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The SHARON-anammox process for the treatment of ammonium-rich liquid residues produced by the anaerobic digestion of municipal solid wastes: a preliminary evaluation.

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“*The only source of knowledge is experience.”*

Albert Einstein

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RESUMO

Este estudo tem como objetivo fornecer uma avaliação preliminar do processo SHARON-anammox quando este é aplicado ao tratamento de resíduos líquidos ricos em amónio produzidos pela digestão anaeróbica (AD) dos resíduos sólidos municipais.

Os dois reatores, SHARON e anammox, foram alimentados com um influente sintético de forma a promover a aclimatização da biomassa; O SHARON foi alimentado com NH_4-N (1500 mg/L), enquanto o anammox foi alimentado com NH_4-N e NO_2-N (698 e 802 mg/L, respetivamente) num rácio molar de 1.15 (NO_2-N/NH_4-N). A performance do processo foi avaliada com diferentes condições operatórias: no reator SHARON, o Tempo de Retenção Hidráulico (HRT) foi progressivamente reduzido de 1.5 para 1 dias; no reator anammox, o *Nitrogen Loading Rate* (NLR) foi aumentado gradualmente de 1 para 1.5 kgN/m^3·d.

Esta avaliação foi realizada através de medições de amónio (NH_4-N), nitritos (NO_2-N) e nitratos (NO_3-N) no efluente usando um método colorimétrico modificado (IRSA-CNR) e cromatografia iónica. Estas medições provaram que ambos os processos se adaptam muito bem às diferentes condições operatórias incluindo as mais severas (HRT de 1 dia para o SHARON; NLR de 1.5 kgN/m^3·d para o anammox): obtendo uma conversão de amónio de 46.4± 2.3% para o SHARON e uma eficiência de remoção de azoto de 89.0± 0.9% para o anammox.

Também foi monitorizada a evolução dos conteúdos sólidos dentro do reator anammox, obtendo um VSS (sólidos voláteis suspensos) com uma média de 4.78± 0.67gVSS/L para o alvo pretendido. Foram realizados ainda ensaios cinéticos de forma a determinar-se as taxas volumétricas e especificas para o consumo de NH_4-N e NO_2-N e a produção de NO_3-N. As taxas específicas obtidas para um NLR de 1.5 kg N/m^3·d foram 0.26± 0.03 gNO_2-N/gVSS·d, 0.20± 0.02 gNH_4-N/gVSS·d e $2.15 \cdot 10^{-3} \pm 9.30 \cdot 10^{-4}$ gNO_3-N/gVSS·d. A concentração da biomassa não mudou significativamente durante o período experimental e os ensaios cinéticos demonstraram que as taxas consumo de NH_4-N e NO_2-N e produção de NO_3-N seguiram uma tendência de crescimento, indicando uma boa atividade e aclimatização da biomassa.

Tanto para o SHARON como para o anammox, os resultados são promissores e encorajam a substituição do influente sintético para águas residuais reais.

Palavras-Chave: Água residual sintética; Amónio; Digestão Anaeróbica; Nitrito; Remoção de Azoto; SHARON-anammox

ABSTRACT

This study aims to provide a preliminary evaluation of the SHARON-anammox process applied to the treatment of ammonium-rich liquid residues produced by the anaerobic digestion (AD) of municipal solid wastes.

A synthetic influent was fed to both SHARON and anammox reactors, to promote biomass acclimation; the SHARON reactor was fed with NH_4-N (1500 mg/L), while the anammox reactor with NH_4-N and NO_2-N (698 and 802 mg/L, respectively) in a 1.15 molar ratio (NO_2-N/NH_4-N). Process performance was evaluated with different operating conditions: as to the SHARON, the hydraulic retention time (HRT) was progressively reduced from 1.5 to 1 days; as to the anammox, the nitrogen loading rate NLR was gradually increased from 1 to 1.5 kg $N/m^3 \cdot d$.

The analysis of ammonium (NH_4-N), nitrites (NO_2-N) and nitrates (NO_3-N) concentrations in the effluent proved that both the SHARON and the anammox processes can adapt very well to the different operating conditions, including the harshest ones (target HRT of 1 day for SHARON; NLR of 1.5 kg $N/m^3 \cdot d$ for anammox): an ammonium conversion of 46.4±2.3% for SHARON and a nitrogen removal efficiency of 89.0±0.9% for anammox were achieved.

Moreover, the evolution of solids content in the anammox reactor was monitored, getting an average VSS (volatile suspended solids) concentration of 4.78±0.67 gVSS/L. The kinetic assays were also carried out to determine NH_4-N and NO_2-N removal and NO_3-N production volumetric and specific rates. The specific rates obtained for a NLR of 1.5 kg $N/m^3 \cdot d$ were 0.26±0.03 gNO_2-N/gVSS·d, 0.20±0.02 gNH_4-N/gVSS·d and $2.15 \cdot 10^{-3} \pm 9.30 \cdot 10^{-4}$ gNO_3-N/gVSS·d. Biomass concentration did not change significantly during the experimental campaign, and the kinetic assays demonstrated an increasing trend, indicating the process may be capable of enduring a higher NLR.

For both the SHARON and anammox processes, results are promising and encourage the replacement of the synthetic influent with real wastewater.

Keywords: Anaerobic digestion; Ammonium; Nitrite; Nitrogen removal; SHARON-anammox; Synthetic wastewater

ABBREVIATIONS

AD- Anaerobic Digestion

Anammox- Anaerobic AMMOnium OXidation

AOB- Ammonium Oxidizing Bacteria

BOD- Biological Oxygen Demand

CANON- Completely Autotrophic Nitrogen Removal Over Nitrite

COD- Chemical Oxygen Demand

C_{ORG}- Organic Carbon

CSTR- Continuous Stirred Tank Reactors

DO- Dissolved Oxygen

EGSB- Expanded Granual Sludge Bed

HRT- Hydraulic Retention Time

Hy.Me.Ca project- Hydrogen, Methane, Compost and Ammonium project

MBR- Membrane Bio-Reactor

MSW- Municipal Solid Waste

NAS- New Activated Sludge

NitDR- Nitrite Discharge Rate

NLR- Nitrogen Loading Rate

NOB- Nitrite Oxidizing Bacteria

NRE-Nitrogen Removal Efficiency

NRR- Nitrogen Removal Rate

ORP- Oxidation Reduction Potential

PN/A- Partial Nitritation/ Anammox

SBR- Sequencing Batch Reactor

SHARON- Single reactor System for High Ammonia Removal Over Nitrite

SNRR- Specific Nitrogen Removal Rate

SRT- Sludge Retention Time

TSS- Total Suspended Solids

UASB- Up flow Anaerobic Sludge Blanket

VSS- Volatile Suspended Solids

WWTP- Wastewater Treatment Plant

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1. INTRODUCTION

1.1 The Hy.Me.C.A. project

The Hy.Me.C.A. (Hydrogen, Methane, Compost and Ammonia) project aims at the development of an integrated system for the biological combined anaerobic production of H_2 and CH_4 from the organic fraction of municipal solid wastes (MSW), the valorisation of solid residues by aerobic bio-oxidation (composting) and the treatment of ammonium-rich liquid residues by an advanced biological process based on double stage partial nitritation/anaerobic ammonium oxidation (SHARON-anammox).

In particular, the possibility to force the conventional anaerobic digestion (AD) of MSW for the sequential recovery of energy as H_2 and CH_4, rather than only CH_4, would strongly improve the net environmental benefit; moreover, final solid residues can achieve an economical value by going through aerobic composting, after being dried and mixed with a bulking material. Although it represents an interesting option, there are still several drawbacks related to the AD process, like the instability due to the intrinsic heterogeneity of organic residues, the difficult hydrolysis of solid and complex substrates in the early stage of the process, the need for water recirculation (in wet systems) and, last but not least, the resulting ammonium-rich liquid residues (approx. 1,000-1,500 mgN/L) which must be properly treated before their discharge in the environment.

1.2 The SHARON-anammox process

For such ammonium-rich liquid streams, conventional biological treatments based on nitrification and denitrification are not suitable. On the other hand, chemical-physical processes (e.g., steam stripping) are very expensive. For these reasons, the research activity is currently focusing on the development of advanced biological treatments based on double stage partial nitritation (SHARON, Single reactor for High activity Ammonium Removal Over Nitrite) and anaerobic ammonium oxidation (anammox).

In the SHARON process, only 50% of the influent ammonium is oxidized to nitrite; the effluent from the SHARON reactor is fed to the anammox reactor, where the residual ammonium and nitrite are anaerobically converted to dinitrogen gas (N_2), with a small production of nitrate.

This double stage process has a higher nitrogen removal efficiency and lower energy costs (-1.8 kWh/kgN), CO_2 emissions (-4 kgCO_2/kgN) and surplus sludge production (80-90% less) compared to the conventional nitrification-denitrification process. Moreover, since this process is completely autotrophic, there is no need for external carbon sources. The combination of partial nitritation and anammox therefore represents an interesting alternative to chemical-physical treatments like steam stripping (-6 €/kgN).

1.3 Goals of this Thesis

The general aim of this Thesis is to provide a preliminary evaluation of the applicability of the SHARON-anammox process to the treatment of the ammonium-rich liquid fraction of the AD digestate from the organic fraction of municipal solid wastes. The results will contribute in setting the starting point for the implementation of compact treatment systems with low environmental impact to support the energy recovery of AD process, in order to minimize its polluting emissions.

The results were achieved by starting up the SHARON and the anammox reactors under controlled conditions in terms of temperature, pH, nitrogen loading rate, and using a synthetic influent to simulate the real wastewater. An evaluation of the effects of different hydraulic retention times (HRT) and nitrogen loading rates (NLR) on the overall process performance in terms of nitrogen removal efficiencies was carried out.

1.4 Structure of the Thesis

Following this introduction, an overview of conventional methods used to treat wastewaters containing ammonium is provided in Chapter 2, together with a deeper description of the advanced SHARON-anammox process.

The methodology and materials used to carry out the experimental activity are presented in Chapter 3.

In Chapter 4, the results of the experimental activity are presented and discussed.

The general conclusions are given in Chapter 5, together with recommendations for further research activity.

2. LITERATURE REVIEW

Nitrogen in wastewater is of great concern and represents a serious environmental problem, since its accumulation can lead to eutrophication and cause oxygen depletion in coastal waters, lakes and rivers (Ahn 2006). Reducing the nitrogen concentration to an acceptable level is of extreme importance.

Presently there are several methods to remove nitrogen from wastewater. Some of the traditional methods are biological nitrification and denitrification, ammonia stripping, chemical precipitation with magnesium ammonium phosphate and electrochemical conversion, with the first two being the most important (Lin et al. 2009).

2.1 Conventional nitrification-denitrification

Biological treatment for nitrogen removal is performed through two steps: in the first step (nitrification, Equation 1) ammonium is converted into nitrite by ammonium oxidizing bacteria (AOB, e.g. *Nitrosomonas* sp.) and subsequently into nitrate by nitrite oxidizing bacteria (NOB, e.g. *Nitrobacter* sp.); in the second step (denitrification, Equation 2) nitrate is converted into dinitrogen gas by heterotrophic bacteria. The overall reaction is described by (Equation 3) (Van Hulle et al. 2010; Metcalf & Eddy 1991; Wang et al. 2006).

$$NH_4{}^+ + 2O_2 + 2HCO_3{}^- \rightarrow NO_3{}^- + 3H_2O + 2CO_2 \quad \text{Eq. 1}$$

$$5C + 4NO_3{}^- + 2H_2O \rightarrow CO_2 + 4HCO_3{}^- + 2N_2 \quad \text{Eq. 2}$$

$$4NH_4{}^+ + 8O_2 + 5C + 4HCO_3 \rightarrow 2N_2 + 10H_2O + 9CO_2 \quad \text{Eq. 3}$$

Figure 1 presents a simplified block scheme of a typical compact WWTP: the wastewater is mechanically pre-treated, and denitrification is performed before nitrification to enhance the efficiency of the process and reduce the operating costs, since a post-denitrification would require a higher supply of external organic carbon (C_{ORG}) (Metcalf & Eddy 1991; ENVI-PUR n.d.).

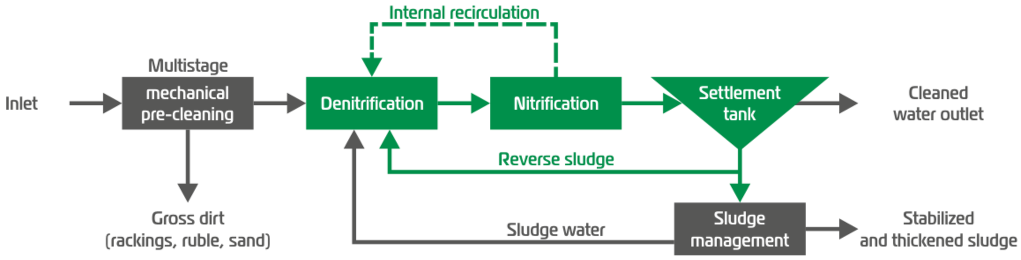


Figure 1- Simplified block scheme of a typical compact WWTP with pre-denitrification and nitrification tanks.

In such system, nitrates produced in the aerobic nitrification tank are recirculated through the internal recirculation line into the anoxic denitrification reactor, where they are reduced to N_2 gas by heterotrophic bacteria (which use the organic carbon contained in the wastewater as carbon source, and nitrates as the electron acceptors). In the aerobic nitrification tank, the oxidation of ammonium to nitrate occurs, together with the oxidation of the residual organic matter contained in the wastewater. This biological oxidation of organic compounds and ammonia nitrogen takes place in presence of dissolved oxygen and in one single reactor (to cut costs related to the process). The activated sludge is then sent to the settling tank where the cleaned water is separated from the activated sludge solids (Metcalf & Eddy 1991; ENVI-PUR n.d.).

This process was proved to be very advantageous due to its high potential efficiency, high process stability and reliability, relatively easy control, and moderate costs. All of this made conventional biological nitrification-denitrification the most common method to remove nitrogen from wastewater; however, this method can only be applied to treat low nitrogen loading rates, since high concentrations of free ammonia (NH_3) are toxic for the microorganisms (Milia et al. 2015a). Moreover, high concentration of ammonium would require more energy to oxidize ammonium to nitrate, and a larger amount of external carbon source may be needed for the heterotrophic denitrification, therefore the cost-effectiveness of this process would be lower. Another drawback associated with this process is that it is relatively slow, due to a low microbial activity and yield (Ahn 2006; Van Hulle et al. 2010).

Since this process is not very efficient when wastewater has a high content of ammonium, chemical-physical methods are usually employed.

2.2 Ammonia Stripping

Ammonia stripping is one of the most commonly applied chemical-physical methods for the treatment of wastewaters rich in ammonium. In ammonia stripping, ammonium is removed from the wastewater through volatilization as free ammonia (NH_3). The liquid phase (wastewater) is brought in contact with a gas, usually air, that drags undesirable substances present in the liquid. To enhance the conversion of ammonium into a gaseous form, the process needs to be operated using lime to increase pH (10.5-11.5) (Wang et al. 2006).

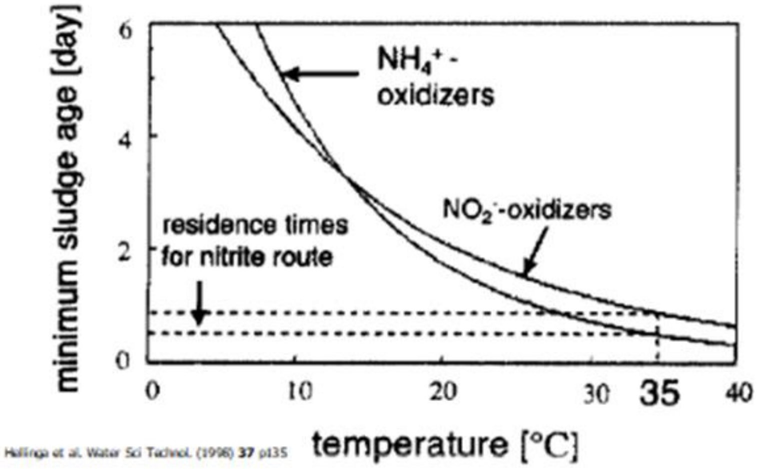
This process has a simple concept but also several advantages: it can be controlled for selected ammonia removals, may be able to meet total nitrogen standards, it is not sensitive to toxic substances and it’s the most applicable process when it’s required to combine a lime system for phosphorus removal. (Metcalf & Eddy 1991).

However ammonia stripping also has serious drawbacks, like it’s sensitivity to temperature (due to the increase of ammonia solubility with lower temperatures), the occurrence of fogging and icing in cold weather, air pollution (which may be caused by the reaction between ammonia and sulfur dioxide), the requirement of lime for controlling the pH, which increases the treatment cost and lime-related operating and maintenance problems, carbonate scaling of packing and piping and potential noise and aesthetic problems (Metcalf & Eddy 1991). The ammonia stripping also has elevated costs in the investment period and during equipment life, turning this into a very expensive method who is best used when the wastewater has a high content of ammonia.

