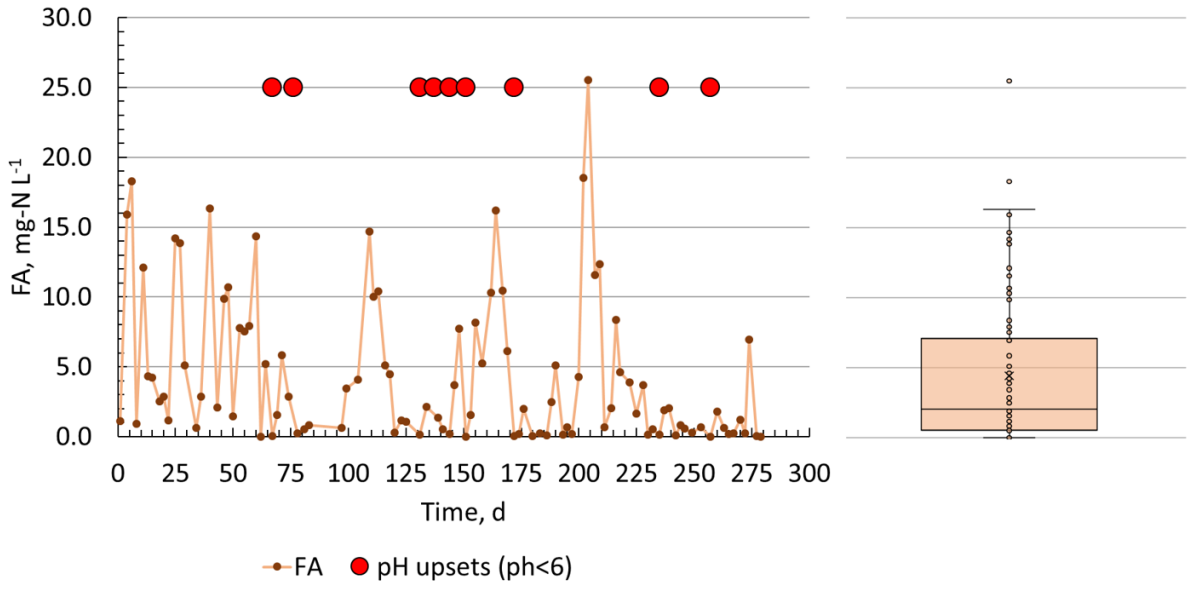


Figure 5-8 Free ammonia (FA) concentration and pH upsets during operation of the pilot MBBR.

Ammonia oxidizing bacteria (AOB), however, are more susceptible to FA inhibition than anammox. Anthonisen (1977) reported AOB inhibition at 7 mg NH_3-N L^{-1}. Nitrite oxidizers are even more susceptible to FA toxicity than AOB (Qian et al., 2017; Torà et al., 2010) with inhibition reported at concentrations as low as 0.1 to 1.0 mg NH_3-N L^{-1} (Turk and Mavinic, 1986). Piculell et al. (2016), observed inhibitory effects of reject water on NOB at low biofilm thickness. The authors believe that better nitritation was observed in thinner biofilms because NOB are more susceptible to FA toxicity and the FA diffusion is better in thin biofilms. The depth of FA penetration into the biofilm is directly proportional to the concentration in bulk and in thin biofilms FA can penetrate the whole depth of the biofilm hence successfully

inhibiting NOB metabolism. In thicker biofilms, NOB communities in inner layers would be protected from exposition to FA.

The afore-mentioned process upsets strongly correlated with low pH and FA concentrations lower than 2 mg NH_3-N L^{-1}. Consequent production of nitrate was also always observed. These results suggest that NOB activity in biofilm deammonification systems is effectively suppressed by FA rather than just by selective washout. Moreover, NO_2-N was never observed to accumulate in the reactor after the process upsets, however, NH_4-N oxidation decreased every time. This suggests that while AOB activity was inhibited, anammox was not. Anammox was, therefore, effectively more resilient to pH perturbation and FA toxicity than AOB confirming previous observations.

5.4.5. Accelerating the start-up time

Due to process upsets described in section 3.4 the time required to reach the design removal rate of 2.5 g-N m^{-2} d^{-1} took approximately 200 days (Fig. 2). However, based on the observed trends in the SNRR during periods of stable operation a theoretical start-up time was calculated to be much shorter. Figure 5-9 shows positive linear trends of nitrogen removal rates during six different periods when the reactor operated without any process upsets. During the first 3 months of operation the SNRR increased at an average rate of 0.038 ± 0.006 g-N m^{-2} d^{-1}. Afterwards when more anammox biomass had accumulated on the carriers the SNRR increased at an average rate of 0.078 ± 0.001 g-N m^{-2} d^{-1}.

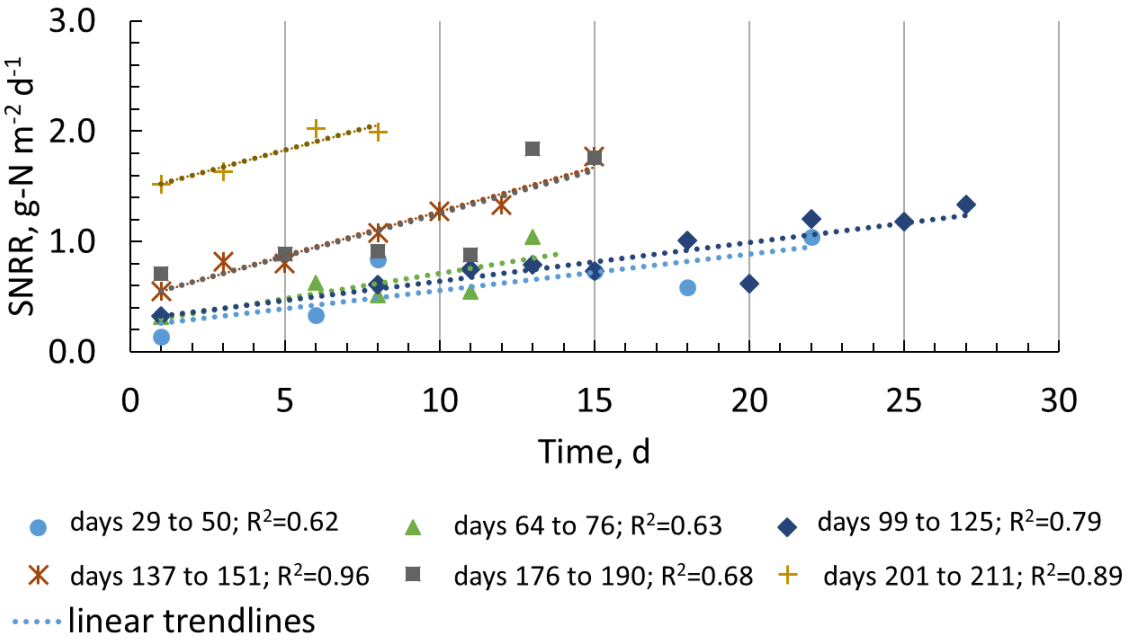


Figure 5-9 Linear trends of surface nitrogen removal rates (SNRR) during six different periods when the reactor operated without process upsets. Dotted lines reflect linear trends.

Based on Figure 5-9, it was assumed that in the beginning the SNRR would increase at the lower rate and after reaching nitrogen removal of 0.75 g-N m^{-2} d^{-1} it would increase at the higher rate due to build-up of anammox biomass. Considering also the 2 weeks required to establish partial nitritation, the total time required to reach the design removal rate of 2.5 g-N m^{-2} d^{-1} would come up to only 56 days (Figure 5-10). Compared to the reported start-up times averaging 4 months the results obtained from this study show that the partial nitritation-anammox MBBR reactor could be started-up in half that time.

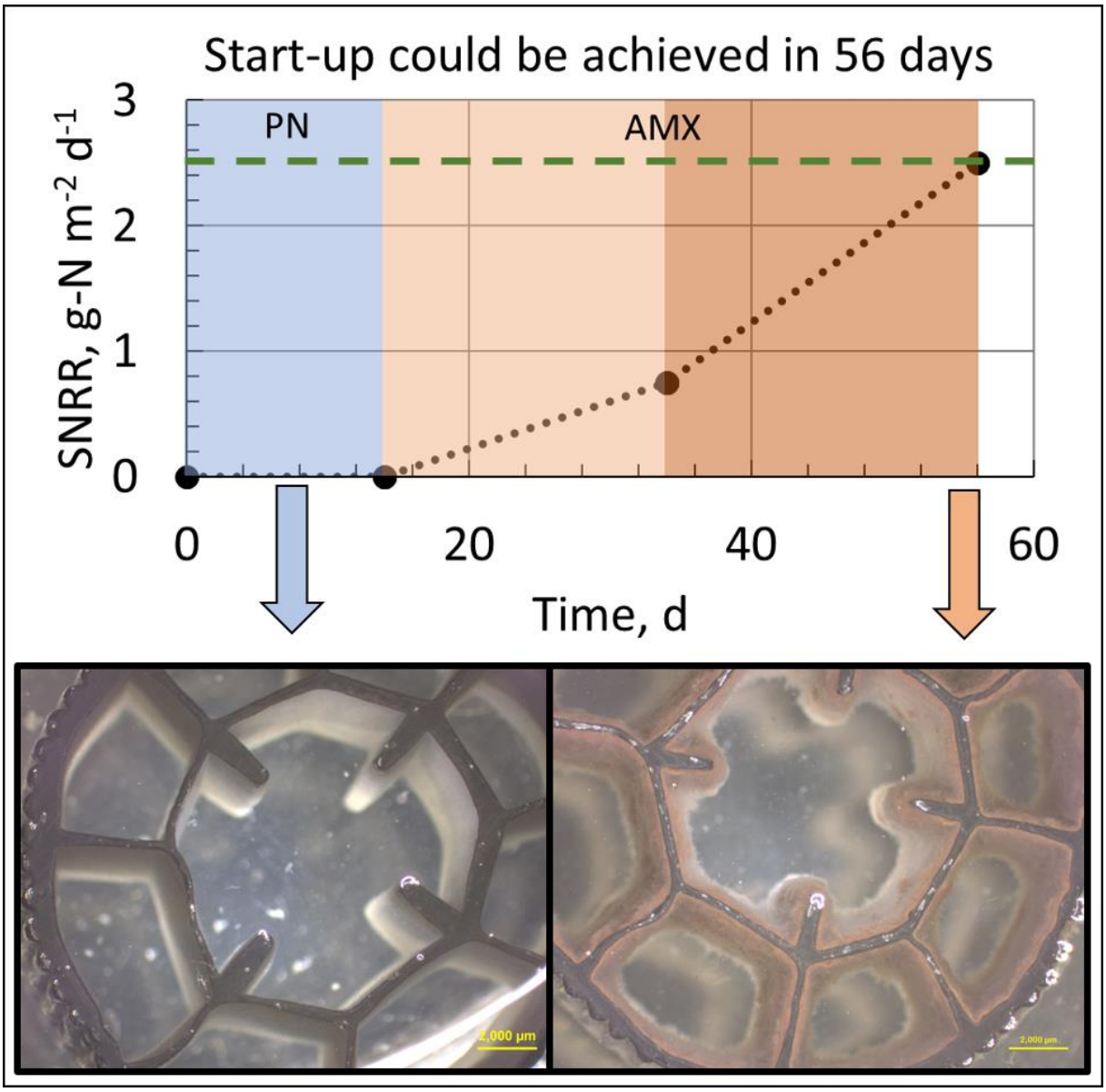


Figure 5-10 Accelerated start-up time of anammox MBBR.

5.5. Conclusions

Pilot partial nitritation-anammox MBBR treating centrate from a full-scale dewatering facility was successfully started-up and operated for 475 days. Development of the anammox process and the performance of the rector was closely monitored. The key findings of the study were:

1) Very fast start-up of partial nitritation-anammox process could be achieved by developing partial nitritation biofilm first and then seeding with anammox biomass.

Incorporating previously suggested bio-primer technique a SNRR of 2.5 g-N m^{-2} d^{-1} in a pilot MBBR was achieved in as little as 56 days.

2) FA was thought to be the predominant inhibitor of NOB activity rather than selective wash-out of nitrifying biomass. A minimum FA concentration of 2 mg NH_3-N L^{-1} was suggested for optimal performance of partial nitritation and to prevent a build-up of nitrate.

3) Biomass from partial nitritation-anammox reactor treating centrate had 75% higher SpAA than seed biomass from anammox-only reactor fed with synthetic wastewater.

4) AnAOB were effectively protected from process perturbations and inhibitors by outer layers of heterotrophic/nitrifying biofilm.

5) Microbial diversity in a partial nitritation-anammox reactor treating centrate may have a significant positive effect on the anammox biomass activity and nitrogen removal performance.

5.6. Acknowledgements

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6. Chapter 6: Effective nitrogen removal in a two-stage partial nitritation-anammox reactor treating municipal wastewater

6.1. Abstract

In order to investigate the feasibility of partial nitritation and anammox process in treatment of municipal wastewater a novel reactor configuration was piloted for 250 days. Primary effluent from full-scale municipal wastewater treatment plant was treated in a two-stage biofilm system incorporating innovative process control method for cold partial nitritation. Partial nitritation was combined with carbon removal in an MBBR to achieve high-rate treatment and nitritation was obtained with DO/TAN ratio control and FA for inhibition of nitratation. Effluent from MBBR was directed to an IFAS reactor where nitrogen was effectively removed via anammox and endogenous denitrification. MBBR achieved partial nitritation at 2.0±0.3 g-N m^{-2} d^{-1} and nitrogen removal in the IFAS reactor reached 0.45±0.1 g-N m^{-2} d^{-1} (55 g-N m^{-3} d^{-1}). The process performed well at 19±3 °C with an average effluent TIN concentration of 11±4 mg L^{-1}. The results reported in this study provide evidence that mainstream PNA is a feasible process although needs further development. Control of partial nitritation was possible, however, achieving stable operation requires a dynamic on-line control system.

