The Influence of Nitrite and Free Ammonia on Nitrogen Removal Rates in Anoxic Ammonium Oxidation Reactors

by

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A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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Winnipeg

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Abstract

This research focuses on anoxic ammonium oxidation (anammox). The anammox process for treating high ammonium and low organic carbon wastewater can reduce operational costs to a greater extent than the conventional autotrophic/heterotrophic treatment process can.

The process has been widely researched because of its potential economic benefits. However, during long-term reactor operation, sudden reductions of nitrogen removal rates have been reported; maximum nitrogen removal rates in different reactor configurations could not approach values predicted based on mathematical modeling; and the crucial stability parameter, such as nitrite, did not have defined threshold concentration. It was hypothesised that free ammonia (FA) increase is the precursor of the instability of the anammox reactor. If it is true that nitrite up to about 200 mg N/L should stimulate nitrogen removal rate inside of the anammox reactor, when FA is kept below the inhibition threshold concentration. The research presented in the thesis argues that FA plays a larger role than has been previously considered in the instability of the anammox reactor.

This study found FA inhibited nitrogen removal rates (NRR) at concentrations exceeding 2 mg N/L. In the pH range 7 to 8, the decrease in anammox activity was independent of pH and related only to the concentration of FA. Nitrite concentrations of up to 200 mg N/L did not negatively affect nitrogen removal rate. This study further found that low nitrite provided stable anammox

reactor performance, but that high nitrite was not necessarily the cause for reactor destabilization.

During the research high nitrogen removal rate was achieved when low FA was provided. During regular reactor operation at pH 6.5, the NRR at about 6.2 g N/Ld was archived. This value was never achieved before till this study was conducted. Conducted research showed controlling FA at low level is required to approach high rates in anammox reactors. Achieving high rates in anammox reactors allow significant reduction in reactor volume which saves resources.

Further studies will be required to identify the FA effect on different microbial interactions, and that may provide more in-depth understanding of the nitrite and FA effect than observations based on NRR alone.

It is not important to be altogether in the right which is depended on assumptions,

however pursuing the Truth is the ultimate reason of the research

Lukasz Jaroszynski

This thesis is dedicated to:

My mother, Katarzyna, my wonderful mom always loving and believing in me

My father, Tymoteusz, my great teacher of the research

My wife, Fatima, for her love and companionship

And my beloved children, Michalina and Patryk

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Nomenclature and abbreviations

Anammox activity – This refers to the nitrogen conversion according to anammox stoichiometry via the anammox consortium, which is assumed to be done mostly by anammox organisms under the assistance of other microorganisms such as oxygen utilisers. In this study and in the vast majority of other anammox studies, anammox activity is related to the nitrogen removal rate or gas production rate when the ammonium is converted into dinitrogen gas, along with nitrite removed and nitrate produced according to anammox stoichiometry.

Anammox stoichiometry – the overall nitrogen removal balance at the ratio of [NH_4-N conversion: NO_2-N conversion: NO_3-N production] signifying [1:(1.32):(0.26)], respectively. This stoichiometric ratio comes from the first published anammox study and it has been accepted in all the literature as typical for the anammox consortium.

FA – free ammonia, un-ionized form of ammonium

FA inhibition threshold concentration - the FA concentration above which the anammox activity is hindered

Gas production rate (GPR) –the amount of gas produced over a specific time in a testing reactor. GPR can represent anammox activity based on the gas produced when nitrogen was removed according to anammox stoichiometry.

MBBR – moving bed biofilm reactor

Michaelis-Menten NRR – It was observed that nitrite stimulated the NRR under low FA concentrations, and the Michaelis-Menten equation can describe the relation between the NRR and nitrite under non limiting TA concentrations. Therefore, it is possible to calculate Michaelis-Menten NRR based on the actual nitrite concentration inside of anammox MBBRs and the estimated saturation function kinetic parameters such as max NRR and $K_{NO2\text{-}N}$.

Nitrogen removal rate (NRR) – This is the amount of nitrogen removed per day and per unit volume inside of the anammox reactor. In this study, it was assumed that the major mechanism involved in nitrogen removal was due to anammox activity. This parameter, the NRR, was used mostly for MBBRs stability analysis under variable pH, TA, FA and nitrite conditions.

Specific nitrogen removal rate (sNRR) – This is the amount of nitrogen removed per day and per unit volatile suspended mass of solids inside of the anammox reactor. In this study, it was assumed that the major mechanism involved in nitrogen removal was due to anammox activity. This parameter, the sNRR, was used mostly for SBR stability analysis under variable pH, TA, FA and nitrite conditions.

NTC – nitrite inhibition threshold concentration, the nitrite concentration above which the anammox activity is hindered

Stable reactor operation condition – Steady state anammox reactor operation was assumed when the NRR varied no more than 10% under a constant nitrogen loading rate. Additionally, NRR, which was achieved inside of the reactor and based on the mass balance, was compared with Michaelis-Menten NRR for the actual nitrite concentration inside of the reactor. The performance of the reactor was considered stable when the difference between NRR and Michaelis-Menten NRR was below 10%.

Semi-continuous fed SBR – This refers to the sequential performance of the reactor where, instead of batch feed mode (classical sequential batch reactor – SBR), the feed was introduced inside of the reactor over either all or part of the reaction phase.

TA – total ammonia. TA is also termed “ammonium.”

Acknowledgements

I would like to thank prof Jan Oleszkiewicz for giving me the opportunity to do Ph.D. research in Canada, and reviewing this thesis.

I would like to express my appreciations to academic staff, professors and students for a friendly environment and help during my work: Dominika Celmer-Repin, Jiazhong Chen, prof Nazim Cicek, Damian Kruk and his wife Karolina, Jong Hyuk Hwang, Kerry, Stanislaw Lozecznik, Donna Parkhurst, Bartek Puchajda, Jastin Rak-Banvill, Bob Romance, prof Richard Sparling, Nicholas Szoke, Arman Vahedi, Victor Wei, Connie Wenzoski, Qiuyan Yuan, Teresa Zolyniak, and many others.

I would like to thank to my family for supporting me during my studies. Thank you Fatima, Dad and Mom, grandfather Adam, Mateusz, Agnieszka and Jakub.

The work was funded by a Discovery Grant from Natural Sciences and Engineering Research Council (NSERC) of Canada, a joint grant from Sustainable Development Innovations Fund of the Manitoba Department of Conservation and the City of Winnipeg's Water and Waste Department (WWD) to J A Oleszkiewicz. The support from the staff at WWD and the North End Water Pollution Control Center in Winnipeg are gratefully acknowledged.

INTRODUCTION

Conventional biological nitrogen removal has been widely applied in full scale reactor operations in different configurations all over the world. In 1995, Mulder et al. (1995) discovered a new pathway: anoxic ammonium oxidation (anammox) for transforming ammonium to dinitrogen gas. Intensive research followed leading to full scale process applications (Wett, 2006; van der Star et al., 2007). A list of different process names in various configurations is presented in Table 1.

Most of the research on anammox has been conducted either in Europe or in Asia. Some research has been conducted in the USA (Musabyimana et al., 2008) with very limited research in Canada (Kosari, 2011); the present research was the first one on this topic at the time it was started. There are three wastewater treatment plants (WWTP) in Winnipeg, Canada. One of them, the North End Water Pollution Control Centre (NEWPCC) facility, has been equipped with two SBR reactors for nitrogen removal from anaerobic digestion reject waters (centrate). Currently, the conventional nitrification-denitrification process is used with significant use of chemicals: methanol and alkalinity. The present research was also targeting the application of alternative technology such as anammox process for treating raw centrate.

Table 1 Process configurations for anammox process (acc. to van der Star et al., 2007)

Configuration	Original name	Reference
Two reactors	SHARON-ANAMMOX[1]	van Dongen et al., 2001
	Two stage OLAND[2]	Wyffels et al., 2004
	Two stage deammonification	Trela et al., 2004
One reactor	Aerobic deammonification	Hippen et al., 1997
	OLAND	Kuai et al., 1998
	CANON[3]	Third et al., 2001
	Aerobic/anoxic deammonification	Hippen et al., 2001
	Deammonification	Seyfried et al., 2001
	SNAD[4]	Lan et al., 2011
	DEMON[5]	Wett 2006
	DIB[6]	Ladiges et al., 2006

[1] *High Ammonia Removal Over Nitrite – Anaerobic AMMonium Oxidation*

[2] *Oxygen Limited Autotrophic Nitrification Denitrification*

[3] *Completely Autotrophic Nitrogen removal Over Nitrite*

[4] *Simultaneous partial nitrification, anammox and denitrification*

[5] *DEaMONification*

[6] *Deammonification in Interval-aerated Biofilm*

The anammox process has become a technically and economically feasible alternative to conventional nitrification-denitrification. The practitioners and researchers have investigated different configurations as well as different parameters which could potentially contribute to reactor stability. Nitrite has been considered a potential destabilizing agent; however, no consistency in

nitrite inhibition threshold concentration has been identified. The objective of the present study, then, was to conduct basic research on nitrite inhibition threshold concentration and try to identify how and when it becomes a destabilizing agent. The FA was hypothesised to be precursor of anammox reactor instabilities. If it is true that nitrite up to about 200 mg N/L should stimulate nitrogen removal rate inside of the anammox reactor, when FA is kept below the inhibition threshold concentration, then it is FA and not nitrite level alone that affects the stability or instability of the anammox reactor

1. LITERATURE REVIEW

1.1. Basics of biological nitrogen removal

Nitrogen at wastewater treatment plants (mostly in the form of total ammonia - TA) can be removed biologically. It has been shown that ammonium concentrations below 2000 mg TA/L (such as reject waters after the anaerobic digestion process, of particular interest for this research) has usually been treated biologically (Mulder, 2003).

The conventional (biological) system for nitrogen removal involves autotrophic nitrification and heterotrophic denitrification. In such a system, nitrogen is removed in two steps: TA is oxidized to nitrate, during the aerobic zone/phase of the bioreactor followed by denitrification with organic carbon utilization. However, this very reliable strategy becomes expensive when high nitrogen loaded and biodegradable carbon deficient streams have to be treated (van Loosdrecht et al., 2006). Processes such as SHARON-ANAMMOX, CANON, OLAND, and DEMON become more economical than conventional autotrophic/heterotrophic processes.

Autotrophic processes which apply partial nitritation and anammox, in some cases, may allow achieving wastewater treatment plant energy self-sufficiency (Wett et al., 2007). Comparing the conventional autotrophic/heterotrophic process with the completely autotrophic nitrogen removal process in terms of oxygen and carbon need for the same amount of TA to be removed, overall, approximately 60% of oxygen consumption can be

reduced without the need for organic carbon addition (when carbon deficient wastewater has to be treated, Ahn Y. H., 2006). Due to the fact that the process is autotrophic, 85% reduction in biomass production can be achieved.

Nitrification

Nitrification is a biologically mediated process where nitritation is carried out by ammonium oxidizing biomass (AOB) such as *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrocystis*, and others (Voytek et al., 1995), while nitratation is carried out by nitrite oxidizing biomass (NOB) such as *Nitrospira*, *Nitrobacter*, *Nitrospina*, *Nitrococcus* (Watson et al.,1986; Bartosch et al., 1999). AOBs and NOBs use molecular oxygen to oxidize TA and nitrite, respectively. Nitritation and nitratation are shown by Reaction 1 and Reaction 2, respectively, where overall nitrification with biomass synthesis is shown by Reaction 3 (Orhon et al., 1994)

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + H_2O + H^+ \qquad \text{(Reaction 1)}$$

$$NO_2^- + 0.5O_2 \longrightarrow NO_3^- \qquad \text{(Reaction 2)}$$

$$NH_4^+ + 1.856O_2 + 1.98\,HCO_3^- \longrightarrow$$

$$0.021\,C_5H_7NO_2 + 0.98\,NO_3^- + 1.876CO_2 + 2.92H_2O \qquad \text{(Reaction 3)}$$

Due to the fact that nitritation and nitratation are carried out by two different groups of microorganisms, it has been demonstrated that nitritation can be achieved by the proper environment parameters manipulation such as

dissolved oxygen (DO) control (Aslan et al., 2009; Bae et al., 2002; Ciudad et al., 2005), free ammonia (FA) or free nitric acid (FNA) control (Ganigué et al., 2007; Vadivelu et al., 2007; Van Hulle et al., 2007; Zhang et al., 2010), or SRT control (Van Hulle et al., 2007).

Anammox

Anammox organisms belong to the phylum of the Planctomycetes (Strous et al., 2006). Planctomycetes are probably rooted in the deepest branching bacterial phylum and/or as a bacterial phylum related to the intracellular parasites of the Chlamydiae (Strous et al., 2006). Until now, the following anammox organisms have been identified: *Candidatus Brocadia anammoxidans, Candidatus Kuenenia stuttgartiensis, Candidatus Scalindua brodae, Candidatus Scalindua wagneri, Candidatus Scalindua sorokinii and Anammoxglobus propionicus* (*candidatus* stands for proposed name, Kartal et al., 2007). However, researchers have reported more unknown anammox organisms in their reactors; therefore, there is a potential for more being discovered (Yang et al., 2011; Yamamoto et al., 2011; Yapsakli et al., 2011). In 2006, the genome sequence of the representative anammox was published Strous et al. (2006). Because of the lack of pure culture, the genome of *Candidatus Kuenenia stuttgartiensis* contained many partial genomes from other microorganisms as well. These genome studies allowed to prove already known metabolic pathways but also allowed to discover more about anammox enzymes performance (van de Vossenberg et al., 2012).

The colour of the anammox bacteria is reddish-brown, probably due to the high cytochrome contents (Jetten et al., 1999).

Anammox organisms have been reported to tolerate a pH range from 6.7 to 8.3 (with an optimum at 8.0) and a temperature range from 20 °C to 43 °C with an optimum at 40 °C (Jetten et al., 1999). They have been shown to be very sensitive to oxygen, causing complete inhibition when above 0.5% oxygen saturation (Jetten et al., 1999). However, many researchers have demonstrated that anammox consortia can be cultivated in an oxygen limited environment. It has been suggested that oxygen-sensitive anammox organisms occupy places inside of flocks/granules while oxygen utilizers dominate closely adjacent sites, thereby protecting the anammox (Third et al., 2001; Sliekers et al., 2003).

Anammox organisms oxidize ammonium according to Reaction 4 (Strous et al., 1998).

$$1\ \mathrm{NH_4^+} + 1.32\ \mathrm{NO_2^-} + 0.066\ \mathrm{HCO_3^-} + 0.13\ \mathrm{H^+}$$
$$\rightarrow 1.02\mathrm{N_2} + 0.26\mathrm{NO_3^-} + 0.066\mathrm{CH_2O_{0.5}N_{0.15}} + 2.03\mathrm{H_2O} \qquad \text{(Reaction 4)}$$

Anammox organisms have a unique structure with a membrane-bound organelle called the anammoxosome; this is surrounded by lipids called ladderanes (Sinninghe Damste et al., 2002). A very dense carbon atom arrangement serves as a diffusion barrier. The purpose of this structure was hypothesized to protect the bacteria interior environment from the toxic

anammox reaction intermediates such as hydroxylamine and hydrazine (Jetten et al., 2003). The anammoxosome's enzyme, which is hydroxylamine oxidoreductase (HAO), is responsible for the oxidation of hydrazine to dinitrogen gas (Lindsay et al., 2001). Based on the studies conducted by Strous et al. (2006), the anammox reaction proceeds in two major steps, as described by Reaction 5 and Reaction 6.

$$NO_2^- \longrightarrow NO \qquad \text{(Reaction 5)}$$

$$NO + NH_4^+ \longrightarrow N_2\ H_4 \longrightarrow N_2 \qquad \text{(Reaction 6)}$$

Bothe (2007) pointed out that the loss of even small amounts of anammox intermediates might have an impact on the biomass yield, due to the fact that endogenous electron donors have to be regenerated by reversed electron transport and CO_2 fixation, both of which are very costly energy-wise. Therefore, growth in biofilm structure may decrease the negative effect of losses in the intermediates out of the microorganism due to significantly lower surface to volume ratio (single cell versus cell agglomerates).

Anammox bacteria may use different metabolic pathways. They are able to reduce nitrate to nitrite and nitrite to ammonium, followed by the conversion of ammonium and nitrite to dinitrogen gas through the anammox pathway. This may allow for overcoming the ammonium limitation (Bakermanset al., 2009).

Another alternative pathway demonstrated by Kartal et al. (2007) shows that anammox organisms were able to co-oxidize organic carbon (propionate) and TA, and out-compete denitrifiers.

It has also been demonstrated that iron and manganese oxides were respired with formate as an electron donor (Strous et al., 2006).

The wide variety of possible metabolic pathways demonstrates the versatile lifestyle of anammox organisms, which links the biological nitrogen cycle with the carbon and metal cycle in new ways (Strous et al., 2006).

1.2. Anammox systems' stability and performance

Generally, the anammox technology has been considered a good option for wastewater treatment plant (WWTP) upgrade in two scenarios: 1) when either not enough organic carbon has been available for denitrification, or 2) when extra nitrogen had to be removed to meet more stringent effluent quality for total nitrogen in the effluent when existing reactor capacity does not allow achievement of this goal (van Loosdrecht & Salem, 2006). Therefore, anammox system stability is crucial for overall WWTP performance to meet effluent quality. Additionally, the anammox system has been considered an economical option for treating TA-rich and organic carbon-poor wastewaters, because it provides a significant saving in aeration and organic carbon addition for denitrification (Mulder, 2003). Therefore, developing methods to

predict nitrogen removal rates under variable operational conditions (which will become tools for better control and design of anammox technologies) is in the best interest of plant operators and designers.

1.2.1. Nitrogen concentrations treated in anammox systems and nitrogen inhibition threshold concentrations for anammox consortia

Generally, anammox systems have been applied for treating TA-rich and organic carbon-poor wastewaters (Gut 2006). An example of such a wastewater is centrate, the anaerobic digester reject water, which was used in the current study. Table 1-1 illustrates the centrate characteristics — total ammonia (TA), temperature (T) and pH in studies ranging from 1998 to the present. At the same time, various lab and full scale experiments have demonstrated high NRRs in different configurations, where short hydraulic retention times (HRT) were required to sustain high loading rates (Tang et al., 2011; Tsushima et al., 2007). The consequence of high concentrate feed and

Table 1-1 Nitrogen concentrations in reject waters coming from anaerobic digestion process

TA [mg N/L]	T [°C]	pH [-]	Reference
1000	30	8.1-8.4	Hellinga et al., 1998
1180	-	6.7-6.8	van Dongen et al., 2001
750	30	-	Salem et al., 2003
598-713	23 – 30	7.2-7.9	Fux et al., 2002
500-1500	30-37	7-8.5	Jetten et al., 1999
1200	30	7.2	Beier et al.,1998
1830	28	-	Wett 2006
436-797	-	7.4-7.9	Musiał 2000
600-750	-	7.6 – 8.0	This study NEWPCC (undiluted)

low HRT is fast substrates accumulation when biomass inhibition occurs (Rosenthal et al., 2009). The commonly-reported, sudden anammox activity reductions are presented in the subchapter 1.2.4 (page 16). This fact of substantial variability in substrates (mostly TA but also nitrite) in anammox reactors was researched intensively from the very beginning of research on anammox technology. This intensive research, however, has not provided a satisfactory answer for substrate inhibition threshold concentration.

Among different nitrogen components in the wastewater being treated in anammox systems, the most significant are nitrite and TA, substrates for anammox organisms. Generally, the higher substrate concentration in the bioreactor, the higher overall nitrogen removal rate, due to lack of substrate

limitation (Caffaz *et al.,* 2006; van der Star *et al.,* 2007). Alternatively, too high nitrogen concentrations may lead to substrate inhibition which is possible due to the nature of the wastewater being treated.

Nitrite has been reported as a prime destabilizing nitrogen component among nitrogen forms such as nitrite, nitrate and TA (Strous et al., 1999). Nitrite inhibition threshold was extensively researched, being reported in a wide range among different studies between 5 mg N/L and 274 mg N/L (Wett et al., 2007; Kimura et al., 2010). On the other hand, it has been reported that, despite maintaining low nitrite concentration inside of an anammox reactor, the reactors stability could not be guaranteed (Rosenthal et al., 2009). Rosenthal et al. (2009) suggested that there must be another factor affecting anammox reactor stability, rather than nitrite alone. Indeed, there have been some studies which demonstrated stable reactor operation despite elevated nitrite (Kaldate et al., 2009; Kimura et al., 2010). Nitrite inhibition will be further reviewed in subchapter 1.3 (page 17).

Based on the reported wide nitrite inhibition range in the literature, it seems that the nitrite inhibition investigation should be placed in a wider research context. Most of anammox reactors have been designed for low nitrite (van der Star et al., 2007) due to nitrite inhibitiory nature and maximization of the nitrogen removal efficiency. At the same time, TA has been allowed to vary in a wide range between about 5 mg N/L and 150 mg N/L (Fux et al., 2002; Joss et al., 2009; Abma et al., 2007; Strous et al., 1998; Szatkowska et al., 2007).

In some studies, FA was investigated as a potential inhibitor due to variable TA in a wide range and ambient pH varying between 7 and 8.5 (Fernández et al., 2010; Jung, et al., 2007). However, pH variations themselves have not been considered important in regards to anammox reactors stability (Fux et al., 2004), due to reported wide pH optimum range for anammox organisms (Jetten et al., 1999). The FA inhibition will be reviewed in subchapter 1.4 (page 27).

1.2.2. Nitrogen removal rates in anammox systems

In the literature, a very wide range of nitrogen removal rates (NRR) between 0.2 g N/L d and 45.2 g N/L d has been presented (Szatkowska et al., 2007; Tang et al., 2010). It has varied for different anammox reactor configurations, but also within the same configuration (Table 1-2). Although some NRRs were very high such as 45.2 g N/L d for anammox stage, or 1.5 g N/L d for one-biomass SBR reactor, they are relatively low when compared with theoretical calculations conducted by van der Star et al. (2007). According to these authors, maximum NRRs in two biomass systems for granular and MBBR systems should be as high as 78 and 6.1, respectively, where maximum NRRs

Table 1-2 Lab and pilot/full-scale maximum nitrogen removal rates (NRR) in anammox systems

Reactor type	max NRR (for anammox stage) Two-biomass process [g N/L d]	max NRR One-biomass process [g N/L d]
Granular sludge	45.2[(1)]; 15.4[(2)]; 6.5[(3)]; 1.5-1.8[(4)];	1.5[(5)]
Biofilm moving bed	0.2[(6)]	0.32 [(7)]; 0.4-0.7[(8)]
SBR	0.6-2.4[(9)]; 0.9[(10)]; 0.75[(11)]	0.1[(12)]; 0.3 [(13)]; 0.5[(14)]; 0.35-0.55[(15)]; 1.1[(16)]; 0.5-1.5[(17)]

[(1)] Tang et al., 2010; [(2)] Tang et al., 2009, [(3)] van de Graaf, 1996, [(4)] Strous et al., 1997a, [(5)] Sliekers et al., 2003, [(6)] Szatkowska et al., 2007, [(7)] Helmer et al., 2001 , [(8)] Rosenwinkel et al., 2005, [(9)] Fux et al., 2002; , [(10)] Strous et al., 1998, [(11)] van Dongen et al., 2001, [(12)] Third et al., 2001, [(13)] Sliekers et al., 2002, [(14)] Vlaeminck et al., 2009, [(15)] Clippeleir et al., 2009, [(16)] Joss et al., 2009, [(17)] Wett, 2006

in one-biomass system for granular and MBBR systems should be as high as 7 and 1.1, respectively. None of those NRRs have been found to be achieved during long term, stable reactor operations. Therefore, it is important to identify limiting factors which did not allow achieving high NRRs. One of the possible explanations is in nitrite inhibition, where anammox reactors had to be operated at low nitrite without exceeding inhibitory concentrations. At the same time, low nitrite concentration inside of the anammox reactor requires a low loading rate and small loading variations, which were demonstrated to be important for the reactors' stability (Gut et al., 2006). A further literature review on reactor stability will be presented in the following sections.

1.2.3. Nitrogen loading rate variability effect on system stability

The feed to the reactor may vary in terms of loading rate but also in terms of composition, such as TA to alkalinity ratio, which determines the amount of TA or nitrite in the effluent from the anammox reactor. In literature, all of those parameters were shown to have a negative effect on the NRR in anammox systems.

The negative effect of varying nitrite to ammonium ratio on anammox process was shown by Gut et al. (2006). The highest nitrogen removal efficiency was recorded when the influent nitrite to ammonium ratio was in the range between 1 and 1,5 mg NO_2-N/mg TA. The nitrogen removal efficiency changed from 86% – 98% (average 87%), for the optimum ratio, to the 74% – 93%, out of optimum range. Authors reported that an increase in nitrogen loading rate could be achieved only in slow and stepwise increments, to allow anammox bacteria to adjust to the new conditions.

Caffaz et al. (2006) reported that a sudden change in the nitrogen loading rate caused rapid loss in the NRR. This occurred when nitrite and TA build-up was up to 40 mg N/L and to 13 mg N/L, respectively.

In the study conducted by Szatkowska et al. (2007), it was shown that overloading the anammox rector caused an increase in nitrite concentration over 70 mg N/L. The recovery period took 4 months after the reactor destabilization. It was observed that nitrite concentration in the range from 20 to 30 mg N/L decreased the nitrogen removal efficiency by 40%.

The feeding rate was considered as a very important factor to keep the process stable in the one-biomass DEMON process (O'Shaughnessy *et al*., 2008). It was reported that the loading rate has to be balanced with AOBs and anammox kinetics, to prevent nitrite build up inside of the DEMON reactor.

Generally, when increasing the loading rate, nitrogen accumulates inside of the reactor as a result of exceeding the reactor's capacity for nitrogen removal. Therefore, it would be more accurate to investigate nitrogen concentrations variability inside of the reactor and their effect on NRR, rather than the loading rate itself, due to a better link between NRR and substrate inhibition threshold concentrations for anammox consortia. Knowing the substrate concentration range inside of the anammox reactor and its effect on biomass activity (NRR), a more accurate reactor design could be achieved.

1.2.4. Sudden activity losses

Unexpected NRRs losses have been reported in the literature for anammox systems. They were not directly related to nitrite accumulation. Fux et al. (2002) reported sudden reduction in anammox activity in the anammox SBR which caused nitrite accumulation up to 60 mg N/L. The reactor recovered its activity, but a 50% reduction in load was required.

Activity losses up to 50% and 90% were reported by Caffaz et al., (2006) in the anammox SBR. Slow recovery of the NRR was reported after a drop of biomass activity.

Joss et al. (2009) reported a sudden loss in a SBR one-biomass system, hypothesized to be a toxic component in the feed. The reduction in the activity was observed during one month's period. At the end of that period, the reactor was stopped for 3 days, and the exponential net biomass growth was back to the usual rate of 0.024 d^{-1}.

Rosenthal et al. (2009) reported unexpected NRR losses in anammox SBR. Authors could not explain them through nitrite inhibition as the reactor was always operated at very low nitrite concentrations. The authors concluded that there must be another factor which was causing reactor destabilization.

Sudden activity losses presented in the literature are very hard to interpret in terms of possible causes and mechanisms. Although some authors did not directly relate NRR losses to nitrite, sudden nitrite accumulation was considered as a sign of the reactor's destabilization.

1.3. Nitrite inhibition

1.3.1. General introduction to nitrite inhibition

In literature, generally, nitrite has been shown to exhibit strong toxicity on bacteria's growth and respiration processes (Rowe et al., 1979; Yarbrough et al., 1980). However, not only different microorganisms have exhibited different

nitrite inhibition threshold concentrations (NTC) but also environmental conditions have appeared to be important when NTC has been investigated.

In the research conducted by Saito et al. (2004), the authors have investigated nitrite inhibition on phosphate uptake rate in the culture containing phosphorus accumulating organisms under anoxic and aerobic conditions. Results have shown that phosphate uptake rate was inhibited when nitrite concentrations of 4 mg $N\text{-}NO_2$/L and about 1 mg $N\text{-}NO_2$/L were exceeded under anoxic and aerobic conditions, respectively. In the same study, a nitrite concentration of about 12 mg N/L was shown to cause complete deactivation of the biomass under aerobic conditions. Regardless of the condition, low nitrite concentration was required to destabilize the biomass activity. Low NTC was also presented by Meinhold et al. (1999), where activated sludge from the enhanced biological phosphorus removal (EBPR) system shown NTC in the nitrite range between 5 – 8 mg N/L, under anoxic conditions. Contrary to what was presented by Saito et al. (2004) and Meinhold et al. (1999), Zeng et al, (2011) presented results which indicated that nitrite concentration up to 30 mg N/L did not have a significant effect on aerobic P-uptake rate. Zeng et al, (2011) were investigating aerobic P-uptake rate during biological phosphorus removal in a sequencing batch reactor, treating domestic wastewater. The authors explained this phenomenon showing the limitation of carbon source (VFA) as cause for P-uptake rate cessation. In the batch tests, when sufficient carbon source was provided, no adverse effect of nitrite up to 30 mg N/L (higher concentrations were not investigated) on poly-b-hydroxyalkanoate

(PHA) storage was observed. The study conducted by Zeng et al, (2011) was well in agreement with an earlier study conducted by Weon et al. (2002). The Acinetobactor, as a model phosphorus accumulating bacteria, was studied, where the growth and P-uptake rate was investigated. The biomass was cultivated under aerobic conditions, where no limitation in organic carbon and oxygen was secured. The authors found nitrite 10% inhibition concentration (IC_{10}) to be at about 151 mg N/L.

Presented studies suggest that nitrite inhibitory effect on either P-uptake or P-release may vary due to experimental methods. The observed nitrite inhibitory effect, correlated with biomass inhibition, may not be the true cause for biomass activity cessation. Batch testing turned out to be a very useful strategy for the biomass inhibition testing.

Nitrite inhibition was also investigated during denitrification in activated sludge process, where Abeling and Seyfried (1992) found that nitrogen removal was completely stopped when nitrite reached 100 mg N/L. Beccari et al. (1983) demonstrated that nitrite at 20 mg N/L contributed significantly to the reduction of the nitrogen removal rate. In that study, nitrite inhibition threshold concentration was estimated to be at nitrite concentration below 10 mg N/L and it was increasing along with biomass concentration. The biomass concentration tested was in the range between 500 mg VSS/L and 1100 mg VSS/L. Additionally, the authors observed sudden nitrogen removal cessation when NTC (10 mg N/L) was exceeded.

Much higher nitrite concentrations were observed in the study conducted by Chen et al. (1991). The authors showed that nitrite concentration up to the investigated level of 2000 mg N/L (pH is unknown) was tolerable by denitrifying organisms in the biofilm system performing denitrification. Such a significant difference in nitrite effect on nitrogen removal between presented studies may be due to the cultivation method. Low NTC was observed in systems with flocculated (suspended growth) biomass, whereas higher nitrite levels were tolerated in the biofilm system. Therefore, the form of biomass: attached or suspended seems to significantly impact the NTC.

Nitrification is a two-step process where each step is mediated by two different groups of microorganisms. In the research conducted by Vadivelu et al. (2007), the nitrite effect on catabolic and anabolic processes of *Nitrosomonas* and *Nitrobacter* was investigated in batch tests. Researchers observed that enriched *Nitrosomonas* culture experienced 50% inhibition of energy production and 100 % of growth inhibition at about 2300 mg NO_2-N/L. On the other hand, in the enriched *Nitrobacter* culture, complete inhibition of catabolic and anabolic processes was observed at about 110 mg NO_2-N/L. The inhibition threshold concentration of growth processes was found to be about 60 mg NO_2-N/L. This significant difference in nitrite tolerance between these two groups of microorganisms was first observed by Anthonisen et al. (1976) which later turned out to be good strategy for achieving partial nitritation (Ganigué et al., 2007; van Hulle et al., 2007; Zhang et al., 2010). Under high nitrite concentration, more sensitive nitrite oxidizing bacteria were

washed out leading into consistent nitrite accumulation. This suggests that different groups of microorganisms may have different nitrite inhibition threshold concentrations.

Based on the reviewed literature, different microorganisms responded differently to nitrite. Growth conditions such as biofilm were shown to enhance resistance to nitrite inhibition, thereby affecting NTC.

1.3.2. Nitrite inhibition in anammox systems

Nitrite inhibition of anammox rates was intensively studied in anammox systems over the past decade. The first research on nitrite inhibition in anammox culture was conducted by Strous et al. (1999), where long- and short-time inhibition was tested. In that study, the nitrite inhibition threshold concentration was shown to be in the range of 150 – 200 mg N/L and 60 – 90 mg N/L, respectively for short- (not specified duration time) and long-term exposure time (up to 50 hour incubation period). Additionally, the authors observed that nitrite demand for ammonium oxidation was increasing along with increasing nitrite concentration, up to the investigated nitrite concentration of 200 mg N/L. The authors stated that the anammox process was completely inhibited by nitrite when nitrite concentrations were greater than 100 mg N/L; however, no data and methods were presented to support this statement.

The next study on nitrite inhibition in the anammox system, which was the first study where experimental methods were presented more clearly, was conducted by Dapena-Mora et al. (2007). The nitrite concentration inhibitory effect on anammox biomass activity was investigated based on the nitrogen gas produced, calculated from the overpressure in the headspace using the ideal gas law equation. The data suggested a nitrite inhibition threshold concentration in the rage of 140 – 210 mg N/L for about 6.5 hours exposure time. The authors reported that their data was considerably different than that reported by Strous et al. (1999), where anammox activity was completely lost of higher than 100 mg N/L of nitrite.

The nitrite inhibition was extensively evaluated during long-term reactor operation; however, a significant discrepancy can be observed between different studies. Szatkowska et al. (2007), Fernández et al. (2010) and Jung et al. (2007) reported that nitrite concentrations exceeding 10 – 20 mg N/L, 16 mg N/L and 35 mg N/L, respectively, were causing reactor destabilizations. Reactor destabilization was characterised by nitrogen removal efficiency and nitrite accumulation in the reactor, respectively for Szatkowska et al. (2007) and Fernández et al. (2010), and Jung et al. (2007). On the other hand, all of these authors did not present the nitrite effect on nitrogen removal rate which could point directly to inhibition. In the study conducted by Tsushima et al. (2007), the authors investigated the inhibitory nitrite effect on nitrogen removal rate, by increasing nitrite concentrations inside of the anammox reactor up to about 225 mg N/L, through nitrogen loading rate variation. Results showed

that nitrite concentrations up to about 117 mg N/L were stimulating NRRs, where the 25% decrease in NRR was observed at nitrite concentration of 224 ± 10 mg N/L. The same as in previous studies (Szatkowska et al., 2007 and Jung et al., 2007), the increase in the nitrogen loading rate was affecting negatively the nitrogen removal efficiency and nitrite build up; however, this was not necessarily correlated with diminishing NRR (up to about 117 mg N/L).

In the studies conducted by Waki et al. (2007), Kimura et al. (2010) and Kaldate et al.(2009), where, in different tests, nitrite concentrations were in the range from close to 0 to 430 mg N/L, the nitrite concentration up to about 200 mg N/L did not cause either reactor destabilization or anammox rate deterioration. Rosenthal et al. (2009) reported that sudden anammox activity deteriorations occurred in their study during anammox SBR reactor operation. The authors conducted a series of batch tests to investigate the nitrite inhibitory nature. They found out that a sudden failure in the reactor's operation could not be caused by nitrite inhibition to anammox organisms due to biomass high tolerance to nitrite. They observed that nitrite inhibition threshold concentration was at about 200 mg N/L for one day sample incubation. They concluded that there had to be other mechanisms responsible for sudden anammox rates deteriorations than nitrite alone.

Nitrite exceeding 4.8 mg N/L was reported to decrease anammox activity (NRR) over a longer period of time in a pilot scale SBR reactor (DEMON

process, Wett, et al., 2007). However, further studies on nitrite inhibition on the biomass from the same reactor showed that nitrite concentrations exceeding 50 – 100 mg N/L became inhibitory to the anammox consortium (Musabyimana et al., 2008). Therefore, nitrite accumulation and reactor deterioration were probably caused by other factors rather than nitrite alone.

The latest study conducted by Bettazzi et al. (2010) reported 25% activity losses at nitrite concentrations higher than 60 mg N/L (single nitrite spike), where repeated addition of nitrite higher than 30 mg N/L caused losses of activity. This study did not show very low nitrite concentrations as inhibitory; however, this study still contradicted some earlier studies which showed higher anammox biomass tolerance to nitrite (Table 1-3).

Table 1-3 The nitrite inhibition threshold concentration (NTC) reported in the literature

Short-term tests					**Long-term tests**				
NTC [mgN/L]	Nitrite grown condition [mg N/L]	Speciation	FA/ pH mg N/L	Reference	**NTC [mgN/L]**	Nitrite grown condition [mg N/L]	Speciation	FA/pH mg N/L	Reference
274	10-50	*Can. Kuenenia stuttgartiensis and others*	-/ -	Kimura et al., 2010	**(200)**	10-50	*Can. Kuenenia stuttgartiensis and others*	-/ -	Kimura et al., 2010
200	Very low	*Can. Brocadia anam.*	-/ -	Rosenthal et al., 2009	**(200)**	About 150	*Can. Brocadia and Can. Kuenenia*	-/7.6±0.26	Kaldate et al., 2009
(200)	1.7-11	unknown	-/6.0-7.6	Waki et al., 2007	**117 - 224**	unknown	*Can. Brocadia anam.*	-/7-7.5 (feed)	Tsushima et al., 2007
200	Very low	*Can. Brocadia anam.*	-/7-7.8	Strous et al., 1999	**60 - 90**	Very low	*Can. Brocadia anammoxidans*	-/-	Strous et al., 1999
140-210	Very low	(*Can. Brocadia anamm. and Can. Kuenenia stutt*)	3.5/7.8	Fernandez et al., 2008	**35**	Very low	unknown	1.7-30/7.8-8.5	Jung et al., 2007
140-210	25-50	*Can. Kuenenia stutt.*	3.5/7.8	Dapena-Mora et al., 2007	**10 - 20**	Low about 10	*Can. Brocadia anammoxidans*	~4/8.1-8.2	Szatkowska et al., 2007
50-100	Very low	Unknown (*Can. Brocadia anammoxidans*)	-/7.3-7.4	Musabyimana et al., 2008	**16**	Very low	*Can. Broc. anammoxidans and Can. Kuen. stuttgartiensis*	-/8.2-8.8	Fernandez et al., 2008
30-60	Very low	*46% Can. Broc. anammoxidans* + 2 others (12%+12%)	0.6-9.4/7.6-8.0	Bettazzi et al., 2010					

In Table 1-3, a summary of nitrite inhibition threshold concentrations for different studies are presented. They vary in a significant nitrite range from about 16 to about 274 mg N/L. Generally, speciation, biomass acclimation to high substrate concentration, or other environmental factors, were shown to induce biomass tolerance to toxic substances (Muyssen & Janssen, 2001; Jiang et al.,2009; Zhou et al., 2011). However, based on the information provided in Table 1-3, nitrite acclimation and speciation did not clearly point to a dominant cause for such a wide nitrite inhibitory range. Although anammox organisms have not been completely classified and identified, *Can. Brocadia anammoxidans* and *Can. Kuenenia stuttgartiensis* have been the dominant species in most studies. Nitrite concentrations, under which anammox consortia have been cultivated, vary in different studies. In short-term tests, no correlation between nitrite inhibition threshold concentrations and nitrite under which biomass was cultivated was observed. In a long-term test, some correlation could be identified for high NTC; however, NTC range between 16 and 90 mg N/L cannot be justified solely based on the nitrite under which the biomass was cultivated.

In Table 1-3, nine studies out of fifteen, shows that the nitrite inhibition threshold concentration could be close to 200 mg N/L. However, this needs to be verified. Lack of adequate and consistent information on the nitrite inhibition threshold NTC points to the need for fundamental research which could explain the mechanism involved in this phenomenon.

1.4. Free ammonia inhibition

1.4.1. General introduction to free ammonia inhibition

Generally, free ammonia (FA) has an inhibitory effect on microorganisms (Borys et al.,1994; Villaverde et al., 2000; Anthonisen et al., 1976; Hansen et al., 1998; Martinelle et al., 1996; Jenkins et al.,1998; Vadivelu et al., 2007 Torà, et al., 2010; Dapena-Mora et al., 2007). It was reported that FA diffuses through the cell membrane into the cell and changes the cytoplasmic pH (Martinelle et al., 1996), neutralizing the membrane potential, which may cause cell death. With a pKa of 9.24, the proportion of FA relative to ammonium (NH_4^+) is pH-dependent, and increases greatly (about twenty four times) as the pH increases from 7 to 8.5.

It was reported that free ammonia, rather than ammonium ion, has been responsible for inhibition (Anthonisen et al., 1976; van Hulle et al., 2007).

The FA was shown to affect negatively both growth and respiration processes. In the research conducted by Vadivelu et al. (2007), where free ammonia effect on anabolic and catabolic processes of *Nitrosomonas* and *Nitrobacter* were investigated, FA was demonstrated as an inhibitor. *Nirosomonas* was shown to not be affected by FA up to an investigated concentration of 16 mg N/L, which was in agreement with previous studies (Hellinga et al.,1999; van Hulle et al., 2007). Hellinga et al. (1999) and van Hulle et al. (2007) reported FA inhibition threshold concentration for the SHARON process at above 300 and about 93 mg N/L, respectively. The

difference between threshold concentrations was hypothesised due to salinity difference. The first study investigated the FA effect on biomass activity under pH 7.0, while the other, under pH 8.0, thereby achieving the same FA concentration under significantly different salt concentrations. Contrary to what was observed for *Nitrosomonas, Nitrobacter* was shown to be immediately inhibited (both growth and respiration processes) at FA concentrations greater than 1.0 mg N/L (Vadivelu et al., 2007).

Different microorganisms may have various tolerances to FA. Very high FA inhibition threshold concentration was shown by Hansen et al. (1998). The methane yield and biomass growth was inhibited, when FA was greater than 1100 mg N/L during batch testing.

In the research conducted by Villaverde et al. (2000), nitrite oxidizing biomass, cultivated in the biofilm, was shown to increase tolerance to FA through acclimation. Within six months, FA could be increased from 0.2 to 0.7 mg N/ g VSS (constant VSS concentration during the test) without negative effects on the nitrification rate.

1.4.2. Free ammonia inhibition in anammox systems

Free (FA) or un-ionized ammonia (NH_3), has been suggested to have a negative effect on anammox systems (Cema et al., 2005). Among different studies (Jung et al., 2007; Tang et al., 2010; Fernandez et al., 2010), the

lowest FA inhibition threshold concentration of 1.7 mg N-NH_3/L was found by Jung et al. (2007). In a number of studies (Strous et al., 1998; Fux et al., 2002; Fux et al., 2004b; Wyffels et al., 2004, Szatkowska et al., 2007; van der Star et al., 2007), the value of 1.7 mg N /L was consistently or intermittently exceeded. If the FA inhibition threshold concentration of 1.7 mg N /L is true, most of the anammox studies may have been operating near the threshold FA concentration for inhibition (Figure 1-1). Indeed, Figure 1-1 indicates that concentrations of total ammonia (TA) needed to approach the threshold for FA inhibition is well above the concentrations of TA typically used in standard operations (typical temperature was in the range 30 – 37 ^{0}C).

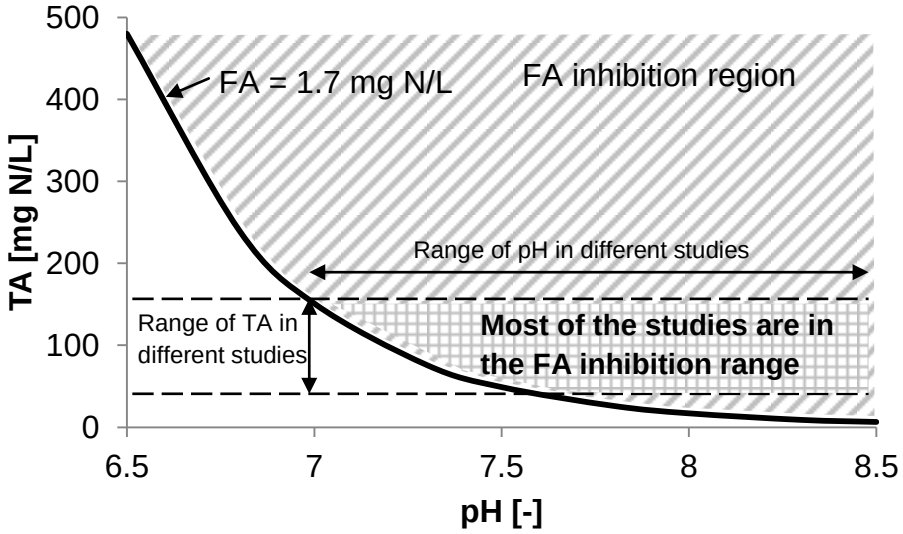


Figure 1-1 Relationship between pH and total ammonia concentration needed to reach the threshold of FA inhibition of 1.7 mg N/L at 35 ˚C (Jaroszynski et al., 2012)

Most of the reported anammox reactors were operated without pH control at a self stabilizing pH between 7 and 8.5, which has been reported as the optimum range for anammox consortia (Strous et al., 1998). Therefore, fluctuations of pH within this range have not been considered important with respect to the stability issue (Fux et al., 2004b).

Among different anammox studies, various specific nitrogen removal rates (sNRR) were reported. In Table 1-4, the relationship between pH, FA and SAA in different anammox reactors is reported. It was noticed that the lower the pH

Table 1-4 Relationship between pH, free ammonia (FA) and the specific nitrogen removal rate (SAA) in different anammox reactors

Reactor type	pH	FA [mg N/L]	NO_2-N [mg N/L]	sNRR [g N/g VSS d]	Reference
UASB(1)	7.9-8.2	1.2 - 2.0	15 - 50	1.8	Tang et al., 2010
FBR(2)	8	Around 8	Very low	0.15-0.18	Strous et al., 1997b
FBR(2)	7	Below 0.8	Very low	1.0	van de Graaf et al, 1996
SBR	7-8	1-10	Very low	1.9	Strous et al., 1998
Attached growth	7.0-7.5	unknown	224	1.6	Tsushima et al., 2007

(1) upflow anaerobic sludge blanket

(2) fluidized bed reactor

and FA, the higher the sNRRs. These studies were selected for lab scale reactors using synthetic wastewater, thereby inert solids (coming from the feed) accumulation was minimized. It was difficult to compare reactors based solely on anammox mass, due to insufficient information provided in those publications about anammox biomass abundance.

There has been very limited research on FA in anammox reactors, showing incoherent results. The first study, where negative FA effect on biomass activity was shown, was conducted by Jung et al. (2007). The authors observed biomass activity losses under high pH and TA. The authors reported that a correlation coefficient between biomass activity and FA was not so high (not specified); however, a good relationship was observed during the reactors' start-up period. Jung et al. (2007) concluded that FA below 1.7 mg N/L was playing a key role in stimulating anammox activity during the start-up period.

Contrary to what was presented by Jung et al. (2007), Tang et al. (2010) showed that the FA threshold concentration was at about 30 mg N/L. The authors operated their UASB anammox reactor at high FA concentrations in the range between 20-30 mg N/L. Although lower FA concentrations were not investigated in this reactor, a similar reactor was operated at lower pH and lower FA concentrations (below 12 mg N/L). Comparing those two reactors based on the maximum NRR achieved, a 94% difference in NRR was

observed (similar VSS concentration was recorded for both reactors), suggesting that FA should be as low as possible.

The immediate anammox biomass activity response to TA was tested in the batch tests by Dapena-Mora et al. (2007). The authors demonstrated a clear correlation between TA and anammox activity under constant pH and temperature. Taking into account that FA is most probably responsible for biomass activity deterioration (Anthonisen et al., 1976), FA greater than about 3 mg N/L was showing a negative effect on biomass activity. A similar study to Dapena-Mora et al. (2007) was conducted by Fernández et al. (2010), which demonstrated a very similar trend. However, both studies did not investigate lower FA concentrations; therefore, the FA inhibition threshold concentration was not identified.

Long-term FA effect on nitrogen removal efficiency in the SBR reactor was investigated by Fernández et al. (2010). The anammox biomass was grown on zeolite particle carriers under nitrite limitation conditions. The loading rate was up to 50% lower than the reactor's capacity for nitrogen removal. The authors concluded that FA should be below 35 - 40 mg N/L for stable reactor performance. Although the authors did not comment on the FA inhibition threshold concentrations, their data suggested a threshold in the range between 5 and 10 mg N/L. This could be observed based on the maximum nitrogen removal rate capacity tested along the reactor operation.

In all studies which investigated the inhibitory effect of FA on the anammox activity, tests were conducted under nitrite limitation conditions, due to generally accepted nitrite inhibitory nature and maximizing nitrogen removal efficiency of the reactor. On the other hand, most of the anammox systems applied different forms of biofilm such as granular sludge or attached biofilm to carrier media. In such systems substrate, limitation was shown to significantly affect the biomass kinetics (Cema et al., 2005; Ni et al., 2010; Chen et al., 2011; Dunn et al., 1985); generally, the higher the substrate concentrations, the higher the rates. Therefore, when analysing reactor behaviour for inhibition, the conditions where no inhibitions occur should first be well defined (such as Michaelis-Menten relation for variable substrate concentrations if applicable). This, however, has been lacking in all studies where FA was studied. This points to the need for in-depth investigation of the FA inhibition from the perspective of nitrite stimulation/inhibition.

1.4.3. Anammox in the moving bed biofilm reactor (MBBR)

Biofilm processes have proved to be reliable for nitrogen removal having some advantages over suspended growth activated sludge processes (Yang *et al.,* 2009). The moving bed biofilm reactor (MBBR) originated from Europe and was preliminary designed for cold climate operation, where slow growing organisms were protected from wash-out (Ødegaard, 2006). The fundamental principle of the MBBR is to immobilize biomass on carrier media,

eliminating the need for sludge settling and return in a continuous operation system.

MBBRs have been used in past research to investigate a variety of operational strategies for nutrient removal systems. Studies included the evaluation of energy recovery options through mechanical mixing (Phattaranawik *et al*., 2011) and assessing the effect of aeration on the concentration of extracellular polymeric substances (EPS) (Rahimi *et al*., 2011). Reactor stability, under changing hydraulic residence time (HRT) (Li *et al*., 2011) or the presence of concentrated organic substrates (Wang *et al*., 2009), was also investigated in the literature.

There has been limited research utilizing MBBRs for anammox processes (Thole *et al*., 2005; Szatkowska *et al.,* 2007). These studies focused on the overall feasibility of nitrogen removal in MBBR reactor systems using anammox organisms, without emphasis on process optimization. Important operating parameters, which affect system performance and stability, such as pH, free ammonia concentration, and the nitrite concentration, have not been studied in sufficient detail.

1.5. Conclusions

The following conclusions were identified based on the literature review:

1. Nitrite was cited as the most important stability parameter in the anammox process and it was reported to be maintained at low concentrations inside an anammox reactor to provide stable reactor operation. This reportedly crucial parameter did not have a clearly defined inhibition threshold concentration which was found varying in a wide nitrite range. Additionally, when researched, either unclear methodology was provided or variable total ammonia (TA) concentrations were present during testing, which could affect the final results achieved.

2. Among many studies, different nitrogen removal rates were achieved in different configurations. However, they were significantly lower, compared to those which were estimated, based on the theoretical calculations, suggesting some form of unknown limitation.

3. The sudden and unexpected anammox activity deteriorations were recorded for anammox reactors. None of the reviewed studies was able to identify a clear source of reactor disturbance.

4. The literature review suggested that the lower the pH, the higher the sNRR, which pointed indirectly to FA inhibition.

1.6. Summary and research needs

The anammox process has been considered as an attractive option for treating wastewaters rich in ammonia and poor in biodegradable organic carbon. Compared with conventional nitrogen removal, significant saving in aeration and organic carbon (mostly petrochemicals such as methanol) can be achieved. The anammox process in different configurations was widely researched and demonstrated high nitrogen removal rates; thus, installation can be small and compact.

The anammox technology has been applied mostly for concentrated nitrogenous wastewaters containing a significant amount of TA. At the same time, partial nitritation produces nitrite, required by metabolism of anammox consortium. Nitrite is commonly accepted as an inhibitor. The negative effect of nitrite on NRR was intensively researched showing wide nitrite inhibitory range (nitrite inhibition threshold concentration or NTC). However, reactor destabilizations were reported despite maintaining low nitrite inside of reactors, suggesting there may be another destabilizing factor acting on biomass, than just nitrite.

Among the studies which investigated FA inhibition in anammox systems, very low FA threshold concentration was reported. The literature review showed that FA can vary in a significant range and may be the cause of reactors' destabilization. Indeed, when reactors were operated at low nitrite concentrations, FA could fluctuate in a wide range due to variable TA and pH.

The proportion of FA relative to ammonium (NH_4^+) is pH-dependent, and increases greatly (about twenty four times) as the pH increases from 7 to 8.5; that is why the reactor can be destabilized regardless of the nitrite concentration. At the same time, the negative effect of FA is intensified when either the loading rate is suddenly increased or a change in pH occurs under elevated TA. This may lead to NRR deterioration, regardless of the nitrite concentrations.

The NRRs archived in different studies were much lower compared to theoretical calculations, suggesting an unknown limitation. The NRR in anammox biofilm systems should be stimulated along with increasing TA and nitrite concentrations, due to diffusion limitation in those systems. The literature review suggested that the lower the pH, the higher the sNRR, pointing indirectly to FA inhibition. Such a trend should not be observed based on the studies where pH alone should not have an important impact on NRR, within a pH range of 7 – 8.5.

Based on this literature review, it was suggested that the inhibitory effect of nitrite on NRR should be reinvestigated from the perspective of FA. It was suggested that the FA may have a greater inhibitory impact on NRR than nitrite, under regular reactor operations (low or medium nitrite concentrations inside of the anammox reactor).

2. PROBLEM STATEMENT, HYPOTHESIS AND OBJECTIVES

2.1. Problem statement

The anammox system has been considered an economical option for treating TA-rich and organic carbon-poor wastewaters, because it provides a significant saving in aeration and organic carbon addition for denitrification. When it is part of the treatment process, anammox system stability is crucial for overall WWTP performance, to meet effluent quality. Therefore, developing methods to predict nitrogen removal rates under variable operational conditions (which will become tools for better control and design of anammox technologies) is in the best interest of plant operators and designers.

In the literature, a very wide range of NRRs have been presented. It has varied for different anammox reactors configurations, but also within the same configuration. Additionally, nitrite concentration inside of anammox reactor, which has been considered a critical the design and operational parameter, has not been clearly defined and has varied in a significant nitrite range. Based on the reported wide nitrite inhibition range in the literature, it seems that the nitrite inhibition investigation should be placed in a wider research context. Indeed, it has been reported that, despite maintaining low nitrite concentrations inside of anammox reactors, the reactors stability could not be guaranteed. It has been suggested that there must be another factor affecting anammox reactor stability, rather than nitrite alone. There have been some studies which demonstrated stable reactor operation, despite elevated nitrite.

TA has been allowed to vary in a wide range between about 5 mg N/L and 150 mg N/L. In some studies, FA was investigated as a potential inhibitor due to variable TA in a wide range and ambient pH varying between 7 and 8.5; however, pH variations, themselves, have not been considered important in regards to anammox reactors stability, due to reported wide pH optimum range for anammox organisms. At the same time, there has been very limited research on FA in anammox reactors, showing incoherent results.

Sudden activity losses presented in the literature are very hard to interpret in terms of possible causes and mechanisms. This is due to lack of variety of parameters such as TA, FA, pH, temperature, biomass activity and nitrite concentration, which has to be known for the accurate problem investigation. Most of the anammox studies may have been operating near the threshold FA concentration for inhibition. It was noticed that the lower the pH and FA, the higher the biomass activity inside of anammox reactors. This, however, has to be clearly demonstrated under controlled conditions. This points to the need for in-depth investigation of the FA inhibition from the perspective of nitrite stimulation/inhibition.

2.2. Hypothesis statement

Among inorganic nitrogen forms (nitrite, total ammonia and free ammonia), un-ionized form of ammonium – free ammonia increase (FA) is the precursor of the instability of the anammox reactor. Nitrite up to about 200 mg N/L, should stimulate the nitrogen removal rate inside of the anammox reactor when FA is kept below the inhibition threshold concentration. The low nitrite inhibition threshold concentrations, which were identified in the literature review, were caused mostly by FA.

2.3. Research objectives statement

- To show FA impacts the stability and performance in the anammox reactor based on the nitrogen removal rate (NRR, in biofilm MBBR reactor) and specific nitrogen removal rate (sNRR, in suspended flocculated SBR reactor)
- To show that nitrite stimulate the NRR in an anammox reactor
- To show that high nitrite concentrations in anammox reactors can be mitigated when FA is maintained at an adequately low level
- To define the stability of an anammox reactor under variable nitrogen loading rates and variable FA and nitrite conditions

3. MATERIALS AND METHODS

This chapter describes reactor configurations, influent characteristics and methods for experiments performed during this study. Some additional details about methods are also provided in corresponding chapters where experimental results and discussions are described.

3.1. Reactors configuration

During the research, a two-reactor configuration was used for the anammox process as shown in Figure 3-1, where the first part of the system consisted of one semi continuously-fed (feed occurred during reaction phase) sequencing batch reactor (SBR) for the partial nitritation process. Due to the sequential performance of the partial nitritation reactor, an equalization tank was placed between nitritation and anammox. The second part of the system consisted of three reactors for the anammox stage, two continuously fed moving bed biofilm reactors (MBBR) and one semi continuously-fed suspended growth SBR.

The entire system was placed in the walk-in environmental chamber set at 35 °C.

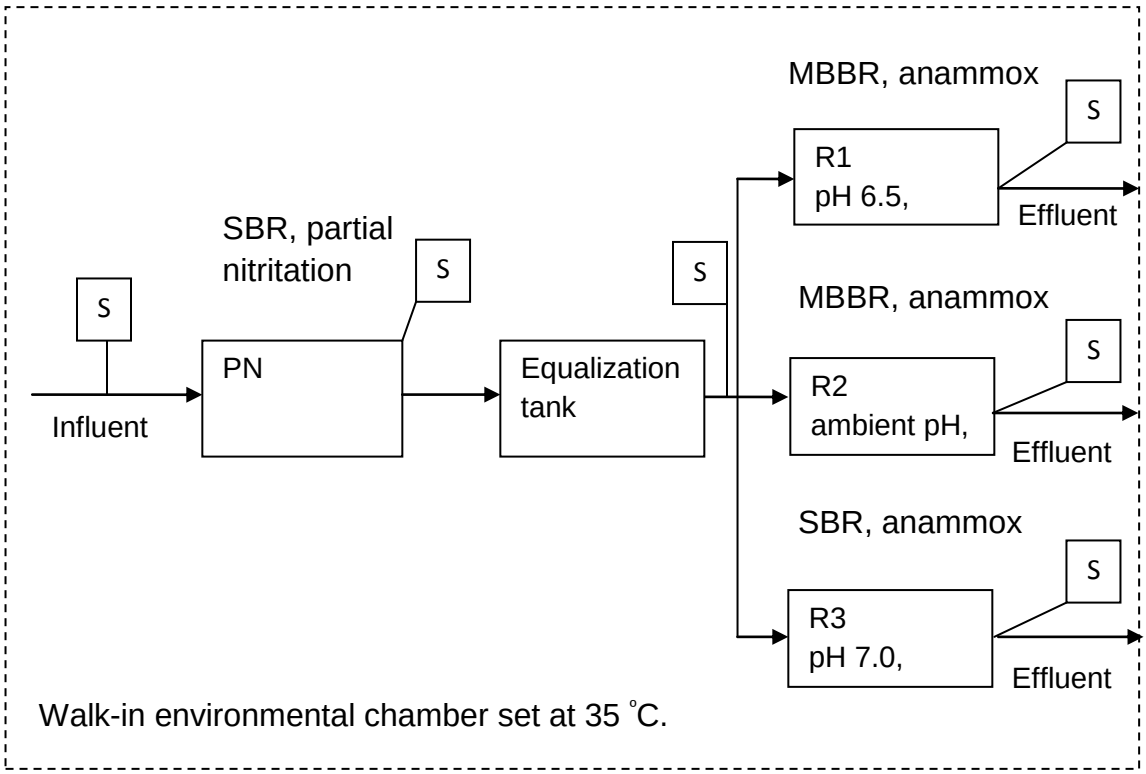


Figure 3-1- Schematic of the two-reactor configuration for anammox process (S – sampling points).

3.1.1. Influent

Anaerobic digester reject water (centrate) from a local wastewater treatment plant (North End Water Pollution Centre NEWPCC, Winnipeg, MB, Canada) was used as feed for the anammox process. Centrate was delivered twice a week, settled to remove solids, and stored at a constant temperature of 5 ^{o}C in the walk-in environmental chamber.

Centrate fed to the partial nitritation reactor had an average total ammonia concentration of 743 mg N/L (std. deviation 58). Alkalinity and VSS was at about 3950 mg $CaCO_3$/L (std. deviation 41) and 85 mg VSS/L (std. deviation

9), respectively. When centrate was occasionally diluted by flushing waters at the wastewater treatment plant (final effluent from the carbonaceous BOD removing wastewater treatment plant), TA and alkalinity were corrected to achieve the average concentrations of undiluted centrate. Other chemical parameters of the centrate were not monitored. During the research a phosphorus limitation was observed (Test 6 – R1), therefore phosphorus was also checked and sodium phosphate was added when phosphate was below 5-10 mg P-PO_4/L.

3.1.2. Partial nitritation

Partial nitritation reactor, PN reactor, had a working volume of 20 L - polycarbonate carboy was used (Figure 3-2). Masterflex peristaltic pumps were used to feed centrate to the reactor, decant the pre-treated centrate and to waste the excess biomass. Three air pumps with six air-stone-diffusers were used to provide oxygen for the nitritation process and mixing. The reactor had a foam collecting system, due to occasional intensive foam production during the aeration phase. The origin of the foam was not investigated; however, it was assumed to be caused by the flocculant used during the dewatering process at the wastewater treatment plant. Technological parameters for partial nitritation process are presented in the Table 3-1. The schematic of the reactor is depicted in Figure 3-2.

Table 3-1 Technological parameters for partial nitritation process in PN reactor

Parameter	Unit	Value
HRT	hours	7 - 40
SRT	days	5 - 15
Dissolved oxygen	mg O_2/L	0.5 – 2.0
pH	-	6.8 – 7.0
Total reactor volume	L	20
Exchange volume	L	1 - 6
Number of cycles	n	12
Feeding/Reacting	h	1.5
Settling	h	0.17
Decanting	h	0.33

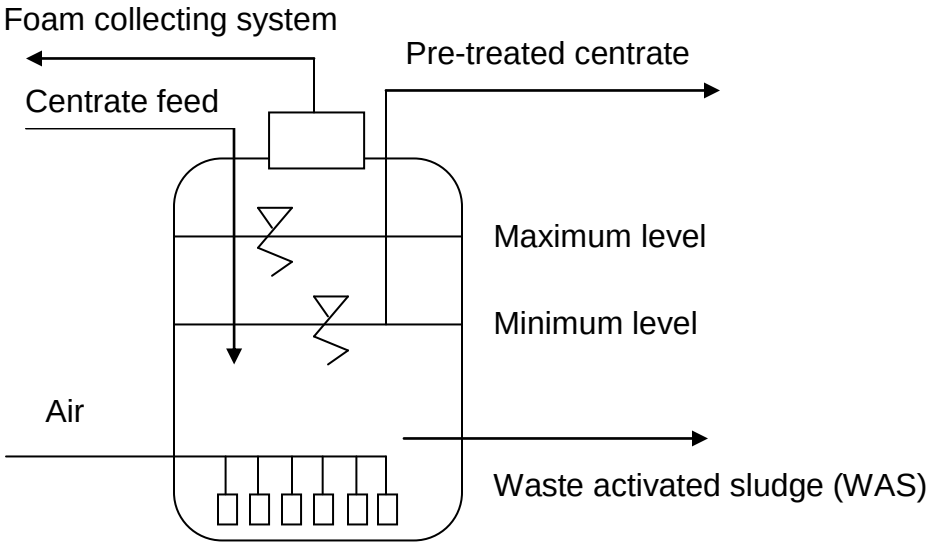


Figure 3-2 Schematic of the partial nitritation reactor, PN reactor

3.1.3. Equalization tank

The equalization tank had a working volume of 6L and it was made of a glass jar. It dampened the flow variations related to the sequencing performance of PN and allowed it to achieve a constant source of pre-treated centrate for the anammox stage (R1, R2, and R3). Nitrogen gas was used to flush the tank, preventing oxygen penetration. A schematic of the equalization tank is depicted in Figure 3-3.

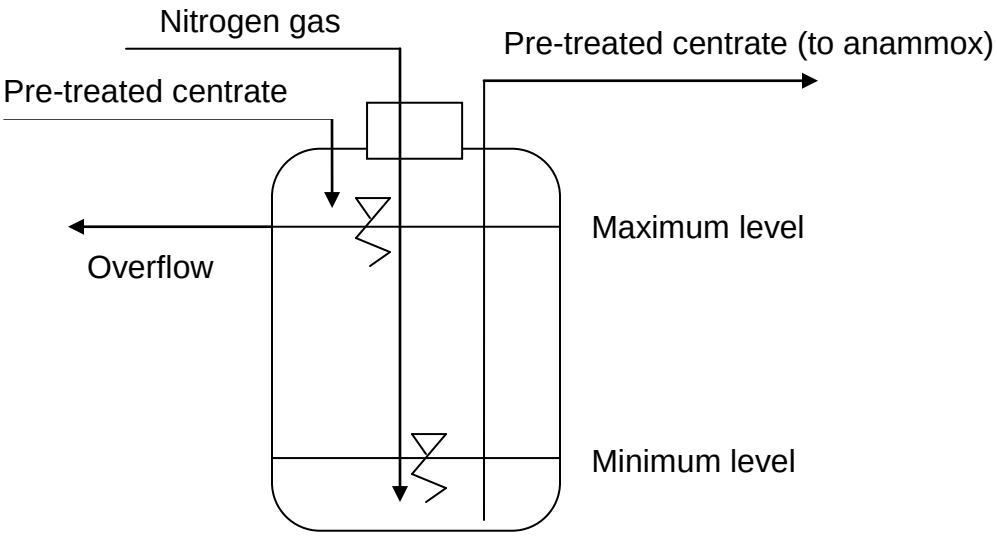


Figure 3-3 Schematic of the equalization tank

3.1.4. Anammox in MBBRs

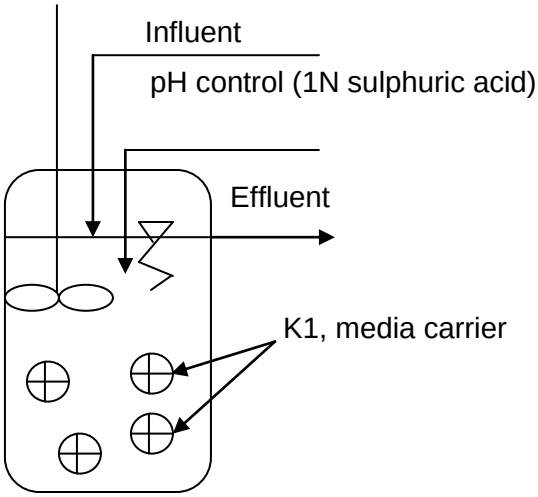
The MBBR reactors, R1 and R2, had a working volume of 3L each and were made of glass jar (Figure 3-4). Masterflex peristaltic pumps were used to

feed pre-treated centrate to the reactors. Biomass was cultivated in a form of biofilm attached to the carrier media (Kaldnes media K1). Reactors were filled 50% by volume with the carrier, providing a surface area of 250 m^2/m^3. Visual investigation of the media and solids mass balance (99% of all solids were in attached form) showed that anammox consortia were mostly growing on the protected area. Propeller mixers were used to mix reactors' content. The effluent from each reactor overflowed through the outlet port. Technological parameters for the MBBR anammox process, R1 and R2, are presented in the Table 3-2. The reactor configurations are presented in the Figure 3-4.