In recent years new biological methods to address the removal of ammonia from wastewater have been studied, some of them include nitritation (e.g. SHARON), anammox and a combination of these two.

2.3 The SHARON process

The SHARON process (Single reactor system for High Ammonia Removal Over Nitrite) was developed at the Technical University of Delft, to stop the ammonium oxidation at nitrite (nitritation). This process is also the first successful commercial technique by which nitrification/denitrification occurs with nitrite as intermediate under stable conditions (Van Kempen et al. 2001). This process uses a single aerated reactor without any biomass retention and recirculation (chemostat), and operates at high temperature (above 25 ºC). The high temperature has an important advantage, since it enables high specific growth rate so that no sludge retention is required (the reactor can be operated as a chemostat, therefore the solids retention time and the hydraulic retention time are the same), turning this process very stable. Above 25 °C (optimal range is 35-45 °C), the maximum specific growth rate of the desired ammonium oxidizer bacteria (AOB) is higher than that of the undesired nitrite oxidizer bacteria (NOB), therefore the selection of AOB can be achieved by properly regulating the hydraulic retention time (which is equal to the solids retention time), as can be seen in Figure 2 (Hellinga et al. 1998).



Hellinga et al. Water Sci Technol. (1998) 37 p135

Figure 2- Effect of temperature on minimum sludge age.

In order to obtain a stable nitritation (Equation 4), the operating variables (namely temperature, pH, hydraulic retention time, substrate concentration and dissolved oxygen) are strictly controlled (Hellinga et al. 1998; Randall et al. 1984). However, the control of these variables can be difficult in large-scale operations. The denitrification (with added methanol) in the SHARON process is normally required for pH control and alkalinity production, allowing for a partial compensation of the acidifying effect in the nitrification phase (Equation 5) (Ahn 2006; Grontmij n.d.(a)).

The Sharon-denitrification stoichiometric reactions are given below (Equations 4, 5 and 6):

$$2NH_4{}^+ + 3O_2 \rightarrow 2NO_2{}^- + 4H^+ + 2H_2O \text{ Eq.4}$$

$$2NO_2{}^- + 4.8g\ COD + 2H^+ \rightarrow N_2 + 1.8g\ sludge \text{ Eq.5}$$

$$2NH_4{}^+ + 3O_2 + 4.8g\ COD\ \rightarrow N_2 + 2H^+ + 1.8\ g\ sludge \text{ Eq.6}$$

Even though this process is not suited to treat all wastewaters due to a high temperature dependency, it is ideally suited to remove nitrogen from wastewaters with high concentration of ammonia (>0.5g N/L) and can be operated in a simple continuous stirred tank reactor. Moreover, the SHARON process is suitable for the pre-treatment of several highly concentrated industrial wastewaters (Hellinga et al. 1998; Jetten et al. 1997a; Van Dongen et al. 2001). Figure 3 shows a schematic representation of how a SHARON reactor operates (Notenboom et al. 2002).

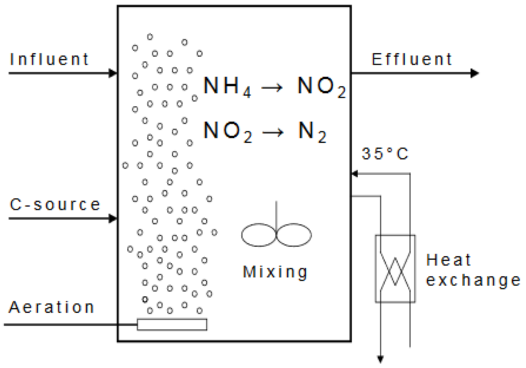


Figure 3- Schematic representation of a SHARON reactor.

The control of pH is very important to prevent the inhibition of the process, since the oxidation of ammonium is an acidifying reaction (Van Kempen et al. 2001) and the nitrite oxidizers are extremely sensitive to a changing pH. If the pH drops below 6.5, the ammonium oxidation will no longer occur because of the pH-dependent equilibrium between the concentrations of NH_3 (which is the actual substrate for AOB) and NH_4^+: at low pH, the free ammonia concentration is insufficient for the growth of the ammonium oxidizers (Hellinga et al. 1998; Khin & Annachhatre 2004). On the other hand, in case the pH is above 8, there are higher amounts of NH_3, who are apparently toxic for ammonium oxidizers and nitrite oxidizers (Anthonisen AC et al. 1976). According to Van Dongen et al. (2001), it is possible to change the ammonium/nitrite ratio in the effluent of the SHARON by properly changing the pH set point in the reactor.

Compared to the ammonium oxidizers, the nitrite oxidizers have a lower affinity for oxygen, so a low dissolved oxygen (DO) concentration is more restrictive for the growth of the nitrite oxidizers (Van Hulle et al. 2010). Different concentrations of ammonium in the effluent are achieved depending on the aerobic retention time (Van Kempen et al. 2001). The ammonia oxidizers are able to tolerate high concentrations of nitrite (>0.5 g NO_2-N/L) at a neutral pH (Jetten et al. 1997a; Van Dongen et al. 2001).

The sludge retention time (SRT) is equal to the hydraulic retention time (HRT) which means that there is no need for any external control for the mixed liquor suspended solids concentration. SHARON is also able to adjust to strong variations in feed conditions without the intervention of an operator and recover from process disturbances in hours. As long as the operations are carried at elevated temperatures and within a certain pH (pH above 7), the SHARON is the most practicable process for reducing significantly the concentration of ammonium in ammonium-rich wastewaters, achieving a nitrogen removal efficiency above 95% (Grontmij n.d.(a)) (Grontmij n.d. (b)).

Some disadvantages of this process are due to the low affinity of AOB for ammonium. In addition this ammonium oxidizers can be inhibited by concentrations higher than 0.2 mg of HNO_2. But compared to other processes (like for example air stripping and conventional nitrification/denitrification), the SHARON was found to be less complex and more flexible, with considerably lower investment and operational costs (Khin & Annachhatre 2004). For example, the cost for aeration required for nitritation, the carbon source (e.g., methanol) required for the denitrification and the sludge production are lower (-25%, -40% and -30%, respectively) when compared to the conventional nitrification/denitrification process, due to the conversion of ammonium to nitrite (not to nitrate) involved in this process.(Grontmij n.d.; Van Hulle et al. 2010; Grontmij (2) n.d.)

The SHARON process has proven to be successful when applied to further improve the main WWTP nitrogen effluent quality.

2.4 The anammox process

ANaerobic AMMonium OXidation (anammox) is a recently discovered microbial pathway in the biological nitrogen cycle and a new cost-effective way to remove ammonia from wastewater (Khin & Annachhatre 2004). It is also a lithoautotrophic biological conversion process (Ahn et al. 2006). Mulder et al. (1995) observed unexplainable nitrogen losses in denitrifying fluidized bed reactors and put forward the idea of this being attributed to anammox. Previously, the oxidation of ammonium was thought to be caused by ammonium oxidizers under aerobic and oxygen-limited conditions. However it was reported that ammonium was oxidized by ammonium oxidizers under anoxic conditions, when gaseous nitrogen dioxide (NO_2) was present (Schmidt & Bock 1997). Also, the ammonium oxidizers are able to denitrify, with ammonium as the electron donor under oxygen-limited conditions (hydrogen or organic compounds act as electron donors when denitrification occurs under anoxic conditions). Lately, through Equations 7 and 8, it was observed that the nitrite was the preferred electron acceptor for the anammox process. The ΔG0' is negative for both reactions (Equation 7 and Equation 8) meaning that they proceed spontaneously (van de Graaf et al. 1995; Bock et al. 1995; Khin & Annachhatre 2004).

$$5NH_4{}^{+} + 3NO_3{}^{-} \rightarrow 4N_2 + 9H_2O + 2H^{+} \text{ Eq. 7}$$

$$\Delta G^{0\prime} = -297\, kJ\, (mol\, NH_4{}^{+})^{-1}$$

$$NH_4{}^{+} + NO_2{}^{-} \rightarrow N_2 + 2H_2O \text{ Eq.8}$$

$$\Delta G^{0^{\prime}} = -358\, kJ\, (mol\, NH_4{}^{+})^{-1}$$

Although N_2 is the main product of anaerobic ammonium oxidation, 10% of the N-feed is converted to $NO_3{}^{-}$. The $NH_4{}^{+}$: $NO_2{}^{-}$ conversion ratio is 1:1.31 ±0.06, and the $NH_4{}^{+}$: $NO_3{}^{-}$ conversion ratio is 1:0.22 ±0.02 (van de Graaf et al. 1996). The overall mass stoichiometry reaction of anammox was determined by making the mass balances over different anammox enrichment cultures, and it is shown by Equation 9 (Van Hulle et al. 2010):

$$NH_4{}^{+} + 1.32\ NO_2{}^{-} + 0.066\ HCO_3{}^{-} + 0.13\ H^{+} \rightarrow 1.02\ N_2 + 0.26\ NO_3{}^{-} + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad \text{Eq.9}$$

This process involves the oxidation of ammonium into dinitrogen gas in absence of oxygen (Strous et al. 1998). Both hydrazine and hydroxylamine were identified as important intermediates (Khin & Annachhatre 2004; Van Hulle et al. 2010). Also this process is based on energy conservation from anaerobic ammonium oxidation with nitrite as the electron acceptor and with no addition of external carbon source, since anammox bacteria are autotrophic (Jetten et al. 1997a; Jetten et al. 2001). So the main carbon source for the growth of anammox bacteria is carbon dioxide (van de Graaf et al. 1996).

Anammox activity was reported in different wastewater treatment plants, from installations which treat wastewater with high nitrogen load at low dissolved oxygen concentrations, to even municipal wastewater treatment plants (Siegrist et al. 1998; Chouari et al. 2003). Anammox is also responsible for contributing significantly to the world's nitrogen cycle as it is found in several seas and rivers (Van Hulle et al. 2010). It can be attributed to anammox up to 70% (depending on the organic load) of the N_2 production in marine sediments (Dalsgaard & Thamdrup 2002). The anaerobic ammonium oxidation reaction can be carried out by different types of bacteria as seen in Table 1. These organisms have a very similar optimum pH and temperature (Van Hulle et al. 2010; Strous et al. 1999).

Table 1- Anammox bacterial species.

Genus	Species	Source
Brocadia	*Candidatus Brocadia anammoxidans*	Wastewater
	Candidatus Brocadia fulgida	Wastewater
Kuenenia	*Candidatus Kuenenia stuttgartiensis*	Wastewater
Scalindua	*Candidatus Scalindua brodae*	Wastewater
	Candidatus Saclindua wagneri	Wastewater
	Candidatus Scalindua sorokinii	Seawater
	Candidatus Scalindua Arabica	Seawater
Others	*Candidatus Jettenia asiatica*	Not reported
	Candidatus Anammoxoglobus propionicus	Wastewater

Anammox biomass has a natural ability to form granules and can be recognised by its intense red colour caused by specific enzymes (Figure 4) (Abma et al. 2007; Tang et al. 2013). According to Schmid et al. (2003) the anammox organisms have a doubling time of 11 days and a biomass yield of 0.13 g dry weight per g NH_3-N oxidized. This low growth rate strongly hindered anammox research. The anammox activity is twenty five times higher than the aerobic nitrifying bacterial oxidation of ammonium under anoxic conditions when using nitrite as the electron acceptor (Jetten et al. 1998) however is more than seven times slower than aerobic ammonia oxidation (Strous et al. 1998).

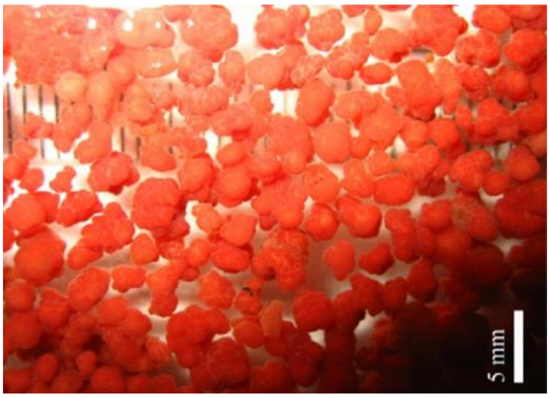


Figure 4- Anammox granules.

Several parameters must be considered when dealing with anammox: for instance, nitrite can inhibit anammox activity, therefore nitrite concentration is an important parameter to be controlled (there is no uniformity in the threshold values of nitrite inhibition, since the different anammox genera seem to have different tolerance for nitrite) (Dapena-Mora et al. 2007; Strous et al. 1999). The anammox bacteria are strictly anaerobic and are strongly inhibited by dissolved oxygen, although inhibition caused by low oxygen concentration was shown to be reversible (Egli et al. 2001). As previously mentioned, anammox bacteria have a very slow growth rate, therefore high biomass retention is extremely important in order for them to be applicable in wastewater treatment (Abma et al. 2007). There is a consensus that the optimum temperature and pH for the operation of anammox bacteria are around 30-40 ºC and 7-8, respectively (Strous et al. 1999; Egli et al. 2001).

The anammox process is particularly suitable to treat effluents coming from an anaerobic treatment step, where biogas is produced. These effluents are known to be traditionally rich in ammonium, since it is formed in the anaerobic step during the degradation of nitrogen-containing organics. Also these effluents are very poor in terms of BOD (Biological Oxygen Demand), since the biodegradable organic matter is used to produce the biogas. Conventional nitrogen cycle cannot be performed when there is high ammonia and low BOD concentrations, without adding an external BOD source (like for example methanol) and high aeration demand (Remi et al. 2015).

The main disadvantage of this process lies on the low growth rate of anammox bacteria. It can occur also inhibition by substrates and exogenous compounds including biodegradable COD and slow start up (Fernández et al. 2012). Another disadvantage may be that is not common to find effluents with the required composition to be treated by the anammox process (Vázquez-Padín et al. 2009). However, in case the anammox process is combined with a partial nitritation step (for example SHARON), only a part of the ammonium is converted to nitrite, and the anammox process can oxidize the remaining ammonium to produce both dinitrogen gas and, to a lesser extent, nitrate, using nitrite as the electron acceptor (Jetten et al. 1997b; Khin & Annachhatre 2004).

2.5 The SHARON-anammox process

As mentioned before anammox can eliminate nitrogen compounds, but ammonium (about 50-60%) must be partly pre-oxidized to nitrite before being fed to the anammox reactor. This partial nitritation can be achieved in a SHARON reactor, under proper operating conditions, according to the following stoichiometry (Equation 10) (Ahn 2006).

$$NH_4{}^{+} + HCO_3 + 0.75O_2 \rightarrow 0.5NH_4{}^{+} + 0.5\ NO_2{}^{-} + CO_2 + 1.5\ H_2O \text{ Eq.10}$$

As shown in Figure 5 (Jetten et al. 2002) and Eq.10 (Khin & Annachhatre 2004), the principle of the combined SHARON and anammox process is that only 50% of influent NH_4-N is oxidized to NO_2-N in the SHARON; this mixture of nitrite and ammonium is suitable to be fed to the anammox reactor, where the residual ammonium is converted to dinitrogen gas using nitrite as the electron acceptor, with negligible nitrate production (Grontmij n.d.(b)).

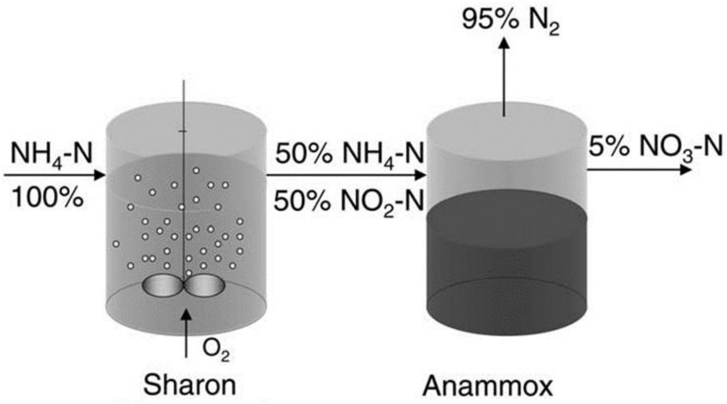


Figure 5- Schematic representation of SHARON-anammox.

The ammonium to nitrite ratio required for anammox influent (1.32) can be achieved with or without any pH control, depending on the concentration of bicarbonates in the SHARON influent. If the bicarbonate to ammonium molar ratio in the influent is 1, all the alkalinity is consumed and pH drops consequently as half of the ammonium in the influent is oxidized to nitrite, preventing any further nitritation (according to nitritation stoichiometry, two moles of bicarbonate are required to oxidize one mole of ammonium) (Jetten et al. 2002).