6.2. Introduction

Incorporating anammox (AMX)technology into the mainstream wastewater treatment train is proving extremely difficult mainly due to low wastewater temperatures. Municipal wastewater in temperate regions (much of North America, Europe and the northern parts of Asia) with meso-thermal climates is subjected to seasonal temperature variations between approximately 20°C and 9°C and anammox conversion rates have been shown to decrease rapidly below 20°C with removal rates falling down to 0.03 kg N m^{-3} d^{-1} (Hendrickx et al.,

2014; Sánchez Guillén et al., 2016). Nevertheless, the growth and enrichment of anammox bacteria at low temperatures is feasible as shown by Lotti et al. (2014) or Hu et al. (2013).

More problematic and a current bottleneck, however, is the control of partial nitritation (PN) which has proven to be even more challenging than low anammox rates (De Clippeleir et al., 2013). At lower temperatures (<20°C), nitrite oxidizing bacteria (NOB) have a kinetic advantage over ammonium oxidizing bacteria (AOB) with maximum specific growth rates higher than AOB (Kaelin et al., 2009; Sin et al., 2008; Wett and Rauch, 2003). For that reason, NOB must be constantly and selectively washed-out over AOB (and/or anaerobic ammonium oxidizing bacteria - AnAOB) in one-stage partial nitritation-anammox systems operating at low temperatures. Else, NOB activity should be supressed such that they cannot propagate within the system. Over the last several years, there has been some research into simultaneous partial nitritation-anammox with granular sludge. Lotti et al. (2014) reported very good TN removal of 86% at 20°C but the removal decreased below 40% at 10°C mainly due to nitrate production. Controlling residual ammonium concentration has been recently shown to partially supress NOB activity (Poot et al., 2016). Isanta et al. (2015) achieved stable partial nitritation in a granular sludge system at 12.5 °C and Reino et al. (2016) at 10 °C by maintaining an adequate ratio between dissolved oxygen (DO) and ammonium concentrations in the bulk liquid. The synthetic wastewater used in those studies, however, did not contain any or very little organic carbon and nitrogen concentration was still relatively high compared to a typically very weak (40 - 50 mg TN L^{-1}) North American wastewater (Metcalf & Eddy et al., 2013) .

On the other hand, moving bed biofilm reactors (MBBRs) have been shown to perform better and retain more anammox biomass at low temperatures (Eva M Gilbert et al., 2015; Gilbert et al., 2014). However, there has been little research so far on mainstream partial nitritation-anammox using biofilm rather than granular systems. Malovanyy et al. (2015a, 2015b) piloted one-stage partial nitritation-anammox MBBR (25°C) treating pretreated primary

effluent and showed significant overproduction of nitrate even at the relatively very high temperature. Piculell et al. (2016) reported partial nitritation with thin biofilms (biofilm thickness controlled by the carriers topology) when intermittently fed with centrate. Bian et al. (2017) reported successful partial nitritation at 15°C in an MBBR by controlling DO/TAN (TAN being total ammonium nitrogen) ratio, however, only with synthetic wastewater without organic carbon. New research, on the other hand, suggests that simple aeration control is not enough to successfully control partial nitritation on wastewater containing organic carbon. Kowalski et al. (2019b) showed that cold partial nitritation at high COD/N ratio was possible only when DO/TAN ratio control was combined with free ammonia (FA) inhibition of NOB activity. So far, there has been no consensus in literature on successful control strategy for partial nitritation in biofilms for the treatment of real municipal wastewater.

This study has examined for the first time the feasibility of mainstream partial nitritation-anammox (PNA) treating primary effluent from full-scale municipal wastewater treatment plant incorporating a two-stage biofilm system with an innovative process control method for partial nitritation.

6.3. Materials and Methods

6.3.1. Reactor configuration and operation

The study was conducted in a two-stage configuration. Partial nitritation combined with carbon removal was conducted in an HDPE tank with 250 L working volume (PN-MBBR). The reactor was filled to 12% with HeadworksBio 450 type media (protected surface area of 402 m^2/m^3). The reactor was constantly aerated and fed with raw primary effluent (PE). The HRT of the reactor was controlled with a peristaltic pump (Masterflex L/S, Cole-Parmer). pH was monitored with a general-purpose pH probe (Oakton®, USA) and controlled (Oakton pH/ORP controller, USA) at 8.90±0.02 – 9.20±0.02 by dosing aqueous solution of sodium hydroxide

(NaOH). DO was measured with an optical probe (Orion RDO, Thermo Scientific, USA) and portable DO meter (Orion 3 Star, Thermo Scientific, USA).

Effluent from PN-MBBR was directed to an anammox IFAS rector (AMX-IFAS) with 100 L working volume filled to 40% with HeadworksBio 450 type media connected to a circular clarifier with 120 L volume with a recycle rate of 0.5. The average HRT of the reactor was equal to 13.2 h. The reactor was not aerated during the first 215 days. From day 215 the reactor was aerated to achieve microaerophilic conditions (0.01-0.1 mg-O_2 L^{-1}) and reduce the concentration of excess ammonium. pH was monitored with a general-purpose pH probe (Oakton®, USA) and controlled at 7.8±0.1 by dosing aqueous solution of sulfuric acid (H_2SO_4). Figure 6-1 presents the schematic diagram of the experimental configuration.

Practical benefits of two-stage configurations for PNA process had been previously shown in sidestream nitrogen removal (Jaroszyński, 2017; Jaroszynski and Oleszkiewicz, 2011) but this approach has been very limited so far in mainstream wastewater treatment mainly due to inability to control partial nitritation.

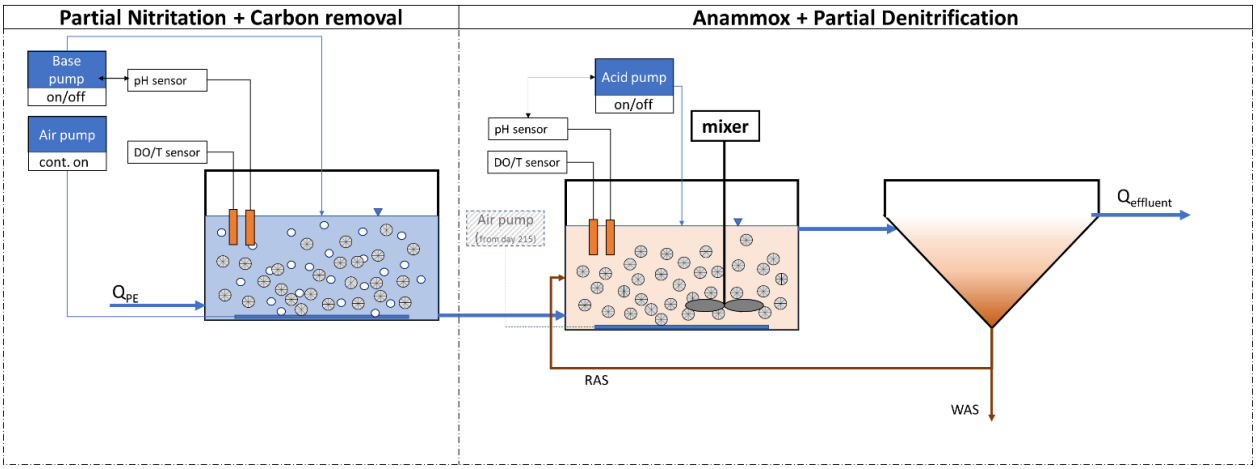

Figure 6-1 Schematic representation of the experimental set-up treating primary effluent.

6.3.2. Source of inoculum

The PN-MBBR was inoculated with conventional activated sludge from a full-scale biological nutrient removal domestic wastewater treatment plant located in Winnipeg, Canada. The full-scale process operates a Westbank-type configuration with carbon removal, nitrification, and denitrification for total nitrogen (TN) removal, and enhanced biological phosphorus removal (EBPR) achieving effluent quality of <15 mg-TN L^{-1} and <1 mg-TP L^{-1} (TP is total phosphorus). Fermentation of primary sludge produces bsCOD (biodegradable soluble COD) for the EBPR process. The inoculum was collected from the return activated sludge (RAS) line and introduced such that the solids concentration in the PN-MBBR reached >2 g-TSS L^{-1} (TSS is total suspended solids).

The AMX-IFAS was inoculated with waste sludge from a sidestream MBBR treating centrate at 34 °C (Kowalski et al., 2019a). Initially, 10 L (at 8.5±0.2 g-TSS L^{-1}) of the seed was put in the pilot reactor. The reactor was then regularly bioaugmented with the waste sludge.

Specific anammox activity (SpAA) of the seed was measured in batch tests prior to seeding. Specific anammox activity equaled to 250 ± 30 mg-N g-VSS^{-1} d^{-1} at 34°C.

6.3.3. Wastewater characteristics

Primary effluent from West End Water Pollution Control Centre in Winnipeg (Manitoba, Canada) was supplied to the PN-MBBR. The PE was collected in a 0.6 m^3 equalization tank and from there continuously fed to the PN-MBBR. To mitigate fermentation the equalization tank was emptied and cleaned 2-3 d per week. The equalization time provided was 2.5-6.5 h depending on how much flow was being drawn for the PN-MBBR and other pilot processes. Characteristics of the primary effluent were quantified on the samples from the inlet to the reactor. The average COD and ffCOD were 347 ± 53 mg-COD L^{-1}, 99 ± 17 mg-ffCOD L^{-1}, respectively. The average TAN concentration was 38 ± 7 mg-NH_4-N L^{-1}. Total nitrogen was

not measured directly but estimated based on PE characterisation. COD/N ratio was on average equal to approximately 6.5 ± 1.5 (without fermentate from primary fermenters). It is noteworthy that the TAN concentration in the PE was relatively low. The PE had very low average solids concentration due to the additional settling in the equalization tank. Average TSS and VSS concentration equaled to 56 ± 50 mg-TSS L^{-1} and 46 ± 35 mg-VSS L^{-1}, respectively.

6.3.4. AMX activity batch tests

To assess the contribution of anammox to the overall nitrogen removal, the consumption of NH_4-N and NO_2-N was measured in the absence of organic carbon source and under anoxic conditions. All activities were measured in ex situ batch tests conducted at 20 °C in triplicates in glass bottles. Each bottle had 30 carriers and was filled with 0.5 L of synthetically prepared medium. NH_4-N and NO_2-N were supplied as NH_4Cl and $NaNO_2$ at a concentration of approximately 36 and 43 mg-N L^{-1}, respectively. The bottles were sealed and constantly mixed. Samples were taken periodically and analysed for NH_4-N, NO_2-N and NO_3-N. SpAA was reported as mg N removed per g VSS per day. Surface anammox activity (SuAA) was reported as mg N removed per square meter per day. N removed was calculated as the sum of NH_4-N and NO_2-N removed. Volumetric consumption rates were calculated by linear regression of off-line measurements of three to four consequent samples of bulk liquid phase. The batch tests were repeated for suspended biomass separately and activities compared.

6.3.5. Analytical methods

Nitrite, nitrate and ammonium concentrations in the samples were measured three times a week by QuickChem flow injection analyser (Lachat QuikChem 8500, HACH, CA). Total Inorganic Nitrogen (TIN) was calculated as the sum of nitrite, nitrate and ammonium nitrogen. Chemical oxygen demand (COD), filtered-flocculated COD (ffCOD), volatile suspended solids (VSS) and total suspended solids (TSS) were measured according to Standard Methods (APHA,

2005). Pictures of the biofilm were taken using a stereoscopic microscope (Zeiss Stereo Discovery.V6, USA). Biofilm thickness was measured using CMEIAS©, an open-source image analysis software. Biofilm solids on the plastic carriers were measured after scraping the biomass off with a cotton swab and de-ionized water. All samples that required filtration were run through medium porosity Q5 filter paper (Fisher Scientific, CA). One-way analysis of variance (VassarStats, USA) was used to determine if data was significantly different (i.e., $\alpha = 0.05$).

Free, un-ionized ammonia (FA) was calculated according to Anthonisen et al. (1977):

$$FA\ (mg\ NH_3 - N\ L^{-1}) = \frac{TAN_R * 10^{pH}}{e^{6344^{(273+T)}} + 10^{pH}}\ , \quad (1)$$

where: TAN_R - total ammonium nitrogen in the reactor, T - temperature.

Surface nitrogen lading rate (SNLR) was calculated based on the following equation:

$$SNLR\ (g\ N\ m^{-2}d^{-1}) = \frac{TN_{in} * Q}{SA}, \quad (2)$$

and surface nitrogen removal rate (SNRR):

$$SNRR\ (g\ N\ m^{-2}d^{-1}) = \frac{(TN_{in} - TIN_{out}) * Q}{SA}; \quad (3)$$

where: TN_{in} – total nitrogen in the PE; TIN_{out} – total inorganic nitrogen in the effluent; Q – flow, SA – total protected surface area of the moving bed.

Surface organic loading rate (SOLR) and surface organic removal rate (SORR) were calculated analogically.