Table 3-2 Technological parameters for MBBR anammox process in R1 and R2

Parameter	Unit	Value	
		R1	R2
HRT	hours	Variable (0.9 - 6)	Variable (0.9 - 6)
Media filling ratio	%	50	50
Dissolved oxygen	mg O_2/L	Below detection	Below detection
pH	-	6.5	7.5-8.2
Total reactor volume	L	3.0	3.0
Mixing speed	RPM	200	200

a)



b)

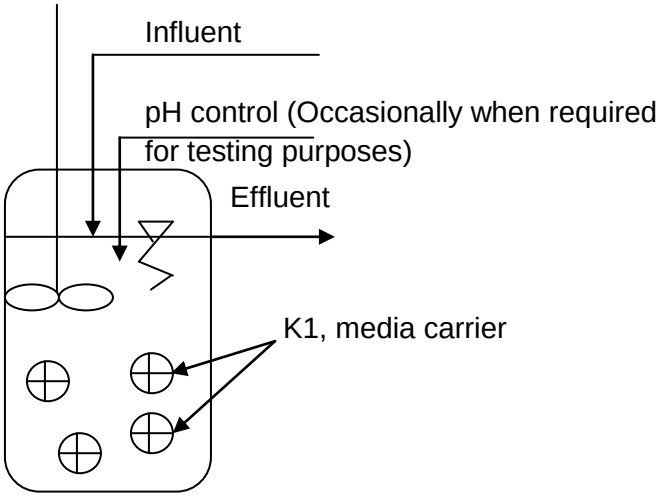


Figure 3-4 Schematic of the anammox MBBRs, a) R1 and b) R2

3.1.5. Anammox in SBR

The SBR anammox reactor, R3, had a working volume of 3L and was made of a glass jar (Figure 3-5). Masterflex peristaltic pumps were used to feed the pre-treated centrate to the reactor, decant the treated centrate and

waste the excess biomass. The biomass was cultivated in the form of flocks. A magnetic steering-bar mixer was used to mix the reactor's content. The reactor was closed with a gas-tight lid and dinitrogen gas was flushed through the gas head space during decant and waste periods, preventing oxygen penetration inside of the reactor. Technological parameters for partial anammox process are presented in the Table 3-3.

Table 3-3 Technological parameters for anammox process in reactor R3

Parameter	Unit	Value
HRT	hours	mostly 4 (100 – 4)
SRT	days	10 - 15
Dissolved oxygen	mg O_2/L	Below detection
pH	-	7.0
Total reactor volume	L	3
Exchange volume	L	1.5
Number of cycles	n	12
Feed time	h	0.75
React (incl. feeding) time	h	1.75
Settle time	h	0.08
Decant time	h	0.17

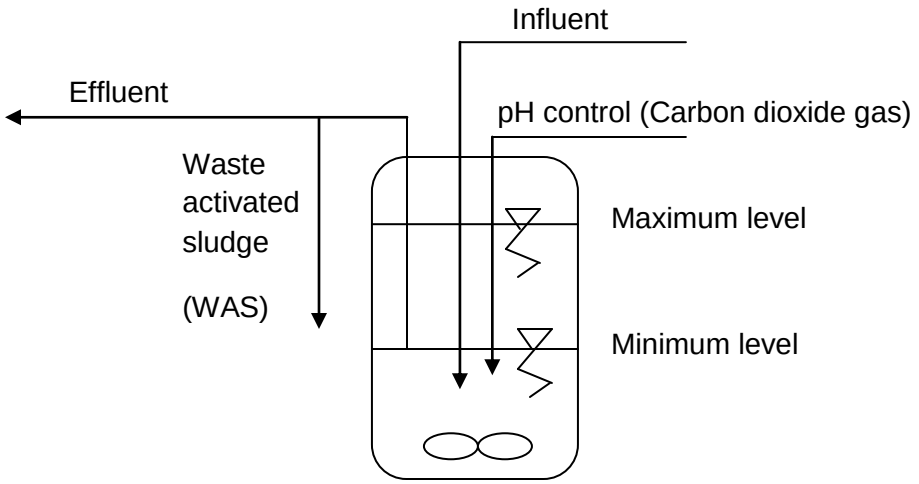


Figure 3-5 Schematic of the anammox SBR, R3 with suspended growth culture

3.1.6. Chemicals for pH control

The pH control was used to achieve desired pH conditions in R1, R2 and R3. The following chemicals were used:

- Sulphuric acid 1N (mostly R1)
- Sodium hydroxide 1N (mostly R2)
- Carbon dioxide gas (only R3)

3.1.7. Synthetic nutrient medium

A nutrient medium base was used in batch testing, according to Table 3-4. Nitrogen components, nitrite and total ammonia, were varying depending on the conducted experiment objectives. A more accurate description will be presented for the particular test described in the procedures section; however, the nutrient medium base was always the same during each batch test.

Table 3-4 A mineral composition for synthetic nutrient medium base

Nutrient component	Unit	Value
Alkalinity $NaHCO_3$	mg $CaCO_3$/L	350
KH_2PO4	mg/L	0.7
$MgSO_4*7H_2O$	mg/L	1.3
$CaCl_2*2H_2O$	mg/L	4
$ZnSO_4*7H_2O$	mg/L	1.25
$MnSO_4*H_2O$	mg/L	5
$CoCl_2*6H_2O$	mg/L	0.3
$FeSO_4*7H_2O$	mg/L	2
$CuSO_4$	mg/L	0.1
Na_2MoO_4	mg/L	0.35
KCl	mg/L	7
$NaNO_2$	mg N/L	50

Synthetic nutrient medium base was prepared according to the following procedure:

- Nutrient bottle was clean with laboratory cleaners and detergents and rinsed thoroughly,
- Nutrient bottle was filled with eight litres of deionised water
- Chemicals were added as presented in Table 3-4 (except alkalinity which was added after nutrient media deoxidation)
- Nutrient medium was deoxidised by nitrogen gas bubbling through a stone diffuser for at least 15 minutes to remove dissolved oxygen. Nutrient media was checked for dissolved oxygen and deoxidation process was repeated when any oxygen was detected.
- Alkalinity, nitrogen (nitrite and TA) and 20 mg/L L-cysteine (97%) were added to the nutrient medium
- Nutrient medium temperature was raised either by heating in the water bath or allowed to reach 35 °C by leaving it in the environmental chamber to equilibrate the temperature
- pH was adjusted to the desired value, just before the test was conducted

3.2. Analytical methods

3.2.1. Analysis of nitrogen species, pH, DO

Total ammonia TA, NO_2-N and NO_3-N were measured using an automatic flow injection analyser (QuichChem8500, Lachat Instruments) on a daily basis. The pH was measured at constant sampling points by an Accumet portable AP61 pH-meter with an Ag/AgCl electrode. Samples for nitrogen analysis were filtered through a 0.45 µm filter. Alkalinity and bulk dissolved oxygen were measured occasionally according to Standard Method 2320B.5, with potentiometric titration to end-point pH 4.5, and by the SensIon 378 HACH DO-meter, respectively. Samples were collected from the feed tank, the equalization tank, and the anammox reactors. Free, un-ionized ammonia FA was calculated based on Anthonisen et al. (1976) using indirect method:

$$\mathbf{FA} = \frac{\mathbf{TA} \cdot \mathbf{10^{pH}}}{\mathbf{e^{6344/(273+T)}} + \mathbf{10^{pH}}} \quad (\mathbf{mg\ N/L}) \quad \textbf{(Equation 3-1)}$$

where:

FA – free ammonia [mg N/L] - calculated

TA – total ammonia [mg N/L] - measured

T – temperature [ºC] – measured

pH - measured

Among different anammox studies (Jung et al., 2007; Tang et al., 2010; Fernández et al., 2010; Plaza et al., 2011) and studies where FA effect on

biomass activity was studied other than on anammox (Anthonisen et al., 1976; Martinelle et al., 1996; Villaverde et al. 2000; van Hulle et al., 2007; Vadivelu et al., 2007; Hellinga et al.,1999), FA was calculated using dissociation constant. Therefore, in this study, this method was used as a fast and reliable.

3.2.2. Procedure for TSS and VSS analysis

Total suspended solids (TSS) and volatile suspended solids (VSS) were measured occasionally for all reactors. Samples from PN and R3 were collected during the aeration cycle or mixing cycle, respectively. Such obtained samples were analyzed according to Standard Method 2540D and 2540E respectively, for TSS and VSS.

Solids accumulated on the moving media in R1 and R2 were estimated based on randomly chosen rings and subsequent scraping of the biomass from the media, using metal wire and de-ionised water (Two rings were scraped into separate crucible, biofilm from each ring into its own crucible. Results were averaged and had the standard deviation no greater than 10%). Such prepared samples were used to estimate solids content in reactors according to Standards Methods (1998). Given the total number of rings in the reactor, TSS and VSS accumulated on the carrier media were calculated according to the following formula:

$$\mathbf{TSS\ or\ VSS} = \mathbf{M} * \mathbf{N} \quad (\mathbf{mg\ TSS\ or\ mg\ VSS}) \qquad \textbf{(Equation 3-2)}$$

Where:

TSS – total suspended solids [mg TSS]

VSS – volatile suspended solids [mg VSS]

M – mass of solids (TSS or VSS) on a single ring [mg]

N – total number of rings in the reactor (R1 or R2) – 1900 rings

3.2.3. Procedure for gas production rate (GPR) measurement in respirometry tests

The Challenge AER-200 respirometer (Challenge technology) system was used for gas production rate measurements (GPR). The system consisted of the following parts:

- Water bath maintaining constant temperature of 35 °C by Refrigerating and Heating Recirculating Chillers (Polyscience)
- Eight test bottles equipped with cap and septa, each
- Eight flow cells equipped in bubble detector, each, for gas volume measurements.
- Tubing connecting test bottles with flow cells
- Challenge data acquisition program

Before each test on GPR was conducted, the following procedure was used for sample preparation:

- Test bottles were clean with laboratory cleaners and detergents and rinsed thoroughly,
- Test bottles were filled with nitrogen gas to remove oxygen,
- Nutrient medium was transferred from the nutrient bottle to the testing reactors in a way that no oxygen penetration was allowed inside of the nutrient bottle and testing reactors.
- Nitrogen components such as nitrite and TA were adjusted when required
- Biomass was transferred from reactor (R1, R2 or R3) to the test bottle and screw cap with butyl rubber septum was placed immediately.
- Testing bottles were placed to the water bath maintaining 35 °C
- 30 minutes equilibration time was provided before starting gas measurements.

3.3. Experimental procedures and methods

During the research period, anammox nitrogen removal rate (NRR) was investigated under different experimental conditions. Additionally, anammox

rates under varying nitrite and FA concentrations were evaluated through respirometry tests.

3.3.1. Representation of anammox activity

In this research, anammox activity was defined either by NRR or gas production rate (respiration tests). Anammox activity refers to overall nitrogen removed inside of reactors R1, R2 and R3 by a cultivated unknown culture. Although a detailed microbial analysis was not performed during this study, anammox activity was observed based on the nitrogen conversion stoichiometry, which was consistent with published literature. Additionally, the MBBR reactor was operated under anoxic conditions and fed with partially nitritated centrate, with a nitrogen balance of nitrite to ammonia ratio at about 1:1. These conditions have been described in literature as suitable for anammox consortium enrichment (Caffaz et al., 2006); therefore, nitrogen conversions achieved in this current study suggests anammox.

Nitrogen removal rate

The nitrogen removal rate (NRR) was a main parameter which was monitored during the entire study and it was calculated as the difference between the nitrogen load to and from the anammox reactor, according to the following equation:

$$\mathbf{NRR} = (\mathbf{Load\ N_{in}} - \mathbf{Load\ N_{out}}) / \mathbf{V}\ [\mathbf{mg\ N/Ld}] \qquad \textbf{(Equation 3-3)}$$

where:

Load N_{in} – sum of nitrogen coming into the reactor (TA, nitrite, nitrate) [mg N/d];

Load N_{out} – sum of nitrogen coming out of the reactor (TA, nitrite, nitrate) [mg N/d];

V – volume of the anammox reactor [L].

Specific nitrogen removal rate

The specific nitrogen removal rate (sNRR) was a main parameter which was monitored during reactor R3 operation under different testing conditions. It was calculated based on the mass balance during feeding and reaction time, respectively, according to the following equations:

sNRR in R3 during feeding time (0 – 45 min)

$$\mathbf{sNRR} = \mathbf{24} * \mathbf{60/t} * (\mathbf{TN_{in\ at\ time\ 0}} + \mathbf{Load\ N_{in\ at\ time\ t}} - \mathbf{TN_{in\ at\ time\ t}}) / \mathbf{VSS_{Total}}\ [\mathbf{g\ N/g\ VSS\ d}] \quad \textbf{(Equation 3-4)}$$

sNRR in R3 during reaction time when feeding pump was turned off (45 – 105 min):

$$\mathbf{sNRR} = \mathbf{24} * \mathbf{60/t} * (\mathbf{TN_{in\ at\ time\ 0}} - \mathbf{TN_{in\ at\ time\ t}}) / \mathbf{VSS_{Total}}\ [\mathbf{g\ N/g\ VSS\ d}] \quad \textbf{(Equation 3-5)}$$

Where:

24 * 60 / t – time coefficient where t is time interval between sampling in minutes (in most tests it was 10 minutes)

$TN_{\text{in at time 0}}$ – the sum of nitrogen mass (TA, nitrite, nitrate) inside of R3 at time 0 [g N];

Load $N_{\text{in at time t}}$ – the sum of nitrogen mass (TA, nitrite and nitrate) fed in to the reactor between time 0 and time t [g N];

$TN_{\text{in at time 0}}$ – the sum of nitrogen mass (TA, nitrite, nitrate) inside of R3 at time t [g N];

VSS_{Total} – the biomass inventory inside of reactor [g VSS]

Anammox nitrogen utilisation ratio (anammox stoichiometry)

The nitrogen utilisation ratio was the only parameter which suggested anammox, in this current study. Therefore, this parameter was regularly controlled during the entire research period and for each test conducted in this study. Anammox stoichiometry, the overall nitrogen conversion ratio [TA conversion: NO_2-N conversion: NO_3-N production], was calculated according to the following equation:

$$\mathbf{TA\ conversion}:\mathbf{NO2-N\ conversion}:\mathbf{NO3-N\ production} = \mathbf{1}:(\mathbf{nitrite\ removed/TA\ removed}):(\mathbf{nitate\ produced/TA\ removed}) \quad \textbf{(Equation 3-6)}$$

where:

TA removed, nitrite removed and nitrate produced – the difference between nitrogen load to and from the anammox reactor [mg N], during reactors operation. However, for the batch testing and during sNRR calculation for R3, they were calculated from the mass balance between sampling, at time 0 and at time t;

Michaelis-Menten based nitrogen removal rate

The saturation function, Michaelis-Menten based nitrogen removal rate (MNRR), was used to predict the NRR, based on the nitrite concentration where ammonium was assumed to not be the NRR substrate limiting parameter. The kinetic parameters, such as maximum nitrogen removal rate (maxNRR) and saturation constant for nitrite (K_{NO2}), were estimated based on the nitrite concentrations and corresponding NRR values, under varying nitrogen loading rates (Procedure 3). The MNRR was primarily developed for the MBBR anammox reactor due to high NRR sensitivity to nitrite concentration, where nitrite was stimulating the NRR in a wide nitrate range (0 – 200 mg N/L). The MNRR was calculated according to the following equation:

$$\mathbf{MNRR} = \mathbf{max\,NRR}\frac{\mathbf{S_{NO2}}}{\mathbf{S_{NO2}+K_{NO2}}}\ [\mathbf{mg\,N/Ld}] \qquad \textbf{(Equation 3-7)}$$

where:

MNRR – Michaelis-Menten based nitrogen removal rate [mg N/Ld]

max NRR – maximum nitrogen removal rate [mg N/Ld]

S_{NO2} – nitrite concentration [mg NO_2-N/L]

K_{NO2} – saturation constant for nitrite [mg N/L]

3.3.2. Steady state system operation

Steady state anammox reactor operations were assumed when the NRR was varying no more than 10% under constant nitrogen loading rate. Additionally, NRR achieved inside of the reactor based on the mass balance, was compared with NRR calculations based on the Michaelis-Menten relation for the actual nitrite concentration inside of the reactor. The performance of reactor was considered stable when the difference between the NRR and Michaelis-Menten NRR was below 10%.

3.3.3. Batch testing

The purpose of batch testing was to investigate the initial (or immediate) response to changes in FA, total ammonia, pH and nitrite. During batch testing, anammox rates were estimated either based on the nitrite depletion rate or dinitrogen gas production rate (respirometry tests).

3.3.4. Experimental procedures

The outline of the conducted research, which involved a series of different tests, followed thirteen procedures. The compilation of research periods,

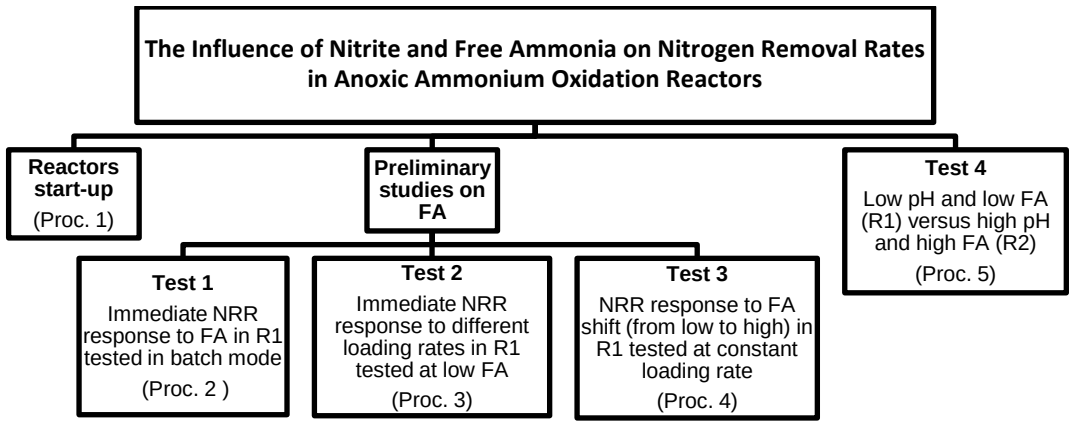
procedures, tests and its raw data location in appendices is presented in the Table 3-5. A simplified version of the table is depicted in the Figure 3-6. To navigate the reader through the result and discussion section, appropriate chapter sections corresponding with particular parts of the Figure 3-6 will be presented at front of each results and discussion chapter.

Table 3-5 The outline of the research periods, procedures with its description and corresponding tests, and location of the raw data in the appendixes

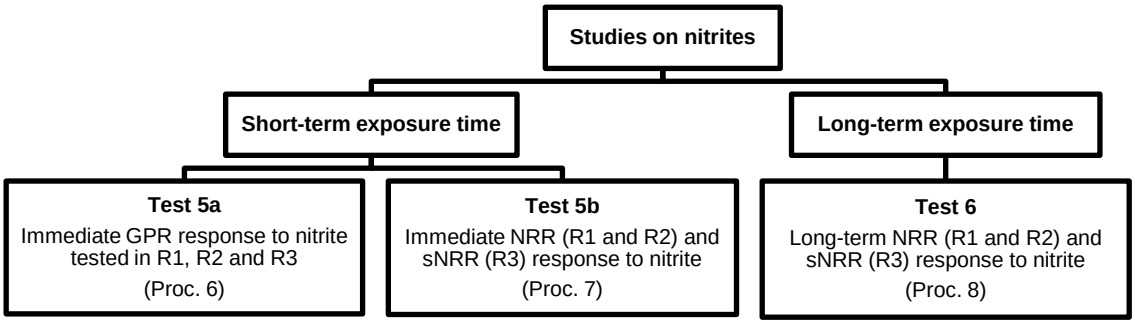
Research period		Procedure with its description	Test number	Location of the raw data in the appendix
Reactors start-up		**Procedure 1** – Reactors start-up	-	Page 234
Primary studies on FA		**Procedure 2** - Immediate nitrogen removal rate (NRR) response to different free ammonia (FA) concentrations up to 14.6 mg N/L in reactor R1 (variable pH) operated in the batch mode during the test	Test 1	Page 252
		Procedure 3 - Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 0.8 mg N/L in reactor R1 (constant pH set at 6.5) operated in the continuous feed mode at variable loading rates during the test	Test 2	Page 257
		Procedure 4 - Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 11.9 mg N/L in reactor R1 (self maintaining pH in the range of 6.9 and 8.2) operated in the constant continuous feed mode during the test	Test 3	Page 259
Low pH and low FA versus high pH and high FA		**Procedure 5** - Low pH and low FA versus high pH and high FA – long term anammox reactors operation	Test 4	Page 261
Studies on nitrite	**Short-term exposure time**	**Procedure 6** – Immediate anammox rate response to nitrite tested based on gas production rate (GPR) response to nitrite	Test 5a – R1; Test 5a – R2; Test 5a – R3	Page 269 Page 273 Page 277
		Procedure 7 - Immediate anammox rate response to nitrite tested based on NRR response to nitrite for R1 and R2, and sNRR response to nitrite for R3	Test 5b – R1; Test 5b – R2; Test 5b – R3	Page 281 Page 282 Page 285
	Long-term exposure time	**Procedure 8** – Long-term anammox response to nitrite, tested based on NRR response to nitrite for R1 and R2, and sNRR response to nitrite for R3	Test 6 – R1; Test 6 – R2; Test 6 – R3	Page 292 Page 295 Page 298

Table 3.5 continuation				
Research period		**Procedure with its description**	**Test number**	**Location of the raw data in the appendix**
Studies on free ammonia	**Short-term exposure time**	**Procedure 9** - Immediate nitrogen removal rate (NRR) response to FA tested in R1	Test 7a – R1	Page 312
		Procedure 10 - Immediate gas production rate (GPR) response to free ammonia (FA) tested in R3	Test 7b – R3	Page 344
		Procedure 11 - Immediate specific nitrogen removal rate (sNRR) response to free ammonia (FA) tested in R3	Test 7c – R3	Page 347
	Long-term exposure time	**Procedure 12** - Long-term nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R2	Test 8a –R2	Page 350
		Procedure 13 - Long-term nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R1 and R2	Test 8b – R1 and R2	Page 352

a) Preliminary studies



b) Detailed studies of nitrite impact



c) Detailed studies of free ammonia impact

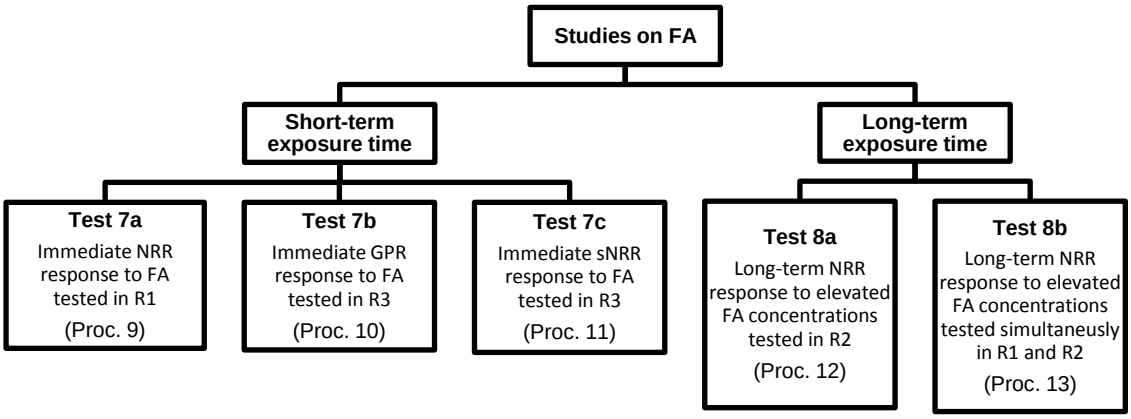


Figure 3-6 The outline of the research periods, procedures with its description and corresponding tests

3.3.4.1. Procedure 1 – Reactors start-up

The main purpose of this procedure was to start-up anammox MBBR reactors, R1 and R2 and anammox SBR, R3. Additionally, it was intended to investigate reactors' NRR performance in face of changing nitrogen loading rate. Figure 3-7 presents Procedure 1 in the overall preliminary studies outline.

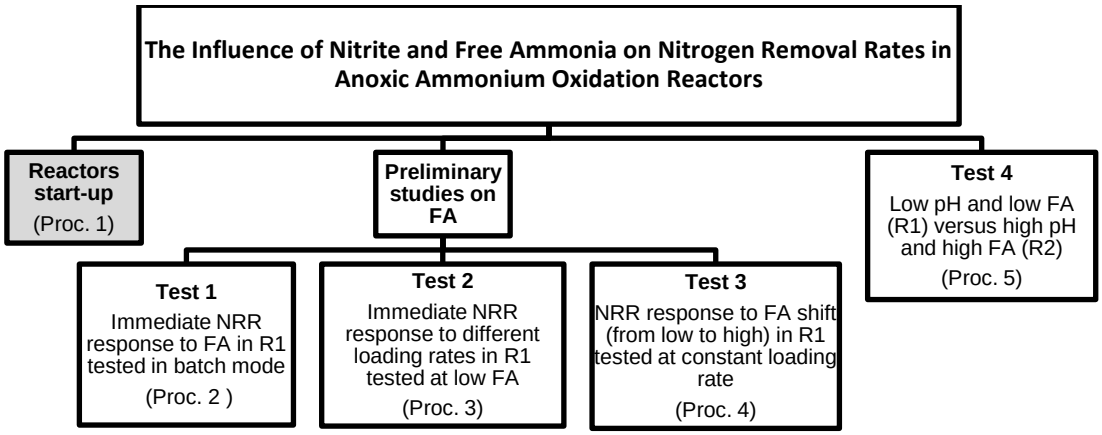


Figure 3-7 Procedure 1 in the overall preliminary studies outline

During the first phase of reactors start-up, R1 and R2, the nitrogen loading was increased over time. Nitrogen loading to reactors was increased stepwise based on the nitrite concentration inside of the reactor; this was maintained below the literature suggested inhibitory concentration of 10 mg N/L (Szatkowska 2007). After achieving NRR (about 240 mg N/Ld), similar to this one at the pilot plant from which the biomass originated, the nitrogen loading rate was varying in a wide range (90 – 260 mg N/Ld). The purpose of such reactors' operation was first, to test reactors' stability in face of variable

loading rate, second, to verify reactors identity in face of changing nitrogen loading conditions. Reactors similarity was analysed from the perspective of NRR, nitrogen concentrations inside of reactor, pH and stoichiometry. During this time, both MBBR reactors were operated without pH control.

During the second phase, pH in reactor R1 was set at 6.5, while R2 remained without pH control. Similar to the first phase of reactors start-up, the nitrogen loading was increased over time, allowing for an increase in NRR up to about 3000 mg N/Ld. As before, nitrite was maintained at a level no greater than 10 mg N/L. When NRR of about 3000 mg N/Ld was achieved in R1, the loading rate was lowered, achieving NRR of about 246 mg N/Ld. Then, the nitrogen loading rate was increased rapidly in a way that NRR was increased daily, over a 6 day period, on an average rate of 466 mg N/Ld per day. The purpose of such a reactor R1 operation was to verify its stability in the face of sudden nitrogen loading rate changes compared to R2, where the reactor loading rate was increased slowly (over a 25 day period, NRR was increased daily on an average of 100 mg N/Ld per day). Additionally, pH 6.5 was checked, to see whether it was possible to operate the anammox MBBR reactor under such a low pH (not studied in literature).

During the first phase of reactor R3 start-up, the pH was not controlled. Nitrogen loading rate was increased slowly, allowing NRR to increase on an average rate of 23 mg N/Ld per day over a 23 day period. After reaching an NRR of 368 mg N/Ld, the nitrogen loading rate was not changed for 35 days.

Then, the nitrogen loading rate was increased again, allowing NRR to increase at 244 mg N/Ld per day over a 3 day period.

During the second phase of reactor R3 start-up, the pH was controlled at 7.0. The same as before, the nitrogen loading rate was increased slowly, allowing NRR to increase on an average rate of 19 mg N/Ld per day over a 32 day period. After reaching an NRR of 1002 mg N/Ld, the nitrogen loading rate was not changed for 63 days. Then, the nitrogen loading rate was increased again, allowing the NRR to increase at 167 mg N/Ld per day, over an 11 day period.

3.3.4.2. Procedure 2 – Immediate nitrogen removal rate (NRR) response to different free ammonia (FA) concentrations up to 14.6 mg N/L in reactor R1 (variable pH) operated in the batch mode during the test (Test 1)

The main purpose of this procedure was to investigate immediate NRR response to FA in reactor R1. Figure 3-8 presents Procedure 2 in the overall preliminary studies outline.

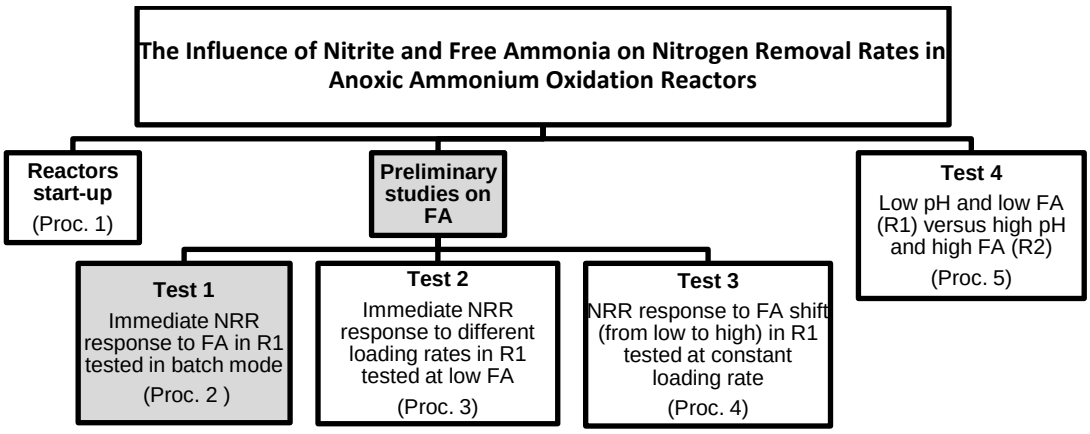


Figure 3-8 Procedure 2 in the overall preliminary studies outline

A series of kinetic tests were carried out with various FA concentrations under different pH conditions (Table 3-6). These tests were carried out in batch mode, with the starting concentration of FA and nitrite of 0.3 to 14.6 mg N/L and about 30 mg N/L, respectively; samples were taken every 5 minutes. After the depletion of nitrite, it was added again to reach the bulk concentration about 30 mg N/L. The pH was constant during the test and it was controlled by H_2SO_4 addition. During the test, the feed to the reactor was stopped. First, the pH was set at a desired value; second, TA was added to reach the desired FA concentration (calculated according to Equation 3-1); lastly, nitrite in the form of $NaNO_2$ powder was added to reach about 30 mg N/L. The test was started after 5 minutes from nitrite addition. This time was arbitrarily chosen, assuming that NRR reached its equilibrium.

Table 3-6 The FA and pH set values at constant temperature of 35 °C during Test 1

FA [mg N/L]	pH value
0.3 - 2.3	7.0
5.7	7.5
5.9	8.0
6.2	8.0
10.3	8.0
12.5	8.0
14.6	7.5

The feed composition to reactor R1 and its performance during this research period is presented in Table 3-7.

Table 3-7 - The feed composition and overall operation of anammox MBBR during the Test 1

Parameter	Parameter value
	Feed composition to the anammox MBBR
pH	about 6.80
TA [mg N/L]	333.7 ± 35.7
Nitrite [mg N/L]	276.1 ± 21.5
Nitrate [mg N/L]	0.4 ± 1.2
Dissolved oxygen [mg O_2/L]	below 0.5
Alkalinity [mg $CaCO_3$/L]	185 ± 25
VSS [mg VSS/L]	40 ± 8
	Operation of MBBR
pH	6.50 ± 0.01
TA [mg N/L]	117.0± 7.4
Nitrite [mg N/L]	3.3 ± 0.5
Nitrate [mg N/L]	44.1 ± 5.4
Dissolved oxygen [mg O_2/L]	below detection limit
Alkalinity [mg $CaCO_3$/L]	104± 17
NRR [mg N/Ld]	1611 ± 223
VSS effluent [mg VSS/L]	about 80
Overall nitrogen balance [TA conversion: NO_2-N conversion: NO_3-N production]	1:(1.22±0.05):(0.18±0.02)

3.3.4.3. Procedure 3 - Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 0.8 mg N/L in reactor R1 (constant pH set at 6.5) operated in the continuous feed mode at variable loading rates during the test (Test 2)

The purpose of this test was to evaluate the effect of nitrogen load change and associated nitrite accumulation inside of R1 on NRR during a continuous feed operation. Secondly, it was intended to describe NRR by Michaelis-Menten relation (Equation 3-7). Figure 3-9 presents Procedure 3 in the overall preliminary studies outline.

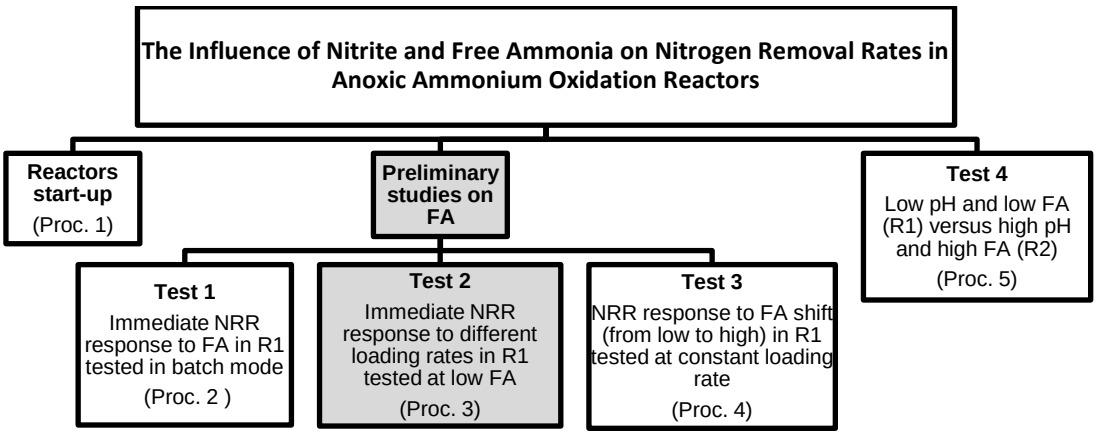


Figure 3-9 Procedure 3 in the overall preliminary studies outline

Desired loads (11.2, 14.2 and 19.0 g N/Ld) were achieved by changing the flow to the reactor (HRT was set at 1.54, 1.20 and 0.91 h, respectively). The test was continued as long as stable nitrogen concentrations in the effluent and NRR were achieved (parameter changes no greater than 10% within one

hour of the test). The test was conducted under pH control set at 6.5, to provide low FA (below 1.7 mg N/L). The operational stability of the reactor was assessed in response to the variable loads and the effect of nitrite concentration on maximum NRR was evaluated. The data points from this test were compared to NRR obtained under long-term reactor operation (Test 4). The effect of nitrite on NRR was evaluated using the Michaelis-Menten relation, where NRR could be stimulated by nitrite as long as no nitrite inhibition occurs. Additionally, the Michaelis-Menten based NRR was used to evaluate the anammox rate losses during the pH and associated FA change on NRR in Test 3.

The feed composition to reactor R1 and its performance during this research period is presented in the Table 3-8.

Table 3-8 - The feed composition and overall operation of anammox MBBR during Test 2

Parameter	Parameter value
	Feed composition to the anammox MBBR
pH	about 6.80
TA [mg N/L]	385.7 ± 35.1
Nitrite [mg N/L]	359.1 ± 14.7
Nitrate [mg N/L]	1.2 ± 3.8
Dissolved oxygen [mg O_2/L]	below 0.5
Alkalinity [mg $CaCO_3$/L]	192 ± 16.4
VSS [mg VSS/L]	33 ± 5
	Operation of MBBR
pH	6.50 ± 0.01
TA [mg N/L]	132.5 ± 26.9
Nitrite [mg N/L]	32.6 ± 8.6
Nitrate [mg N/L]	58.0 ± 5.2
Dissolved oxygen [mg O_2/L]	below detection limit
Alkalinity [mg $CaCO_3$/L]	88.0 ± 22.1
NRR [mg N/Ld]	6185 ± 414
Overall nitrogen balance [TA conversion: NO_2-N conversion: NO_3-N production]	1:(1.24±0.05):(0.22±0.02)

3.3.4.4. Procedure 4 - Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 11.9 mg N/L in reactor R1 (self maintaining pH in the range of 6.9 and 8.2) operated in the constant continuous feed mode during the test (Test 3)

The purpose of this procedure was to evaluate NRR stability in the face of variable pH and associated FA changes, during 26.5 hours of the continuous feed to reactor R1. Figure 3-10 presents Procedure 4 in the overall preliminary studies outline.

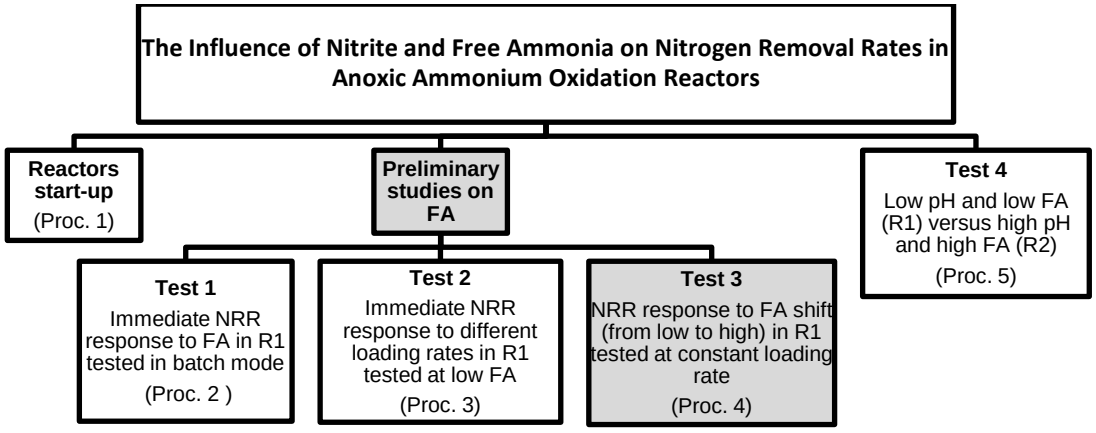


Figure 3-10 Procedure 4 in the overall preliminary studies outline

Changes in the NRR were evaluated during the continuous feed period over 26.5 hours when the pH control was turned off at time 0 and FA was accumulating. Following this period, the pH was adjusted back to 6.9 and NRR was monitored up to 2.5 hours. This was compared to the NRR obtained under low FA concentrations during Test 2 and long-term reactor operation

(Test 4) using the Michaelis-Menten equation for specific nitrite concentrations. The biomass rate was evaluated based on the comparison between the NRR achieved inside the reactor and Michaelis-Menten based NRR, for a specific nitrite concentration. It was assumed that any difference between those two values would represent the anammox rate loss (actual NRR versus Michaelis-Menten based NRR). This method was verified at the end of this test period. The maximum predicted Michaelis-Menten based NRR earlier in this test (time 26.5 h, high nitrite) at high FA was compared with the measured NRR during reactor operation at a similar nitrite concentration, but low FA conditions.

Before Test 3 was conducted, the pH set point was changed from 6.5 to 6.9. Consistent NRR was recorded at 4630 ± 120 mg N/Ld for 22 days after the pH changed from 6.5 to 6.9. An overall nitrogen balance once again indicated anammox activity (Table 3-9). Despite a temporary pH change from pH 6.5 to 6.9, no significant effect on NRR was observed. This was verified based on an NRR comparison between Michaelis-Menten based NRR and actual NRR, inside the MBBR reactor. The Michaelis-Menten based NRR for average nitrite concentrations of 18.2 ± 0.8 mg NO_2-N/L was 4634 mg N/Ld which is very close to the theoretical value achieved during this research period. This provides confidence in the values obtained from Michaelis-Menten based NRR predictions in relation to nitrite concentration.

The feed composition to reactor R1 and its performance during this research period is presented in the Table 3-9.

Table 3-9 - The feed composition and overall operation of anammox MBBR during Test 3

Parameter	**Parameter value**
	Feed composition to the anammox MBBR
pH	about 6.80
TA [mg N/L]	438.5 ± 24.7
Nitrite [mg N/L]	339.8 ± 12.7
Nitrate [mg N/L]	1.3 ± 1.8
Dissolved oxygen [mg O_2/L]	below 0.5
Alkalinity [mg $CaCO_3$/L]	181 ± 15
VSS [mg VSS/L]	37 ± 2
	Operation of MBBR
pH	6.90 ± 0.01
TA [mg N/L]	148.3 ± 12.7
Nitrite [mg N/L]	18.2 ± 0.8
Nitrate [mg N/L]	53.9 ± 2.9
Dissolved oxygen [mg O_2/L]	below detection limit
Alkalinity [mg $CaCO_3$/L]	about 114.0
NRR [mg N/Ld]	4630 ± 120
Overall nitrogen balance [TA conversion: NO_2-N conversion: NO_3-N production]	1:(1.11±0.04):(0.18±0.01)

3.3.4.5. Procedure 5 - Low pH and low FA versus high pH and high FA – long term anammox reactors operation (Test 4)

The purpose of this procedure was to evaluate NRR stability in two parallel reactors operated under low pH set at 6.5 (R1) and ambient pH (R2), in face of three nitrogen loading rates scenarios and associated three nitrite concentrations. The nitrite was also evaluated as an inhibitor. Figure 3-11 presents Procedure 5 in the overall preliminary studies outline.

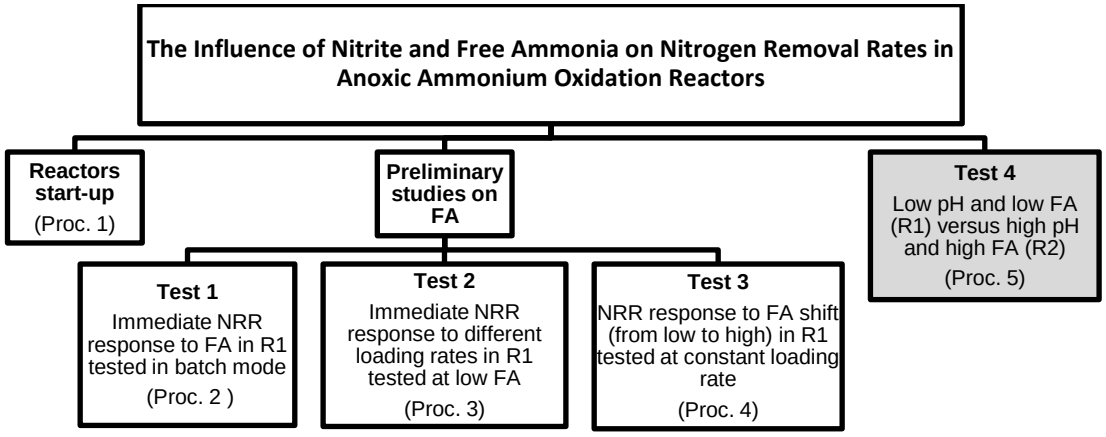


Figure 3-11 Procedure 5 in the overall preliminary studies outline

Two parallel reactors were operated for 168 days and 102 days, respectively, for R1 and R2. The R1 had pH set at 6.5 and R2 was naturally maintaining pH between 7.5 and 8.1, without pH control.

The two reactors were operated at the medium loading rate maintaining nitrite concentration at about 15 mg N/L for one year, before tests were

conducted on stability. During these tests, the reactors' response to three (low, medium and high) nitrogen loads and (low, medium and high) nitrite concentrations under the two different pH conditions was investigated. The low and medium loading rates were set in a way that they would not cause nitrite accumulation inside of the reactors greater than about 10 mg N/L. The high loading rate was set in way that nitrite would be greater than 10 mg N/L but no more than about 30 – 50 mg N/L. During low and medium loading rate periods, nitrogen loading rates to both reactors were the same. However, during the high loading period, the loading rate to R2 had to be lowered due to NRR instabilities and nitrite accumulations exceeding 50 mg N/L.

3.3.4.6. Procedure 6 – Immediate anammox rate response to nitrite tested based on gas production rate (GPR) response to nitrite (Test 5a – R1; Test 5a – R2; Test 5a – R3)

The purpose of this procedure was to test immediate anammox rate response to nitrite, based on the gas production rate. Figure 3-12 presents

Procedure 6 in the overall detail nitrite studies outline.

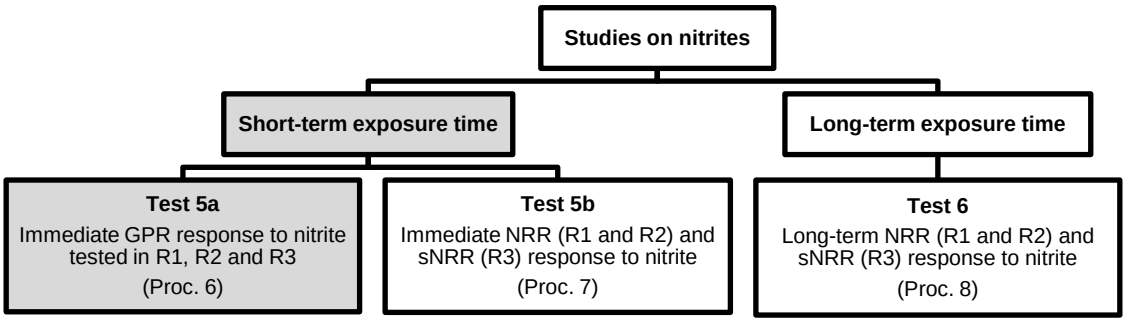


Figure 3-12 Procedure 6 in the overall detail nitrite studies outline

This test was conducted in duplicate. The GPR was obtained according to the following procedure:

- Sampling bottles were placed in the water bath maintaining 35 °C and gas tubing were connected.
- The cumulative gas volumes from sampling bottles were recorded over the two hours of the experiment (baseline condition, where nitrite concentration was 50 mg N/L for each sampling reactor. The GPR was calculated based on the cumulative gas volume recorded during the last hour of the experiment)
- At time 2 hours, the desired nitrite concentration was adjusted by adding concentrated nitrite solution into each sampling bottle, except control one (first sampling bottle, where starting nitrite concentration

remained the same as at the end of first phase). The 5 mL sodium nitrite solution was injected. Each test had its own concentrated nitrite solution; therefore the injected volume into specific testing reactor was constant.

- The cumulative gas volumes from sampling bottles were recorded over three and a half hours of the experiment (inhibition condition, however results from the last hour of the experiment were used for GPR calculation).

The nitrite concentrations, which were tested through this procedure, were as follows: about 50 mg N/L (control sample), 50 mg N/L, 75 mg N/L, 100 mg N/L, 150 mg N/L, 200 mg N/L, 300 mg N/L, 400 mg N/L.

The GPR_0 and GPR for each respective batch test (baseline and nitrite test) were calculated based on the gas production rate according to Equation 3-8.

$$GPR_0 \text{ and } GPR = \alpha V \text{ [m L/ reactor d]} \quad \textbf{(Equation 3-8)}$$

where:

αV – slope of the linear regression for volume [m L/reactor d];

GPR_0 - is the gas production rate during the last hour of the first two hours run (baseline);

GPR - is the cumulative gas production rate during last hour of the second three and a half hours run (test under elevated nitrite condition).

Then, the activity percentage for each test in duplicate, % GPR, which represented the change in the activity between control sample and the sample where nitrite to be tested, was calculated based on the following equation:

$$\%\,\mathbf{GPR} = \left(\frac{\mathbf{GPR}}{\mathbf{GPR_0}}\right) \cdot \mathbf{100}\ [\%] \qquad \textbf{(Equation 3-9)}$$

After obtaining results from the duplicate under the same nitrite condition, the % GPR was averaged. The same calculation method was used for all nitrite conditions (in all tests).

3.3.4.7. Procedure 7 - Immediate anammox rate response to nitrite tested based on NRR response to nitrite for R1 and R2, and sNRR response to nitrite for R3 (Test 5b – R1; Test 5b – R2; Test 5b – R3)

The purpose of this procedure was to test the immediate NRR and sNRR response to nitrite in R1 and R2, and R3, respectively. Figure 3-13 presents Procedure 7 in the overall detail nitrite studies outline.

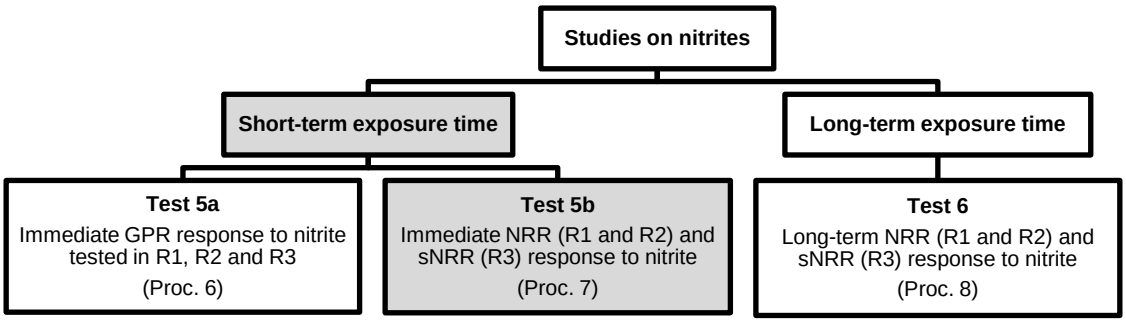


Figure 3-13 Procedure 7 in the overall detail nitrite studies outline

The testing procedure for tests Test 5b – R1 and Test 5b – R2 was similar to Procedure 3, where elevated nitrite was achieved through the nitrogen loading rate manipulation. At FA of about 7.5 mg N/L loading rate change from 7.5 to 10.7 g N/Ld caused reactor destabilization. Then, FA was lowered (FA range 0.4 – 0.8 mg N/L; pH set at 6.5) and new loading rates were set (lading rates: 5.4, 8.5, 8.4, 19.9, 15.3, 9.6, 3.5 g N/L d).

During the Test 5b – R3, the elevated nitrite concentrations inside of the reactor were achieved by the addition of extra nitrite (200 mg N/L) into the feed just before the test was conducted.

3.3.4.8. Procedure 8 – Long term anammox response to nitrite, tested based on NRR response to nitrite for R1 and R2, and sNRR response to nitrite for R3 (Test 6 – R1; Test 6 – R2; Test 6 – R3)

This procedure was used to investigate the long-term nitrite inhibitory effect on NRR during reactors operation, R1, R2 and R3. Figure 3-14 presents Procedure 8 in the overall detail nitrite studies outline.

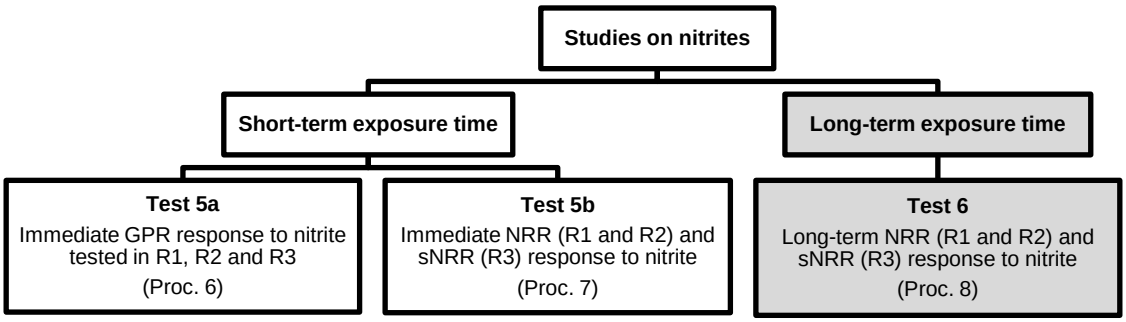


Figure 3-14 Procedure 8 in the overall detail nitrite studies outline

During the test, first, reactors were operated under the regular reactor operation mode (low nitrite inside of reactor) for 35 days. The purpose of the first phase was to document stable NRR and sNRR performance under regular nitrite concentrations within a nitrite range up to 50 mg N/L. Then, during the second phase of the testing, nitrite concentrations were increased inside of the reactors by adding nitrite into the feed. The purpose of the second phase was to find the nitrite inhibition threshold concentration which

would hinder the NRR and sNRR, respectively, for R1 and R2, and R3. Additionally, it was intended to demonstrate the inhibitory nature of nitrite during long-term reactors operation.

3.3.4.9. Procedure 9 - Immediate nitrogen removal rate (NRR) response to FA tested in R1 (Test 7a – R1)

Batch tests were used to investigate the initial response of the reactor to sudden changes in TA, pH and FA. The FA inhibition threshold concentration for these consortia was also determined through these tests. Figure 3-15 presents Procedure 9 in the overall detail free ammonia studies outline.

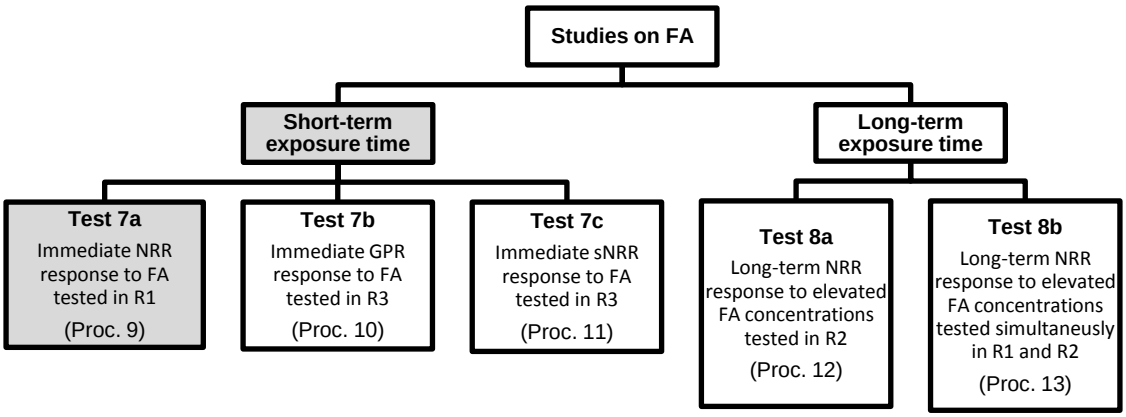


Figure 3-15 Procedure 9 in the overall detail free ammonia studies outline

Six, 1.2 L reactors from R1 to R6 (1 L liquid volume, 0.2 L gas head space volume for each reactor) were set-up in the walk-in environmental chamber maintaining a constant temperature of 35^0C. Reactors had rubber caps to prevent oxygen penetration. Strict anoxic conditions were maintained by a N_2 gas atmosphere in the head space and L-cysteine (97%) addition (20 mg/L) in the bulk liquid. One L of synthetic medium was used in each test (Chapter 3.1.7, page 50). In total, twenty eight tests were conducted with FA (0.5, 1.0, 2.0, 5.0, 10.0, 25.0, 50.0 mg N/L) and pH (6.5, 7.0, 7.5, and 8.0) adjusting total ammonia in each individual test for pH and FA according to Equation 3.1. During the tests, the pH was maintained by a pH controller using 1N sulphuric acid. The acid addition was below 10 mL for each testing reactor to sustain desired pH condition.

Each test, conducted in triplicate, consisted of two phases. First, eight pieces of carrier media for each testing reactor (R1, R2 and R3 - first triplicate) were transferred from the MBBR anammox reactor. Then the specific anammox activity (SAA_0 - baseline) was obtained under conditions identical to those in the MBBR (pH 6.5 and total ammonia 150 mg N/L). The duration of this test was 150 minutes. Then, the fixed film culture (eight pieces of carrier media for each bottle) was transferred into new bottles R4, R5 and R6 (from R1 into R4, from R2 into R5, and from R3 into R6 - second triplicate) where the specific anammox activity (SAA) was tested under the new FA and pH condition to be tested. The duration of this test was 180 minutes. Samples in

all tests were taken every 30 minutes (5 mL with a syringe) and analysed for nitrogen species.

The SAA_0 and SAA for each respective batch test (baseline and FA test) were calculated based on the nitrite depletion rate according to Equation 3-10.

$$SAA_0 \text{ and } SAA = \alpha NO_2 \text{ [mg N/L d]} \quad \textbf{(Equation 3-10)}$$

where: αNO_2 – slope of the linear regression for nitrite [mg N/Ld]; SAA_0 is the specific anammox activity obtained in the first run (baseline); SAA is the specific anammox activity obtained in the second run (test under defined FA and pH condition).

The activity percentage for each test in triplicate, % SAA, was then calculated based on the following equation:

$$\%\,\mathbf{SAA} = \left(\frac{\mathbf{SAA}}{\mathbf{SAA_0}}\right) \cdot \mathbf{100}\ [\%] \quad \textbf{(Equation 3-11)}$$

After obtaining results from triplicates under the same FA and pH condition, the % SAA was averaged (average %SAA). The same calculation method was used for all FA and pH conditions (in total twenty eight tests).

Eventually, relative average % SAA to maximum average %SAA obtained under any condition was calculated according to Equation 3-10. Therefore, the relative % SAA, was used to describe the effect of total ammonia (TA = NH_4^+-N + NH_3-N), free ammonia (NH_3-N) and pH on anammox activity.

$$\mathbf{relative}\ \%\mathbf{SAA} = \left(\frac{\%\mathbf{SAA}}{\mathbf{maximum}\ \%\mathbf{SAA}}\right) \cdot \mathbf{100}\ [\%] \quad \textbf{(Equation 3-12)}$$

The procedure for this test was modified from Dapena-Mora et al. (2007). The purpose of the modification was to compensate for the difference between the baseline test at pH 6.5 and that at pH 7.0 tests. Otherwise, values for %SAA at pH 7 would be greater than 100% (the maximum SAA at pH 7.0 was greater than SAA_0 at pH 6.5).

The specific anammox activity (SAA) represents the specific nitrogen removal rate obtained inside of testing reactors, where eight pieces of carrier media were placed. The SAA was calculated based on the nitrite depletion rate. However, specific nitrogen removal rate (sNRR) represents the specific nitrogen removal rate obtained inside of anammox SBR reactor, where biomass concentration was known. The sNRR was calculated based on the TA, nitrite and nitrate mass balance.

The feed composition to reactor R1 and its performance during this research period is presented in Table 3-10.

Table 3-10 - The feed composition and overall operation of anammox MBBR during Test 7a – R1

Parameter	Parameter value
	Feed composition to the R1
pH	about 6.80
TA [mg N/L]	385.7 ± 35.1
Nitrite [mg N/L]	359.1 ± 14.7
Nitrate [mg N/L]	1.2 ± 3.8
Dissolved oxygen [mg O_2/L]	below 0.5
Alkalinity [mg $CaCO_3$/L]	192 ± 16.4
VSS [mg VSS/L]	33 ± 5
	Operation of R1
pH	6.50 ± 0.01
TA [mg N/L]	132.5 ± 26.9
Nitrite [mg N/L]	32.6 ± 8.6
Nitrate [mg N/L]	58.0 ± 5.2
Dissolved oxygen [mg O_2/L]	below detection limit
Alkalinity [mg $CaCO_3$/L]	88.0 ± 22.1
NRR [mg N/Ld]	6185 ± 414
Overall nitrogen balance [TA conversion: NO_2-N conversion: NO_3-N production]	1:(1.24±0.05):(0.22±0.02)

Additionally, the immediate NRR response to TA under FA concentrations below 2 mg N/L at constant pH of 6.5 was tested. Desired TA concentrations (12.5 mg N/L, 25 mg N/L, 50 mg N/L – baseline, 100 mg N/L) were achieved by changing the flow to the reactor. The test was conducted as long as stable nitrogen concentrations in the effluent and NRRs were achieved (parameter

change no greater than 10% within one hour of the test). Results of this test are presented in Appendix 5 (page 341).

3.3.4.10. Procedure 10 - Immediate gas production rate (GPR) response to free ammonia (FA) tested in R3 (Test 7b – R3)

The purpose of this procedure was to test immediate anammox rate response to FA based on the gas production rate, tested on the biomass from reactor R3. Figure 3-16 presents Procedure 10 in the overall detail free ammonia studies outline.

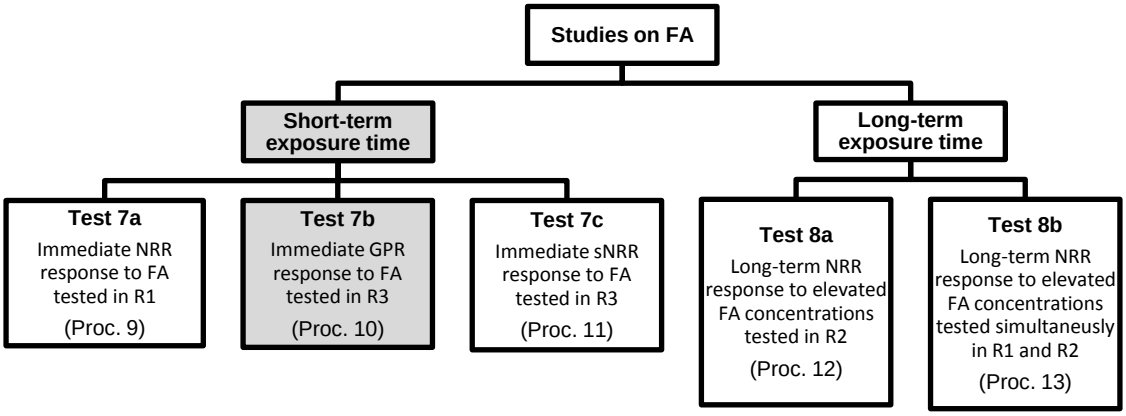


Figure 3-16 Procedure 10 in the overall detail free ammonia studies outline

This procedure was used to test the following FA concentrations in duplicates: 1.1 mg N/L (control sample – 100%, FA concentration was the

same as during reactor R3 operation), 0.3 mg N/L, 0.6 mg N/L, 3.3 mg N/L, 6.6 mg N/L, 15.5 mg N/L, 28 mg N/L. The pH was set at about 7.0 using the HEPAS buffer; however, the FA concentration was calculated for the measured pH at the end of the test, for each testing bottle individually. The amount of TA was calculated based on Equation 3-1. The calculated amount of TA in a form of ammonium chloride powder was added to each sampling bottle at the beginning of the test.

The test was conducted in a single run. Testing bottles were placed in the water batch and gas volume was measured during four hours. The gas volume measurements from the last hour were used to calculate GPRs according to Equation 3-8, where GPR_0 was the gas production rate achieved in the control sample, and GPR was the gas production rate achieved during all other testing reactors. Then, the activity percentage for each test in duplicate, % GPR, was calculated according to Equation 3-9. Such calculated % GPRs were averaged for particular FA concentration and were used to represent the FA effect on the anammox rate.

3.3.4.11. Procedure 11 - Immediate specific nitrogen removal rate (sNRR) response to free ammonia (FA) tested in R3 (Test 7c – R3)

The purpose of this procedure was to test sNRR response to FA (under three pHs) inside of reactor R3 during three SBR cycles. Figure 3-17 presents Procedure 11 in the overall detail free ammonia studies outline.

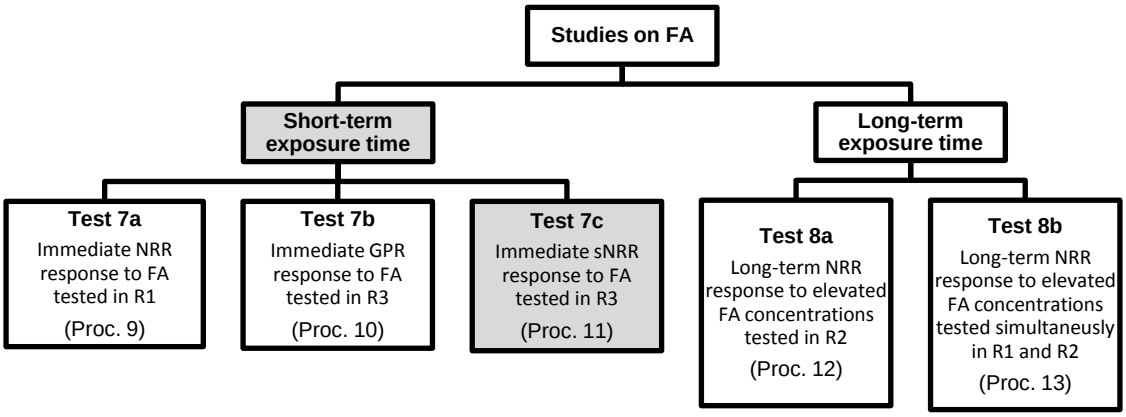


Figure 3-17 Procedure 11 in the overall detail free ammonia studies outline

Three tests were conducted during three SBR cycles one by one. During each test, the sNRR was monitored based on the mass balance (Equation 3-4 and Equation 3-5). The first test was conducted under regular pH of 7.0. Then at the beginning of the second SBR cycle, the pH was changed to pH 7.5. Finally, during the third SBR cycle, the pH was changed to 8.0. As a result of

pH changes, the reactor was operated under three different FA ranges. TA was not controlled.

3.3.4.12. Procedure 12 - Long-term specific nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R2 (Test 8a –R2)

The purpose of this procedure was to test NRR response to sudden pH change from 8.0 to 7.0 (FA change from high to low) under a reactor destabilization scenario where high nitrite (up to 247.8 mg NO_2-N/L) accumulation occurred over a 5 day period. Figure 3-18 presents Procedure 12 in the overall detail free ammonia studies outline.

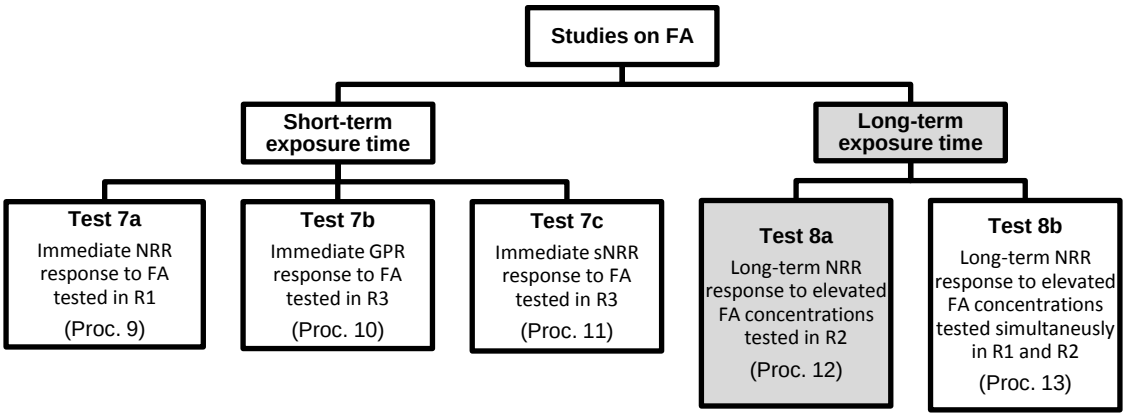


Figure 3-18 Procedure 12 in the overall detail free ammonia studies outline

Before the test was conducted, reactor R2 was operated at an NRR of about 2173 ± 10 mg N/L d and a self-maintained pH of about 7.8. The reactor was destabilized by shifting FA as a result of pH change from 7.8 to 8.0 under constant TA. Then, the reactor was operated as long as a substantial NRR loss occurred (up to 72 %) and high nitrite accumulation was achieved. When NRR destabilization occurred, the pH was changed from 8.0 to 7.0 and the NRR was monitored as long as the original NRR level was achieved.

3.3.4.13. Procedure 13 - Long-term nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R1 and R2 (Test 8b – R1 and R2)

The purpose of this procedure was to test NRRs responses to elevated FA concentrations in reactors R1 and R2, which were operated under the same pH and loading conditions. Additionally, acclimation to FA was tested; the question was whether biomass grown under medium FA concentrations (R2) would be more resistant to elevated FA concentrations compared to biomass grown under low FA concentrations (R1). Figure 3-19 presents Procedure 13 in the overall detail free ammonia studies outline.