The combined partial nitritation-anammox process can be accomplished in one single step (one reactor) or by two separated reactors (two-step). This two-step system, where partial nitritation and anammox are separated in space, compared to the single–reactor (CANON, Completely Autothrophic Nitrogen Removal Over Nitrite) provides more flexibility and a more stable process performance since both steps can be controlled separately (Wyffels et al. 2004; Hao et al. 2002; Veys et al. 2010). Moreover, the application of two reactors is important when toxic and biodegradable compounds are present in the wastewaters, since these compounds can inhibit the anammox bacteria; in a two-step system, such compounds may be degraded in the partial nitritation step, avoiding anammox inhibition (Vázquez-Padín et al. 2009; Lackner et al. 2008).

The two-stage process represents a reliable, cost-effective and energetically efficient alternative for the treatment of ammonium-rich wastewaters (Milia et al. 2015a). There are various advantages which prove the superiority of partial nitritation-anammox compared to conventional nitrification-denitrification: the aeration requirement is decreased by 60% (1.9 kg O_2/kg N instead of 4.6 kgO_2/kg N), the sludge production is decreased by 90% (0.08 instead of 1 kg VSS/kg N), CO_2 emissions are reduced by more than 100% since anammox actually consumes CO_2 (Van Loosdrecht & Jetten 1998), there is no need for external carbon source (methanol is not required, since the process is completely autotrophic). Moreover, the two-stage process is a compact system with high achievable loads (> 2kgN/m^3.d) (Remi et al. 2015).

To sum up, the SHARON-anammox process is 90% less expensive than the conventional nitrification-denitrification process (Khin & Annachhatre 2004). The biggest disadvantage of this process is related to the low growth rate of anammox. When compared to the Canon process, investment costs are obviously higher.

2.6 The CANON process

As previously mentioned, CANON is a process where partial nitritation and anammox are performed in a single aerated reactor, making two groups of bacteria (grown as biofilms or granules) cooperate in the whole process: *Nitrosomomas*-like aerobic bacteria and *Planctomycete*-like anaerobic ammonium-oxidizing bacteria. (Ahn 2006). These autotrophic cultures convert ammonia directly into dinitrogen gas with nitrite as an intermediate (Khin & Annachhatre 2004). The aerobic nitrifiers oxidize, under oxygen-limited conditions, ammonium (Equation 11), consuming oxygen and creating anoxic conditions needed for the anammox process to proceed. However the Canon process is very sensitive to dissolved oxygen concentration, nitrogen surface-load, biofilm thickness and temperature (Ahn 2006).

After the AOB oxidize half of the influent ammonium to nitrite, the anaerobic oxidizers convert the residual ammonium to dinitrogen gas and small amounts of nitrate (Equation 12), as previously described. The formation of nitrate in the reaction is coupled with growth since the nitrite serves as an electron donor for the formation of biomass from carbon. The combination of Equation 11 and Equation 12 results in nitrogen removal as shown in Equation 13 (Strous 2000).

$$NH_4{}^+ + 1.5\ O_2 \rightarrow NO_2{}^+ + 2\ H^+ + H_2O \text{ Eq.11}$$

$$NH_4{}^+ + 1.3\ NO_2{}^- \rightarrow 1.02\ N_2 + 0.26\ NO_3{}^- + 2\ H_2O \text{ Eq.12}$$

$$NH_4{}^+ + 0.85\ O_2 \rightarrow 0.435\ N_2 + 0.13\ NO_3{}^- + 1.3\ H_2O + 1.4\ H^+ \text{ Eq.13}$$

A concentration of dissolved oxygen (DO) up to 0.5 mg/l does not affect the ammonium oxidation, however the anammox bacteria are reversibly inhibited by low concentrations of oxygen (0.5% air saturation).

Since this process is completely autotrophic and does not require the addition of organic carbon, it represents an efficient and economic option for wastewater treatment, mainly wastewaters with a high concentration of ammonium but without any (or with very low amount of) organic matter. Comparatively with the conventional nitrification-denitrification process, the CANON consumes 63% less oxygen and 100% less reducing agents. Table 2 shows a comparison between the processes (Khin & Annachhatre 2004; Ahn 2006).

Table 2- Comparison between the SHARON-anammox, CANON and the conventional nitrification-denitrification processes.

	Conventional nitrification/ denitrification	**SHARON/ anammox**	**CANON**
Number of reactors	2	2	1
Conditions	Oxic/anoxic	Oxic/anoxic	Oxygen limited
Oxygen	high	Low/none	low
pH control	yes	None	none
Biomass retention	none	none/yes	yes
COD requirement	yes	None	none
Sludge production	high	Low	low
Alkalinity (g $CaCO_3$/g N)	7.14/ 3.57*	3.57/ 0.24	3.68

**Denitrification produces alkalinity.*

2.7 SHARON-anammox: full-scale applications

The majority of the partial nitritation-anammox (PN/A) installations are located in Europe, although the interest in this system seems to be increasing in North America. The early implementations used a two-step system, but recently the focus has shifted mainly towards single-stage systems. Figure 6 shows a comparison between the number of publications related to PN/A and the number of installations since 1995. Analysing the graph it is possible to see that there was a short delay between the discovery and early publications and the first full scale implementations. The steady increase in new plants in recent years has resulted in more than 100 operating installations in 2014. Most of the installations adopted the one-stage system configuration (88%) and were applied to municipal wastewater treatment (75%) (Lackner et al. 2014).

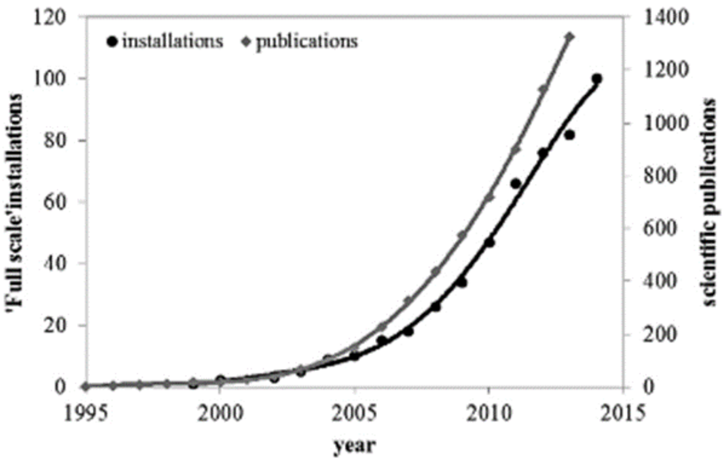


Figure 6- Development of full-scale partial nitritation-anammox installations and scientific publications on anammox/deammonification.

One of the first systems to be implemented was the two-stage SHARON-anammox process in Rotterdam (NL), where anammox was operated as a granular sludge bed in two reactor compartments on top of each other (Figure 7) (van der Star et al. 2007). The second stage converted 90-95% of nitrogen load of >10 $kg/m^3 \cdot d$ after 3.5 years of start-up (W. R. Abma et al. 2007).

At this treatment site, the sludge is thickened and digested with a residence time of 30 days and a temperature between 32–33 °C. The centrifuged digestate (containing 1200 mg/l NH_4^+–N) can be heated or cooled and is fed to the SHARON reactor. The temperature of the nitritation reactor is kept at 33 °C and the reactor can be operated with nitritation alone, or with nitritation–denitrification. Nitrite oxidation to nitrate is avoided by controlling aerobic residence time (the aeration period): this “aerated hydraulic retention time” is set long enough to enable the growth of ammonium oxidizers, but short enough to lead to the washout of (undesired) nitrite oxidizers. The effluent of the nitritation reactor serves (after passing a tilted plate settler) as influent for the anammox reactor. The effluent of the anammox reactor is then returned to the influent of the main WWTP (van der Star et al. 2007).

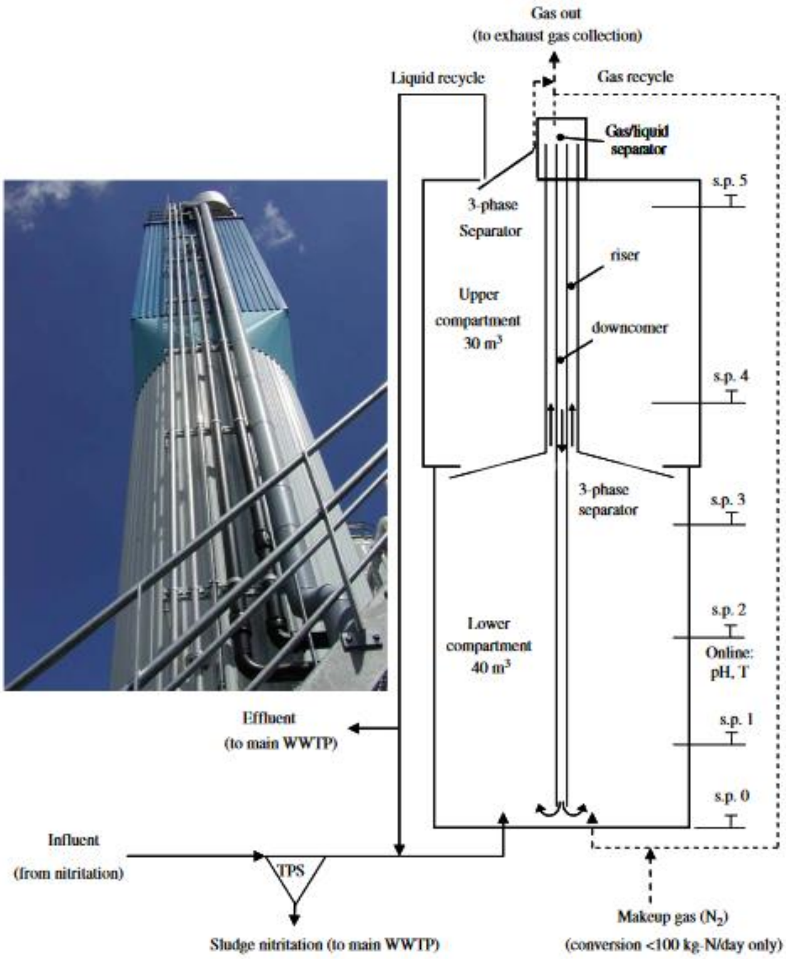


Figure 7- Full scale anammox reactor (70 m^3) in Rotterdam, designed to treat 500 kg-N/d.

Although most of the installations adopted are single stage, facilities using suspended sludge in two or multi-stage configurations have been also implemented. For example, two facilities of Aquaconsult in Germany use a two-stage configuration with suspended sludge, consisting of an aerated reactor, a mixed reactor and settlers. Also the new activated sludge concept (NAS) by Colsen uses suspended solids too and is based on a multi-stage principle. These installations consist of aerobic and anoxic, stirred compartments relying on a hybrid combination of PN/A and nitrification/denitrification process (Lackner et al. 2014).

Lackner et al. (2014), made a survey on 14 full-scale PN/A plants including multi-stage ones with SHARON-anammox technologies. The results for SHARON-anammox are shown in Table 23 in Appendix I.

3. MATERIALS AND METHODS

3.1 SHARON setup

The SHARON was carried out in a glass CSTR with a working volume of 2 L, continuously stirred by mechanical mixing and without any biomass retention (chemostat). The temperature was kept constant at 35±0.5ºC with a water jacket and a thermostatic bath (model HAAKE K) and the pH was maintained at 7.1±0.5 using 1M HCl to avoid unexpected pH variations during the reactor operation. Air was supplied by a membrane pump and introduced via a fine bubble aerator at bottom of the reactor, in order to keep the dissolved oxygen concentration above 2 mg/L, since under this value nitritation is strongly limited. The SHARON reactor has been inoculated with 2L of nitrifying activated sludge drawn from the municipal wastewater treatment plant of Is Arenas (Cagliari).

A schematic representation of the SHARON reactor is given in Figure 8, which shows the influent and effluent peristaltic pumps (Cellai, mod. 503U and Velp, mod. SP311 respectively), the pH (XS sensor) and the DO (WTW, mod. Oxi 320) probes, the peristaltic pumps (Velp, mod. SP311) for pH control with acid and base (the latter was never used in this experiment), the mechanical stirrer and the outlet for sampling. Figure 9 shows the actual reactor.

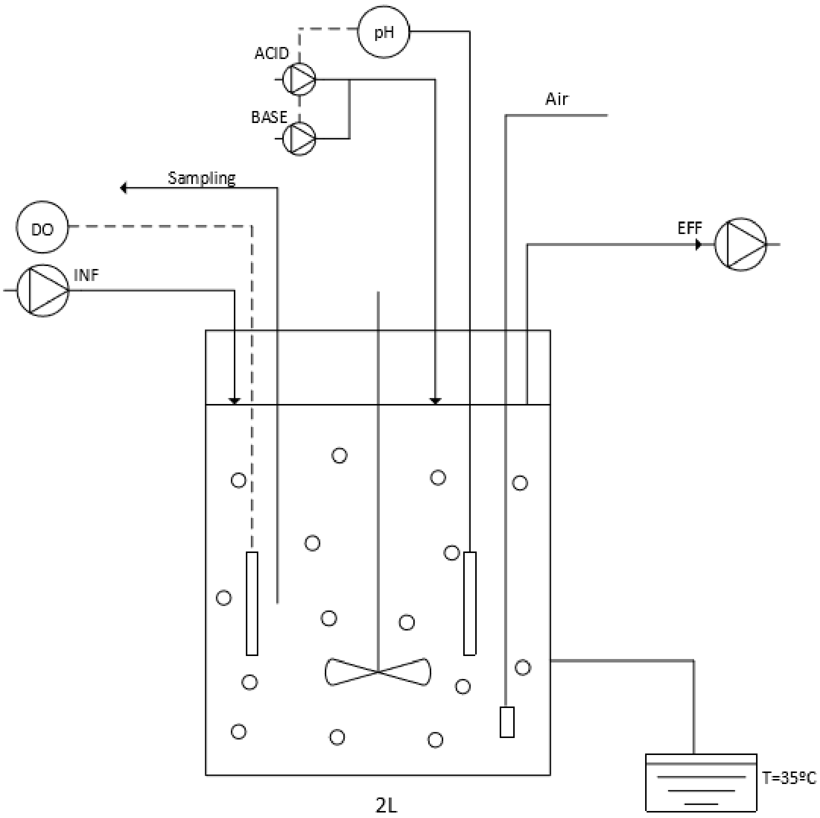


Figure 8- Set up of the SHARON reactor.

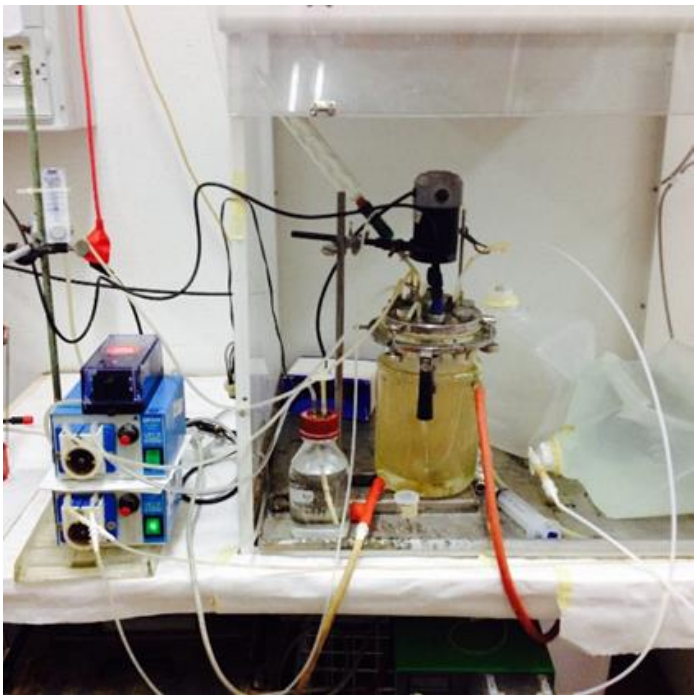


Figure 9- The SHARON reactor used in the experiment.

3.1.1 Operating and feeding strategy

The SHARON reactor was fed with a synthetic influent simulating the liquid stream produced by the two-step anaerobic digestion of the organic fraction of MSW. In order to determine the lowest applicable hydraulic retention time (a lower HRT implies higher treatable flowrates or smaller reactor volumes), three long term tests were carried out using HRTs of 1.5, 1.25 and 1 d (Table 3). Every time the HRT was changed, the SHARON reactor was started-up with fresh activated sludge drawn from Is Arenas.

Table 3- Summary of each phase with the most important operating conditions used in SHARON.

Phase	**HRT** **d**	**Flow Rate** **mL/min**	**NLR** **kgN/m^3·d**	**Phase duration** **days**
1	1.5	0.93	1	60
2	1.25	1.11	1.2	98
3	1.0	1.39	1.25	117

The synthetic medium (Tables 4 and 5) used in this study was prepared following the recipes reported in Milia et al. (2015a) and Milia et al. (2012). Each time a fresh synthetic medium was made, a sample was taken for analytical characterization.

Table 4- Composition of SHARON feeding bag.