The SRT in AMX-IFAS reactor was estimated according to Equation 4. It should be noted that the mass of solids in the clarifier was neglected for SRT estimations, and therefore actual SRTs would be higher than estimated. Net observed yield (Y_{net}) was estimated according to Equation 5.

$$SRT = \frac{MLSS_{reactor} \cdot V_{reactor} + M_{biofilm}}{Q_{influent} \cdot TSS_{effluent}} \quad (4)$$

$$Y_{net} = \frac{VSS_{effluent}}{(COD_{influent} - COD_{effluent})} \quad (5)$$

It should be noted that Q refers to the flow, V refers to volume, and M refers to total mass of biofilm in the reactor.

6.3.6. Modeling Software

There are many wastewater treatment modelling and simulation programs currently available on the market, like Biowin, GPS-X, SIMBA, SUMO etc. All of them are based on the same integrated Activated Sludge Models (ASM1, ASM2d and ASM3 by the International Water Association). Biowin is a comprehensive simulation tool for biological wastewater treatment plant design and analysis that is most widely used in commercial applications in North America. The software was developed with the primary objective of providing a powerful tool to aid both the process designers and operators of these facilities. The user can define and analyze behavior of complex treatment plant configurations with single or multiple wastewater inputs.

A crucial component of BioWin is the biological process model. The BioWin model is unique in that it merges both activated sludge and anaerobic biological processes. Additionally, the model integrates pH and chemical phosphorus precipitation processes. BioWin is a very powerful analysis tool. The program has been evaluated against an extensive data set and has been demonstrated to provide accurate simulation results for a range of systems. BioWin is used and referenced in papers throughout the industry (EnviroSim Associates Ltd).

A simulation has been set up based on default kinetic parameters in Biowin version 5.3 using the data gathered during partial nitritation experiments and compared to the observed data. Complete information regarding the modelling package and ASM3 parameters can be found on EnviroSim Associates Ltd website (https://envirosim.com/).”

6.4. Results and discussion

6.4.1. Stage one: PN-MBBR

Prior to the introduction of nitritation, the MBBR operated as a high-rate carbon removal reactor at SOLR of 31±1 g-COD m^{-2} d^{-1}. Subsequently, the SOLR was appropriately adjusted (lowered to allow for nitrification) and the PN-MBBR reactor was working at an average SOLR of 14±4 g-COD m^{-2} d^{-1}. Figure 6-2B shows the trends in SOLR and SORR during the whole period of the study. During the first 50 days the SOLR was controlled at approximately 20 g-COD m^{-2} d^{-1} but nitrification was limited, consequently, the loading was further reduced during the next 50 days. The optimal loading rate for partial nitrification was found at around 12 g-COD m^{-2} d^{-1} and was controlled at this level from day 100.

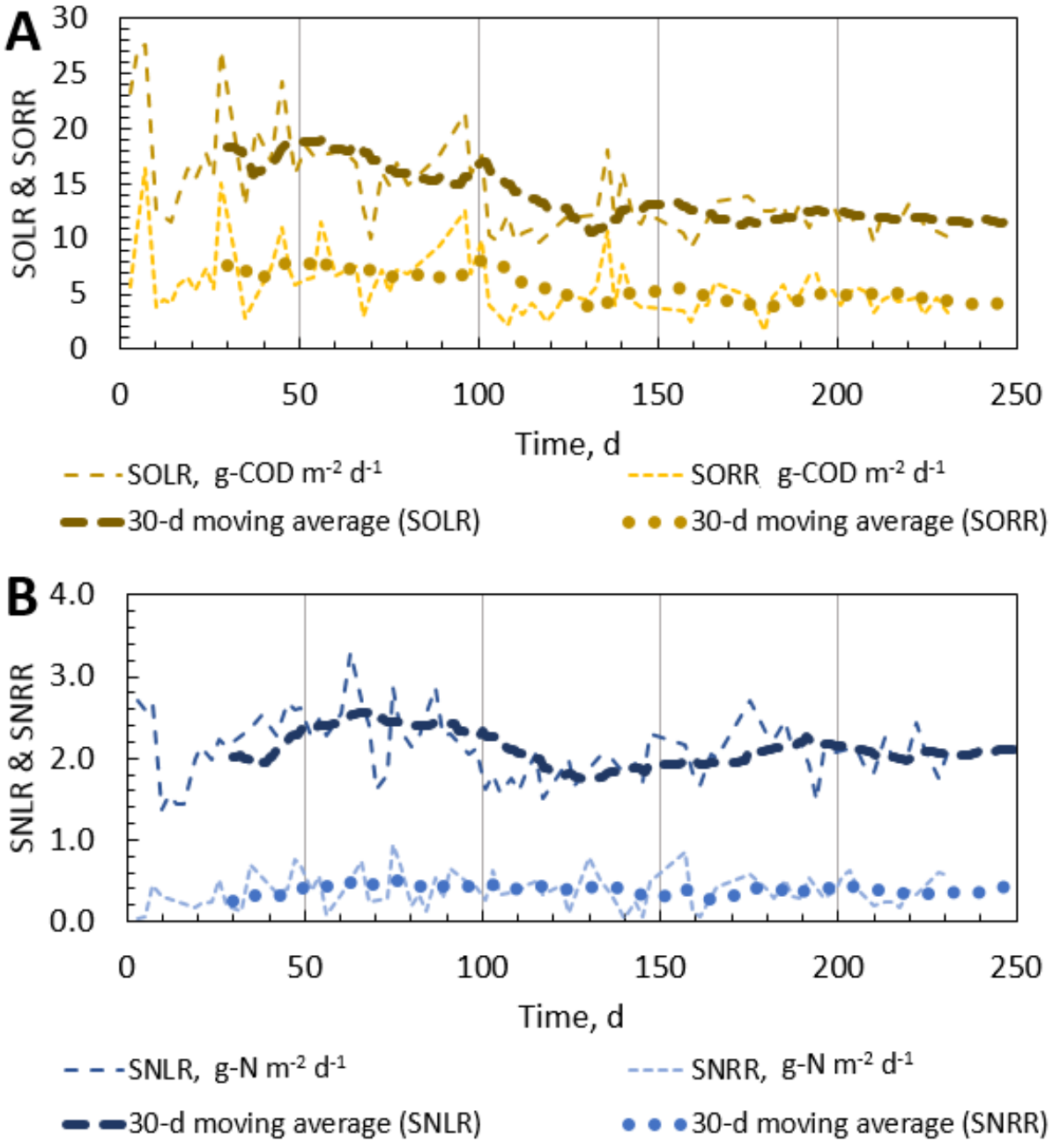

Figure 6-2 A - Surface organic lading and removal rates (SOLR &SORR) in PN-MBBR; B – Surface nitrogen loading rate (SNLR).

SNLR is shown in Figure 6-2B together with a 30-day moving average trends. During the first 100 days the average SNLR was 2.3±0.4 g N m^{-2} d^{-1} and decreased to 2.0±0.3 g N m^{-2} d^{-1} when the organic loading rate to the reactor was lowered after day 100. Less than 20% of TN was removed in the PN-MBBR, the removal was associated with assimilation of nitrogen to new biomass.

Partial nitritation was controlled in the MBBR based on the method described previously in Kowalski et al. (2019b) which was shown successful at 10 °C and at COD/N ratios >2. Target level of ammonium oxidation was achieved by controlling DO/TAN ratio and nitritation was obtained by FA inhibition. During the operation of PN-MBBR treating raw primary effluent with COD/N ratio of approximately 8, partial nitritation was more difficult to control but still achievable. Previously, cold partial nitritation with biofilms was shown only with synthetic wastewater and/or at very low COD/N ratios. Piculell et al. (2016) reported successful partial nitritation at 15°C in an MBBR reactor treating synthetic municipal wastewater by maintaining thin biofilm and alternating feeding of synthetic centrate. Laureni et al. (2016) reported successful NOB suppression at micro-aerophilic conditions (0.15 - 0.18 mg-O_2 L^{-1}) at 15°C. Bian et al. (2017) achieved stable partial nitritation through maintaining a constant ratio of 0.17 between DO and total ammonium nitrogen (TAN) concentration at 6 - 16°C, similarly to Isanta et al. (2015) and Reino et al. (2016) who have successfully run the process in suspended growth reactors.

During this study, partial nitritation with a NO_2-N/NH_4-N ratio of 1.1±0.1 was obtained at DO/TAN ratio of 0.22±0.09 and at FA concentration of 3.5±1.2 mg NH_3-N L^{-1} and the optimal performance was observed between days 105 – 155 (Figure 6-3). Although steady state partial nitritation was challenging it was shown possible. Figure 6-3 also displays fractionation of inorganic nitrogen species in the effluent from PN-MBBR. On average 14% of TIN in the effluent was present in the form of NO_3-N. Similar observation for the residual nitrate was made previously in a lab scale reactor (Kowalski et al., 2019) where certain fraction (although smaller) of TIN was still converted to nitrate. One possible explanation could be that NOBs were not totally inhibited or that some fraction of nitrifiers was able to perform a comammox process. Further metagenomic study is necessary to clarify these observations.

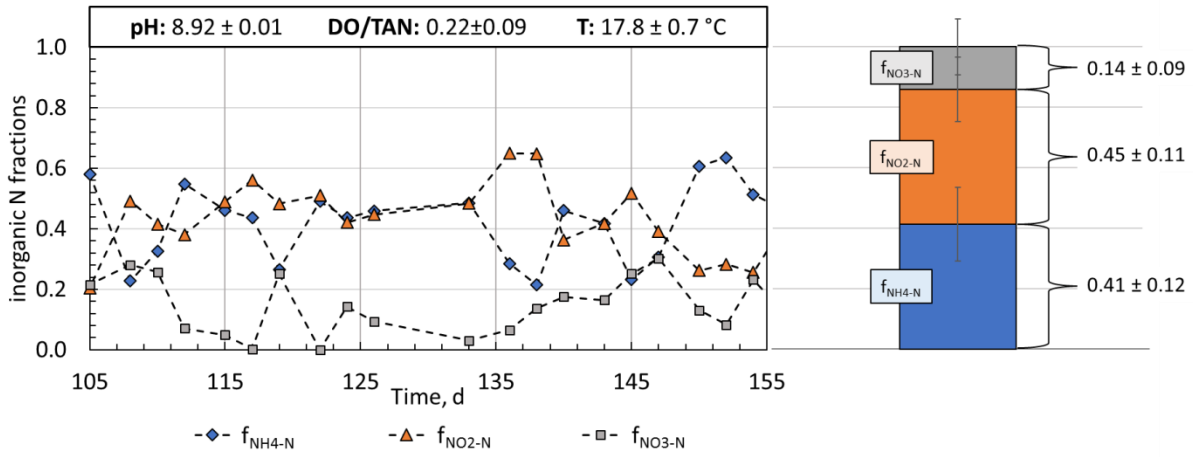

Figure 6-3 Fractionation of inorganic nitrogen species in the effluent from PN-MBBR.

In order to achieve high FA concentration in the reactor the pH was controlled at an elevated level, on average 8.9 (see

Figure 6-4 for complete FA and pH trends). It is well known that pH can have a large impact on the morphology and metabolism of biofilms. Sudden shifts in pH can be very detrimental to metabolic activity and exert biocidal effects. Microorganisms, however, possess mechanisms that allow them to adapt to gradual changes in pH (Garrett et al., 2008). Indeed, the appearance of the biofilm changed significantly when the MBBR reactor was switched to high pH conditions. The colour of the biofilm became darker and the morphology of the biofilm appeared denser or more compact (see Figure 6-5 in supplementary material for microscopic comparison). The activity of the biofilm, however, did not diminish. Another side-effect observed after increasing the pH was significantly lower biomass production. The observed biomass yield in the MBBR reactor prior to pH control was equal to 0.55-g VSS g-COD^{-1} which is very well within the range of 0.4-0.6-g VSS g-COD^{-1} reported for aerobic CAS processes (Metcalf & Eddy et al., 2013). After the pH control was placed on-line the yield decreased to only 0.40-g VSS g-COD^{-1} which is almost 40% lower. This constitutes an important additional benefit associated with sludge handling and management.

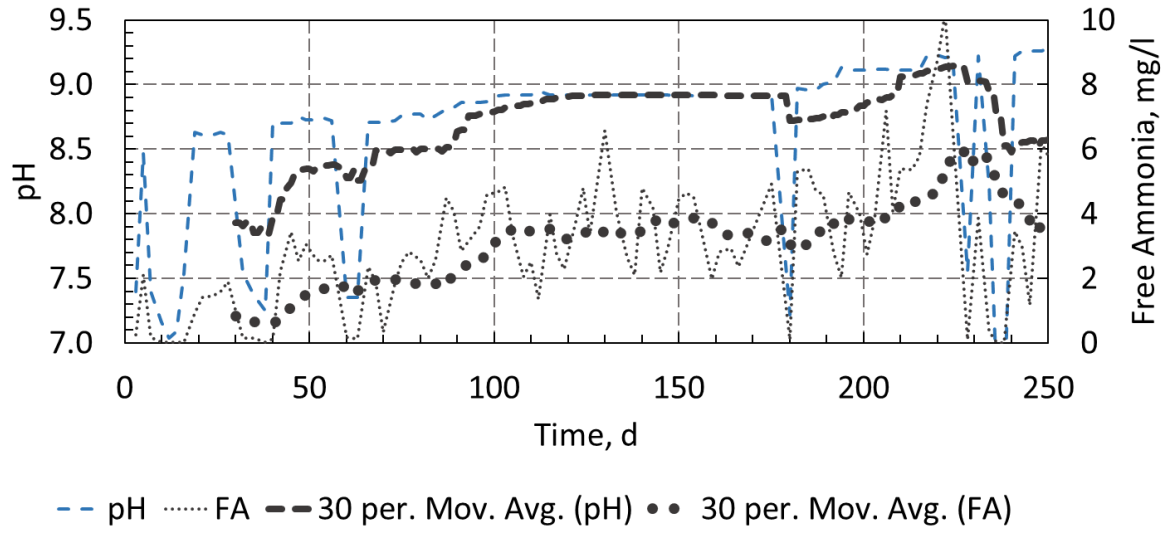

Figure 6-4 pH and free ammonia trends during 250 days of operation of PN-MBBR reactor.