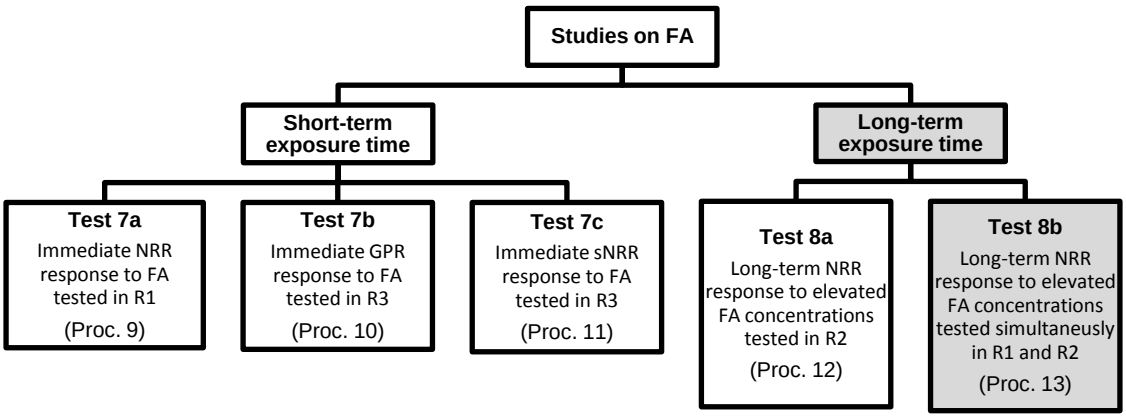


Figure 3-19 Procedure 13 in the overall detail free ammonia studies outline

Before the test was conducted, both reactors were operated under the same pH of 6.5 and nitrogen loading rate. They were achieving a very similar NRR at about 2795 ± 20 mg N/Ld and 2790 ± 14 mg N/Ld, respectively, for R1 and R2. The pH in R2 was changed at the beginning of the Test 5b – R2 and lasted for about two weeks, prior to the time when the current test was conducted (Test 8b – R1 and R2).

When the NRR was lost in both reactors, a pH shift from pH 8.0 to pH 6.5 was made in both reactors, to reduce FA concentrations. This, however, did not recover the NRR within one day; therefore, another pH shift was made from pH 6.5 to 7.0. This operation was also not helpful in NRR restoration. The NRR was restored eventually when 100 mg of hydrazine sulphite powder was added.

3.3.5. Statistical analysis

All statistical analysis such as average, standard deviation and regressions were obtained using Microsoft Excel tools.

4. RESULTS AND DISCUSSION

This chapter presents results and discussion in five subchapters. The first subchapter describes the anammox reactor performance during the start-up period. During that period, it was observed that nitrogen was removed according to well documented anammox stoichiometry for nitrogen conversion. Since there was no continual analysis of any microbial speciation on the biomass inside the reactors, anammox stoichiometry was checked regularly during the complete research project.

In the second subchapter, preliminary results on free ammonia (FA) inhibitory effect on nitrogen removal rate (NRR) are described. Three basic tests were performed to investigate the role of FA and nitrite during typical reactor operation scenarios such as pH and nitrogen concentration changes (FA, TA, and nitrite) under constant and variable loading rates.

In the third subchapter, results from the operation period of two moving bed biofilm reactors (MBBRs) are described and discussed. These reactors were operated under two different pH conditions where biomass consortia were exposed to significantly different FA concentrations. The role of FA and nitrite on NRRs was analyzed.

The detailed analysis of nitrite inhibitory effect on NRR during short- and long-term tests is the main topic presented and described in the fourth subchapter. This section explains the anammox rate responses to nitrite during different testing conditions.

Finally, the fifth subchapter presents a detailed analysis of FA inhibitory effect on NRR during short- and long-term tests describing the anammox rate responses to FA that were investigated during different testing configurations, including full scale scenarios.

The raw data collected from all the tests are presented in five appendices. Each appendix corresponds to one subchapter of this chapter. Appendices utilize the same titles as the subchapters and performed tests.

4.1. Reactors start-up

4.1.1. Start-up of the partial nitritation (PN) reactor

At the beginning of the research, the seed for the partial nitritation reactor was obtained from bench-scale SBR reactors, one performing nitrification and denitrification and second reactor performing nitrification only (Dytczak et al., 2008). They were operated at 24 ± 1°C with a solids residence time (SRT) of 12 d and a hydraulic residence time (HRT) of 36 h. They were fed with a synthetic wastewater containing beef and yeast extract as a carbon source and ammonium chloride as ammonia source.

At the end of the research, partial nitritation needed to be reseeded due to sudden partial nitritation cessation. The cause of this phenomenon was identified as a phosphorus limitation. Reseeded biomass originated from a bench-scale nitritation-denitritation SBR, a different reactor than before. This

reactor was operated at 27 ± 1°C with an SRT of 10 d and an HRT of 3 d. They were fed with a synthetic wastewater containing beef and yeast extract as a carbon source and ammonium chloride as ammonia source. Methanol was used for denitrification during the anoxic phase.

The start-up of the partial nitritation reactor (PN) was simple and fast and did not involve any sophisticated control methods for nitrite oxidizing bacteria (NOB) suppression, such as dissolved oxygen (DO) control (Aslan et al., 2009; Bae et al., 2002; Ciudad et al., 2005), free ammonia (FA) or free nitric acid (FNA) control (Ganigué et al., 2007; Vadivelu et al., 2007; Van Hulle et al., 2007; Zhang et al., 2010), or SRT control (Van Hulle et al., 2007). Within two weeks, the PN reactor achieved its desired performance which was a nitrite production rate of about 1 g NO_2-N/L d at that time. NOBs were not growing in significant amount as no long-term nitrate was recorded during or after the start-up period. Figure 4-1 depicts the example of the PN reactor start-up after the biomass was reseeded. During the entire research process, the PN reactor was operated under nitrogen loading conditions which varied depending on the required nitrogen load to the anammox reactors (between 2 and 50 g N/d).

During the start-up period (first two weeks), the sludge was not wasted, thus allowing nitrifiers to grow. It was observed that the nitrite oxidising bacteria (NOB) did not grow in the system. This may have been due to high nitrite (about 400 mg NO_2-N/L at the steady state) and moderate concentration

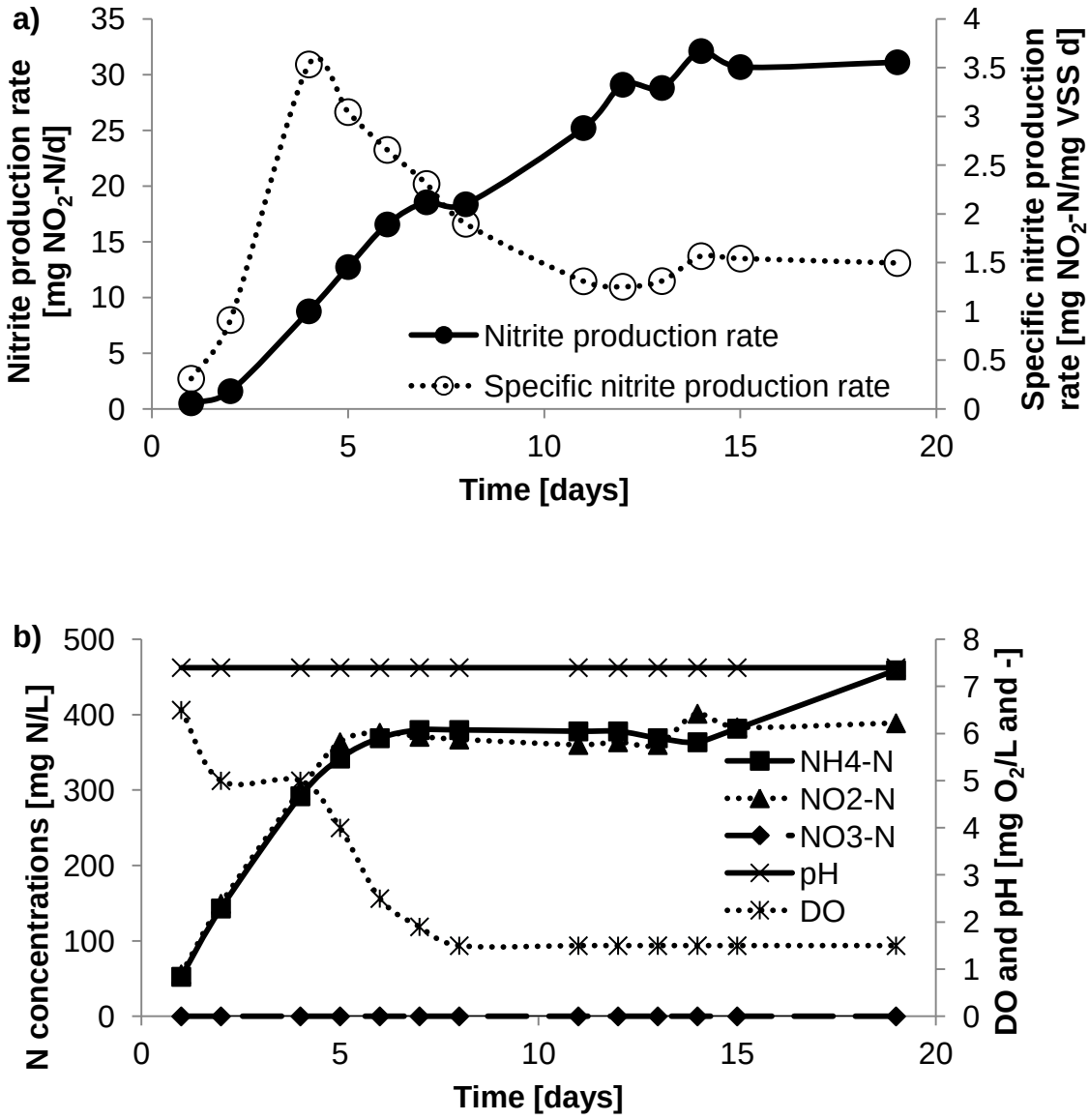


Figure 4-1 Time schemes of a) nitritation rate and b) nitrogen concentrations, DO and pH during partial nitritation reactor start-up

of free ammonia (in the range between 4 and 11 mg N/L at the steady state), which were reported to have an inhibitory effect on NOB (Anthonisen et al., 1976; Vadivelu et al., 2007). On the other hand, the start-up period without

sludge wasting was short enough for possible NOB acclimation to nitrite and free ammonia (Fux et al., 2004; Villaverde et al., 2000). Later on during the course of the experiment, biomass was wasted from the reactor, maintaining the SRT in the range between 5 and 15 days, depending on the desired nitritation rate.

At the beginning of the start-up, the dissolved oxygen concentration was very high reaching the DO value of 6.5 mg O_2/L. During the course of the start-up period, the DO gradually decreased due to the increasing demand for oxygen and limited transfer capacity for oxygen diffusers. The DO was about 1.5 mg O_2/L, when the partial nitritation reached its steady state.

4.1.2. Start-up of the anammox MBBR reactors

Two, moving-bed, biofilm reactors (MBBR) were started-up with the Kaldnes media K1, which originated from the deammonification pilot plant in Stockholm, courtesy of Dr J. Trela from the Royal Institute of Technology in Stockholm, Sweden. At that pilot plant, the anammox reactor was started up by inoculation with activated sludge from a nitrification basin at Himmerfjarden WWTP. In that process, the anammox reactor was operated in a batch mode, then connected to the partial nitritation reactor where the anammox culture was established (Trela et al., 2004). *Can. Brocadia anammoxidans* was identified by Cema (2009) in that pilot plant as the dominant anammox organism (anammox biomass in anammox reactor of two MBBR configuration

was tested in 2005. For this experiment, the biomass was obtained in 2007 when the pilot plant in Stockholm was switched into a one reactor configuration).

After the medium with anammox biofilm was transferred from Sweden for the present study, two MBBRs were operated as continuous flow reactors under nitrite limiting conditions, similar to those at the pilot plant in Sweden. The nitrogen load was controlled and varied occasionally by adjusting the flow rate in such a way as to maintain the nitrite concentration inside the reactors at a level no greater than 10 mg NO_2-N/L. The pH was allowed to vary naturally in both reactors. This research period provided a preliminary result, confirming successful establishment of anammox obtained after the inoculation. The anammox NRRs achieved in reactors R1 and R2 were similar to those observed in the pilot plant in Sweden and typical anammox activities reported in the literature (nitrite to ammonium conversin ratio: from 1.04 Bettazzi et al., 2010 and 1.17 Isaka et al., 2007 to 1.3 Strous et al., 1998). Results from this experimental period are presented in Table 4-1.

Table 4-1 The pH, conversion of nitrogen, and microbiological speciation in R1 and R2 compared with first study on anammox and results from the pilot plant from which carrier media originated

Parameter	unit	First study reported on anammox[1]	Stockholm pilot plant	This study[4]	
				R1	R2
pH	[-]	Controlled in the range 7.0 – 8.0	8.21 ± 0.2 [2]	7.65 ± 0.47	7.66 ± 0.52
NO_2-N/NH_4-N	[mg N/ mg N]	1.32	1.21 ± 0.32 [2]	1.20 ± 0.27	1.23 ± 0.22
Identified organism responsible for anammox reaction	-	*Can. Brocadia anammoxidans*	*Can. Brocadia anammoxidans*[3]	Unknown	Unknown

[1] *after Strous et al. (1998)*

[2] *after Szatkowska (2007)*

[3] *after Cema (2009)*

[4] *Appendix 1*

After the inoculation, NRRs in both reactors were at similar rates as those observed in the pilot plant, from which the carrier media originated. The nitrogen removal rates (NRR) reported by Szatkowska (2007) were in the range between 10 and 230 mg N/L d, where in this study, R1 and R2 achieved NRR in the range between 30 and 240 mg N/L d (Figure 4-2).

Both R1 and R2 had similar NRRs, nitrogen conversion ratios of NO_2-N to NH_4-N and nitrogen removal dynamics when nitrogen load varied. Meanwhile, nitrogen concentrations detected inside the reactors R1 and R2 had similar values (Figure 4-3). These parameters suggested that both reactors performed in a similar way when exposed to variable loading rates.

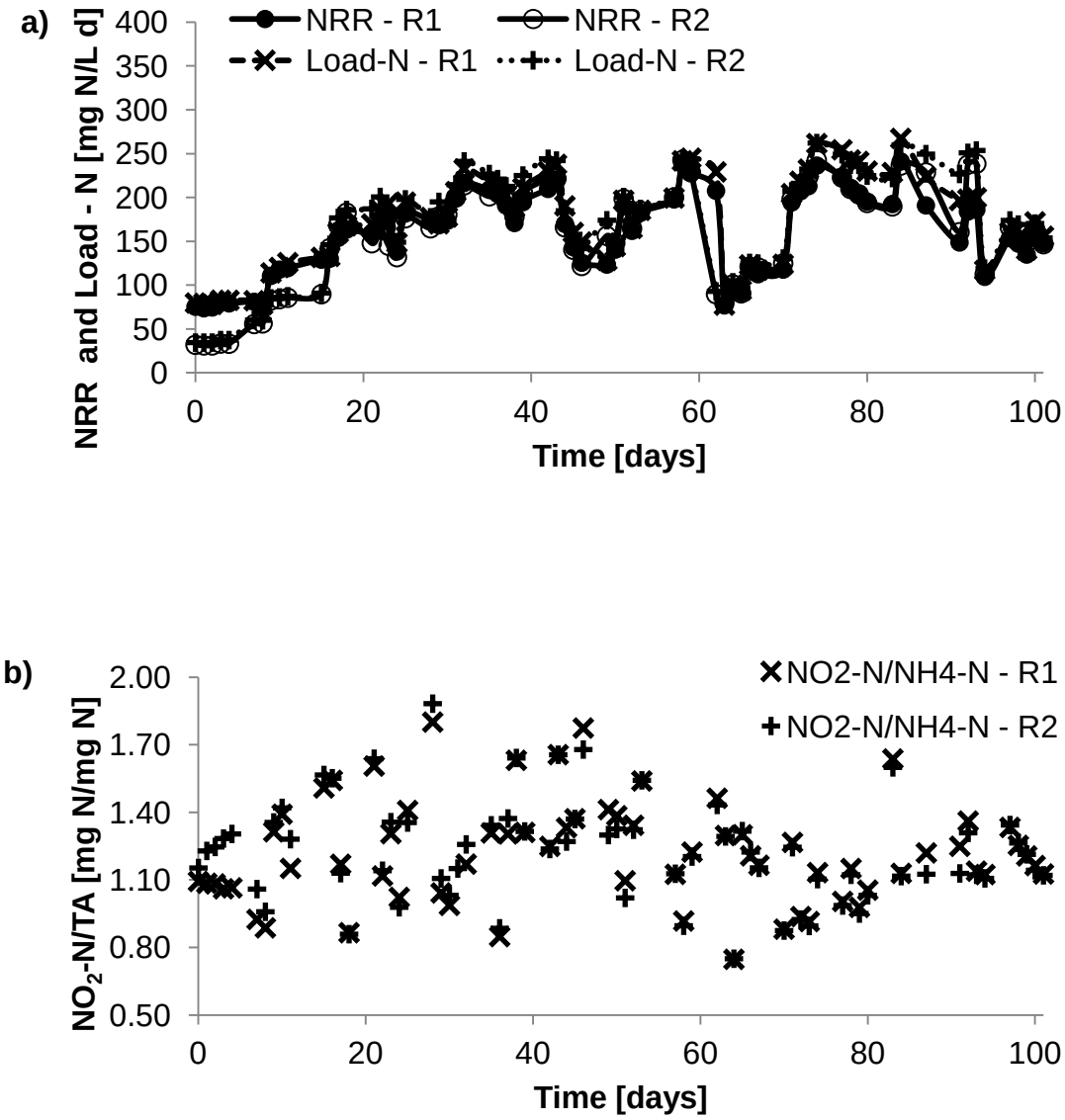


Figure 4-2 Histographs of a) nitrogen removal rates (NRR), nitrogen loading (Load –N), and b) nitrogen conversion rations NO_2-N to NH_4-N with its dynamics in R1 and R2 after the start-up with carrier media from the pilot plant in Stockholm (on day 0)

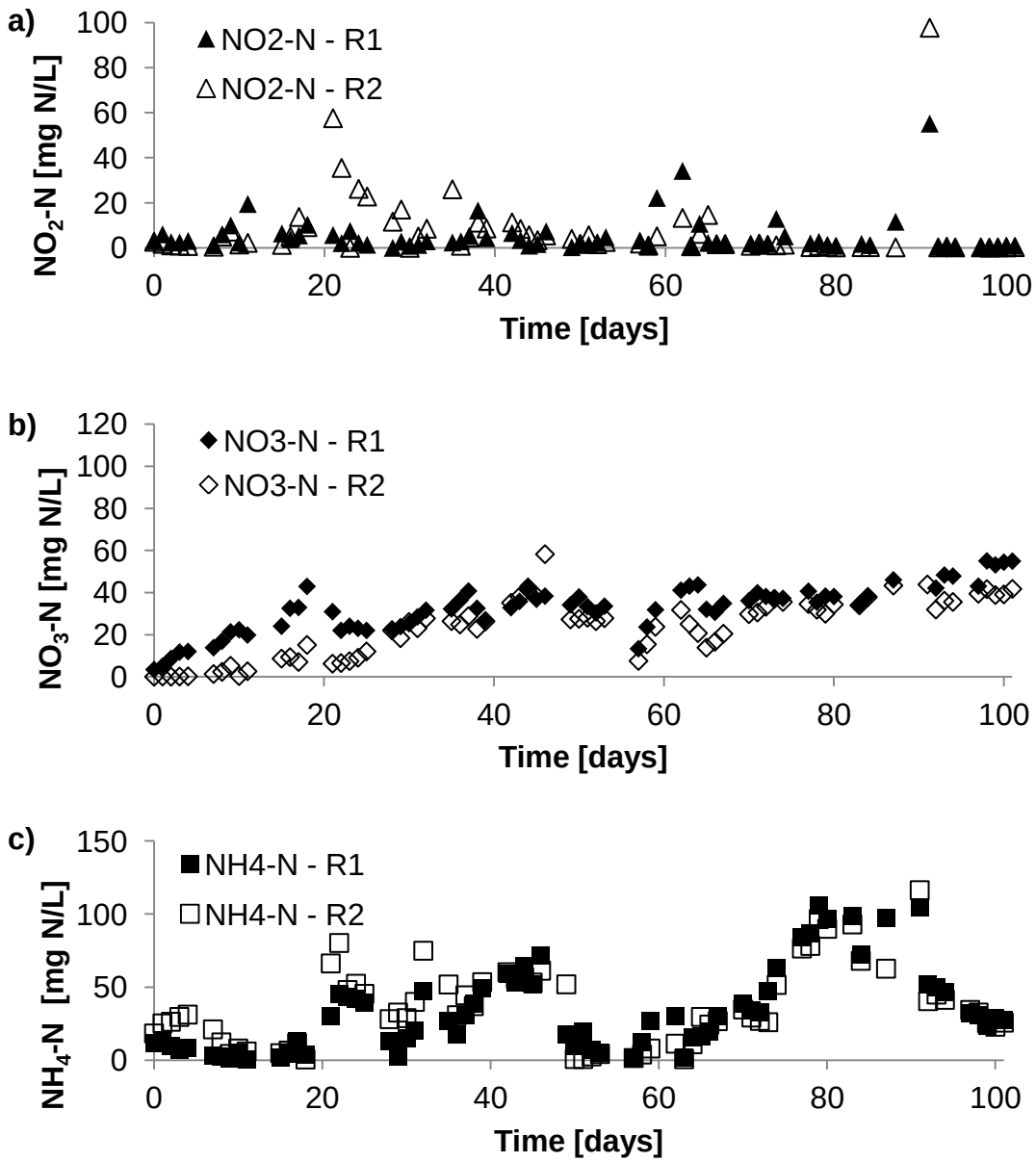


Figure 4-3 Histographs of nitrogen concentrations (a, nitrite, b, nitrates, c, TA) in R1 and R2 after the inoculation with carrier media from the pilot plant in Stockholm

During the second phase of the start-up period, the pH-control set at pH 6.5 was introduced in R1 while in R2, pH was allowed to at self stabilize at pH between 7.5 and 8.1, without pH control. This period lasted about one year, after which studies on pH and FA were conducted (described in chapter 4.2). Results from this experimental period are presented in Table 4-2, showing that

Table 4-2 The pH, conversion of nitrogen ratio and nitrogen removal rate (NRR) in R1 and R2 after the addition of pH control in R1 set at 6.5

Parameter	unit	This study[1]	
		R1	R2
pH	[-]	6.5	7.84 ± 0.1
NO_2-N/NH_4-N	[mg N/ mg N]	1.17 ± 0.08	1.10 ± 0.10
NRR	[mg N/L d]	2900 ± 370	2600 ± 350

[1] *Appendix 2*

there was no difference in the anammox stoichiometry when the pH in R1 was lowered, compared with R2 under nitrite below 10 mg N/L. Additionally, NRRs were comparable, showing no negative effect of pH 6.5 on anammox rates (Figure 4-4). No destabilization occurred when low loading rate with corresponding low NRR was tested during a 10 day period. In addition, a significant increase in NRRs was achieved in both reactors when the loading rates were increased at the end of this testing period. During this increased loading period, special attention was paid to nitrite which had been reported to have a strong adverse effect on anammox performance in the pilot plant in

Stockholm (Szatkowska et al., 2007). Therefore, nitrite concentrations were maintained inside of the reactors at a level no greater than 10 mg NO_2-N/L. Nitrogen concentrations detected inside the reactors had similar values (Figure 4-5), confirming the reactors' similarity.

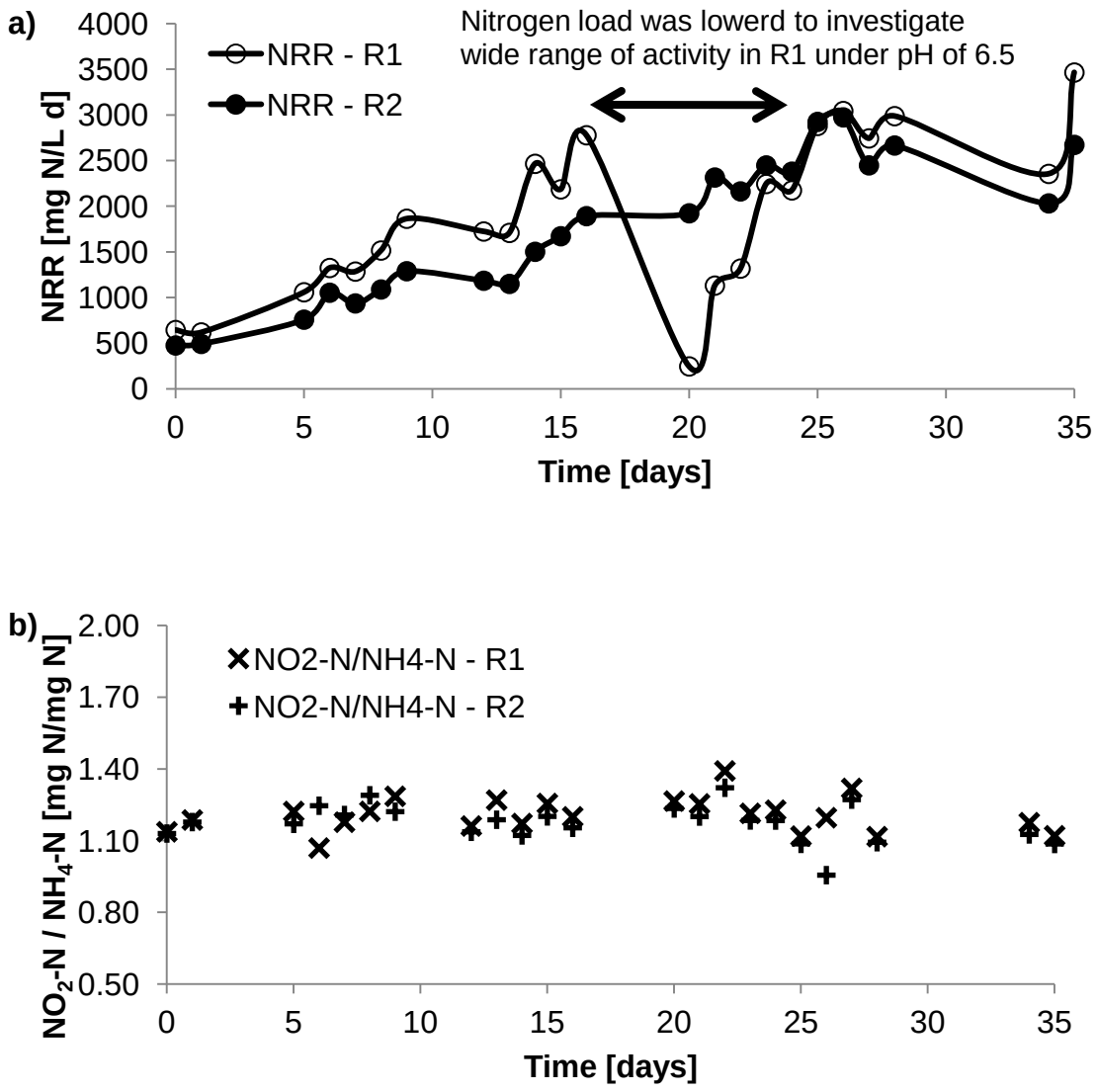


Figure 4-4 Histographs of a) nitrogen removal rate (NRR) and b) nitrogen conversions rations NO_2-N to NH_4-N in R1 and R2 after the addition of pH control in R1 set at 6.5

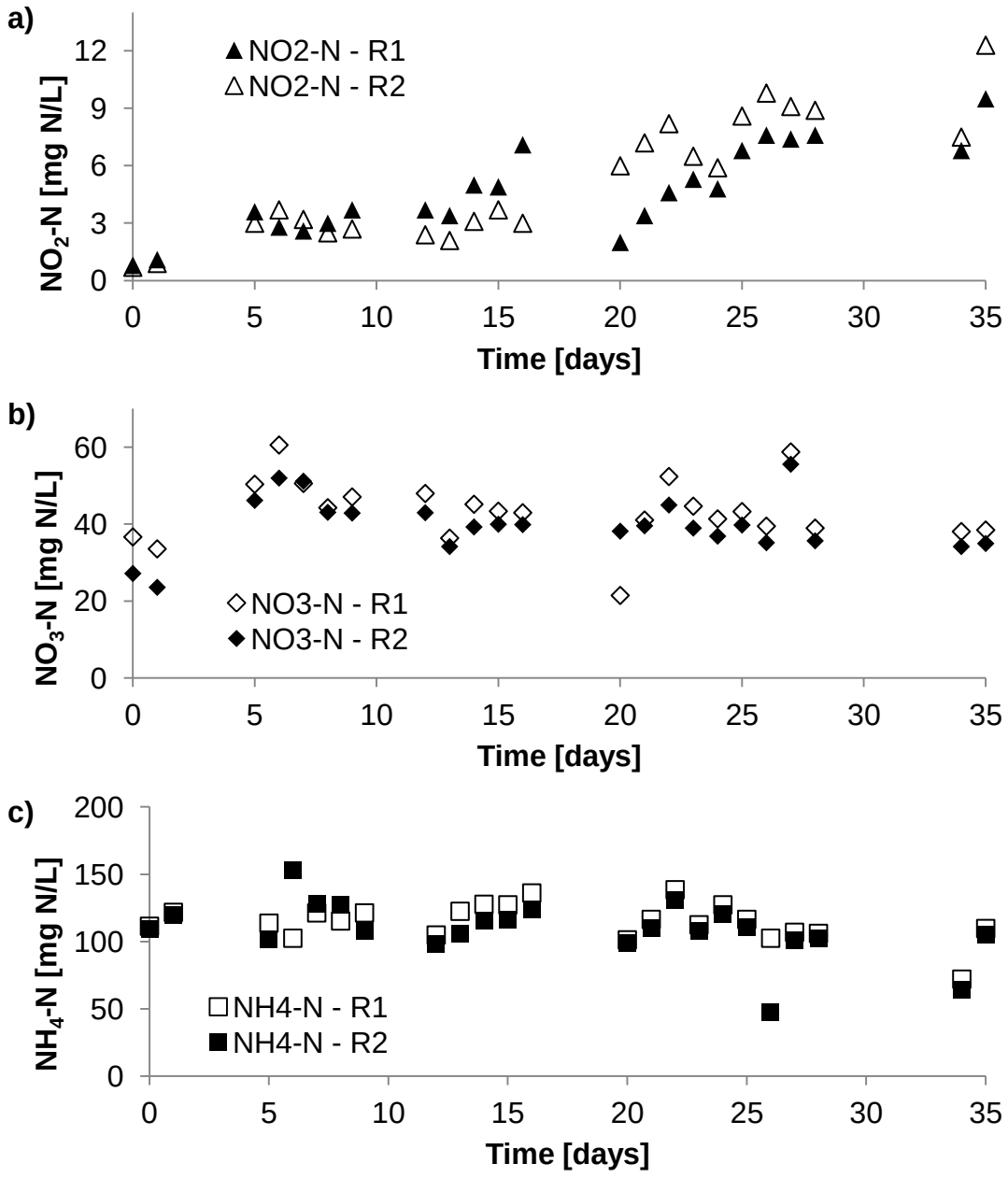


Figure 4-5 Histographs of nitrogen concentrations (a, nitrite, b, nitrate, c, ammonium) and its dynamics in R1 and R2 after the addition of pH control in R1 set at 6.5

At the end of the start-up period, carrier media in both reactors were similar in appearance, with abundant biofilm accumulation on the protected surface (Figure 4-6). There was no visible biofilm on the outside surface of the carrier media. The biofilm had an intense reddish-brown colour which is typical in anammox systems (Jetten et al., 1999) and has been considered a sign of anammox organisms (Kaldate et al., 2009; Tsushima et al., 2007; Cho et a., 2010; Innerebner et al., 2007; Ahn et al., 2004).

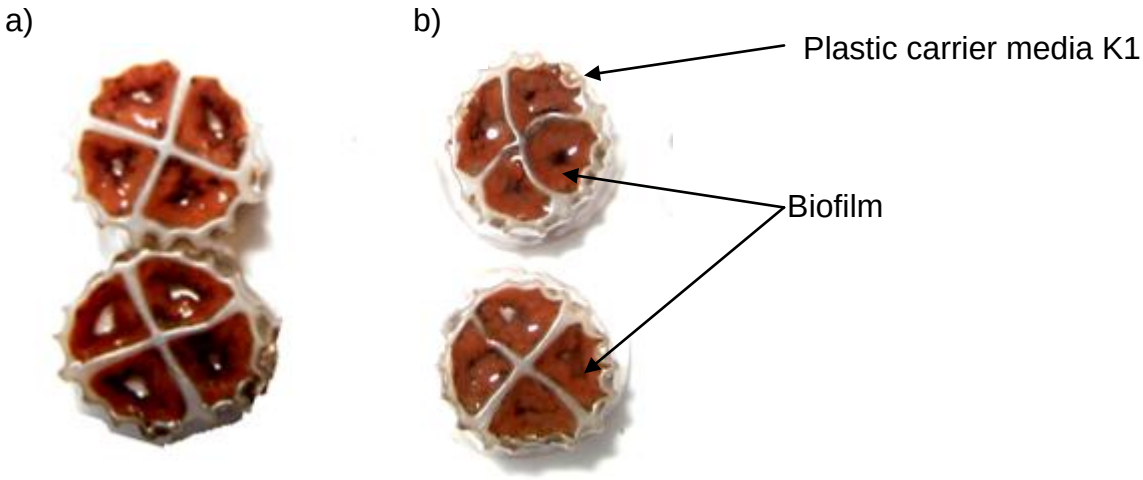


Figure 4-6 Carrier media K1 with accumulated biofilm in a) R1 and b) R2 at the end of start-up period

4.1.3. Start-up of the suspended anammox SBR reactor

The seed for the suspended flocculated anammox reactor (R3) was obtained from the effluent of anammox MBBRs. Solids from MBBR effluent

were settled and introduced to the new SBR reactor. After such a reactor inoculation, the starting NRR was about 160 mg N/Ld.

The nitrogen was removed according to anammox stoichiometry, typical to those presented in the literature for anammox reactors (Strous et al., 1998). Table 4-3 summarises the anammox stoichiometry obtained after the inoculation and introduction of pH control on day 67.

Table 4-3 The pH, conversion of nitrogen and microbiological speciation in R3 compared with first study on anammox

Parameter	unit	First study reported on anammox	This study[2]	
			R3 before pH control	R3 after pH control
pH	[-]	Controlled in the range 7.0 – 8.0[1]	7.49 ± 0.29	7.00 – 7.03
NO_2-N/NH_4-N	[mg N/ mg N]	1.32[1]	1.09 ± 0.13	1.16 ± 0.08
Identified organism responsible for anammox reaction	-	*Can. Brocadia anammoxidans*[1]	Unknown	Unknown

[1] *after Strous et al. (1998)*

[2] *Appendix 1*

Originally, the anammox SBR reactor was operated without pH control and at a self-maintained pH of about 7.49 ± 0.29. During that period, sudden activity losses were observed. After implementation of pH-control set at pH

7.00, with a pH bandwidth of 0.03, anammox NRR became stable, which allowed for a further increase in nitrogen load to the reactor (Figure 4-7).

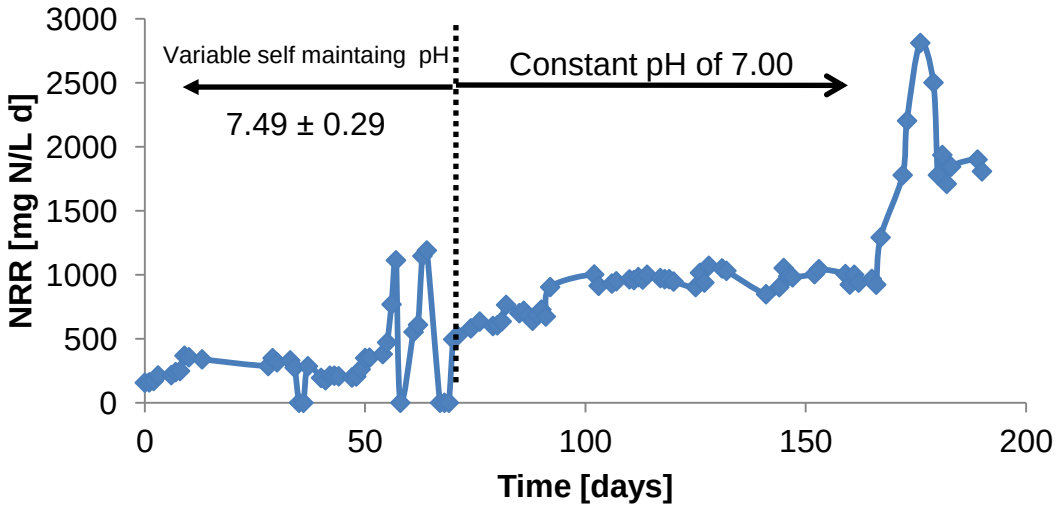


Figure 4-7 Histographs of nitrogen removal rate (NRR) in reactor R3 during the start-up before and after the addition of pH control set at 7.00

4.2. Preliminary studies on anammox

During this research period, all conducted tests were using raw centrate obtained from the local wastewater treatment plant (NEWPCC), Winnipeg, Canada. It was pre-treated in the partial nitritation reactor prior to feeding the anammox MBBR, reactor R1 (pH 6.5). This preliminary study was targeting the free ammonia (FA) inhibitory effect on nitrogen removal rate (NRR), with biomass being exposed to various FA concentrations and pH. The purpose of

this study was (Test 1, Test 2 and Test 3) to document whether FA could play an important destabilizing role in the anammox system.

The FA range applied in several tests (FA up to 15 mg N/L) was typical and can be observed in pilot and full scale systems (van der Star et al., 2007; Szatkowska et al., 2007). This range was reported by a majority of researchers to not have a significant effect on anammox organisms during long-term reactor operation (Fernández et al., 2010; Tang et al., 2010; Plaza et al., 2011). The significant difference between this study and studies presented in the literature review was that, in this study, nitrogen removal rates with high FA (FA greater than 2 mg N/L) were compared with rates obtained under low FA concentrations (FA below 2 mg N/L).

Additionally, two NRRs were compared for actual FA inhibition representation. The first one was calculated on the basis of the nitrogen mass balance which represented actual activity inside of the reactor. The second NRR was calculated on the basis of the measured nitrite concentrations which were adjusted using Michaelis-Menten relationships – Michaelis-Menten based NRR (approximation of biomass rates free of FA inhibition). The reason of such a Michaelis-Menten based NRR representation was to include significant stimulating dependency of NRR on bulk nitrite concentration, which was widely reported in the literature for anammox biofilm systems (Cema et al., 2005; Ni et al., 2010; Chen et al., 2011)

4.2.1. Immediate nitrogen removal rate (NRR) response to different free ammonia (FA) concentrations up to 14.6 mg N/L in reactor R1 (variable pH) operated in the batch mode during the test (Test 1).

The Test 1 in the overall preliminary studies outline is presented in Figure 4-8.

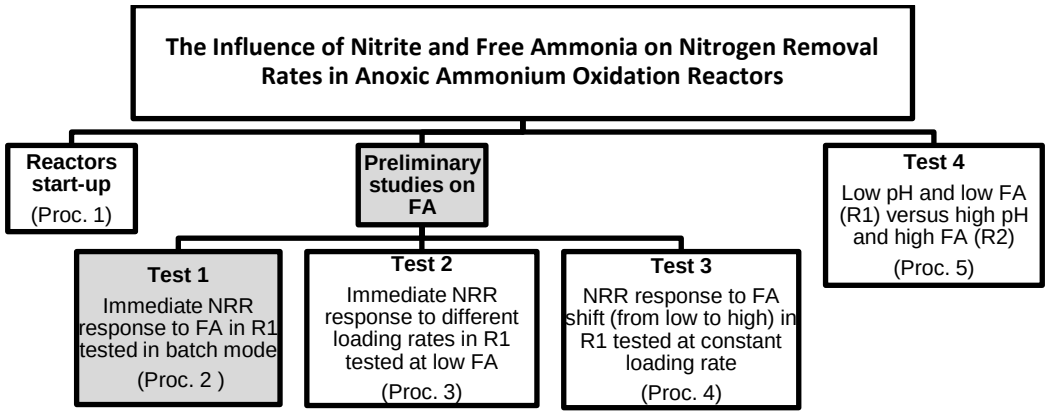


Figure 4-8 Test 1 in the overall preliminary studies outline

During this test, NRR was not affected negatively by increasing FA in the range between 0.6 and 2.1 mg N/L. A negative effect of FA on NRR was observed when FA was greater than 2.1 mg N/L, leading to a 54% reduction in NRR at an FA concentration about 14.6 mg N/L, compared with the maximum NRR recorded at FA concentration of 2.1 mg N/L. Graphical representation of immediate NRR response to FA is presented in Figure 4-9.

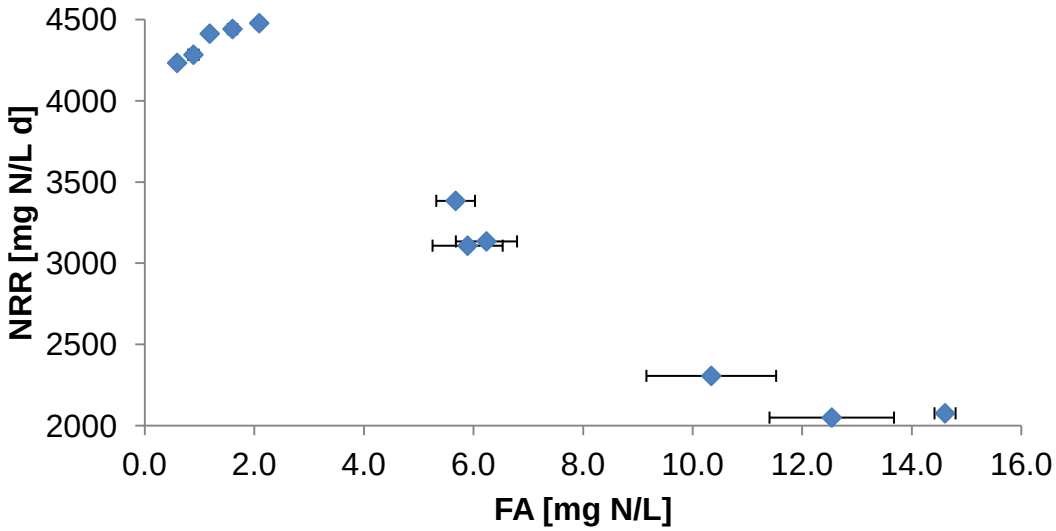


Figure 4-9 Immediate nitrogen removal rate (NRR) response to free ammonia (FA) in reactor R1

The experimental results are in close agreement with similar studies where immediate response of anammox activity to FA was investigated. In studies conducted by Dapena-Mora et al. (2007) and Fernández et al. (2010), the negative effect of FA on anammox activity was observed when FA was greater than 3.5 mg N/L, up to an investigated maximum FA concentration of 135 mg N/L. In those two studies, lower FA concentrations than 3.5 mg N/L were not investigated; therefore FA inhibition at lower FA concentrations had been unknown in those two studies.

The only study which has reported the inhibitory effect of FA at concentrations below 3.5 mg N/L was the study conducted by Jung et al.

(2007). During long-term reactor operation, the authors recorded FA concentrations greater than 1.7 mg N/L as inhibitory.

During this test, nitrogen conversion ratios were similar to those presented in the literature for anammox reactors (Tang et al., 2011); however, variable nitrogen conversion ratios were recorded, as well. It was observed that anammox activity was reaching an overall nitrogen balance at the ratio of [NH_4-N conversion: NO_2-N conversion: NO_3-N production] of [1:(1.39±0.63):(0.23±0.06)]. Similar observations with variable conversion ratios were recorded by Cema et al. (2005), indicating instability could be caused by transferring samples outside of the mother reactor. The authors noticed that the testing reactor could not well represent real conditions in the mother reactor. In this study, this should not be a problem, because the entire reactor was sampled, and the only variable was FA, which was adjusted by pH and ammonium concentration, where nitrite was spiked each time the test was conducted. This phenomenon may be due to insufficient stabilization periods (pH and substrate concentrations), which were 30 minutes, before tests were conducted. On the other hand, this was in agreement with the observation made by Fernamdez et al. (2008) and Strous et al. (1999) that, change in the nitrite to ammonium utilization ratio was the symptom of anammox activity deterioration.

During this test, FA concentrations above 2.1 mg N/L were investigated under high pH (7.5 and 8) where the lower FA concentrations were

investigated under pH 7 – full-scale reactor operation case. High FA concentrations occur naturally at high pH, according to the equilibrium constant. It was observed that FA had a greater impact on NRR than pH alone (or other pH related equilibriums which were not investigated in this work). The pH in the range of 7.0 – 8.5 was recorded to have minimal effect on anammox activity (Strous et al., 1997), which was confirmed during Test 7a – R1 (page 176) for the pH range of 7.0 – 8.0.

4.2.2. Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 0.8 mg N/L in reactor R1 (constant pH set at 6.5) operated in the continuous feed mode at variable loading rates during the test (Test 2)

The Test 2 in the overall preliminary studies outline is presented in Figure 4-10.

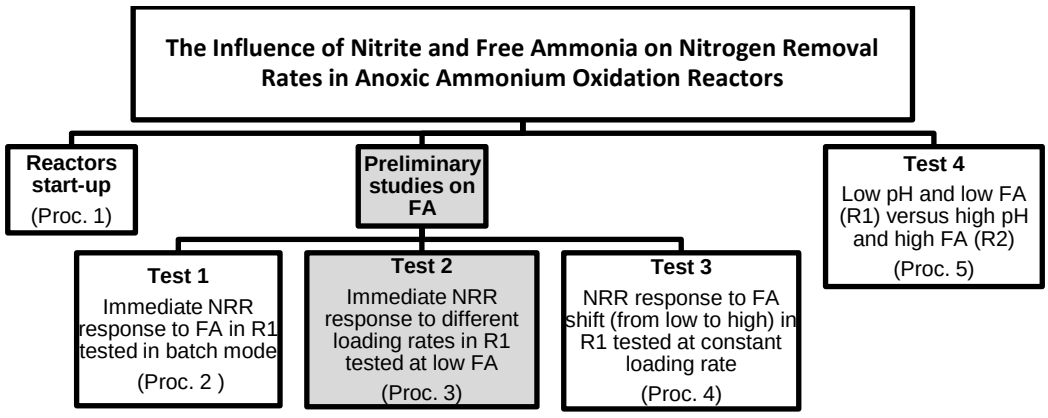


Figure 4-10 Test 2 in the overall preliminary studies outline

During the entire test, reactor R1 had the pH control turned on, and set at pH 6.5, which provided low FA (however FA was variable between 0.6 – 0.8 mg N/L) despite variable total ammonia (TA). Steady state operation was assumed after each increase of nitrogen loading, based on observed stable nitrite and total ammonia concentrations (Appendix 2, page 257). Three different nitrogen loading levels were tested consecutively (Table 4-4). These increased loads and associated FA concentrations within the tested range of 0.6 – 0.8 mg N/L did not appear to result in reactor destabilization during any test period, which was up to 2.8 h. In contrast, it was observed that NRR increased as nitrogen loading increased. Low concentrations of FA within the tested range showed no negative effect on NRR, a result which is consistent with results obtained during Test 1. Table 4-4 and Figure 4-11 summarize the results of these tests. The baseline values represent stable reactor operation (for almost 3 months) at constant nitrogen loading of 9.1 g N/Ld prior to this experiment.

Table 4-4 Reactor R1 operational parameters and performance during load change test at pH 6.5 within low free ammonia (FA) range

Parameter / Test	FA [mg N/L]	N load [g N/Ld]	HRT [h]	NRR [g N/Ld]
Baseline	0.56	9.1	1.94	6.0
N-Load 1	0.63	11.2	1.54	6.9
N-Load 2	0.68	14.2	1.2	8.1
N-Load 3	0.79	19.0	0.91	9

As shown in Figure 4-11, NRRs obtained under varying nitrogen loading conditions, closely followed the Michaelis-Menten model, where Ks for nitrite was 25 mg N/L and max NRR was 11 g N/L d (This trend was confirmed also in other tests such as Test 5b - R1, Test 5b - R2, Test 6 - R1 having the difference in NRRs below 10%). The best fit was achieved by changing Ks and max NRR using Microsoft Excel tool. It was assumed that the concentration of total

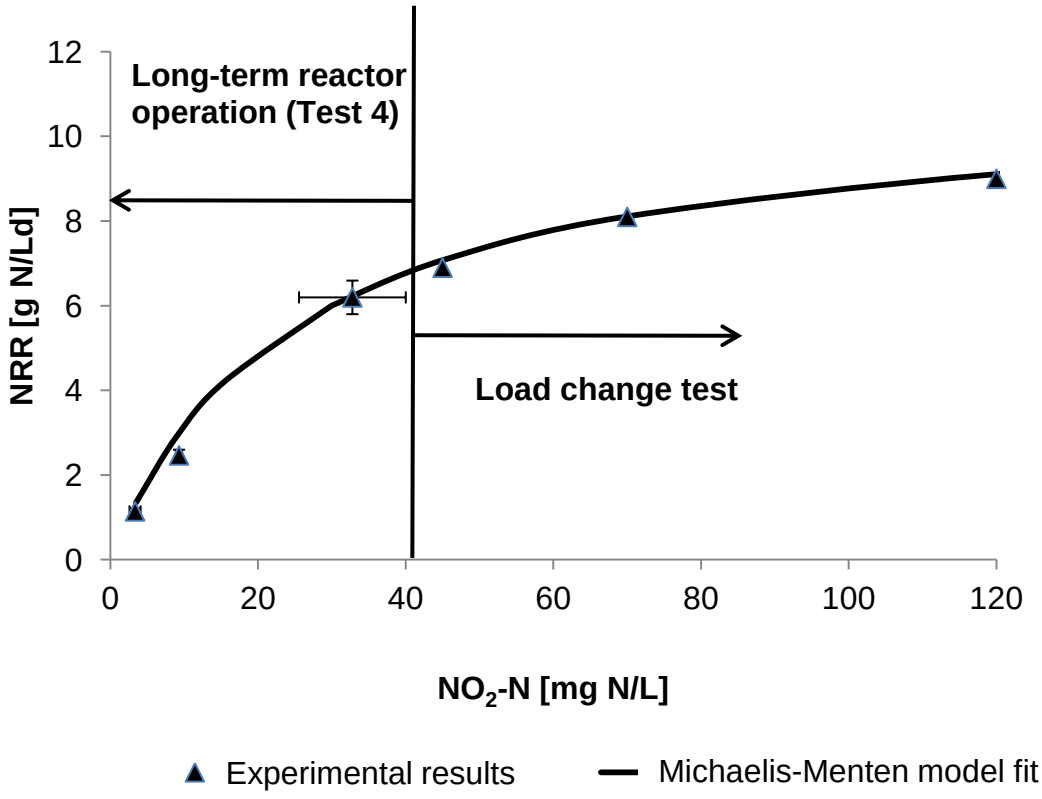


Figure 4-11 Experimental results with the Michaelis-Menten model fit for NRR under different nitrite concentrations (kinetic parameters: max NRR = 11 g N/Ld, and K_{NO2} = 25 mg N/L). Experimental rates for the NRR were obtained under steady state reactor operation (Test 4) and load change test within low free ammonia range of 0.6 – 0.8 mg N/L.

ammonia was not a limiting factor in these tests, which was confirmed by the test presented in Appendix 5 (page 341).

The results of these experiments suggest that elevated nitrite concentration (up to the investigated limit of 120 mg N/L, Figure 4-11) or an increase in nitrogen loading are not the main destabilizing factors in the anammox system when FA is kept low. This is different from the study presented by Gut et al. (2006) where an increase in nitrogen load could be achieved only in slow step-by-step increments, to allow anammox bacteria to adjust to the new conditions. The only significant operational difference between that study and the current research is the pH, and therefore, the FA levels within the reactors. The lower pH in this study resulted in about a 10 fold lower FA than that in the work of Gut et al. (2006), allowing for nitrogen load variations, without system destabilization and loss of anammox activity.

During each nitrogen loading test, nitrogen conversion ratios were very close to the theoretical anammox conversion ratio presented in the literature, thereby confirming anammox reactor stability despite variable loading rates. It was observed that overall anammox activity during different FA levels was reaching an overall nitrogen balance at ratio of [NH_4-N conversion: NO_2-N conversion: NO_3-N production] of [1:(1.29±0.04):(0.25±0.01)].

This test also demonstrated a significant dependency of NRR on the bulk nitrite concentration when below 30 mg NO_2-N/L. This is the result of high K_s value for this configuration which was estimated to be at about 25 mg NO_2-

N/L. Such a substrate dependency on biofilm biomass activity is a typical phenomenon for anammox and other biofilm systems (Cema et al., 2005; Ni et al., 2010; Chen et al., 2011; Dunn et al., 1985). This suggests, when analyzing changes of NRR related to inhibitory component, substrate limiting concentrations may greatly affect NRR and misdirect the data interpretation.

4.2.3. Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 11.9 mg N/L in reactor R1 (self maintaining pH in the range of 6.9 and 8.2) operated in the constant continuous feed mode during the test (Test 3)

The Test 3 in the overall preliminary studies outline is presented in Figure 4-12.

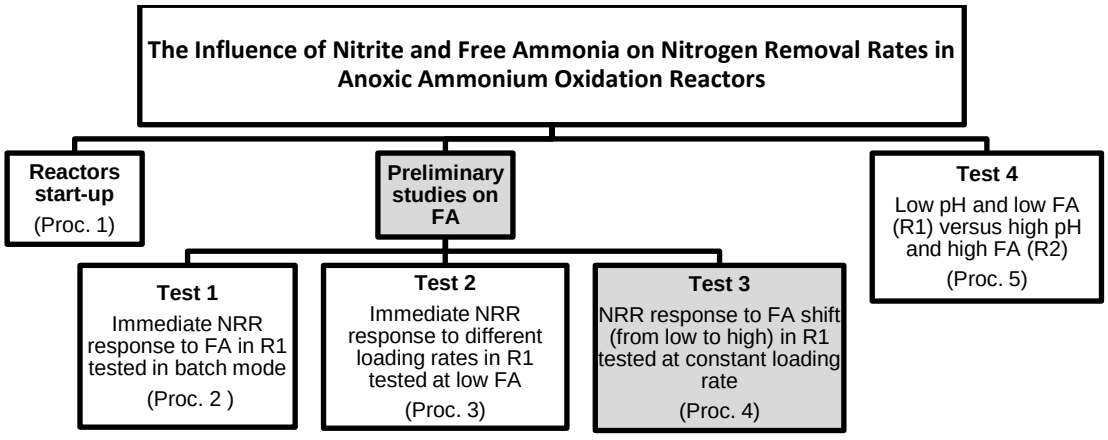


Figure 4-12 Test 3 in the overall preliminary studies outline

In order to evaluate the effect of elevated FA concentration on the NRR under constant continuous feed mode (constant nitrogen load), the reactor pH control was turned off, thereby allowing pH to rise gradually up to pH of 8.2. A subsequent FA concentration increase (as a result of pH and total ammonia) was correlated with nitrite and total ammonia increase, and NRR decrease. It was observed that at FA of 11.9 mg N/L, the NRR was hindered (based on the nitrogen mass balance calculation) by 12.6%, compared with FA at 0.3 mg N/L. However, when the pH control was turned on, the bulk pH and FA reached the original values of 6.9 and 0.3 mg N/L, respectively, while NRR recovered in 99.5% within 2.5 h.

Figure 4-13 presents the dynamics of the reactor during the test.

Nitrogen conversion ratios, during the test, remained very close to the theoretical anammox conversion ratio presented in the literature. It was observed that overall anammox activity during the test was maintaining an overall nitrogen balance at a ratio of [NH_4-N conversion: NO_2-N conversion: NO_3-N production] of [1:(1.26±0.06):(0.20±0.01)].

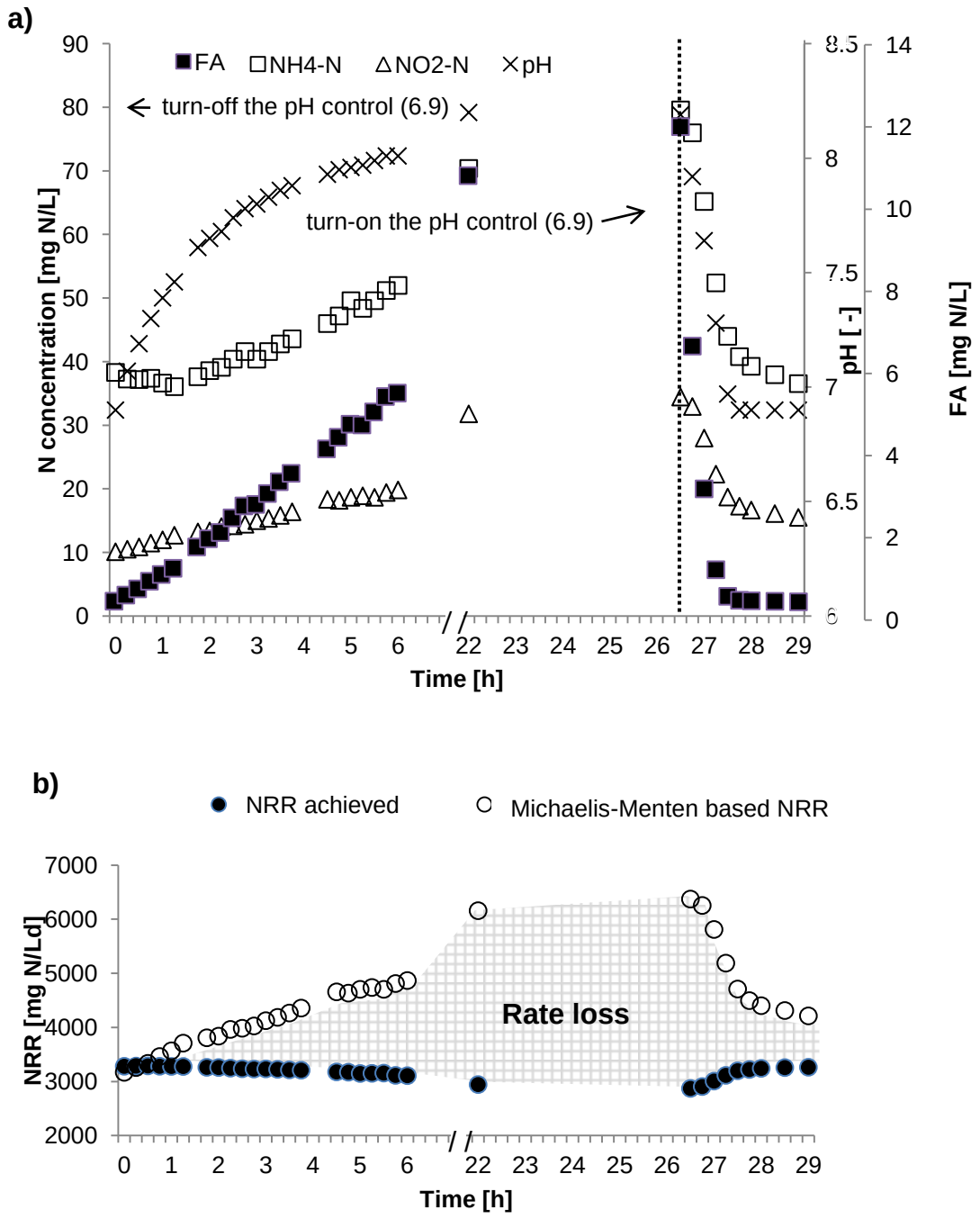


Figure 4-13 The MBBR anammox reactor performance during the test with pH and FA change with constant load a) nitrogen concentrations, pH, and FA during the test; b) nitrogen mass balance based NRR and Michaelis-Menten based NRR (darkened area represents the reduction in anammox rate)

Changes in the nitrogen concentrations and pH caused a decrease in the nitrogen removal rate of about 12.6% (based on the nitrogen mass balance,

Figure 4-13b) which was unexpected, according to the results presented by Strous et al. (1997). Strous et al. (1997) showed that the pH in the range of 7 and 8 was little stimulating the anammox activity. Furthermore, ammonium and nitrite concentrations up to 1000 mg NH_4-N/L and 60 mg NO_2-N/L, respectively, were reported not to have a negative effect on the nitrogen removal rate (pH was not provided, Strous et al., 1998). This indicated that a mechanism, other than pH and total nitrogen concentration, was involved in the destabilization of the process. It is possible that FA - the unionized form of ammonia - was responsible for the reduction in anammox activity.

During the first part of this test (time 0 – 26.5 h), nitrite concentration was increasing from 10.1 to 34.4 mg N/L, while NRR was decreasing. This was in contrast to Test 2, where nitrite significantly stimulated the NRR within that range (Figure 4-11). Comparing those two tests, it can be noticed that the only significant difference is the FA level (based on Test 7a – R1, the pH variations should not have a significant inhibitory effect on NRR) which indeed may play an important destabilizing role. If so, due to the fact that both tests were conducted on the same biomass and the NRR response to nitrite under no inhibitory FA concentrations was known, it was possible to present the actual biomass rate loss under elevated FA.

The 12.6% NRR loss at FA of 11.9 mg N/L during 26.5 hrs was the result of a significant biomass activity loss which can be obtained by comparing two NRR values, before (Test 2) and after inhibition (Test 3), on the nitrite concentration bases. The first NRR, Michaelis-Menten based NRR, represents the biomass rate potential which allows achieving the maximum NRR under no inhibition scenario and actual nitrite concentration, and was calculated using the Michaelis-Menten relation between the NRR and nitrite concentration estimated in Test 2. The second NRR, mass balance based NRR, represents the estimation of biomass rate under the elevated FA concentration and actual nitrite concentration inside the reactor, and was calculated on the basis of the actual performance of the reactor. As a result of such an analysis, a 55% decrease of biomass rate was recorded when FA concentration was about 11.9 mg N/L (Figure 4-13b).

There is a difference between the NRR loss (12.6%) and the biomass rate loss (55%) which is probably due to the comparison of NRRs under nitrite limiting concentrations. At the beginning of the test (low FA), the NRR was driven by substrate limitation (excess of biomass), while at the high FA, the NRR was driven by inhibition. This suggests that the negative effect of FA can be mitigated by excess biomass inventory. Indeed, Wett et al. (2010) reported that excess biomass inventory allows coping with variable biomass activity,

thereby increasing the reactor's stability without compromising nitrogen removal efficiency (constant nitrogen concentrations in the effluent).

Jung et al. (2007) reported that FA levels greater than 1.7 mg N/L were required to destabilize the reactor under low nitrite concentrations and continuous reactor operation mode. This low FA inhibitory concentration agrees with the FA range under which the highest NRR was recorded in this test.

Results obtained in this study are in opposition to those reported in some studies, where FA up to 15 mg N/L has not been considered important for reactor stability (Fernández et al., 2010; Tang et al., 2010; Plaza et al., 2011). The reason for such a discrepancy can be related to the testing methodology.

In the research conducted by Fernández et al. (2010), the authors determined FA inhibition based on the nitrite removal efficiency. The SBR reactor was operated under nitrite limiting concentration with 30 minutes reaction time, without feeding at the end of the cycle, allowing complete nitrite utilization. Such a reactor operation was under loaded as the authors showed by comparing effective nitrogen loading and maximum nitrogen removal capacity. They concluded that FA up to 20 mg N/L (significantly different than observed in this research, which was about 2 mg N/L) does not affect the nitrite removal efficiency in such a configuration. However, such a method cannot be representative of the effect of FA on the biomass activity. In that study, up to about 50% of biomass activity can be lost without a negative

effect on the nitrogen removal efficiency. Some indication of FA effect on biomass activity can be observed based on the nitrogen removal capacity, which was affected significantly, when FA was greater than about 8 mg N/L. However, it is not clear if this FA concentration was already inhibitory or not because of a significant system stability problem. In the study presented by Fernández et al. (2010), the nitrogen removal capacity increased during the test, which does not allow for an accurate comparison between nitrogen removal rate before and after the inhibition, during the 200 days of the test. On the other hand, it becomes evident that system stability (despite elevated FA) can be greatly improved by keeping the load low in the reactor. Research presented by Fernández et al. (2010) cannot provide reliable information about FA inhibition threshold concentration for biomass activity, when the system would be operated at its maximum capacity.

In the research conducted by Tang et al. (2010), the negative effect of FA was hypothesized on the basis of the NRR. The authors observed that pH change could not be responsible for the activity deterioration, but that FA could. They reported that a 22% decrease in NRR was correlated with an FA increase up to about 83 – 130 mg N/L and high nitrite up to 115 mg NO_2-N/L during 4 days of the test. This was a very high FA concentration and it led to only moderate NRR deterioration. The reason for that can be related to the test baseline condition which was nitrite limiting concentration close to 0 mg NO_2-N/L and FA in the rage of 20 - 30 mg N/L. At the same time, biomass concentration was high at about 25 g VSS/L, providing enough capacity for

reactor stability. In the research conducted by Tang et al. (2010), the reactor which was used to test FA had a maximum NRR at about 2.5 g N/Ld. A similar reactor (the same study), which operated under FA far below 12.3 mg N/L concentration (probably FA about 1.2 – 2 mg N/L), and nitrite of about 15 - 50 mg NO_2-N/L, achieved an NRR of about 45 g N/Ld. Comparing these two reactors (they had similar VSS concentrations) it is noteworthy that the overall FA (far below 12.3 mg N/L, versus 20 – 30 mg N/L) and nitrite concentrations (about 10 - 30 versus close to 0) caused a 94% difference in NRRs between those two reactors. This suggests that FA should be studied not solely based on the NRR comparison calculated, but that the nitrite effect on NRR should be also considered. Although the authors did not present the relation between NRR and nitrite, they stated that increasing nitrogen loading (and associated nitrite increases) and a step-by-step increase in ammonium concentration had a stimulating role in achieving a high NRR.

Finally, in the research conducted by Plaza et al. (2011), the negative FA effect in the anammox system was narrowed down to the nitritation step (one biomass system). The authors did not observe any FA inhibition up to 15 mg N/L on anammox. Nitrite was readily consumed when produced and was reported to be at very low concentrations (probably at detection limit). Therefore, the reported FA value may be accurate only for the investigated configuration when the anammox rate is substrate limited, but it cannot be considered valid in the case of a description of FA effect on anammox activity, because such an activity was not investigated.

In those three publications discussed earlier (Fernández et al., 2010; Tang et al., 2010; Plaza et al., 2011), high FA concentrations were required for system destabilization; however, achieved FA threshold concentrations can be questioned due methodology which did not involved stimulating role of nitrite on anammox rates. The current test provided additional insight, suggesting significant FA negative impact on biomass activity starting at very low FA concentrations (shown on Figure 4-13 where some NRR deterioration could be ignored without Michaelis-Menten based NRR presentation), which was further explored in this study.

Additionally, the results suggest that systems maintaining pH close to neutral (and below) may have greater NRR stability due to low FA concentrations.

Another very important observation is that the biomass activity (NRR) did not recover completely within 2.5 h. Based on

Figure 4-13b, only a 77.5% NRR was recovered. This means that such a biomass cannot be used for immediate testing, as its activity is limited.

4.2.4. Conclusions

Preliminary studies investigated the short-term effect of FA on the NRR. Immediate response to FA was tested in batch and continuous feeding modes. Obtained results suggest the following conclusions:

- FA may have an immediate significant impact on the NRR when greater than about 2 mg N/L
- Controlling FA at low concentrations may increase the NRR stability (buffer capacity) to variable abiotic parameters
- Nitrite stimulate the NRR within tested range of 3 – 120 mg N/L, when FA is low (below 2 mg N/L)
- Nitrite inhibition may not be as severe as described in the literature when FA is kept low (below 2 mg N/L)
- NRR in anammox MBBR reactor is strongly dependent on the nitrite concentrations when below 30 mg N/L, when FA is low (below 2 mg N/L)

4.3. Low pH and low FA versus high pH and high FA – long term anammox reactors operation (Test 4)

The Test 4 in the overall preliminary studies outline is presented in Figure 4-15.