Compound	**Concentration (mg/L)**
NH_4-N (as NH_4HCO_3)	1500 (8466)
$MgSO_4 \cdot 7\ H_2O$	100
KH_2PO_4	1000
Trace Elements solution	10 mL/L

Table 5- Composition of SHARON trace elements solution.

Trace Elements Solution	**Concentration (g/L)**
EDTA disodic salt	19.11
$FeSO_4 \cdot 7\ H_2O$	1.5
$ZnCl_2$	0.72
$CuCl_2 \cdot 6\ H_2O$	0.6
$MnSO_4 \cdot H_2O$	1.9
$CoCl_2 \cdot 6\ H_2O$	0.34
$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	0.48
$CaCl_2$	1.48

3.2 Anammox setup

Anammox was carried out in a glass SBR (Sequencing Batch Reactor) with a working volume of 2 L. The Sequencing Batch Reactor is an activated sludge process designed to operate under non-steady state conditions (Abreu & Estrada n.d.). The operation of an SBR is based on a fill-and-draw principle, which consists of five steps—fill, react, settle, decant, and idle. These steps can be altered for different operational applications (Poltak 2005). The SBR was operated in fed-batch mode with a 6-hour cycle (200-267 min of filling, 350 min of reaction (including filling), 5 min of settling, 5 min of decantation and 0 min of idling). Mechanical mixing was provided by a marine impeller (110±5 rpm). At the beginning of each working cycle, inert N_2 gas was flushed for 2-5 minutes, in order to assure anaerobic conditions inside the SBR. The temperature was kept constant at 35±0.5 ºC by a water jacket and a thermostatic bath (mod. HAAKE K), while the pH was maintained at 6.8±0.3 pH using 1M HCl and 1M NaOH. The reactor also was completely covered with aluminium foil, in order to avoid any penetration of light that would hinder anammox activity. Process timing and control were performed via NI (National Instrument) Compact Rio. The SBR reactor was started-up using 1L of anammox granular biomass drawn from the Dokhaven-Sluisjesdijk wastewater treatment plant in Rotterdam (The Netherlands).

A schematic representation of the anammox reactor is given in Figure 10, which shows the influent and effluent peristaltic pumps (Watson Marlow, mod. 1200 and Watson Marlow, mod. 505S, respectively), the pH probe (Mettler Toledo, mod. InPro3253i), for pH control with acid and base (the latter was never used in this experiment), the mechanical stirrer and the outlet for sampling. Figure 11 shows the actual reactor.

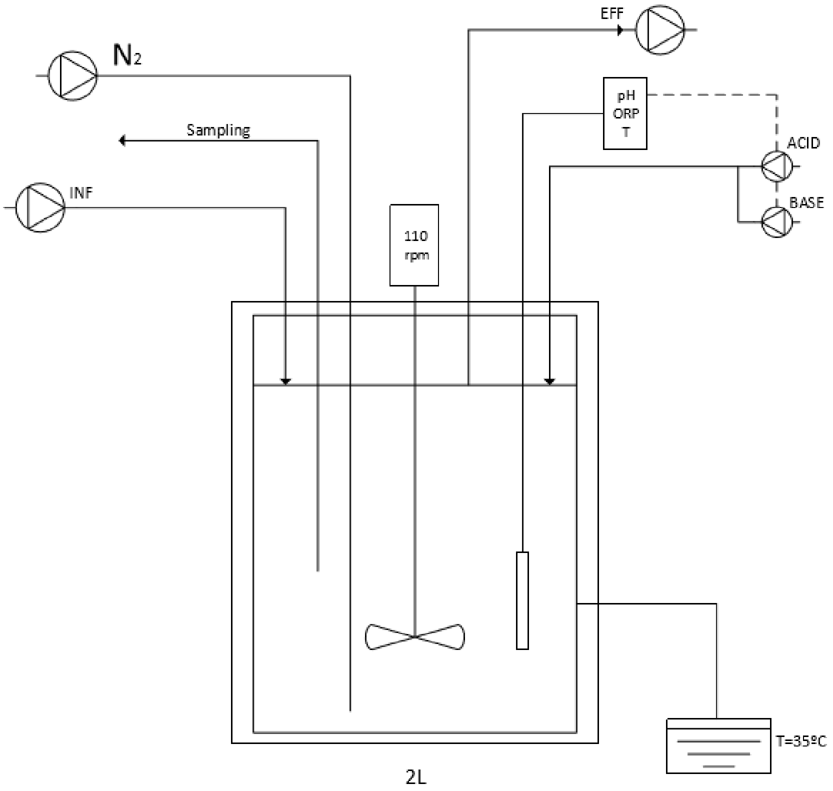


Figure 10- Set up of the anammox reactor.

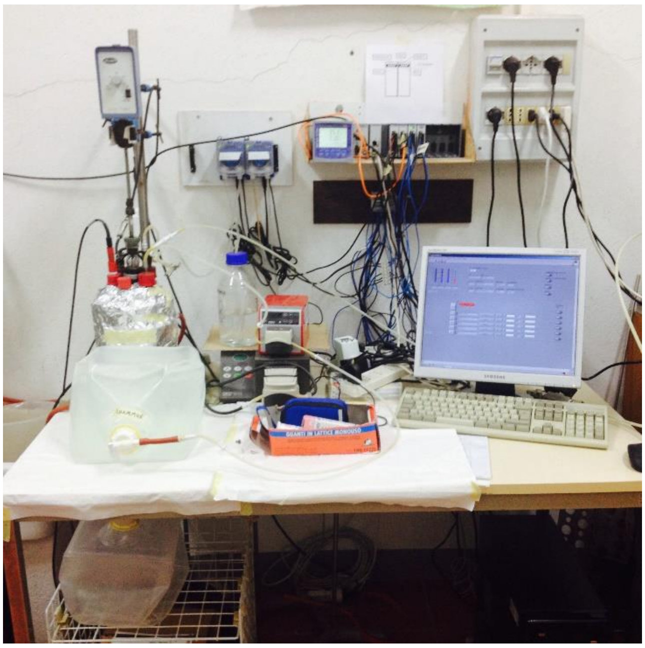


Figure 11- The anammox reactor used in the experiment.

3.2.1 Operating and feeding strategy

A synthetic medium simulating the effluent from the SHARON reactor was fed to the granular anammox SBR. This experiment had three phases with three different NLR that were 1, 1.2 and

1.5 ($kgN/m^3\cdot d$). The most important operating conditions of each phase are summarised in Table 6 and extensively in Table 24 in Appendix II. The volumetric exchange ratio is the feed volume for each cycle divided by the working volume. Each change of NLR was made gradually so the bacteria could get acclimated.

Table 6- Summary of each phase with the most important operating conditions used in anammox.

Phase	NLR $kgN/m^3\cdot d$	Flow rate mL/min	HRT target days	Feed duration min	Volume fed per cycle mL	Volumetric exchange ratio	Duration days
1	0.8→1.0	1.2	1.5	208-267	250-320	0.17	35
2	1.0→1.2	1.5	1.25	213-267	320-400	0.20	43
3	1.2→1.5	2.0	1.0	200-267	400-533	0.25	134

Table 7, 8 and 9 show the compounds needed for the preparation of the feeding bag. The concentrations of NH_4HCO_3 and $NaNO_2$ were chosen to obtain the desired NO_2-N/NH_4-N molar ratio of 1.15, lower than the optimal value of 1.32 (Strous 2000) required for anammox (an excess of NH_4-N was dosed, in order to avoid any possible accumulation of NO_2-N). The concentrations of the other chemicals were chosen according to van de Graaf et al. (1996).

Table 7- Composition of anammox feeding bag.

Compound	Concentration (mg/L)
NH_4-N (as NH_4HCO_3)	698 (3938)
NO_2-N (as $NaNO_2$)	802 (3952)
$MgSO_4\cdot 7\,H_2O$	200
KH_2PO_4	6.25
$CaCl_2$ anidrous	300
Trace Elements solution	1.25 mL/L
Iron Solution	2.50 mL/L

Table 8- Composition of anammox trace elements solution.

Trace Elements Solution	Concentration (g/L)
EDTA disodic salt	19.11
$ZnSO_4\cdot 7\,H_2O$	0.43
$CoCl_2\cdot 6\,H_2O$	0.24
$MnCl_2\cdot 2\,H_2O$	0.99
$CuSO_4\cdot 7\,H_2O$	0.25
$Na_2MoO_4\cdot 2\,H_2O$	0.22
$NiCl_2\cdot 6\,H_2O$	0.19
$Na_2SeO_4\cdot 10\,H_2O$	0.21
H_3BO_4	0.014

Table 9- Compounds for the anammox iron solution.

Iron Solution	**Concentration (g/L)**
EDTA disodic salt	5.00
$FeSO_4 \cdot 7\ H_2O$	5.00

3.3 Ammonium, nitrites, nitrates

Nitrite (NO_2-N) and nitrate (NO_3-N) concentrations were measured by ion-chromatography, using a DIONEX ion-chromatograph (mod. ICS-90, Figure 12) equipped with an anion exchange pre-column (DIONEX, mod. IonPac AG14A) and column (DIONEX, mod. IonPac AS14A). The samples were filtered and properly diluted using de-ionized water before analysis. The filters used were Sartorius Stedim Biotech GmbH with a pore size of 0.45µm. Each sample was analysed twice.

Figure 12- The DIONEX ICS-90 ion-chromatograph used for the analysis.

Ammonium (as NH_4-N) was measured by a modified colorimetric method (IRSA-CNR), using a spectrophotometer (HITACHI, mod. U-2000) in the 420 nm wavelength (Figure 13). The samples were filtered and properly diluted (the method is precise if NH_4-N concentration stays within the range 0.5-5.0 mgNH4-N/L). The filters used were Sartorius Stedim Biotech GmbH with a pore size of 0.45µm.

Figure 13- The spectrophotometer used for the analysis.

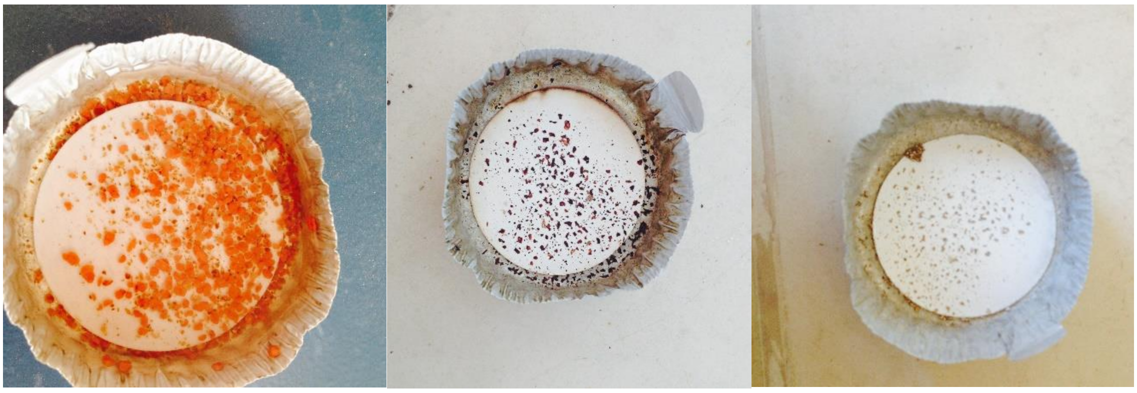
Samples for NH_4-N quantification were prepared in 50 ml bottles with distilled water. In each bottle, 3 drops of potassium sodium tartrate and 1 ml of Nessler reagent were added (Figure 14). Depending on the concentration of ammonium, the colour of the sample switches from transparent to yellow. Samples were analysed via spectrophotometer 15-30 minutes after adding the Nessler reagent. Measurements were made in triplicate to guarantee a more statistically precise measurement.

Figure 14- Exemplification of samples analysed with the spectrophotometer.

3.4 Suspended solids

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) concentrations in the reactors and in the effluents were measured, according to the following procedure:

1. An aluminium cup and a glass microfiber filter (ALBET Ø 47 mm, nominal porosity size 1.2 µm) were put in a dehumidifier for 15 minutes, then weighted with a precision balance. The "empty weigth", M_0 (g), was determined;
2. A known volume of sample (V, expressed in litres) was drawn (from the reactors or from the effluents) and filtered. After sample filtration, the aluminium cup containing the filter+sample (Figure 15 a) was put in a 105 ºC oven for 12 hours, then weighted with a precision balance. The "dry weight", M_1 (g) was determined;
3. The cup containing the filter+sample (Figure 15 b) was subsequently put in a 550 °C oven for 2 hours. The cup containing the filter+sample (Figure 15 c) was weighted with a precision balance, and the "ash content", M_2 (g), was determined.



a) *b)* *c)*

Figure 15- The aluminium cup with the filter+sample before putting it on the 105 ºC oven; b) The aluminium cup with the filter+sample before putting it on the 550 ºC oven (Dry); c) The aluminium cup with filter+sample after putting it on the 550 ºC oven (Ash).

Final calculations were (Equations 14 and 15):

$$TSS = \frac{M_1 - M_0}{V} \qquad \left[\frac{g_{TSS}}{L}\right] \text{ Eq.14}$$

$$VSS = \frac{M_1 - M_2}{V} \qquad \left[\frac{g_{VSS}}{L}\right] \text{ Eq. 15}$$

As to the anammox reactor, granules density was also measured.

1. A known volume of sample (V) was drawn, and filtered as described for TSS determination;

2. Granules were collected from the filter and put in a 5 mL cylinder, which had been previously half-filled;

3. The specific volume of granules (SV_{gran}, as L_{gran}/L_{sample}), was calculated as the difference between the final volume (measured after adding granules) and the initial volume (5 mL) in the cylinder, divided by the sample volume (V),

4. TSS concentration was determined as previously described, and granules density was calculated according to Equation 16.

$$\rho_{\text{gran}} = \frac{TSS}{SV_{\text{gran}}} \qquad \left[\frac{g_{TSS}}{L_{\text{gran}}}\right] \text{Eq.16}$$

3.5 Mass Balances

After the measurements it is possible to do the mass balances for anammox. All the mass balance equations are expressed below. To do the mass balances it is also necessary to use the Equation 12 described in the chapter 2.

Starting with nitrogen removal efficiencies:

$$Total\ Removed\ NH_4 - N\ (mg/L) = NH_{4_{INF}} - NH_{4_{EFF}}\ \text{Eq.17}$$

$$Total\ Removed\ NO_2 - N\ (mg/L) = NO_{2_{INF}} - NO_{2_{EFF}}\ \text{Eq.18}$$

$$Total\ Production\ NO_3 - N\ (mg/L) = NO_{3_{EFF}} - NO_{3_{INF}}\ \text{Eq.19}$$

$$Observed\ NH_4 - N\ removal\ efficiency\ (\%) = \frac{Total\ Removed\ NH_4{-}N}{NH_{4_{INF}}}\ \text{Eq.20}$$

$$Observed\ NO_2 - N\ removal\ efficiency\ (\%) = \frac{Total\ Removed\ NO_2{-}N}{NO_{2_{INF}}}\ \text{Eq.21}$$

$$NRE\ (Nitrogen\ Removal\ Efficiency)\ (\%) = \frac{(NH_{4_{INF}}+NO_{2_{INF}}+NO_{3_{INF}})-(NH_{4_{EFF}}+NO_{2_{EFF}}+NO_{3_{EFF}})}{(NH_{4_{INF}}+NO_{2_{INF}}+NO_{3_{INF}})}\ \text{Eq.22}$$

Next are the equations belonging to the Nitrate production:

$$Theorethical\ production\ considering\ NO_2 - N\ consumption\ (mg\ NO_3 - N) = \frac{Total\ Removed\ NO_2{-}N \times Feed\ Volume\ per\ cycle}{Full\ Reactor\ Volume} \times \frac{0.26}{1.32}\ \text{Eq.23}$$

$$Theorethical\ production\ considering\ NH_4 - N\ consumption\ (mg\ NO_3 - N) = \frac{Total\ Removed\ NH_4{-}N \times Feed\ Volume\ per\ cycle}{Full\ Reactor\ Volume} \times 0.26\ \text{Eq.24}$$

$$Measured\ production\ (mg\ NO_3-N)=\frac{Total\ Production\ NO_3-N\times Feed\ Volume\ per\ cycle}{Full\ Reactor\ Volume}\quad \text{Eq.25}$$

Nitrogen Loading Rate (NRL):

$$NRL\ Expected\ (\%)=\frac{Theorethical\ Nitrogen\ Influent\times Number\ of\ cycles\ per\ day\times Feed\ Volume\ per\ cycle}{Full\ Reactor\ Volume}\quad \text{Eq.26}$$

$$NRL\ Measured\ (\%)=\frac{Measured\ Nitrogen\ Influent\times Number\ of\ cycles\ per\ day\times Feed\ Volume\ per\ cycle}{Full\ Reactor\ Volume}\quad \text{Eq.27}$$

Nitrite Discharge Rate (NitRD):

$$NitRD\ (kg\ NO_2-N/m^3\cdot d)=\frac{NO_{2_{EFF}}\times Number\ of\ cycles\ per\ day\times Feed\ Volume\ per\ cycle}{Full\ Reactor\ Volume}\quad \text{Eq.28}$$

Nitrogen Removal Rate (NRR):

$$NRR\ (kg\ N/m^3\cdot d)=\frac{(Total\ Removed\ NO_2+Total\ Removed\ NH_4)\times Number\ of\ cycles\ per\ day}{Full\ Reactor\ Volume}\quad \text{Eq.29}$$

Molar Ratios:

$$\frac{Removed\ NO_2-N}{Removed\ NH_4-N}=\frac{Total\ Removed\ NO_2-N}{Total\ Removed\ NH_4-N}\quad \text{Eq.30}$$

$$\frac{Produced\ NO_3-N}{Removed\ NH_4-N}=\frac{Total\ Produced\ NO_3-N}{Total\ Removed\ NH_4-N}\quad \text{Eq.31}$$

$NH_{4_{INF}}$-Concentration of NH_4-N in the influent (mg/L);

$NH_{4_{EFF}}$- Concentration of NH_4-N in the effluent (mg/L);

$NO_{2_{INF}}$- Concentration of NO_2-N in the influent (mg/L);

$NO_{2_{EFF}}$- Concentration of NO_2-N in the effluent (mg/L);

$NO_{3_{INF}}$- Concentration of NO_3-N in the influent (mg/L);

$NO_{3_{EFF}}$- Concentration of NO_3-N in the effluent (mg/L);

$(NH_4+NO_2)_{INF}$- Concentration of NH_4-N and NO_2-N in the influent (mg/L);

$(NH_4+NO_2)_{EFF}$- Concentration of NH_4-N and NO_2-N in the influent (mg/L).