Biofilm reactors are traditionally considered long solids retention time (SRT) systems. The SRT of the PN-MBBR was, however, much shorter than expected. Based on the effluent solids it was estimated at only 1.6 days. This was somewhat lower than the theoretical minimum SRT of 2 days for nitrification at the operational temperature of around 18°C, as well as substrate and DO concentration[7]. One possible explanation could be that the biofilm was not uniformly sloughing off from the media and there were layers of the biofilm that were replaced at a slower rate than others (deeper layers could be replaced slower having higher SRT) and the average SRT might not have represented the actual SRT of the AOBs and/or NOBs.

[7] Estimated based on $\mu_{AOB} = \mu_{max,AOB,18°C}\left(\frac{S_{NH4}}{S_{NH4}+K_{NH4}}\right)\left(\frac{S_O}{S_O+K_{O,AOB}}\right) - b_{AOB,18°C}$

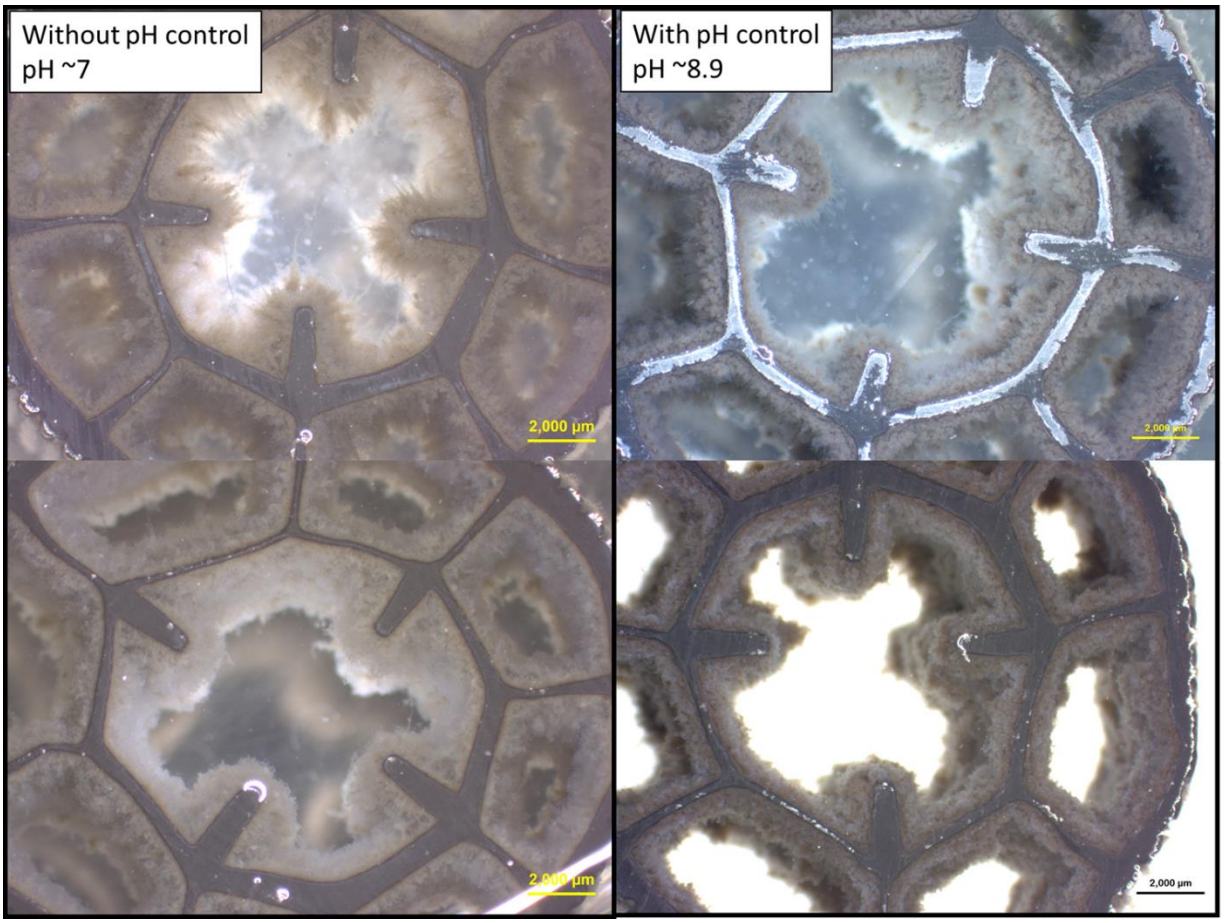

Figure 6-5 Comparison of biofilm appearance between no pH control (left) and pH controlled at 8.9 (right).

The difficulties in achieving stable partial nitritation originated mainly from the fact that the temperature and primary effluent characteristics varied significantly. The set-points for DO/TAN ratio and pH had to be always manually adjusted at the pilot facility which created significant difficulties. These observations proved that automatic process control is necessary to achieve stable partial nitritation at variable process conditions. Nevertheless, enough data was collected during the period of the study to define the accurate values of control set-points for DO/TAN ratio and FA to achieve high quality final effluent. All results with final effluent TIN<11 mg L^{-1} (n=37) were compiled and checked for the observed FA concentration and the actual DO/TAN ratio in the PN-MBBR reactor. The results revealed that for the best nitrogen removal performance in the configuration the set-points in PN-MBBR should be controlled for FA at 3.6±1.8 mg NH_3-N L^{-1}, and for DO/TAN at 0.26±0.9. These values are valid for the

design loading rates and temperature in the PN-MBBR and would change under different conditions. The relatively high standard deviation in the observed set-points means that they do not need to be controlled extremely tightly and that the AMX-IFAS reactor had enough buffering capacity before the drop in nitrogen removal performance was observed.

Application of MBBR for mainstream partial nitritation is additionally beneficial because of the high rates that can be achieved in the reactor. This is mainly because nitritation is not limited by low DO concentrations which are necessary to control NOBs in one-stage systems (Isanta et al., 2015b; Reino et al., 2018). Moreover, the presented system pre-treats the wastewater (by removing sBOD) and partially nitrifies in one reactor which simplifies the treatment. It is therefore possible to achieve high rate carbon removal, produce nitrite and significantly lower biomass production within one compact reactor. All are major advantages over low DO or transient anoxia one-stage configurations which rely on microaerophilic conditions to achieve NOB suppression.

6.4.2. Chemical requirement

The chemical requirement needed to artificially increase pH is an additional operating cost associated with the proposed control strategy. Sodium hydroxide is easy to handle, allows 50% solutions and has been a proven chemical of choice used in full-scale applications for pH control, sidestream phosphorus recovery or sludge treatment. The average consumption of NaOH was estimated at 0.2 kg/m^3 of wastewater. The cost of industrial grade NaOH varies greatly depending on the purity, however, assuming the average price of 200-400 $/tonne gives approximately 0.04-0.08 $ per m^3 of wastewater in additional chemical cost. It is not easy to precisely estimate the operational costs of traditional BNR plants as they vary greatly depending on process design, scale, energy costs, location etc. According to Gratziou and Chrisochoidou (2011) the total annual operating cost of an activated sludge process with pre-denitrification

was 1.54[8] $/m^3. Molinos-Senante et al. (2010) reported much lower operating costs estimated at 0.265[1] $/m^3. Similarly, Zessner et al. (2010) who showed that the cost of operating BNR plants in Austria ranges from 0.154 to 0.225[1] $/m^3. Rodriguez-Garcia et al. (2011) reported moderately higher range of 0.271-0.320[1] $/m^3.

Integration of anammox into mainstream process is associated with potentially significant savings in operating costs. Assuming that energy and sludge disposal (including chemical costs and maintenance) make up around 60% of total operational costs of the treatment plant (Molinos-Senante et al., 2010), the authors believe that the additional chemical costs could be easily offset by the gained savings.

6.4.3. Stage two: AMX-IFAS

In order to accelerate the attachment of anammox bacteria to the virgin carrier material and subsequently shorten the start-up time, a layer of biofilm was grown on the virgin media in the AMX-IFAS reactor prior to inoculation with anammox biomass. This method was previously shown effective in a lab-scale reactor (Kowalski et al., 2017) and by other authors (Klaus et al., 2016). The AMX-IFAS reactor was then seeded with waste anammox sludge from sidestream MBBR treating centrate at 34 °C and was periodically bioaugmented, usually 1-3 times per week with small amounts of waste sludge, approximately 1.5 L (at 8 g-VSS L^{-1}) at a time, simulating bioaugmentation from the full-scale sidestream reactor. Figure 6-6 shows the nitrogen removal performance of AMX-IFAS reactor during 250 days of operation. Effluent TIN concentration decreased below 15 mg L^{-1} after the first 2 weeks of operation and remained below that limit for most of the study. Considerable and prolonged drop in nitrogen removal performance was only observed once between days 200 and 215 when the effluent TIN

[8] The price was converted to USD and the inflation from the year of original publication was taken into account.

concentration increased to about 18 mg L^{-1}. This was mainly due to operational problems with PN-MBBR reactor and subsequent limited nitrite supply to the AMX-IFAS reactor.

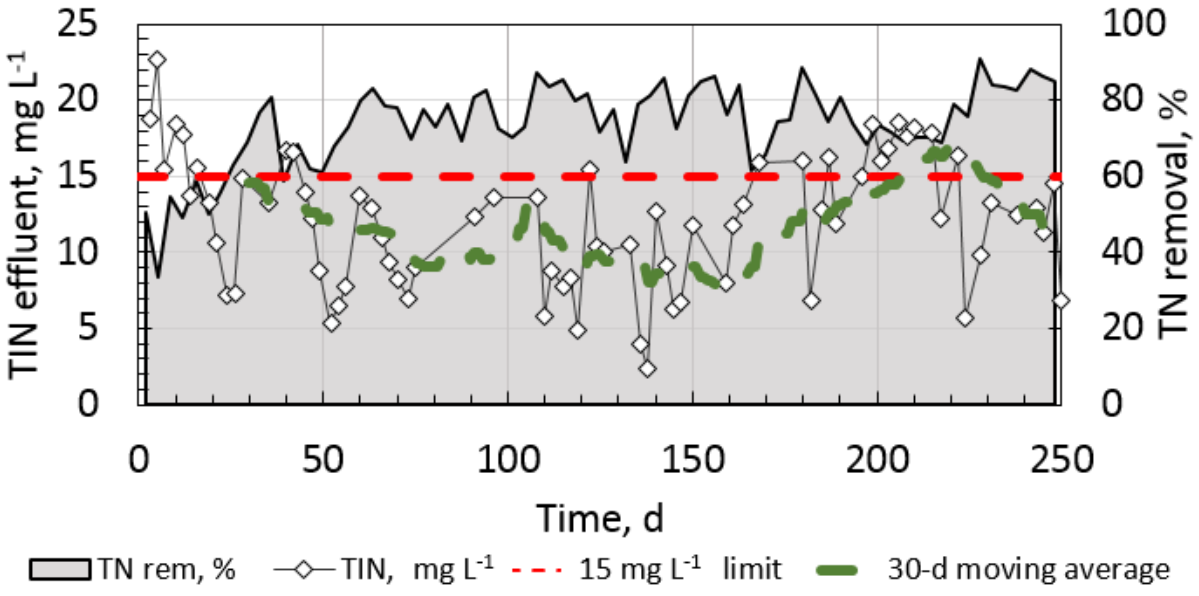

Figure 6-6 Combined performance of the two-stage partial nitritation/anammox configuration.

The average SNLR in AMX-IFAS reactor during the whole period was equal to 0.50±0.11 g-N m^{-2} d^{-1} at an average temperature of 19±3 °C. High quality final effluent was achieved with an average TIN concentration of 11.4±4.0 mg L^{-1}, COD of 62±26 mg L^{-1}, and TSS of 26±28 mg L^{-1}.

The average SNRR reached 0.45±0.10 g-N m^{-2} d^{-1} which is significantly higher than previously reported nitrogen removal rates in mainstream anammox MBBRs. Gilbert et al. (2014) showed a removal of only 0.01 g-N m^{-2} d^{-1} at 10°C. Malovanyy et al. (2015) reported surface removal rates of approximately 0.1 g-N m^{-2} d^{-1} at 25°C. They have also studied a one-stage IFAS reactor and reported approximately 3 times higher volumetric removal rates than observed in MBBR (also at 25°C). Most of the research to date, however, was focused on granular systems (see Table 6-1) rather than MBBRs or IFAS and comparison of volumetric nitrogen removal rates between the two is problematic. This is because in case of suspended growth reactors the removal rate is only a function of suspended sludge concentration and

biomass activity while in IFAS reactors; media filling fraction, specific surface area of the media and biofilm must be also taken into account. In the studied AMX-IFAS reactor a volumetric nitrogen removal rate of 55 g-N m^{-3} d^{-1} was observed. This value fits well within the range of values reported by Malovanyy et al. (2015) in their IFAS reactor, or by Laureni et al. (2016) in the SBBR who reported removal rates of 47 g-N m^{-3} d^{-1} at 15°C. Lotti et al. (2015) and Hoekstra et al. (2108) reported significantly higher removal rates in granular SBRs with rates ranging from 200 to 400 g-N m^{-3} d^{-1}. It is, however, important to consider that the volumetric removal rate in the studied AMX-IFAS reactor could be significantly increased just by using media with higher specific surface area. The observed thickness of the biofilm was very low during the whole period of the study and as such media with specific surface area of more than 1000 m^{-2} m^{-3} could be easily used without clogging issues. By doing so and keeping the same media fill in the reactor the volumetric removal rate could be almost tripled to approximately 0.15 kg-N m^{-3} d^{-1}. The reported nitrogen removal rates for mainstream PNA are therefore comparable with typical values achieved in conventional wastewater treatment employing nitrification-denitrification process (Lotti et al., 2014).