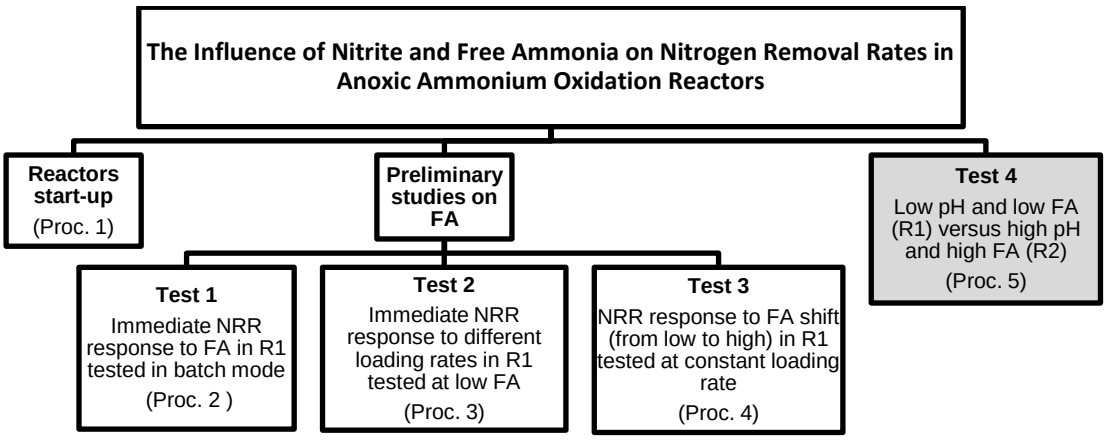


Figure 4-14 Test 4 in the overall preliminary studies outline

4.3.1. Reactors operation and observations

In the long-term reactor operation test, two MBBRs, reactor 1 with pH 6.5 (R1 – pH set at 6.5) and reactor 2 with the ambient pH naturally occurring at about 7.8±0.24 (which reported optimum range in the literature, R2), were compared. In both reactors, similar nitrogen conversion ratios were observed, with an overall nitrogen balance at a ratio of NH_4-N conversion to NO_2-N conversion to NO_3-N production of 1:(1.23±0.06):(0.21±0.02) and 1:(1.15±0.09):(0.13±0.03) for R1 and R2, respectively. These nitrogen conversions are typical for anammox, indicating that the dominant reaction was driven by anammox.

Over 99% of the total measured VSS in the MBBR reactors were in attached form. Suspended solids concentration in the effluent of the MMBRs was 0.1 g VSS/L and 0.06 g VSS/L for R1 and R2, respectively, while

suspended solids concentration in the influent to the anammox MBBRs was 0.04 g VSS/L. Some of these incoming solids originate from the nitritation reactor, with the balance coming from the feed centrate. It is possible that oxygen utilizing organisms from the nitritation reactor scavenged any dissolved oxygen present in the anammox reactor. This could assist the anammox process by eliminating the well studied inhibitory effects of dissolved oxygen (Strous et al., 1997; Third et al., 2001; Cema et al., 2005; Liu, et al., 2008; Chen et al., 2009).

Both reactors R1 and R2 had stable nitrite concentration and similar NRR, when they were operated under low and medium load and nitrite concentrations (days 0 to 54 for R1 and R2, Figure 4-15, Table 4-5). Subsequently, under the high load and nitrite concentrations tested (days 100 – 168 for R1 and days 55 – 102 for R2), it was observed that R1 had a relatively stable nitrite concentration and high volumetric specific nitrogen removal rate (NRR, Figure 4-15a, Table 4-5), while R2 experienced some NRR instability, which was correlated to nitrite concentrations (Figure 4-15b).

High nitrogen loads in reactor R2 at first enhanced the NRR, reaching the highest activity at 3746 mg N/Ld, and subsequently hindered the NRR. This is in agreement with similar observations in the literature (Szatkowska et al., 2007; Okabe et al., 2011). During the high loading period, the loading rate to R2 had to be lowered due to NRR instabilities and nitrite accumulations

exceeding 50 mg N/L. Therefore, the maximum loading rate for R2 was adjusted in a way that nitrite would not exceed 50 mg N/L.

Table 4-5 – Operational results of reactors R1 and R2 under various loads and nitrite concentrations

Parameter	Units	Low load and low nitrite condition		Medium load and medium nitrite condition		High load[(1)] and high nitrite condition	
Reactor	-	R1	R2	R1	R2	R1	R2
Research period (Fig. 5.13)	d	34-54	34-54	0-19	6-19	96-168	55-102
NH_4-N	mg N / L	42.0 ±15.0	26.1 ±14.0	66.4 ±17.1	54.5 ±19.5	126.7 ±24.4	68.1 ±28
NO_2-N	mg N / L	3.3 ±0.4	3.1 ±0.6	9.3 ±0.8	11.0 ±2.3	32.8 ±7.2	23.4 ±14
pH	-	6.5 ±0.01	7.46 ±0.07	6.5 ±0.01	7.92 ±0.14	6.5 ±0.01	7.92 ±0.16
N-load	mg N / L d	1313 ±163	1208 ±90	2981 ±96	2836 ±83	8777 ±519	3055 ±484
NRR	mg N / L d	1143 ±135	1090 ±75	2466 ±74	2405 ±82	6199 ±396	2448 ±580
FA	mg N / L	0.2 ±0.1	0.8 ±0.4	0.4 ±0.5	4.8 ±2.2	0.5 ±0.1	6.4 ±4.7
NO_2-N / NH_4-N	mg N / mg N	1.16 ±0.05	1.10 ±0.05	1.19 ±0.09	1.13 ±0.09	1.23 ±0.06	1.14 ±0.11
NO_3-N / NH_4-N	mg N / mg N	0.15 ±0.04	0.12 ±0.01	0.16 ±0.04	0.14 ±0.04	0.21 ±0.02	0.12 ±0.03

[(1)]High load means maximum nitrogen load to the anammox reactor which will increase nitrite concentration inside of the reactor but will not cause nitrite accumulation to greater than 30 – 50 mg N/L

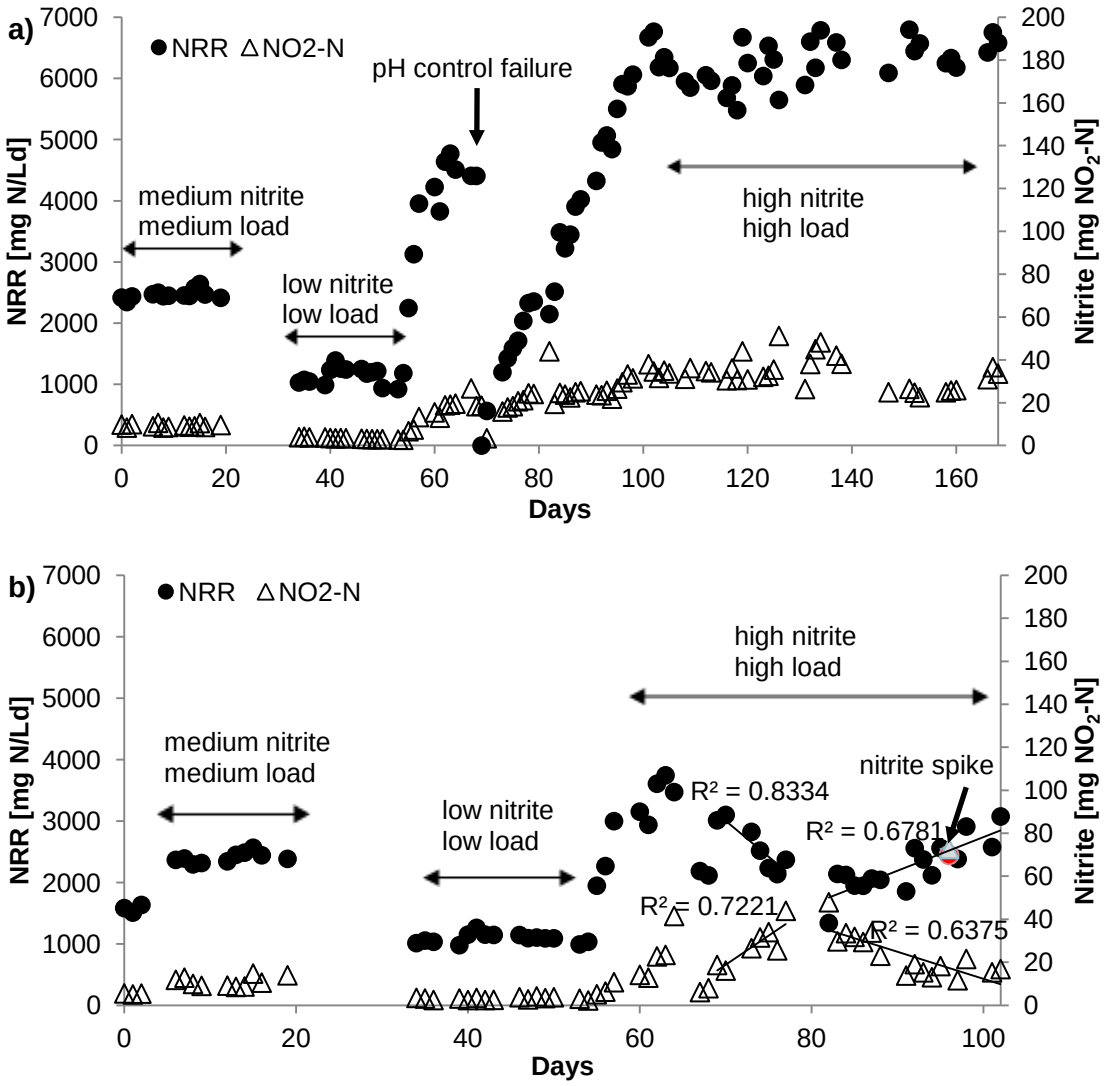


Figure 4-15 The NRR and nitrite during long-term reactors operation in a) R1 at pH = 6.5 and b) R2 at an ambient pH = 7.8±0.24. Point indicated by an arrow was not included in the linear regression (sudden nitrite spike)

A significant increase in NRR occurred under high nitrogen load and nitrite conditions for R1, as compared to R2. Indeed the average NRR obtained in R1 was 61% greater than that obtained in R2 (Table 4-5). Such a high NRR

observed in R1 has never been reported for an anammox MBBR system, although it agrees with the theoretical prediction proposed by van der Star et al. (2007).

4.3.2. Impact of nitrite concentration on NRR

Low nitrite concentration (below 5 - 20 mg NO_2-N/L) has been considered critical to preventing anammox bacteria inhibition (Szatkowska et al., 2007; Wett et al., 2007; Bettazzi et al., 2010). In this study, the NRR was plotted against nitrite concentration for both reactors (Figure 4-16), to evaluate any inhibitory effect of elevated nitrite levels. For R1 and R2, nitrite did not cause inhibition within the range tested, since a clear declining trend for NRR was not observed. For R1, higher nitrite concentrations resulted in higher NRR, until a plateau was reached at 25 mg N/L. For R2, the nitrite concentration was linearly correlated with the NRR (similar to R1), but at levels above 10 mg N/L, the NRR reached a scattered plateau. While the two reactors were inoculated from the same source, it was possible that the differences in pH regime for R1 and R2 during the acclimation process, which lasted 1 year, caused the selection of different anammox populations with different pH optima and tolerances to nitrite. It was unknown whether different microorganisms were in both reactors; however, both reactors performed very similar nitrogen conversion ratios and they had the same NRR, when exposed to the same pH and FA conditions (Test 5b – R2 and Test 8b – R1 and R2). These results

suggest that anammox reactors can be operated at higher loading rates and high nitrite levels, when the pH is kept low.

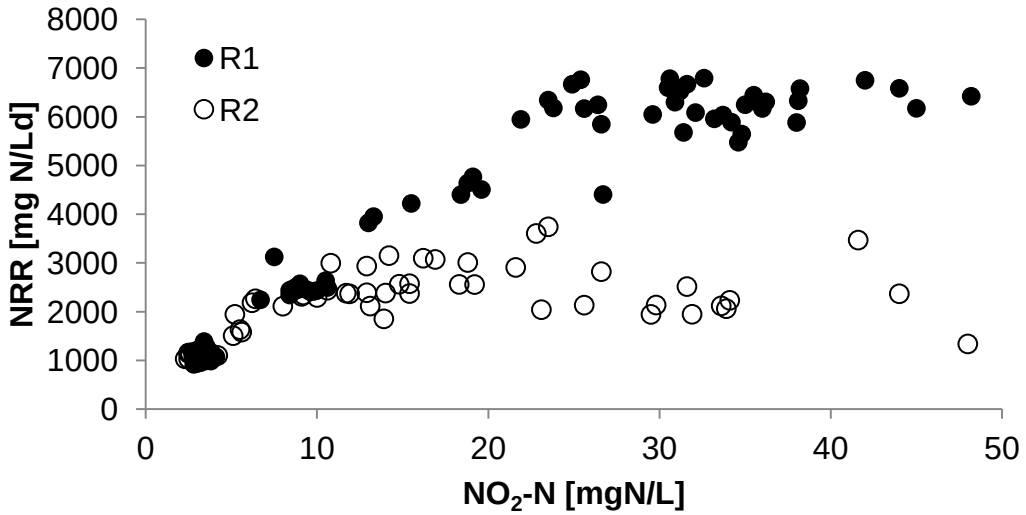


Figure 4-16 - The relation between the nitrite concentration and the NRR in R1 with low pH and R2 with ambient pH for the entire research period.

Within the tested range, it is unlikely that nitrite was responsible for the NRR deterioration in R2 at nitrite levels exceeding 20 mg NO_2-N/l, as observed in Figure 4-15. Lack of operational stability, reflected in sudden spikes of nitrite and changes in NRR, was reported by Rosenthal et al. (2009). Those authors could not explain sudden NRR deterioration by using the nitrite inhibition concept reported in the literature, since their reactor was operated under a low nitrite concentration (below 1 mg N/L). They also concluded that there must be another parameter which caused sudden NRR fluctuations.

Szatkowska et al. (2007) claimed a nitrite inhibitory tendency, based on the nitrogen removal efficiency, which in the light of these results is rather doubtful (any nitrogen concentration increase in nitrite or ammonium, or both at the same time, will result in nitrogen removal efficiency decrease). However, other researchers (Rosenthal et al., 2009; Okabe et al., 2011) recorded a clear correlation between NRR and nitrite, although maximum nitrite concentrations were below majority threshold concentrations (described in the Literature Review). On the other hand, such a positive correlation between elevated nitrite and hindered NRR may show that nitrite is a very clear indicator of system destabilization (NRR losses) during reactor operation. It should be noted that most anammox reactors are designed for nitrite limiting conditions (van der Star et al., 2007). Therefore, any nitrite increase above the designed concentration, but within nitrite non-inhibitory range, is truly related to an unstable situation (other than nitrite inhibition), which is widely documented in the literature (Dapena-Mora et al., 2004; Fux et al., 2002; Zhang et al., 2010; Dosta et al., 2008; Arrojo et al., 2006; Wett et al., 2007).

4.3.3. The pH-related free ammonia effect on NRR

According to the literature, the reported optimum pH range for anammox organisms is 7 to 8.5 (Strous et al., 1997). In this study, low bulk pH provided better performance under high-load and high-substrate concentrations. Because of the potentially toxic nature of FA and its pH dependent fluctuation

at constant total ammonia concentration, it is difficult to distinguish pH effects from FA effects. Therefore, FA concentration was investigated as a potential factor, which may have greater impact on the anammox rates than pH alone. During the entire research period, R1 had a pH set at 6.5 with the liquid calculated FA averaging 0.4 ± 0.3 mg N/L; R2 had a naturally occurring pH of 7.81 ± 0.24, with the liquid calculated FA averaging 4.5 ± 3.2 mg N/L, a concentration that is typically observed in full and pilot scale anammox reactors (van der Star et al., 2007; Szatkowska et al., 2007).

The lowest FA toxicity threshold concentration provided in the literature was 1.7 mg N/L (Jung et al., 2007), which was lower than the values obtained for R2. Therefore, FA was tested as a potential cause for NRR deterioration. When the NRR was plotted versus FA (Figure 4-18), an inverse correlation was observed between NRR and FA, supporting the contention that FA is the

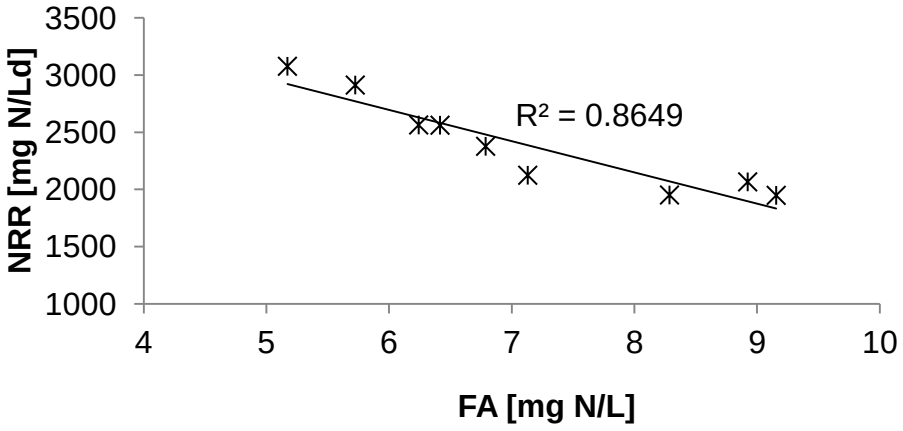


Figure 4-17 - The relation between the FA concentration and the NRR in R2 with ambient pH between day 67 and 102. Regression refers to points for TA above 70 mg N/L and nitrite in the range of 15 – 50 mg N/L.

inhibitor. The R^2 = 0.86 refers to points where nitrite and ammonium concentrations range above 70 mg NH_4-N/L and 15-50 mg NO_2-N/L, respectively, between day 67 and 102 of the experiment.

R1, operating at low pH and FA, outperformed R2, operating at ambient pH and high FA. This trend is also reflected in the literature (Table 4-6). In Table 4-6, only anammox reactors with synthetic feed were presented as a comparison to this study, in order to eliminate the effect of the inert solids coming to the system with the feed and to minimize the influence of other

Table 4-6 – Relationship between pH, free ammonia (FA) and the specific nitrogen removal rate (sNRR) in different anammox reactors

Reactor type	pH	FA [mg N/L]	NO_2-N [mg N/L]	sNRR [g N / g VSS d]	Reference
UASB[(1)]	7.9-8.2	1.2 - 2.0	15 - 50	1.8	Tang *et al.,* 2010
FBR[(2)]	7	Below 0.8	Close to 0	1.0	van de Graaf *et al*, 1996
FBR	8	Around 8	Close to 0	0.15-0.18	Strous *et al.,* 1997
SBR	7-8	1-10	Close to 0	1.9	Strous *et al.,* 1998
Attached growth	7.0-7.5	-	224	1.6	Tsushima *et al.,* 2007
R1[(3)]	**6.5**±0.01	**0.5**±0.1	**30(120)**	**0.7(1.1)**	**This study**
R2[(3)]	**7.8**±0.2	**8.3**±5.1	**30**	**0.3**	**This study**

[(1)] upflow anaerobic sludge blanket

[(2)] fluidized bed reactor

[(3)] VSS was not monitored on regular basis; result based on one VSS measurement from two randomly chosen rings

microorganisms. It was observed that high specific anammox activity was associated with low FA. This observation, together with the collected data from the conducted experiment, suggests that FA concentration may be critical to anammox process stability, while nitrite toxicity may be overestimated in some cases in the literature.

4.3.4. Engineering significance

This test demonstrated that high and stable NRR can be achieved when low FA was provided. During regular reactor operation at pH 6.5, the NRR at about 6.2 g N/Ld was archived. This value was never achieved before till this study was conducted. One particular study found based on theoretical calculations that MBBR reactor should remove nitrogen at rate close to 6.1 g N/Ld. Conducted research showed that controlling FA at low level is required to approach high rates in anammox reactors. Achieving high rates in anammox reactors allow significant reduction in reactor volume which saves money.

Conducted test showed that controlling FA at low level is required to approach high rates in anammox reactors. Achieving high rates in anammox reactors allow significant reduction in reactor volume, thereby, designing compact reactors

Current full-scale plants are greatly overdesigned when NRR achieved compared to NRR obtained in current study. At the same time, they have high

potential for process optimization when greater nitrogen loading has to be treated.

During this research period, pH control failure occurred, which caused irreversible NRR reduction up to about 1200 mg N/Ld. However, during 24 days, the NRR was possible to recover completely (about 6000 mg N/Ld). Sudden upsets may appear during full scale reactors operation (van der Star et al., 2007). Fast NRR recovery was possible due to operation reactor at high nitrite (prevent from significant substrate limitation) and low FA which allowed to operate reactor at high nitrite (at high FA, high nitrite were inhibiting NRR in R2).

4.3.5. Conclusions

The aim of this test was to study the effect of nitrite and pH on the anammox organism activity in moving bed biofilm reactors (MBBR). Based on the nitrogen mass balance, the nitrogen removal rate (NRR) was calculated to assess the anammox rates. The following observations and conclusions were made:

- Reactor operating under ambient pH of about 7.5 – 8.0 had a 61% lower NRR than a reactor with a lower pH 6.5.

- The reactor stability was not affected by the periodically changed loads when the reactor was operated under the low pH of 6.5.
- It was confirmed that low nitrite provided stable anammox reactor performance; however, high nitrite concentrations were not found to cause process destabilization.
- It is hypothesized that free ammonia is a very important stability parameter in anammox systems, where nitrite inhibition may be overestimated. Indeed, different studies have suggested that elevated nitrite was correlated with activity losses; however, the losses were unlikely to be caused by nitrite.

4.4. Anammox rate response to nitrite

This chapter focused on anammox response to nitrite (Test 5, Procedure 5 and Procedure 6). Biomass consortia were cultivated in a form of biofilm on a plastic carrier media (R1 – low pH of 6.5 and R2 – ambient pH) and in suspended flocculated form (R3 – pH 7.0). The purpose of this part of the research was to present the rates of anammox response to nitrite concentration in a wide nitrite range, thereby finding nitrite inhibition threshold concentration (NTC). The anammox rate response to nitrite was also investigated under different exposure times. Short-term tests investigated biomass immediate response to nitrite (below 1 day), whereas long-term tests

investigated biomass behaviour during reactor operation at elevated nitrite (greater than 1 day). The intention was to show that nitrite can be mitigated if FA is kept low (below 2 mg N/L) or constant.

The nitrite range (up to 400 mg NO_2-N/L) and exposure time (below one day and above one day) applied in the current tests were similar to those applied in different studies (Wett et al., 2007; Bettazzi et al., 2010; Kaldate et al., 2009; Fernández et al., 2010; Cho et al., 2010; Jung et al., 2007; Kimura et al., 2010; Musabyimana et al., 2008; Rosenthal et al., 2009; Strous et al., 1999; Tsushima et al., 2007; Dapena-Mora et al., 2007). The drastic differences in results presented in the literature were confronted and appeared to be FA related. This current study was consistent with the majority of studies, which demonstrated a lack of nitrite inhibition up to 150 – 200 mg NO_2-N/L. The significant difference between this study and some previous studies, as presented in the Literature Review chapter, was that literature studies investigated biomass activities responses to different nitrite concentrations under variable FA concentrations greater than 2 mg N/L.

4.4.1. Immediate anammox rate response to nitrite (Test 5)

The Test 5 in the overall nitrite detail studies outline is presented in Figure 4-18.

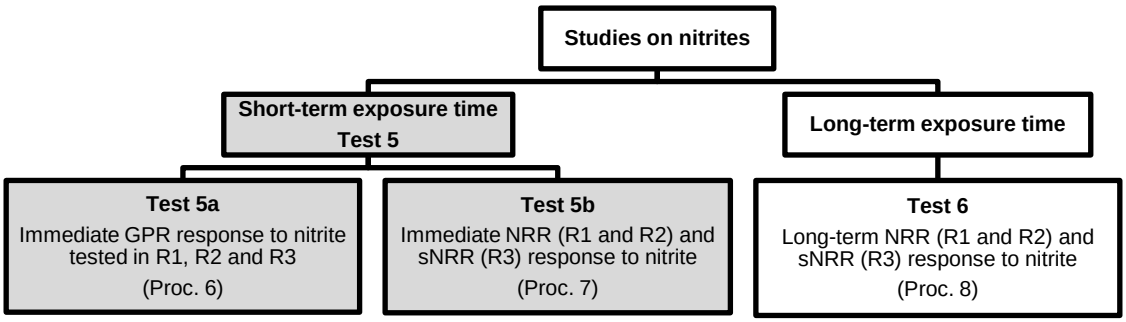


Figure 4-18 Test 5 in the overall nitrite detail studies outline

Immediate anammox response to nitrite was investigated in two different test series. The first test series, Test 5a, investigated nitrite inhibitory effect on biomass activity, based on the gas respiration rate using respirometry. Samples were tested using the identically defined nutrient media with only one variable which was nitrite in the range from about 33 to about 420 mg NO_2-N/L. The second test series, Test 5b, investigated the inhibitory effect of nitrite on nitrogen removal rate inside the reactor, based on the nitrogen mass balance. During Test 5b, pre-treated centrate in the partial nitritation reactor was used.

4.4.1.1. Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R1 (Test 5a - R1)

During the respiration test, the anammox rate was significantly increased, starting at 53% at 34 mg NO_2-N/L, and reaching 100% (the maximum gas

production rate – GPR, during the test) at about 138 mg NO_2-N/L. Subsequently, a 5% minor decrease in GPR was observed at 193 mg NO_2-N/L, followed by a significant 49% decrease in GPR at 416 mg NO_2-N/L. The results of this test are depicted in Figure 4-19. Although gas composition was not analyzed, it was assumed that nitrogen gas had been respired during the anammox reaction. Based on the nitrogen removed in the control sample (the lowest nitrite concentration tested), typical anammox nitrogen conversions were recorded (1:1.30±0.03:0.21±0.00). According to a study conducted by Bettazzi et al. (2010) and Ni et al. (2010), the anammox reaction generates nitrogen gas as a final product and it can be a good representation of anammox activity, when ammonium and nitrite utilization and nitrate production follow anammox stoichiometry.

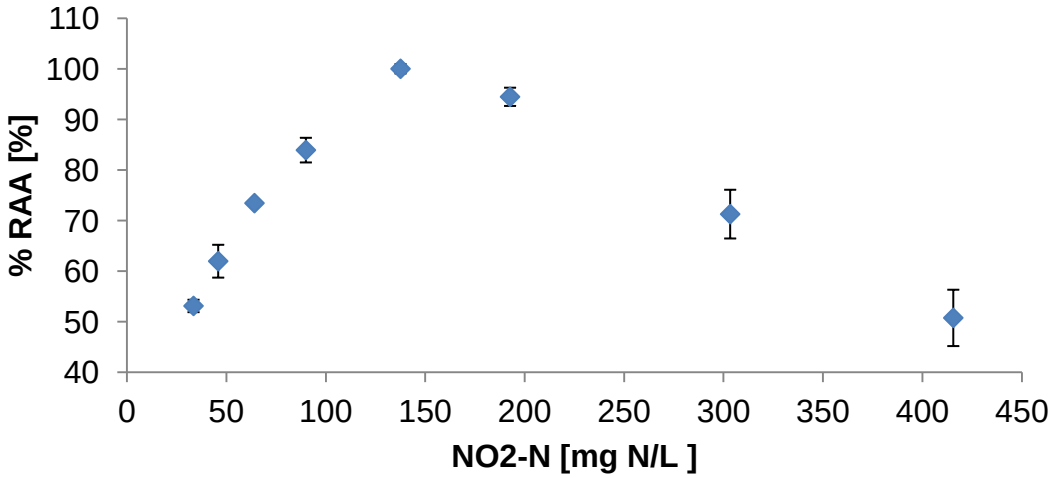


Figure 4-19 Immediate response of anammox activity (biomass originated from R1) to nitrite measured by gas production rate. Percentage represents relative anammox activity (%RAA) to the highest observed gas production rate (GPR) under any nitrite concentration.

These results were in very close agreement with most of the studies which investigated the immediate anammox activity response to nitrite based on the nitrogen gas production rate (Dapena-Mora et al., 2007; Fernández et al., 2010) and nitrogen consumption rate (Strous et al., 1999). In all of these studies, nitrite greater than 200 mg NO_2-N were required to achieve significant reduction in anammox rate.

4.4.1.2. Immediate NRR response to nitrite tested in R1 (Test 5b - R1 is equal to Test 2)

The results of these tests were described in the Chapter 4.2, preliminary study (Test 2). Although higher nitrite concentrations than 200 mg NO_2-N/L were not investigated, the primary goal was achieved showing no inhibition of nitrite concentrations up to the investigated level. In Figure 4-20, nitrite stimulated NRR the same as they stimulated gas production rate observed in the Test 5a – R1 (Figure 4-19). FA during this test was always below 2 mg N/L.

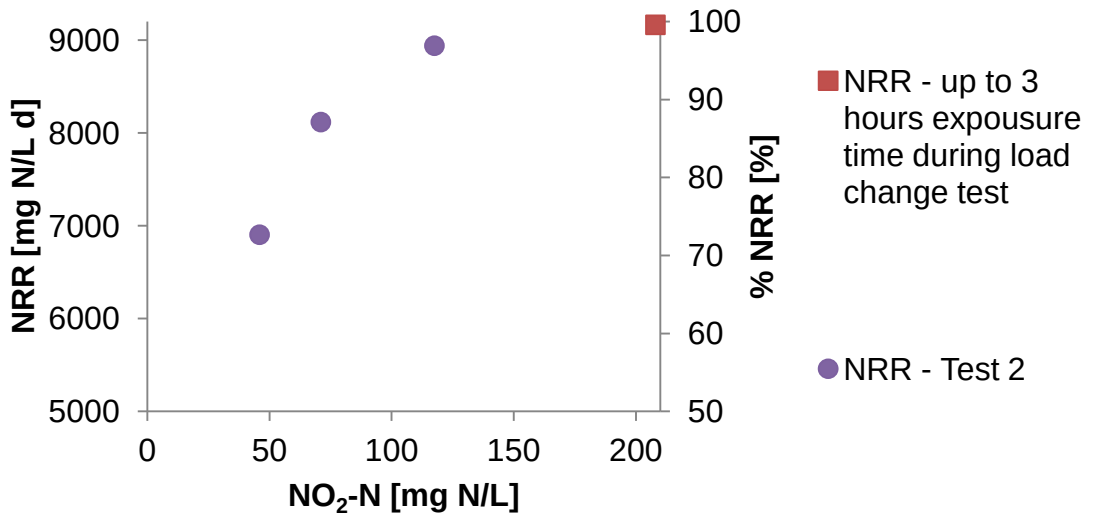


Figure 4-20 Immediate response of nitrogen removal rate (NRR) to nitrite measured by nitrogen mass balance. Percentage represents relative NRR (%NRR) to the highest NRR observed during any nitrite concentration. Tests were conducted in reactor R1.

These results show that the NRR was stimulated by nitrite up to 117.5 mg N/L without signs of inhibition, for up to 3 hours of exposure time. Nitrite as high as 208 mg N/L did not further stimulate the NRR and did not show signs of inhibition when compared to Michaelis-Menten based NRR for the same nitrite. The NRR achieved (9.2 g N/Ld) was very close to predicted value (9.4 g N/Ld).

Nitrite as high as 208 mg N/L did not negatively affect the NRR. This result was in close agreement with the majority of results, where nitrite inhibition was investigated under either constant or low FA concentrations (constant FA at 3.5 mg N/L Fernández et al., 2010; batch testing with constant pH and TA

Rosenthal et al., 2009; batch testing with constant pH and TA Strous et al., 1999; constant FA at 3.5 mg N/L Dapena-Mora et al., 2007). This supports the contention that nitrite can stimulate or exhibit no inhibitory effect on NRR, as long as FA remains below the inhibition threshold concentration of about 2 mg N/L.

4.4.1.3. Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R2 (Test 5a – R2)

During the respiration test, the anammox activity was almost constant within nitrite concentrations of 54 – 151 mg NO_2-N/L, followed by a significant 45% decrease in GPR at 422 mg NO_2-N/L. The results of this test are depicted in Figure 4-21. Although gas composition was not analyzed, it was assumed that nitrogen gas had been produced as end product during the anammox reaction. Based on the nitrogen removed in the control sample (the lowest nitrite concentration tested), typical anammox nitrogen conversions were recorded (1:1.18±0.04:0.34±0.05). According to the study conducted by Bettazzi et al. (2010) and Ni et al. (2010), anammox reaction generated nitrogen gas as a final product and it can be a good representation of anammox activity when ammonium and nitrite utilization and nitrate production follow anammox stoichiometry.

These results agreed with most of the studies that investigated the immediate anammox activity response to nitrite based on the nitrogen gas

production rate (Dapena-Mora et al., 2007; Fernández et al., 2010) and nitrogen consumption rate (Strous et al., 1999).

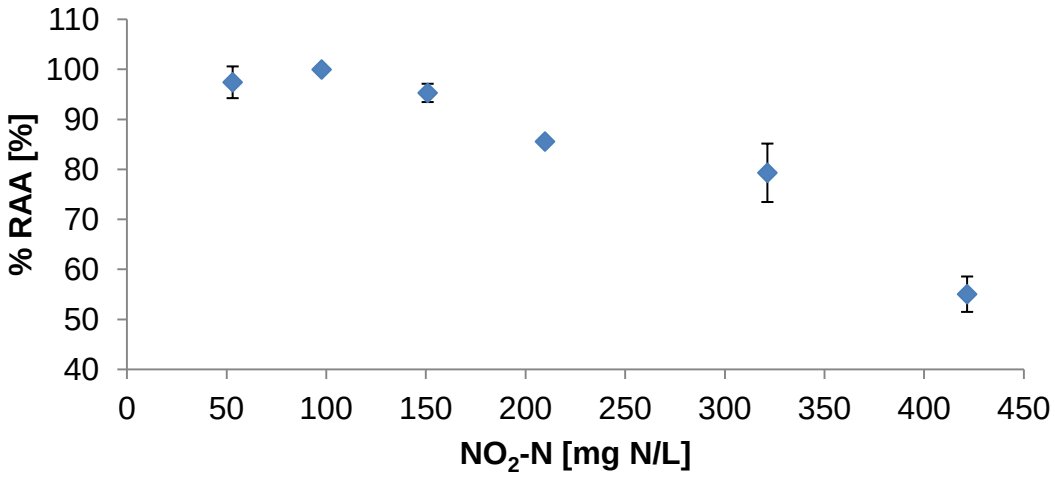


Figure 4-21 Immediate response of anammox activity (biomass originated from R2) to nitrite measured by gas production rate. Percentage represents relative anammox activity (%RAA) to the highest observed gas production rate (GPR) under any nitrite concentration.

These results of %RAAs, within nitrite rage of 54 – 151 mg NO_2-N/L, are significantly different from %RAAs in R1, within a similar nitrite range. In R2, %RAAs were almost constant where in R1 they had a significant increase. Such a %RAA response in R2 can be related to FA inhibition residue observed in Test 3 for R1. Although FA was below 2 mg N/L during the respirometry test, the biomass was grown under elevated FA at about 5 – 10 mg N/L, as similar values were recorded during Test 3. Test 5b – R2 further supports the FA inhibition residue hypothesis. In that test, when FA was low, NRR in R2 was stimulated by nitrite, the same as it was observed for R1.

4.4.1.4. Immediate nitrogen removal rate (NRR) response to nitrite tested in R2 (Test 5b – R2)

Test 4 demonstrated that nitrite did not stimulate NRR when nitrite was greater than 10 mg NO_2-N/L. In the current test, the loading rate was increased by 44% (achieved by increasing the flow rate) at FA of 7.5 mg N/L, which caused ammonium and nitrite accumulation. The steady state where ammonium and nitrite were increasing was not observed until the end of the test (in total 26 hours). NRR achieved at the end of this test was similar to the one recorded at the beginning of this test. This test showed a lack of nitrite stimulating effect (up to 123.2 mg NO_2-N/L) on NRR similar to those observed during Test 4, but within a lower nitrite range (up to 50 mg NO_2-N/L). Results of this part of the Test 5b – R2 are presented in Appendix 5 (page 281).

It was decided that a new test should be conducted. During this new test, the FA was lowered below 2 mg N/L, by adjusting the pH inside of the reactor at 6.5, similar to the pH in R1. After that, different nitrogen loading rates were set to provide desired nitrite concentrations. The purpose of such an FA concentration was to eliminate the inhibitory FA effect on NRR observed during Test 1 and Test 3 for R1. Test 5b – R2 was conducted according to the same procedure as Test 2.

Figure 4-22 reports the NRR response to different nitrite concentrations. The test showed that nitrite stimulated the NRR up to the investigated concentration of 108 mg NO_2-N/L.

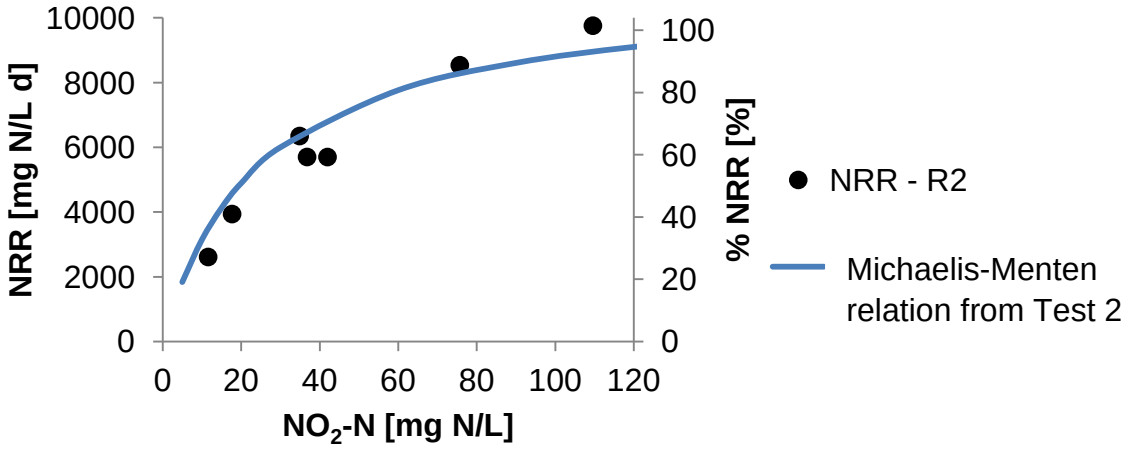


Figure 4-22 Immediate response of nitrogen removal rate (NRR) to nitrite measured by nitrogen mass balance. Percentage represents relative NRR (%NRR) to the highest NRR observed during any nitrite concentration. Tests were conducted in reactor R2.

Although higher nitrite concentrations than 108 mg NO_2-N/L were not investigated, the primary goal was achieved, showing no inhibition of nitrite concentrations up to the investigated level. Additionally, this test showed that nitrite can stimulate NRR when FA is low.

In Figure 4-22, nitrite stimulate NRR in R2 the same as they stimulated the gas production rate observed in the respirometry test in R1 (Test 5a – R1, Figure 4-19) and NRR in R1 at elevated nitrite during loading changes (Test 5b – R1, Figure 4-20). This suggests that R2 can perform NRR as well as R1 can, as long as FA is kept low; this shows the importance of the FA on the reactor performance, despite growing biomass under different pH and FA conditions. It was unknown whether the anammox species or multiorganism

consortium composition responsible for the reaction was the same or different but certainly they could perform the same NRR under the same nitrite, pH, and FA conditions. This is important from the engineering point of view where reactor stability can be controlled effectively by environmental parameters in which acclimation time to new environmental conditions (pH and FA) may not be significant. Test 8b – R1 and R2 provided further evidence supporting this important observation that FA has the dominant role in achieving high NRR regardless of the acclimation time to FA.

4.4.1.5. Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R3 (Test 5a – R3)

During the test, the anammox activity increased starting at 71% at 44.2 mg NO_2-N/L and reaching 100% (the maximum gas production rate – GPR, during the test) at about 206.1 mg NO_2-N/L. After reaching maximum activity, a 64% drastic decrease in GPR at 415.5 mg NO_2-N/L occurred. The results of this test are depicted in Figure 4-23. Although gas composition was not analyzed, it was assumed that nitrogen gas had been produced during the anammox reaction. Based on the nitrogen removed in the control sample (the lowest nitrite concentration tested), typical anammox nitrogen conversions were recorded (1:1.33±0.04:0.10±0.06). According to a study conducted by Bettazzi et al. (2010) and Ni et al. (2010), anammox reaction generates nitrogen gas as a final product and a clear representation of anammox activity can be

observed when ammonium and nitrite utilization and nitrate production follow anammox stoichiometry.

These results were in very close agreement with most of the studies which investigating the immediate anammox activity response to nitrite based on the nitrogen gas production rate (Dapena-Mora et al., 2007; Fernández et al., 2010) and nitrogen consumption rate (Strous et al., 1999).

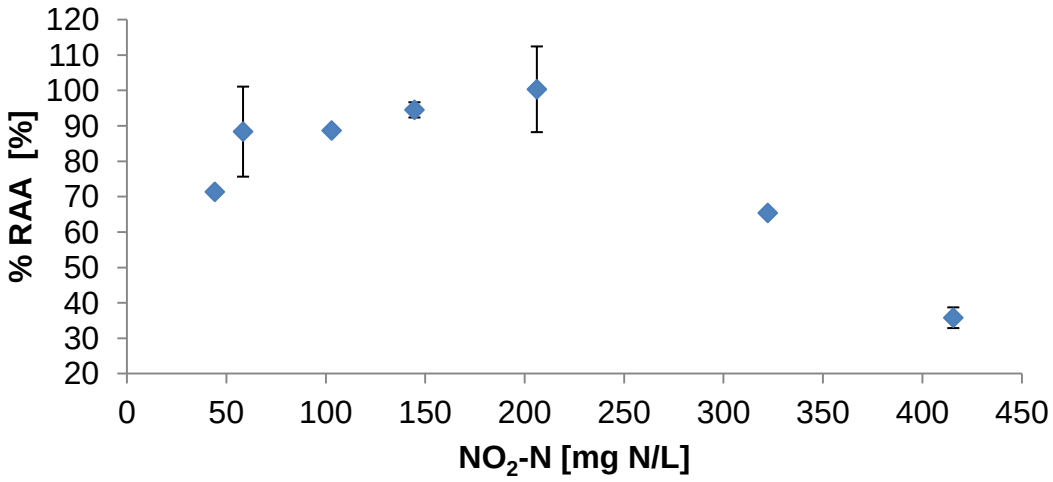


Figure 4-23 Immediate response of anammox activity (biomass originated from R3) to nitrite measured by gas production rate. Percentage represents relative anammox activity (%RAA) to the highest observed gas production rate (GPR) under any nitrite concentration.

During this test, similar to those for biofilm, Test 5a – R1 and Test 5a – R2, nitrite concentrations in the range 150 – 200 mg NO_2-N/L were shown to be the threshold concentration. Just as before, a threshold concentration achieved in the current study was similar to those reported in the literature.

These results further support the contention that nitrite, up to investigated concentration in the range of 150 – 200 mg NO_2-N/L, should not have a negative inhibitory effect on biomass activity.

Batch tests during this test and previous tests were very useful method for testing the immediate biomass activity response to nitrite. They allowed evaluating nitrite and FA effect on biomass activity without the threat of losing the biomass activity in the main reactor.

4.4.1.6. Immediate specific nitrogen removal rate (sNRR) response to nitrite tested in R3 (Test 5b – R3)

During this test, only two maximum nitrite concentrations were tested, first up to 91.0 mg NO_2-N/L and then up to 143.5 mg NO_2-N/L. These results were compared to the sNRR achieved during long-term reactor operation. As shown in Figure 4-24, the sNRR was not affected negatively by nitrite up to the investigated level, since no declining trend for sNRR was observed.

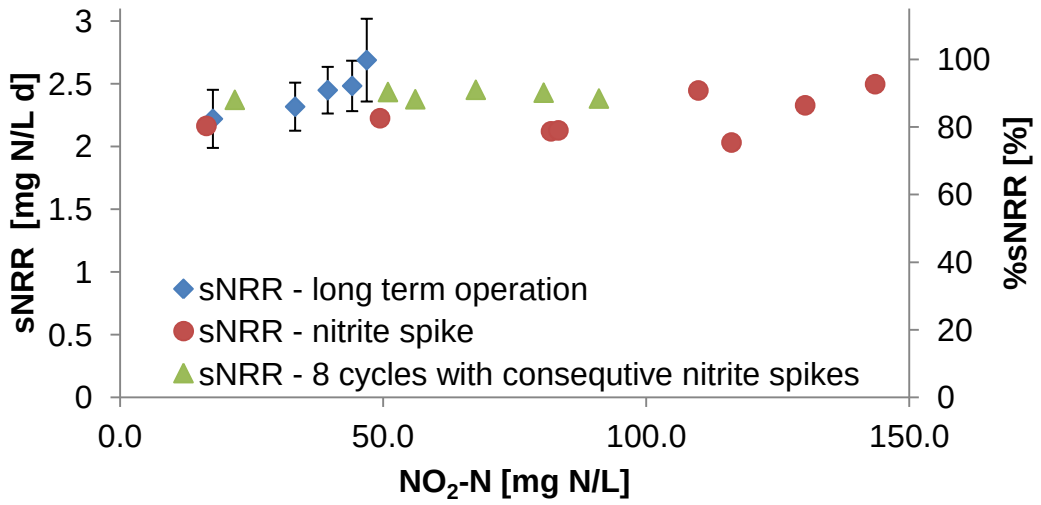


Figure 4-24 Immediate response of specific nitrogen removal rate (sNRR) to nitrite measured by nitrogen mass balance during three different SBR cycles. Percentage represents relative sNRR (%sNRR) to the highest sNRR observed during any nitrite concentration. Tests were conducted in reactor R3.

The results of this test agree with the previous results obtained during the respirometry test (Test 5a – R3), where nitrite did not have an inhibitory effect on biomass activity within a similar nitrite range. Although higher nitrite concentrations than 143.5 mg NO_2-N/L were not investigated, the primary goal was achieved, showing no inhibition of nitrite concentrations up to the investigated level of about 150 mg NO_2-N/L.

4.4.2. Long-term anammox response to nitrite (Test 6)

The Test 6 in the overall nitrite detail studies outline is presented in Figure 4-25.

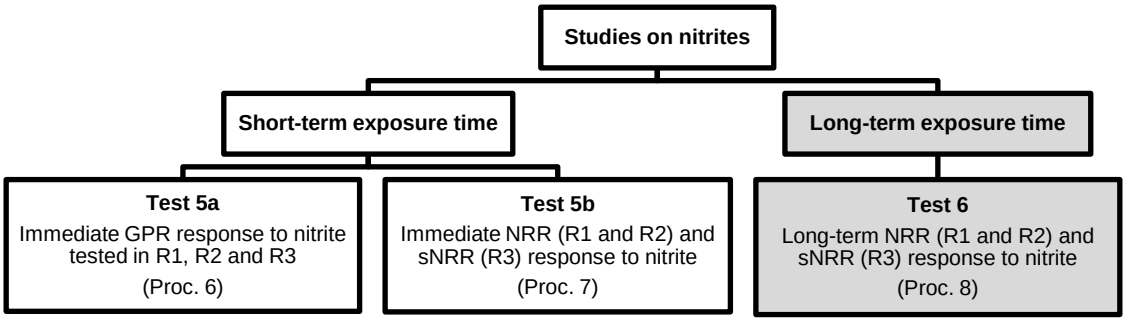


Figure 4-25 Test 6 in the overall nitrite detail studies outline

In Test 5, immediate nitrogen removal rate (NRR) and gas production rate (GPR) response to nitrite showed that the nitrite inhibition threshold concentration was likely to be rather high at about 150 – 200 mg NO_2-N/L. However, Strous et al. (1999) demonstrated significant anammox activity deterioration when exposure time was greater than 10 hours. Therefore, in Test 6, elevated nitrite concentrations were tested during a long-term reactor operation (greater than one day).

Elevated nitrite concentrations inside anammox reactors were achieved by adding nitrite to the feed. Nitrogen feed composition, centrate (feed to the

partial nitritation reactor) and pre-treated centrate (feed to anammox reactors), are presented in Appendix 5.

4.4.2.1. Long-term nitrogen removal rate (NRR) response to elevated nitrite concentrations tested in R1 (Test 6 – R1)

During this test, reactor R1 was operated at a constant flow rate. For the first 34 days of the test, the reactor was operated at regular nitrogen load and nitrogen concentrations. The NRR was stable at about 5401 ± 542 mg N/Ld with nitrite at about 34.0 ± 5.3 mg NO_2-N/L. The nitrogen conversions were very similar to anammox activity reported in the literature and were (1:1.29±0.02:0.24±0.01). The nitrite and ammonium in the feed (the pre-treated centrate in the partial nitritation reactor) were at a ratio of about 0.95 ± 0.10 mg NO_2-N/mg NH_4-N. The reactor was operated at nitrite limiting condition (typical situation during anammox reactors operation, van der Star et al., 2007). In this study, the FA was about 0.5 ± 0.1 mg N/L, which is below the inhibition threshold concentration (Test 7a – R1). Figure 4-26 depicts reactor R1 performance during the test.

On day 28, a sudden 63% NRR reduction occurred with nitrite accumulation up to 268.8 mg NO_2-N/L. The source of this interruption was identified as a phosphorus limitation (PO_4-P below detection limit). After raising the phosphorus concentration up to about 10 mg PO_4-P/L, the NRR recovered within two days with no further effect on reactor performance. After

that, the phosphorus feed concentration was monitored and it was supplemented, when necessary, by adding sodium phosphate to maintain arbitrarily chosen inorganic phosphorus concentrations between 5 and 10 mg PO_4-P/L. Phosphorus limitation occurred only in this test and it was caused by the sudden onset of ferric chloride dosing at the wastewater treatment plant. Usually high phosphorus concentration exists in the centrate due to phosphorous release from biomass during anaerobic digestion process. Based on this experience, where chemicals binding P are added to centrate, phosphorus should be checked on regular basis.

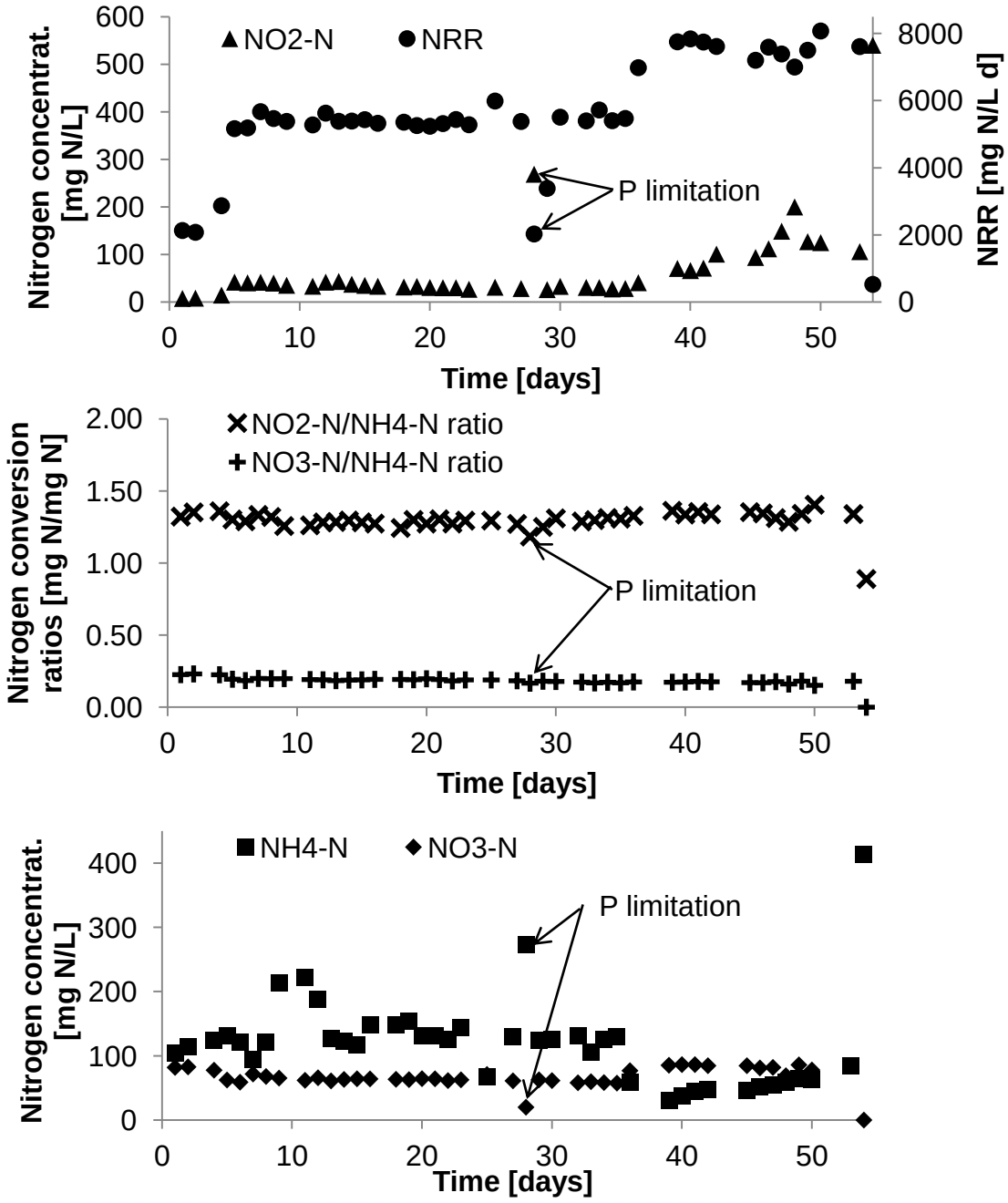


Figure 4-26 Histographs of a) nitrogen removal rate (NRR) and nitrite; b) nitrogen conversions rations NO_2-N to NH_4-N and NO_3-N to NH_4-N; and c) ammonium and nitrite in R1 during Test 6 – R1

On day 34, the nitrite concentration in the centrate feed (Appendix 5) was raised by adding sodium nitrite. This caused an increase in nitrite inside the anammox reactor on day 35 and the following 20 days. Because of the addition of nitrite in the feed, the nitrogen loading rate increased, and that led to a higher NRR.

During two periods of minimum 3 consecutive days 39 – 41 and 42 – 46, nitrite concentrations of about 69.4 ± 2.8 mg NO_2-N/L and 102.2 ± 9.2 mg NO_2-N/L, respectively, caused no reactor destabilization. On the contrary, during those times, a very high NRR was achieved with approximately 7779 ± 52 mg N/Ld and 7469 ± 232 mg N/Ld, respectively, for days 39 – 41 and 42 – 46. At the same time, nitrogen conversion ratios were at very similar ratios (1:1.35±0.01:0.24±0.00 and 1:1.34±0.01:0.23±0.00, respectively) to these, during reactor operation at lower nitrite (period 0 – 36). The FA was below 0.2 mg N/L. These results are in full agreement with previous studies where anammox reactors were operated at elevated nitrite (Tsushima et al., 2007; Kaldate et al., 2009; Kimura et al., 2010) and no negative effects of nitrite on NRR were observed. These results show that the nitrite inhibition threshold concentration is above 100 mg NO_2-N/L.

During days 47 and 48, it was observed that elevated nitrite up to 149 mg NO_2-N/L and 200 mg NO_2-N/L did not destabilize the NRR (NRR variations below 9% were recorded) and nitrogen conversion ratio (1:1.31:0.23 and 1:1.29:0.21, respectively). After this test period, nitrite were lowered to about

119.3 ± 11.6 mg NO_2-N/L, with the purpose of longer reactor operation at this nitrite concentration. Just as had been observed during days 39 – 46, there was no significant negative effect of elevated nitrite on NRR during this period.

After five days of operation (period 49 – 54) at nitrite of about 119.3 ± 11.6 mg NO_2-N/L, sudden NRR failure was observed on day 54, causing nitrite accumulation up to 540 mg NO_2-N/L. There is no indication of what caused this phenomena, as there was no evidence of system malfunction such as leakage of reactor content, exposure to atmospheric air, or breakage of tubing, and the feed was the same as in the three previous days (feed toxicity and/or nutrient problem).

Overall, the entire system performed as expected, except for the necessary change in the mixing speed (which was made on day 41). Based on visual observation, an overgrowth of the biofilm on the carrier media was observed, showing a decrease in the carrier aperture. As shown in Figure 4-27, the increase in mixing speed caused an increase in the concentration of solids in the effluent, which then led to the decrease in the apparent SRT (during the research, the solids mass balance may not represent true biomass SRT accumulated on the carrier media due to non-uniform biomass detachment from the carrier media).

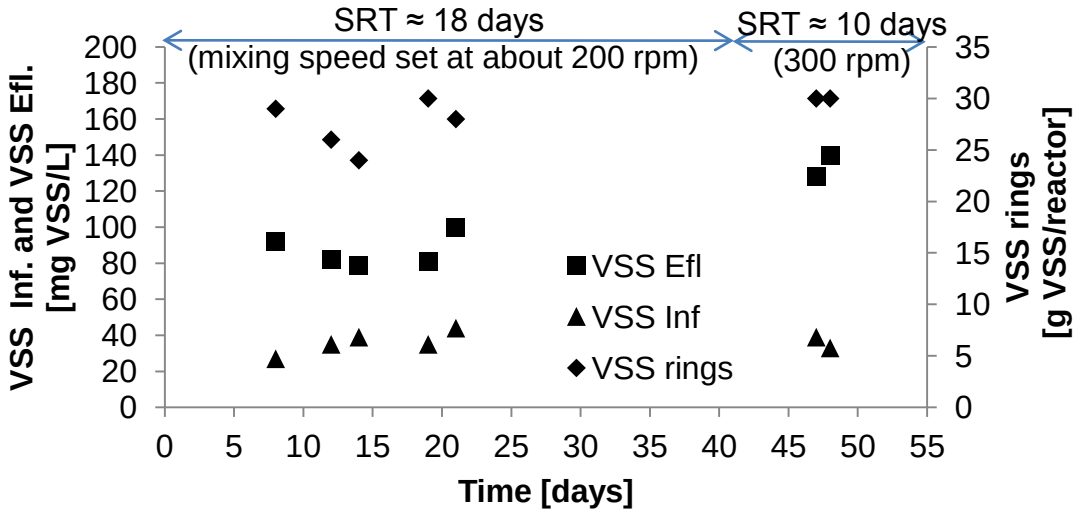


Figure 4-27 The volatile suspended solids (VSS) mass balance and apparent SRT during Test 6 – R1

Although, a constant amount of biomass was accumulated on the carrier media and stable NRR was recorded during the previous days (1 – 53), on day 54, the high NRR was lost. The reason for this sudden NRR loss is not clear; however, it is unlikely that it was caused directly by nitrite alone. Shortening the SRT could be one of the possible reasons for this sudden NRR loss, since in that process, either the anammox organism or other microorganisms, such as oxygen or an anammox by-product utilizer, would have been washed out of the system. The doubling time for anammox organism observed in pilot and full scale systems was between 10 and 40 days (Abma, et al., 2007; Caffaz, et al., 2006; Joss, et al., 2009). Therefore, operating reactor at 10 days SRT could washout anammox. The widely reported presence of nitrifiers (mostly

AOB) in anammox systems (Jetten et al., 1999; Cho et al., 2010; Schmid et al., 2000) may suggest their importance for the anammox consortium. Cho et al. (2010) conducted a microbial distribution study in the granular anammox consortium. Results clearly demonstrated that anammox organisms were surrounded by oxygen utilizers such as Chloroflexi-like filamentous bacteria and betaproteobacterial ammonia oxidizing bacteria. Of all the biomass, 80.6 ± 4.2% was anammox organisms with a small amount of other organisms thought to be crucial for oxygen removal. In this current test, when the shear force was increased through the mixing speed at day, the outer layer of the biofilm could be washed out which was protecting anammox consortium against oxygen.

During this test, NRR was tested under elevated nitrite concentrations where multi organism consortium was not analysed from the perspective of possible interaction between different microorganisms. The stability of anammox biochemistry relies on anammox organisms as well as ammonium oxidizing bacteria and others. This however was not analysed which was beyond the scope of this study. It is possible, that NTC could be exceeded for microorganism, which assisted the anammox, therefore sudden NRR deterioration was observed. The observed phenomenon, calls for interdisciplinary research, where microbiologists, having advanced molecular techniques, would be able to track and define complex interaction in the multi organism matrix.

This test did show that a anammox reactor can be operated at elevated nitrite levels with consecutive nitrite accumulation up to 200 mg NO_2-N/L, over 10 days time interval, without NRR reduction. Unfortunately, this test did not provide clear evidence for nitrite inhibition threshold concentration during long term reactor operation (more than 10 days), due to unclear sudden NRR reduction. This shows the complexity of nitrite inhibition which needs to be further studied.

4.4.2.2. Long-term nitrogen removal rate (NRR) response to elevated nitrite concentrations tested in R2 (Test 6 – R2)

In this test, reactor R2 was operated at fixed pH set at 8.0. Between day 5 and 12 (Figure 4-28), when the loading rate was being adjusted by the flow rate, strong variations in NRR were observed due to FA inhibition (high FA values up to 21.4 mg N/L were recorded). At the same time, a correlation between FA and NRR was found with R^2 equal to 0.75. When the load was adjusted, the NRR became more stable, although a progressive declining trend was observed during the course of the test. This declining NRR trend correlated with accumulated mineral inorganic fraction on the carrier media (Appendix 5). A similar phenomenon was observed by Trigo et al. (2006) who noted that mineral precipitation was limiting the NRR. In that study of 20 days, biomass activity was reduced by 90%. Although inorganic interference was observed, the study was continued for the purpose of nitrite inhibition

threshold concentration identification. It was assumed that, despite slow ongoing NRR reduction, rapid NRR reduction will occur when nitrite inhibition becomes the dominant driver of the reaction.

During Test 6, the nitrogen conversion was similar to the anammox activity recorded in the literature (1:0.9±0.12:0.21±0.05). FA was about 7.1 ± 3.4 mg N/L, which was above the inhibition threshold concentration estimated for R1 (Test 7a - R1). Reactor R2 performance during the test is depicted in Figure 4-28.

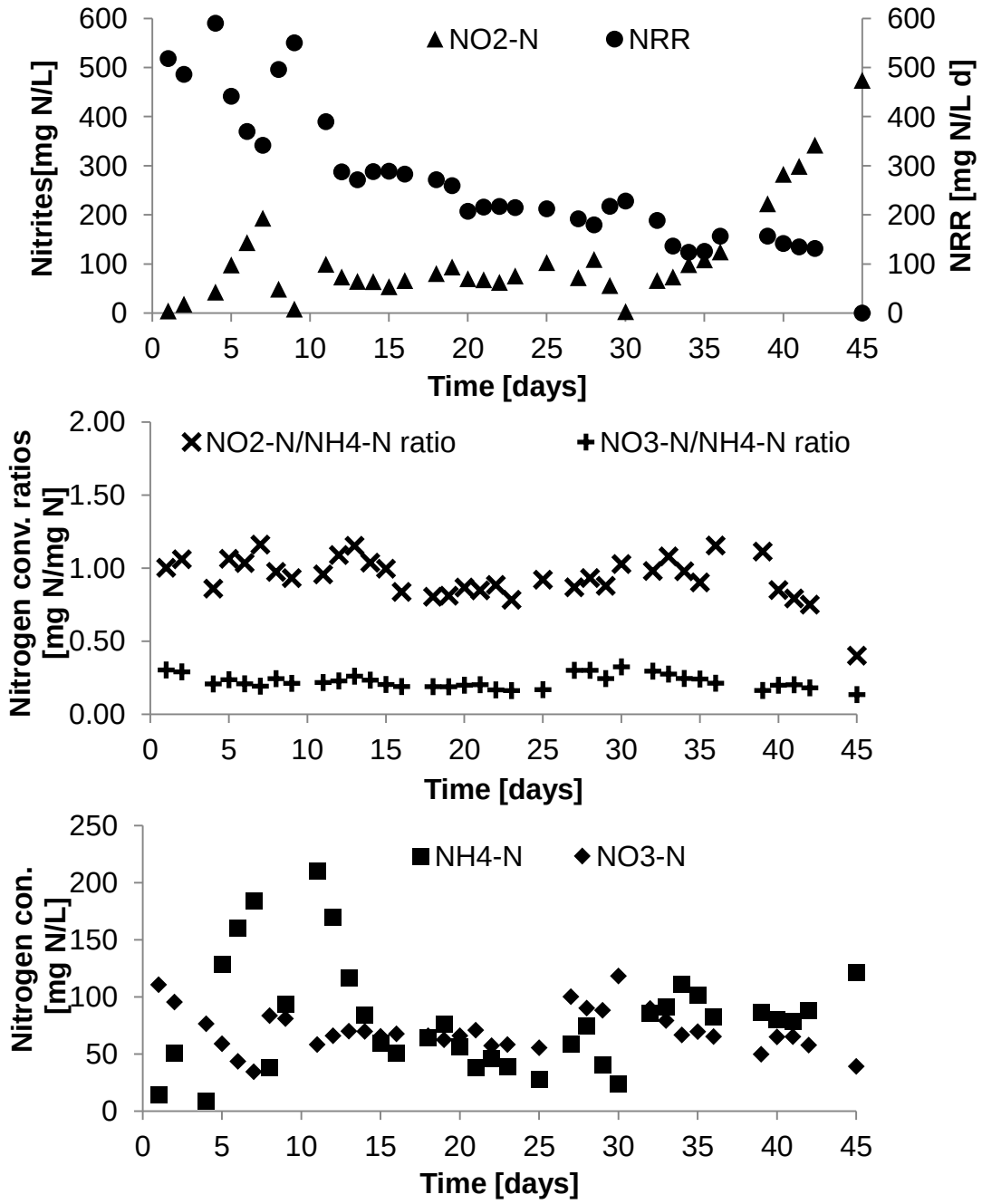


Figure 4-28 Time courses of a) nitrogen removal rate (NRR) and nitrite; b) nitrogen conversions ratios NO_2-N to NH_4-N and NO_3-N to NH_4-N; and c) ammonium and nitrite in R2 during Test 6 – R2

Between days 11 and 28, nitrite concentrations of about 72.3 ± 16.6 mg NO_2-N/L did not destabilize the NRR. More to the point, when the reactor was operated at elevated nitrite with consecutive nitrite accumulation up to 342 mg NO_2-N/L over 10 days time interval (days 32 - 42), the NRR did not show any sudden deterioration tendency. At that time, the FA was almost constant at about 8.2 ± 1.1 mg N/L. On day 45, a complete loss of NRR was observed, which caused nitrite accumulation up to 474 mg NO_2-N/L.

The last recorded nitrite concentration, with an unchanged NRR, was 342 mg NO_2-N/L. On the other hand, nitrite at 474 mg NO_2-N/L caused complete loss of NRR. This data suggests that nitrite concentrations greater than about 340 mg NO_2-N/L should be avoided.

Throughout this test, a gradual increase in nitrite concentration (days 32 – 42) had no negative effect on the NRR, at constant FA. This result further supports the observation from Test 4 that the correlation between NRR and nitrite in R2 was driven by FA (chapter 4.3.3, page 135). At that time, the FA followed the nitrite trend, due to a constant nitrite to ammonium ratio in the feed to R2 and constant pH inside of R2.

4.4.2.3. Long-term specific nitrogen removal rate (sNRR) response to elevated nitrite concentrations tested in R3 (Test 6 – R3)

Reactor R3 was operated at a constant batch feed rate throughout Test 6 – R3. For the first 35 days of the test, the reactor was operated at the regular nitrogen load of about 4440 ± 144 mg N/Ld. The NRR was stable at about 3584 ± 146 mg N/Ld (sNRR 2.43 ± 0.27 g N/g VSS d) with nitrite inside the SBR reactor at about 46.9 ± 18.8 mg NO_2-N/L (maximum nitrite concentration during the SBR cycle). The ratio of substrate used was very similar to anammox ratios recorded in the literature (1:1.24±0.05:0.18±0.03). During this research period, maximum FA in the SBR operation was about 1.0 ± 0.3 mg N/L. Reactor R3 performance is depicted in Figure 4-29.