3.6 Kinetic Assay

The kinetic assay is done in order to determinate the NO_2-N and NH_4-N consumption and the

NO_3-N production rates in the anammox reactor. This assay was made, normally, when the reactor was full (immediately after feeding). A pre-determined volume (25-50 mL) of a solution containing ammonium and nitrite (Table 10) was dosed into the anammox reactor, and a sample was drawn (t_0). New samples were taken every 10 minutes for 50 minutes, as well as at the end of the working cycle (effluent). These samples were filtered and analysed for ammonium, nitrite and nitrate quantification, as previously described.

Table 10- Composition of the kinetic solution.

Kinetic Assay Solution	**Concentration (gNVL)**
NO2-N (as $NaNO_2$)	2.00
NH4-N (as NH_4Cl)	2.00

After analytical measurements, Microsoft EXCEL was used to plot NO_2-N, NH_4-N and NO_3-N concentration profiles over time. A linear interpolation of the experimental data was made in order to determine the maximum slope of each concentration profiles, which corresponded to the maximum removal (for nitrite and ammonium) or production (for nitrate) rates (mgN/L h). Such rates were divided by the VSS concentration to calculate the specific removal (or production) rates.

4. RESULTS AND DISCUSSION

4.1 General Aspects

4.1.1 SHARON

The experiment with SHARON lasted for 275 days and the different operating conditions for each Phase are described in detail in Table 3 in Chapter 3.1.1. Main parameters used to assess process performance were:

- Influent and Effluent ammonium concentration and conversion efficiency;
- Effluent nitrite concentration and nitrite/ammonium ratio;
- Effluent nitrate concentration and NO_3 % production.

4.1.2 Anammox

As described in Table 6 the experiment was divided into three phases, each with a different NLR. Each Phase was divided into two periods, one with a gradual change of NLR and one with a stable NLR that normally lasted for at least three weeks. The NLR increase was performed according to an exponential law, where the exponential factor (μ_{ap}=0.022 d^{-1}) was lower than the anammox bacteria maximum specific growth rate (μ_{max}=0.065 d^{-1}). The use of such conservative value for the μ_{ap} was to ensure a successful anammox biomass enrichment, without nitrite accumulation, however it implied a lower increase rate. This exponential increase was chosen since according to the literature this strategy proved to be more appropriated when compared to others (López et al. 2008). Equation 32 shows the exponential function used for the increase:

$$NRR \propto NLR = A \cdot e^{\mu_{ap} \cdot t} \qquad \text{Eq.32}$$

Where: A is a pre-exponential factor at time zero, corresponding to the NLR at the beginning of each step (t=0). μ_{ap} is the operational factor, while t is the time in days, so the change of the NLR is made by day and not by cycle.

The experiment lasted for a total of 214 days and the influent was always a synthetic feed that simulated the SHARON effluent. It is important to take note that the first step was not the start-up of this experiment, which is not discussed in this chapter.

In order to discuss the process performance during the experiment, the following parameters were used:

- Nitrogen Loading Rate (NLR), Nitrogen Removal Rate (NRR) and Nitrite Discharge Rate (NitDR);
- Observed NH_4-N removal efficiency, Observed NO_2-N removal efficiency and Nitrogen Removal Efficiency (NRE);
- Stoichiometric ratios;
- Suspended solids;
- Specific NO_2-N and NH_4-N removal rates, as well as specific NO_3-N production rate.

4.2 SHARON Performance

4.2.1 Influent and effluent ammonium concentration and conversion efficiency

Analysing Figure 16, the concentrations on the acclimation period seems to have a better behaviour when the HRT is 1.25d (Figure 16 b). This acclimation period is associated to behaviour of the biomass right after a change in the operating conditions (in this case HRT) is made. However few samples were taken during that period possibly giving a misleading view. All the graphs show a very stable trend in the influent concentrations which was expected since the influent was synthetic.

The target concentration of ammonium in the influent was 1500 mg/L as specified in Table 4; this value was practically achieved as stated in Table 11 with very minor differences. Also the ammonium concentrations in the effluent should be similar to what is described in Table 7 in order to be an appropriated influent for the anammox process. As reported in Table 11 the effluent when the HRT is 1.25 d is the best suited as the anammox influent.

This ammonium conversion efficiency is very important to evaluate the SHARON process and, as stated in Chapter 2, the effluent (in the SHARON-anammox process) should have 50% ammonium and 50% nitrite. The closest efficiency to the expected value was achieved when the process was working with a 1.25 d HRT (Phase 2); however the efficiencies obtained for the other HRTs are very similar so the process may work with any of this HRT.

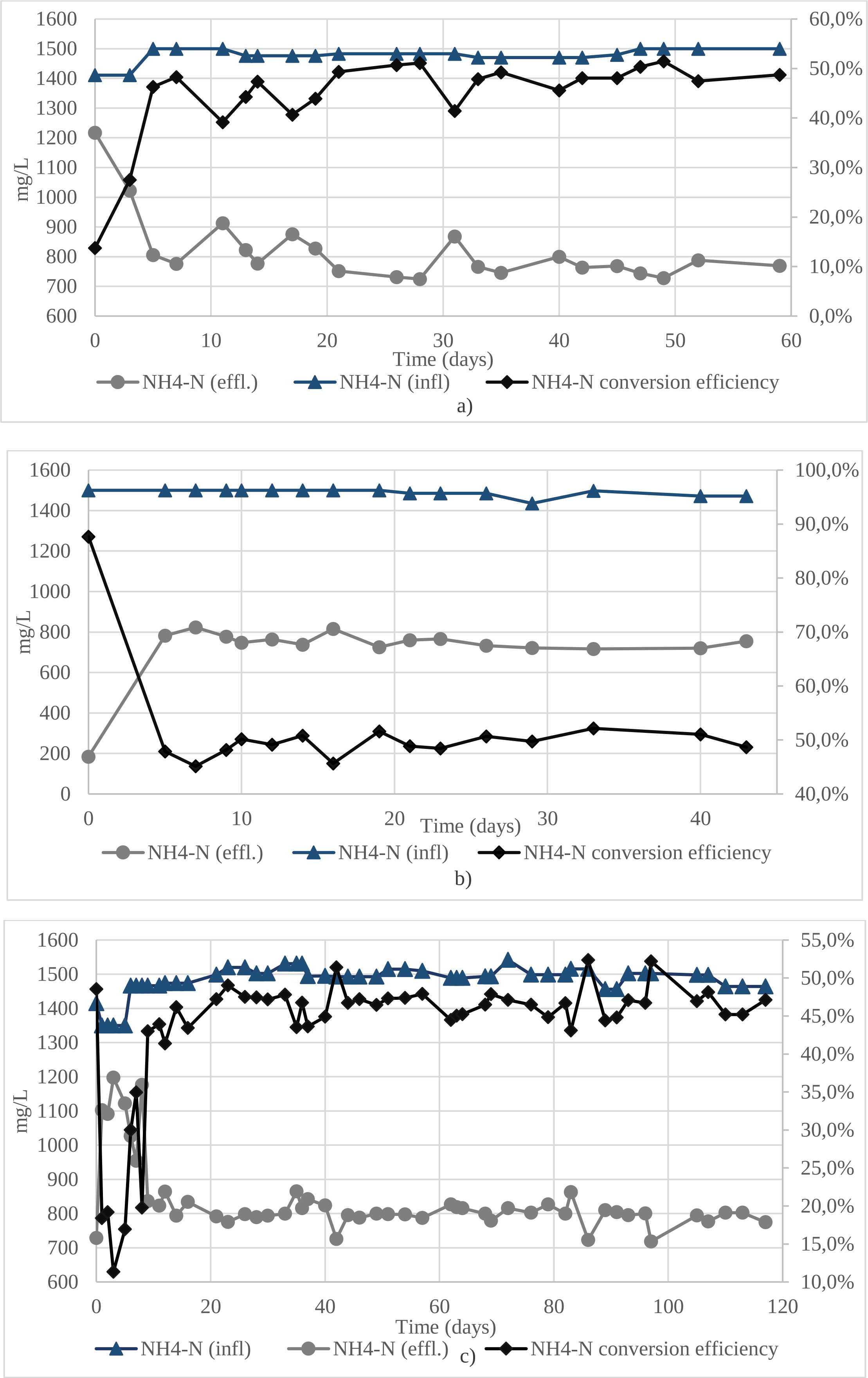


Figure 16- Influent and effluent ammonium concentration and conversion efficiency for different HRT a) HRT=1.5d; b) HRT=1.25d; c) HRT=1d.

Table 11- Concentrations of ammonium in the influent and effluent and conversion efficiency for each HRT, observed under steady conditions.

Phase	**NH_4-N Influent mg/L**	**NH_4-N Effluent mg/L**	**NH_4-N conversion efficiency %**
1	1478.09± 24.63	787.27± 51.68	47.0± 3.5%
2	1489.44± 17.79	756.17± 32.91	49.2± 2.0%
3	1481.05± 45.10	801.83± 31.34	46.4± 2.3%

4.2.2 Effluent nitrite concentration and NO_2-N/NH_4-N ratio

Both the effluent nitrite concentration and NO_2-N/NH_4-N ratio have a similar trend as seen in Figure 17: this behaviour was expected since the two parameters are strictly connected. Although the NO_2-N/NH_4-N ratio in the synthetic bag used to feed the anammox reactor was 1.15 (a precaution in order to avoid any possible accumulation of nitrite), the SHARON effluent showed a different ratio, which was still acceptable for the subsequent treatment by anammox (Figure 17).

Analysing Table 12 the nitrite concentrations in the effluent seem to be variable for each phase, with the highest and the most desirable value, for the anammox feeding according to Table 7, belonging to Phase 2. Again the Phase 2 seems to have the best operating conditions to obtain the intended objectives (in this case the nitrite effluent concentration and ratio). A ratio a little lower than 1.15 may not provoke adverse effects.

In overall, the SHARON process seems to work better when the HRT is 1.25 d. However the other HRTs do have similar results and can also be implemented.

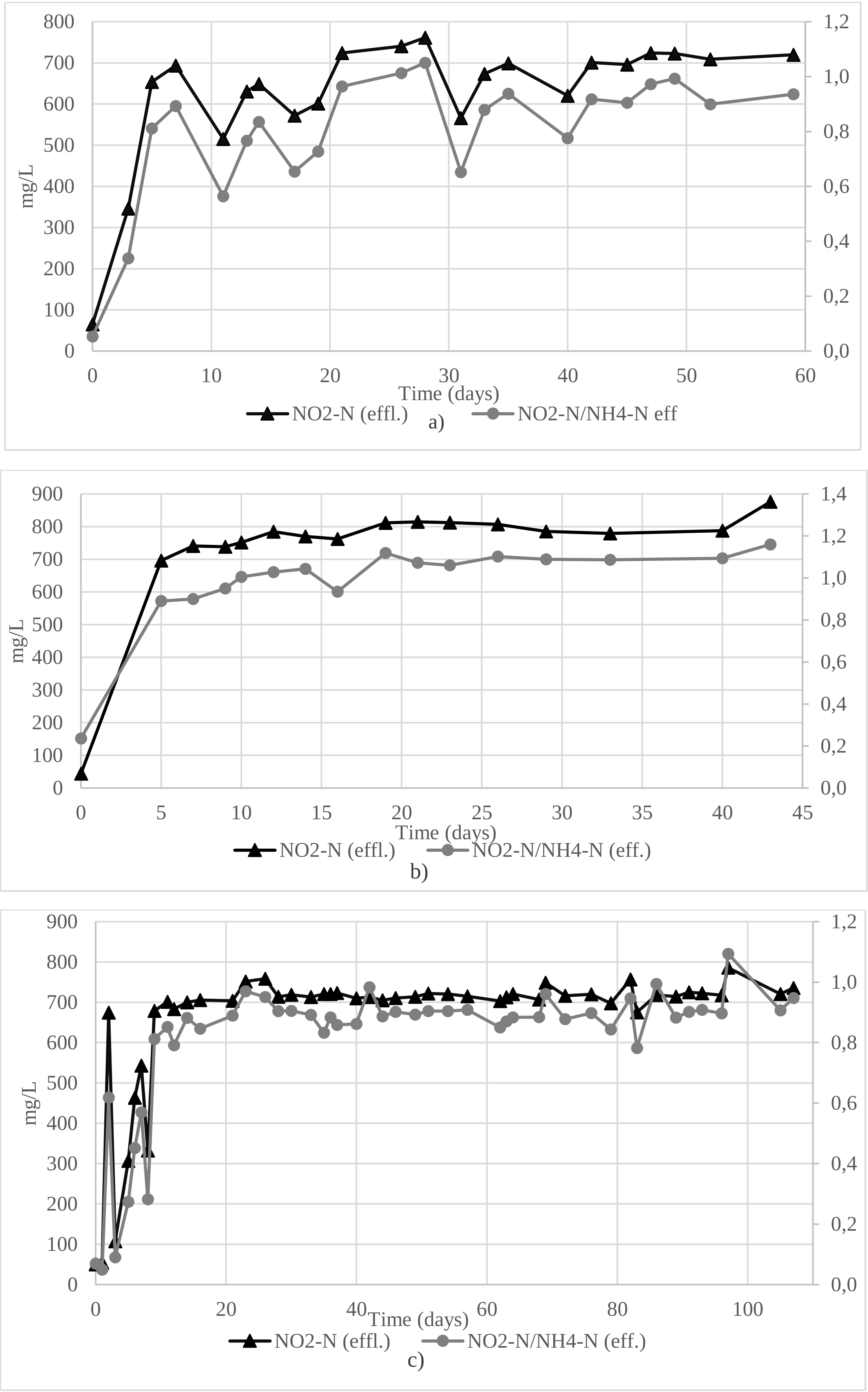


Figure 17- Effluent nitrite concentration and NO_2-N/NH_4-N ratio for different HRT a) HRT=1.5d; b) HRT=1.25d; c) HRT=1d.