SRT in the AMX-IFAS reactor was controlled only during the first month of operation and then stopped. This was due to very low yields in both reactors as well as problems with the performance of the clarifier. Accumulation of suspended sludge in the bioreactor was very low and occasional floating sludge in the clarifier caused additional wash-out. From the second month forward the SRT was only controlled by a natural wash-out of biomass from the clarifier. This led to higher concentration of suspended sludge in the bioreactor. Based on average effluent solids, biofilm and suspended biomass the average operational SRT of the reactor was estimated at 24.1 days. The MLSS in the reactor varied significantly with an average concentration of 760±450 mg-TSS L^{-1} (see

Figure 6-7 for complete MLSS profile). Interestingly, there was no clear correlation between MLSS and effluent TIN concentration (R^2=0.0576).

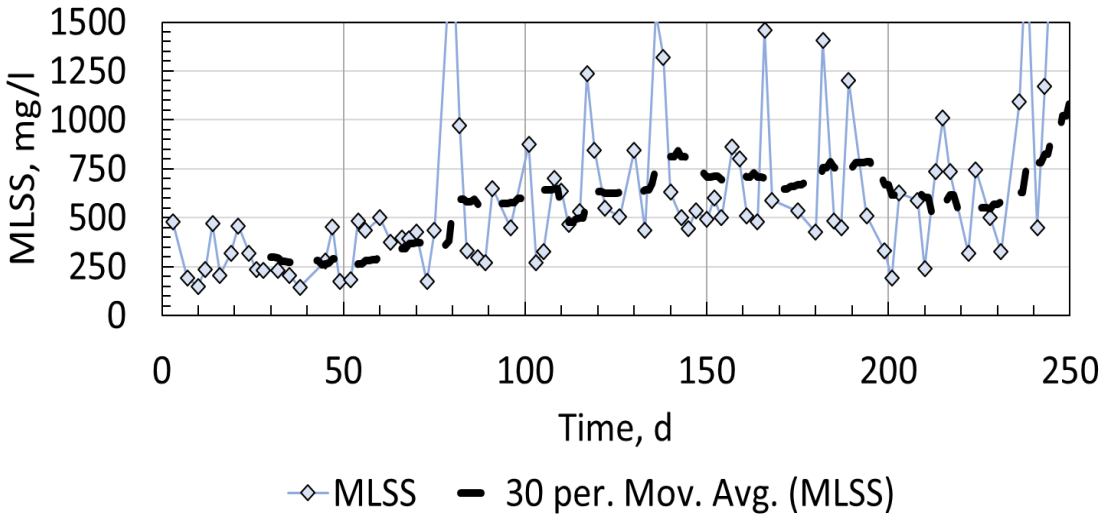

Figure 6-7 MLSS profile in the AMX-IFAS reactor.

The media in the bioreactor were periodically microscopically inspected and the biofilm thickness as well as biofilm solids were measured. Throughout the whole period of the study the biofilm remained very thin with a thickness of around 100-200 µm. The amount of biomass on the carriers equaled to 19±1 mg-TSS per piece. Both suspended sludge and biofilm appeared black or very dark in colour. This agrees with observations made by other authors and mainstream anammox studies.

Figure 6-8A-C shows TN, COD and ffCOD profiles between PE, effluent from PN-MBBR and effluent from AMX-IFAS as well as relative percent removals in PN-MBBR and total removals in the configuration. Cumulative nitrogen removal in the configuration reached 77±8 % with approximately 19±5 % being removed in the first stage (PN-MBBR). TN concentration was reduced from 54±9 to 13±4 mg-N L^{-1}. Approximately 40±5 % of COD and 65±16 % of ffCOD was removed in the first stage with total removals averaging 86±9 % and 81±7 %, respectively.

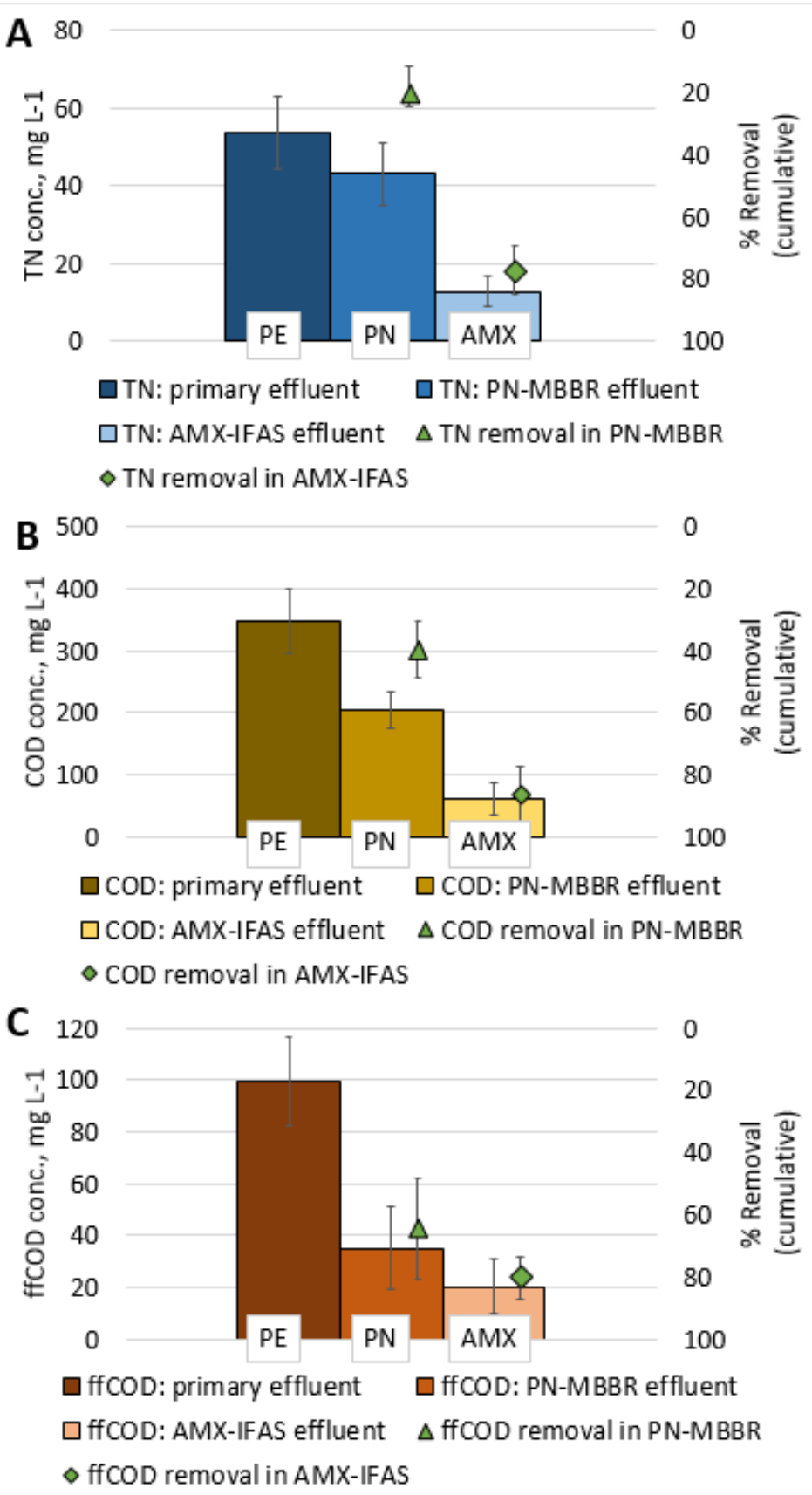

Figure 6-8 A – total nitrogen (TN) profile and percent removal within the configuration; B – chemical oxygen demand (COD) profile and percent removal within the configuration; C - filtered-flocculated COD (ffCOD) profile and percent removal within the configuration.

Table 6-1 Comparison of reported nitrogen removal rates and specific anammox activities in different mainstream partial nitritation-anammox configurations and temperatures.

Reactor type	Biomass type	Process	Wastewater type	Temp.	NRR	SpAA	Comment	Reference
MBBR	biofilm	PNA	Synthetic 50 mg N L^{-1}	20	NA	0.14-1 g-N kg-TSS^{-1} d^{-1}	NO2/NO3 accumulation	(Gilbert et al., 2014)
				10	5-11 mg-N m^{-2} d^{-1} 8-16 g-N m^3 d^{-1}			
SBR	granules	PNA	Synthetic NA	20	NA	0.1/0.8 g-N g-VSS^{-1} d^{-1}	Non-acclimated/acclimated	(Lotti et al., 2015)
				10		0/0.2 g-N g-VSS^{-1} d^{-1}		
Gas lift reactor with MBR module	granules	Anammox only	Synthetic 60 mg N L^{-1}	10	NA	39 mg-N g-VSS^{-1} d^{-1}	>300d acclimation	(Hendrickx et al., 2014)
MBBR	biofilm	PNA	Pretreated primary effluent 40 mg N L^{-1}	25	0.04-0.13 g-N m^{-2} d^{-1} 18 g-N m^{-3} d^{-1}	NA	NO3 overproduction	(Malovanyy et al., 2015b)
IFAS	biofilm	PNA	Pretreated primary effluent 40 mg N L^{-1}	25	55 g N m^3 d^{-1}	NA	NO3 overproduction	(Malovanyy et al., 2015a)
SBR	granules	Anammox only	Synthetic 40 mg N L^{-1}	13	30 g N m^3 d^{-1}	15.1 mg-N g-VSS^{-1} d^{-1}	Small granules decreased to 150 microns	(Sánchez Guillén et al., 2016)
RBC	biofilm	PNA	Pretreated primary effluent 30-60 mg N L^{-1}	15	435 g N m^3 d^{-1}	NA	NO2 and NO3 accumulation	(De Clippeleir et al., 2013)

SBR	granules	Anammox only	Synthetic 60 mg N L^{-1}	10	NA	92 mg-NH_4-N g-VSS^{-1} d^{-1}	High anammox activity at 10 °C	(De Cocker et al., 2018)
SBR	granules	PNA	Pretreated primary effluent 15-30 mg N L^{-1}	23.2	223 ± 29 g N m^3 d^{-1}	70 mg N g-VSS^{-1} d^{-1} (20°C)	Observed unstable removal in situ	(Hoekstra et al., 2018)
				13.4	97 ± 16 g N m^3 d^{-1}			
SBR	granules	PNA	Synthetic 60 mg N L^{-1}	10	90 g N m^3 d^{-1}	NA	Unstable operation at 10°C	(T Lotti et al., 2014)
				15	400 g N m^3 d^{-1}			
UASB	granules	Anammox only	Treated wastewater amended with nitrite 70 mg N L^{-1}	11	1.2 ± 0.5 kg N m^{-3} d^{-1}	70 mg N g-VSS^{-1} d^{-1}	High NRR due to high solids (VSS=17 g L^{-1})	(Reino et al., 2018)
SBR	biofilm	PNA	Pretreated municipal wastewater 21 mg N L^{-1}	15	30 g-N m^{-3} d^{-1}	103 ± 18 mg N L^{-1} d^{-1} (max anammox activity)	anammox activity suppression during operation at 11 °C.	(Laureni et al., 2016)
SBR	granules	Anammox only	Pretreated municipal wastewater amended with nitrite 20 mg N L^{-1}	12.5 °C	46 g N m−3·d−1	NA	Anammox growth was 2 to 3 times slower on pre-treated compared to synthetic wastewater.	(Laureni et al., 2015)
IFAS **40% fill**	**biofilm**	**Anammox and partial denitrification**	**Partially nitritated primary effluent;** **40 mg N L^{-1}**	**19**	**0.45 g-N m^{-2} d^{-1}** **55 g N m^{-3} d^{-1}**	**187±46 mg-N g-VSS^{-1} d^{-1}** **0.85 g-N m^{-2} d^{-1} (both at 20°C)**	**Dynamic online control of partial nitritation is required**	**This study**

6.4.4. SpAA batch tests

A set of ex-situ batch tests were run (after day 250) to measure specific anammox activity (SpAA) of the biofilm and suspended sludge as well as the specific surface anammox activity (SuAA) on the plastic media. The results (presented in Figure 6-9) showed that SpAA of the biofilm was equal to 187±46 mg-N g-VSS^{-1} d^{-1} which was more than 5 times higher than the anammox activity of the MLSS (only 34±6 mg-N g-VSS^{-1} d^{-1}). These results confirm previous observations that the MLSS concentration had relatively low influence on the nitrogen removal. SpAA observed in this study was much higher than the activity observed by Hoekstra et al. (2018) who reported specific anammox activity of 70 mg-N g-VSS^{-1} d^{-1} in a one-stage SBR reactor. Lott et al. (2015) showed an anammox activity as high as 800 mg-N g-VSS^{-1} d^{-1} at the same temperature (20°C). The reactor, however, treated synthetic wastewater and it has been shown that anammox growth is much lower on real wastewater than it is on a synthetic medium (Laureni et al., 2016).