Reactor R3 was operated in a sequential batch mode. Such a reactor operation was different than the continuous feed in R1 and R2, resulting in significant nitrogen concentration variation during the R3 operation. Long-term nitrite inhibitory effect on biomass activity was, therefore, investigated on the basis of sequential (repeated) nitrite spikes appearing during the SBR cycles. Such a methodology (repeated addition of nitrite spikes) was used to test the long-term nitrite effect on the anammox biomass, which had been studied by Bettazzi et al. (2010).

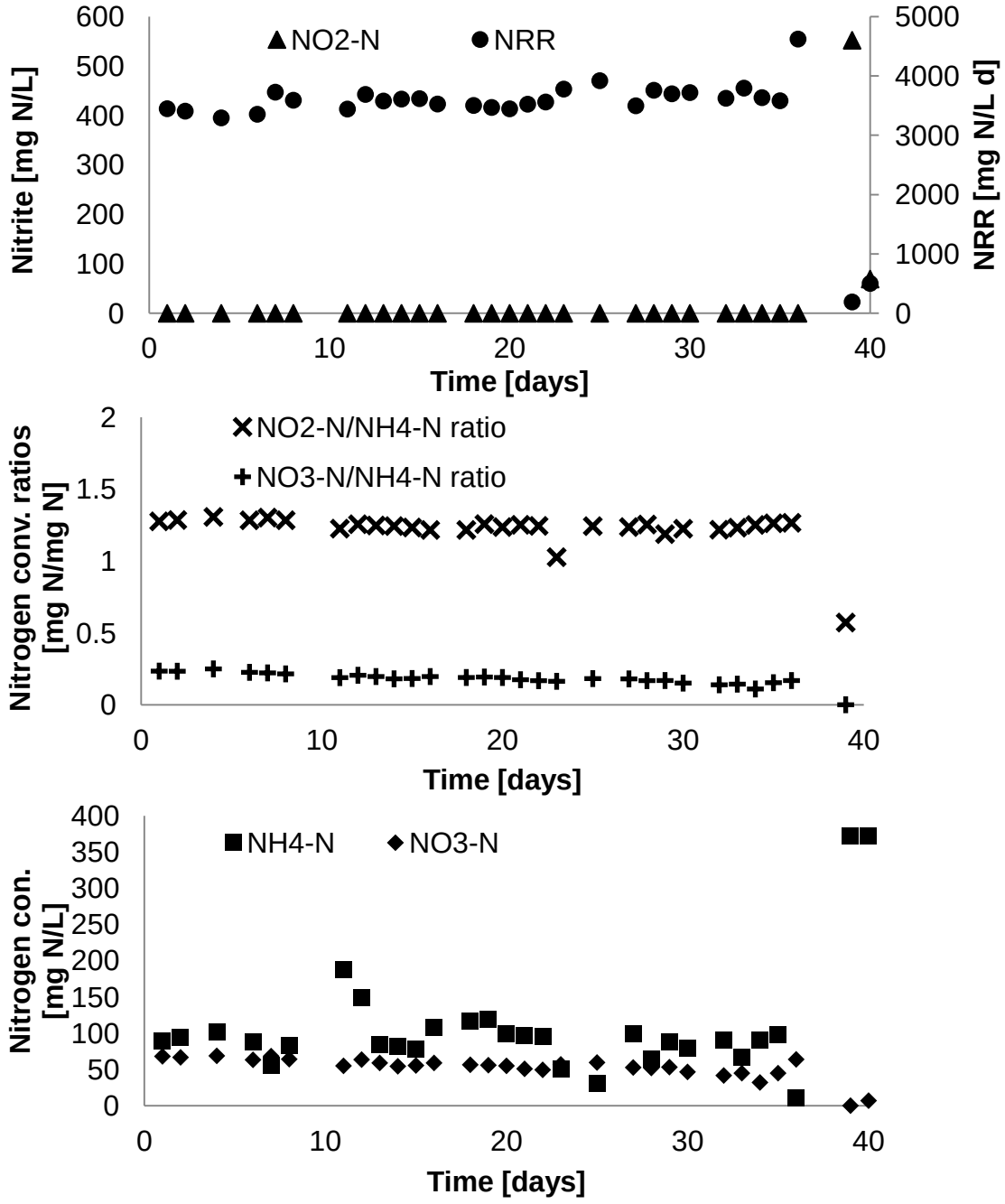


Figure 4-29 Histographs of a) nitrogen removal rate (NRR) and nitrite; b) nitrogen conversions rations NO_2-N to NH_4-N and NO_3-N to NH_4-N; and c) ammonium and nitrite in R2 during Test 6 – R2.

An additional consequence of the sequential performance of reactor R3 was that NRR was not a representative parameter (not sensitive enough) for testing the nitrite inhibitory effect on biomass activity. This was caused by the fixed set-up of maximum reaction duration (105 minutes), where the actual reaction time had to be shortened and was depending on biomass activity inside of reactor. In order to not exceed the fixed maximum reaction duration, reactor was under-loaded. This was required for achieving good biomass settling, where the biomass activity had to be stopped in order to cease nitrogen gas production and prevent biomass flotation (biomass washout). Biomass activity could be stopped only by achieving either nitrite or ammonium depletion.

The specific nitrogen removal rate (sNRR) was used to analyse the nitrite inhibitory effect on biomass activity during the nitrite dynamics for each reactor cycle. The sNRR and nitrogen concentrations dynamics recorded during regular reactor operation are depicted in Figure 4-30. During that time, nitrite was the reaction driver and ammonium was always in excess at all times. There was an insignificant change in sNRR within nitrite range of 15 – 65 mg NO_2-N/L, but this showed no signs of substrate limitation or inhibition.

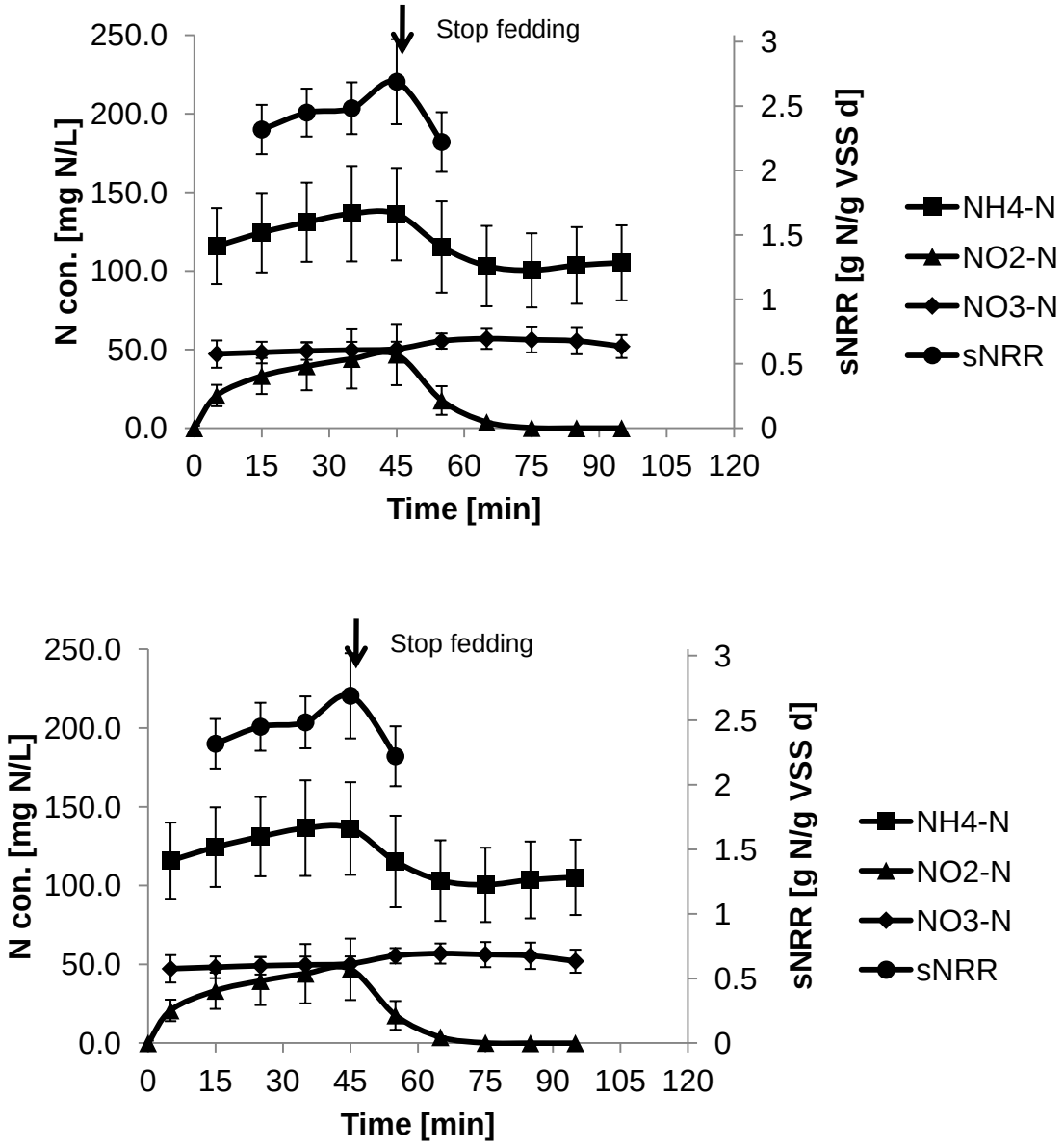


Figure 4-30 Histographs of specific nitrogen removal rate (sNRR) ammonium, nitrite and nitrates in one SBR cycle in R3 during Test 6 – R3.

On day 34, the nitrite concentration in the centrate feed was increased, which caused increases in loading rate with consecutive increase in NRR on

day 35. At the same time, a nitrite spike (the maximum nitrite concentration achieved during the feeding phase) of 80.5 mg NO_2-N/L was recorded on the 8^{th} cycle. It showed no inhibitory effect on sNRR (Test 5b – R3). Additionally, very low ammonium concentration at the end of the cycle was recorded. This was caused by similar substrate ratio utilized by anammox consortium (nitrite to ammonium) to nitrogen substrate ratio in the feed to the reactor (little TA residue). After that, on day 36, nitrite in the feed centrate was further increased.

On day 39, the biomass was washed out and nitrite accumulated up to 552 mg NO_2-N/L inside of the reactor. The biomass washout was related to a decrease in sNRR. Since there was incomplete substrate utilization at the end of the reaction cycle, the biomass was unable to settle due to gas formation inside the flocks. Additional reactor inspection did not identify system malfunction.

After the increase in nitrite concentration in the centrate feed on day 36, the nitrite concentration remaining should not exceed 140 mg NO_2-N/L during the SBR cycles. Based on the typical biomass nitrogen conversion stoichiometry in R3 and known nitrogen concentrations in the feed, the maximum nitrite concentration during the cycle would not exceed 140 mg NO_2-N/L. This nitrite concentration was not identified as an inhibitory concentration during short-term tests (Test 5a – R3).

The last measured maximum nitrite concentration of 80.5 mg NO_2-N/L and predicted nitrite of 140 mg NO_2-N/L suggest that the nitrite threshold concentration would be within this range, unless a mechanism other than nitrite inhibition was involved in system destabilization. On day 33, the ammonium depletion scenario, rather than the typical reactor performance under excess of ammonium, was tested. Previous studies have reported that under such a situation, the reactor tends to become unstable. In the study conducted by Third et al. (2001), described earlier in Test 6 – R1, biomass inactivation was observed under ammonium limitation due to excess of oxygen inside of the reactor. In the current study, as shown in Figure 4-31, measured sNRR was very low at the beginning of the cycle. It was possible to increase sNRR by adding 20 mg of hydrazine sulfite powder; however, the sNRR was not as high as it had been before the test. Although high nitrite was recorded at the beginning of the cycle (100 mg NO_2-N/L, exposure time of about 15 minutes), nitrite was shown to have no negative effect on the sNRR and GPR (Test 5a – R3 and Test 5b R3). Therefore, a 90% reduction in sNRR was probably due to a factor other than the inhibitory nature of nitrite. The addition of hydrazine sulphide at time 25 minutes allowed an increase in sNRR, but a 59% reduction of its maximum sNRR was still observed, which was opposite to that reported by Strous et al. (1999). In the current study, a lack of immediate recovery after nitrite inhibition (opposite to that shown by Strous et al.,1999) suggests a mechanism other than nitrite inhibition (for example

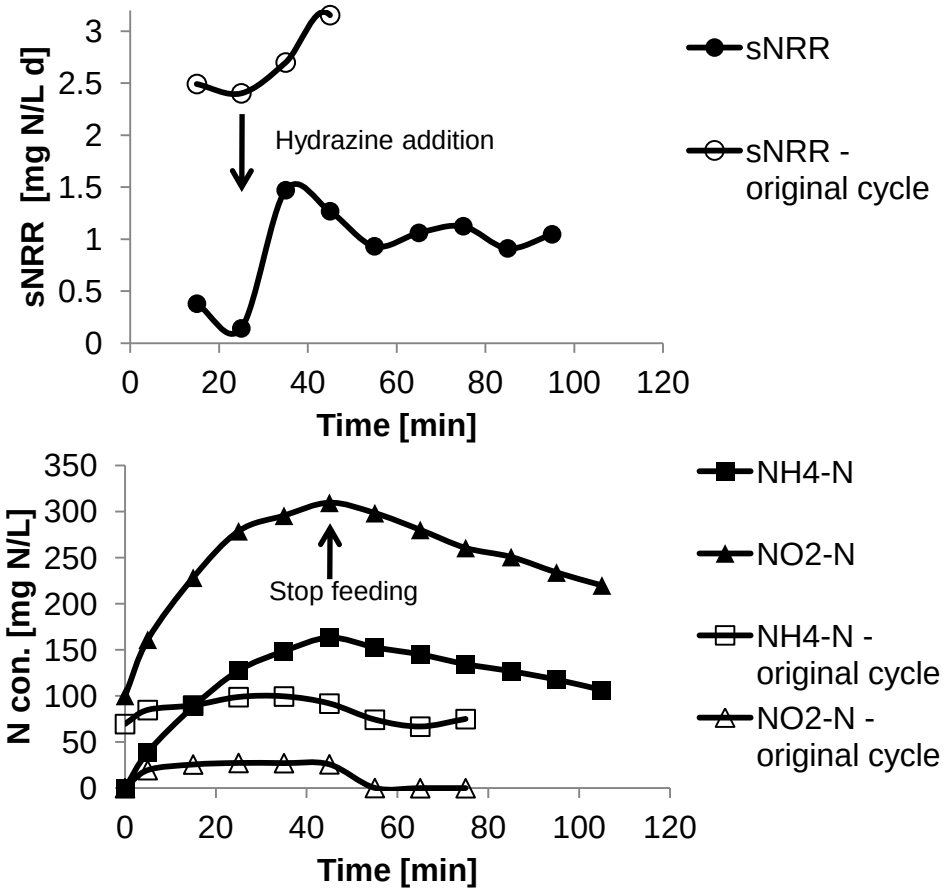


Figure 4-31 Specific nitrogen removal rate (sNRR) ammonium and nitrite with no ammonia at the beginning of SBR cycle compared to original cycle during Test 6 – R3.

oxygen inhibition). More in-depth research is, therefore, needed in order to fully understand this phenomenon.

Although, this test did not provide clear evidence for nitrite inhibition threshold concentration during long term reactor operation, it did show sudden NRR destabilization when the feed nitrite to ammonium ratio was greater than 1.3. This feed ratio is consistent with that in the literature, where sudden

reactor destabilizations were recorded (Vazquez-Padin et al., 2009). At the same time, a very short time was required to build up nitrite concentrations which may further intensify inhibition process. It seems that studying nitrite inhibition in an SBR, by changing the nitrite to ammonium ratio in the feed at greater values than required by anammox stoichiometry, may not be a useful method due to periods without ammonium inside the reactor and the unknown stability effect on the performance of the anammox system.

4.4.3. Conclusions

The short- and long-term nitrite inhibitory effect on anammox rates were investigated on the basis of the nitrogen removal rate (NRR) and the gas production rate (GPR). The following conclusions were formulated:

- Nitrite stimulated anammox rates during short- and long-term reactor operation, as long as a sufficient amount of ammonium was provided and free ammonia was below the inhibitory concentration of about 2 mg N/L.
- Results were consistent with those in the literature, where nitrite inhibition was investigated under constant free ammonia or low free ammonia concentrations during testing.
- During short-term tests, sudden anammox rate reductions were not observed.

- During long-term tests, sudden anammox rate losses were recorded, but the origin of these losses was not identified.

- Nitrite was shown to either stimulate NRR (or GPR) or be neutral for NRR (and sNRR) up to the investigated level of 150 – 200 mg NO_2-N/L, as long as low FA concentrations (below 2 mg N/L) were provided.

- Similar NTC was observed for fixed biofilm in MBBR reactors and suspended flocculated biomass in SBR reactor

- Batch tests were very useful method for testing the immediate biomass activity response to nitrite. They allowed evaluating nitrite effect on biomass activity without the threat of losing the biomass activity in the main reactor.

4.5. Anammox response to FA

In this research, biomass consortia were cultivated in a form of biofilm on a plastic carrier media (R1 – low pH of 6.5 and R2 – ambient pH) and in a suspended flocculated form (R3 – pH 7.0). The purpose of this research was to present the response of the anammox rate to FA concentration in a wide FA range, thereby finding the FA inhibition threshold concentration. The response of the anammox rate to FA was also investigated under different exposure times. Short-term tests (< 1 day) investigated the immediate response of biomass to nitrite, whereas long-term tests (> 1 day) investigated biomass

behaviour when the reactors operated at an elevated FA. It was intended to show that FA may have a greater inhibitory effect on the NRR than nitrite. A further investigation focused on biomass acclimation at elevated FA concentrations.

The FA range (up to 50 mg FA-N/L) and exposure time (less than one day and above one day) applied in the current tests were within the concentration and exposure time ranges tested in previous research studies (Fernández et al., 2010; Tang et al., 2010; Jung et al., 2007; Dapena-Mora et al., 2007). This current study was consistent with studies where low a FA threshold concentration (about 2 mg N/L) was demonstrated.

4.5.1. Immediate anammox response to FA (Test 7)

The Test 7 in the overall free ammonia detail studies outline is presented in Figure 4-32.

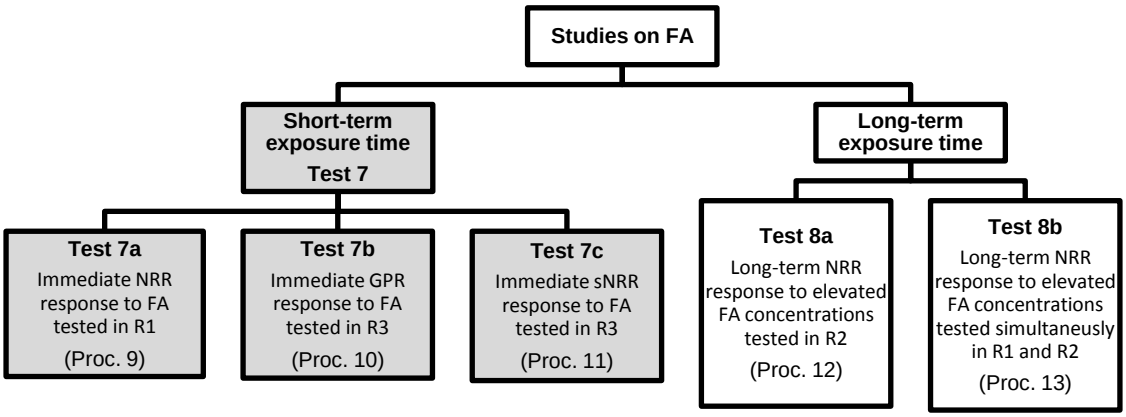


Figure 4-32 Test 7 in the overall free ammonia detail studies outline

Immediate anammox response to FA was investigated using biomass from reactors R1 and R3. The biomass from reactor R2 was not used during this research period due to previously described FA inhibition residue where biomass needs a time to recover its activity after its exposure to FA above the inhibition threshold concentration (Test 3 for R1; and Test 5a – R2 and Test 5b – R2 for R2).

4.5.1.1. Immediate nitrogen removal rate (NRR) response to FA tested in R1 (Test 7a – R1)

In these tests, the initial response to changes in FA, TA and pH was investigated. FA threshold concentrations which result in inhibition were determined. Additionally, the form of nitrogen (free or total ammonia) and pH were investigated as a cause of NRR reductions.

Although changes in NRR were estimated, based only on the nitrite depletion inside of the test reactors, verification of actual anammox activity was based on the complete nitrogen mass balance. The overall nitrogen balance ratio [TA conversion: NO_2-N conversion: NO_3-N production] of [1:(1.29±0.12):(0.23±0.06)], based on seven random tests, confirmed the anammox reaction.

When specific anammox activity (SAA) was not limited by total ammonia concentration (which is the general operating condition for anammox

systems), the major factor governing SAA appeared to be FA concentration (Figure 4-33). It was observed that SAA was strongly correlated to FA regardless of different operating pH values. This relationship was observed for points within the previously reported optimum pH range (7.0-8.5) for anammox organisms (Strous et al., 1997). At a pH of 7, FA concentrations greater than 2 mg NH_3-N/L resulted in a significant decline of SAA (Figure 4-33b). Tested activity at a pH of 6.5 showed that an FA concentration of about 2 mg N/L was an inhibition threshold (Figure 4-33b). Also, the maximum activity was about 20% lower relative to a pH of 7 (Figure 4-33b). This may indicate that other factors come into play with respect to controlling the anammox activity at pH values below 7, which should be studied further. Operation at a pH of 6.5 in this study provided the greatest stability of anammox activity against fluctuations in total ammonia up to 560 mg N/L (Figure 4-33a),

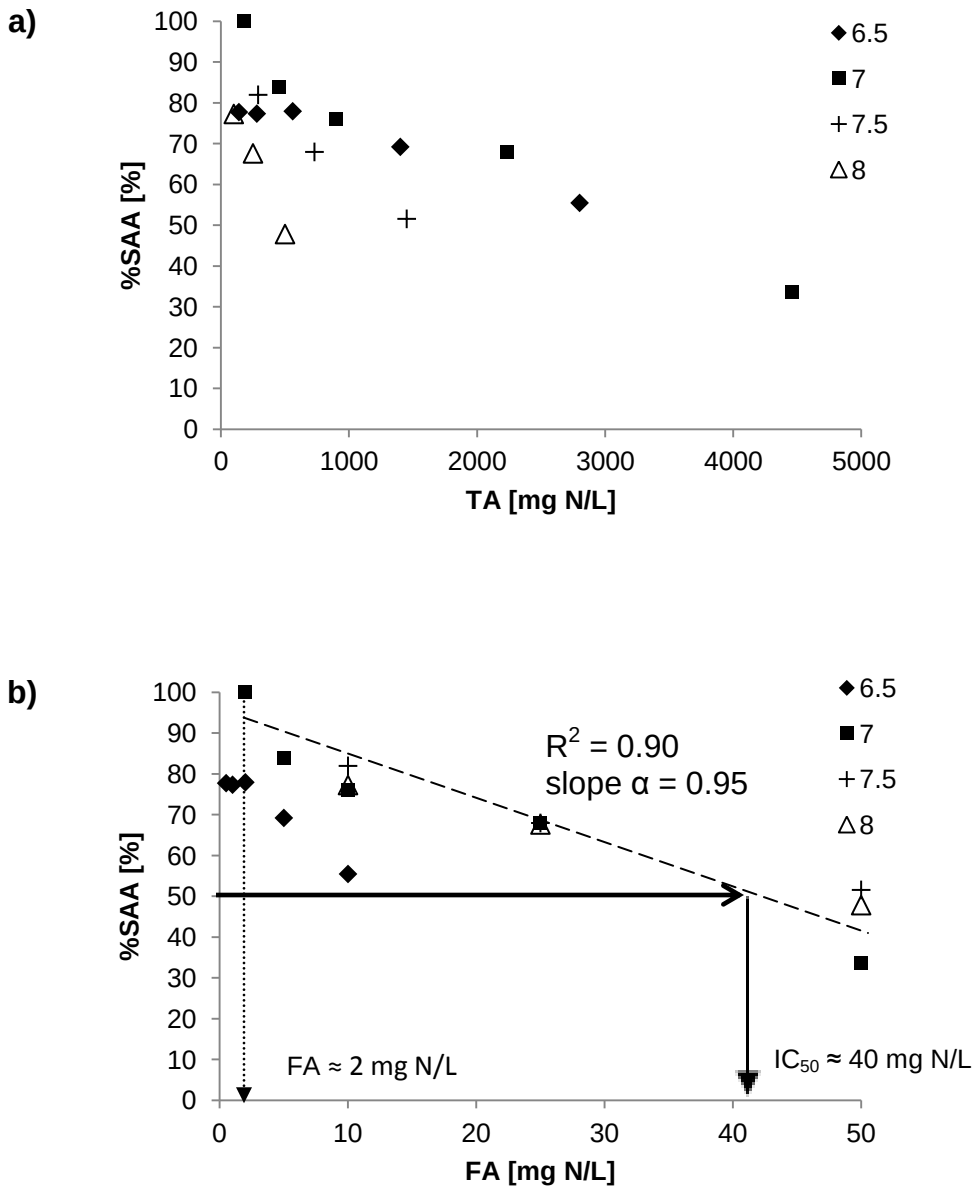


Figure 4-33 The effect of a) the total ammonium (TA) and b) the free ammonia (FA) and its inhibition threshold concentration and 50% inhibition concentration (IC_{50}) on specific anammox activity (SAA) under different pH (non limiting conditions for TA, $SAA_{100\%}$ = 15 mg NO_2/m^2d). Test results showed small variations between the triplicates, resulting in average standard deviations of 2.3% for each point. SAA dependency on FA regardless of the pH (7.0, 7.5 and 8.0) is represented by a dash line.

These results (Figure 4-33) were in clear agreement with results obtained by Dapena-Mora et al. (2007) and Fernández et al. (2010). The FA inhibition concentration, which caused a 50% reduction in activity (IC_{50}) in both studies, was about 40 mg N/L in the pH range between 7 and 8. The slope (α), which describes the correlation between NRR and FA, was also similar and was calculated to be α = 0.95. The lowest observed FA inhibition threshold concentration of 2 mg N/L was similar to what was reported by Jung et al. (2007). The presented results indicate that FA, regardless of operating pH within the range of 7.0 - 8.0, had a significant impact on anammox activity. At a pH of 6.5, the relationship between SAA and FA follows a different curve, and the cells appear to be more sensitive to FA. However, at FA threshold concentration (about 2 mg N/L), TA would be required at about 560 mg N/L which was not observed in anammox reactors.

In the current experiment, the TA required to reach the maximum NRR was in the range between 80 and 180 mg N/L, regardless of the pH (Appendix 5, pages 339 and 340). The TN range required to reach high NRR was also confirmed during the test, which was conducted under different loadings and a constant pH of 6.5. This was similar to tests for nitrite (Test 5b – R1 and Test 5b – R2) and showed strong NRR dependency on TA concentration (Appendix 5, page 341). The wide TN range required to achieve a high NRR in the current study was most likely related to FA inhibition, where TA could not stimulate the NRR due to an exceeded FA inhibition threshold concentration.

A similar phenomenon was observed during Test 4 (page 128), where nitrite could stimulate NRR only at the lower concentrations.

Batch tests were very useful for testing the immediate biomass activity response to FA. They allowed evaluation of the FA effect on biomass activity without the threat of losing the biomass activity in the main reactor.

4.5.1.2. Immediate gas production rate (GPR) response to free ammonia (FA) tested in R3 (Test 7b – R3)

During this test, the gas production rate (GPR) were obtained under constant pH of 6.85 and a constant temperature of 35 ^{0}C, using a synthetic medium. The anammox rates were comparable (standard deviation about 5%) within an FA range of 0.3 – 1.1 mg N/L, showing no inhibitory effect on the GPR. A 14% minor decrease in GPR was observed at 3.3 mg FA-N/L followed by a significant 72% decrease in GPR at 28 mg FA-N/L. Results of this test are depicted in Figure 4-34. Although gas composition was not analysed, it was assumed that N_2 was produced during the anammox reaction. Carbon dioxide was unlikely to be respired during the test in a significant amount, due to lack of organic carbon presence in the synthetic medium. During this test, anammox activity was not verified based on stoichiometry due to consistent anammox stoichiometry obtained during Test 5a – R3 and Test 7c – R3, both

of which were conducted during the same time period. According to a study conducted by Bettazzi et al. (2010) and Ni et al. (2010), the anammox reaction generates nitrogen gas as a final product and it can be a good representation of anammox activity, when ammonium and nitrite utilization and nitrate production follow anammox stoichiometry.

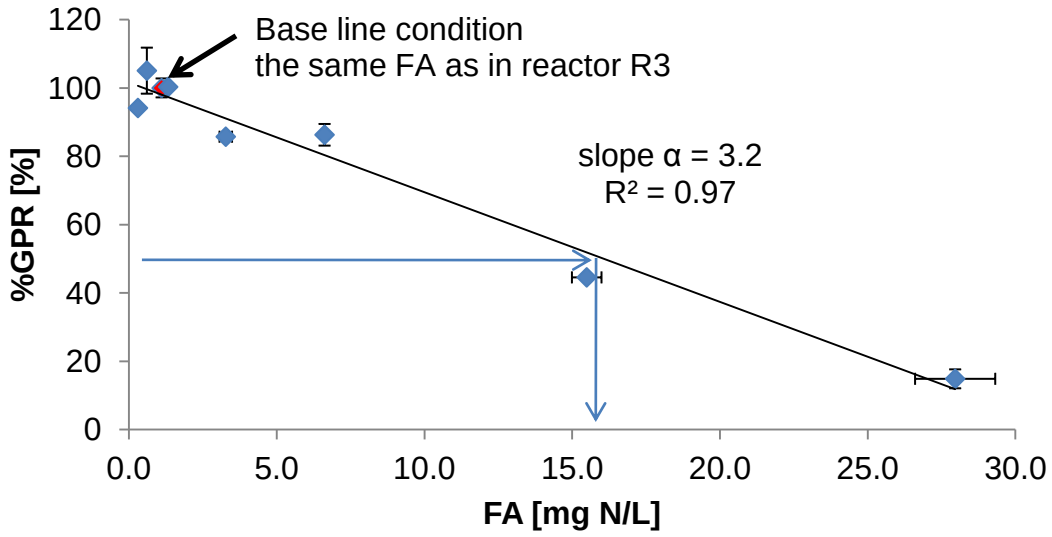


Figure 4-34 Immediate response of anammox activity (biomass originated from R3) to free ammonia (FA) measured by gas production rate (pH = 6.85, T = 35 ^{0}C). Percentage represents relative gas production rate (%GPR) to the baseline GPR obtained at the same FA conditions as in reactor R3 (indicated by an arrow).

These results were in some agreement with studies which investigated the immediate anammox activity response to nitrite, based on N_2 production rate (Dapena-Mora et al., 2007; Fernández et al., 2010). All studies recorded

declining biomass activity correlated with increasing FA concentration. However, the IC_{50} in the current study was about 15 mg FA-N/L, whereas Dapena-Mora et al. (2007) and Fernández et al. (2010) reported an IC_{50} of about 40 mg FA-N/L. This difference in IC_{50} may be related to biomass structure. In the current study, biomass in reactor R3 was flocculated, whereas biomass in studies conducted by Dapena-Mora et al. (2007) and Fernández et al. (2010) were in a granular form and an attached biofilm on natural zeolite media, respectively. Additionally, in both studies, biomass was cultivated under medium FA concentrations of up to 5 mg FA-N/L, which could have had a negative effect on the obtained results (Test 3 and Test 5b – R2 show this tendency).

During the current study, although the FA inhibition threshold concentration was not clearly defined, it became clear that low FA concentrations were required for stable biomass activity. This was in close agreement with the study conducted by Jung et al. (2007), where an FA greater than 1.7 mg N/L was causing a decrease in NRR. However, in that study, FA concentrations lower than 1.7 mg N/L were not investigated.

4.5.1.3. Immediate specific nitrogen removal rate (sNRR) response to free ammonia (FA) tested in R3 (Test 7c – R3)

In order to verify observations recorded during Test 7a - R1, where pH within the range of 7.0 – 8.0 did not have a significant effect on biomass NRR, similar tests were conducted for biomass cultivated in R3. Three consecutive tests were performed during three SBR cycles. During each cycle, a different pH was set as follows: the first cycle operated at a regular pH of 7.0, then pH was changed to 7.5, and during the third SBR cycle, pH was set to 8.0. As a result of the pH changes, the reactor was operated consecutively under three different FA concentrations, from the lowest to the highest.

Under each pH condition, the typical anammox stoichiometry was recorded. Based on the nitrogen mass balance, the following nitrite to ammonia and nitrate to ammonia ratios were recorded: for pH 7.0 - (1:1.30±0.03:0.21±0.08), for pH 7.5 - (1:1.31±0.09:0.22±0.08), and for pH 8.0 - (1:1.54±0.38:0.18±0.12).

During this test, the relative anammox activity (%RAA) was correlated with FA concentrations which were obtained under three pHs within the FA range of 1.5 – 20.5 mg N/L (Figure 4-35). By comparing slopes (α) obtained during this test and during Test 7a – R3 (Figure 4-34), it was observed that they were the same (the correlation between %RRA and FA, slope α = 3.2); this proved

a strong %RAA correlation with FA regardless of different pHs (within pH range of 7.0 – 8.0) and showing, once again, the inhibitory nature of FA.

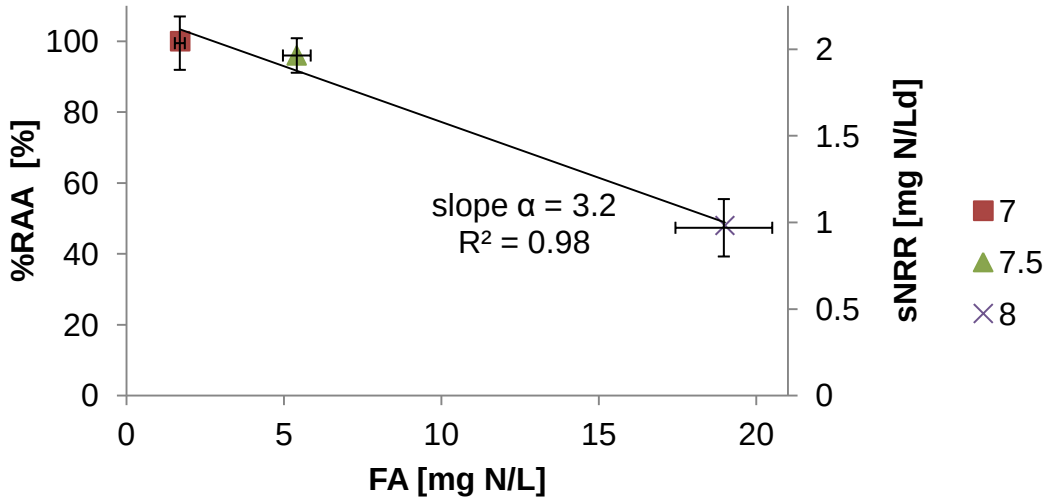


Figure 4-35 The effect of FA on the sNRR and relative SAA (%RAA) under pH 7.0, 7.5, and 8.0 tested during three consecutive SBR cycles.

These results suggest that pH does not have a significant effect on NRR within the pH range of 7.0 – 8.0 during short-term tests within the tested FA range. They also suggest that if FA accumulation (greater that about 2 mg N/L) would occur, a decrease in biomass activity should be expected regardless of the pH. However, it should be emphasized that this statement about pH should not be generalized because pH affects all kinds of equilibriums such as mineral precipitation and other correlations between ionized and unionized forms of substances. Therefore, detailed analysis

should be conducted to determine whether a pH shift will be the right choice for system performance improvement in a full scale system. Such an analysis could be conducted using batch tests, where immediate response of biomass activity to different pH would be evaluated.

4.5.2. Long term anammox response to pH and FA (Test 8)

The Test 8 in the overall free ammonia detail studies outline is presented in Figure 4-36.

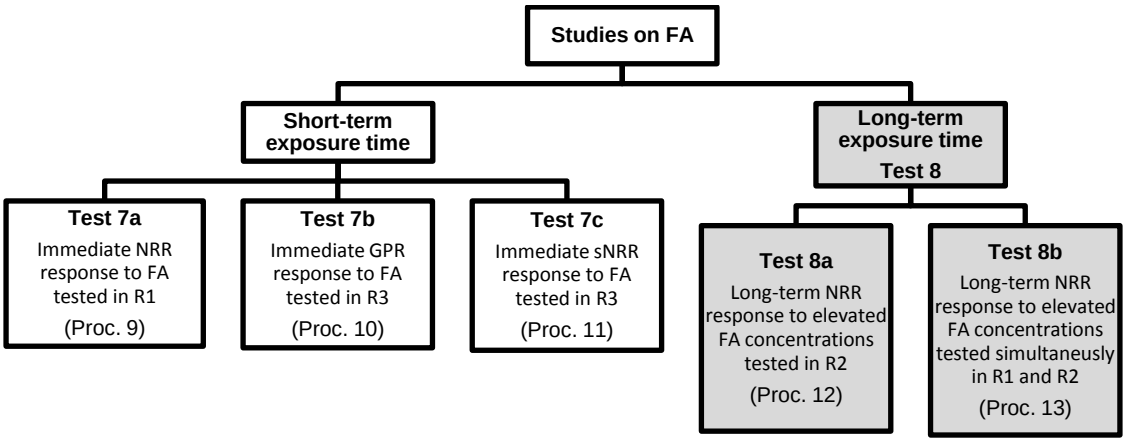


Figure 4-36 Test 8 in the overall free ammonia detail studies outline

During this research period, the long term (> 1 day) response to FA was investigated during two tests on MBBR reactors. The first test, Test 8a –R2, was focussed primarily on the investigation of NRR response to elevated FA and nitrite concentrations. The reactor was destabilised by an FA shift from 5.0

to 7.7 mg N/L (pH shift from 7.8 to 8.0). Afterwards, an FA shift from 31.1 to 3.6 mg N/L (pH shift from 8.0 to 7.0) was investigated to determine whether it could restore NRR despite high nitrite (up to 247.8 mg NO_2-N/L), thereby showing which inhibitory parameter (FA versus nitrite) has the dominant influence on NRR stability. The second test, Test 8b – R1 and R2, was focused primarily on the investigation of NRR to FA in two reactors, R1 and R2, which operated in parallel mode and had the same loading rate. These comparisons were made for the purpose of finding whether anammox consortium grown under medium FA concentrations will be more resistant to elevated FA than anammox consortium grown under low FA concentrations. The resistance of anammox consortia to FA was tested based on the NRR response to FA in R1 and R2.

4.5.2.1. Long-term specific nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R2 (Test 8a –R2)

During this test, under constant load condition, when the pH was changed from self stabilizing pH of 7.8 to pH controlled 8.0, an increase in FA occurred, causing NRR deterioration. This test was similar to Test 3 conducted on R1; however, a longer time was provided, allowing greater NRR loss and higher nitrite accumulation exceeding one day of exposure time. The purpose of such

an experiment was to test the worst case scenario which might possibly occur during full-scale reactor operation. Additionally, the purpose was to prove the research hypothesis that FA has a greater negative effect on NRR than nitrite, under regular reactor operation.

During the first two days of the test, reactor R2 was achieving NRR of 2173 ± 10 mg N/Ld with anammox stoichiometry of (1:1.15±0.07:0.13±0.01). On day two, the pH was changed from self stabilizing pH of 7.8 to pH controlled 8.0. This pH change caused a 54% increase in FA due to equilibrium shift between FA and TA (FA increase from 5.0 to 7.7 mg N/L).

On the following days, NRR decreased where nitrite and FA accumulated, as a result of constant load and incomplete substrate utilization inside of the reactor. After three days of continuous NRR reduction, the nitrite concentration exceeded the inhibition threshold concentration estimated during Test 5a – R2; at the same time, no sudden complete NRR loss was observed, thus agreeing with results obtained during Test 6 – R2.

On day seven, after 5 days of continuous NRR reduction, nitrite and FA accumulated up to 247.8 mg NO_2-N/L and 32.1 mg N/L, respectively. Both nitrite and FA exceeded their inhibition threshold concentrations. At that time, the progressive NRR deterioration could be stopped only if the dominant inhibitor would be eliminated. As a result of the pH change to 7.0, immediate NRR recovery was observed followed by almost complete NRR recovery the next day (2060 ± 5 mg N/Ld). Through the pH shift, FA was changed from 31.1

to 3.6 mg N/L (88% decreases). Results of this test are depicted in Figure 4-37.

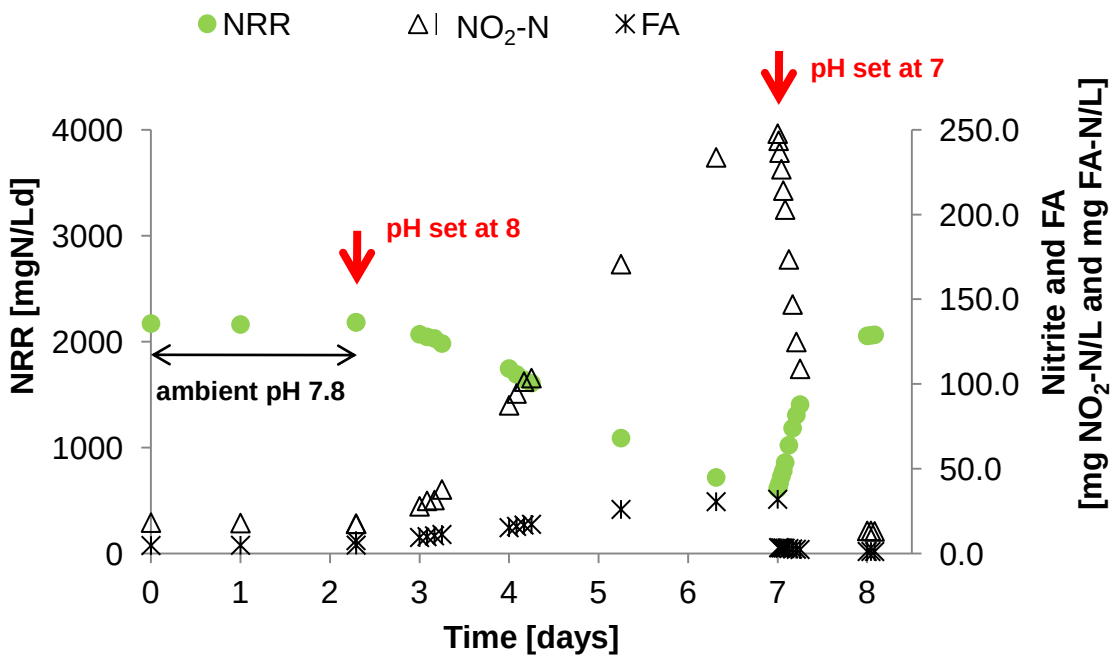


Figure 4-37 NRR response, in R2, to pH set at 8 and consecutive change to 7 under constant N-load conditions during 8 days.

During the test, the NRR response to FA was very consistent, showing high correlation (R^2). Figure 4-38 shows a decrease in NRR along with an increase in FA. Comparing these results (long-term exposure time) with results obtained during Test 7a – R1 (short-term exposure time, Figure 4-34), exposure time seems to have a significant negative effect on the NRR. The slope obtained during this test (slope 2.9, but also during Test 8b – R1 and

R2, slope 3.6) was greater than what was achieved during Test 7a – R1 (slope 0.95).

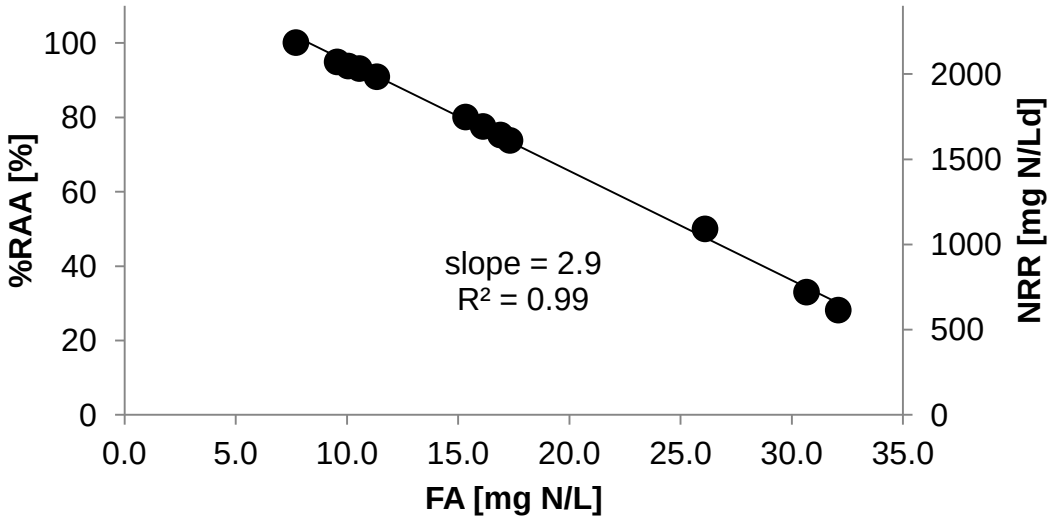


Figure 4-38 The correlation between FA and % of relative anammox activity (RAA) and NRR during Test 8a –R2, where 100% represents reactors NRR at the beginning of the test (2173 ± 10 mg N/Ld)

4.5.2.2. Long-term nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R1 and R2 (Test 8b – R1 and R2)

This test focused on two objectives. The first was to test whether acclimation to FA had occurred. For this, two reactors, R1 and R2, were

compared. In those reactors consortia were cultivated under low (R1) and medium FA (R2) concentrations. The second objective was to test NRR recovery via a FA shift after its complete loss.

Both reactors, R1 and R2, were operated under constant load during this research period. At the beginning of this test, R1 was operated at 2795 ± 20 mg N/Ld, exhibiting very close similarity to Michaelis-Menten based NRR (difference below 3 %, thereby showing its stability and predictability); this test exhibited overall typical anammox stoichiometry with an overall nitrogen balance at a ratio of NH_4-N conversion to NO_2-N conversion to NO_3-N production of 1:1.26±0.02:0.18±0.01. This suggested anammox as a dominant route for nitrogen conversion. Reactor R2 was used during Test 5b – R2 before this test was conducted. However, during day 0 and 1, when R2 was loaded the same as R1, the NRR and nitrogen conversion stoichiometry was very similar to those obtained in R1 achieving 2790 ± 14 mg N/Ld and (1:1.26±0.02:0.18±0.01), respectively.

On day 1, the pH was changed from 6.5 to 8.0 thereby changing the FA from 0.4 mg N/L, in both reactors, to 10.9 mg N/L and 11.4mg N/L, respectively, for R1 and R2.

As is depicted in Figure 4-39, between days 1 and 6, ongoing and comparable NRR reductions was observed in both reactors. The correlation between NRR and FA for R1 and R2 is presented in Figure 4-40. It was observed that there was no significant difference in achieved NRRs. At all

times, NRRs in both reactors were correlated to FA with high R^2 at 0.99 within FA range of 18.5 – 41.4 mg N/L (Figure 4-40). If the anammox consortium for R2 did not change significantly during Test 5b – R2 over a two-week research period (time duration of the Test 5b – R2), this particular result suggests that FA has a superior role in sustaining high NRR over biomass enrichment conditions (low FA versus medium FA). In other words, because of the similar correlations between NRR and FA for R1 and R2, no acclimation to FA occurred.

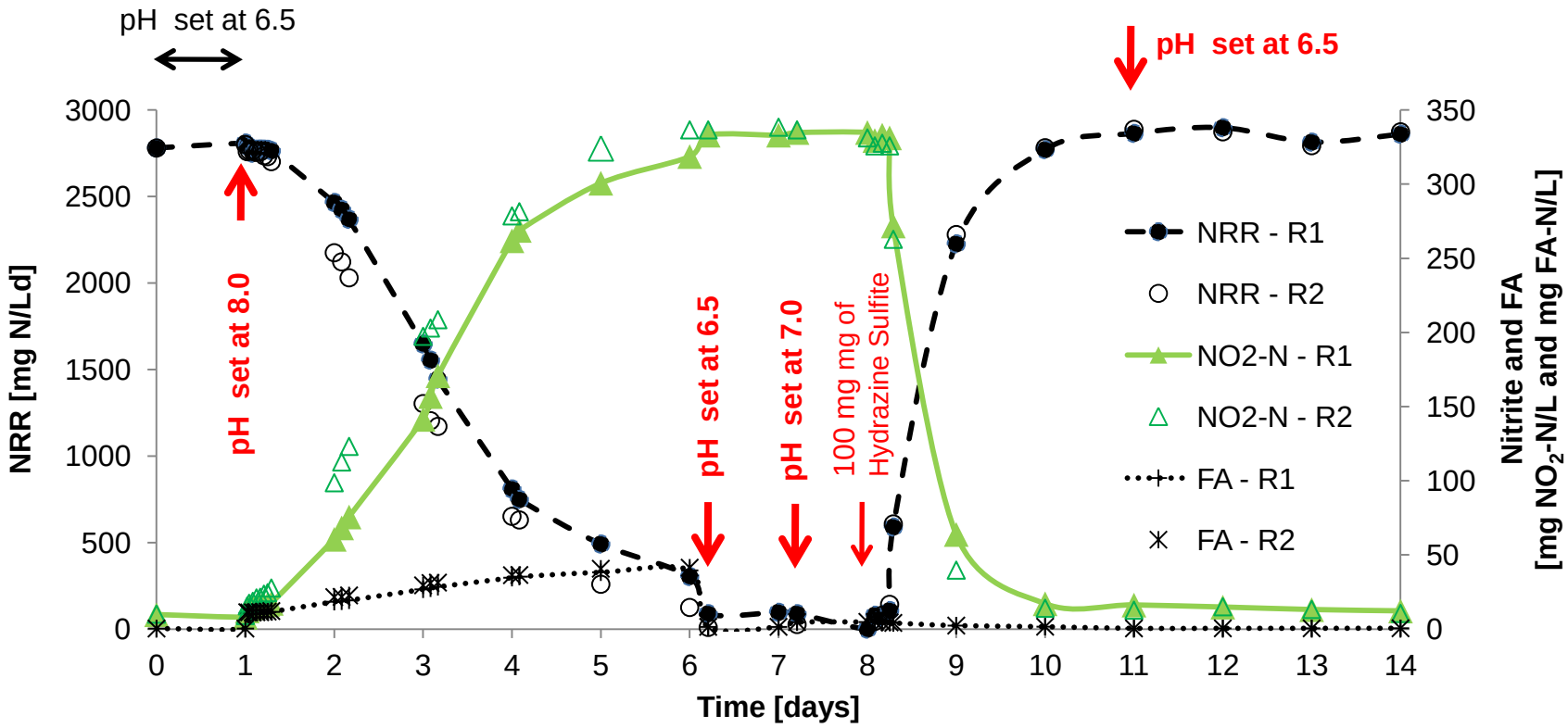


Figure 4-39 NRR response, in R1 and R2, to pH set at 8 and consecutive change to 6.5 followed by 7.0 and 6.5 under constant N-load conditions during 14 days.

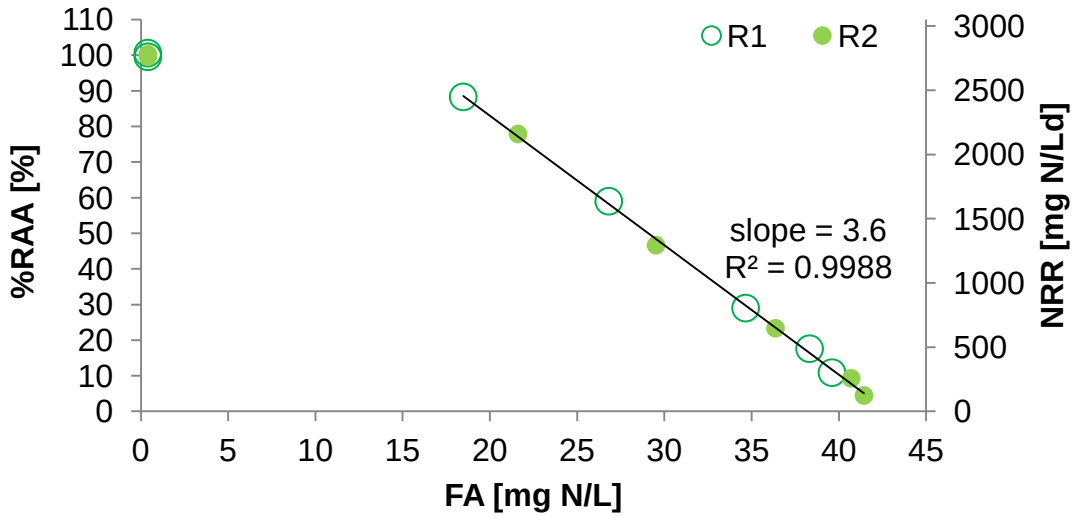


Figure 4-40 The correlation between FA and relative anammox activity (%RAA) and NRR during Test 8b – R1 and R2, where 100% represents reactors NRR before the test start (average for both reactors – 2792 ± 15 mg N/Ld.) The linear regression refers to points, for both R1 and R2, within FA range of 18.5 – 41.4 mg N/L.

With regard to slopes, the correlations between NRR and FA achieved during this test for R1 and R2 (slope α = 3.6) compared with the slope obtained for R2 during Test 8a –R2 (slope α = 2.9), it appears that they represent similar values. These similar slopes suggest that the significant pH shift itself, from pH 6.5 to 8.0 in the current study, did not have a great effect on NRR deteriorations (pH shift during Test 8a –R2 was from 7.8 to 8.0, causing a similar negative effect on NRR as it was observed during current test).

On day 6, almost all activity was lost in both reactors. Consecutive pH changes to 6.5 on day 6 and to 7.0 on day 7 did not elicit sudden NRR

recovery, as opposed to what was observed during Test 8a –R2. On day 8, a 100 mg of hydrazine sulfite was added to each reactor, in order to initiate NRR recovery. This suggests that when anammox activity is lost, the NRR is very difficult to recover within a short period of time, and the only way to recover NRR is by adding anammox intermediates. Wett et al. (2007) reported irreversible damage to anammox activity in one biomass system (DEMON process) when deactivated, due to system control failure. In this thesis research, recovery by adding anammox intermediate reaction products was not investigated. Anammox intermediate reaction product addition in a form of hydroxylamine and hydrazine was studied by Strous et al. (1999) and Bettazzi et al. (2010). They both demonstrated that anammox activity can be recovered after the addition of hydroxylamine.

After the addition of Hydrazine Sulfite on day 8, both reactors recovered NRR within a 2-day period, thus showing robustness and ease of recovery when FA was low.

4.5.3. Conclusions

During this research period, short- and long-term free ammonia (FA) inhibitory effect on anammox rates was investigated on the basis of nitrogen removal rate (NRR) and gas production rate (GPR). The following conclusions were formulated:

- During anammox reactor operation, the pH manipulation can affect overall NRR, either negatively (when being increased causing increase in FA) or positively (when being decreased causing decrease in FA).
- Both nitrite and total ammonia tended to accumulate when NRR decreased under constant load condition.
- The FA, the un-ionized ammonium, was found to be responsible for NRR deterioration when the inhibition threshold concentration exceeded approx. 2 mg N/L.
- The FA inhibition was reversible unless NRR decreased to zero, i.e. was completely lost.
- The FA did not cause sudden loss of NRR during short- and long-term tests.
- The NRR did recover when nitrite inhibition threshold concentration was exceeded for over 2 days (based on the batch tests) - when FA was simultaneously lowered below inhibition threshold concentration.
- The negative effect of FA inhibition on NRR can be overcome either by lowering the pH (affecting the equilibrium between TA and FA up to investigated pH of 6.5) or under-loading the reactor i.e. operating an anammox reactor with anticipation of low NRR.

- Similar FA threshold concentration was observed for fixed biofilm in MBBR reactors and suspended flocculated biomass in SBR reactor
- The FA can be considered a more important inhibitory parameter for NRR than nitrite.
- Batch tests were very useful for testing the immediate biomass activity response to FA. They allowed evaluation of the FA effect on biomass activity without the threat of losing the biomass activity in the main reactor.

5. CONCLUSIONS

The present research led to the following conclusions:

- Among inorganic nitrogen forms, un-ionized form of ammonium – free ammonia (FA) increase, was shown to be the main precursor of the instability of moving bed biofilm reactors (MBBRs) and suspended growth SBR reactors.

- For the best MBBR stability, FA should be kept below 2 mg N/L, as per the testing within the pH range of 6.5 and 8.0.

- For the best suspended growth SBR stability, FA should be kept below 2 mg N/L, as per testing within the pH range of 7.0 and 8.0.

- FA exhibited a very similar inhibitory effect on anammox NRR regardless of whether FA impact was tested on the long-term operation of the anammox consortia cultivated in MBBR reactors or whether consortia cultivated at lower FA in an MBBR reactor were exposed to high FA outside of the reactor (immediate response).

- Nitrite stimulated anammox rates during short- and long-term reactor operation, as long as free ammonia was below the inhibitory concentration of about 2 mg N/L.

- Nitrite during short-term exposure (less than a 1 day) was not necessarily the cause for reactor destabilization.

- The short-term nitrite inhibition threshold concentration (NTC) was estimated at approx. 150 – 200 mg NO_2-N/L, regardless of reactor configuration

- The long-term nitrite inhibition threshold, NTC, was not achieved in applied configurations due to other, possible, inhibitory mechanisms than nitrite inhibition on anammox alone.

- Batch tests were very useful for testing the immediate biomass activity response to FA and nitrite. They allowed evaluation of the nitrite and the FA effect on biomass activity without the threat of losing the biomass activity in the main reactor.

- Reactor stability was not affected by the periodically changed loads when the reactor was operated under the FA below 2 mg N/L.

- Similar FA and nitrite threshold concentrations were observed for fixed biofilm in MBBR reactors and suspended flocculated biomass in SBR reactor

- Conducted research showed that controlling FA at low level is required to approach high nitrogen removal rates in anammox reactors. Achieving high rates in anammox reactors allows significant reduction in reactor volume

- Current full-scale plants are significantly over-designed when their achieved nitrogen removal rates NRR were compared to the NRR obtained in this study.

6. ENGINEERING SIGNIFICANCE

When designing an anammox system, it is important to identify a loading rate for the reactor and system operational conditions which allow stable and predictable reactor performance. To achieve this goal, the NRR and associated destabilizing parameters have to be known and identified (Wett et al., 2007; Bowden et al., 2007; Wett et al., 2008; van der Star et al., 2007; O'Shaughnessy et al., 2008). This research, which focused on the response of anammox NRR to FA and nitrite, is therefore of wide interest to process designers and system operators.

Design parameters in this research project emphasized the stability of anammox reactor performance from the perspective of FA. The results of this project, in conjunction with a study of previous research, allow us to propose a conceptual design of the anammox process for the existing centrate facility at the local wastewater treatment plant.

6.1. Design tools achieved through the research

This research project provided important tools for the design, optimization and operation of an anammox MBBR. The key finding was that the anammox reactor can be effectively controlled and that NRR can be accurately predicted when FA concentrations are below 2 mg N/L, under regular reactor operation (when other disturbances in NRR have not occurred). At the same time, nitrite

was shown to play a very important stimulating role on the achievable NRR. By conducting several experiments, it was possible to obtain and validate the correlation between NRR and nitrite concentration, as described by the Michaelis-Menten equation. Table 6-1 summarises the design criteria for an MBBR anammox reactor.

Table 6-1 Design criteria for anammox MBBR reactor obtained during the research

Description of the design parameter	Design parameter
Saturation equation for nitrite[(1)] - ammonium non-limiting condition, - 50% filing ratio with media K1 - tested in pH range 6.5 – 7.0 - tested at temperature 35 ^{0}C - equation is valid when FA does not exceed 2 mg N/L inside of anammox reactor	$\mathrm{NRR} = \mathrm{maxNRR} \cdot \frac{S_{NO2-N}}{K_{NO2-N} + S_{NO2-N}}$ [mg N/Ld]
	K_{NO2} = 25 mg N/L
	Theoretical max NRR = 11 000 mg N/Ld
Saturation equation for TA[(2)] - nitrite non-limiting condition - 50% filing ratio with media K1 - tested at pH 6.5 - tested at temperature 35 ^{0}C - equation is valid when FA does not exceed 2 mg N/L inside of anammox reactor	$\mathrm{NRR} = \mathrm{maxNRR} \cdot \frac{S_{TA-N}}{K_{TA-N} + S_{TA-N}}$ [mg N/Ld]
	K_{TA} = 76 mg N/L
	Theoretical max NRR = 16 000 mg N/Ld
Free ammonia inhibition threshold concentration	2 mg N/L
Suggested nitrite inhibition threshold concentration	150 – 200 mg N/L
Suggested operational pH	6.5 – 7.0
Suggested SRT	at or above 18 days

(1) Equation obtained through Test 2

(2) Equation obtained through Test 7a – R1 (page 343)

6.2. Determining optimum FA conditions for anammox reactor operation

Based on the results of these experiments, it was possible to assess the optimum conditions (in terms of pH and FA) for the anammox process in an anammox MBBR and anammox SBR. A number of studies have shown variation in total ammonia in anammox systems, varying between 30 and 150 mg N/L (van der Star et al., 2007; Wett 2007; Szatkowska et al*.,* 2007; Joss et al*.,* 2009). Because of the relationship between FA and pH, it is difficult to control the total ammonia concentrations in anaerobic digestion liquors in order to maintain FA at a level below the inhibition threshold concentration (estimated to be 2 mg N/L) without pH control. This is especially problematic because the anammox process tends to increase the pH (in the range between pH 7 and 8.5) in a two-reactor system, causing an increase in FA at a constant level of TA. To achieve system stability, pH control by acid addition or carbon dioxide injection would be required. It would be necessary to maintain a bulk pH of approximately 7 to enhance ammonia oxidation while maintaining FA concentrations below inhibitory levels. In the case of a one biomass system, such as the DEMON process (Wett 2007) or single-sludge SBR (Joss et al., 2009), where the operating pH is around 7.0, this might not be needed. System stability, under varying nitrogen loading conditions, is achieved because the naturally lower pH of operation might be an important advantage of the one-biomass process, over the two-biomass configuration (Jaroszynski and Oleszkiewicz 2011; Joss et al., 2009, van Hulle et al., 2010). Additionally,

maintaining a pH at 7 or below to provide FA below inhibitory levels will raise the operational cost of nitrogen removal, in the two-biomass configurations. This additional cost, however, can be reasonable when savings in reactor volume can be achieved, where high NRR can be achieved without compromising reactors stability. Indeed, Test 4 demonstrated superior performance of the anammox MBBR when pH was controlled compared to MBBR with self-maintain pH above 7. Findings of this research may help to evaluate system choices, based on the additional parameter related to FA.

6.3. Rate limiting step in anammox systems

Anoxic ammonium oxidation requires two processes for nitrogen removal, the first one is nitritation and the second one is anammox. These processes can be split into separate reactors (steps) or they occur simultaneously. Based on the conducted research, it was observed that nitrite stimulate the NRR, therefore, nitrite at about 25 - 30 mg N/L (Test 2 and Test 4) allows achieving high NRRs at about 6 g N/Ld. In different reactor configurations such as up-flow granular reactors, NRR up to 45 g N/L can be achieved (Tang et al., 2010). Such a high NRRs require high nitrogen loading rates and high nitritation rates (first step). Although nitritation was not researched in this study, nitritation rate was achieved at about 1 g NO_2-N/Ld (without optimization). In literature, nitritation reactors were able to achieve nitritation rates up to about 2.2 g NO_2-N/Ld (Chen et al., 2010). Comparing maximum nitrogen conversion rates between nitritation stage and anammox stage,

nitritation is a limiting step due to much greater reactor volume required for nitritation to sustain high NRR in anammox reactor based on achieved rates in bench scale reactors (nitritation reactor volume to anammox reactor volume ratio: 3.5:1 for current research data and 10:1 for literature data). Indeed, researchers found nitritation step as a limiting step during long term reactor operation (Fux, et al., 2002; O'Shaughnessy, et al., 2008; Szatkowska et al., 2007).

Ongoing research (Aslan et al., 2009; Li et al., 2012; Zanetti et al., 2012, Zhang et al., 2010) on nitritation demonstrated high potential for nitritation rate improvement in nitritation reactors, proper manipulation of reactors operational parameters as dissolved oxygen, pH, FA, FNA showed to stimulate nitritation. This, however, was shown to be difficult when operating one stage process (Jaroszynski & Oleszkiewicz, 2011), where complex interactions between AOBs, NOBs and anammox bacteria allow for limited process flexibility. It was shown that very complex strategy for HRT and DO control has to be applied for the control of one stage anammox process to avoid either limitations or inhibitory phenomena (Vazquez-Padin at al., 2010a)

In lab scale reactors, maximum growth rates for AOBs and anammox organisms were shown to be different, where doubling time about 1 day (Gali et al., 2007) and about 4.3 day (Tsushima et al., 2007a) were observed, respectively (similar temperatures). These values were achieved under non limiting substrate concentrations. During long term reactors operation,

observed doubling time for anammox organism was about 3.6 – 25 days (van der Star et al., 2007; Joss et al., 2009; Tsushima et al., 2007b; Tang et al., 2011). This significant difference in growth rates, between maximum and observed, was most likely related to substrate limitation. Although it is easy to increase substrate concentrations in anammox reactor by increasing nitrogen loading rate, where substrates are in dissolved forms, it is difficult to increase oxygen concentration in nitration reactor at high temperatures due to diffusion limitation.

Achieving high nitritation rates and anammox rates requires high biomass concentration. It was shown that granulation provide high biomass concentration inside of reactor and high surface area for substrate diffusion (van der Star et al., 2007; Vázquez-Padín et al., 2010).

Anammox organisms are very sensitive to oxygen therefore oxygen concentration in one stage process has to be controlled at low concentration, preferably below 0.3 mg O_2/L (Wett et al., 2007) and up to maximum 1 mg O_2/L (Joss et al., 2009). Such low oxygen concentrations were shown to limit significantly nitritation rate in granular reactor (0.2 g N/Ld at DO 2 mg O_2/L versus 0.8 g N/Ld at DO 4 mg O_2/L - Vázquez-Padín et al., 2010) and nitritation in one stage anammox reactor (Sliekers et al., 2003).

Limiting step in anammox systems seems to be related to nitritation and oxygen concentration. Intensifying the nitritation rate, it is possible to achieve

high overall nitrogen removal rate, which seems to be easier in a two-stage process.

6.4. MBBR reactor versus SBR reactor operation comparison

MBBR reactor contains biomass on the carrier media, immobilizing it and preventing washout. SBR reactor contains biomass in suspension, where the separation of wastewater from the biomass occurs through sedimentation and subsequent liquid withdrawal. However, for the accurate process performance, reaction has to be completed, before the sedimentation occurs. During reactor operation advantages and disadvantages were identified (Table 6-2).

Table 6-2 Advantages and disadvantages of MBBR and SBR anammox reactors

MBBR		**SBR**	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Continuous flow system	Difficult SRT control	Easy SRT control	Sequential flow system limits the loading rate
Easy loading manipulation	High mixing speed was causing not uniform biofilm slaughtering	Low sNRR dependency on substrate concentration	Reaction completion required for sedimentation to occur
High biomass accumulation	High NRR dependency on substrate concentrations		Easy biomass washout
	Carrier media clogging when NRR greater than 6 g N/Ld		

6.5. Conceptual design of a two-reactor system for autotrophic nitrogen removal from centrate at the NEWPCC, Winnipeg, Canada

The North End Water Pollution Control Centre (NEWPCC), a wastewater treatment plant, is located in the northern part of Winnipeg, Canada. It treats about 70% of Winnipeg's wastewater and has an average annual flow of about 200 000 m^3/d (Baker et al., 2009). It uses a high purity oxygen activated sludge. The treated effluent is disinfected and discharged into the Red River.

Waste activated sludge (WAS) solids and primary solids from the NEWPCC are co-thickened in the primary clarifiers and then blended with the raw mixture of WAS and primary solids trucked from two smaller plants in the City. Blended solids are stabilized in mesophilic anaerobic digesters operated at 38^{o}C. The digested solids are dewatered in centrifuges.