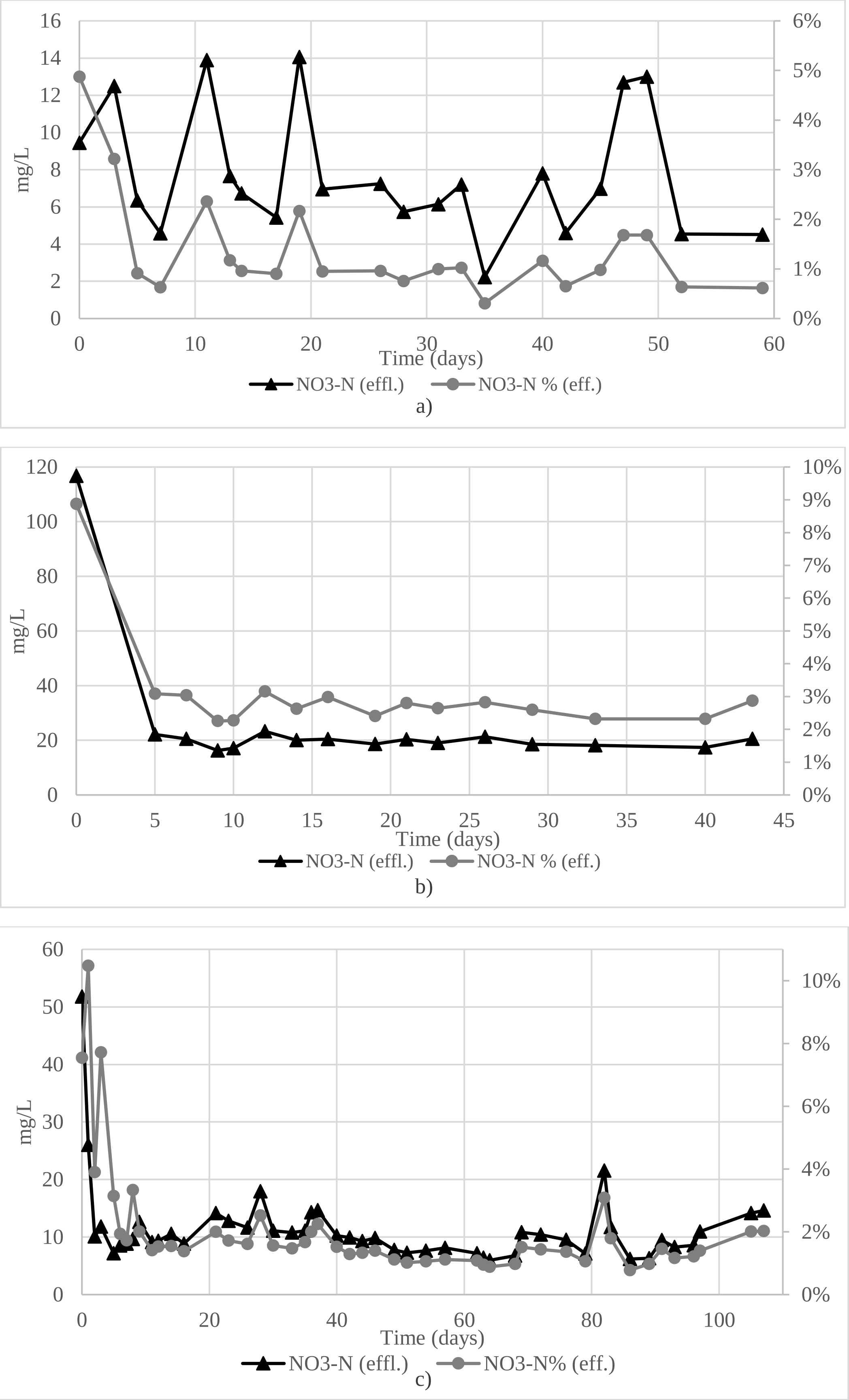
Table 12- Concentration for nitrite in the effluent and NO_2-N/NH_4-N ratio for each HRT, observed when the process stabilized.

Phase	**NO_2-N effluent mg/L**	**NO_2-N/NH_4-N effluent**
1	668.69± 65.95	0.86± 0.13
2	781.06± 42.08	1.04± 0.08
3	708.97± 21.07	0.90± 0.06

4.2.3 Effluent nitrate concentration and NO_3 % production

The nitrate concentrations were always very low (Figure 18) in the effluent. The highest value for the concentration of nitrate was obtained in Phase 2 (Figure 18 b) corresponding to 116.73 mgNO_3-N/L. However this value was obtained during the acclimation of the biomass to the change of HRT and normally these values are more erratic. So, as expected, after this acclimation phase the nitrate concentration returned to the normal values (more or less 20 mg NO_3-N/L).

Interestingly the highest average values for both the nitrate concentration in the effluent and production were on Phase 2 (Table 13) which until now presented the best results. As mentioned on Chapter 2 the SHARON process was developed to stop the ammonium oxidation at nitrite, so having nitrate can mean that this wasn't completely achieved. However the nitrate production was really low (no more than 3% of the total effluent) and so it can be considered acceptable.



a)

b)

c)

Figure 18- Effluent nitrate concentration and NO_3 % production for different HRT a) HRT=1.5d; b) HRT=1.25d; c) HRT=1d.

Table 13- Concentration of nitrate in the effluent and nitrate production for each HRT practised and calculated when the process stabilized.

Phase	**NO_3-N effluent mg/L**	**NO_3-N production %**
1	7.42± 3.37	1.1± 0.5%
2	19.58± 1.94	2.7± 0.3%
3	10.34± 3.28	1.5± 0.5%

4.3 Anammox Performance

4.3.1 Nitrogen Loading Rate, Nitrogen Removal Rate, Nitrite Discharge Rate

The Nitrogen Loading Rate (NLR), Nitrogen Removal Rate (NRR) and Nitrite Discharge Rate (NitDR) profiles are shown in Figure 19.

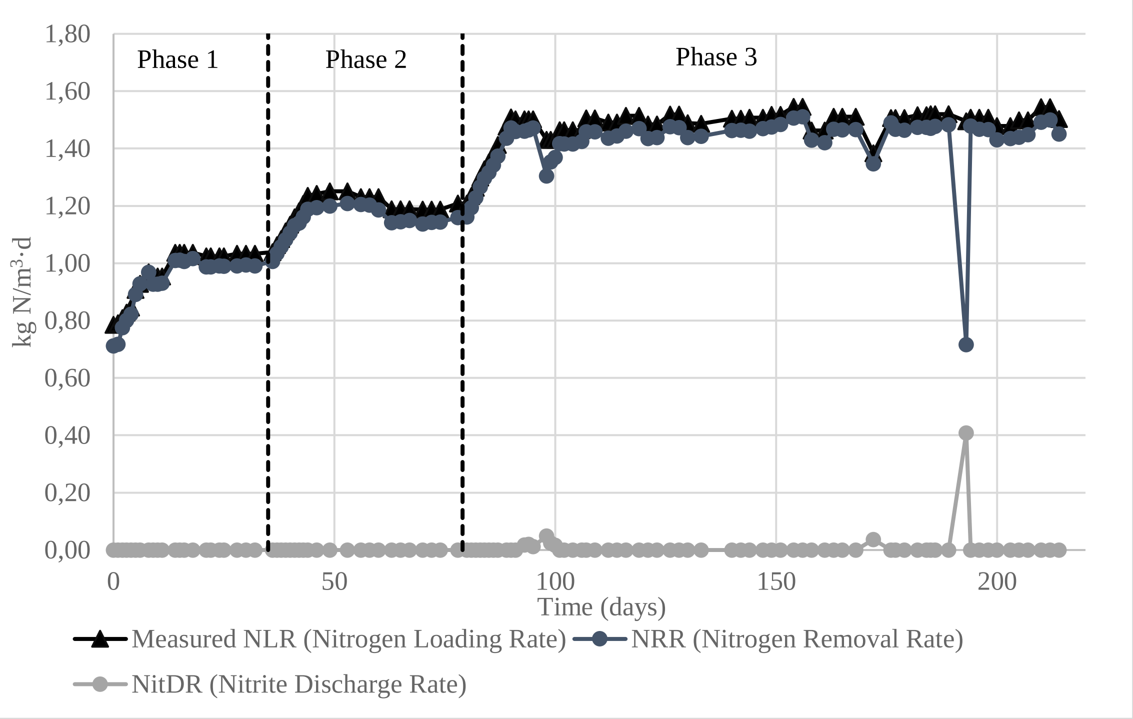


Figure 19- The NRL, NRR and NitDR profiles observed during the experiment.

According to the literature (López et al. 2008) the NLR and NRR curves tend to coincide when the process reaches stable conditions. During this experiment both curves generally coincided, indicating a good process performance. The NitDR was always very low, mostly zero, except in one moment where it reached a value of 0.41 kg N/m^3·d (day 193), which coincided with an abrupt decrease of NRR. The reason behind this was a system malfunction; however process performance was recovered in the next days.

During the first Phase of the experiment (day 0 to day 35) both NLR and NRR values were similar, however the NRR was lower than NLR (the value of NRR/NLR was 96.7± 2.2%); during the same period the NitDR was always zero. Since an almost complete removal of nitrogen occurred during this Phase, it can be assumed that the biomass already had a good acclimation for this operating conditions.

For both Phases 2 (day 36 to day 79) and 3 (day 80 to day 214) the NLR and NRR had a similar behaviour to what was explained for the Phase 1, except in Phase 3 but this was mostly due to a malfunction that occurred in that period. The NRR/NRL ratio was 96.7± 0.7% and 96.2± 6.1% for Phases 2 and 3, respectively. The last Phase had a smaller ratio than the rest of the Phases, mainly due to some minor problems and the system malfunction. The NitDR remained zero during all Phase 2, while in Phase 3 even though it was not always zero it, the values were very low with an average of $2.72 \cdot 10^{-3} \pm 8.9 \cdot 10^{-3}$kg N/m^3·d.

The average values during the whole experiment for both the NRR/NLR and the NitDR were 96.4± 4.8% and $1.62 \cdot 10^{-3} \pm 6.95 \cdot 10^{-3}$ kg N/m^3·d respectively. Taking all into account, it is possible to claim that no substantial difference was observed among all Phases in terms of NRR/NLR and NitDR.

4.3.2 NH_4-N, NO_2-N and Nitrogen Removal Efficiencies

Figure 20 shows the ammonium and nitrite removal efficiencies observed during the experiment. During Phases 1 and 2 the observed NO_2-N removal efficiency was always 100%, while the ammonium removal efficiency was 93.5 ± 4.1% and 93.1 ± 1.4%, respectively. The presence of ammonium in the effluent was expected since an excess of NH_4-N was dosed during feeding (in order to avoid the accumulation of NO_2-N). The NRE for both Phases was 88.4± 2.3% and 88.7± 0.6%, respectively. This shows that the process had a very high and stable nitrogen removal efficiency throughout both Phases.

During Phase 3, NO_2-N and NH_4-N removal efficiencies, as well as NRE did not change significantly. On day 193 a system failure caused a nitrite and ammonium accumulation up to 409 and 370 mgN/L, respectively, corresponding in 47.5%, 48.4% and 44.7% nitrogen removal efficiencies for ammonium, nitrite and NRE, respectively. As correct system operating conditions were restored, biomass quickly recovered its removal capability and process performance switched back to its previous level. Not taking into account the anomalous values from day 193, the average removal efficiencies were 94.0±1.6%, 99.6±1.2% and 89.0±0.9% for NH_4-N, NO_2-N and NRE, respectively. A study using a SBR reactor (Dapena-Mora et al. 2004a) with a NLR= 0.70 kg N/m^3·d and synthetic feeding achieved a 78% nitrogen removal efficiency and a complete nitrite removal. Again Dapena-Mora et al. (2004b) obtained a nitrogen removal of 82% using a SBR with a NRL=1.4 kg N/m^3·d. In 2008 López et al. elaborated a study also with a SBR reactor applying an NLR of 1.60 kg N/m^3·d: they also used a synthetic feeding with a total nitrogen concentration of 2929.40 mg N/L. The ammonium and nitrite efficiency they obtained was 99.9% and 99.5%, respectively. More recently, Ni et al. (2011) reported similar removal efficiencies using a UASB (Up flow Anaerobic Sludge Blanket) reactor with a nitrogen concentration of 1036 mg N/L in a synthetic feeding and a NLR of 1.03 kg N/m^3·d. They obtained a NH_4-N and NO_2-N efficiency of 99.3% and 100% respectively, and total nitrogen removal efficiency was 89.6± 0.96%. Taking into account the literature values obtained for the SBR, the nitrogen removal efficiency seems better when higher NLR is applied.

Despite the ammonium removal efficiencies of this study being a little lower when compared to recent studies, it should be taken into account factors like the excess of ammonium dosed during feeding and some minor problems that occurred (provoking anammox inhibition) and caused the efficiencies to drop. But overall this shows that the process, independently of the NLR applied, is very stable and has a high nitrogen removal efficiency.

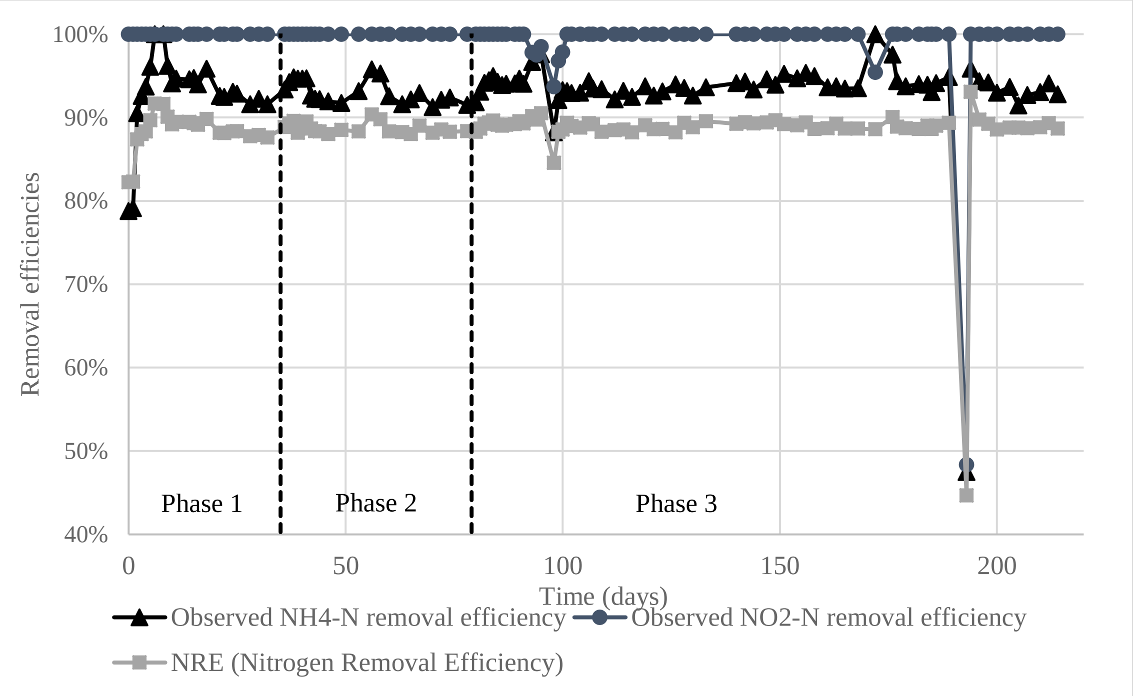


Figure 20- Ammonium and nitrite removal efficiencies during the experiment.

4.3.3 Stoichiometric ratios

4.3.3.1 NO_2-N and NH_4-N consumption

The anammox process consists, as written in the previous chapter, of an anaerobic oxidation of ammonium using nitrite as electron acceptor (Equation 33) according with the stoichiometry described by Strous et al. (1998):

$$NH_4{}^+ + 1.32\, NO_2{}^- + 0.066\, HCO_3{}^- \rightarrow 1.02\, N_2 + 2.03\, H_2O + 0.066\, CH_2O_{1.5}N_{0.15} + 0.26\, NO_3{}^- \quad \text{Eq.33}$$

As stated by Lotti et al. (2016), Strous obtained the stoichiometry by mass balancing on 200 days of experimental data and on an estimated 90% retention of growing biomass in the reactor, however the evaluation of the stoichiometry was affected by a 50% of uncertainty in the volatile solids measurements (Strous et al. 1998). Lotti (2016) also referred that the electron balance of the conversion rates used by Strous for data reconciliation had also a significant error, so a different stoichiometry for the anammox process was proposed by them.

Their study assessed the availability of a high purity anammox culture actively growing in a controlled system (MBR), enabling an accurate identification of anammox macro-chemical reaction equation. The biomass yield was calculated during the *kinetic characterization experiment* made in the study. From the obtained yield and the elemental composition of the biomass, the stoichiometry of the anammox process was calculated considering the ammonium as the N-source and nitrite/nitrate as the electron-donor couple as shown in Equation 34:

$$NH_4{}^+ + 1.15\, NO_2{}^- + 0.071\, HCO_3{}^- \rightarrow 0.99\, N_2 + 2.0\, H_2O + 0.071\, CH_{1.74}O_{0.31}N_{0.2} + 0.16\, NO_3{}^- \quad \text{Eq.34}$$

The influent mass concentrations of NO_2-N and NH_4-N had a ratio of 1.15, equivalent to molar ratio.

From the mass balances it is possible to assess the observed NO_2-$N_{removed}$ to NH_4-$N_{removed}$ stoichiometric ratio and compare the results to the theoretical ratios proposed by Lotti or Strous (Figure 21).