The AMX-IFAS reactor had developed a SuAA of approximately 0.85±13 g-N m^{-2} d^{-1} which is noteworthy since the reactor was never seeded with plastic media containing anammox biomass but was only bioaugmented with waste suspended sludge from the sidestream MBBR. This means that anammox bacteria had successfully attached to the surface of the carriers. These observations prove that anammox biofilm can be grown successfully under mainstream conditions and on real wastewater. A supply of wastewater with adequate NO_2-N/NH_4-N ratio from PN-MBBR was crucial for a successful operation of the AMX-IFAS reactor and a cultivation of anammox biofilm.

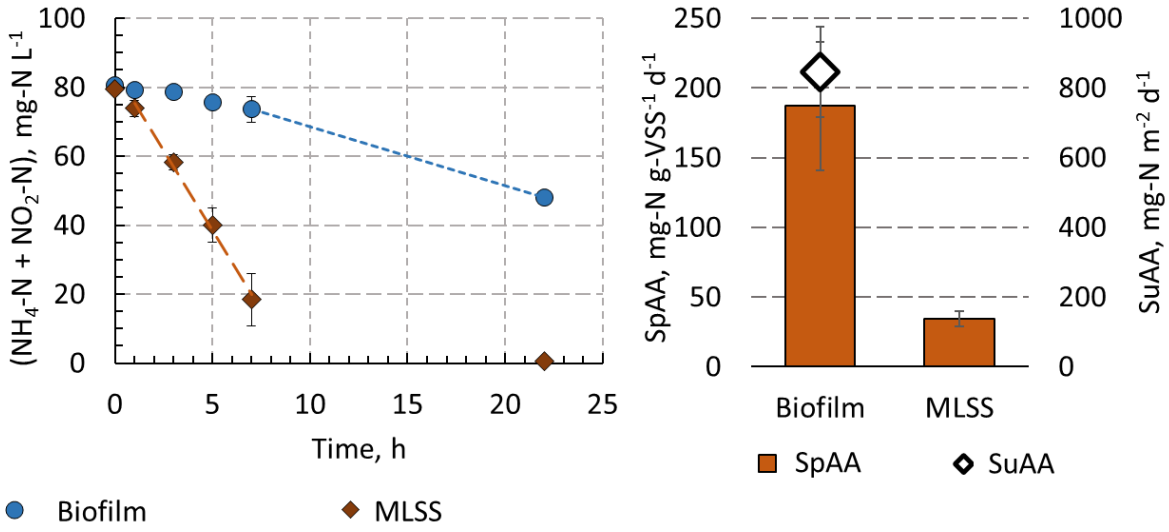

Figure 6-9 Left - Volumetric nitrogen consumption rates in biofilm and MLSS samples taken from AMX-IFAS reactor; Right – Specific anammox activity (SpAA) of the biofilm and MLSS as well as surface anammox activity (SuAA) of the biofilm. Rates and activities were measured in ex-situ batch tests at 20 °C. Error bars represent one standard deviation.

6.5. Partial nitritation in Biowin 5.3

Results presented in Chapter 4 showed that cold partial nitritation can be achieved only when DO/TAN ratio control is combined with inhibitory (for NOBs) concentration of FA achieved by artificially raised pH. When DO/TAN ratio was the only control parameter nitratation was uninhibited. A simulation has been set up based on Activated Sludge Model No. 3 (ASM3) default kinetic parameters in Biowin version 5.3 simulation software using the data gathered during a period when only DO/TAN was controlled. This was done in order to check if Biowin can correctly predict partial nitritation kinetic in a MBBR. The results showed (Table 6-2) that the model cannot correctly predict the fractionation of nitrogen in the effluent using default kinetic parameters for NOBs (μ_{max}=0.7 d^{-1} and K_{DO}=0.5 mg L^{-1}). In fact, under the studied conditions the model predicts no nitratation whatsoever with complete inhibition of NOB activity. This is in huge contrast with the observed data.

In order to achieve values comparable with the observed data the kinetic parameters of nitrite oxidizers had to be modified to match those of ammonium oxidizers (μ_{max}=0.9 d^{-1} and K_{DO}=0.25 mg L^{-1}).

Table 6-2 Comparison of kinetic parameters for AOB and NOB.

PARAMETER	UNITS	OBSERVED DATA	BIOWIN (DEFAULT)		BOWIN (FITTED)	
		Chapter 4	AOB	NOB	AOB	NOB
μ_{max} at 20°C	d^{-1}	-	0.9	0.7	0.9	0.9
θ	-	-	1.072	1.063	1.072	1.063
K_{DO}	mg L^{-1}	-	0.25	0.50	0.25	0.25
f_{NH4-N}	-	0.37±0.07	0.27		0.39	
f_{NO2-N}	-	0.02±0.01	0.73		0.01	
f_{NO3-N}	-	0.61±0.06	0.00		0.60	
DO/TAN	-	0.08	0.18		0.13	

Figure 6-10 presents a comparison of nitrogen fractionation between the observed data, default Biowin model and a model with corrected parameters.

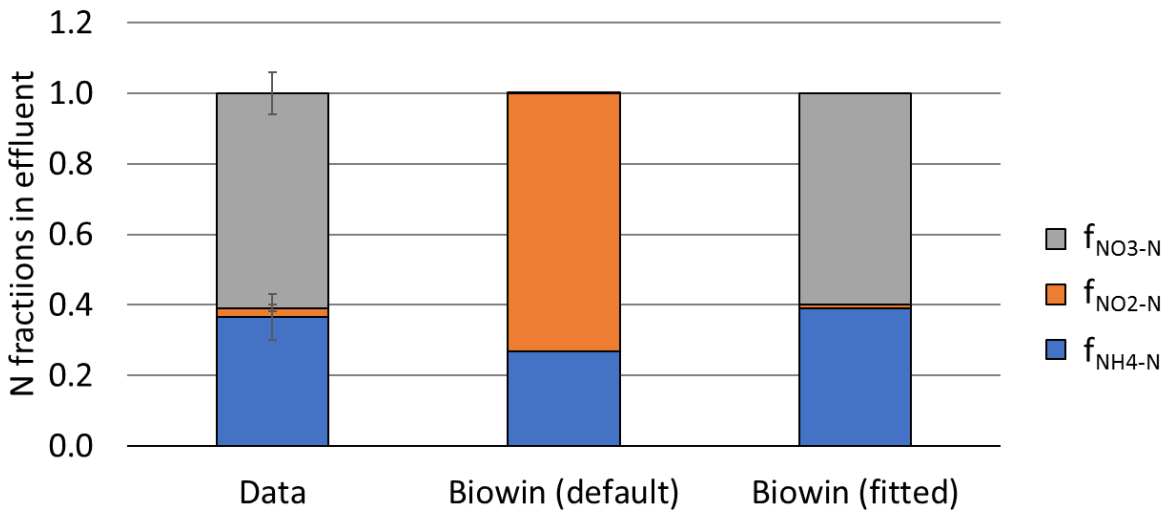

Figure 6-10 Fractionation of inorganic nitrogen species in the effluent from PN-MBBR (data from Chapter 4) vs default and fitted Biowin model.

6.6. Temperature effect on AMX conversion rates in AMX-IFAS

A set of ex-situ batch tests at different temperatures were run to measure specific anammox activity (SpAA) as well as the specific surface anammox activity (SuAA) on the plastic media in the AMX-IFAS reactor. The tests were run 40 days after the regular bioaugmentation of the reactor was stopped. The results (presented in Figure 6-11) showed that SpAA of the biofilm at 20°C was equal to 78±4 mg-N g-VSS^{-1} d^{-1} which was more than 2 times lower than the anammox activity measured during bioaugmentation period. These results confirm that bioaugmentation can be used to enhance anammox activity in a mainstream biofilm reactor relatively easy even if suspended biomass is used for bioaugmentation.

The results further revealed significant decrease in the anammox activity at lower temperatures (Figure 6-11). In fact, SpAA was 46% lower at 15°C (42±4 mg-N g-VSS^{-1} d^{-1}) and as much as 72% lower at 10°C (22±1 mg-N g-VSS^{-1} d^{-1}).

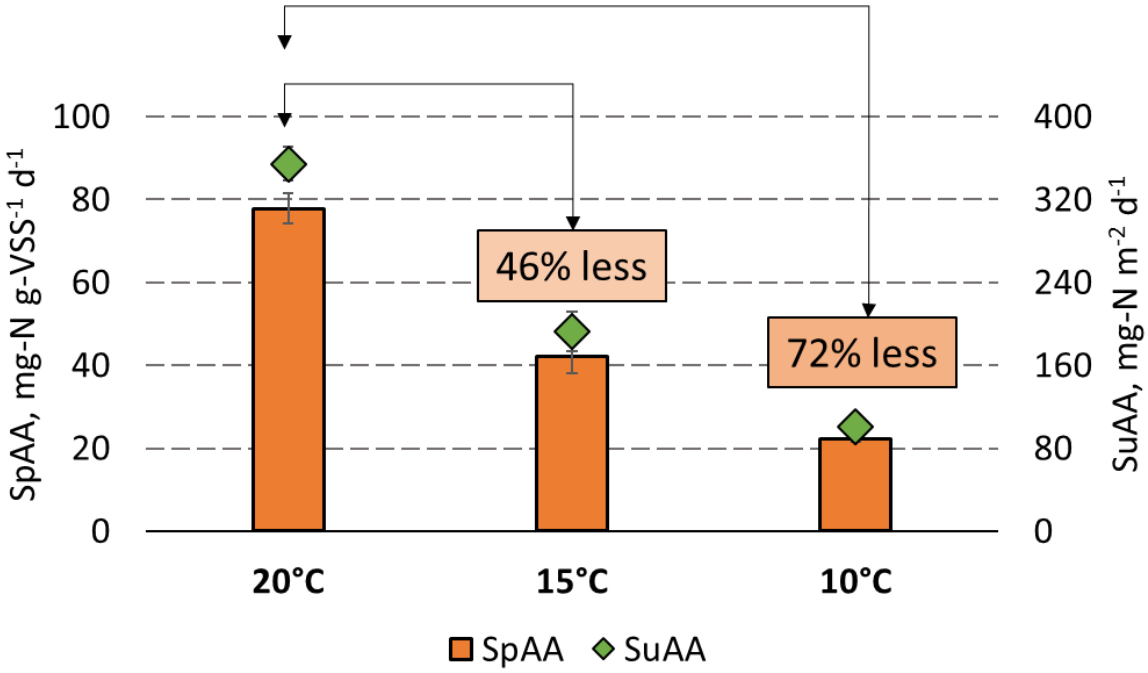

Figure 6-11 Specific anammox activity (SpAA) surface anammox activity (SuAA) of the biofilm. Rates and activities were measured in ex-situ batch tests at 20°C, 15°C and 10°C. Error bars represent one standard deviation.

On the basis of these results a temperature dependence of the anammox conversion rates was developed. The temperature-activity coefficient θ was estimated based on the slope of a linear regression of modified Arrhenius plot (ln(SpAA) vs temperature in °C) according to the equation:

$$\theta = e^{a}$$

where a is the slope of a linear regression.

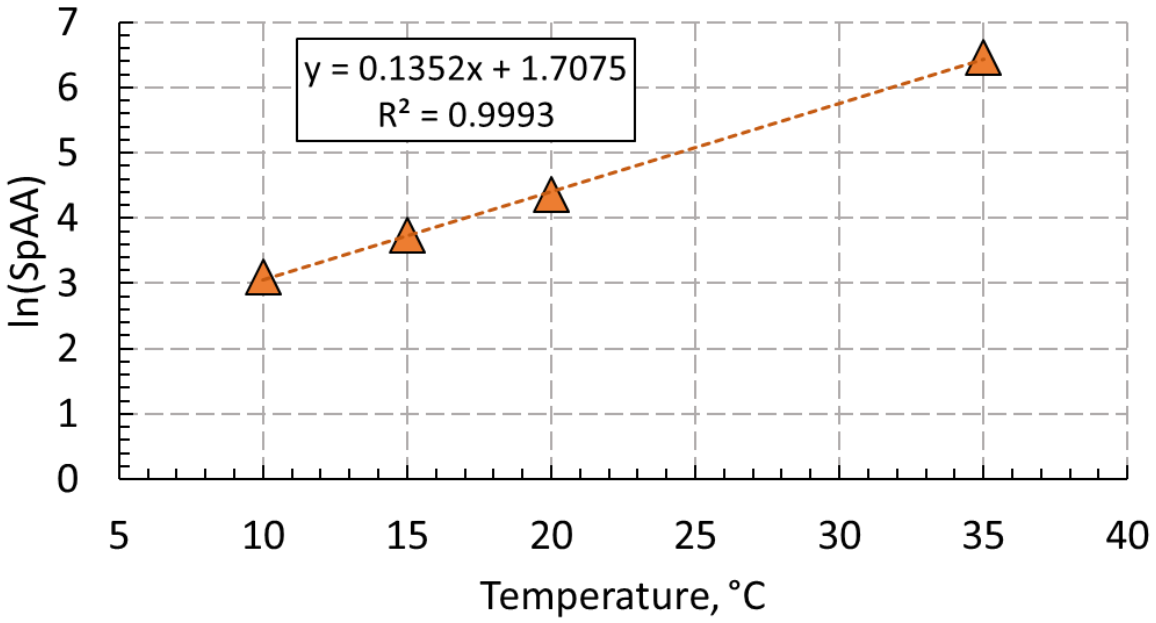

Figure 6-12 Modified Arrhenius Plot for the Specific Anammox Activity (SpAA) measured at different temperatures.