The centrate from the centrifuges is discharged directly to the head of the plant. Although the centrate flow averages only 2 000 m^3/d (one percent of the total flow to the plant), it contributes about 30 % of the total nitrogen load to the plant. The return phosphorus load to the plant is very low due to ferric chloride added before the sludge dewatering.

The NEWPCC has a short SRT (about 2 days)in mainstream reactors, since it was originally designed for carbon removal only. As a result, no

nitrification occurs. The province of Manitoba decided to implement nutrient control measures which obligated the City of Winnipeg to reduce its nitrogen load to the Red River by 13%, for now. However, further reductions in nitrogen load will be required in the future (Environment Act Licences for year 2014). This need resulted in the construction of a centrate facility for side-stream total nitrogen (TN) removal, completed in August 2008.

The centrate facility consists of two SBR reactors having a volumetric capacity of 5800 m^3 each. They were designed to remove approximately 1 600 kg TN/d at an average daily flow rate of 2 000 m^3/d. In the following conceptual design, the available volume of reactors was compared to the volume that is required to achieve total nitrogen removal of 1 600 kg N/d, by an anammox process with the existing configuration.

For the purpose of conceptual design, two options of a two-reactor system were considered, as follows:

- Option 1 - Continuous flow system – chemostat for partial nitritation followed by an MBBR for anammox (with pH control when required)
- Option 2 - Sequential flow system – SBR for partial nitritation followed by another SBR for anammox (with pH control when required)

Option 1

This option provides continuous flow through the system. In this option, centrate flows directly to the first reactor where partial nitritation occurs. Then, treated centrate flows directly to the second reactor, where nitrogen is removed through an anammox process. This configuration does not require SRT control and solids handling. Incoming solids and biosolids growing in the chemostat are passed through the entire system without retention. Anammox biomass is attached to the carrier media which provides a sufficient amount of time for slow growing anammox organisms. No sophisticated process control is required except dissolved oxygen control in the chemostat for partial nitritation and pH control if required (current plant is equipped with a tank which would allow acid dosage). Although such a process configuration is not reported in a pilot or full-scale system, similar ones are operated at the Dokhaven wastewater treatment plant in Rotterdam, Holland (van der Star, et al., 2007). Instead of an MBBR reactor, a UASB reactor is used at that plant.

The minimum reactor sizing for the NEWPCC centrate facility required for TN removal is presented in Table 6-3. The current research, together with a study of the literature, found that the existing centrate facility has enough reactor volume to treat 1600 kg N/d.

Table 6-3 Reactors sizing for Option 1

Process	Chemostat partial nitritation	MBBR anammox
Required nitrogen conversions	(1)900 kg N/d	(2)1600 kg N/d]
Biomass activity based on Gali et al. (2007) for chemostat and experimentation for anammox	(3)0.9 kg N/kg VSS d (3)0.35 kg N/m^3d at 35 ^{0}C	(4)4.8 kg N/$m^3_{media\ K1}$ at 35 ^{0}C no pH control
Minimum reactor volume required	(5)2570 m^3	(6)700 m^3
Reactor volume available	(7)5800 m^3	(7)5800 m^3

(1) Required TA to be oxidised to nitrite, as based on anammox stoichiometry (1.3 kg NO_2-N/kg TA-N)

(2) Required nitrogen to be removed in the anammox process

(3) The observed nitritation rate based on the results presented by Gali et al. (2007) which are similar to those observed in full scale reactors (Jaroszynski & Oleszkiewicz, 2011)*, not tested in this research*

(4) The observed nitrogen removal rate based on the tested lab scale anammox reactor (Test 4, page134)

(5) Minimum reactor volume required based on the volumetric nitrite production rate verified for a minimum HRT of 1 day at a dissolved oxygen concentration of 3.0 mg O_2/L and temperature of 35 ^{0}C

(6) Minimum reactor volume required based on the 50% filling ratio with media K1 (333 m^3 of carrier media K1)

(7) Available reactor volume at the existing facility

Option 2

This option provides sequential flow through the system, a flow that is similar to the one at the existing centrate facility. In this option, first, centrate flows to the equalization tank, then the first reactor is fed and partial nitritation occurs. Second, during decanting in the partial nitritation reactor, the pre-treated centrate flows to the second reactor where nitrogen is removed through the anammox process. After three hours of reaction, the centrate is discharged to the head of the plant. This configuration requires SRT control similar to that of the existing centrate facility. Minimum SRT for partial nitritation and anammox should be above 5 days and 20 days, respectively. Some process control is required, such as reactor phase control, dissolved oxygen control in the partial nitritation reactor, and SRT control. Although such a process configuration has not been reported in a full-scale system, it has been tested in pilot scale reactors (Fux et al., 2002).

The minimum reactor sizing required for TN removal at the NEWPCC centrate facility required for TN removal is presented in Table 6-4. The present research found that the existing centrate facility has enough reactor volume to treat 1600 kg N/d.

Table 6-4 Reactors sizing for Option 2

Process	SBR partial nitritation	SBR anammox
Required nitrogen conversions	(1)900 kg N/d	(2)1600 kg N/d]
Biomass activity based on experimentation	(3)1.4 kg N/kg VSS d (3)0.9 kg N/m^3d at 35 ^{0}C	(4)0.5 kg N/m^3d at 35 ^{0}C no pH control
Minimum reactor volume required	(5)1000 m^3	(6)3200 m^3
Reactor volume available	(7)5800 m^3	(7)5800 m^3

(1) Required TA to be oxidised to nitrite, based on anammox stoichiometry (1.3 kg NO_2-N/kg TA-N)

(2) Required nitrogen to be removed in anammox process

(3) The observed nitritation rate based on the tested lab scale partial nitritation reactor (page 97 and page 289)

(4) The observed nitrogen removal rate based on the tested lab scale anammox reactor (page 108)

(5) Minimum reactor volume required, based on the following assumptions: SBR (3 cycles - 5 h aeration/reaction time, 1 h settling time, 2 hours decanting/feeding to the anammox reactor), solids concentration VSS = 1.0 kg VSS/m^3, dissolved oxygen concentration in the range 1 – 3 mg O_2/L, and temperature of 35 ^{0}C

(6) Minimum reactor volume required, based on the following assumptions: SBR (3 cycles - 2 h feeding time, 4 h reacting time, 2 h settling and decanting time), solids concentration VSS ≈ 3.0 kg VSS/m^3, and temperature of 35 ^{0}C

(7) Available reactor volume at the existing facility

Comparison of Options

In a comparison of the two options from the perspective of ease of operation and reliability, Option 1 appears to be preferable. This option includes fewer parameters requiring control, and the slow growing anammox biomass is secured to the carrier media through an attached biofilm. The only significant disadvantage is the need for the carrier media, which has to be purchased. Other advantages and disadvantages, between Options 1 and 2, are presented in Table 6-5.

During the research period, it was noticed that phosphorus limitations may occur. Such a problem was identified during the last period of the research (page 155), and it caused significant anammox NRR deterioration. Online phosphorus monitoring is recommended for enhanced system stability.

Table 6-5 Options comparison with their advantages and disadvantages

Option 1		**Option 2**	
Advantages	Disadvantages	Advantages	Disadvantages
Continuous flow system	Need for K1 media purchase	No investment cost	Sequential flow system
No SRT control			SRT control
More flexible configuration			Anammox sludge flotation risk
Requires less total reactor volume of 3 270 m^3			Requires more total reactor volume of 4 200 m^3

6.6. Conclusions

This research project fulfilled its engineering significance because it was able to identify design parameters for the anammox process. Key inhibitors, such as FA and nitrite, were investigated through full-scale reactor operation scenario tests. It was confirmed that the raw centrate used through the entire research period was treatable, using a two reactor anammox configuration for nitrogen removal. Phosphorus limitation was identified during the course of the research, and therefore, online phosphorus monitoring should be conducted to ensure stable anammox system performance.

The existing configuration for centrate treatment at NEWPCC has sufficient reactor volume to implement the anammox process. The suggested option (above) provides continuous flow throughout the system. The suggested configuration proposes partial nitritation in a chemostat reactor, with MBBR configuration for the anammox process.

Replacing existing conventional system with an anammox based system will reduce (or even completely eliminate) the need for chemical addition. Additionally, plant carbon footprint will be reduced due to reduction of CO_2 emission to the atmosphere as no longer methanol will be added for denitrification.

Comparing the existing centrate facility reactor volume for conventional nitrogen removal with volume required for anammox-based process (Option 1), 72% of the total volume could be saved. When designing such a new

facility the capital costs savings would amount to about 25 M$ (assuming cost of 1 m^3 of the reactor at about 3000 $).

7. FUTURE WORK

This study identified FA as a very important stability parameter for an anammox reactor. In order to gain a more in-depth understanding of the pH associated FA inhibitory effect on an anammox consortium, however, further research will need to be undertaken. The following are suggestions for future projects that could make invaluable contributions toward such an understanding:

- **Findings of this research should be verified in the pilot or full scale systems for centrate and/or other industrial wastewater.** The results of current research suggest that a pH within the range of 7.0 and 8.0 does not have a significant effect on nitrogen removal rate (NRR), when FA was the inhibition reaction driver within the pH range tested. However, various wastewaters have different compositions which may not allow for such a pH manipulation for FA control. Therefore, anammox reactor operation should be investigated under different pH levels, from the perspective of various ionic forms existing in industrial wastewaters.

- **The role of microbial diversity and their interaction within the anammox consortium on anammox system stability.** In the current study, it was observed that within the nitrite and FA range tested, NRR was stimulated by nitrite, when nitrite was changing from 3 mg NO_2-N/L to about 200 mg NO_2-N/L and while FA was below 2 mg N/L. It was

also observed that FA had an adverse effect on NRR when it exceeded 2 mg N/L. It should be noted that NRR is the result of complex interactions between many groups of organisms, such as anammox organisms (primarily responsible for nitrogen removal), oxygen utilizers (provide nitrite for the anammox process and protect anammox organisms from oxygen inhibition), and other organisms whose existence may be vital for the anammox consortium. In order to gain a better understanding of the effect of FA on the anammox process, anammox consortium community structure should be tracked along with FA changes.

- **The FA inhibitory effect on anammox kinetic parameters such as growth rate and decay***.* In the literature, there is a lack of reliable data about the growth and decay parameters for anammox microorganisms. These data could be used for modeling purposes, and therefore, research that would target this issue is needed.

- **The inhibitory effect of nitrite on anammox kinetic parameters such as growth rate and decay below FA inhibition concentration.** Results of current research suggest that FA has an inhibitory effect on NRR when it exceeds 2 mg N/L. Also total ammonia (TA) limitation, associated with elevated nitrite, was suggested to be responsible for NRR cessation. Hence, an investigation into kinetic parameters under elevated nitrite levels at low FA and under no TA limitation would have great value for further anammox technology development and modeling purposes.

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9. APPENDIX

Appendix 1 – Reactors start-up

Partial nitritation start-up

Time	NH_4-N	NO_2-N	NO_3-N	pH	DO	TSS total	VSS total	Specific nitrite production rate	Nitrite production rate
[day]	[mg N/L]	[mg N/L]	[mg N/L]	[-]	[mg O_2/L]	mg TSS	mg VSS	[mg NO_2-N $_{prod.}$/ mg VSS d]	[mg NO_2-N $_{prod.}$/ d]
1	52.5	55.8	0	7.4	6.5	1664	1622	0.31	0.5
2	143.1	149.4	0	7.4	5.0	2160	1740	0.91	1.6
4	292.0	300.0	0	7.4	5.0	3376	2480	3.53	8.8
5	342.0	363.6	0	7.4	4.0	5753	4181	3.04	12.7
6	369.0	376.2	0	7.4	2.5	8569	6232	2.66	16.6
7	379.8	370.8	0	7.4	1.9	11248	8037	2.31	18.5
8	379.8	367.2	0	7.4	1.5	14079	9671	1.90	18.4
11	378.0	360.0	0	7.4	1.5	28440	19260	1.31	25.2
12	378.0	363.6	0	7.4	1.5	34440	23220	1.25	29.1
13	369.0	360.0	0	7.4	1.5	33000	21980	1.31	28.8
14	363.6	401.4	0	7.4	1.5	30780	20500	1.57	32.1
15	381.6	383.4	0	7.4	1.5	29680	19900	1.54	30.7
19	459.0	388.8	0	7.4	1.5	30524	20800	1.50	31.1

R1 – reactor start-up after the inoculation with carrier media

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0	7.54	215	12.0	3.1	3.4	0.84	75	1.09	0.03	0.5
1	7.28	205	13.7	6.0	5.0	0.84	74	1.08	0.04	0.3
2	7.27	-	10.1	2.5	8.7	0.84	75	1.08	0.07	0.2
3	7.10	-	7.3	2.4	11.7	0.90	80	1.06	0.09	0.1
4	7.29	188	8.5	3.1	12.0	0.90	79	1.06	0.09	0.2
7	7.20	-	3.1	1.2	13.8	0.90	81	0.92	0.09	0.1
8	7.35	-	2.3	6.0	16.9	0.86	75	0.89	0.11	0.1
9	7.62	185	1.2	10.0	21.3	0.88	111	1.31	0.12	0.1
10	7.72	-	5.4	2.4	22.3	0.93	118	1.39	0.13	0.3
11	8.08	213	0.5	19.5	19.8	0.93	120	1.15	0.11	0.1
15	8.08	230	1.8	6.4	24.0	0.93	129	1.51	0.10	0.2
16	8.02	-	4.8	3.8	32.5	0.92	130	1.54	0.18	0.5
17	8.04	-	13.2	5.5	32.9	0.92	154	1.17	0.13	1.5
18	8.17	220	4.2	10.3	42.9	0.90	163	0.86	0.09	0.6
21	8.11	-	30.4	5.7	30.9	0.90	159	1.61	0.09	3.9
22	7.85	-	45.3	2.1	22.0	0.90	171	1.12	0.08	3.4
23	8.07	-	43.6	7.5	24.0	0.89	178	1.30	0.04	5.1
24	8.04	-	42.3	2.2	23.0	0.66	138	1.02	0.07	4.7
25	8.03	-	39.5	1.4	22.0	0.95	182	1.41	0.09	4.3

Table continuation - R1 – reactor start-up after the inoculation with carrier media										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
28	7.71	185	13.2	0.0	22.7	0.95	173	1.80	0.11	0.7
29	7.83	136	2.8	2.8	23.8	0.88	168	1.04	0.08	0.2
30	7.75	-	15.1	0.9	25.2	0.80	174	0.99	0.07	0.9
31	7.62	-	20.3	1.3	28.4	0.95	200	0.06	-1.40	0.9
32	7.57	132	47.6	2.9	31.6	0.95	217	1.17	0.10	1.9
35	7.22	85	26.7	2.4	32.2	0.95	209	1.31	0.11	0.5
36	7.20	-	17.8	2.9	36.3	0.95	204	0.85	0.10	0.3
37	7.28	-	31.1	5.3	40.7	0.95	191	1.30	0.15	0.7
38	7.57	-	39.0	16.6	32.5	0.95	171	1.63	0.15	1.6
39	8.38	-	49.3	4.3	27.0	0.90	195	1.31	0.09	10.5
42	7.85	110	59.5	6.6	32.8	0.90	210	1.25	0.06	4.4
43	7.84	-	53.1	3.5	35.8	0.94	220	1.66	-0.05	3.9
44	7.80	-	64.8	1.0	43.0	0.94	170	1.33	0.07	4.3
45	7.70	-	52.15	1.7	36.8	0.94	143	1.37	0.16	2.8
46	7.62	-	71.5	7.3	38.3	0.90	126	1.77	0.16	3.2
49	6.82	-	17.7	0.3	34.1	0.80	124	1.41	0.10	0.1
50	7.19	-	9.6	1.5	37.9	0.95	141	1.38	0.19	0.2
51	7.96	-	19.4	1.4	33.3	0.95	190	1.10	0.11	1.8
52	8.23	193	7.1	2.5	30.3	0.86	162	1.34	0.08	1.1
53	8.40	-	5.3	4.7	33.5	0.86	183	1.54	-0.34	1.2
57	8.06	-	2.2	3.3	13.3	0.86	198	1.12	-0.11	0.3
58	8.23	-	12.4	0.6	23.6	0.86	238	0.92	-0.28	2.0
59	8.08	197	27.2	22.2	31.8	1.03	228	1.22	-0.01	3.3

Table continuation - R1 – reactor start-up after the inoculation with carrier media										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
62	8.11	-	30.5	34.2	41.1	1.00	208	1.46	-0.40	3.9
63	7.07	-	1.7	0.8	43	0.40	77	1.30	0.16	0.0
64	7.45	-	15.6	10.7	43.6	0.40	97	0.75	-0.07	0.5
65	8.11	283	16.5	2.2	32	0.39	92	1.30	-0.01	2.1
66	8.13	-	20	1.7	30.6	0.49	120	1.21	0.01	2.7
67	7.09	466	30	1.2	34.8	0.44	113	1.16	0.04	0.4
70	8.06	533	39	2	36.1	0.44	118	0.88	0.01	4.5
71	7.48	-	34.2	1.2	39.9	0.85	194	1.27	0.12	1.1
72	7.98	-	33	1.8	38.1	0.87	209	0.94	0.08	3.2
73	8.09	-	47.1	12.9	37.2	0.97	213	0.91	0.07	5.8
74	8.07	719	63.3	5.1	37.2	1.10	237	1.13	0.11	7.4
77	7.15	-	84.3	2	40.6	1.07	224	1.00	0.11	1.3
78	8.13	-	87	2.7	35.4	1.07	211	1.15	0.08	11.5
79	7.30	-	106.2	1.3	38.3	1.07	203	0.98	0.07	2.4
80	8.14	-	96.9	0.9	38.1	1.07	195	1.05	0.08	13.1
83	7.67	-	99	1.8	34.2	1.07	193	1.64	0.16	5.0
84	7.71	-	72.5	1.2	38.4	1.07	241	1.13	0.08	4.0
87	6.68	330	97.5	11.6	46	0.90	191	1.22	0.05	0.5
91	6.25	240	104.4	55.2	120.3	0.90	149	1.25	0.19	0.2
92	7.90	-	52.2	0.5	42.1	0.80	184	1.36	-0.04	4.3
93	7.00	-	50.1	0.9	48.3	0.80	186	1.14	0.02	0.6
94	7.31	-	46.5	0.2	47.8	0.48	110	1.12	0.06	1.1
97	7.37	-	32.4	0.2	43	0.65	154	1.33	-0.35	0.8

Table continuation - R1 – reactor start-up after the inoculation with carrier media										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
98	7.33	-	30.6	0.5	55	0.65	147	1.25	-0.10	0.7
99	7.35	-	23.9	0.3	53.1	0.65	135	1.21	0.05	0.6
100	7.70	127	28.7	1.1	54.4	0.85	164	1.16	0.01	1.5
101	6.20	217	27.8	0.9	54.9	0.85	148	1.13	0.20	0.0

R2 – reactor start-up after the inoculation with carrier media

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0	7.34	181	18.4	2.7	0.0	0.36	32	1.15	0.00	0.4
1	7.25	184	25.3	1.8	0.1	0.36	31	1.23	0.00	0.5
2	7.32	-	26.4	1.1	0.0	0.36	31	1.25	0.00	0.6
3	7.31	-	29.7	1.0	0.1	0.39	33	1.28	0.00	0.7
4	7.36	200	31.3	0.8	0.2	0.39	33	1.30	0.00	0.8
7	7.49	-	21.2	0.5	1.3	0.63	56	1.06	0.01	0.7
8	7.52	-	12.3	5.0	2.3	0.63	56	0.96	0.02	0.4
9	7.67	198	4.4	7.2	5.2	0.63	83	1.35	0.03	0.2
10	7.67	-	8.1	1.5	0.3	0.63	84	1.42	0.00	0.4
11	7.89	218	6.1	2.2	2.6	0.62	86	1.28	0.01	0.5
15	8.29	239	4.9	1.3	8.6	0.62	90	1.57	0.01	0.9
16	8.36	-	6.7	5.0	9.3	0.96	142	1.55	0.05	1.4
17	8.44	-	11.8	13.7	7.0	0.97	169	1.13	0.03	2.8
18	8.32	225	0.6	9.2	15.1	0.95	183	0.86	0.00	0.1
21	8.38	-	66.3	57.6	6.2	0.95	148	1.64	-0.03	14.2
22	7.99	-	80.3	35.5	6.5	0.95	164	1.14	0.03	8.0
23	8.05	-	48.1	0.0	7.4	0.70	145	1.36	-0.02	5.4
24	8.12	-	52.4	26.2	9.0	0.65	132	0.98	0.03	6.8
25	8.14	-	45.5	22.8	12.0	0.95	176	1.35	0.05	6.2
28	8.07	184	28.2	11.6	22.0	0.95	165	1.88	0.12	3.3
29	8.14	-	32.7	17.0	18.3	1.00	179	1.11	0.07	4.4

Table continuation – R2 – reactor start-up after the inoculation with carrier media										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
30	8.23	-	28.9	0.0	26.0	0.85	181	1.03	0.08	4.7
31	7.60	-	40.0	5.0	23.0	0.98	200	1.15	0.10	1.7
32	8.02	136	75.0	8.5	27.7	0.98	214	1.26	0.09	8.0
35	7.94	-	51.9	26.0	26.4	0.98	201	1.34	0.10	4.7
36	7.55	-	30.9	0.9	24.8	0.98	211	0.88	0.07	1.2
37	7.74	-	44.5	4.6	28.8	0.98	197	1.37	0.11	2.6
38	7.75	-	37.1	11.2	22.7	0.96	178	1.64	0.10	2.2
39	7.95	-	53.5	8.6	26.3	0.96	205	1.31	0.09	4.9
42	7.82	-	60.3	11.3	35.1	0.96	221	1.24	0.06	4.2
43	7.76	-	56.0	8.4	38.0	0.96	222	1.66	-0.04	3.4
44	7.69	-	56.8	5.6	41.6	0.91	166	1.27	0.06	3.0
45	7.63	-	53.2	3.4	38.3	0.93	140	1.37	0.17	2.5
46	7.32	-	61.3	5.5	58.2	0.89	122	1.68	0.27	1.4
49	6.78	-	52.0	4.0	27.2	0.96	156	1.30	0.06	0.0
50	7.09	-	0.9	1.8	27.5	0.96	148	1.33	0.13	0.0
51	7.29	-	0.9	5.6	28.0	0.96	199	1.02	0.09	0.0
52	7.62	-	2.7	1.6	26.4	0.86	165	1.32	0.07	0.1
53	7.90	-	4.3	2.6	27.8	0.86	185	1.54	-0.36	0.4
57	7.86	-	1.6	1.9	7.5	0.86	200	1.13	-0.13	0.1
58	8.15	-	4.0	1.0	15.4	0.86	243	0.90	-0.30	0.6
59	8.26	197	8.1	5.1	23.7	0.97	229	1.20	-0.04	1.4
62	8.34	-	11.4	13.3	31.7	0.40	90	1.43	-0.41	2.3
63	7.99	-	0.8	0.7	25.0	0.40	80	1.29	0.09	0.1

Table continuation – R2 – reactor start-up after the inoculation with carrier media										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
64	8.26	-	11.0	6.2	20.7	0.40	101	0.75	-0.12	1.9
65	8.39	-	29.7	14.6	13.8	0.39	91	1.32	-0.07	6.5
66	7.45	-	24.5	1.6	16.6	0.49	121	1.22	-0.04	0.8
67	7.78	453	26.8	1.7	20.4	0.46	120	1.15	0.00	1.7
70	7.78	396	34.8	0.9	29.7	0.46	125	0.87	-0.01	2.2
71	8.04	-	29.3	1.9	30.5	0.85	198	1.24	0.09	3.2
72	7.46	-	27.0	1.3	33.2	0.86	208	0.92	0.07	0.9
73	7.73	-	26.1	1.1	37.0	0.97	224	0.89	0.07	1.5
74	8.03	889	51.6	1.4	35.5	1.10	243	1.10	0.10	5.6
77	7.67	-	76.5	0.3	34.5	1.04	223	0.99	0.09	3.9
78	7.99	-	78.3	0.9	31.8	1.04	210	1.12	0.07	7.8
79	6.94	-	96.6	0.4	29.9	1.04	204	0.95	0.04	0.9
80	7.34	-	90.0	0.2	34.3	1.04	194	1.03	0.07	2.2
83	7.67	-	93.0	0.3	33.9	1.04	190	1.60	0.15	4.7
84	7.28	-	68.1	0.3	37.8	1.04	236	1.12	0.08	1.4
87	6.35	-	62.7	0.2	43.3	1.00	229	1.12	0.03	0.2
91	5.94	-	116.4	97.8	43.8	0.93	161	1.13	-0.13	0.1
92	7.26	-	40.5	0.2	31.9	1.00	238	1.31	-0.07	0.8
93	7.29	-	45.0	0.5	36.1	1.00	239	1.12	-0.02	1.0
94	7.50	-	41.4	0.2	35.5	0.48	112	1.11	0.02	1.4
97	6.69	-	34.5	0.2	39.4	0.70	166	1.34	-0.36	0.2
98	6.91	-	32.7	0.1	41.6	0.70	162	1.26	-0.14	0.3
99	7.22	-	25.5	0.2	38.8	0.70	148	1.21	0.00	0.5

Table continuation – R2 – reactor start-up after the inoculation with carrier media										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
100	7.55	177	23.0	0.2	39.1	0.82	164	1.14	-0.05	0.9
101	6.28	233	25.8	0.3	41.7	0.82	147	1.12	0.15	0.1

R1 – Reactor operation after the addition of pH control set at 6.5

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0	6.40	-	111.3	0.8	36.7	3.60	644	1.14	0.14	0.3
1	6.50	-	121.6	1.1	33.6	3.60	618	1.19	0.13	0.4
5	6.50	-	113.6	3.6	50.4	5.90	1058	1.22	0.16	0.4
6	6.50	-	102.4	2.8	60.6	6.00	1324	1.07	0.17	0.4
7	6.50	-	121.2	2.6	50.6	7.70	1285	1.18	0.20	0.4
8	6.50	-	115.2	3.0	44.3	9.40	1516	1.22	0.19	0.4
9	6.50	92	121.2	3.7	47.1	11.40	1864	1.29	0.20	0.4
12	6.50	-	104.8	3.7	48	10.60	1724	1.16	0.19	0.4
13	6.50	-	122.4	3.4	36.4	11.00	1709	1.27	0.16	0.4
14	6.50	-	127.6	5.0	45.2	16.10	2465	1.17	0.19	0.5
15	6.50	-	127.2	4.9	43.4	14.60	2186	1.26	0.20	0.5
16	6.50	93	136.0	7.1	43.0	18.70	2778	1.20	0.19	0.5
20	7.00	-	101.2	2.0	21.5	1.50	246	1.27	0.08	1.1
21	6.50	-	116.4	3.4	41.1	7.20	1131	1.25	0.16	0.4
22	6.50	-	138.4	4.6	52.4	9.20	1318	1.39	0.26	0.5
23	6.50	128	112.4	5.3	44.7	14.40	2244	1.21	0.19	0.4
24	6.50	-	127.2	4.8	41.4	14.40	2174	1.23	0.19	0.5
25	6.50	-	116.4	6.8	43.3	17.30	2880	1.12	0.17	0.4
26	6.50	-	102.4	7.6	39.5	19.90	3044	1.20	0.17	0.4
27	6.50	-	106.8	7.4	58.8	20.20	2744	1.32	0.27	0.4
28	6.50	-	106.0	7.6	39.0	20.20	2988	1.12	0.16	0.4
34	6.50	-	72.0	6.8	38.1	18.00	2355	1.17	0.19	0.3

Table continuation – R1 – Reactor operation after the addition of pH control set at 6.5										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
35	6.50	-	109.6	9.5	38.5	22.70	3466	1.12	0.16	0.4

R2 – Reactor operation after the addition of pH control set at 6.5 in R1

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0	6.40	-	109.5	0.7	27.2	2.60	475	1.13	0.10	0.3
1	6.90	-	119.6	0.9	23.6	2.80	492	1.18	0.09	1.1
5	7.64	-	101.6	3.0	46.2	4.10	758	1.17	0.14	4.8
6	7.88	-	152.8	3.7	52.0	5.10	1053	1.25	0.17	12.1
7	7.86	-	128.0	3.2	51.2	5.70	936	1.21	0.21	9.7
8	7.60	-	127.2	2.5	43.1	6.90	1089	1.29	0.19	5.5
9	7.62	151	108.0	2.7	42.9	7.60	1289	1.22	0.17	4.9
12	7.80	-	98.4	2.4	43.0	7.10	1185	1.14	0.17	6.6
13	7.60	-	106.0	2.1	34.2	7.10	1150	1.19	0.14	4.6
14	7.47	-	115.6	3.1	39.3	9.40	1501	1.12	0.16	3.7
15	7.53	-	116.4	3.7	40.0	10.80	1672	1.20	0.17	4.3
16	7.50	175	123.6	3.0	39.9	12.20	1892	1.15	0.17	4.3
20	7.80	-	98.8	6.0	38.2	12.20	1924	1.23	0.15	6.6
21	7.66	-	109.6	7.2	39.6	14.60	2316	1.20	0.15	5.4
22	7.50	-	130.4	8.2	45.0	14.70	2163	1.32	0.21	4.5
23	7.51	223	107.6	6.5	39.0	15.40	2448	1.18	0.17	3.8
24	7.57	-	120.0	5.9	36.9	15.40	2380	1.18	0.16	4.9
25	7.67	-	110.4	8.6	39.8	17.30	2925	1.09	0.15	5.6
26	7.95	-	47.6	9.8	35.2	17.30	2974	0.96	0.12	4.4
27	7.87	-	100.8	9.1	55.6	17.70	2449	1.27	0.25	7.8
28	7.83	-	102.4	8.9	35.7	17.80	2666	1.09	0.14	7.3
34	7.80	-	64.0	7.5	34.2	15.10	2032	1.13	0.17	4.3

Table continuation – R2 – Reactor operation after the addition of pH control set at 6.5 in R1										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
35	7.90	-	104.8	12.3	35.0	17.30	2673	1.09	0.15	8.7

R3 – reactor start-up after the inoculation with the biomass from R1 and R2

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0	7.51	-	68.0	3.2	32.2	0.70	157	1.13	0.09	2.4
1	7.61	-	66.4	1.2	34.5	0.70	159	1.16	0.10	2.9
2	7.56	-	70.0	0.0	46.1	0.85	170	0.98	0.03	2.8
3	7.57	248	67.2	0.0	44.2	1.15	214	1.03	0.06	2.7
6	7.48	-	50.4	0.0	47.5	1.15	216	1.25	0.15	1.7
7	7.08	-	38.0	0.0	33.5	1.15	239	1.10	0.06	0.5
8	7.18	242	33.0	0.0	28.7	1.15	248	1.18	0.08	0.6
9	7.40	-	11.0	0.0	11.7	1.60	368	1.11	0.03	0.3
10	7.61	-	2.9	0.0	0.0	1.60	356	1.11	0.00	0.1
13	7.41	-	15.8	0.0	8.0	1.60	339	1.13	0.02	0.4
28	7.30	-	1.1	0.0	45.5	1.00	287	1.15	0.13	0.0
29	8.12	-	6.3	0.0	36.4	1.43	349	1.04	0.09	0.8
30	7.71	-	0.6	0.0	29.8	1.22	316	1.01	0.08	0.0
33	8.46	-	8.6	0.0	16.2	1.43	331	1.17	0.05	2.1
34	8.11	-	65.2	43.8	16.4	1.41	275	1.10	0.06	8.3
35	7.51	-	163.6	146.0	0.0	0.00	0	-	-	5.8
36	7.14	-	38.2	1.4	13.2	0.00	0	-	-	0.6
37	7.90	-	52.4	17.8	21.5	1.40	285	1.20	0.07	4.3
40	6.96	-	33.2	24.7	39.7	0.77	194	1.10	0.12	0.3
41	7.53	-	13.8	0.0	28.6	0.67	176	1.06	0.09	0.5
42	7.38	200	14.0	0.0	22.7	0.77	211	0.96	0.06	0.4
43	7.40	-	21.0	0.0	20.8	0.77	211	0.99	0.06	0.6

Table continuation – R3 – reactor start-up after the inoculation with the biomass from R1 and R2										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
44	7.23	-	25.2	0.0	29.1	0.77	207	1.02	0.08	0.5
47	7.01	-	11.6	0.0	30.8	0.77	197	1.13	0.10	0.1
48	7.15	-	22.9	0.0	35.5	0.77	205	1.05	0.10	0.4
49	7.30	183	29.2	0.0	40.2	1.20	261	1.11	0.12	0.6
50	7.31	-	19.7	0.0	30.4	1.80	350	1.06	0.08	0.4
51	7.40	-	7.8	0.0	30.4	1.78	352	1.00	0.06	0.2
54	7.05	-	1.9	0.0	16.5	1.80	380	1.03	0.05	0.0
55	7.64	-	23.0	0.0	26.5	2.15	471	1.11	0.08	1.1
56	7.50	-	100.4	68.4	29.4	5.00	769	0.92	0.07	3.5
57	7.32	-	42.0	0.0	12.8	5.00	1113	1.07	0.00	1.0
58	7.50	-	44.8	0.0	1.0	0.00	0	-	-	1.6
61	7.50	-	20.4	0.0	0.2	1.70	553	1.07	0.00	0.7
62	7.60	-	20.6	0.0	6.0	2.00	609	1.11	0.02	0.9
63	7.80	-	29.3	0.0	35.0	5.20	1147	0.98	0.04	2.0
64	7.80	-	24.4	0.0	35.2	5.20	1190	1.00	0.09	1.6
67	7.50	-	199.2	212.8	2.4	0.00	0	-	-	6.9
68	7.60	-	84.0	32.7	7.3	0.00	0	-	-	3.6
69	7.50	-	81.6	0.0	16.0	0.00	0	-	-	2.8
70	7.50	-	59.6	0.0	22.4	2.00	495	1.49	0.09	2.1
71	7.41	-	54.0	32.4	24.8	2.00	525	1.37	0.09	1.5
74	7.93	-	90.4	0.0	40.4	2.00	581	0.98	0.11	8.0
76	7.80	-	14.6	2.6	17.5	2.80	634	1.56	0.08	1.0
79	7.60	-	29.3	5.0	14.0	2.00	600	0.90	0.03	1.3

Table continuation – R3 – reactor start-up after the inoculation with the biomass from R1 and R2										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
80	7.10	-	27.4	2.1	18.1	2.00	605	0.88	0.05	0.4
81	7.46	-	16.9	0.0	20.7	2.00	636	0.95	0.05	0.5
82	7.61	-	30.2	0.0	27.0	3.00	765	1.11	0.08	1.3
85	7.40	-	32.4	1.0	34.3	2.50	700	1.05	0.10	0.9
86	7.40	-	50.4	0.0	28.3	2.50	718	0.98	0.08	1.4
87	7.40	-	50.4	4.0	39.2	2.50	681	1.06	0.12	1.4
88	7.40	-	47.2	1.4	41.8	2.50	641	1.12	0.14	1.3
Introduction of pH control applying sparging CO_2 gas										
89	7.00	-	52.0	0	32.7	2.50	689	1.05	0.10	0.7
90	7.00	-	38.3	0	5.8	2.50	728	1.03	0.02	0.5
91	7.00	-	40.4	0	7.3	2.50	674	1.16	0.02	0.5
92	7.00	-	45.6	0	26.9	4.20	904	1.23	0.09	0.6
102	7.00	-	48.0	0	24.3	4.50	1002	1.05	0.07	0.6
103	7.00	-	70.8	0	13.5	4.50	914	1.11	0.04	0.9
106	7.00	-	88.8	0	39.6	5.00	931	1.15	0.13	1.1
107	7.00	-	80.4	0	36.2	5.00	949	1.17	0.12	1.0
110	7.00	-	98.0	0	31.6	5.00	964	1.07	0.10	1.2
111	7.00	-	112.0	0	24.9	5.00	959	1.08	0.08	1.4
112	7.00	-	86.4	0	3.0	5.00	982	1.11	0.01	1.1
113	7.00	-	89.6	0	9.1	5.00	963	1.22	0.03	1.1
114	7.00	-	55.6	0	12.4	5.00	1000	1.17	0.04	0.7
117	7.00	-	31.3	0	7.5	5.00	974	1.17	0.03	0.4
118	7.00	-	44.4	0	24.0	5.00	965	1.16	0.08	0.6

Table continuation – R3 – reactor start-up after the inoculation with the biomass from R1 and R2										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
119	7.00	-	60.8	0	30.9	5.00	966	1.05	0.09	0.8
120	7.00	-	88.4	0	33.5	5.00	947	1.07	0.11	1.1
125	7.00	-	64.0	0	23.0	5.00	904	1.12	0.08	0.8
126	7.00	-	68.8	0	34.8	5.00	1015	1.12	0.05	0.9
127	7.00	-	68.4	0	44.5	5.00	939	1.26	0.11	0.9
128	7.00	-	84.0	0	40.0	5.00	1070	1.04	0.08	1.1
131	7.00	-	27.1	0	30.0	5.00	1050	1.15	0.08	0.3
132	7.00	-	31.6	0	25.4	5.00	1031	1.10	0.08	0.4
141	7.00	-	113.2	0	25.7	4.00	848	1.28	0.09	1.4
144	7.00	-	86.8	0	8.5	4.00	903	1.21	0.03	1.1
145	7.00	-	91.6	0	8.8	5.00	1053	1.11	0.03	1.2
146	7.00	-	92.4	0	11.8	5.00	987	1.11	0.04	1.2
147	7.00	-	90.4	0	12.9	5.00	980	1.06	0.04	1.1
152	7.00	-	94.4	0	5.0	5.00	1005	1.11	0.02	1.2
153	7.00	-	93.2	0	4.5	5.00	1043	1.06	0.01	1.2
159	7.00	-	103.2	0	15.7	5.00	1005	1.11	0.05	1.3
160	7.00	-	93.2	0	37.8	5.00	923	1.21	0.13	1.2
161	7.00	-	83.2	0	26.4	5.00	1000	1.09	0.08	1.0
162	7.00	-	54.4	0	25.5	5.00	939	1.18	0.09	0.7
165	7.00	-	25.7	0	31.3	5.00	967	1.22	0.10	0.3
166	7.00	-	22.6	0	38.5	5.00	923	1.23	0.13	0.3
167	7.00	-	26.2	0	48.3	7.00	1292	1.25	0.17	0.3
172	7.00	-	90.0	0	42.4	10.00	1778	1.18	0.15	1.1

Table continuation – R3 – reactor start-up after the inoculation with the biomass from R1 and R2										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
173	7.00	-	100.0	0	53.0	12.60	2203	1.24	0.19	1.3
176	7.00	-	100.8	0	53.0	14.00	2811	1.15	0.16	1.3
179	7.00	-	60.4	0	69.6	14.00	2501	1.24	0.24	0.8
180	7.00	-	60.0	0	60.0	10.00	1779	1.29	0.21	0.8
181	7.00	-	90.0	0	68.2	10.00	1936	1.21	0.21	1.1
182	7.00	-	75.2	0	65.6	10.00	1710	1.43	0.25	0.9
183	7.00	-	29.6	0	40.6	9.00	1845	1.21	0.12	0.4
189	7.00	-	127.2	0	35.5	10.00	1900	1.19	0.12	1.6
190	7.00	-	130.0	0	34.2	10.00	1808	1.26	0.12	1.6

Appendix 2 - Preliminary studies on FA in anammox reactor R1

Immediate nitrogen removal rate (NRR) response to FA in reactor R1 (Test 1)

First FA level								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	212.0	27.6	46.6	2.5	7			
5	207.0	17.1	49.4	2.5	7.01			
10	199.0	9.2	49.4	1.7	6.86			
			Average:	2.3		4118	1.41	0.21
			Std. Dev.:	0.5				
0	191.5	27.1	51.4	2.0	6.95			
5	181.0	18.0	53.1	2.1	6.98			
10	175.0	9.5	54.5	2.1	7.01			
			Average:	2.1		4478	1.07	0.19
			Std.Dev.:	0.1				
0	140.0	28.1	58.3	1.7	7.00			
5	132.0	18.2	60.4	1.6	7.01			
10	123.0	9.8	62.8	1.5	7.01			
			Average:	1.6		4442	1.08	0.27
			Std.Dev.:	0.1				

Table continuation - First FA level - Immediate NRR response to FA in reactor R1								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	109.1	28.6	62.0	1.2	6.96			
5	99.6	18.8	64.6	1.2	7.01			
10	93.0	10.0	66.1	1.1	7.01			
			Average:	1.2		4414	1.16	0.26
			Std.Dev.:	0.0				
Second FA level								
0	81.6	27.5	67.3	1.0	7.00			
5	75.0	17.9	68.5	0.9	7.00			
10	67.2	9.4	70.1	0.8	6.99			
			Average:	0.9		4284	1.26	0.20
			Std.Dev.:	0.1				
Third FA level								
0	55.7	28.5	70.5	0.7	7.01			
5	48.2	18.4	72.8	0.6	7.01			
10	41.3	9.5	74.5	0.5	7.00			
			Average:	0.6		4233	1.32	0.28
			Std.Dev.:	0.1				
Fourth FA level								
0	29.7	28.1	75.1	0.3	6.97			
5	22.9	17.9	78.1	0.3	7.02			
10	15.8	9.2	79.6	0.2	7.02			
			Average:	0.3		4070	1.37	0.33
			Std.Dev.:	0.1				

Table continuation – Fifth FA level - Immediate NRR response to FA in reactor R1								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	160.0	30.9	55.1	5.9	7.50			
5	161.0	23.9	58.4	6.1	7.51			
10	151.0	15.3	58.5	5.7	7.51			
15	146.0	8.3	58.7	5.5	7.51			
20	140.0	3.9	59.8	5.2	7.50			
			Average:	5.7		3310	1.27	0.18
			Std.Dev.:	0.4				
Sixth FA level								
0	71.2	30.4	21.8	6.7	7.96			
5	66.6	23.3	23.5	6.4	7.97			
10	59.6	16.6	24.2	5.8	7.98			
15	53.8	13.0	25.3	5.5	8.00			
20	49.1	5.6	25.5	5.1	8.01			
			Average:	5.9		3108	1.05	0.16
			Std.Dev.:	0.6				
Seventh FA level								
0	70.8	30.2	44.8	6.8	7.97			
5	63.6	22.9	45.5	6.2	7.98			
10	63.6	15.5	46.9	6.5	8.00			
15	54.8	8.6	48.1	5.5	7.99			
			Average:	6.2		3134	1.51	0.24
			Std.Dev.:	0.6				

Table continuation – Eights FA level - Immediate NRR response to FA in reactor R1								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	106.8	33.2	38.3	11.5	8			
5	106.8	27.8	40.0	12.0	8.02			
10	101.4	23.8	40.2	10.5	7.98			
15	99.0	19.6	40.9	10.5	7.99			
20	89.4	13.6	42.1	9.9	8.01			
25	85.8	11.0	43.4	8.9	7.98			
30	83.4	6.1	43.8	9.0	8.00			
			Average:	10.3		2306	1.01	0.20
			Std.Dev.:	1.2				
Ninth FA level								
0	127.2	42.8	56.2	13.0	8.00			
5	126.4	38.5	56.9	13.4	8.02			
10	120.6	33.8	58.0	12.3	8.00			
15	141.0	29.4	58.8	14.1	7.99			
20	112.8	25.1	58.3	11.3	7.99			
25	110.4	20.1	59.1	11.2	8.00			
30	109.8	16.2	60.6	11.4	8.01			
			Average:	12.5		2049.6	1.37	0.20
			Std.Dev.:	1.1				

Table continuation - Tenth FA level - Immediate NRR response to FA in reactor R1								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	394.2	41.9	55.3	14.6	7.50			
5	393.3	34.7	57.1	14.8	7.51			
10	390.1	28.8	56.7	14.7	7.51			
15	388.8	22.0	58.0	14.7	7.51			
20	387.0	16.3	59.1	14.3	7.50			
25	385.2	12.2	58.5	14.5	7.51			
			Average:	14.6		2076	3.22	0.36
			Std.Dev.:	0.2				

Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 0.8 mg N/L in reactor R1 (constant pH set at 6.5) operated in the variable continuous feed mode during the test (Test 2)

Test 2 – Nitrogen loading 1								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	150.3	20.8	64.5	0.5	6.5	-	-	-
10	155.7	33.0	60.5	0.6	6.5	-	-	-
20	162.9	39.2	58.5	0.6	6.5	-	-	-
30	165.6	43.3	56.9	0.6	6.5	-	-	-
45	169.2	45.5	54.4	0.6	6.5	-	-	-
60	169.2	46.1	56.8	0.6	6.5	-	-	-
75	174.6	46.4	56.4	0.6	6.5	-	-	-
90	171.9	45.8	56.1	0.6	6.5	6918	1.33	0.26
120	173.7	45.9	56.0	0.6	6.5	6890	1.34	0.26
			Average	0.6	6.5	6904	1.33	0.26
			SD	0.0	0	20	0.01	0.00
Nitrogen concentrations and pH in the feed during the test								
-	387.0	331.2	0	-	6.8			

Table continuation - Test 2 – Nitrogen loading 2								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	164.7	46.2	52.9	0.6	6.5	-	-	-
10	174.6	56.0	51.4	0.6	6.5	-	-	-
20	177.3	61.3	51.5	0.6	6.5	-	-	-
40	187.2	68.8	46.8	0.7	6.5	-	-	-
70	188.1	71.2	48.2	0.7	6.5	8146	1.27	0.24
130	190.8	70.7	48.8	0.7	6.5	8090	1.29	0.24
			Average	0.7	6.5	8118	1.28	0.24
			SD	0.0	0.0	40	0.01	0.00
Nitrogen concentrations and pH in the feed during the test								
-	390.6	327.6	0	-	6.8			
Test 2 - Nitrogen loading 3								
0	193.5	67.9	51.1	0.7	6.5	-	-	-
10	201.6	80.5	46.6	0.7	6.5	-	-	-
20	199.8	85.8	45.8	0.7	6.5	-	-	-
50	216.0	100.8	45.0	0.8	6.5	-	-	-
80	216.0	108.9	41.7	0.8	6.5	-	-	-
110	217.8	112.5	41.7	0.8	6.5	-	-	-
140	223.2	117.0	40.6	0.8	6.5	8941	1.26	0.24
170	222.3	117.9	40.6	0.8	6.5	8941	1.25	0.24
			Average	0.80	6.5	8941	1.25	0.24
			SD	0.00	0.0	0	0.01	0.00
Nitrogen concentrations and pH in the feed during the test								
-	390.6	327.6	0	-	6.8			

Nitrogen removal rate (NRR) response to free ammonia (FA) concentrations up to 11.9 mg N/L in reactor R1 (self maintaining pH in the range of 6.9 and 8.2) operated in the constant continuous feed mode during the test (Test 3)

Test 3								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[h]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	38.3	10.1	52.7	0.3	6.90	3281	1.23	0.20
0.25	37.3	10.5	52.7	0.5	7.07	3286	1.22	0.20
0.5	37.2	10.9	51.9	0.6	7.19	3289	1.22	0.20
0.75	37.4	11.5	52.1	0.8	7.30	3282	1.22	0.20
1	36.6	12.0	52.8	1.0	7.39	3279	1.21	0.20
1.25	36.1	12.7	52.9	1.1	7.46	3278	1.20	0.20
1.75	37.6	13.2	53.2	1.7	7.61	3262	1.21	0.20
2	38.6	13.4	52.6	1.9	7.65	3258	1.21	0.20
2.25	39.1	14.1	53.1	2.0	7.68	3247	1.21	0.20
2.5	40.4	14.2	53.4	2.4	7.74	3236	1.22	0.21
2.75	41.6	14.4	52.8	2.7	7.78	3230	1.22	0.20
3	40.4	15.0	53.0	2.7	7.80	3233	1.22	0.20
3.25	41.6	15.4	52.6	3.0	7.83	3225	1.22	0.20
3.5	42.8	15.8	52.6	3.2	7.86	3215	1.23	0.20
3.75	43.6	16.4	52.0	3.5	7.88	3209	1.23	0.20

Test 3 – Table continuation								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[h]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
4.5	46.0	18.4	52.4	4.0	7.93	3177	1.23	0.21
4.75	47.2	18.2	52.2	4.3	7.95	3172	1.24	0.21
5	49.6	18.7	52.9	4.6	7.96	3148	1.25	0.21
5.25	48.4	18.9	53.5	4.6	7.97	3151	1.24	0.21
5.5	49.6	18.7	51.7	4.9	7.99	3156	1.25	0.21
5.75	51.2	19.4	55.8	5.3	8.01	3114	1.25	0.23
6	52.0	19.8	55.4	5.4	8.01	3108	1.26	0.22
22	70.4	31.8	46.2	10.7	8.20	2944	1.38	0.20
26.5	79.6	34.4	45.2	11.9	8.19	2872	1.44	0.20
26.75	76.0	33.0	45.0	6.6	7.92	2907	1.42	0.20
27	65.2	28.0	45.6	3.1	7.64	3008	1.37	0.19
27.25	52.4	22.3	48.1	1.1	7.28	3114	1.32	0.19
27.5	44.0	18.7	47.3	0.5	6.97	3199	1.28	0.18
27.75	40.8	17.3	48.3	0.4	6.90	3222	1.27	0.18
28	39.3	16.7	47.3	0.4	6.90	3243	1.27	0.18
28.5	38.0	16.1	47.1	0.3	6.90	3257	1.26	0.18
29	36.6	15.5	48.1	0.3	6.90	3264	1.26	0.18
						Average	1.26	0.20
						Std. Dev.	0.06	0.01
Nitrogen concentrations and pH in the feed during the test (0 – 6 hours)								
	282.6	309.6	3.6	-	6.80			
Nitrogen concentrations and pH in the feed during the test (22 – 29 hours)								
	273.6	313.2	5.4	-	6.80			

Appendix 3 - Low pH and low FA versus high pH and high FA – long term anammox reactors operation (Test 4)

Reactor R1 (low pH and low FA)

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0	6.50	62	67.0	9.8	59.0	11.8	2418	1.21	0.19	0.2
1	6.50	-	54.0	8.4	62.4	11.8	2345	1.27	0.22	0.2
2	6.50	-	84.4	10.0	67.2	11.8	2437	1.22	0.22	0.3
6	6.50	-	83.2	9.0	60.2	11.9	2473	1.17	0.19	0.3
7	7.30	-	86.0	10.6	58.2	11.9	2499	1.21	0.19	1.9
8	6.50	-	84.4	8.4	58.8	11.9	2440	1.00	0.07	0.3
9	6.50	72	76.0	8.7	59.3	11.9	2449	1.04	0.11	0.3
12	6.50	-	73.6	9.4	54.6	11.9	2453	1.32	0.19	0.3
13	6.50	-	74.0	8.8	50.0	11.0	2446	1.20	0.12	0.3
14	6.50	95	50.4	9.0	55.4	11.0	2575	1.22	0.16	0.2
15	6.50	-	40.8	10.5	56.7	11.0	2640	1.18	0.16	0.1
16	6.50	-	44.0	8.7	55.3	11.0	2468	1.23	0.17	0.2
19	6.50	-	45.6	9.6	52.4	11.0	2415	1.21	0.16	0.2
34	6.50	-	17.8	3.9	56.1	4.5	1031	1.20	0.17	0.1
35	6.50	-	25.0	4.1	54.3	4.5	1074	1.09	0.15	0.1
36	6.50	-	26.4	3.8	55.0	4.5	1047	1.08	0.15	0.1
39	6.50	-	26.3	3.8	53.8	4.5	989	1.22	0.17	0.1
40	6.50	-	46.4	3.5	50.3	5.9	1237	1.17	0.16	0.2
41	6.50	-	47.2	3.4	49.0	5.9	1390	1.19	0.14	0.2
42	6.50	-	39.6	3.5	45.8	5.9	1259	1.18	0.15	0.1

Table continuation – Test 4 – R1 (low pH and low FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
43	6.50	-	42.0	3.4	46.2	5.9	1243	1.21	0.15	0.2
46	6.50	-	40.4	3.2	49.6	5.9	1250	1.19	0.16	0.1
47	6.50	-	51.6	3.1	47.4	5.9	1176	1.18	0.16	0.2
48	6.50	92	61.6	3.0	49.2	5.9	1199	1.09	0.16	0.2
49	6.50	-	64.0	3.0	43.4	5.9	1217	1.12	0.14	0.2
50	6.50	-	67.2	3.0	42.2	4.6	940	1.16	0.14	0.2
53	6.50	-	31.6	2.8	48.0	4.6	922	1.19	0.16	0.1
54	6.50	-	42.4	2.7	46.5	5.6	1183	1.11	0.14	0.2
55	6.50	119	57.6	6.7	51.7	11.1	2247	1.19	0.17	0.2
56	6.50	-	53.2	7.5	56.5	15.3	3127	1.15	0.17	0.2
57	6.50	-	64.4	13.3	53.9	19.8	3955	1.15	0.14	0.2
60	6.50	-	51.2	15.5	29.0	20.6	4225	1.15	0.10	0.2
61	6.50	-	64.4	13.0	61.8	20.2	3826	1.23	0.22	0.2
62	6.50	-	90.4	18.8	58.8	24.5	4640	1.06	0.17	0.3
63	6.50	-	106.4	19.1	57.4	24.5	4771	1.26	0.16	0.4
64	6.50	-	107.2	19.6	48.2	23.9	4511	1.35	0.15	0.4
67	6.50	-	78.8	26.7	48.1	22.7	4408	1.20	0.17	0.3
68	6.50	-	78.8	18.4	47.6	22.7	4407	1.28	0.17	0.3
69	2.00	-	100.8	18.6	50.6	0.0	0	1.16	0.10	0.0
70	7.00	-	252.0	3.4	65.4	3.6	565	2.43	0.42	2.8
73	7.00	-	49.2	15.9	36.5	5.7	1199	1.25	0.12	0.6
74	7.00	-	33.7	17.8	47.0	7.2	1427	1.14	0.14	0.4
75	7.00	-	39.2	18.4	47.6	8.4	1590	1.19	0.17	0.4

Table continuation – Test 4 – R1 (low pH and low FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
76	7.00	-	48.8	20.7	46.9	9.6	1713	1.35	0.19	0.5
77	7.00	-	77.6	21.2	50.0	10.8	2037	1.56	0.21	0.9
78	7.00	-	134.8	24.4	54.0	11.7	2328	1.08	0.17	1.5
79	7.00	-	148.0	24.1	53.1	12.0	2354	1.07	0.17	1.7
82	6.50	-	14.2	44.0	47.2	13.0	2148	1.37	0.21	0.1
83	6.50	-	96.8	19.5	50.5	14.1	2515	1.30	0.20	0.3
84	6.50	-	109.6	24.6	52.6	17.8	3484	1.08	0.17	0.4
85	6.50	-	113.2	23.8	54.6	17.7	3224	1.09	0.18	0.4
86	6.50	-	116.0	22.5	51.9	18.7	3447	1.08	0.18	0.4
87	6.50	-	96.8	24.9	55.1	19.8	3906	1.11	0.18	0.3
88	6.50	-	78.0	25.4	57.8	21.6	4023	1.21	0.21	0.3
91	6.50	-	94.8	23.8	57.0	23.0	4326	1.21	0.20	0.3
92	6.50	-	100.8	23.5	56.9	25.3	4954	1.07	0.18	0.4
93	6.50	-	102.4	25.6	58.0	27.1	5068	1.17	0.20	0.4
94	6.50	-	91.6	21.9	55.7	27.1	4845	1.24	0.21	0.3
95	6.50	-	112.0	26.6	52.6	29.7	5502	1.18	0.19	0.4
96	6.50	-	114.4	29.6	50.8	32.3	5907	1.21	0.19	0.4
97	6.50	-	92.4	33.2	48.0	33.8	5872	1.26	0.19	0.3
98	6.50	-	84.4	31.4	53.0	33.1	6062	1.29	0.20	0.3
101	6.50	-	109.2	38.0	50.8	35.3	6672	1.15	0.18	0.4
102	6.50	-	111.2	34.6	53.5	35.2	6764	1.31	0.20	0.4
103	6.50	-	113.2	31.6	54.9	34.7	6185	1.23	0.21	0.4
104	6.50	-	148.8	35.0	50.9	35.3	6346	1.10	0.18	0.5

Table continuation – Test 4 – R1 (low pH and low FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
105	6.50	-	144.8	33.7	51.4	35.3	6172	1.21	0.20	0.5
108	6.50	-	126.8	31.2	52.0	35.0	5950	1.25	0.21	0.5
109	6.50	-	130.4	36.2	52.2	35.4	5850	1.23	0.21	0.5
112	6.50	-	130.4	34.8	53.5	34.6	6052	1.21	0.20	0.5
113	6.50	-	124.4	34.2	54.1	33.7	5962	1.24	0.21	0.4
116	6.50	-	154.8	30.5	53.7	32.3	5683	1.18	0.20	0.6
117	6.50	-	157.2	36.0	50.1	33.5	5886	1.13	0.18	0.6
118	6.50	-	149.6	30.6	50.3	32.4	5481	1.28	0.21	0.5
119	6.50	-	115.6	44.0	50.8	38.2	6672	1.18	0.19	0.4
120	6.50	-	101.6	30.9	54.5	34.6	6251	1.26	0.21	0.4
123	6.50	-	86.0	32.1	55.2	35.6	6039	1.30	0.23	0.3
124	6.50	-	92.8	32.6	56.2	37.4	6533	1.25	0.22	0.3
125	6.50	-	133.2	35.5	55.5	37.5	6310	1.21	0.22	0.5
126	6.50	-	181.8	51.2	52.3	36.7	5648	1.28	0.23	0.6
131	6.50	-	110.8	26.4	51.2	35.7	5893	1.23	0.21	0.4
132	6.50	-	121.8	38.1	63.7	36.0	6601	1.20	0.17	0.4
133	6.50	-	103.6	45.0	63.8	35.6	6174	1.26	0.20	0.4
134	6.50	-	143.5	48.2	62.6	35.3	6786	1.08	0.17	0.5
137	6.50	-	91.7	42.0	58.6	35.4	6588	1.27	0.20	0.3
138	6.50	-	101.5	38.2	61.1	35.2	6303	1.25	0.23	0.4
147	6.50	-	131.6	24.9	57.7	33.4	6090	1.27	0.22	0.5
151	6.50	-	152.6	26.5	64.1	37.4	6797	1.27	0.24	0.5
152	6.50	-	142.8	24.7	60.0	37.1	6447	1.23	0.23	0.5

Table continuation – Test 4 – R1 (low pH and low FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
153	6.50	-	141.4	22.6	45.1	36.9	6572	1.18	0.17	0.5
158	6.50	-	149.8	24.9	50.2	35.3	6249	1.25	0.19	0.5
159	6.50	-	158.9	25.7	52.0	35.0	6333	1.22	0.19	0.6
160	6.50	-	163.1	25.9	57.3	35.0	6178	1.27	0.22	0.6
166	6.50	-	137.2	31.0	59.0	38.0	6425	1.30	0.24	0.5
167	6.50	-	139.3	36.6	54.2	38.0	6753	1.18	0.20	0.5
168	6.50	-	85.4	33.6	55.0	38.0	6582	1.19	0.21	0.3

Reactor R2 (high pH and high FA)

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0	7.90	123	41.7	5.6	52.4	7.3	1584	1.13	0.15	3.5
1	7.85	-	37.5	5.1	57.7	7.3	1510	1.21	0.19	2.8
2	7.64	-	48.8	5.5	54.9	7.3	1635	1.10	0.16	2.3
6	8.00	-	77.6	11.9	57.3	11.3	2369	1.14	0.17	7.9
7	8.00	-	79.0	12.9	57.9	11.3	2392	1.17	0.18	8.1
8	7.98	-	73.6	10.0	57.2	11.0	2295	0.96	0.06	7.2
9	7.84	140	63.6	9.1	56.5	11.0	2318	1.00	0.09	4.6
12	7.85	-	56.8	9.2	50.4	11.0	2345	1.25	0.16	4.2
13	7.60	-	51.6	8.6	46.0	10.6	2451	1.13	0.10	2.2
14	7.82	148	51.6	8.8	53.1	10.6	2486	1.22	0.16	3.6
15	8.08	-	36.8	14.8	50.1	10.6	2566	1.15	0.14	4.4
16	8.06	-	27.5	10.6	51.6	10.6	2443	1.16	0.15	3.2
19	7.96	-	26.9	14.0	50.2	10.6	2385	1.13	0.15	2.5
34	7.50	-	9.1	3.5	45.3	4.3	1014	1.17	0.13	0.3
35	7.48	-	11.8	3.2	48.0	4.3	1055	1.05	0.13	0.4
36	7.51	-	10.3	2.5	47.9	4.3	1035	1.03	0.13	0.4
39	7.56	-	14.0	3.2	42.4	4.3	980	1.18	0.13	0.6
40	7.47	-	22.6	2.5	39.5	5.2	1152	1.09	0.12	0.7
41	7.49	-	27.8	3.5	46.9	5.2	1262	1.13	0.13	0.9
42	7.36	-	22.8	2.5	39.1	5.2	1152	1.12	0.12	0.6
43	7.37	-	21.8	2.6	37.4	5.2	1147	1.14	0.11	0.6
46	7.51	-	24.6	3.8	39.8	5.2	1145	1.13	0.12	0.9

Table continuation – Test 4 – R2 (high pH and high FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
47	7.47	-	27.4	2.8	38.4	5.2	1095	1.09	0.12	0.9
48	7.46	173	42.0	4.2	40.2	5.2	1104	1.03	0.12	1.3
49	7.40	-	52.8	3.4	43.0	5.2	1092	1.08	0.13	1.5
50	7.44	-	54.4	3.8	37.4	5.2	1092	1.11	0.12	1.7
53	7.51	-	20.4	3.1	40.1	4.8	993	1.15	0.13	0.7
54	7.30	-	29.0	2.3	46.9	4.8	1036	1.06	0.14	0.6
55	7.61	195	47.2	5.2	49.2	9.4	1948	1.16	0.16	2.1
56	7.66	-	31.8	6.4	50.4	10.6	2268	1.08	0.14	1.6
57	7.85	-	49.2	10.8	46.0	14.4	2999	1.10	0.11	3.7
60	7.50	-	34.4	14.2	27.4	14.9	3154	1.09	0.09	1.2
61	7.93	-	49.2	12.9	53.5	14.9	2939	1.16	0.18	4.3
62	8.00	-	86.4	22.8	51.2	18.8	3608	1.03	0.14	8.8
63	8.00	-	98.0	23.5	47.9	18.8	3746	1.20	0.12	10.0
64	8.01	-	113.2	41.6	38.1	19.0	3473	1.29	0.12	11.8
67	7.80	-	39.5	6.2	34.6	10.0	2186	1.12	0.11	2.6
68	7.88	-	48.8	8.0	35.6	10.0	2116	1.19	0.12	3.9
69	7.98	-	73.6	18.8	50.4	14.4	3012	1.06	0.09	7.2
70	7.98	-	75.2	16.2	54.6	14.4	3101	1.10	0.16	7.4
73	8.19	-	31.8	26.6	41.8	13.4	2825	1.15	0.13	4.8
74	8.22	-	37.6	31.6	38.0	12.9	2519	1.11	0.11	6.0
75	8.25	-	44.8	34.1	35.2	12.0	2236	1.16	0.13	7.5
76	8.12	-	35.2	25.6	33.3	11.5	2137	1.26	0.13	4.6
77	8.12	-	83.6	44.0	39.8	13.0	2371	1.50	0.17	10.9

Table continuation – Test 4 – R1 (low pH and low FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
82	7.94	-	7.8	48.0	22.8	7.7	1341	1.32	0.10	0.7
83	7.90	-	66.4	29.8	22.2	11.0	2140	1.13	0.08	5.5
84	7.84	-	98.0	33.6	30.0	10.4	2123	1.01	0.09	7.1
85	7.89	-	102.4	31.9	30.1	10.2	1950	1.03	0.10	8.3
86	7.93	-	104.0	29.5	31.5	10.1	1947	1.01	0.10	9.2
87	8.00	-	87.6	33.9	33.7	10.1	2065	1.05	0.11	8.9
88	8.00	-	52.0	23.1	37.7	10.1	2044	1.12	0.12	5.3
91	7.91	-	68.4	13.9	38.9	9.0	1856	1.14	0.13	5.8
92	7.91	-	76.0	19.2	43.8	12.2	2560	1.00	0.13	6.4
93	7.96	-	72.4	15.4	39.4	11.5	2376	1.10	0.13	6.8
94	8.00	-	62.4	13.1	41.7	10.8	2118	1.14	0.14	6.4
95	7.92	-	72.4	18.3	41.3	12.5	2563	1.06	0.13	6.2
96	7.90	-	116.8	72.8	26.0	13.9	2445	1.06	0.10	9.7
97	7.71	-	57.6	11.7	34.7	12.1	2383	1.19	0.12	3.2
98	7.92	-	66.4	21.6	44.0	14.9	2911	1.24	0.16	5.7
101	7.76	-	79.6	15.4	36.2	12.2	2577	1.12	0.11	4.9
102	7.76	-	84.4	16.9	51.1	14.8	3075	1.26	0.17	5.2

Appendix 4 Anammox response to nitrite

a) Immediate anammox response to nitrite (Test 5)

Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R1 (Test 5a - R1)

	Nitrate range							
Test A	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
Start / End N-NH4 [mg N/L]	154.1/140.7	-/137.9	-/134.4	-/137.2	-/135.8	-/139.7	-/142.7	-/150.6
Start / End N-NO2 [mg N/L]	51.3/33.7	-/45.6	-/67.7	-/91.0	-/137.9	-/192.6	-/304.2	-/408.0
Start / End N-NO3 [mg N/L]	0/2.8	-/3.1	-/3.5	-/3.7	-/3.5	-/1.8	-/-	-/3.0
pH start	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95
pH end	7.1	7.09	7.09	7.09	7.08	7.08	7.06	7.05
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell A-1	Cell A-2	Cell A-3	Cell A-4	Cell A-5	Cell A-6	Cell A-7	Cell A-8
0.00	0	0	0	0	0	0	0	0
0.17	0.88	0.91	0.86	0.27	0.86	0.93	0.85	0.94
0.33	1.5	1.5	1.49	0.32	1.4	1.56	1.43	1.61
0.50	2.08	2.09	2.04	0.36	1.9	2.09	1.92	2.23
0.67	2.56	2.55	2.54	0.36	2.35	2.58	2.37	2.77
0.83	3.01	2.96	2.94	0.36	2.71	3.03	2.77	3.21
1.00	***3.32***	***3.32***	***3.31***	***0.36***	***3.03***	***3.43***	***3.13***	***3.61***
1.17	***3.62***	***3.64***	***3.67***	***0.36***	***3.34***	***3.83***	***3.44***	***3.97***
1.33	***3.93***	***3.96***	***3.99***	***0.36***	***3.62***	***4.14***	***3.75***	***4.33***
1.50	***4.29***	***4.32***	***4.35***	***0.36***	***3.93***	***4.54***	***4.11***	***4.73***