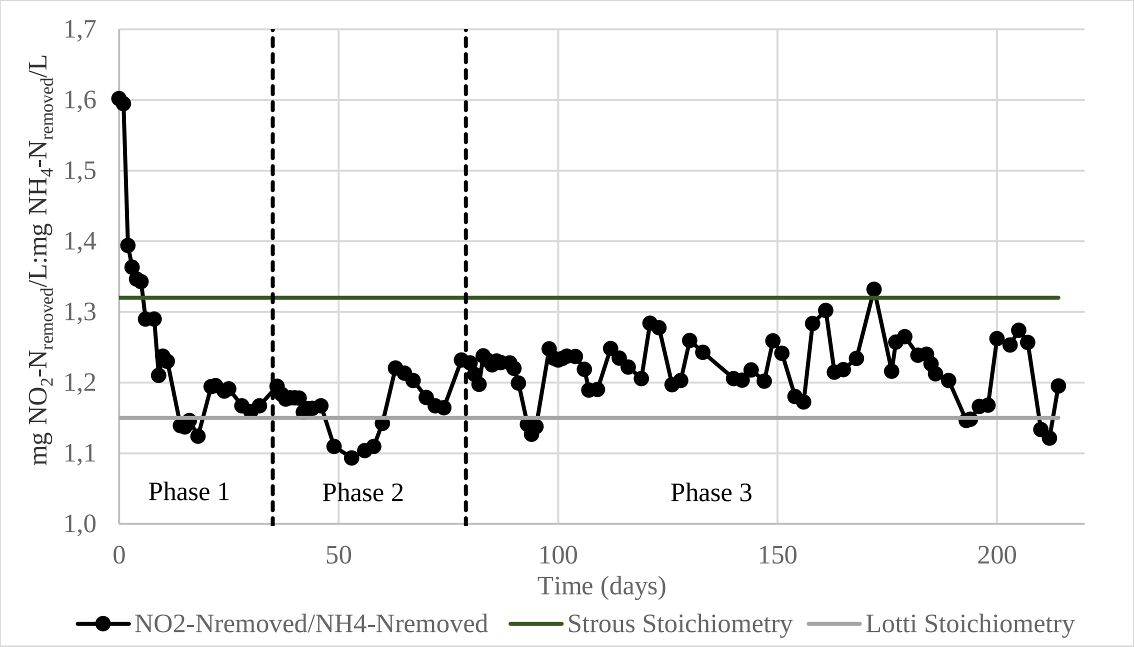


Figure 21- Comparison between the experimental NO_2-$N_{removed}$ to NH_4-$N_{removed}$ stoichiometry ratio and the theoretical values proposed by Strous and Lotti.

During the course of the experiment the ratio remained within the values 1.1-1.6. The values tended to be more similar to the theoretical value from the Lotti stoichiometry equation, but the graph seems to indicate that on Phase 3 the values have a tendency to increase and apparently become a little closer to the theoretical ratio proposed by Strous.

The overall average is 1.22 ± 0.08 which is more or less half the way between Lotti and Strous theoretical values, therefore it can be considered acceptable. The process also showed a slightly higher depletion of nitrite than expected, according to the theoretical values proposed by Lotti.

4.3.3.2 NO_3-N production

In Figure 22 the ratio between the production of NO_3-N and the consumption of the NH_4-N throughout the whole experiment is presented. Moreover a comparison is also made with the values reported in literature.

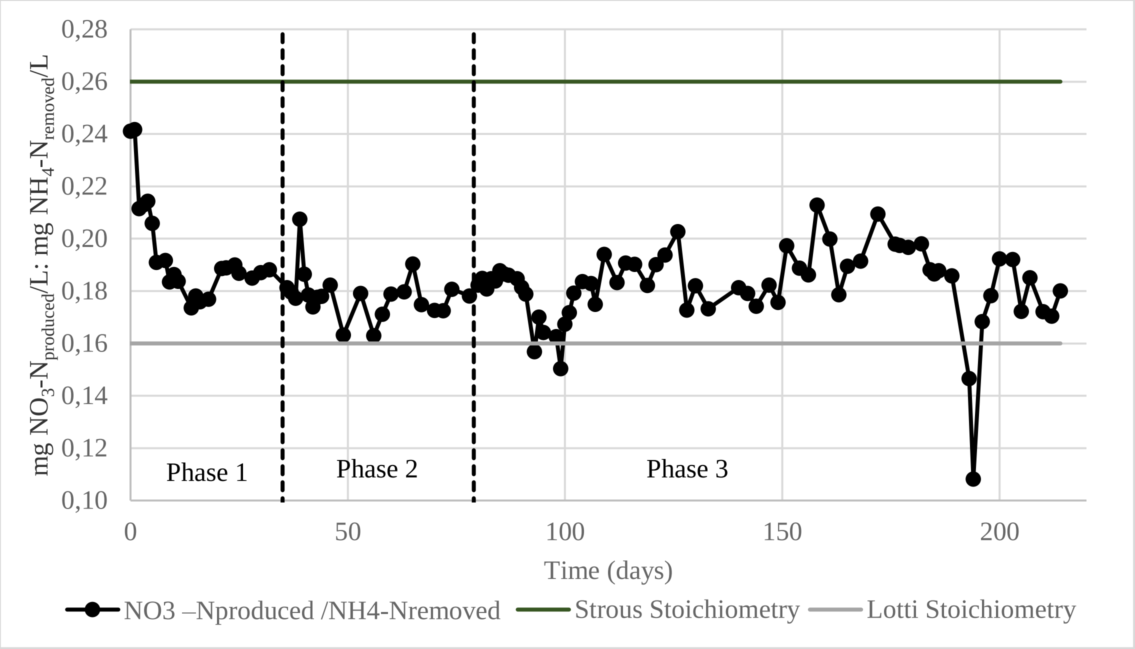


Figure 22- Comparison between the experimental NO_3–$N_{produced}$ /NH_4-$N_{removed}$ molar ratio and the theoretical values proposed by Strous and Lotti.

It is possible to see that the experimental values are closer to the Lotti stoichiometry, never reaching the value proposed by Strous (0.26). In Phase 1 the average for the ratio was 0.19± 0.02, not very different from Phase 2 and 3 that had an average of 0.18± 0.01 and 0.18± 0.02 respectively; the overall average is 0.18±0.02. So the experimental ratio was constant despite the change of the NLR applied which proves that the process has a great adherence to Lotti stoichiometry.

Table 14 shows the experimental (average) and some literature values for the parameters previously discussed:

Table 14- The experimental and theoretical values (obtained with synthetic medium) for the production of nitrate and the consumption of ammonium and nitrite.

References		**NLR** **kg N/m^3·d**	**NO_2-$N_{removed}$/ NH_4-$N_{removed}$**	**NO_3-$N_{produced}$/ NH_4-$N_{removed}$**	**Reactor**
This study	Phase 1	1	1.26± 0.14	0.19± 0.02	SBR
	Phase 2	1.2	1.17± 0.04	0.18± 0.01	
	Phase 3	1.5	1.22± 0.04	0.18± 0.02	
Strous et al. (1998)		1	1.32	0.26	SBR
Lotti (2016)		0.5-1.06	1.15	0.16	MBR
Dapena-Mora et al. (2004b)		0.7	1.11	0.2	SBR
López et al. (2008)		0.02-1.6	1.32± 0.05	0.23± 0.05	SBR
Ni et al. (2011)		1.03	1.26± 0.02	0.26± 0.01	UASB
Milia et al. (2015b)		0.13-0.35	1.07-1.67	-	SBR
Yang et. Al (2009)		0.16	1.15	-	UASB

The observed NO_2-$N_{removed}$ to NH_4-$N_{removed}$ stoichiometric ratio was closest to the one obtained by Ni et al. (2011) that used UASB reactor. When it comes to the ratio between the production of NO_3-N and the consumption of the NH_4-N the closest values were from Dapena-Mora et al (2011) and Lotti (2016). Interestingly some of the literature values using a SBR reactor are the most different from the ones obtained in this study. Another interesting observation was that with a lower NLR (like the one used by Milia et al. (2015b) and Yang et al. (2009)) the NO_2-$N_{removed}$ to NH_4-$N_{removed}$ ratio obtained was normally lower than the one obtained when the NLR applied was higher (ex. 1 kg $N/m^3 \cdot d$).

4.3.4 Suspended Solids

The solids analysis was performed only for the biomass inside the reactor, since the effluent did not have, for all the duration of the experiment, enough quantity of biomass to make a reliable measurement. The measurements were made normally every three weeks. Figure 23 is a graphic representation of the total suspended solids (TSS), volatile suspended solids (VSS) and the percentage ratio between the two (VSS/TSS).

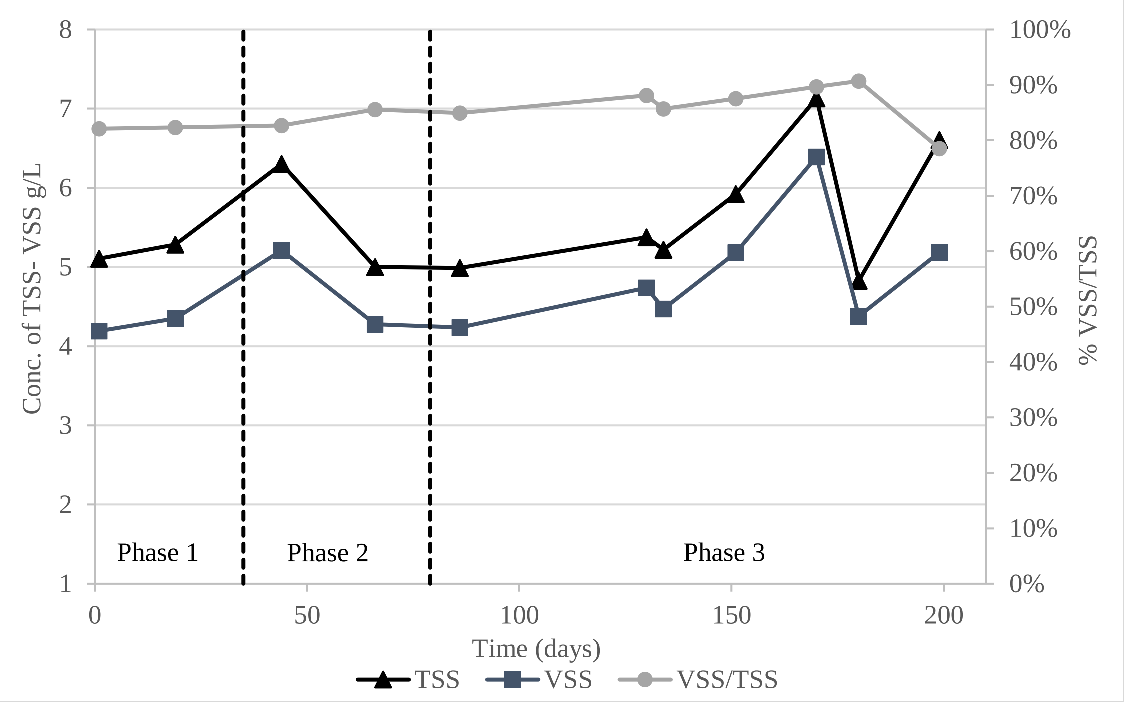


Figure 23- Solids concentrations during the course of the experiment.

The solids concentration was quite stable throughout the experiment, however the data does seem to be disperse. During Phase 3, an increasing trend was observed, but on day 180 the values dropped from 7.14 and 6.40 g/L to 4.83 and 4.33 g/L for TSS and VSS, respectively. This may be ascribed to a minor malfunction that caused, on day 172, a nitrite accumulation up to 37.0 mgNO_2-N/L, and a washout of a small quantity of biomass from the reactor together with the effluent withdrawal.

Since the discharged biomass was then recirculated inside the reactor, solids concentration increased again.

A comparison between the average values of TSS, VSS and VSS/TSS ratio of both Phase 1 and 2 was made with Phase 3, which resulted in the Table 15:

Table 15- Comparison of the solids concentrations between the phase 1&2 and the phase 3.

Phase	**Conc. TSS g TSS/L**	**Conc. VSS g VSS/L**	**VSS/TSS Ratio %**
1 & 2	5.42± 0.60	4.51± 0.47	83± 1.6%
3	5.72± 0.87	4.94± 0.74	86± 4.0%

Taking into account the averages, there is an increase in solids concentration from Phase 1 and 2 to Phase 3, yet this increase is very small and is accompanied by a significant standard deviation.

The density of the granules was also measured (Figure 24) and much like TSS and VSS, the density appears to be slighting increasing in Phase 3.

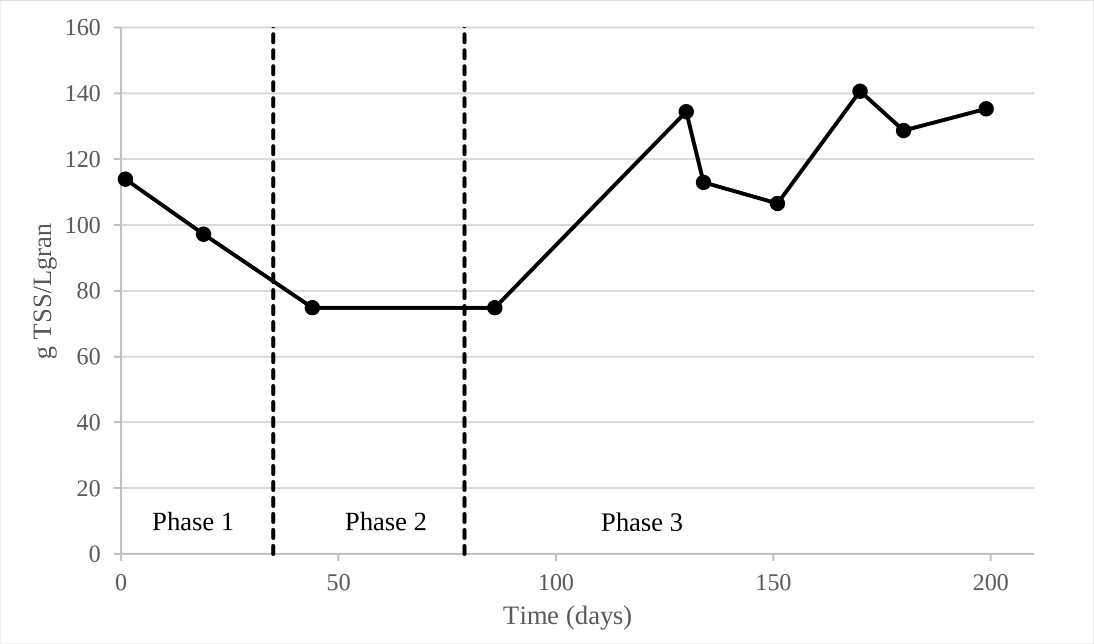


Figure 24- Density of the granules during the experiment.

In Table 16 the average values of TSS and VSS concentration, VSS/TSS ratio and granules density, calculated throughout the whole experiment, are reported. Overall, they all showed a mostly stable trend and results that are in good agreement with the slow growth of autotrophic and anaerobic organisms like anammox.

Table 16- Average of the total suspended solids concentration, volatile suspended solids concentration, ratio between the two of them, and density.

Conc. TSS **g TSS/L**	**Conc. VSS** **g VSS/L**	**VSS/TSS Ratio** **%**	**Density** **g TSS/L_{gran}**
5.62 ± 0.76	4.78 ± 0.67	85.2 ± 3.7%	111.94 ± 23.95

4.3.5 Specific NO_2-N and NH_4-N removal rates, as well as specific NO_3-N production rate

A series of tests was made to determine the volumetric removal rate of NO_2-N and NH_4-N, as well as the volumetric production rate of NO_3-N, expressed as mg N/l·h. By relating those values to the corresponding volatile suspended solids concentration in the reactor it was possible to calculate the specific NO_2-N and NH_4-N removal rates, as well as the specific NO_3-N production rates, expressed in g N/g VSS·d. These tests were performed when the NLR was stable.

4.3.5.1 NO_2-N removal rates

In Figure 25 a typical example of a valid ($R^2 \geq 0.96$) kinetic test for NO_2-N removal is shown.

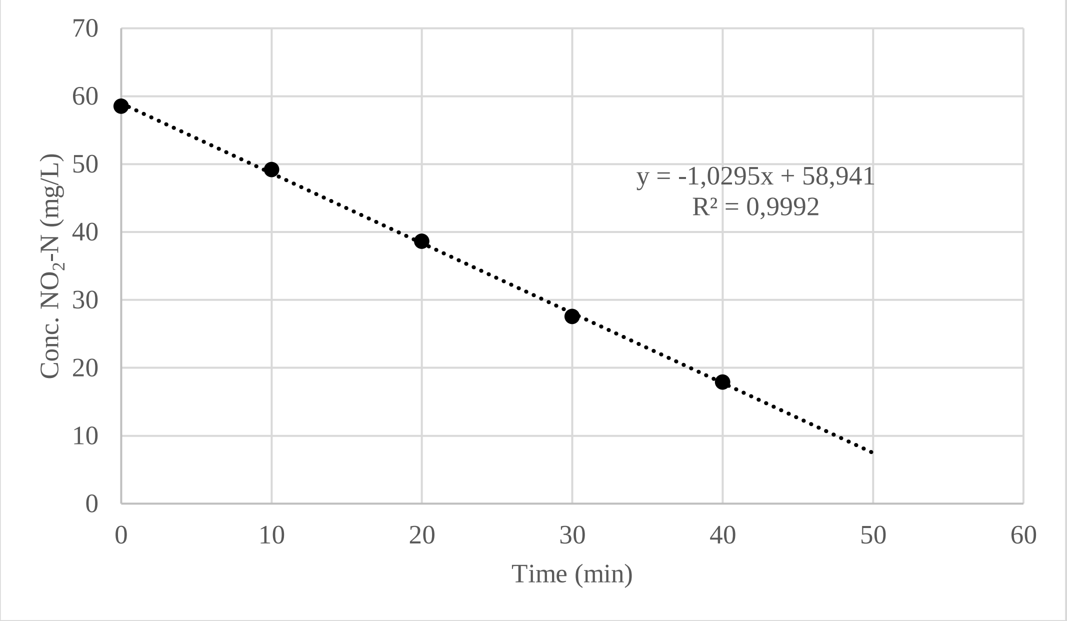


Figure 25- Volumetric removal rate of nitrite (day 212).