Distribution of the data in Figure 6-12 showed that the value of the coefficient was very constant throughout the tested temperature range. In fact, based on the best fit of linear regression, θ for temperature in the range of 10 to 35°C[9] was estimated at 1.145.

Figure 6-13 was prepared using θ of 1.145 to show van't Hoff-Arrhenius relationship between SpAA and temperature in the discussed range.

[9] SpAA at 35°C was measured using biomass from the sidestream MBBR (Chapter 5)

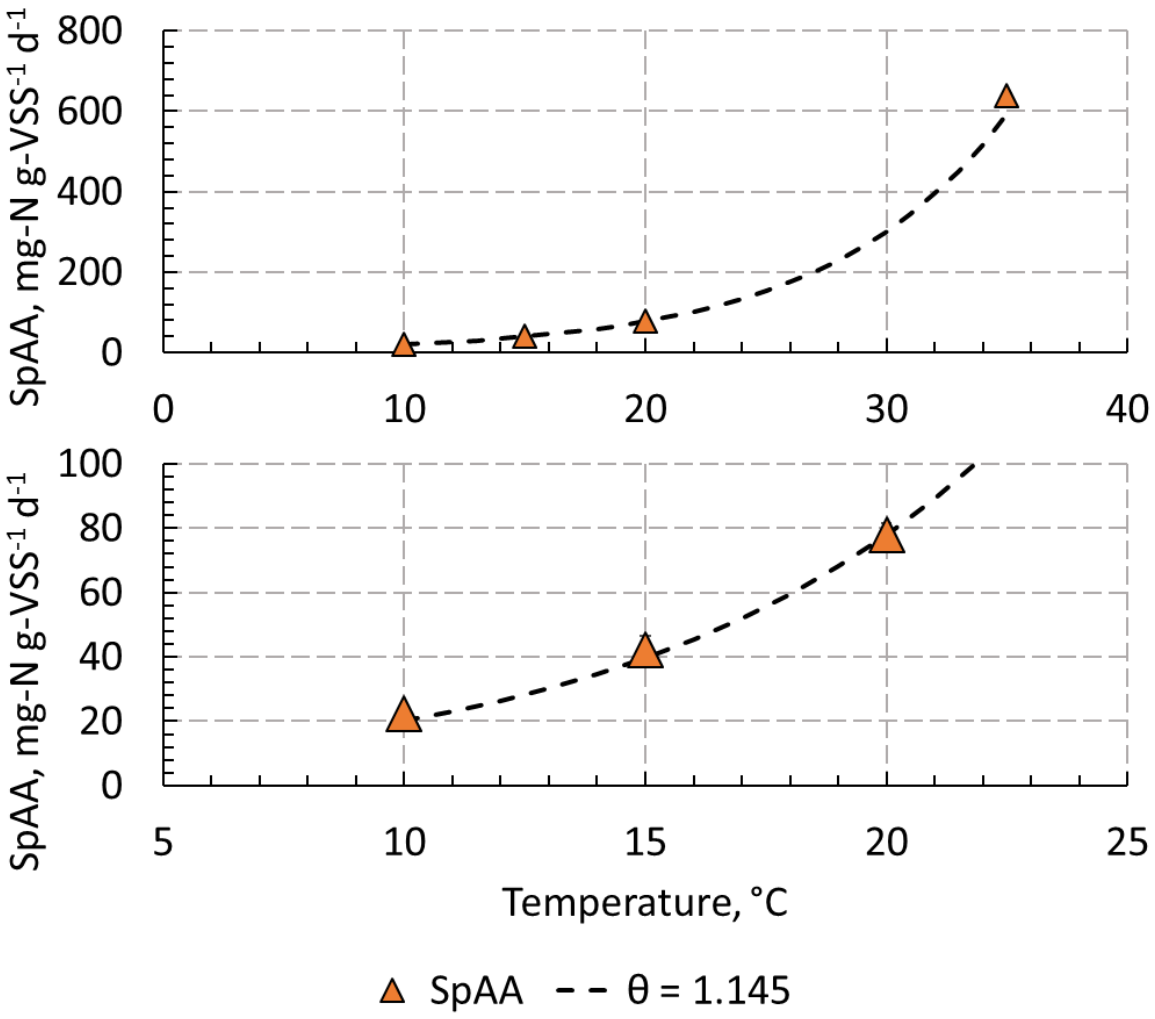

Figure 6-13 Specific Anammox Activity (SpAA) versus Temperature.

6.7. AOB, NOB and AnAOB growth rates

Finally, growth rates of AOB, NOB and AnAOB have been modeled based on kinetic coefficients elucidated in the previous subchapters (Figure 6-14). The maximum growth rates of AOB and NOB at 20°C were assumed the same at 0.9 d^{-1} with θ values of 1.072 and 1.063, respectively (based on modelling results from subchapter 6.5). What it means is that AOB growth rates increase with temperature at a higher rate than the growth rates of NOB. That kinetic advantage over NOB is used in sidestream PNA reactors to selectively wash-out NOB by aggressive SRT control. On

the other hand, however, NOB have a kinetic advantage over AOB in a temperature range below 20°C, and this is what makes mainstream partial nitritation impossible to control just by using SRT.

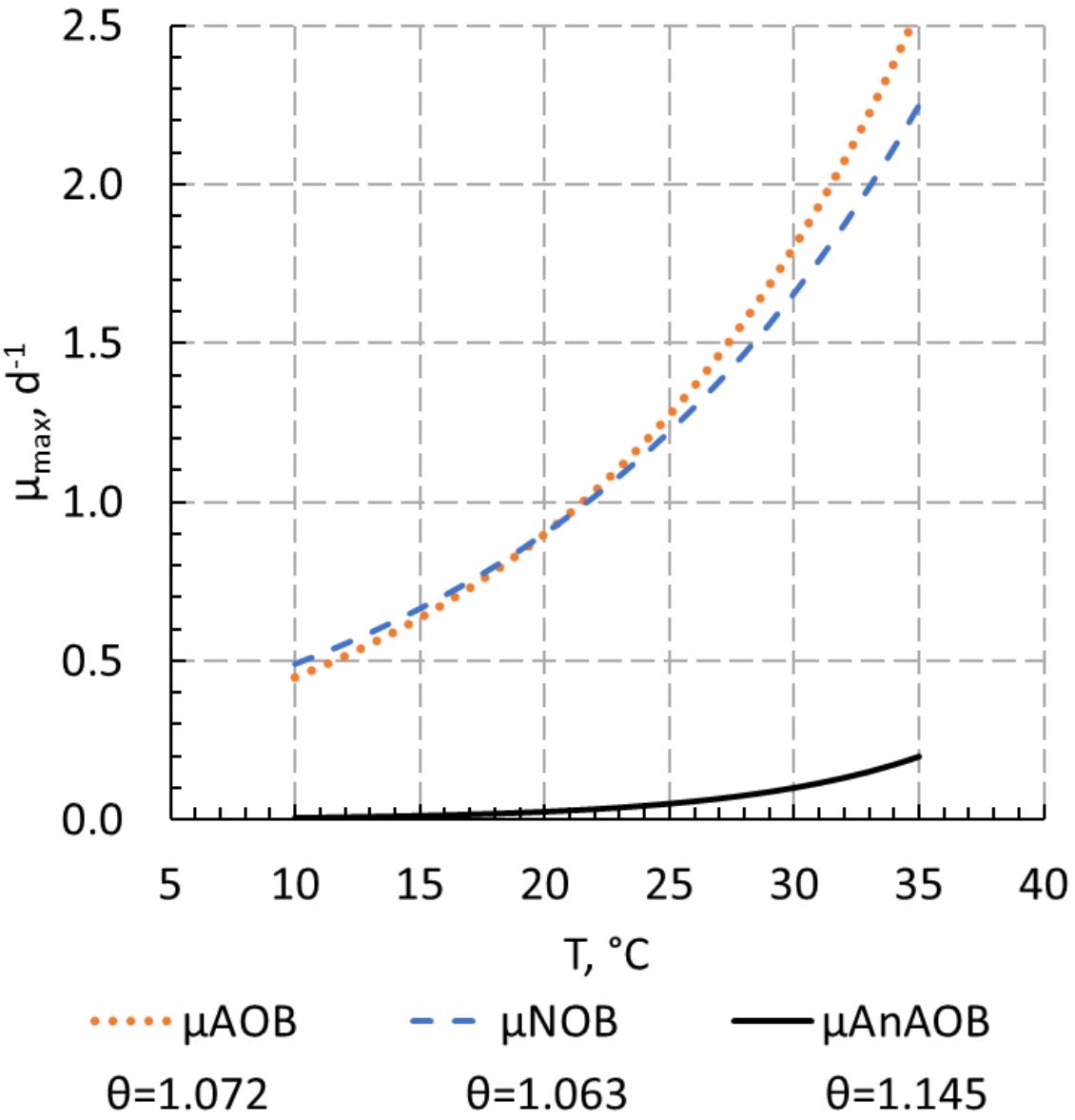

Figure 6-14 Maximum specific growth rates of ammonium and nitrite oxidizers, and anammox bacteria versus temperature.

6.8. PN-MBBR operational parameters

Figure 6-15A was developed based on the operation of the mainstream PN-MBBR. Values of the two control parameters (DO/TAN ratio and FA) collected during 250 days of reactor operation were plotted against observed nitrite concentrations in the effluent and showed as bubbles (the bigger the bubble the higher the concentration). The data was further divided into two groups, first group presents nitrite concentrations higher than 10 mg L^{-1} (green bubbles) and second group shows nitrite concentrations lower than 10 mg L^{-1} (red bubbles). It was observed that at least 10 mg NO_2-N L^{-1} was necessary to guarantee good performance of the AMX-IFAS reactor. It can be seen in Figure 6-15A that the bubbles form a specific distribution pattern on the graph and separation of green and red bubbles can be observed. In fact, red bubbles tend to appear above the line that the green bubbles form. This suggests that the main cause of low nitrite concentration in PN-MBBR was an elevated ratio of DO/TAN.

In order to define optimum values for DO/TAN and FA parameters and the relationship between them, the data was filtered (by removing red bubbles and outliers) and Figure 6-15B was prepared. Data shows strong correlation between DO/TAN, FA and nitrite production in the PN-MBBR. In fact, the relation can be modeled by a negative exponential function with a high correlation factor (R^2=0.7563). This relationship could be used to optimize process control strategy and could help develop an automatic online control system.

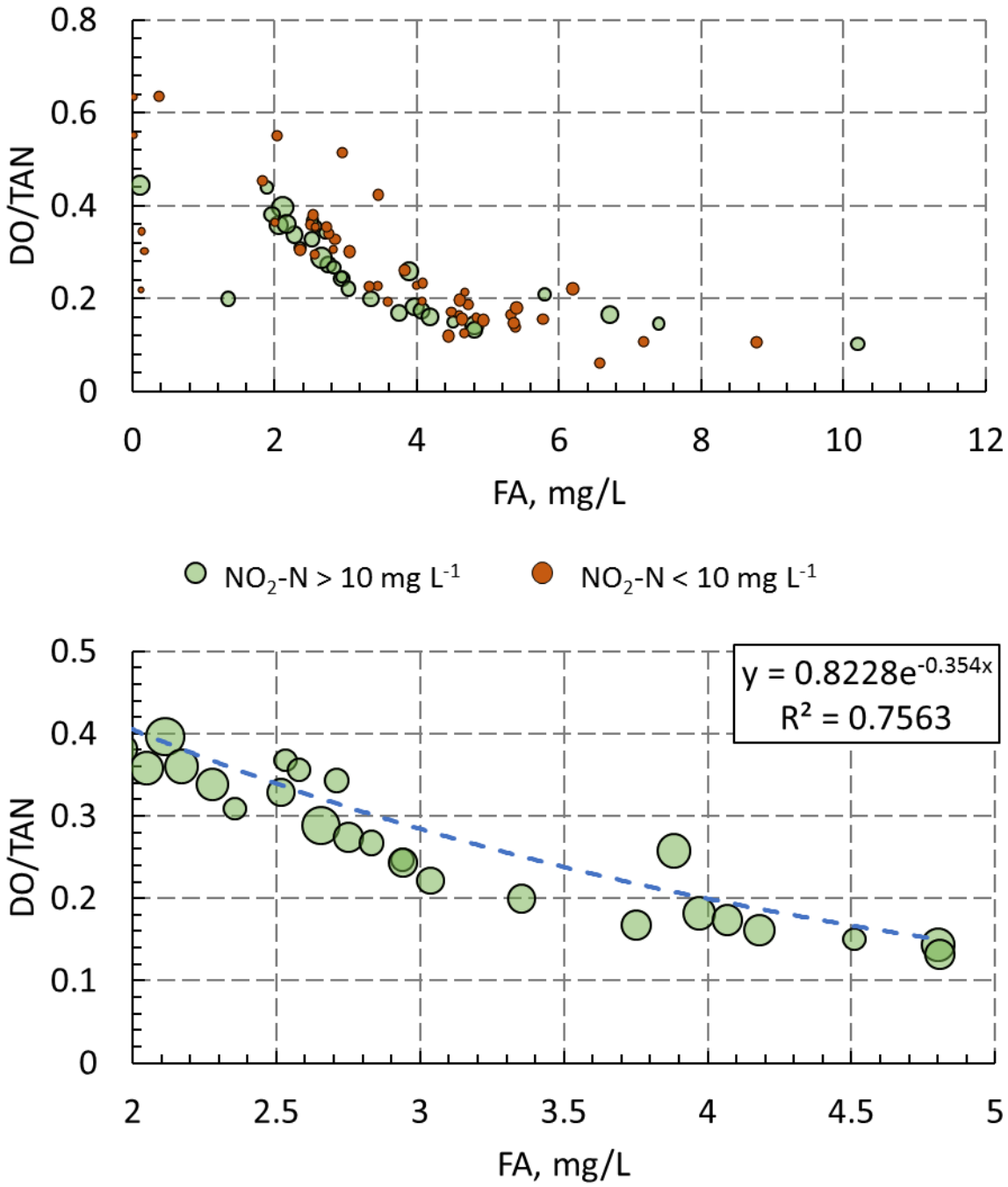

Figure 6-15 A - Distribution of the partial nitritation control parameters (DO/TAN and FA) vs nitrite concentration. Nitrite concentrations higher than 10 mg L^{-1} are represented by green bubbles and nitrite concentrations lower than 10 mg L^{-1} are represented by red bubbles. B – filtered control parameters data representing the optimum operational correlation between DO/TAN and FA.