Table continuation: Test A								
Time [hours]	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
1.67	***4.51***	***4.55***	***4.62***	***0.36***	***4.16***	***4.81***	***4.34***	***5.00***
1.83	***4.82***	***4.87***	***4.94***	***0.36***	***4.43***	***5.16***	***4.65***	***5.35***
2.00	***5.08***	***5.19***	***5.25***	***0.36***	***4.7***	***5.47***	***4.96***	***5.71***
2.17	5.3	7.69	7.79	2.35	7.19	7.92	7.51	8.25
2.33	5.7	8.1	8.34	2.49	7.73	8.46	8.05	8.74
2.50	5.92	8.37	8.74	2.53	8.14	8.9	8.4	9.05
2.67	6.23	8.74	9.24	2.62	8.59	9.39	8.81	9.37
2.83	6.5	9.01	9.69	2.71	9.04	9.83	9.12	9.63
3.00	6.76	9.37	10.19	2.85	9.49	10.32	9.52	9.95
3.17	7.03	9.69	10.65	2.98	9.94	10.81	9.83	10.21
3.33	7.29	10.01	11.1	3.07	10.4	11.3	10.19	10.48
3.50	7.56	10.33	11.55	3.21	10.85	11.79	10.55	10.75
3.67	7.82	10.65	12.05	3.34	11.35	12.28	10.86	11.06
3.83	8.09	10.97	12.5	3.48	11.8	12.77	11.22	11.33
4.00	8.35	11.24	12.96	3.57	12.25	13.26	11.58	11.55
4.17	8.57	11.56	13.41	3.71	12.7	13.79	11.89	11.82
4.33	8.84	11.83	13.82	3.8	13.15	14.28	12.2	12.04
4.50	***9.11***	***12.15***	***14.27***	***3.93***	***13.61***	***14.82***	***12.56***	***12.35***
4.67	***9.37***	***12.47***	***14.72***	***4.07***	***14.06***	***15.31***	***12.87***	***12.62***
4.83	***9.64***	***12.79***	***15.22***	***4.2***	***14.55***	***15.84***	***13.23***	***12.89***
5.00	***9.9***	***13.1***	***15.67***	***4.34***	***15.01***	***16.38***	***13.59***	***13.16***
5.17	***10.17***	***13.42***	***16.13***	***4.52***	***15.5***	***16.91***	***13.95***	***13.47***
5.33	***10.39***	***13.7***	***16.58***	***4.61***	***15.91***	***17.44***	***14.26***	***13.69***
5.50	***10.65***	***14.01***	***17.03***	***4.75***	***16.41***	***17.98***	***14.62***	***14.00***

Numbers in bold and italic format were used for GPR calculation

Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R1 (Test 5a – R1)

	Nitrate range							
Test B	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
Start / End N-NH4 [mg N/L]	157.6/143.5	-/138.6	-/138.6	-/134.4	-/133.7	-/141.3	-/144.5	-/153.3
Start / End N-NO2 [mg N/L]	51.2/33.2	-/46.1	-/64.1	-/88.9	-/137.2	-/192.6	-/302.4	-/423
Start / End N-NO3 [mg N/L]	0/2.9	-/3.7	-/3.9	-/3.5	-/3.5	-/3.6	-/-	-/-
pH start	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95
pH end	7.11	7.12	7.12	7.12	7.11	7.1	7.07	7.07
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell A-1	Cell A-2	Cell A-3	Cell A-4	Cell A-5	Cell A-6	Cell A-7	Cell A-8
0.00	0	0	0	0	0	0	0	0
0.17	0.75	0.77	0.77	0.77	0.72	0.71	0.72	0.76
0.33	1.28	1.32	1.31	1.31	1.22	1.29	1.25	1.34
0.50	1.86	1.87	1.9	1.9	1.76	1.87	1.79	1.96
0.67	2.39	2.32	2.36	2.4	2.26	2.4	2.28	2.45
0.83	2.83	2.73	2.81	2.85	2.67	2.85	2.68	2.94
1.00	***3.09***	***3.05***	***3.13***	***3.16***	***2.94***	***3.16***	***2.99***	***3.26***
1.17	***3.4***	***3.32***	***3.44***	***3.48***	***3.25***	***3.47***	***3.26***	***3.52***
1.33	***3.71***	***3.64***	***3.76***	***3.8***	***3.53***	***3.78***	***3.53***	***3.84***
1.50	***4.15***	***4***	***4.17***	***4.2***	***3.93***	***4.18***	***3.89***	***4.19***
1.67	***4.33***	***4.23***	***4.39***	***4.47***	***4.11***	***4.41***	***4.11***	***4.46***
1.83	***4.64***	***4.55***	***4.71***	***4.79***	***4.43***	***4.72***	***4.38***	***4.73***
2.00	***4.99***	***4.87***	***5.03***	***5.11***	***4.7***	***5.03***	***4.65***	***5.04***
2.17	5.22	7.6	7.52	7.59	7.19	7.39	6.97	7.58
2.33	5.66	8.05	8.06	8.14	7.73	7.92	7.51	8.12

Table continuation: Test B								
Time [hours]	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
2.50	5.92	8.33	8.43	8.54	8.14	8.37	7.87	8.43
2.67	6.23	8.64	8.83	9.04	8.63	8.81	8.22	8.79
2.83	6.54	8.96	9.24	9.49	9.09	9.26	8.63	9.05
3.00	6.85	9.33	9.65	9.94	9.58	9.75	8.98	9.37
3.17	7.16	9.65	10.06	10.44	10.08	10.19	9.34	9.41
3.33	7.43	9.96	10.42	10.85	10.53	10.64	9.66	9.41
3.50	7.74	10.28	10.83	11.35	11.03	11.08	10.01	9.99
3.67	8	10.6	11.23	11.8	11.53	11.57	10.37	10.3
3.83	8.35	10.97	11.64	12.29	12.02	12.06	10.73	10.57
4.00	8.62	11.24	12.05	12.7	12.48	12.55	11.04	10.84
4.17	8.88	11.56	12.41	13.15	12.97	12.99	11.4	11.11
4.33	9.11	11.88	12.77	13.61	13.42	13.48	11.71	11.33
4.50	***9.41***	***12.19***	***13.18***	***14.06***	***13.92***	***13.97***	***12.07***	***11.64***
4.67	***9.72***	***12.51***	***13.59***	***14.51***	***14.42***	***14.51***	***12.43***	***11.91***
4.83	***10.03***	***12.92***	***14***	***15.01***	***14.92***	***15***	***12.78***	***12.22***
5.00	***10.3***	***13.24***	***14.41***	***15.46***	***15.41***	***15.53***	***13.14***	***12.49***
5.17	***10.61***	***13.56***	***14.77***	***15.87***	***15.91***	***16.02***	***13.45***	***12.76***
5.33	***10.83***	***13.83***	***15.13***	***16.32***	***16.36***	***16.46***	***13.81***	***13.02***
5.50	***11.14***	***14.15***	***15.54***	***16.77***	***16.86***	***17***	***14.17***	***13.29***

Numbers in bold and italic format were used for GPR calculation

Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R2 (Test 5a – R2)

	Nitrate range							
Test A	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
Start / End N-NH4 [mg N/L]	143/133	-/132.3	-/134.4	-/129.5	-/133.7	-/134.8	-/135	-/134.1
Start / End N-NO2 [mg N/L]	51/38.9	-/53.9	-/74.9	-/97.3	-/147	-/212.4	-/324	-/426
Start / End N-NO3 [mg N/L]	0/3.7	-/3.4	-/3.5	-/3.5	-/2.2	-/2.8	-/3.6	-/-
pH start	7.05	7.05	7.05	7.05	7.05	7.05	7.05	7.05
pH end	7.28	7.26	7.25	7.26	7.24	7.23	7.21	7.2
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell B-1	Cell B-2	Cell B-3	Cell B-4	Cell B-5	Cell B-6	Cell B-7	Cell B-8
0	0	0	0	0	0	0	0	0
0.166667	0.57	0.55	0.54	0.54	0.54	0.53	0.54	0.54
0.333333	0.93	0.91	0.86	0.9	0.86	0.89	0.85	0.85
0.5	1.28	1.27	1.18	1.27	1.18	1.2	1.16	1.16
0.666667	1.64	1.64	1.54	1.63	1.54	1.56	1.52	1.52
0.833333	1.86	1.82	1.72	1.85	1.72	1.74	1.7	1.69
1	***2.12***	***2.09***	***1.99***	***2.08***	***1.99***	***1.96***	***1.92***	***1.92***
1.16667	***2.39***	***2.37***	***2.22***	***2.35***	***2.21***	***2.18***	***2.15***	***2.19***
1.33333	***2.56***	***2.5***	***2.36***	***2.53***	***2.4***	***2.36***	***2.28***	***2.32***
1.5	***2.87***	***2.78***	***2.67***	***2.85***	***2.67***	***2.63***	***2.55***	***2.63***
1.66667	***3.09***	***3***	***2.9***	***3.12***	***2.94***	***2.85***	***2.77***	***2.85***
1.83333	***3.36***	***3.23***	***3.13***	***3.39***	***3.16***	***3.07***	***2.99***	***3.08***
2	***3.58***	***3.46***	***3.35***	***3.62***	***3.34***	***3.25***	***3.17***	***3.26***
2.16667	3.71	6.42	6.02	6.46	6.15	6.05	6.12	6.11
2.33333	3.98	6.64	6.25	6.78	6.42	6.27	6.39	6.38

Table continuation: Test A								
Time [hours]	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
2.5	4.11	6.78	6.39	6.92	6.6	6.41	6.57	6.56
2.66667	4.33	6.96	6.57	7.19	6.78	6.59	6.75	6.73
2.83333	4.51	7.1	6.75	7.37	6.96	6.76	6.93	6.87
3	4.69	7.28	6.93	7.59	7.14	6.94	7.11	7.05
3.16667	4.86	7.46	7.11	7.82	7.32	7.12	7.29	7.23
3.33333	4.99	7.6	7.25	8	7.46	7.21	7.42	7.31
3.5	5.17	7.78	7.43	***8.18***	7.64	7.39	7.55	7.45
3.66667	5.35	7.92	7.57	***8.36***	7.77	7.52	7.73	7.58
3.83333	5.48	8.01	7.7	***8.54***	7.86	7.61	7.82	7.67
4	5.66	8.19	7.88	***8.72***	8.05	7.79	8	7.8
4.16667	5.83	8.33	8.02	***8.9***	8.23	7.92	8.09	7.89
4.33333	6.06	8.51	8.24	***9.13***	8.41	8.1	8.27	8.03
4.5	***6.28***	***8.74***	***8.43***	***9.36***	***8.63***	***8.32***	***8.45***	***8.16***
4.66667	***6.41***	***8.92***	***8.61***	9.4	***8.81***	***8.46***	***8.63***	***8.3***
4.83333	***6.59***	***9.1***	***8.74***	9.4	***8.99***	***8.59***	***8.76***	***8.38***
5	***6.81***	***9.28***	***8.97***	9.45	***9.18***	***8.81***	***8.94***	***8.52***
5.16667	***6.94***	***9.42***	***9.11***	9.45	***9.36***	***8.94***	***9.03***	***8.61***
5.33333	***7.12***	***9.6***	***9.24***	9.49	***9.49***	***9.03***	***9.16***	***8.65***
5.5	***7.25***	***9.74***	***9.38***	10.17	***9.63***	***9.17***	***9.25***	***8.7***

Respiro. problem

Numbers in bold and italic format were used for GPR calculation

Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R2 (Test 5a – R2)

	Nitrate range							
Test B	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
Start / End N-NH4 [mg N/L]	142.4/131.6	-/129.5	-/129.5	-/131.6	-/133.7	-/133.2	-/132.8	-/129.9
Start / End N-NO2 [mg N/L]	53.1/40.7	-/52.1	-/72.8	-/98	-/154.7	-/207	-/318.6	-/417
Start / End N-NO3 [mg N/L]	0/3.3	-/2.7	-/2.8	-/2.8	-/1.8	-/-	-/1.8	-/-
pH start	7.05	7.05	7.05	7.05	7.05	7.05	7.05	7.05
pH end	7.21	7.2	7.21	7.21	7.22	7.19	7.18	7.17
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell B-1	Cell B-2	Cell B-3	Cell B-4	Cell B-5	Cell B-6	Cell B-7	Cell B-8
0	0	0	0	0	0	0	0	0
0.166667	0.57	0.59	0.59	0.54	0.54	0.49	0.58	0.58
0.333333	0.88	0.91	0.91	0.86	0.86	0.8	0.89	0.89
0.5	1.19	1.27	1.27	1.18	1.18	1.07	1.21	1.25
0.666667	1.5	1.68	1.63	1.54	1.54	1.42	1.56	1.61
0.833333	1.72	1.91	1.86	1.72	1.72	1.6	1.79	1.83
1	***1.94***	***2.18***	***2.13***	***1.99***	***1.94***	***1.82***	***2.01***	***2.05***
1.16667	***2.21***	***2.46***	***2.4***	***2.26***	***2.17***	***2.05***	***2.23***	***2.32***
1.33333	***2.34***	***2.64***	***2.58***	***2.4***	***2.31***	***2.18***	***2.41***	***2.5***
1.5	***2.65***	***2.96***	***2.9***	***2.71***	***2.62***	***2.45***	***2.73***	***2.81***
1.66667	***2.92***	***3.23***	***3.13***	***2.98***	***2.8***	***2.67***	***2.91***	***3.03***
1.83333	***3.18***	***3.5***	***3.4***	***3.21***	***3.03***	***2.89***	***3.17***	***3.3***
2	***3.36***	***3.73***	***3.62***	***3.44***	***3.21***	***3.07***	***3.35***	***3.48***
2.16667	3.49	6.55	6.43	6.15	5.79	5.56	6.03	6.02
2.33333	3.85	6.82	6.75	6.37	6.06	5.79	6.35	6.29

Table continuation: Test B								
Time [hours]	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
2.5	4.02	7.01	6.93	6.55	6.19	5.92	6.53	6.47
2.66667	4.2	7.14	7.11	6.73	6.33	6.05	6.7	6.65
2.83333	4.38	7.37	7.29	6.92	6.51	6.19	6.84	6.82
3	4.64	7.6	7.57	7.14	6.73	6.41	7.11	7.05
3.16667	4.82	7.78	7.79	7.32	6.87	6.54	7.24	7.23
3.33333	4.95	7.96	7.97	7.46	7.01	6.68	7.38	7.36
3.5	5.13	8.14	8.15	7.64	7.19	6.81	7.55	7.49
3.66667	5.35	8.33	8.38	7.82	7.32	6.94	7.69	7.63
3.83333	5.53	8.51	8.56	8	7.46	7.08	7.82	7.76
4	5.7	8.74	8.79	8.18	7.64	7.21	8	7.94
4.16667	5.88	8.92	8.97	8.36	7.77	7.34	8.14	8.07
4.33333	6.14	9.15	9.24	8.59	7.96	7.52	8.27	8.25
4.5	***6.41***	***9.42***	***9.56***	***8.81***	***8.18***	***7.74***	***8.54***	***8.47***
4.66667	***6.59***	***9.6***	***9.78***	***8.99***	***8.36***	***7.88***	***8.67***	***8.61***
4.83333	***6.81***	***9.83***	***10.01***	***9.22***	***8.5***	***8.01***	***8.81***	***8.74***
5	***7.07***	***10.06***	***10.28***	***9.45***	***8.72***	***8.23***	***8.98***	***8.88***
5.16667	***7.25***	***10.24***	***10.51***	***9.63***	***8.86***	***8.32***	***9.12***	***8.96***
5.33333	***7.38***	***10.42***	***10.69***	***9.76***	***8.99***	***8.46***	***9.21***	***9.05***
5.5	***7.56***	***10.6***	***10.92***	***9.9***	***9.09***	***8.54***	***9.3***	***9.1***

Numbers in bold and italic format were used for GPR calculation

Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R3 (Test 5a – R3)

	Nitrate range							
Test A	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
Start / End N-NH4 [mg N/L]	151.3/147.7	-/146.3	-/146.3	-/146.3	-/144.9	-/150.1	-/161.5	-/164.1
Start / End N-NO2 [mg N/L]	48.5/43.8	-/58.1	-/77	-/103.6	-/144.9	-/198	-/329.4	-/417
Start / End N-NO3 [mg N/L]	0/0.5	-/0.4	-/0.7	-/-	-/0.7	-/-	-/-	-/-
pH start	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
pH end	6.99	6.98	6.97	6.97	6.96	6.95	6.95	6.94
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell B-1	Cell B-2	Cell B-3	Cell B-4	Cell B-5	Cell B-6	Cell B-7	Cell B-8
0	0	0	0	0	0	0	0	0
0.166667	0.75	0.77	0.18	0.77	0.77	0.85	0.76	0.71
0.333333	1.33	1.37	0.27	1.36	1.36	1.47	1.34	1.29
0.5	1.72	1.77	0.27	1.81	1.76	1.91	1.74	1.69
0.666667	2.03	2.09	0.27	2.08	2.03	2.23	2.06	2.01
0.833333	2.3	2.37	0.27	2.35	2.31	2.54	2.32	2.27
1	2.52	2.55	0.27	2.58	2.53	2.76	2.5	2.5
1.16667	2.65	2.68	0.27	2.71	2.67	2.89	2.64	2.63
1.33333	***2.87***	***2.87***	0.27	***2.94***	***2.85***	***3.07***	***2.86***	***2.85***
1.5	***3.01***	***3***	0.27	***3.07***	***2.98***	***3.25***	***2.99***	***2.99***
1.66667	***3.09***	***3.09***	0.27	***3.16***	***3.07***	***3.34***	***3.08***	***3.08***
1.83333	***3.18***	***3.19***	0.27	***3.25***	***3.16***	***3.43***	***3.17***	***3.17***
2	***3.32***	***3.28***	0.27	***3.34***	***3.3***	***3.56***	***3.31***	***3.3***
2.16667	3.45	5.32	1.63	5.29	5.24	5.43	5.27	5.31
2.33333	3.54	5.37	1.63	5.38	5.33	5.52	5.41	5.44

Table continuation: Test A								
Time [hours]	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
2.5	3.62	5.41	1.63	5.42	5.42	5.61	5.5	5.53
2.66667	3.71	5.51	1.63	5.56	5.51	5.7	5.63	5.66
2.83333	3.8	5.55	1.63	5.65	5.6	5.83	5.77	5.75
3	3.93	5.69	1.63	5.74	5.74	5.96	5.9	5.89
3.16667	4.07	5.78	1.63	5.88	5.88	6.14	6.08	6.02
3.33333	4.15	5.91	1.63	6.01	6.01	6.27	6.21	6.11
3.5	4.24	5.96	1.63	6.06	6.1	6.36	6.3	6.15
3.66667	4.38	6.1	1.63	6.19	6.24	6.54	6.44	6.24
3.83333	4.46	6.14	1.63	6.28	6.37	6.63	6.53	6.29
4	4.6	6.28	1.63	6.42	6.51	6.81	6.66	6.42
4.16667	4.77	6.46	1.63	6.6	6.73	6.99	6.84	6.56
4.33333	4.86	6.55	1.63	6.69	6.83	7.12	6.93	6.6
4.5	***4.99***	***6.69***	1.63	***6.83***	***7.01***	***7.25***	***7.06***	***6.65***
4.66667	***5.08***	***6.78***	1.63	***6.92***	***7.1***	***7.43***	***7.15***	***6.73***
4.83333	***5.26***	***6.92***	1.63	***7.1***	***7.28***	***7.61***	***7.29***	***6.82***
5	***5.3***	***7.01***	1.63	***7.19***	***7.37***	***7.7***	***7.38***	***6.87***
5.16667	***5.39***	***7.1***	1.63	***7.28***	***7.46***	***7.79***	***7.42***	***6.87***
5.33333	***5.53***	***7.23***	1.63	***7.46***	***7.64***	***8.01***	***7.55***	***6.96***
5.5	***5.61***	***7.33***	1.63	***7.55***	***7.77***	***8.1***	***7.64***	***7***

Numbers in bold and italic format were used for GPR calculation

Immediate gas production rate (GPR) response to nitrite tested on the biomass originated from R3 (Test 5a – R3)

	Nitrate range							
Test B	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
Start / End N-NH4 [mg N/L]	154.2/147	-/145.6	-/144.9	-/144.9	-/144.2	-/159.5	-/161.1	-/157.2
Start / End N-NO2 [mg N/L]	54.3/44.5	-/58.6	-/77	-/102.2	-/144.2	-/214.2	-/315	-/414
Start / End N-NO3 [mg N/L]	0/0.4	-/0.41	-/-	-/-	-/-	-/-	-/-	-/-
pH start	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
pH end	6.99	6.99	6.98	6.98	7	6.96	6.96	6.94
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell B-1	Cell B-2	Cell B-3	Cell B-4	Cell B-5	Cell B-6	Cell B-7	Cell B-8
0	0	0	0	0	0	0	0	0
0.166667	0.66	0.73	0.72	0.72	0.68	0.71	0.72	0.71
0.333333	1.24	1.32	1.31	1.27	1.18	1.29	1.3	1.29
0.5	1.59	1.68	1.68	1.67	1.58	1.65	1.7	1.69
0.666667	1.86	2	1.99	1.99	1.9	1.96	2.01	2.01
0.833333	2.12	2.28	2.26	2.21	2.17	2.18	2.28	2.27
1	***2.34***	***2.5***	***2.49***	***2.44***	***2.4***	***2.4***	***2.5***	***2.5***
1.16667	***2.48***	***2.64***	***2.63***	***2.58***	***2.53***	***2.54***	***2.64***	***2.68***
1.33333	***2.65***	***2.82***	***2.85***	***2.76***	***2.76***	***2.71***	***2.82***	***2.85***
1.5	***2.78***	***2.96***	***2.99***	***2.89***	***2.89***	***2.85***	***2.99***	***2.99***
1.66667	***2.87***	***3.09***	***3.08***	***2.98***	***3.03***	***2.94***	***3.08***	***2.99***
1.83333	***2.96***	***3.19***	***3.17***	***3.07***	***3.12***	***3.03***	***3.17***	***2.99***
2	***3.09***	***3.32***	***3.31***	***3.16***	***3.25***	***3.16***	***3.31***	***2.99***
2.16667	3.18	5.19	5.16	4.61	5.11	4.98	5.14	4.86
2.33333	3.32	5.28	5.3	4.75	5.33	5.16	5.36	5.04

Table continuation: Test B								
Time [hours]	**<50**	**50**	**75**	**100**	**150**	**200**	**300**	**400**
2.5	3.36	5.32	5.35	4.79	5.42	5.25	5.45	5.13
2.66667	3.45	5.41	5.44	4.88	5.56	5.38	5.59	5.26
2.83333	3.54	5.51	5.53	4.97	5.7	5.52	5.72	5.35
3	3.67	5.64	5.62	5.06	5.83	5.7	5.9	5.49
3.16667	3.76	5.73	5.75	5.2	6.01	5.87	6.03	5.57
3.33333	3.93	5.87	5.89	5.33	6.19	6.05	6.17	5.71
3.5	3.93	5.91	5.93	5.38	6.28	6.14	6.26	5.71
3.66667	4.07	6.05	6.02	5.51	6.42	6.27	6.39	5.84
3.83333	4.2	6.14	6.16	5.6	6.6	6.45	6.53	5.89
4	4.33	6.32	6.3	5.74	6.73	6.59	6.66	6.02
4.16667	4.51	6.46	6.43	7.55	6.96	6.76	6.79	6.11
4.33333	4.64	6.6	6.57	7.68	7.1	6.94	6.93	6.2
4.5	***4.82***	***6.73***	***6.7***	***7.77***	***7.28***	***7.08***	***7.06***	***6.24***
4.66667	***4.91***	***6.92***	***6.8***	***7.86***	***7.41***	***7.21***	***7.15***	***6.33***
4.83333	***5.04***	***7.05***	***6.93***	***8.05***	***7.59***	***7.39***	***7.29***	***6.42***
5	***5.13***	***7.23***	***7.02***	***8.14***	***7.73***	***7.52***	***7.33***	***6.42***
5.16667	***5.13***	***7.28***	***7.07***	***8.18***	***7.82***	***7.65***	***7.42***	***6.47***
5.33333	***5.35***	***7.46***	***7.25***	***8.41***	***8.05***	***7.83***	***7.55***	***6.6***
5.5	***5.39***	***7.64***	***7.34***	***8.45***	***8.18***	***7.97***	***7.64***	***6.6***

Numbers in bold and italic format were used for GPR calculation

Immediate nitrogen removal rate (NRR) response to nitrite tested in R2 under reactor self maintain pH and high FA (Test 5b – R2)

Time	NH_4-N	NO_2-N	NO_3-N	FA	pH	NRR	NO_2/NH_4	NO_3/NH_4
[h]	[mg N/L]	[mg N/L]	[mg N/L]	[mg N/L]	[-]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]
0	49.0	13.0	44.6	7.6	8.10	2098	1.23	0.16
0.5	48.6	13.2	45.1	7.4	8.09	2097	1.23	0.16
Loading change from 7465 mg N/Ld to 10722 mg N/Ld								
1	59.4	22.5	44.7	8.0	8.03	-	1.24	0.17
1.5	60.5	30.5	42.3	8.2	8.03	-	1.21	0.16
2	79.1	37.0	42.8	10.5	8.02	-	1.28	0.17
3	74.2	46.3	41.9	9.8	8.02	-	1.22	0.17
4	80.5	52.9	40.3	10.7	8.02	-	1.22	0.16
5	84.7	58.5	38.8	11.2	8.02	-	1.22	0.16
6	88.9	63.3	38.2	11.8	8.02	-	1.22	0.16
26	128.8	123.2	23.8	16.4	8.00	2072	1.17	0.12
Nitrogen concentration in the feed to the MBBR								
0 – 6	325.8	352.8	0	-	6.80			
26	320.9	348.4	0	-	6.80			

Immediate nitrogen removal rate (NRR) response to nitrite tested in R2 under low FA (Test 5b – R2)

Test 5b – R2 – Nitrogen loading 1 (5 379 mg N/Ld)								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[hours]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	122.5	17.9	51.8	0.4	6.50	3950	1.19	0.19
1	125.3	18.1	52.4	0.4	6.50	3923	1.20	0.20
2	121.8	17.2	53.0	0.4	6.50	3952	1.19	0.20
	Average	17.7	-	0.4	6.50	3942	1.19	0.20
	SD	0.5	-	0.0	0.00	16	0.01	0.00
Nitrogen concentrations and pH in the feed during the test								
-	388.8	334.8	0	-	6.8			
Test 5b – R2 – Nitrogen loading 2 (8 514 mg N/Ld)								
0	144.9	38.4	51.0	0.5	6.50	-	-	-
1	144.9	40.3	52.0	0.5	6.50	5723	1.21	0.21
2	148.4	44.0	51.2	0.5	6.50	5648	1.21	0.21
3	142.8	41.7	51.4	0.5	6.50	5739	1.19	0.21
	Average	42.0	-	0.5	6.50	5703	1.20	0.21
	SD	1.8	-	0.0	0.00	48	0.01	0.00
Nitrogen concentrations and pH in the feed during the test								
-	388.8	334.8	0	-	6.8			

Test 5b – R2 – Nitrogen loading 3 (8 408 mg N/Ld)								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[hours]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	142.8	37.0	52.8	0.5	6.50	5671	1.27	0.22
2	138.6	36.6	51.6	0.5	6.50	5739	1.25	0.22
	Average	36.8	-	0.5	6.50	5705	1.26	0.22
	SD	0.3	-	0.0	0.00	48	0.01	0.01
Nitrogen concentrations and pH in the feed during the test								
-	378.0	336.6	0	-	6.8			
Test 5b – R2 – Nitrogen loading 4 (19 931 mg N/Ld)								
0	214.2	75.7	49.7	0.8	6.50	-	-	-
1	226.8	94.5	44.5	0.8	6.50	-	-	-
2	242.2	108.9	41.6	0.9	6.50	9720	1.32	0.23
3	240.8	112.8	40.2	0.9	6.50	9692	1.29	0.22
4	239.4	109.6	40.7	0.9	6.50	9798	1.30	0.22
5	240.8	108.4	41.4	0.9	6.50	9776	1.32	0.23
6	239.4	108.1	41.7	0.9	6.50	9812	1.31	0.23
	Average	109.6	-	0.9	6.50	9760	1.31	0.23
	SD	1.9	-	0.0	0.00	52	0.01	0.00
Nitrogen concentrations and pH in the feed during the test								
-	421	345.6	0	-	6.8			

Test 5b – R2 – Nitrogen loading 5 (15 332 mg N/Ld); Measurements were obtain next day after the loading rate was set day before								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[hours]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	214.2	75.7	49.7	0.8	6.50	8539	1.30	0.24
Nitrogen concentrations and pH in the feed during the test								
-	421	345.6	0	-	6.8			
Test 5b – R2 – Nitrogen loading 6 (9590 mg N/Ld); Measurements were obtain next day after the loading rate was set day before								
0	177.8	34.9	49.8	0.6	6.50	6353	1.29	0.20
Nitrogen concentrations and pH in the feed during the test								
-	424.8	352.8	0	-	6.8			
Test 5b – R2 – Nitrogen loading 7 (3543 mg N/Ld); Measurements were obtain next day after the loading rate was set day before								
0	129.5	11.6	46.8	0.5	6.50	2616	1.34	0.19
Nitrogen concentrations and pH in the feed during the test								
-	376.2	342	0	-	6.8			

Immediate specific nitrogen removal rate (sNRR) response to nitrite tested in R3 (Test 5b – R3)

Results for this test are presented in:

- *Nitrite spike, extra nitrite added to the feed, test on day 35 (Page 310)*
- *The sNRR in the SBR cycle measured at 8th cycle after the nitrite where increased in the feed day before (Page 311)*

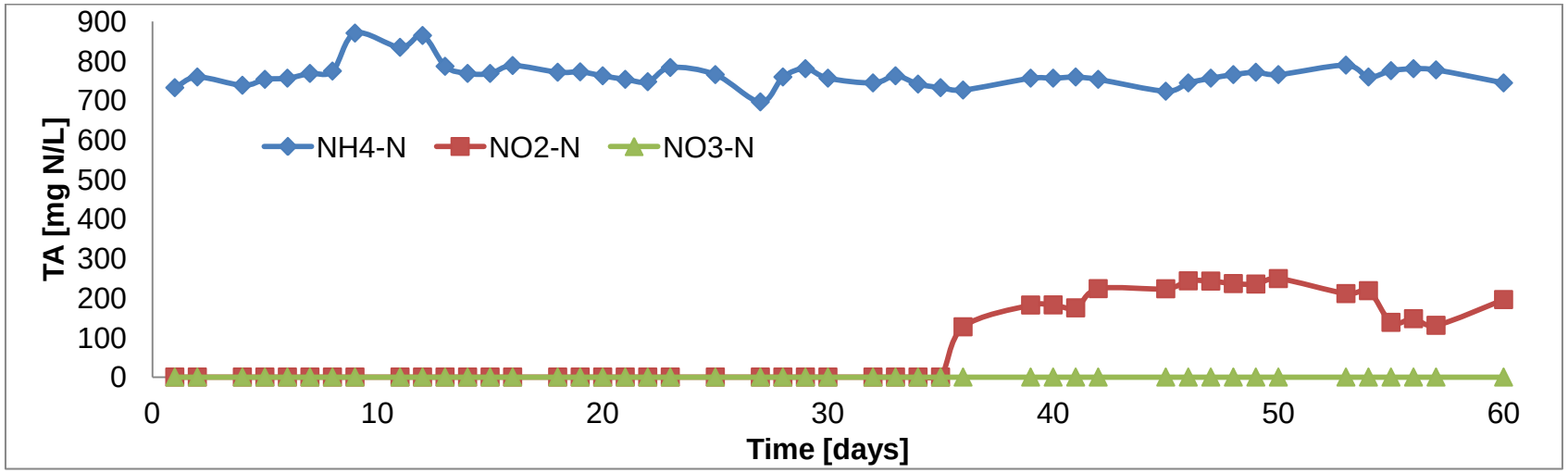
b) Long-term anammox response to nitrite (Test 6)

Centrate feed composition during the test

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	TSS	VSS
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[mg TSS/L]	[mg VSS/L]
1	7.98	-	732	0	0	-	-
2	7.95	-	759	0	0	-	-
4	7.9	-	738	0	0	-	-
5	7.98	-	753	0	0	-	-
6	7.97	-	756	0	0	-	-
7	7.96	-	768	0	0	-	-
8	7.85	3025	774	0	0	103	74.5
9	7.93	-	870	0	0	-	-
11	7.9	-	834	0	0	-	-
12	7.95	2940	864	0	0	102	77.5
13	7.95	-	786	0	0	-	-
14	8.05	2960	768	0	0	128	89
15	8	-	768	0	0	-	-
16	8	-	788	0	0	-	-
18	7.98	-	771	0	0	-	-
19	7.99	2925	772	0	0	106	73
20	8	-	762	0	0	-	-
21	8.01	2935	753	0	0	141	93
22	8	-	747	0	0	-	-
23	8.01	-	783	0	0	-	-

Table continuation - Centrate feed composition during the Test 6							
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**TSS**	**VSS**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg TSS/L]**	**[mg VSS/L]**
25	8.01	-	765	0	0	-	-
27	8	-	696	0	0	-	-
28	8	-	759	0	0	-	-
29	8	-	780	0	0	-	-
30	8	-	756	0	0	-	-
32	8	-	744	0	0	-	-
33	8	3005	762	0	0	-	-
34	8	-	741	0	0	-	-
35	8	-	732	0	0	-	-
36	8	-	726	127	0	-	-
39	8	-	756	182.1	0	-	-
40	8	-	756	182.4	0	-	-
41	8	-	759	174.9	0	-	-
42	8	-	753	223.5	0	-	-
45	8	-	723	223.2	0	-	-
46	7.99	-	744	243.3	0	-	-
47	7.98	-	756	242.7	0	105	88.5
48	7.95	-	765	236.4	0	-	-
49	8	-	771	235.2	0	-	-
50	8	-	765	249	0	-	-
53	8	-	789	211	0	-	-
54	8	-	759	218.4	0	-	-
55	8	-	775	138.3	0	-	-

Table continuation - Centrate feed composition during the Test 6							
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**TSS**	**VSS**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg TSS/L]**	**[mg VSS/L]**
56	8	-	780	147.6	0	-	-
57	8	-	777	130.8	0	-	-
60	8	-	744	195.9	0	-	-

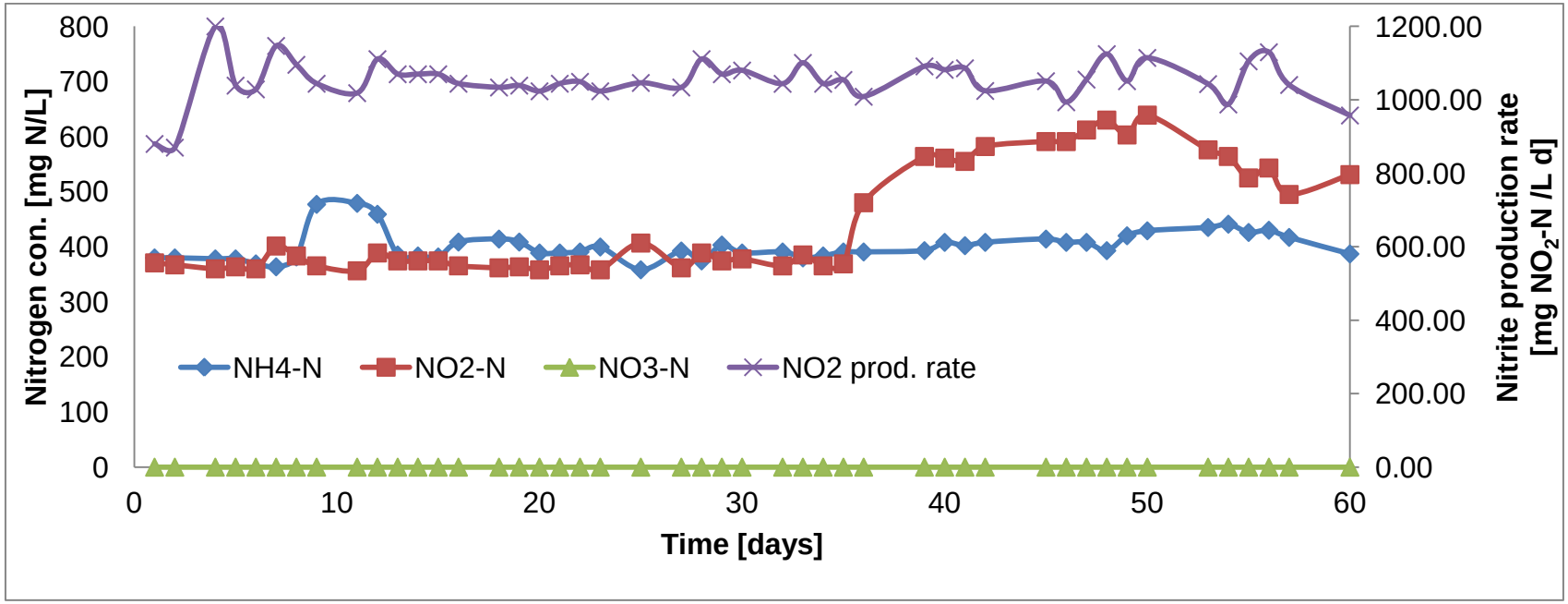


The effluent from the partial nitritation reactor – the feed for anammox stage (R1, R2, R3); Test 6

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Nitrite product. rate	Tot. TSS reactor	Tot. VSS reactor	TSS effluent	VSS effluent
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[mg NO_2-N/L d]	[mg VSS/L]	[mg TSS/L]	[mg TSS/L]	[mg VSS/L]
1	7.4	-	379.8	370.8	0	880	-	-	-	-
2	7.4	-	379.8	367.2	0	870	-	-	-	-
4	7.4	-	378	360	0	1200	-	-	-	-
5	7.4	-	378	363.6	0	1039	-	-	-	-
6	7.4	-	369	360	0	1029	-	-	-	-
7	7.4	-	363.6	401.4	0	1147	-	-	-	-
8	7.4	502	381.6	383.4	0	1095	29680	19900	34	27
9	7.4	-	477	365.4	0	1044	-	-	-	-
11	7.4	-	478.8	356.4	0	1018	-	-	-	-
12	7.4	345	459	388.8	0	1111	34660	23540	40	35
13	7.4	-	385.2	374.4	0	1070	-	-	-	-
14	7.4	510	383.4	374.4	0	1070	29520	20280	48	38.5
15	7.4	-	381.6	374.4	0	1070	-	-	-	-
16	7.4	-	408.6	365.4	0	1044	-	-	-	-
18	7.4	-	414	361.8	0	1034	-	-	-	-
19	7.4	518	408.6	363.6	0	1039	27640	19080	37.5	34.5
20	7.4	-	388.8	358.2	0	1023	-	-	-	-
21	7.4	526	388.8	365.4	0	1044	29479	20049	56.5	44
22	7.4	-	390.6	367.2	0	1049	-	-	-	-
23	7.4	-	399.6	358.2	0	1023	-	-	-	-

Table continuation – The effluent from the partial nitritation reactor – the feed for anammox stage; Test 6										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Nitrite product. rate**	**Tot. TSS reactor**	**Tot. VSS reactor**	**TSS effluent**	**VSS effluent**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg NO_2-N/L d]**	**[mg VSS/L]**	**[mg TSS/L]**	**[mg TSS/L]**	**[mg VSS/L]**
25	7	-	358.2	406.8	0	1046	-	-	-	-
27	7.4	-	392.4	361.8	0	1034	-	-	-	-
28	7.4	-	374.4	388.8	0	1111	-	-	-	-
29	7.4	-	403.2	374.4	0	1070	-	-	-	-
30	7.4	-	388.8	378	0	1080	-	-	-	-
32	7.4	-	390.6	365.3	0	1044	-	-	-	-
33	7.4	518	379.8	385.2	0	1101	26240	20300	40	38.5
34	7.4	-	383.4	365.4	0	1044	-	-	50	33
35	7.4	-	390.6	369	0	1054	-	-	-	-
36	7.4	-	390.6	480	0	1009	-	-	-	-
39	7.4	-	393	564	0	1091	-	-	-	-
40	7.4	-	408	561	0	1082	-	-	-	-
41	7.4	-	402	555	0	1086	-	-	-	-
42	7.4	-	408	582	0	1024	-	-	-	-
45	7.4	-	414	591	0	1051	-	-	-	-
46	7.4	-	408	591	0	993	-	-	-	-
47	7.4	-	408	612	0	1055	-	-	-	-
48	7.4	-	393	630	0	1125	-	-	-	-
49	7.4	-	420	603	0	1051	-	-	-	-
50	7.4	-	429	639	0	1114	-	-	-	-
53	7.4	-	435	576	0	1043	-	-	-	-
54	7.4	-	441	564	0	987	-	-	-	-
55	7.4	-	426	525	0	1105	-	-	-	-

Table continuation – The effluent from the partial nitritation reactor – the feed for anammox stage; Test 6										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Nitrite product. rate**	**Tot. TSS reactor**	**Tot. VSS reactor**	**TSS effluent**	**VSS effluent**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg NO_2-N/L d]**	**[mg VSS/L]**	**[mg TSS/L]**	**[mg TSS/L]**	**[mg VSS/L]**
56	7.4	-	430	543	0	1130	-	-	-	-
57	7.4	-	417	495	0	1041	-	-	-	-
60	7.4	-	387	531	0	957	-	-	-	-



Long-term nitrogen removal rate (NRR) response to elevated nitrite concentrations tested in R1 (Test 6 – R1)

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
1	6.5	-	105	7.6	82	11.5	2131	1.32	0.30	0.4
2	6.5	-	114.1	8.2	82.8	11.5	2077	1.35	0.31	0.4
4	6.5	-	123.9	14.7	77.7	16.5	2869	1.36	0.31	0.5
5	6.5	-	130.9	41.9	62.4	30.6	5165	1.30	0.25	0.5
6	6.5	-	121.1	40.4	59	30.6	5187	1.29	0.24	0.4
7	6.5	-	93.8	41.7	71.7	30.5	5671	1.33	0.27	0.3
8	6.5	180	121.2	39.9	67.9	30.6	5467	1.32	0.26	0.4
9	6.5	-	214.2	35.3	65.4	30.6	5381	1.26	0.25	0.8
11	6.5	-	222.6	33.3	61.9	30.6	5277	1.26	0.24	0.8
12	6.5	120	188.3	41.9	65.9	30.6	5627	1.28	0.24	0.7
13	6.5	-	127.4	43.3	61	30.6	5385	1.28	0.24	0.5
14	6.5	195	123.2	37.5	63.3	30.3	5391	1.29	0.24	0.5
15	6.5	-	116.9	35.1	64.3	30.2	5433	1.28	0.24	0.4
16	6.5	-	147.7	33.2	64.1	30.2	5325	1.27	0.25	0.5
18	6.5	-	148.4	31.7	63.5	30.2	5357	1.24	0.24	0.5
19	6.5	200	154	32.9	63	30.2	5258	1.30	0.25	0.6
20	6.5	-	131.6	30.7	64.5	30.2	5237	1.27	0.25	0.5
21	6.5	211	131.6	30.2	64.3	30.2	5316	1.30	0.25	0.5
22	6.5	-	126	30.2	61.5	30.2	5437	1.27	0.23	0.5
23	6.5	-	143.5	26.9	62.7	30.2	5282	1.29	0.24	0.5

Table continuation – Test 6 – R1 (low pH and low FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
25	6.5	-	68	31.2	71	30.2	5988	1.29	0.24	0.3
27	6.5	-	130.2	28.5	61.1	30.2	5380	1.27	0.23	0.5
28	6.5	-	273	268.8	20	30.2	2027	1.18	0.20	1.0
29	6.5	-	124.6	26.2	62.7	18	3385	1.25	0.23	0.5
30	6.5	-	125.3	33	61.5	30.2	5506	1.31	0.23	0.5
32	6.5	-	130.9	30.8	58.1	30.2	5397	1.29	0.22	0.5
33	6.5	202	106.4	30.5	59.8	30.2	5721	1.30	0.22	0.4
34	6.5	-	126	27.7	58.4	30.2	5403	1.31	0.23	0.5
35	6.5	-	130.2	28.6	57.5	30.2	5469	1.31	0.22	0.5
36	6.5	-	59.5	40.7	76.9	30.2	6981	1.33	0.23	0.2
39	6.5	-	30.8	70.7	85.4	30.2	7752	1.36	0.24	0.1
40	6.5	-	37.7	66.2	86.4	30.2	7839	1.34	0.23	0.1
41	6.5	-	44.8	71.4	86.4	30.8	7745	1.35	0.24	0.2
42	6.5	-	48.2	100.8	84.7	30.2	7613	1.34	0.24	0.2
45	6.5	-	46.5	93.8	84.7	27.7	7202	1.35	0.23	0.2
46	6.5	-	51.6	112	81.2	30.2	7592	1.34	0.23	0.2
47	6.5	-	54.9	149	82	30.2	7390	1.31	0.23	0.2
48	6.5	-	58.5	200	69.1	30.2	7000	1.29	0.21	0.2
49	6.5	-	64.9	127	86	30.2	7501	1.34	0.24	0.2
50	6.5	-	62.9	125	78	30.2	8074	1.40	0.21	0.2
53	6.5	-	84.1	106	84.5	31	7609	1.34	0.24	0.3
54	6.5	-	414	540	0	31	527	0.89	0.00	1.5
55	6.5	-	342	426	3.2	24	1438	1.18	0.04	1.3

Table continuation – Test 6 – R1 (low pH and low FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
56	6.5	-	339	372	15.8	9.4	771	1.88	0.17	1.3
57	6.5	-	315	348	12.6	9.4	741	1.44	0.12	1.2
60	6.5	-	144	236	38.9	9.4	1564	1.21	0.16	0.5

Solids in R1 during Test 6 – R1

Time	**Tot. TSS reactor**	**Tot. VSS reactor**	**TSS effluent**	**VSS effluent**
[day]	**[mg TSS/L]**	**[mg VSS/L]**	**[mg TSS/L]**	**[mg VSS/L]**
8	29830	28690	111	91.5
12	28785	26125	97.5	82
14	26695	23845	90	78.5
19	32585	29735	93	80.5
21	30590	28215	116.5	100
47	30780	29640	158.5	128
48	-	-	169.5	140

Long-term nitrogen removal rate (NRR) response to elevated nitrite concentrations tested in R2 (Test 6 – R2)

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
1	8	-	13.9	4.3	110.5	2.5	518	1.00	0.30	1.5
2	8	-	50.3	18.1	95.3	2.5	486	1.06	0.29	5.3
4	8	-	8.5	42.7	76.3	2.9	590	0.86	0.21	0.9
5	8	-	128.1	98	58.8	2.9	441	1.06	0.24	13.4
6	8	-	159.6	143.5	43.4	2.9	370	1.03	0.21	16.7
7	8	-	184.1	193.2	34.3	2.9	342	1.16	0.19	19.3
8	8	460	37.7	48.7	83.4	2.5	496	0.97	0.24	4.0
9	7.4	-	93.1	8.1	80.8	2.5	550	0.93	0.21	2.7
11	8	-	210	99.4	58.1	2.5	390	0.96	0.22	22.0
12	8	440	169.4	73.5	65.8	1.6	288	1.09	0.23	17.8
13	8	-	116.2	64.6	69.8	1.6	271	1.15	0.26	12.2
14	8	460	84	64.1	69.6	1.6	288	1.04	0.23	8.8
15	8	-	59.3	53.7	65.3	1.5	289	1.00	0.20	6.2
16	8	-	50.8	66.2	67.5	1.44	283	0.84	0.19	5.3
18	8	-	63.8	80.5	65.8	1.44	272	0.80	0.19	6.7
19	8	513	75.6	93.8	62.3	1.44	259	0.81	0.19	7.9
20	8	-	56.1	70	65.8	1.12	207	0.87	0.20	5.9
21	8	520	37.5	67.9	70.7	1.12	216	0.85	0.20	3.9
22	8	-	46.1	62.6	57.1	1.1	217	0.88	0.17	4.8
23	8	-	38.4	75.6	58.1	1.1	215	0.78	0.16	4.0

Table continuation – Test 6 – R2 (ambient pH and high FA)										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
25	8	-	27.8	102.9	55.3	1.1	212	0.92	0.17	2.9
27	8	-	58.6	72.1	100	1.1	192	0.87	0.30	6.1
28	8	-	74.2	109.2	90	1.1	180	0.93	0.30	7.8
29	8	-	40.6	56.1	88.1	1.1	217	0.88	0.24	4.3
30	7.3	-	23.6	3	118.1	1.1	228	1.03	0.32	0.5
32	8	-	85.4	66.4	89.7	1.1	189	0.98	0.29	9.0
33	8	563	91	73.5	79.1	0.785	136	1.08	0.27	9.5
34	8	-	110.6	98.7	66.5	0.785	124	0.98	0.24	11.6
35	8	-	101.5	108.4	69.4	0.785	126	0.90	0.24	10.6
36	8	-	82.6	124.6	65.1	0.785	157	1.15	0.21	8.7
39	8	-	86.1	222.6	49.6	0.785	157	1.11	0.16	9.0
40	8	-	80.1	282.6	64.8	0.785	142	0.85	0.20	8.4
41	8	-	78.3	298.8	65	0.785	135	0.79	0.20	8.2
42	8	-	87.7	342	57.6	0.785	132	0.75	0.18	9.2
45	8	-	121.5	474	39	0	-	-	-	12.7
46	8	-	88.8	489	39	0	-	-	-	9.3
47	8	-	44.4	519	45	0	-	-	-	4.7
48	8	-	24.2	534	52.6	0	-	-	-	2.5
49	8	-	4.8	450	135	0	-	-	-	0.5

Solids in R2 during Test 6 – R2

Time	Tot. TSS reactor	Tot. VSS reactor	TSS effluent	VSS effluent
[day]	[mg TSS/L]	[mg VSS/L]	[mg TSS/L]	[mg VSS/L]
8	56240	28690	115.5	83
12	58330	25631	54	43.5
14	61275	25935	66	54
19	47975	25840	65	50
21	76855	26885	68	52.5

Long-term specific nitrogen removal rate (sNRR) response to elevated nitrite concentrations tested in R3 (Test 6 – R3)

Time	pH	Alkalinity	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg $CaCO_3$/L]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
1	7	-	88.9	0	68	18	3447	1.27	0.23	1.0
2	7	-	93.8	0	66.6	18	3406	1.28	0.23	1.1
4	7	-	102.2	0	68.7	18	3293	1.31	0.25	1.2
5	7	-	-	-	-	-	-	-	-	0.0
6	7	-	88.2	0	63.2	18	3354	1.28	0.23	1.0
7	7	-	55	0	68.3	18	3726	1.30	0.22	0.6
8	7	472	82.6	0	64.1	18	3590	1.28	0.21	1.0
9	7	-	-	-	-	-	-	-	-	0.0
11	7	-	187.6	0	54.9	18	3441	1.22	0.19	2.2
12	7	315	149.1	0	63.5	18	3688	1.25	0.20	1.7
13	7	-	84.7	0	58.9	18	3577	1.25	0.20	1.0
14	7	487	81.9	0	54.3	18	3609	1.24	0.18	0.9
15	7	-	77.7	0	55.4	18	3617	1.23	0.18	0.9
16	7	-	107.8	0	58.9	18	3526	1.21	0.20	1.2
18	7	-	116.2	0	56.4	18	3502	1.21	0.19	1.3
19	7	500	119	0	55.8	18	3469	1.26	0.19	1.4
20	7	-	98.7	0	54.9	18	3446	1.23	0.19	1.1
21	7	505	96.6	0	50.8	18	3523	1.25	0.17	1.1
22	7	-	95.2	0	49.4	18	3561	1.24	0.17	1.1
23	7	-	50.3	0	56.9	18	3778	1.03	0.16	0.6

Table continuation – Test 6 – R3										
Time	**pH**	**Alkalinity**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg $CaCO_3$/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
25	7	-	30.5	0	59.5	18	3919	1.24	0.18	0.4
27	7	-	99.4	0	52.6	18	3497	1.23	0.18	1.2
28	7	-	64	0	52	18	3758	1.25	0.17	0.7
29	7	-	87.5	0	53.1	18	3699	1.19	0.17	1.0
30	7	-	79.8	0	46.5	18	3719	1.22	0.15	0.9
32	7	-	90.3	0	41.6	18	3623	1.22	0.14	1.0
33	7	503	66.8	0	44.8	18	3794	1.23	0.14	0.8
34	7	-	91	0	32	18	3634	1.25	0.11	1.1
35	7	-	98	0	44.8	18	3581	1.26	0.15	1.1
36	7	-	11	0	63.8	18	4621	1.26	0.17	0.1
39	7	-	372	552	0	18	192	0.57	0.00	4.3
40	7	-	372	69.3	6.9	3	504	13.66	0.19	4.3
41	7	-	293.4	153.9	7	3.4	551	3.69	0.06	3.4
42	7	-	125.3	0	0	0	0	2.06	0.00	1.5
45	7	-	82.6	13.2	58.2	0.7	192	1.74	0.18	1.0
46	7	-	67.8	0.7	59.4	0.7	197	1.74	0.17	0.8
47	7	-	209	258	28	1.5	254	1.78	0.14	2.4
48	7	-	192	262	25.8	0.3	53	1.83	0.13	2.2
49	7	-	150	232	34	0	0	1.37	0.13	1.7

Solids in R3 during Test 6 – R3

Time	Tot. TSS reactor	Tot. VSS reactor	TSS effluent	VSS effluent
[day]	[mg TSS/L]	[mg VSS/L]	[mg TSS/L]	[mg VSS/L]
8	2992	2512	35	26.5
12	3230	2774	29.5	26.5
14	3302	2774	41.5	36.5
19	3422	2888	35	31.5
21	3684	3074	44	39
33	3708	2984	-	-
35	3690	2986	-	-
36	3880	3118	-	-

Long-term specific nitrogen removal rate (sNRR) response to elevated nitrite concentrations tested in R3 (Test 6 – R3)

One complete SBR cycle duration was 120 min where 45 min was feeding (from 0 to 45 min), reaction and mixing 105 min (from 0 to 105 min)

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 8								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	102.2	24.4	58.9	1.2	7.0	-	-	-
15	117.6	47	55.2	1.4	7.0	-	-	-
25	130.2	61.9	53.6	1.5	7.0	2.32	1.31	0.24
35	136.5	72.1	51.1	1.6	7.0	2.63	1.21	0.18
45	142.1	79.8	51.1	1.6	7.0	2.57	1.25	0.24
55	123.9	53.6	56.3	1.4	7.0	2.25	1.44	0.29
65	104.3	26.2	62.7	1.2	7.0	-	-	-
75	86.8	1.4	68	1.0	7.0	-	-	-
85	83.3	0	66.1	1.0	7.0	-	-	-
					Average	2.44	1.31	0.24
					SD	0.18	0.10	0.04
Nitrogen concentrations and pH in the feed during the test								
-	381.6	383.4	0	-	7.4			

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 12								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	163.1	19.9	57.8	1.8	7.0	-	-	-
15	173.6	36.3	57.5	1.9	7.0	-	-	-
25	182.7	46.8	56.8	2.0	7.0	2.54	1.26	0.23
35	201.6	55.3	56.7	2.3	7.0	2.15	1.84	0.36
45	194.6	61.9	55.7	2.2	7.0	3.00	0.97	0.17
55	172.9	31.4	61.7	1.9	7.0	2.40	1.41	0.28
65	152.6	3.1	66.6	1.7	7.0	-	-	-
75	148	0	65.8	1.7	7.0	-	-	-
85	151.2	0	64.7	1.7	7.0	-	-	-
95	146.3	0	62.9	1.6	7.0			
					Average	2.52	1.37	0.26
					SD	0.36	0.37	0.08
Nitrogen concentrations and pH in the feed during the test								
-	459	388.8	0	-	7.4			

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 14								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	102.2	19.8	49.8	1.1	7.0	-	-	-
15	108.5	33.1	50.9	1.2	7.0	-	-	-
25	115.5	41.2	50.5	1.3	7.0	2.62	1.26	0.21
35	121.1	47.8	51.6	1.4	7.0	2.59	1.25	0.25
45	125.3	53.6	52.1	1.4	7.0	2.61	1.23	0.23
55	102.9	24.1	56.4	1.2	7.0	2.48	1.32	0.19
65	84	0	59.8	0.9	7.0	-	-	-
75	83.3	0	58.3	0.9	7.0	-	-	-
85	83.3	0	57.3	0.9	7.0	-	-	-
95	80.5	0	54.5	0.9	7.0	-	-	-
					Average	2.57	1.26	0.22
					SD	0.06	0.04	0.02
Nitrogen concentrations and pH in the feed during the test								
-	383.4	374.4	0	-	7.4			

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 19								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	136.5	20	51.4	1.5	7.0	-	-	-
15	144.2	30.2	51.7	1.6	7.0	-	-	-
25	146.3	32	53.4	1.6	7.0	2.71	1.28	0.25
35	150.5	34.1	54.8	1.7	7.0	2.61	1.35	0.26
45	147.7	33.4	55.5	1.7	7.0	2.92	1.21	0.21
55	126.7	2.7	60.6	1.4	7.0	-	-	-
65	121.8	0	59.9	1.4	7.0	-	-	-
75	121.1	0	59.1	1.4	7.0	-	-	-
85	119.7	0	57.3	1.3	7.0	-	-	-
95	119.7	0	55.6	1.3	7.0	-	-	-
					Average	2.75	1.28	0.24
					SD	0.16	0.07	0.03
Nitrogen concentrations and pH in the feed during the test								
-	408.6	363.6	0	-	7.4			

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 21								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	116.2	20.7	45.4	1.3	7.0	-	-	-
15	122.5	30	47.7	1.4	7.0	-	-	-
25	126	32.3	48.9	1.4	7.0	2.54	1.31	0.23
35	126.7	33.3	50.7	1.4	7.0	2.62	1.26	0.23
45	127.4	33.3	52.1	1.4	7.0	2.64	1.29	0.23
55	104.3	2.4	57	1.2	7.0			
65	100.8	0	55.4	1.1	7.0	-	-	-
75	99.4	0	53.9	1.1	7.0	-	-	-
85	98	0	51.5	1.1	7.0	-	-	-
95	98	0	50.5	1.1	7.0	-	-	-
					Average	2.60	1.29	0.23
					SD	0.05	0.02	0.00
Nitrogen concentrations and pH in the feed during the test								
-	388.8	365.4	0	-	7.4			

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 32								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	106.4	20	36.6	1.2	7.0	-	-	-
15	112.7	27.1	38.8	1.3	7.0	-	-	-
25	116.2	30	41.4	1.3	7.0	2.47	1.26	0.23
35	119	31.6	43.3	1.3	7.0	2.49	1.28	0.23
45	119.7	31.3	45	1.3	7.0	2.63	1.27	0.22
55	95.9	0.4	50.4	1.1	7.0			
65	94.5	0	48.4	1.1	7.0	-	-	-
75	92.4	0	46.8	1.0	7.0	-	-	-
85	91	0	43.8	1.0	7.0	-	-	-
95	91	0	42.1	1.0	7.0			
					Average	2.53	1.27	0.23
					SD	0.08	0.01	0.00
Nitrogen concentrations and pH in the feed during the test								
-	390.6	365.4	0	-	7.4			

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 33								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	69.5	0	47.1	0.8	7.0	-	-	-
5	84.7	19.6	39.6	0.9	7.0	-	-	-
15	89.6	25.7	43.1	1.0	7.0	2.49	1.27	0.24
25	98.7	27.4	45.4	1.1	7.0	2.40	1.61	0.28
35	99.4	27.2	47.7	1.1	7.0	2.70	1.34	0.24
45	91.7	25.9	49	1.0	7.0	3.15	1.06	0.17
55	74.2	0.2	54.3	0.8	7.0	-	-	-
65	66.7	0	51.4	0.7	7.0	-	-	-
75	74.9	0	48.7	0.8	7.0	-	-	-
					Average	2.69	1.32	0.24
					SD	0.34	0.23	0.05
Nitrogen concentrations and pH in the feed during the test								
-	370.8	381.6	0	-	7.4			

No ammonium at the beginning of SBR cycle, day 33

Test 6 – R3 – Day 33								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	0	100	55.2	0.0	7.0	-	-	-
5	39	161	47.6	0.4	7.0	-	-	-
15	88.2	228.2	35	1.0	7.0	*0.38*	-	-
25	127.4	278.6	28	1.4	7.0	*0.14*	-	-
35	148.4	295.4	21	1.7	7.0	1.47	-	-
45	163.1	309.4	21	1.8	7.0	1.27	1.62	0.19
55	152.6	298.2	22.4	1.7	7.0	0.93	1.07	0.13
65	144.9	280	25.2	1.6	7.0	1.06	2.36	0.36
75	134.4	260.4	30.8	1.5	7.0	1.13	1.87	0.53
85	126.7	250.6	29.4	1.4	7.0	0.81	1.27	0.09
95	117.6	233.8	33.6	1.3	7.0	1.15	1.85	0.23
105	106.4	219.8	36.4	1.2	7.0	1.08	1.25	0.25
					Average	1.11	1.61	0.26
					SD	0.37	0.45	0.15
Nitrogen concentrations and pH in the feed during the test								
-	396	603	0	-	7.4			

Estimation of sNRR based nitrogen measurements during one SBR cycle

Test 6 – R3 – Day 35								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	115.5	22	38.1	1.3	7.0	-	-	-
15	126.7	36.7	40.3	1.4	7.0	-	-	-
25	133	44.1	42.7	1.5	7.0	2.11	1.28	-0.27
35	137.2	51.4	41	1.5	7.0	2.21	1.18	-0.13
45	141.4	55.8	42.5	1.6	7.0	2.10	1.29	-0.26
55	121.8	26.3	47.2	1.4	7.0	2.05	1.51	-0.24
65	100.8	1.1	50.9	1.1	7.0	-	-	-
75	98	0	49.1	1.1	7.0	-	-	-
85	98.7	0	47.5	1.1	7.0	-	-	-
95	95.9	0	46.6	1.1	7.0			
105	95.2	0	44.9	1.1				
					Average	2.12	1.31	-0.22
					SD	0.07	0.14	0.07
Nitrogen concentrations and pH in the feed during the test								
-	390.6	369	0	-	7.4			

Nitrite spike, extra nitrite added to the feed, test on day 35

Test 6 – R3 – Day 35								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	110.6	39.2	34.3	1.2	7.0	-	-	-
15	122.5	81.9	32.9	1.4	7.0	-	-	-
25	128.8	109.9	28.7	1.4	7.0	2.45	1.21	0.01
35	134.4	130.2	28.7	1.5	7.0	2.33	1.29	0.13
45	138.6	143.5	28	1.6	7.0	2.50	1.39	0.10
55	117.6	116.2	32.2	1.3	7.0	2.03	1.30	0.20
65	95.2	83.3	41.3	1.1	7.0	2.13	1.47	0.41
75	72.8	49.4	49.3	0.8	7.0	2.23	1.51	0.36
85	51.7	16.4	58.5	0.6	7.0	2.17	1.56	0.44
95	39.8	0.7	61.5	0.4	7.0	-	-	-
105	38.8	0	60.6	0.4	7.0	-	-	-
					Average	2.26	1.39	0.23
					SD	0.17	0.13	0.17
Nitrogen concentrations and pH in the feed during the test								
-	399	570	0	-	7.4			

The sNRR in the SBR cycle measured at 8th cycle after the nitrite where increased in the feed day before

Test 6 – R3 – Day 36								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	33.5	27	58.4	0.4	7.0	-	-	-
15	48.7	50.9	57.6	0.5	7.0	2.44	1.20	0.20
25	60.9	67.6	56.3	0.7	7.0	2.45	1.26	0.19
35	70.7	80.5	55.3	0.8	7.0	2.43	1.27	0.19
45	78.4	91	55.3	0.9	7.0	2.38	1.26	0.22
55	53.2	56.1	61.5	0.6	7.0	2.38	1.38	0.25
65	28.1	21.8	67.1	0.3	7.0	2.37	1.37	0.22
75	10.9	0	70.7	0.1	7.0	-	-	-
85	9.3	0	68.3	0.1	7.0	-	-	-
95	8.2	0	66.3	0.1	7.0	-	-	-
105	7.2	0	65.1	0.1	7.0	-	-	-
					Average	2.41	1.29	0.21
					SD	0.03	0.07	0.02
Nitrogen concentrations and pH in the feed during the test								
-	396	498	0	-	7.4			

Appendix 5 Anammox response to FA

a) Immediate anammox response to FA

Immediate nitrogen removal rate (NRR) response to FA tested in R1 (Test 7a – R1)

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	145.6	50.4	0.375	6.5	145.6	51	0.375	6.5	151.2	50.1	0	6.5
30	143.5	48.6	0.75	6.5	-	49.2	-	6.5	-	48.9	-	6.5
60	142.8	46.2	0.75	6.5	-	47.7	-	6.5	-	47.4	-	6.5
90	143.5	44.7	0.75	6.5	-	46.5	-	6.5	-	46.2	-	6.5
120	140.7	42.9	1.125	6.5	-	45	-	6.5	-	45	-	6.5
150	138.6	41.1	1.5	6.5	-	43.5	-	6.5	-	43.5	-	6.5
180	135.8	39.3	1.5	6.5	142.8	42.9	0.75	6.5	146.1	42.6	1.125	6.5
FA test – pH = 6.5; FA = 0.5 mg N/L												
0	-	-	-	-	137.2	49.8	0	6.5	140.7	53.1	0	6.5
30	-	-	-	-	-	47.7	-	6.5	-	51.6	-	6.5
60	-	-	-	-	-	46.5	-	6.5	-	50.4	-	6.5
90	-	-	-	-	-	45.3	-	6.5	-	49.2	-	6.5
120	-	-	-	-	-	44.1	-	6.5	-	48	-	6.5
150	-	-	-	-	-	42.6	-	6.5	-	46.2	-	6.5
180	-	-	-	-	-	39.3	-	6.5	-	44.1	-	6.5
210	-	-	-	-	130.2	37.8	1.5	6.5	134.4	42.9	1.125	6.5

FA test – pH = 7.0; FA = 0.5 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	141.4	51.9	-	6.5	148.4	52.8	-	6.5	154.7	50.1	-	6.5
30	-	50.5	-	6.5	-	51.531	-	6.5	-	49.7	-	6.5
60	-	49.2	-	6.5	-	50.4	-	6.5	-	48.3	-	6.5
90	-	48.3	-	6.5	-	49.5	-	6.5	-	46.5	-	6.5
120	-	46.8	-	6.5	-	48.3	-	6.5	-	46.2	-	6.5
150	139.3	45.6	-	6.5	145.6	47.4	-	6.5	150.5	45.6	-	6.5
FA test – pH = 7.0; FA = 0.5 mg N/L												
0	48.3	51.3	0.3	7.0	48.6	51.6	0.3	7.0	-	52.2	0.6	7.0
30	-	50.1	-	7.0	48.3	50.7	0.6	7.0	48.6	51	-	7.0
60	-	49.2	-	7.0	46.2	49.5	-	7.0	-	50.1	-	7.0
90	-	47.7	-	7.0	45.9	48.6	0.9	7.0	-	49.5	-	7.0
120	-	46.8	-	7.0	45.9	47.4	1.2	7.0	-	48.3	-	7.0
150	-	45.6	-	7.0	45.3	46.2	1.5	7.0	-	47.4	-	7.0
180	-	-	-	7.0	43.5	45.6	1.5	7.0	-	46.2	-	7.0
210	-	42.9	-	7.0	43.2	44.4	1.5	7.0	-	45.3		7.0
240	41.1	41.4	1.8	7.0	42.3	43.2	1.8	7.0	40.5	-	2.1	7.0

FA test – pH = 7.5; FA = 0.5 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	133.7	-	-	6.5	-	47.8	3.2	6.5	133.7	49.2	3.1	6.5
30	131.6	49.0	1.15	6.5	132.3	45.8	-	6.5	-	47.4	-	6.5
60	130.9	47.2	2.1	6.5	-	44.4	-	6.5	-	46	-	6.5
90	132.3	45.8	1.5	6.5	-	42.6	-	6.5	-	44.4	-	6.5
120	127.4	43.6	2.7	6.5	-	41.4	-	6.5	-	43	-	6.5
150	126.0	42.6	2.4	6.5	127.4	39.8	4.3	6.5	130.9	41.6	4.0	6.5
FA test – pH = 7.5; FA = 0.5 mg N/L												
0	18.1	48.6	0.9	7.5	18.4	48.6	0.9	7.5	17.7	49.0	1.3	7.5
30	-	47.2	-	7.5	-	47.4	-	7.5	-	47.2	-	7.5
60	-	46.2	-	7.5	-	45.8	-	7.5	-	46.6	-	7.5
90	-	45.0	-	7.5	-	44.4	-	7.5	-	45.4	-	7.5
120	-	44.0	-	7.5	-	43.4	-	7.5	-	44.4	-	7.5
150	-	43.0	-	7.5	-	41.8	-	7.5	-	43.4	-	7.5
180	10.9	41.6	2.8	7.5	10.5	40.6	2.8	7.5	10.7	40.8	2.8	7.5

FA test – pH = 8.0; FA = 0.5 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	135.1	49.0	0.4	6.5	138.6	48.6	0.6	6.5	138.6	48.8	0.9	6.5
30	-	47.8	-	6.5	-	47.0	-	6.5	-	47.6	-	6.5
60	-	46.2	-	6.5	-	45.2	-	6.5	-	46.4	-	6.5
90	-	44.6	-	6.5	-	43.6	-	6.5	-	44.0	-	6.5
120	-	43.0	-	6.5	-	41.8	-	6.5	135.8	43.2	-	6.5
150	131.6	41.4	2.8	6.5	131.6	40.0	3.2	6.5	-	41.6	2.8	6.5
FA test – pH = 8.0; FA = 0.5 mg N/L												
0	10.0	49.0	0.6	8.0	10.5	49.8	0.2	8.0	10.5	50.0	0.4	8.0
30	-	48.2	-	8.0	-	49.0	-	8.0	-	49.2	-	8.0
60	-	47.4	-	8.0	-	48.2	-	8.0	-	48.8	-	8.0
90	-	46.4	-	8.0	-	47.6	-	8.0	-	48.0	-	8.0
120	-	45.8	-	8.0	-	47.0	-	8.0	-	47.2	-	8.0
150	-	44.6	-	8.0	-	46.4	-	8.0	-	46.8	-	8.0
180	4.3	44.2	2.2	8.0	5.7	46.0	1.5	8.0	5.2	45.8	1.9	8.0

FA test – pH = 6.5; FA = 1.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	137.2	47.2	0.4	6.5	138.6	47.8	0.6	6.5	138.6	47.4	0.6	6.5
30	135.8	45.8	1.3	6.5	137.9	46.2	0.8	6.5	-	46.2	-	6.5
60	135.8	44	1.9	6.5	137.9	44.6	1.5	6.5	-	44.6	-	6.5
90	133.0	42.6	2.1	6.5	134.4	43.2	1.7	6.5	-	43.6	-	6.5
120	133.0	41.2	1.9	6.5	135.8	41.6	2.1	6.5	-	42	-	6.5
150	-	39.8	2.1	6.5	132.3	40.0	2.5	6.5	130.9	41	2.3	6.5
FA test – pH = 6.5; FA = 1.0 mg N/L												
0	-	-	-	6.5	-	-	-	6.5	-	-	-	6.5
30	280.8	46.0	-	6.5	289.8	45.2	-	6.5	297.0	47.4	-	6.5
60	-	44.4	-	6.5	-	44.2	-	6.5	-	46.0	-	6.5
90	-	42.6	-	6.5	-	42.2	-	6.5	-	44.4	-	6.5
120	-	41.4	-	6.5	-	40.6	-	6.5	-	43.4	-	6.5
150	-	39.8	-	6.5	-	39.4	-	6.5	-	42.2	-	6.5
180	270.0	38.8	-	6.5	270	37.6	-	6.5	286.2	40.8	-	6.5

FA test – pH = 7.0; FA = 1.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	133.7	47.8	-	6.5	133.0	46.4	-	6.5	137.2	46.8	-	6.5
30	-	46.2	-	6.5	-	44.8	-	6.5	-	45.6	-	6.5
60	-	44.4	-	6.5	-	43.4	-	6.5	-	43.8	-	6.5
90	-	43.0	-	6.5	-	41.8	-	6.5	-	42.6	-	6.5
120	-	41.4	-	6.5	-	40.4	-	6.5	-	41.4	-	6.5
150	128.8	40.0	-	6.5	128.1	39.4	-	6.5	130.9	40.4	-	6.5
FA test – pH = 7.0; FA = 1.0 mg N/L												
0	-	46.0	-	7.0	-	46.6	-	7.0	-	46.6	-	7.0
30	-	44.6	-	7.0	-	45.4	-	7.0	-	45.6	-	7.0
60	-	43.0	-	7.0	-	44.4	-	7.0	-	44.6	-	7.0
90	-	41.6	-	7.0	-	43.4	-	7.0	-	43.4	-	7.0
120	-	40.2	-	7.0	-	41.8	-	7.0	-	42.0	-	7.0
150	-	38.4	-	7.0	-	40.8	-	7.0	-	40.8	-	7.0
180	-	36.8	-	7.0	-	39.2	-	7.0	-	40.0	-	7.0

FA test – pH = 7.5; FA = 1.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	140.7	46	2.3	6.5	141.4	34.2	1.5	6.5	137.2	46.8	1.9	6.5
30	144.2	44.4	2.6	6.5	-	32.6	-	6.5	-	45.4	-	6.5
60	142.1	42.6	3.4	6.5	-	31.2	-	6.5	-	43.8	-	6.5
90	142.1	40.8	3.4	6.5	-	29.6	-	6.5	-	42.8	-	6.5
120	140.7	39.4	3.8	6.5	-	28.2	-	6.5	-	41.2	-	6.5
150	137.9	38.2	3.8	6.5	137.9	26.6	3.4	6.5	-	39.8	2.6	6.5
FA test – pH = 7.5; FA = 1.0 mg N/L												
0	31.2	46.2	-	7.5	31.0	47.6	0.8	7.5	30.8	47	-	7.5
30	-	44.8	-	7.5	-	45.2	-	7.5	-	45.8	0.4	7.5
60	-	43.4	-	7.5	-	44.2	-	7.5	-	44.4	-	7.5
90	-	42.2	-	7.5	-	42.4	-	7.5	-	42.6	-	7.5
120	-	40.8	-	7.5	-	41.2	-	7.5	-	41.2	-	7.5
150	-	39.2	-	7.5	-	39.4	-	7.5	-	39.4	2.4	7.5
180	25.4	38.4	-	7.5	23.8	37.6	2.8	7.5	24.4	38.6	-	7.5

FA test – pH = 8.0; FA = 1.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	142.1	46.2	1.4	6.5	138.6	47.4	1.8	6.5	139.3	-	0.6	6.5
30	-	44.0	-	6.5	-	45.8	-	6.5	-	45.6	-	6.5
60	-	42.0	-	6.5	-	44.0	-	6.5	-	43.6	-	6.5
90	-	40.7	-	6.5	-	42.4	-	6.5	-	42.2	-	6.5
120	-	38.2	-	6.5	-	41.0	-	6.5	-	40.4	-	6.5
150	137.2	36.0	2.7	6.5	135.1	38.6	3.15	6.5	135.8	-	0.8	6.5
FA test – pH = 8.0; FA = 1.0 mg N/L												
0	13.2	46.6	-	8.0	13.6	44.6	-	8.0	14.1	46.8	0	8.0
30	-	45.4	-	8.0	-	43.8	-	8.0	-	45.2	-	8.0
60	-	44.4	-	8.0	-	42.2	-	8.0	-	44.2	-	8.0
90	-	43.0	-	8.0	-	41.4	-	8.0	-	43.0	-	8.0
120	-	42.0	-	8.0	-	40.0	-	8.0	-	41.8	-	8.0
150	-	40.6	-	8.0	-	39.0	-	8.0	-	41.0	-	8.0
180	6.9	39.2	2.0	8.0	8.0	37.6	1.0	8.0	8.7	40.4	1.5	8.0

FA test – pH = 6.5; FA = 2.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	136.5	46.0	0.8	6.5	136.5	46	0	6.5	133.0	47.6	0	6.5
30	-	44.2	-	6.5	-	43.8	-	6.5	-	46	-	6.5
60	-	42.2	-	6.5	-	41.8	-	6.5	-	44.2	-	6.5
90	-	40.6	-	6.5	-	40.6	-	6.5	-	42.4	-	6.5
120	-	38.8	-	6.5	-	38.2	-	6.5	-	41	-	6.5
150	135.1	37.6	1.2	6.5	131.6	36.6	1.2	6.5	130.2	39	1.2	6.5
FA test – pH = 6.5; FA = 2.0 mg N/L												
0	499.8	44.6	-	6.5	512.4	45.2	1.5	6.5	519.4	45.6	1.0	6.5
30	-	43.4	-	6.5	-	43.4	-	6.5	-	43.8	-	6.5
60	-	41.8	-	6.5	-	41.4	-	6.5	-	42	-	6.5
90	-	40.4	-	6.5	-	39.4	-	6.5	-	40	-	6.5
120	-	38.4	-	6.5	-	37.2	-	6.5	-	38.8	-	6.5
150	-	36.6	-	6.5	-	35.2	-	6.5	-	37.2	-	6.5
180	502.6	35.0	2.0	6.5	512.4	33.6	2.0	6.5	522.2	35.6	2.0	6.5

FA test – pH = 7.0; FA = 2.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	139.3	46.0	0.5	6.5	135.1	43.4	0.9	6.5	137.9	45.2	1.4	6.5
30	-	44.6	-	6.5	-	41.8	-	6.5	-	44	-	6.5
60	-	43.4	-	6.5	-	40.2	-	6.5	-	42.2	-	6.5
90	-	41.6	-	6.5	-	38.4	-	6.5	-	40.4	-	6.5
120	-	40.4	-	6.5	-	37	-	6.5	-	39.2	-	6.5
150	129.5	39.2	1.8	6.5	130.9	35.4	3.2	6.5	133.0	37.8	2.7	6.5
FA test – pH = 7.0; FA = 2.0 mg N/L												
0	171.9	-	-	7.0	171.1	50.2	-	7.0	178.2	52.4	-	7.0
30	-	49.4	-	7.0	-	47.8	-	7.0	-	50.4	-	7.0
60	-	48.4	-	7.0	-	45.8	-	7.0	-	48.4	-	7.0
90	-	46.0	-	7.0	-	43.8	-	7.0	-	47.2	-	7.0
120	-	44.6	-	7.0	-	41.2	-	7.0	-	45.0	-	7.0
150	-	-	-	7.0	-	39.0	-	7.0	-	43.0	-	7.0
180	170.1	40.8	-	7.0	171.1	37.2	-	7.0	168.3	41.2	-	7.0

FA test – pH = 7.5; FA = 2.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	130.9	43.4	1	6.5	133.7	42.8	1.0	6.5	135.1	44.0	1.0	6.5
30	-	42.4	-	6.5	-	41.4	-	6.5	-	42.6	-	6.5
60	-	40.8	-	6.5	-	39.6	-	6.5	-	41.4	-	6.5
90	-	39.6	-	6.5	-	38.0	-	6.5	-	39.4	-	6.5
120	-	38.2	-	6.5	-	36.4	-	6.5	-	38.0	-	6.5
150	129.5	37.6	1.5	6.5	128.1	35.0	2.0	6.5	130.9	36.8	1.5	6.5
FA test – pH = 7.5; FA = mg 2.0 N/L												
0	56.4	45.0	-	7.5	55.6	44.8	0	7.5	54.4	46.0	-	7.5
30	-	43.8	-	7.5	-	43.6	-	7.5	-	44.4	-	7.5
60	-	42.8	-	7.5	-	42.2	-	7.5	-	43.2	-	7.5
90	-	41.4	-	7.5	-	40.2	-	7.5	-	41.8	-	7.5
120	-	40.4	-	7.5	-	38.6	-	7.5	-	40.4	-	7.5
150	-	38.8	-	7.5	-	37.2	-	7.5	-	39.2	-	7.5
180	50.6	37.8	-	7.5	48.2	35.6	1.6	7.5	49.2	37.6	-	7.5

FA test – pH = 8.0; FA = 2.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	-	43.8	-	6.5	-	42.4	-	6.5	-	44.6	-	6.5
30	-	42.4	-	6.5	-	40.6	-	6.5	-	43.0	-	6.5
60	-	40.6	-	6.5	-	38.6	-	6.5	-	41.2	-	6.5
90	-	38.8	-	6.5	-	36.6	-	6.5	-	39.4	-	6.5
120	-	37.0	-	6.5	-	35.2	-	6.5	-	37.8	-	6.5
150	-	35.8	-	6.5	-	-	-	6.5	-	36.6	-	6.5
FA test – pH = 8.0; FA = 2.0 mg N/L												
0	22.8	45.6	-	8.0	22.6	45.4	-	8.0	23.2	46.0	-	8.0
30	-	43.8	-	8.0	-	44.0	-	8.0	-	44.6	-	8.0
60	-	42.6	-	8.0	-	42.8	-	8.0	-	43.4	-	8.0
90	-	41.6	-	8.0	-	41.8	-	8.0	-	42.2	-	8.0
120	-	40.4	-	8.0	-	40.8	-	8.0	-	41.8	-	8.0
150	-	39.0	-	8.0	-	39.2	-	8.0	-	40.2	-	8.0
180	16.2	38.0	-	8.0	16.4	38.0	-	8.0	17.3	39.4	-	8.0

FA test – pH = 6.5; FA = 5.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	141.4	46.0	0.6	6.5	139.3	44.8	0.3	6.5	137.9	46	0.9	6.5
30	-	44.2	-	6.5	-	43.2	-	6.5	-	44.4	-	6.5
60	-	42.8	-	6.5	-	41.6	-	6.5	-	42.8	-	6.5
90	-	41.2	-	6.5	-	40.2	-	6.5	-	40.8	-	6.5
120	-	39.6	-	6.5	-	38.8	-	6.5	-	39.4	-	6.5
150	-	38.2	2.4	6.5	-	37.6	2.4	6.5	135.1	38.2	2.4	6.5
FA test – pH = 6.5; FA = 5.0 mg N/L												
0	1399	47.0	-	6.5	1405	46.8	-	6.5	1443	47.4	-	6.5
30	-	45.8	-	6.5	-	45.6	-	6.5	-	46.0	-	6.5
60	-	44.2	-	6.5	-	44.0	-	6.5	-	44.0	-	6.5
90	-	43.2	-	6.5	-	42.8	-	6.5	-	42.6	-	6.5
120	-	42.2	-	6.5	-	41.6	-	6.5	-	41.4	-	6.5
150	-	40.6	-	6.5	-	40.2	-	6.5	-	40.0	-	6.5
180	1380	39.2	-	6.5	1418	38.8	-	6.5	1449	38.6	-	6.5

FA test – pH = 7.0; FA = 5.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	140.7	46.4	1.3	6.5	142.8	46.2	0.4	6.5	144.2	47	1.3	6.5
30	-	45.0	-	6.5	-	44.6	-	6.5	-	45.4	-	6.5
60	-	43.4	-	6.5	-	43.0	-	6.5	-	43.8	-	6.5
90	-	42.2	-	6.5	-	41.8	-	6.5	-	42.4	-	6.5
120	-	40.6	-	6.5	-	40.4	-	6.5	-	40.8	-	6.5
150	-	39.2	3.1	6.5	-	39.0	2.2	6.5	-	39.2	1.7	6.5
FA test – pH = 7.0; FA = 5.0 mg N/L												
0	455.4	47.4	-	7.0	475.2	47.8	-	7.0	473.4	47.6	-	7.0
30	-	45.4	-	7.0	-	46.2	-	7.0	-	46.0	-	7.0
60	-	44.2	-	7.0	-	44.4	-	7.0	-	44.4	-	7.0
90	-	42.6	-	7.0	-	42.6	-	7.0	-	43.0	-	7.0
120	-	41.0	-	7.0	-	40.2	-	7.0	-	41.0	-	7.0
150	-	39.8	-	7.0	-	38.4	-	7.0	-	39.4	-	7.0
180	453.6	37.4	-	7.0	491.4	36.2	-	7.0	462.6	38.0	-	7.0

FA test – pH = 7.5; FA = 5.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	-	38.8	-	6.5	137.9	45.4	1.0	6.5	-	45.4	-	6.5
30	-	36.8	-	6.5	-	43.8	-	6.5	-	43.8	-	6.5
60	-	35.4	-	6.5	-	42.2	-	6.5	-	41.8	-	6.5
90	-	34.2	-	6.5	-	41.0	-	6.5	-	40.0	-	6.5
120	-	32.6	-	6.5	-	39.4	-	6.5	-	38.2	-	6.5
150	-	31.2	-	6.5	133.7	38.2	1.5	6.5	-	36.6	-	6.5
FA test – pH = 7.5; FA = 5.0 mg N/L												
0	-	43.0	-	7.5	154.0	48.6	-	7.5	-	44.2	-	7.5
30	-	41.8	-	7.5	-	47.4	-	7.5	-	42.4	-	7.5
60	-	40.6	-	7.5	-	46.4	-	7.5	-	41.0	-	7.5
90	-	39.4	-	7.5	-	45.2	-	7.5	-	39.2	-	7.5
120	-	38.2	-	7.5	-	44.0	-	7.5	-	37.8	-	7.5
150	-	36.2	-	7.5	-	42.8	-	7.5	-	36.0	-	7.5
180	-	35.0	-	7.5	149.1	41.0	-	7.5	-	34.6	-	7.5

FA test – pH = 8.0; FA = 5.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	135.8	44.8	1.5	6.5	140.7	44.2	1.1	6.5	137.2	45.2	1.9	6.5
30	-	43.4	-	6.5	-	42.8	-	6.5	-	44.0	-	6.5
60	-	42.2	-	6.5	-	41.0	-	6.5	-	42.4	-	6.5
90	-	40.8	-	6.5	-	39.8	-	6.5	-	40.8	-	6.5
120	-	39.8	-	6.5	-	38.4	-	6.5	-	39.2	-	6.5
150	134.4	38.8	1.9	6.5	136.5	-	1.9	6.5	132.3	38.4	2.3	6.5
FA test – pH = 8.0; FA = 5.0 mg N/L												
0	48.8	47.6	-	8.0	49.6	42.4	-	8.0	49.8	43.4	-	8.0
30	-	46.6	-	8.0	-	41.6	-	8.0	-	42.0	-	8.0
60	-	45.8	-	8.0	-	40.4	-	8.0	-	40.8	-	8.0
90	-	44.8	-	8.0	-	39.2	-	8.0	-	39.4	-	8.0
120	-	43.6	-	8.0	-	38.0	-	8.0	-	38.2	-	8.0
150	-	42.4	-	8.0	-	36.8	-	8.0	-	36.6	-	8.0
180	41.8	41.0	-	8.0	43.4	35.6	-	8.0	43.4	34.8	-	8.0

FA test – pH = 6.5; FA = 10.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	-	43.8	-	6.5	145.6	46.0	-	6.5	148.4	46.4	-	6.5
30	137.9	42.6	-	6.5	-	44.6	-	6.5	-	44.8	-	6.5
60	-	41.0	-	6.5	-	43.2	-	6.5	-	43.8	-	6.5
90	-	40.2	-	6.5	-	41.8	-	6.5	-	42.4	-	6.5
120	-	39.0	-	6.5	-	40.2	-	6.5	-	41.0	-	6.5
150	135.1	37.6	-	6.5	141.4	39.2	-	6.5	141.4	39.8	-	6.5
FA test – pH = 6.5; FA =10.0 mg N/L												
0	2637	48.0	-	6.5	2745	48.8	-	6.5	2889	49.2	-	6.5
30	-	47.4	-	6.5	-	48.0	-	6.5	-	48.2	-	6.5
60	-	46.6	-	6.5	-	47.2	-	6.5	-	47.0	-	6.5
90	-	45.8	-	6.5	-	46.2	-	6.5	-	46.2	-	6.5
120	-	45.0	-	6.5	-	45.4	-	6.5	-	45.2	-	6.5
150	-	43.8	-	6.5	-	44.2	-	6.5	-	43.8	-	6.5
180	2727	43.0	-	6.5	-	43.4	-	6.5	2817	43.2	-	6.5

FA test – pH = 7.0; FA = 10.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	135.8	42.4	-	6.5	137.9	44.0	-	6.5	138.6	44.6	-	6.5
30	-	40.8	-	6.5	-	42.8	-	6.5	-	42.8	-	6.5
60	-	39.8	-	6.5	-	41.0	-	6.5	-	42.0	-	6.5
90	-	38.4	-	6.5	-	39.8	-	6.5	-	40.4	-	6.5
120	-	37.2	-	6.5	-	38.0	-	6.5	-	39.0	-	6.5
150	134.4	36.0	-	6.5	135.1	37.0	-	6.5	135.8	37.6	-	6.5
FA test – pH = 7.0; FA = 10.0 mg N/L												
0	855.0	46.2	-	7.0	877.5	45.0	-	7.0	-	-	-	-
30	-	45.2	-	7.0	-	43.6	-	7.0	-	-	-	-
60	-	44.0	-	7.0	-	42.2	-	7.0	-	-	-	-
90	-	42.6	-	7.0	-	40.8	-	7.0	-	-	-	-
120	-	41.2	-	7.0	-	39.0	-	7.0	-	-	-	-
150	-	40.0	-	7.0	-	38.0	-	7.0	-	-	-	-
180	859.5	39.0	-	7.0	900.0	36.8	-	7.0	-	-	-	-

FA test – pH = 7.5; FA = 10.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	137.9	46.0	2.5	6.5	142.1	46.4	2.5	6.5	141.4	46.8	2.5	6.5
30	-	44.4	-	6.5	-	45.4	-	6.5	-	45.4	-	6.5
60	-	43.0	-	6.5	-	44.0	-	6.5	-	44.2	-	6.5
90	-	41.8	-	6.5	-	42.4	-	6.5	-	43.2	-	6.5
120	-	40.4	-	6.5	-	41.2	-	6.5	-	41.4	-	6.5
150	133.7	39.0	3.0	6.5	137.2	39.6	3.0	6.5	140.0	40.2	3.0	6.5
FA test – pH = 7.5; FA = 10.0 mg N/L												
0	285.1	48.0	-	7.5	286.4	46.6	-	7.5	292.8	46.8	-	7.5
30	-	46.4	-	7.5	-	44.6	-	7.5	-	45.6	-	7.5
60	-	45.2	-	7.5	-	43.6	-	7.5	-	44.2	-	7.5
90	-	43.4	-	7.5	-	41.8	-	7.5	-	42.6	-	7.5
120	-	42.2	-	7.5	-	40.2	-	7.5	-	41.6	-	7.5
150	-	40.8	-	7.5	-	39.0	-	7.5	-	40.2	-	7.5
180	264.5	39.4	-	7.5	273.5	37.4	-	7.5	289.0	38.8	-	7.5

FA test – pH = 8.0; FA = 10.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	137.9	45.8	1.1	6.5	138.6	47.8	1.5	6.5	140.0	46.0	-	6.5
30	-	44.4	-	6.5	-	46.2	-	6.5	-	45.0	-	6.5
60	-	43.0	-	6.5	-	44.8	-	6.5	-	43.6	-	6.5
90	-	41.6	-	6.5	-	43.2	-	6.5	-	42.4	-	6.5
120	-	40.0	-	6.5	-	42.2	-	6.5	-	41.2	-	6.5
150	133.7	39.0	3.0	6.5	133.0	41.0	2.7	6.5	133.7	40.0	-	6.5
FA test – pH = 8.0; FA = 10.0 mg N/L												
0	100.8	46.4	0	8.0	98.0	45.2	-	8.0	98.8	43.2	-	8.0
30	-	45.0	-	8.0	-	44.2	-	8.0	-	42.0	-	8.0
60	-	43.8	-	8.0	-	42.8	-	8.0	-	40.8	-	8.0
90	-	43.0	-	8.0	-	41.6	-	8.0	-	39.8	-	8.0
120	-	41.0	-	8.0	-	40.2	-	8.0	-	38.6	-	8.0
150	-	39.8	-	8.0	-	38.8	-	8.0	-	37.2	-	8.0
180	94.0	37.8	0.5	8.0	92.8	37.4	-	8.0	-	35.8	-	8.0

FA test – pH = 7.0; FA = 25.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	130.2	41		6.5	128.8	42.2	-	6.5	130.2	42.8	-	6.5
30	-	40	-	6.5	-	40.8	-	6.5	-	41.6	-	6.5
60	-	38.6	-	6.5	-	40.2	-	6.5	-	40	-	6.5
90	-	36.8	-	6.5	-	38.2	-	6.5	-	38.8	-	6.5
120	-	35.4	-	6.5	-	36.8	-	6.5	-	37.4	-	6.5
150	119.7	34.4	-	6.5	120.4	35.4	-	6.5	123.2	36.6	-	6.5
FA test – pH = 7.0; FA = 25.0 mg N/L												
0	1926	45.6	-	7.0	2061	45.8	-	7.0	1971	46.2	-	7.0
30	-	44.8	-	7.0	-	44.8	-	7.0	-	45.6	-	7.0
60	-	39.8	-	7.0	-	39.8	-	7.0	-	40.6	-	7.0
90	-	38.6	-	7.0	-	38.6	-	7.0	-	39.4	-	7.0
120	-	37.2	-	7.0	-	37.2	-	7.0	-	38.2	-	7.0
150	1917	36.4	-	7.0	1980	36.2	-	7.0	2025	37.2	-	7.0
180	-	-	-	-	-	-	-	-	-	-	-	-

FA test – pH = 7.5; FA = 25.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	124.6	46.4	0.2	6.5	128.1	47.0	0.2	6.5	119.7	46.6	0.2	6.5
30	-	44.0	-	6.5	-	45.2	-	6.5	-	44.6	-	6.5
60	-	43.0	-	6.5	-	44.0	-	6.5	-	43.4	-	6.5
90	-	41.8	-	6.5	-	42.8	-	6.5	-	41.6	-	6.5
120	-	40.2	-	6.5	-	41.2	-	6.5	-	40.0	-	6.5
150	117.6	39.6	1.6	6.5	123.2	39.7	1.4	6.5	113.4	38.8	2.0	6.5
FA test – pH = 7.5; FA = 25.0 mg N/L												
0	636.0	46.4	0.4	7.5	-	-	-	-	546.0	46.0	1.0	7.5
30	-	45.6	-	7.5	-	-	-	-	-	45.0	-	7.5
60	-	44.4	-	7.5	-	-	-	-	-	44.0	-	7.5
90	-	43.2	-	7.5	-	-	-	-	-	42.2	-	7.5
120	-	42.0	-	7.5	-	-	-	-	-	41.0	-	7.5
150	-	40.8	-	7.5	-	-	-	-	-	39.6	-	7.5
180	615.0	39.6	1.6	7.5	-	-	-	-	612.0	38.0	2.8	7.5

FA test – pH = 8.0; FA = 25.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	138.6	44.4	-	6.5	-	-	-	6.5	141.4	45	-	6.5
30	-	42.8	-	6.5	-	-	-	6.5	-	43.2	-	6.5
60	-	41.2	-	6.5	-	-	-	6.5	-	41.8	-	6.5
90	-	40	-	6.5	-	-	-	6.5	-	40.4	-	6.5
120	-	38.8	-	6.5	-	-	-	6.5	-	39.2	-	6.5
150	133.7	37.2	-	6.5	-	-	-	6.5	135.8	37.6	-	6.5
FA test – pH = 8.0; FA = 25.0 mg N/L												
0	248.4	46.4	-	8.0	-	-	-	8.0	259.2	44.6	-	8.0
30	-	45.0	-	8.0	-	-	-	8.0	-	43.4	-	8.0
60	-	44.0	-	8.0	-	-	-	8.0	-	42.2	-	8.0
90	-	42.8	-	8.0	-	-	-	8.0	-	41.2	-	8.0
120	-	42.2	-	8.0	-	-	-	8.0	-	39.6	-	8.0
150	-	40.4	-	8.0	-	-	-	8.0	-	38.6	-	8.0
180	226.8	38.8	-	8.0	-	-	-	8.0	252.0	37.2	-	8.0

FA test – pH = 7.0; FA = 50.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	130.2	40.6		6.5	127.4	43.2	-	6.5	130.9	43	-	6.5
30	-	38.8	-	6.5	-	41.6	-	6.5	-	41.2	-	6.5
60	-	37	-	6.5	-	40.2	-	6.5	-	39.8	-	6.5
90	-	35.6	-	6.5	-	39	-	6.5	-	38.4	-	6.5
120	-	34.2	-	6.5	-	37.4	-	6.5	-	37	-	6.5
150	123.9	32.6	-	6.5	121.8	36	-	6.5	126.7	35.6	-	6.5
FA test – pH = 7.0; FA = 50.0 mg N/L												
0	3798	46	-	7.0	4068	46.4	-	7.0	3798	46.2	-	7.0
30	-	45.4	-	7.0	-	46	-	7.0	-	45.6	-	7.0
60	-	44.8	-	7.0	-	45.2	-	7.0	-	44.6	-	7.0
90	-	44.4	-	7.0	-	44.8	-	7.0	-	44.4	-	7.0
120	-	43.6	-	7.0	-	43.8	-	7.0	-	43.4	-	7.0
150	-	42.8	-	7.0	-	43.2	-	7.0	-	42.8	-	7.0
180	4032	42.6	-	7.0	4050	42.8	-	7.0	4212	42.8	-	7.0

FA test – pH = 7.5; FA = 50.0 mg N/L

Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	118.3	44.8	0.4	6.5	121.1	46.8	0.6	6.5	121.8	47.2	0.4	6.5
30	-	43.6	-	6.5	-	45.6	-	6.5	-	45.4	-	6.5
60	-	42.0	-	6.5	-	43.8	-	6.5	-	44.0	-	6.5
90	-	40.8	-	6.5	-	42.4	-	6.5	-	42.8	-	6.5
120	-	39.0	-	6.5	-	40.6	-	6.5	-	41.2	-	6.5
150	114.1	38.0	1.8	6.5	117.6	40.0	1.6	6.5	118.3	40.8	1.6	6.5
FA test – pH = 7.5; FA = 50.0 mg N/L												
0	1086	45.2		7.5	1218	45.4	0.6	7.5	-	-	-	-
30	-	44.6	-	7.5	-	44.4	-	7.5	-	-	-	-
60	-	43.6	-	7.5	-	43.8	-	7.5	-	-	-	-
90	-	42.6	-	7.5	-	42.6	-	7.5	-	-	-	-
120	-	42.0	-	7.5	-	41.8	-	7.5	-	-	-	-
150	-	40.6	-	7.5	-	40.8	-	7.5	-	-	-	-
180	1170	39.8	-	7.5	1200	40.0	1.4	7.5	-	-	-	-

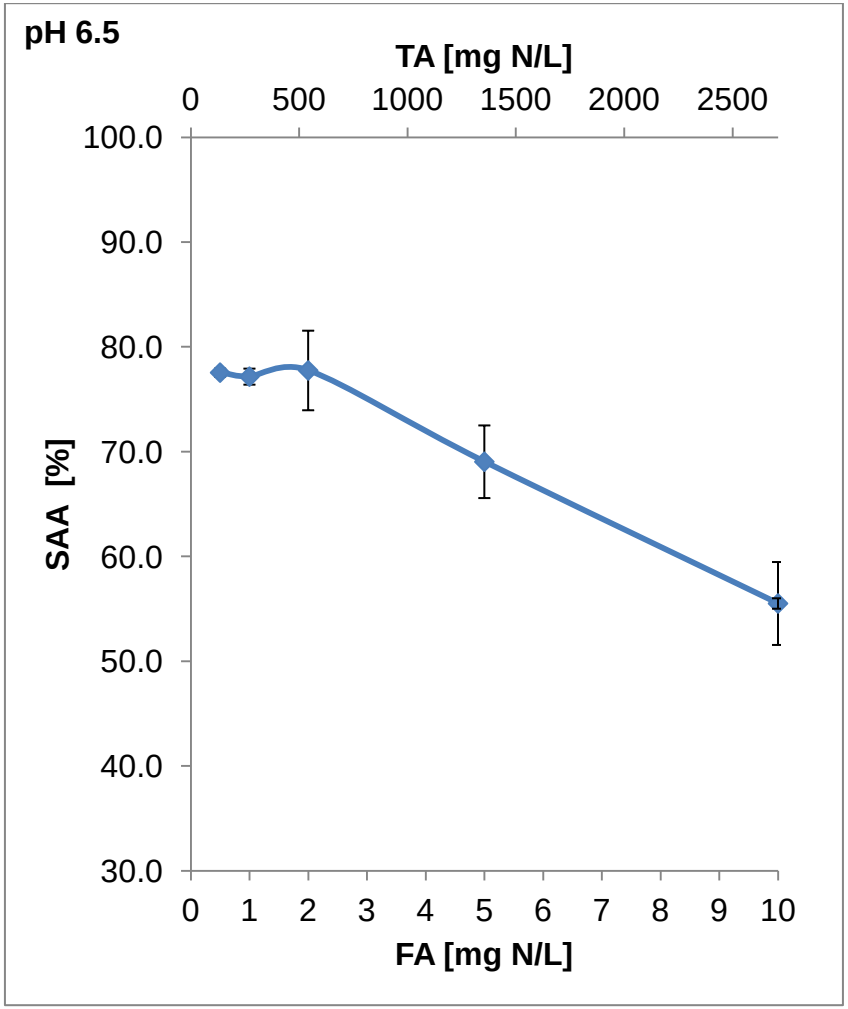
FA test – pH = 8.0; FA = 50.0 mg N/L

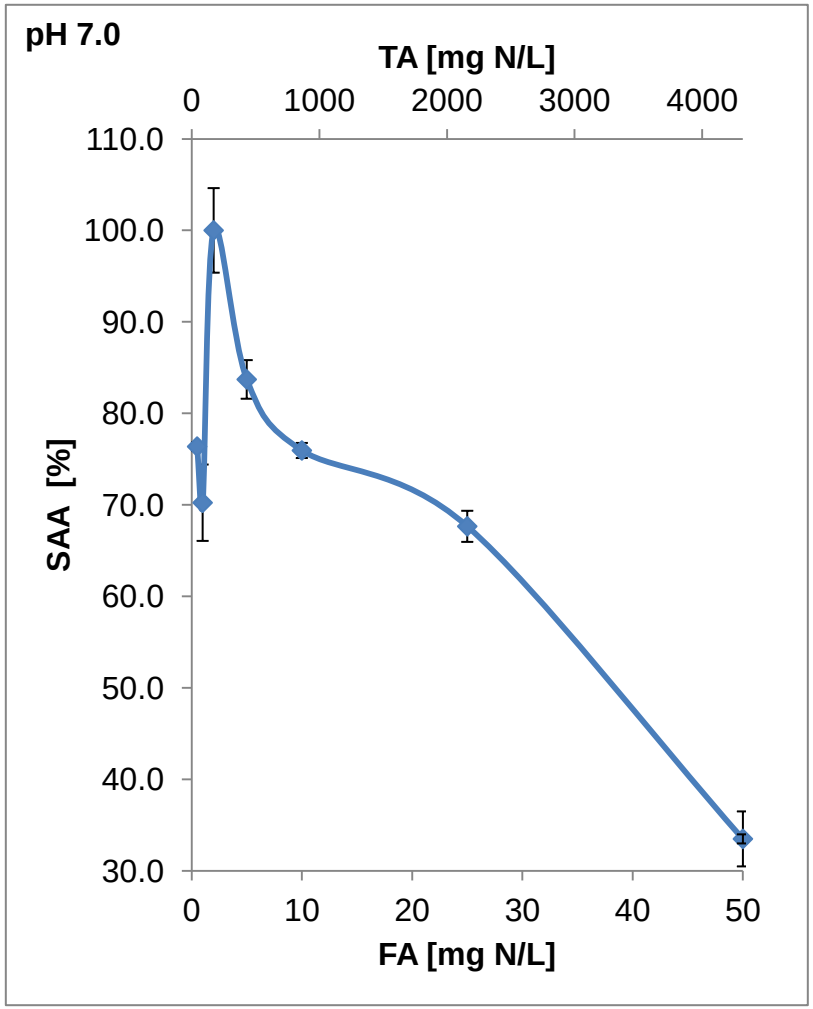
Baseline test												
	1				**2**				**3**			
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**pH**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**
0	138.6	44.0	3.8	6.5	143.5	44.2	-	6.5	144.2	45.0	-	6.5
30	-	42.6	-	6.5	-	43.0	-	6.5	-	43.8	-	6.5
60	-	41.4	-	6.5	-	41.8	-	6.5	-	42.8	-	6.5
90	-	40.0	-	6.5	-	40.4	-	6.5	-	41.8	-	6.5
120	-	38.8	-	6.5	-	39.2	-	6.5	-	40.6	-	6.5
150	135.1	37.4	5.2	6.5	140.0	38.0	-	6.5	140.0	39.2	-	6.5
FA test – pH = 8.0; FA = 50.0 mg N/L												
0	488.3	46.4	-	8.0	508.5	47.8	-	8.0	510.8	45.6	-	8.0
30	-	45.8	-	8.0	-	47.4	-	8.0	-	44.4	-	8.0
60	-	45.0	-	8.0	-	46.2	-	8.0	-	43.4	-	8.0
90	-	44.2	-	8.0	-	45.6	-	8.0	-	42.6	-	8.0
120	-	43.6	-	8.0	-	44.8	-	8.0	-	42.0	-	8.0
150	-	43.0	-	8.0	-	44.0	-	8.0	-	41.4	-	8.0
180	571.5	42.4	-	8.0	603.0	43.4	-	8.0	600.8	40.6	-	8.0

Results of the Test 7a – R1 after normalization in respect to the highest SAA obtained under any condition (pH 7.0, FA 2.0 mg N/L)

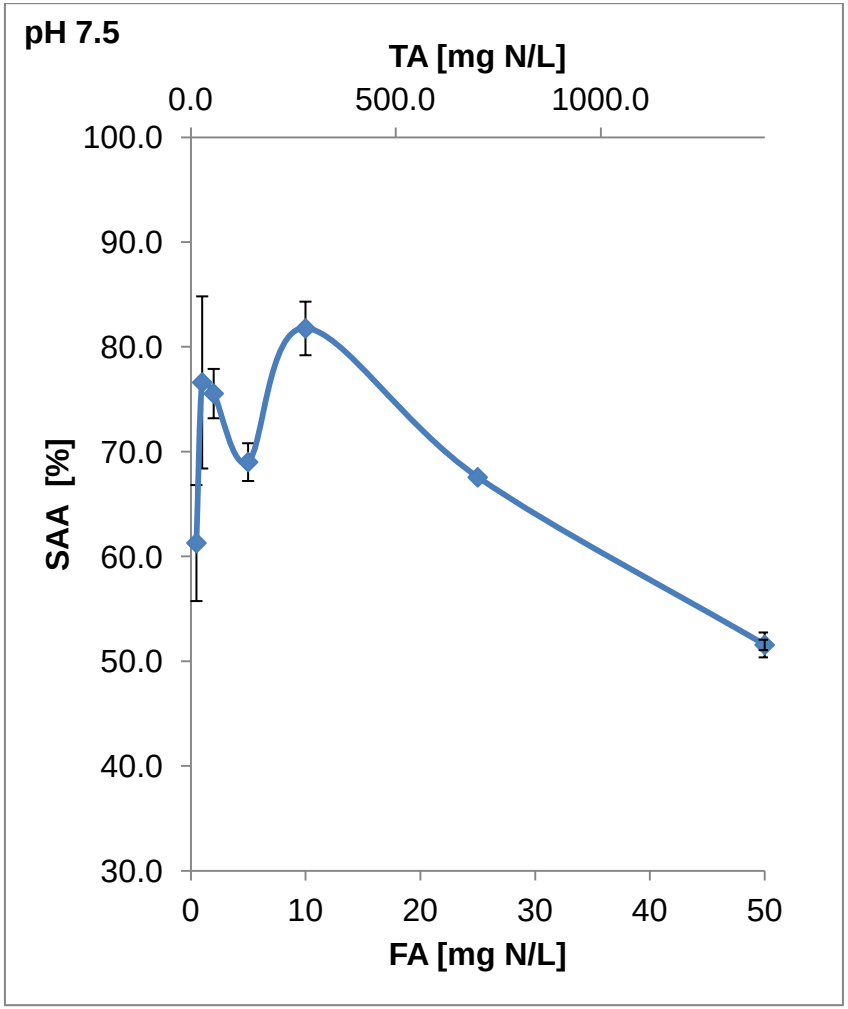
FA	pH 6.5	SD	pH 7	SD	pH 7.5	SD	pH 8	SD
[mg N/L]	[%]	[%]	[%]	[%]	[%]	[%]	[%]	[%]
0.5	77.6	0.4	76.4	0.5	61.3	5.5	35.5	5.3
1	77.2	0.8	70.2	4.2	76.6	8.2	52.2	3.3
2	77.8	3.8	**100.0**	4.6	75.6	2.4	51.5	2.6
5	69.0	3.5	83.7	2.1	69.0	1.8	67.8	4.3
10	55.5	4.0	76.0	0.8	81.8	2.6	77.1	1.3
25	-	-	67.7	1.7	67.6	0.1	67.5	0.9
50	-	-	33.5	3.0	51.6	1.2	47.7	6.0

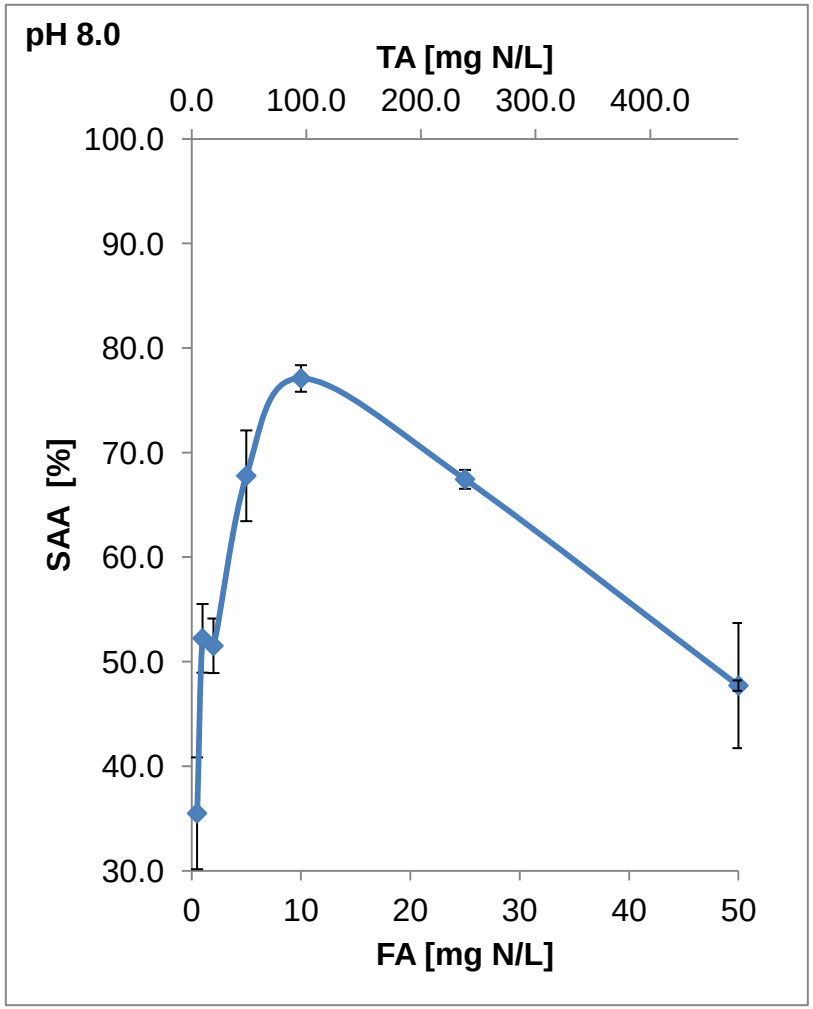
Graphical representation of results from Test 7a – R1 (pH 6.5 and pH 7.0)





Graphical representation of results from Test 7a – R1 (pH 7.5 and pH 8.0)



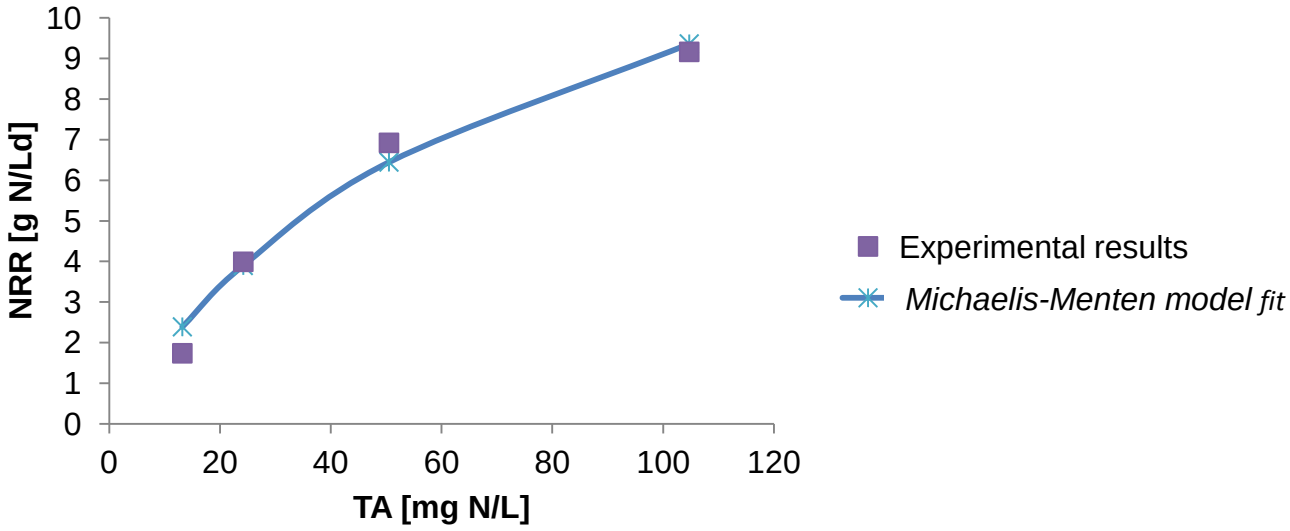


Immediate nitrogen removal rate (NRR) response to TA tested in R1 under FA concentrations below 2 mg N/L at constant pH of 6.5

Nitrogen loading 1 – Regular reactor operation on day 46 during Test 6 – R1								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[hours]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	50.5	112.7	80.5	0.18	6.5	6919	1.39	0.23
Nitrogen loading 2 – set at time 0								
1.5	87.9	173	71	0.31	6.5	-	-	-
2	99.3	190	66	0.35	6.5	-	-	-
2.5	105	201	64	0.38	6.5	-	-	-
3	107	206	64	0.38	6.5	-	-	-
3.5	106	206	62	0.38	6.5	-	-	-
4	107	208	62	0.38	6.5	-	-	-
4.5	106	207	62	0.38	6.5	-	-	-
5	106	207	62	0.38	6.5	-	-	-
5.5	105	207	64	0.38	6.5	9160	1.33	0.22
6	105	209	63	0.38	6.5	9146	1.33	0.22
6.5	104	208	63	0.37	6.5	9175	1.32	0.22
Average	104.7	208.0	63.3	0.37	-	9160	1.33	0.22
SD	0.6	1.0	0.6	0.00	-	15	0.00	0.00

Nitrogen concentrations and pH in the feed during the test								
-	397	596	0	-	7.4			
Table continuation - Nitrogen loading 3								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**
[hour]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**
0	29.8	107.1	85.4	0.11	6.5	-	-	-
0.5	23.9	102.2	87.5	0.09	6.5	-	-	-
1	23.7	100.8	88.2	0.08	6.5	-	-	-
1.5	24	100.1	88.2	0.09	6.5	-	-	-
2	23.3	100.8	87.5	0.08	6.5	3996	1.37	0.24
2.5	24.6	100.1	88.9	0.09	6.5	3986	1.38	0.24
3	24.8	100.1	88.9	0.09	6.5	3984	1.38	0.24
Average	24.2	100.3	88.4	0.09	-	3989	1.38	0.24
SD	0.8	0.4	0.8	0.00	-	6	0.00	0.00
Nitrogen loading 4 – set at time 3								
3.5	14.4	77.7	97.4	0.05	6.5	-	-	-
4	11.2	74.9	98.9	0.04	6.5	-	-	-
4.5	12.5	75.6	96.7	0.04	6.5	1739	1.40	0.26
5	13.5	76.3	97.4	0.05	6.5	1734	1.40	0.26
5.5	13.5	74.9	98.1	0.05	6.5	1736	1.41	0.26
Average	13.2	75.6	97.4	0.05	-	1736	1.40	0.26
SD	0.6	0.7	0.7	0.00	-	3	0.00	0.00
Nitrogen concentrations and pH in the feed during the test								
-	388	602	0	-	7.4			

Graphical representation of results for immediate nitrogen removal rate (NRR) response to TA tested in R1 under FA concentrations below 2 mg N/L at constant pH of 6.5



Kinetic parameters for the Michaelis-Menten model fit: NRR_{max} = 16 g N/Ld; K_{TA} = 76 mg N/L

Immediate gas production rate (GPR) response to free ammonia (FA) tested in R3 (Test 7b – R3)

	FA tested							
Test A	**1.1**	**1.1**	**0.3**	**0.3**	**0.6**	**0.6**	**1.3**	**1.4**
Feed N-NO2 [mg N/L]	56.1							
End N-NO2 [mg N/L]	36.9	37.2	38	37.8	38.1	37.2	37.2	38.4
pH start	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95
pH end	6.86	6.83	6.78	6.77	6.77	6.78	6.8	6.83
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell A-1	Cell A-2	Cell A-3	Cell A-4	Cell A-5	Cell A-6	Cell A-7	Cell A-8
0.00	0	0	0	0	0	0	0	0
0.17	1.46	0.77	0.14	1.4	1.36	1.25	1.43	1.25
0.33	2.52	1.82	1.18	2.4	2.4	2.18	2.55	2.19
0.50	3.4	2.68	1.99	3.25	3.21	3.16	3.44	2.9
0.67	4.11	3.37	2.67	3.98	3.93	3.96	4.16	3.48
0.83	4.77	3.96	3.31	4.57	4.52	4.63	4.78	3.97
1.00	5.3	4.5	3.81	5.11	5.06	5.25	5.32	4.37
1.17	5.79	4.96	4.26	5.56	5.51	5.74	5.81	4.68
1.33	6.28	5.41	4.67	6.01	5.97	6.27	6.3	5
1.50	6.72	5.82	5.12	6.42	6.37	6.76	6.75	5.31
1.67	7.16	6.23	5.57	6.83	6.78	7.21	7.15	5.62
1.83	7.56	6.64	5.89	7.23	7.14	7.65	7.55	5.84
2.00	8	7.01	6.3	7.59	7.55	8.1	7.96	6.15
2.17	8.4	7.37	6.66	7.96	7.91	8.5	8.36	6.38
2.33	8.75	7.78	7.02	8.32	8.32	8.9	8.76	6.6
2.50	9.15	8.14	7.34	8.68	8.63	9.3	9.12	6.82

Table continuation: Test A								
Time [hours]	**1.1**	**1.1**	**0.3**	**0.3**	**0.6**	**0.6**	**1.3**	**1.4**
2.67	9.41	8.42	7.61	8.9	8.95	9.61	9.43	6.96
2.83	9.77	8.74	7.93	9.27	9.31	9.92	9.79	7.14
3.00	***10.08***	***9.05***	***8.24***	***9.54***	***9.63***	***10.28***	***10.06***	***7.27***
3.17	***10.43***	***9.37***	***8.52***	***9.85***	***9.94***	***10.64***	***10.42***	***7.45***
3.33	***10.7***	***9.6***	***8.79***	***10.12***	***10.22***	***10.95***	***10.68***	***7.58***
3.50	***10.96***	***9.83***	***9.01***	***10.31***	***10.44***	***11.17***	***10.91***	***7.67***
3.67	***11.27***	***10.15***	***9.29***	***10.62***	***10.76***	***11.53***	***11.22***	***7.8***
3.83	***11.62***	***10.51***	***9.65***	***10.98***	***11.12***	***11.93***	***11.58***	***8.03***
4.00	***11.93***	***10.83***	***9.92***	***11.25***	***11.44***	***12.28***	***11.89***	***8.16***

Numbers in bold and italic format were used for GPR calculation

	FA tested							
Test B	**3.1**	**3.4**	**6.5**	**6.7**	**15.1**	**15.8**	**27.0**	**28.9**
Feed N-NO2 [mg N/L]	56.1							
End N-NO2 [mg N/L]	40.2	51.9	39.9	38.7	45.9	44.4	51	50.7
pH start	6.95	6.95	6.95	6.95	6.95	6.95	6.95	6.95
pH end	6.79	6.83	6.81	6.82	6.78	6.8	6.73	6.76
	Cumulative volume recorded during the test in mL							
Time [hours]	Cell A-1	Cell A-2	Cell A-3	Cell A-4	Cell A-5	Cell A-6	Cell A-7	Cell A-8
0.00	0	0	0	0	0	0	0	0
0.17	1.24	1.23	1.22	1.22	1.22	1.2	1.3	1.34
0.33	2.12	2.09	2.13	2.12	2.08	2	2.1	2.19

Table continuation: Test B								
Time [hours]	**3.1**	**3.4**	**6.5**	**6.7**	**15.1**	**15.8**	**27.0**	**28.9**
0.50	2.83	2.73	2.85	2.89	2.76	2.63	2.77	2.9
0.67	3.4	3.23	3.44	3.48	3.3	3.07	3.26	3.39
0.83	3.93	3.64	3.94	4.02	3.71	3.47	3.67	3.84
1.00	4.38	3.91	4.39	4.47	4.07	3.78	3.93	4.1
1.17	4.73	4.19	4.76	4.88	4.38	4.05	4.16	4.37
1.33	5.13	4.41	5.16	5.29	4.66	4.27	4.38	4.59
1.50	5.53	4.64	5.57	5.7	4.93	4.54	4.56	4.82
1.67	5.88	4.82	5.93	6.06	5.2	4.76	4.74	5
1.83	6.23	4.96	6.25	6.37	5.38	4.94	4.87	5.13
2.00	6.63	5.19	6.61	6.73	5.6	5.16	5.05	5.31
2.17	6.94	5.32	6.98	7.1	5.83	5.38	5.19	5.49
2.33	7.29	5.46	7.34	7.41	6.06	5.56	5.32	5.57
2.50	7.6	5.55	7.66	7.77	6.24	5.79	5.41	5.71
2.67	7.82	5.6	7.88	8	6.37	5.87	5.45	5.75
2.83	8.13	5.6	8.2	8.27	6.51	6.05	5.5	5.75
3.00	***8.4***	***5.64***	***8.47***	***8.59***	***6.69***	***6.19***	***5.59***	***5.84***
3.17	***8.66***	***5.64***	***8.74***	***8.86***	***6.83***	***6.32***	***5.63***	***5.89***
3.33	***8.93***	***5.64***	***8.97***	***9.09***	***6.96***	***6.45***	***5.63***	***5.93***
3.50	***9.11***	***5.64***	***9.2***	***9.27***	***7.05***	***6.54***	***5.63***	***5.93***
3.67	***9.37***	***5.64***	***9.47***	***9.54***	***7.19***	***6.68***	***5.68***	***5.98***
3.83	***9.68***	***5.64***	***9.78***	***9.85***	***7.37***	***6.85***	***5.81***	***6.11***
4.00	***9.95***	***5.64***	***10.06***	***10.12***	***7.5***	***6.99***	***5.81***	***6.15***

Immediate specific nitrogen removal rate (sNRR) response to free ammonia (FA) tested in R3 (Test 7c – R3)

Test 7c – R3 – pH 7.0 (baseline condition – regular cycle)								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.0	-	-	-
5	123.9	18.6	54.2	-	7.0	-	-	-
15	140.0	37.7	54.7	-	7.0	-	-	-
25	150.5	51.5	53.2	1.7	7.0	1.96	1.27	0.26
35	159.6	62.5	49.9	1.8	7.0	1.99	1.32	0.17
45	166.6	70.7	50.2	1.9	7.0	1.87	1.34	0.33
55	147.0	45.4	53.9	1.6	7.0	2.28	1.29	0.19
65	129.5	23.2	55.9	1.5	7.0	2.08	1.27	0.12
75	112.7	5.6	53.5	-	7.0	-	-	-
85	109.2	0.0	54.0	-	7.0	-	-	-
			Average	1.7	-	2.03	1.30	0.21
			SD	0.2	-	0.15	0.03	0.08
Nitrogen concentrations and pH in the feed during the test								
-	383.4	351	0	-	6.8			

Test 7c – R3 – pH 7.5								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	7.5	-	-	-
5	123.2	17.5	51.7	-	7.5	-	-	-
15	139.3	39.7	49.9	-	7.5	-	-	-
25	151.9	55.9	48.9	5.3	7.5	1.93	1.27	0.28
35	162.4	69.4	45.9	5.6	7.5	2.02	1.26	0.17
45	172.2	79.8	44.3	6.0	7.5	1.99	1.43	0.24
55	156.1	57.6	49.4	5.4	7.5	1.83	1.38	0.31
65	137.9	35.7	51.4	4.8	7.5	2.10	1.20	0.11
75	121.8	14.4	54.3	4.2	7.5	1.90	1.32	0.18
85	109.9	0.0	55.7	-	7.5	-	-	-
			Average	5.2	-	1.96	1.31	0.22
			SD	0.6	-	0.09	0.08	0.08
Nitrogen concentrations and pH in the feed during the test								
-	383.4	351	0	-	6.8			

Test 7c – R3 – pH 8.0								
Time	**NH_4-N**	**NO_2-N**	**NO_3-N**	**FA**	**pH**	**sNRR**	**NO_2/NH_4**	**NO_3/NH_4**
[min]	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[-]**	**[mg N/mg VSS d]**	**[mg N/mg N]**	**[mg N/mg N]**
0	-	-	-	-	8.0	-	-	-
5	124.6	22.1	47.8	-	8.0	-	-	-
15	155.4	60.3	43.2	-	8.0	-	-	-
25	177.1	88.9	37.9	18.0	8.0	1.11	1.18	0.07
35	194.6	111.3	33.5	19.8	8.0	1.21	1.25	0.02
45	210.0	128.8	30.7	21.4	8.0	1.20	1.70	0.16
55	203.0	119.0	31.2	20.7	8.0	0.90	1.40	0.07
65	196.0	107.1	32.5	20.0	8.0	0.97	1.70	0.19
75	189.7	97.3	34.3	19.3	8.0	0.79	1.56	0.29
85	182.7	87.5	35.2	18.6	8.0	0.88	1.40	0.13
95	175.7	75.6	37.9	17.9	8.0	0.89	1.70	0.39
105	171.5	65.5	39.3	17.5	8.0	0.72	2.42	0.32
115	161.7	55.0	41.0	16.5	8.0	1.02	1.06	0.18
			Average	19.0	-	0.97	1.54	0.18
			SD	1.5	-	0.17	0.38	0.12
Nitrogen concentrations and pH in the feed during the test								
-	383.4	351	0	-	6.8			

b) Long-term anammox response to FA

Long-term specific nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R2 (Test 8a –R2)

Time	pH	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0.00	7.8	72.1	18.3	38.4	11.3	2172	1.19	0.14	4.8
1.00	7.8	74.2	18.1	39.0	11.3	2163	1.20	0.14	5.0
2.29	7.8	75.6	17.6	34.4	11.3	2184	1.07	0.12	5.0
2.29	8.0	75.6	17.6	34.4	11.3	2184	1.07	0.12	7.7
3.00	8.0	93.8	27.9	35.9	11.3	2071	1.10	0.13	9.6
3.08	8.0	98.7	31.2	34.2	11.3	2047	1.11	0.12	10.1
3.17	8.0	103.6	31.6	32.8	11.3	2032	1.13	0.12	10.6
3.25	8.0	111.3	37.9	31.6	11.3	1984	1.14	0.12	11.3
4.00	8.0	150.5	87.5	21.7	11.3	1747	1.17	0.10	15.3
4.08	8.0	158.2	94.5	21.7	11.3	1692	1.18	0.10	16.1
4.17	8.0	165.9	101.5	20.3	11.3	1642	1.19	0.10	16.9
4.25	8.0	170.1	103.6	22.4	11.3	1610	1.20	0.11	17.3
5.25	8.0	256.2	170.8	12.4	11.3	1091	1.28	0.09	26.1
6.31	8.0	301.0	233.8	3.1	11.3	720	1.21	0.04	30.7
7.00	8.0	315.0	247.8	3.1	11.3	614	1.25	0.04	32.1
7.01	7.0	317.8	243.6	0.0	11.3	631	1.36	0.00	3.6
7.02	7.0	310.8	236.6	4.6	11.3	667	1.33	0.06	3.5
7.04	7.0	303.8	226.8	4.6	11.3	730	1.33	0.05	3.4

7.06	7.0	299.6	214.2	7.7	11.3	781	1.41	0.09	3.4
7.08	7.0	288.4	203.0	9.3	11.3	860	1.37	0.09	3.2
Table continuation - Test 8a –R2									
Time	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
7.12	7.0	268.8	173.6	14.8	11.3	1024	1.39	0.12	3.0
7.17	7.0	247.8	147.0	19.8	11.3	1184	1.37	0.14	2.8
7.21	7.0	232.4	124.9	24.4	11.3	1308	1.38	0.16	2.6
7.25	7.0	219.8	109.1	26.5	11.3	1407	1.37	0.16	2.5
8.00	7.0	116.2	13.4	49.8	11.3	2056	1.21	0.19	1.3
8.04	7.0	115.5	13.4	49.4	11.3	2061	1.21	0.18	1.3
8.08	7.0	114.1	13.2	49.7	11.3	2066	1.21	0.18	1.3

Long-term nitrogen removal rate (NRR) response to elevated free ammonia (FA) concentrations tested in R1 and R2 (Test 8b – R1 and R2)

- *Results for R1*

Time	pH	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0.00	6.5	110.1	9.7	46.6	14.9	2780	1.28	0.18	0.4
1.00	6.5	107.8	8.1	52.9	14.9	2809	1.24	0.19	0.4
1.02	8.0	107.1	12.3	52.3	14.9	2795	1.23	0.19	10.9
1.04	8.0	108.5	13.7	51.2	14.9	2786	1.23	0.19	11.1
1.08	8.0	116.2	14.5	50.1	14.9	2750	1.26	0.19	11.8
1.13	8.0	111.3	15.5	49.1	14.9	2774	1.23	0.18	11.3
1.17	8.0	111.3	16.0	48.4	14.9	2774	1.23	0.18	11.3
1.21	8.0	112.0	16.4	47.5	14.9	2774	1.23	0.18	11.4
1.25	8.0	112.7	16.7	46.7	14.9	2773	1.23	0.17	11.5
1.29	8.0	114.1	17.4	46.2	14.9	2765	1.24	0.17	11.6
2.00	8.0	181.3	60.6	37.5	14.9	2466	1.26	0.16	18.5
2.08	8.0	184.1	68.3	35.2	14.9	2425	1.24	0.15	18.8
2.17	8.0	190.4	75.6	33.0	14.9	2368	1.24	0.15	19.4
3.00	8.0	263.2	141.4	23.2	14.9	1648	1.44	0.16	26.8
3.08	8.0	270.2	156.8	19.6	14.9	1554	1.40	0.14	27.5
3.17	8.0	280.0	170.8	17.8	14.9	1445	1.40	0.14	28.5
4.00	8.0	340.2	261.8	1.7	14.9	810	1.29	0.02	34.7
4.08	8.0	347.2	268.8	0.0	14.9	749	1.32	0.00	35.4

Table continuation – results for R1 - Test 8b – R1 and R2									
Time	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
5.00	8.0	376.2	300.6	0.0	14.9	492	1.29	0.00	38.3
6.00	8.0	388.8	318.6	0.0	14.9	304	1.43	0.00	39.6
6.21	6.5	417.6	333.0	0.0	14.9	Close to 0	-	-	1.5
7.00	6.5	416.0	333.0	0.0	14.9	Close to 0	-	-	1.5
7.21	7.0	415.8	334.8	0.0	14.9	Close to 0	-	-	4.7
8.00	7.0	424.8	334.8	0.0	14.9	Close to 0	-	-	4.8
8.08	7.0	414.0	329.4	0.0	14.9	Close to 0	-	-	4.6
8.17	7.0	412.2	333.0	0.0	14.9	Close to 0	-	-	4.6
8.25	7.0	406.8	331.2	0.0	14.9	107	1.40	0.00	4.6
8.29	7.0	369.0	271.8	0.0	14.9	590	1.54	0.00	4.1
9.00	7.0	211.4	63.8	41.5	14.9	2227	1.40	0.20	2.4
10.00	7.0	144.9	17.1	57.7	14.9	2771	1.37	0.22	1.6
11.00	6.5	139.3	16.2	61.1	14.9	2865	1.28	0.22	0.5
12.00	6.5	149.8	14.8	56.6	14.9	2898	1.23	0.20	0.5
13.00	6.5	151.2	13.1	59.3	14.9	2814	1.24	0.21	0.5
14.00	6.5	165.2	12.2	60.3	14.9	2860	1.26	0.21	0.6

- *Results for R2*

Time	pH	NH_4-N	NO_2-N	NO_3-N	Flow	NRR	NO_2/NH_4	NO_3/NH_4	FA
[day]	[-]	[mg N/L]	[mg N/L]	[mg N/L]	[L/d]	[mg N/Ld]	[mg N/mg N]	[mg N/mg N]	[mg N/L]
0.00	6.5	109.5	10.1	46.8	14.9	2780	1.27	0.18	0.4
1.00	6.5	109.2	9.2	52.3	14.9	2800	1.25	0.19	0.4
1.02	8.0	112.0	15.7	51.2	14.9	2759	1.24	0.19	11.4
1.04	8.0	110.6	17.4	49.7	14.9	2765	1.22	0.18	11.3
1.08	8.0	111.3	18.8	47.3	14.9	2767	1.22	0.17	11.3
1.13	8.0	112.7	21.1	45.9	14.9	2755	1.22	0.17	11.5
1.17	8.0	112.7	21.5	45.0	14.9	2758	1.22	0.17	11.5
1.21	8.0	115.5	23.6	44.5	14.9	2736	1.22	0.17	11.8
1.25	8.0	116.2	24.6	43.9	14.9	2730	1.22	0.16	11.8
1.29	8.0	119.7	27.7	43.0	14.9	2702	1.23	0.16	12.2
2.00	8.0	212.1	98.7	27.1	14.9	2175	1.26	0.13	21.6
2.08	8.0	213.5	112.7	22.3	14.9	2122	1.20	0.11	21.7
2.17	8.0	223.3	123.2	20.5	14.9	2030	1.21	0.11	22.7
3.00	8.0	289.8	197.4	10.0	14.9	1303	1.29	0.08	29.5
3.08	8.0	305.2	203.0	8.9	14.9	1204	1.43	0.09	31.1
3.17	8.0	308.0	208.6	7.1	14.9	1171	1.42	0.07	31.4
4.00	8.0	357.0	278.6	0.0	14.9	652	1.38	0.00	36.4
4.08	8.0	358.4	281.4	0.0	14.9	631	1.36	0.00	36.5
5.00	8.0	399.6	324.0	0.0	14.9	259	1.64	0.00	40.7
6.00	8.0	406.8	336.6	0.0	14.9	125	2.50	0.00	41.4
6.21	6.5	430.0	336.6	0.0	14.9	Close to 0	-	-	1.5
7.00	6.5	432.0	338.4	0.0	14.9	Close to 0	-	-	1.5

Table continuation – results for R2 - Test 8b – R1 and R2									
Time	**pH**	**NH_4-N**	**NO_2-N**	**NO_3-N**	**Flow**	**NRR**	**NO_2/NH_4**	**NO_3/NH_4**	**FA**
[day]	**[-]**	**[mg N/L]**	**[mg N/L]**	**[mg N/L]**	**[L/d]**	**[mg N/Ld]**	**[mg N/mg N]**	**[mg N/mg N]**	**[mg N/L]**
7.21	7.0	426.6	336.6	0.0	14.9	Close to 0	-	-	4.8
8.00	7.0	437.4	331.2	0.0	14.9	Close to 0	-	-	4.9
8.08	7.0	424.8	325.8	0.0	14.9	Close to 0	-	-	4.8
8.17	7.0	415.8	327.6	0.0	14.9	Close to 0	-	-	4.7
8.25	7.0	405.0	325.8	0.0	14.9	143	1.67	0.00	4.5
8.29	7.0	374.4	262.8	0.0	14.9	608	1.96	0.00	4.2
9.00	7.0	213.5	39.8	52.6	14.9	2280	1.53	0.26	2.4
10.00	7.0	146.3	14.1	57.1	14.9	2782	1.39	0.22	1.6
11.00	6.5	139.3	12.8	59.8	14.9	2888	1.29	0.21	0.5
12.00	6.5	154.7	15.1	56.2	14.9	2874	1.25	0.20	0.6
13.00	6.5	156.8	13.3	57.6	14.9	2794	1.27	0.21	0.6
14.00	6.5	164.5	10.6	59.9	14.9	2874	1.26	0.21	0.6