6.9. Conclusions

A two-stage approach of fixed film partial nitritation and anammox process in the mainstream treatment train has been shown effective on primary effluent wastewater. The proposed configuration comprised of partial nitritation-MBBR and anammox-IFAS has achieved nitrogen removal rates comparable with conventional mainstream TN removal treatment. PN-MBBR achieved partial nitritation at 2.0±0.3 g-N m^{-2} d^{-1} and nitrogen removal reached 0.45±0.1 g-N m^{-2} d^{-1} (55 g-N m^{-3} d^{-1}) in the AMX-IFAS reactor. The process performed well at 19±3 °C with an average effluent TIN concentration of 11±4 mg L^{-1}. The temperature-activity coefficient θ for anammox was estimated at 1.145 based on kinetic tests in the temperature range of 10 to 35°C.

Combining partial nitritation with carbon removal in one MBBR reactor was successful and created significant advantages including high carbon removal and nitritation rates, as well as relatively low sludge production. Partial nitritation was modelled in Biowin and the results showed that to correctly predict the fractionation of nitrogen in the effluent the kinetic parameters for NOBs (μ_{max}=0.7 d^{-1} and K_{DO}=0.5 mg L^{-1}) have to be modified to match those of ammonium oxidizers (μ_{max}=0.9 d^{-1} and K_{DO}=0.25 mg L^{-1}). Maintaining partial nitritation by manually controlling the level of un-ionized ammonia was feasible in the MBBR, however, achieving long-term stable operation would require a dynamic on-line control system. It was found that the relation between DO/TAN and FA parameters can be modeled by a negative exponential function and this relationship could be used to optimize process control strategy and could help develop an automatic online control system.

6.10. Acknowledgements

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7. Chapter 7: Overview and Conclusions

7.1. Final summary

The primary objective of the thesis was to adapt partial nitritation and anammox technology to the challenges of mainstream municipal wastewater treatment. Issues resulting from low temperature, low nitrogen concentration, slow growth and relatively low anammox activity as well as extreme difficulty in controlling partial nitritation under these conditions had to be effectively solved. As a result, a novel two-stage biofilm-based partial nitritation-anammox technology was developed. To meet this goal new methods to increase the attachment rate of anammox bacteria to the biocarriers and rapid anammox MBBR start-up methods were developed in Chapters 2 and 3.

In Chapter 2 a pre-treatment technique was developed in which a layer of fast-growing heterotrophs was cultivated on the media prior to inoculation of the reactor with anammox. Results obtained from the study showed great potential of the developed pre-treatment method in significantly reducing the start-up times of biofilm anammox reactors. Anammox activity was four times higher in the reactor with media that possessed the bioprimer before inoculation as compared to the control. This biofilm acted as a scaffold with EPS matrix that allowed for a very quick attachment of anammox bacteria. This study demonstrated for the first time the ground-breaking advantages of the bioprimer for rapid development of anammox biofilms.

In Chapter 3 it was shown that the start-up of anammox MBBR reactors can be easily achieved by inoculating with waste sludge from granular systems like DEMON® reactors. More than 80% TN removal was achieved in approximately 50 days. This study shows that granular biomass can be transitioned to a biofilm relatively easily which opens a new window of opportunity for starting-up anammox MBBRs or effectively bio-augmenting. Most importantly, granular sludge from

DEMON® reactors is more readily available than seed carriers and the start-up of biofilm reactors does not necessitate the use of proprietary seed carriers from biofarms.

It was also necessary to develop a novel control method for cold partial nitritation suited for biofilm reactors (Chapter 4). Partial nitritation at 10°C, low nitrogen concentration and high COD/N ratio (>2) has been shown to be feasible. Mainstream partial nitritation was achieved by combining two control strategies. The target level of ammonium oxidation was achieved by controlling DO/TAN ratio and nitritation was obtained by FA inhibition of NOBs at high pH. Long term stability was successfully obtained at DO/TAN ratio of 0.06 and at FA concentration of 1.1±0.2 mg NH_3-N L^{-1}. NOB did not acclimate to FA during 40 days of steady state operation and were effectively inhibited. An algorithm for dynamic process control of partial nitritation has been also developed.

Additionally, a sidestream PNA MBBR reactor treating real centrate was required to be operated in order to farm anammox biomass used to bio-augment mainstream reactor (Chapter 5). Pilot partial nitritation-anammox MBBR treating centrate from a full-scale dewatering facility was successfully started-up and operated for 475 days. Development of the anammox process and the performance of the rector was closely monitored. Very fast start-up of partial nitritation-anammox process could be achieved by incorporating previously suggested bioprimer technique. SNRR of 2.5 g-N m^{-2} d^{-1} was achieved in as little as 56 days. Moreover, FA was found to be the predominant inhibitor of NOB activity rather than selective wash-out of nitrifying biomass. A minimum FA concentration of 2 mg NH_3-N L^{-1} was suggested for optimal performance of partial nitritation and to prevent a build-up of nitrate.

Finally, a mainstream PNA reactor incorporating all previous techniques was developed. The proposed configuration comprised of partial nitritation-MBBR and anammox-IFAS reactor has achieved nitrogen removal rates comparable with conventional mainstream TN removal treatment

(Chapter 6). A two-stage approach of fixed film partial nitritation and anammox process in the mainstream treatment train has been shown effective on primary effluent wastewater. PN-MBBR achieved partial nitritation at 2.0±0.3 g-N m^{-2} d^{-1} and nitrogen removal reached 0.45±0.1 g-N m^{-2} d^{-1} in the AMX-IFAS reactor. The process performed well at 19±3 °C with an average effluent TIN concentration of 11±4 mg L^{-1}. Moreover, combining partial nitritation with carbon removal in one MBBR reactor was successful and created significant advantages including high carbon removal and nitritation rates, as well as relatively low sludge production. Maintaining partial nitritation by manually controlling the level of un-ionized ammonia was feasible in the MBBR, however, achieving long-term stable operation would require a dynamic on-line control system.

7.2. Engineering significance

Throughout the course of this work a vast operational experience and process design knowledge was gained from long term operation of the pilot scale reactors that will significantly contribute to the development of new and improved deammonification technologies.

7.2.1. Sidestream PNA

Factors governing successful start-up of sidestream PNA MBBR reactors were identified. A start-up procedure was developed which if implemented in full-scale has a potential to significantly reduce the start-up time of anammox MBBRs. Furthermore, conditions that promote high performance and stable operation of PNA process treating real centrate were determined. Ultimately, biofilm-based PNA process was proven to be feasible and reliable in cost-effectively treating anaerobically digested sludge dewatering centrate from Winnipeg's North End Water Pollution Control Centre (MB, Canada).

Based on the gathered data, fast and successful start-up of sidestream PNA MBBR can be achieved following few simple steps:

1) Start-up of partial-nitritation process and cultivation of a bioprimer. Establishing steady state nitritation.
2) Wash-out of the reactor with process water to reduce the concentration of FA and FNA immediately prior to inoculation with anammox biomass.
3) Anammox inoculation.
4) Stepwise increase of the nitrogen loading based on the observed removal rates.
5) Reaching design nitrogen loading rate and completion of the start-up.

The above procedure guarantees rapid start-up of anammox MBBR that could be achieved in as little as 1 month.

Moreover, in order to achieve and sustain stable performance of sidestream PNA MBBR reactor the following operational conditions were identified:

1) appropriate FA ammonia concentration was crucial to the successful control of partial nitritation. Moreover, FA was found to be the predominant inhibitor of NOB activity rather than selective wash-out of nitrifying biomass. A minimum FA concentration of 2 mg NH_3-N L^{-1} is suggested for optimal performance of partial nitritation and to prevent a build-up of nitrate.
2) pH should be therefore appropriately controlled based on TAN concentration in the reactor. It is, therefore, a recommendation of this work to install pH set-points for the stable performance of deammonification reactors.

3) Anammox bacteria can be effectively protected from process perturbations and inhibitors by outer layers of heterotrophic/nitrifying biofilm.

4) Stratification of the biofilm is governed by the DO concentration in the bioreactor. Higher DO levels enhance biofilm stratification while lower DO levels shift heterotrophs/nitrifiers into suspension.

5) Microbial diversity positively effected the general nitrogen removal performance and can enhance nitrogen removal beyond the theoretical anammox removal threshold due to partial denitrification. DO set-point in the reactor and the thickness of the biofilm play crucial role in this phenomenon.

7.2.2. Mainstream PNA

Feasibility of a two-stage configuration for mainstream PNA was studied in a pilot PN-MBBR/AMX-IFAS system treating real wastewater. Long-term (app. 1 year) operation revealed clear advantage of the proposed reactor configuration as well as identified aspects that need further development.

The advantages of the piloted mainstream PNA technology include:

1) One sludge system. There is no need for high-rate carbon removal activated sludge process prior to PNA in order to pre-treat the primary effluent wastewater and remove carbon.

2) Carbon removal pre-treatment is combined with partial nitritation. The proposed high-rate MBBR with the DO/TAN and FA based process control effectively removes carbon and successfully produces nitrite for anammox.

3) High pH significantly lowers the observed sludge yield during the treatment ultimately producing low sludge quantities.

4) High pH also helps with biofilm control. The morphology of the biofilm is denser and more compact at these conditions which prevents clogging even at high organic loading rates.

5) High DO setpoint allows for high nitrification rates and high surface loading rates hence compact reactor design.

6) Lack of solids separation unit between PN-MBBR and AMX-IFAS, instead the solids from the first stage serve as a source of slowly hydrolysable BOD for partial denitrification in the AMX reactor. Partial denitrification enhances nitrogen removal by providing additional nitrite for anammox.

7) Ideally, AMX-IFAS would be fully anoxic with no aeration requirements.

In the face of all the benefits listed above, there is only one significant drawback associated with the piloted partial nitritation control system. The high pH setpoint requirement creates a need for external dosing of alkali solution to artificially increase pH. This creates additional costs associated with operation and maintenance of the chemical dosing system. The proposed method should be, however, still economically viable considering the inherent benefits of PNA process and advantages of the piloted configuration.

All in all, this work will allow to design PNA reactors with confidence, knowing the parameters that make the process work. The research provides information relevant to rapid start-up of PNA systems, as well as other autotrophic processes. Furthermore, a novel control for NOB out-selection was developed. The Canadian economy will benefit in the short-term as utilities will be moving towards adopting the lower cost, more effective and efficient nitrogen removal PNA technology; easing the burden on the taxpayer.

7.3. New research directions

The experimental work completed during research as well as extensive literature review revealed the need to proceed with further investigations in the following areas:

1) Optimization of alkali dosing. The potential of minimizing alkali dosing should be explored, and the effects of semi-continuous dosing should be investigated. If the activity of NOB is effectively inhibited by FA, there should be a delay between pausing of alkali dosing and the recovery of the NOB activity. This window could be used to minimize the chemical requirements.

2) Identification of N_2O emissions. Greenhouse gas emissions have become a new concern in wastewater treatment. N_2O is both a by-product and an intermediate during nitritation with greenhouse gas potential 300 times higher than CO_2. The level of N_2O emissions from the proposed configuration should be measured and compared with traditional treatment methods.

3) Development of dynamic online control system with predictive algorithms. Dynamic online control system for partial nitritation has been proposed with a novel control algorithm. The system should be tested and further refined.

4) Determination of the effects of shock temperature drops below 15 °C and prolonged operation at 15 °C on the performance of AMX-IFAS. Performance of the anammox process in the AMX-IFAS reactor should be further assessed at temperatures lower than studied so far.

5) Metagenomic study of microbial communities in high PN-MBRR and AMX-IFAS. The characterisation of the microbial communities in the PN-MBBR may provide new

information on the speciation of AOBs under high pH conditions and show the relative abundance of NOB or comammox bacteria. In case of AMX-IFAS the dominant anammox species would be identified and potentially the degree of partial denitrification could be assessed on total nitrogen removal performance.

6) Integration of EBPR into the system. Phosphorus removal is a necessary part of the modern wastewater treatment train and must be implemented in some form to the proposed treatment train in order to meet P discharge limits. EBPR would be a very good fit because rbCOD in PE could be easily utilized to fuel the process. This would, however, necessitate a two-sludge system which would complicate the configuration. CEPT, on the other hand, is a simpler solution but requires chemicals. The trade-offs between the two options should be assessed.

7) Full-scale verification of the proposed wastewater treatment technology. Ultimately, full-scale pilot would be necessary in order to asses the feasibility of the process under real conditions and to asses impacts of scalability and dynamic wastewater flows and loads on the process control.