In Figure 26 the volumetric nitrite removal rates measured during the experimentation are shown. Despite values seemed to be affected by a certain variability, an increasing trend can be detected, especially during Phase 3. It seems that even though the NLR was increased that had not a negative impact on the removal rate of the nitrite. This can be due to the gradual change in the NLR that allowed the bacteria to better acclimate to the increase of nitrogen loading or be due to a biomass increase (more biomass can consume nitrite faster).

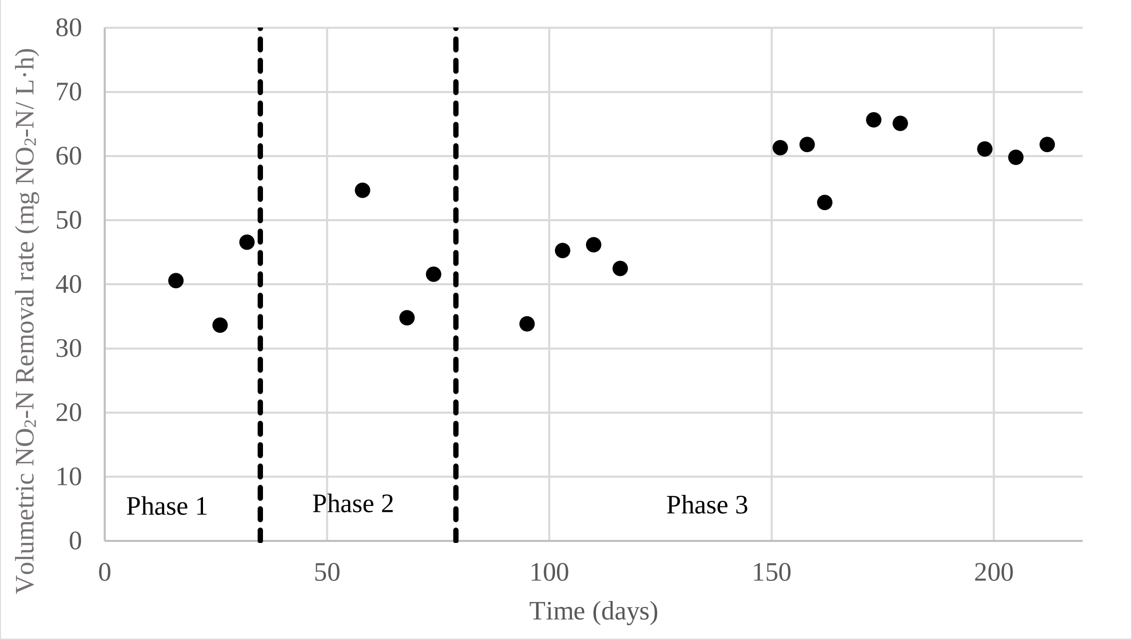


Figure 26- The nitrite volumetric removal rates throughout the experiment.

According to Table 17 the removal rate increased in each phase, which indicates that the performance of the anammox bacteria is not affected by higher nitrogen loading rates. However the values corresponding to the first two phases are very similar with a high standard deviation which can mean that there is not really an increase from Phase 1 to 2 but more a stabilization. Despite a not negligible variability of the data, during Phase 3 a clear increasing trend was detected.

Looking closer to the duration of each phase this behaviour may be explained. Both Phases 1 and 2 had more or less the same duration time, but Phase 3 had more than the double of the duration, which signifies that the bacteria had more time to get acclimated to the NLR used in that phase. But this conclusion cannot be done looking only into this parameter, since it does not take into account the solids concentration inside the reactor (VSS).

Table 17- Average volumetric nitrite removal rate for each Phase.

NLR kg N/m^3·d	NO_2-N Removal rate mg NO_2-N/L·h
1	40.24± 6.54
1.2	43.66± 10.10
1.5	54.74± 10.40

The specific removal rate is a better method to correlate the removal of the NO_2-N inside the reactor with biomass activity. So, as Figure 27 demonstrates, the specific removal rate follows the trend of the removal rate as expected, yet the increase appears to be lower.

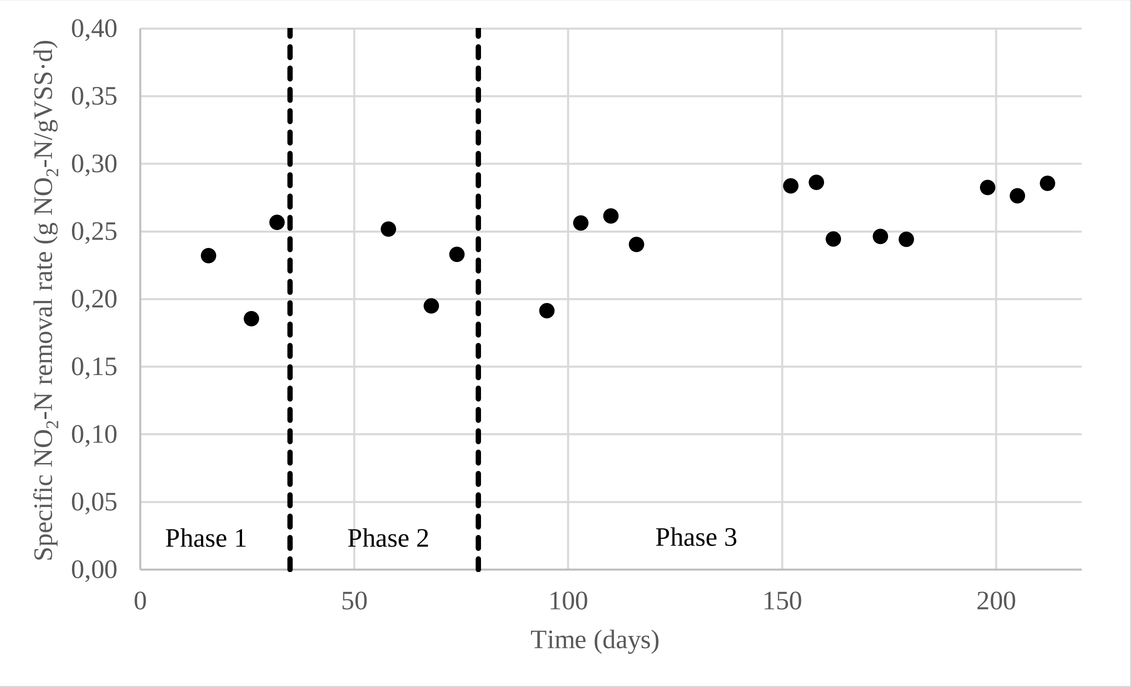


Figure 27- The specific nitrite removal rates during all the experiment.

Table 18 shows the average nitrite specific removal rate for the different phases. The specific removal rates are very similar, despite the increase of NLR. This means that the anammox activity was not hindered, indicating a very good biomass acclimation.

Table 18- Specific nitrite removal rates during the experiment.

NLR kg N/m^3·d	NO_2-N specific removal rate gNO_2-N/gVSS·d
1	0.22± 0.04
1.2	0.23± 0.03
1.5	0.26± 0.03

The highest value obtained for this parameter was 0.29 gNO_2-N/g VSS·d while the average for the whole experiment was 0.25± 0.03 gNO_2-N/g VSS·d.

These results can be compared to those reported in the literature. Strous et al. (1996) obtained, using a fluidized bed reactor with a synthetic medium, a nitrite specific removal rate of 0.1 g NO_2-N/ g VSS·d. Chen et al. (2011b) reported the kinetic characteristics of anammox in a EGSB (Expanded Granular Sludge Bed) reactor after feeding with high ammonium-containing synthetic wastewater (95-500 mg NH_4-N/L and 90-520 mg NO_2-N/L) and the values obtained for the specific removal rate were between 0.04-0.95 gNO_2-N/gVSS·d, very low when compared to the ones obtained in this study. Before Chen et al. (2011a) had performed a kinetic assay, also with a EGSB reactor, however they applied an even higher concentration of both ammonium and nitrite (300-700 mg NH_4-N/L and 300-770 mg NO_2-N/L), obtaining values for the specific removal rate between 0.16-0.3 gNO_2-N/gVSS·d, very similar to the ones obtained in this study. Interestingly Chen had better (and closer to the ones obtained in this study) specific nitrite removal rate when the influent concentrations were higher.

4.3.5.2 NH_4-N removal rates

In Figure 28 a typical volumetric ammonium removal rate measurement is shown.

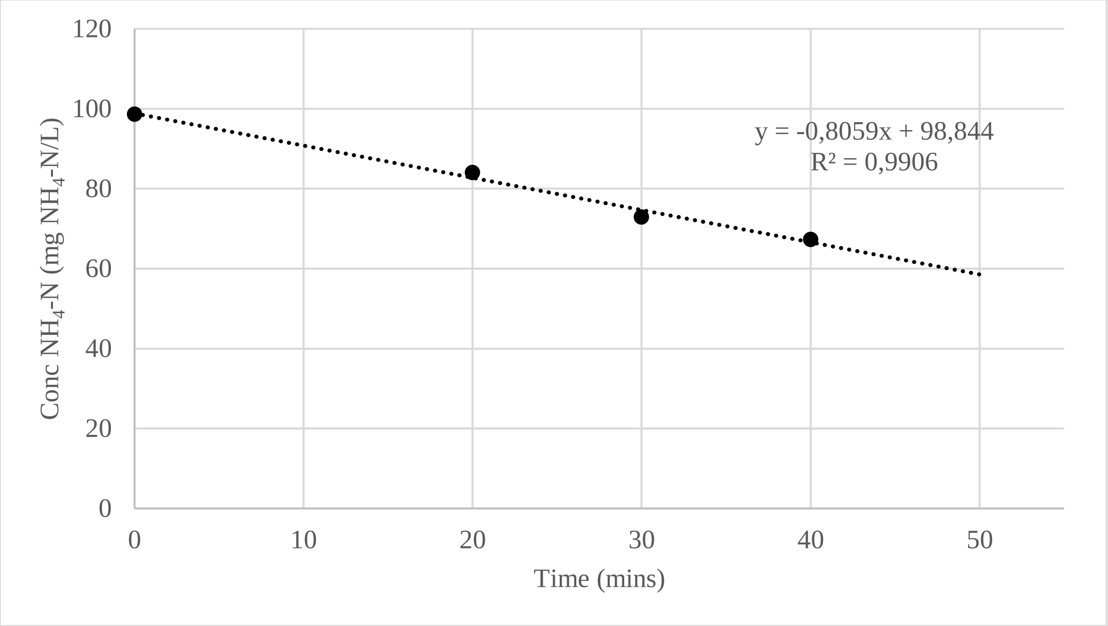


Figure 28- Volumetric ammonium removal rate (day 212).

In Figure 29, the specific ammonium removal rates measured during the experiment are reported. The values seem to have stabilized in Phase 3 after an increase in Phase 2. Table 19 was created with the average values of volumetric and specific removal rates in order to have a better understanding of these parameters.

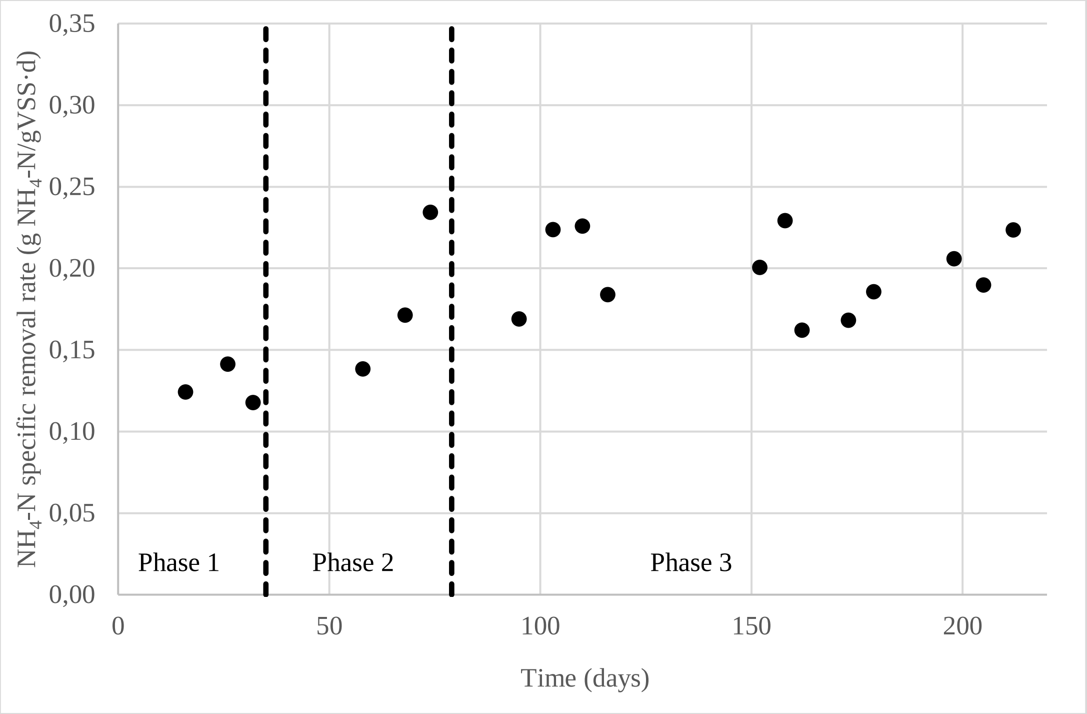


Figure 29- The specific ammonium removal rates during all the experiment.

As shown in Table 19 both volumetric and specific rates increased during the experiment, with a bigger difference from Phase 1 to Phase 2. Moreover, the ratio between NO_2-N and NH_4-N specific removal rates stabilized at 1.33±0.18 during Phase 3, close to the expected stoichiometric value proposed by Strous et al. (1998), indicating that ammonium and nitrite removal were due to anammox activity.

The values obtained for both the ammonium volumetric and specific removal rates are lower when compared to the corresponding values obtained for the nitrite, as expected if anammox is the only metabolic process occurring. Table 20 shows the average SNRR (Specific Nitrogen Removal Rate) values obtained for each phase of the experiment.

Table 19- Volumetric and specific removal rates of ammonium for each Phase.

NLR **kg N/m^3·d**	**Removal NH_4-N rate** **mg NH_4-N/l·h**	**Specific NH_4-N removal rate** **gNH_4-N/gVSS·d**
1	22.89± 2.36	0.13± 0.01
1.2	34.14± 6.64	0.18± 0.05
1.5	41.48± 6.50	0.20± 0.02

Table 20- Specific nitrogen removal rates for each Phase.

NLR **kg N/m^3·d**	**Specific nitrogen removal rate** **gN/gVSS·d**
1	0.35± 0.05
1.2	0.41± 0.08
1.5	0.46± 0.06

Dapena-Mora et al. (2004a b) obtained a SNRR of 0.5 g N/g VSS·d and a NH_4-N specific removal rate of 0.18 g NH_4-N/ g VSS·d. Van Dongen (2001) however obtained a SNRR of 0.82 gN/gVSS·d, while under anoxic or oxygen-limiting conditions the SNRR dropped to 0.08 gN/gVSS·d. Milia et al. (2015b) obtained a value for the SNRR of 0.49 gN/gVSS·d and for NH_4-N specific removal rate 0.2 g NH_4-N/ g VSS·d using a SBR reactor. Chen et al. (2011a) obtained values for the specific ammonium removal rate within the range 0.14-0.28 gNH_4-N/gVSS·d, and for the SNRR within the range 0.30-0.57 g N/ g VSS·d. Later that year Chen et al. (2011b), using the same reactor but with a lower nitrogen concentration on the influent, obtained values for the specific ammonium removal rate within the range 0.04-0.74 gNH_4-N/gVSS·d, and for the SNRR within the range 0.08-1.65 g N/ g VSS·d. These values are comparable with those obtained in this work; moreover, it is possible to observe that Chen et al. (2011 a,b) obtained a similar trend with the specific nitrite removal rate being always higher than the specific ammonium removal rate.

4.3.5.3 NO_3-N production rates

Figure 30 shows an example of a typical measurement of nitrate production rate. The concentration profile followed a linear trend, as previously observed for ammonium and nitrite consumption.

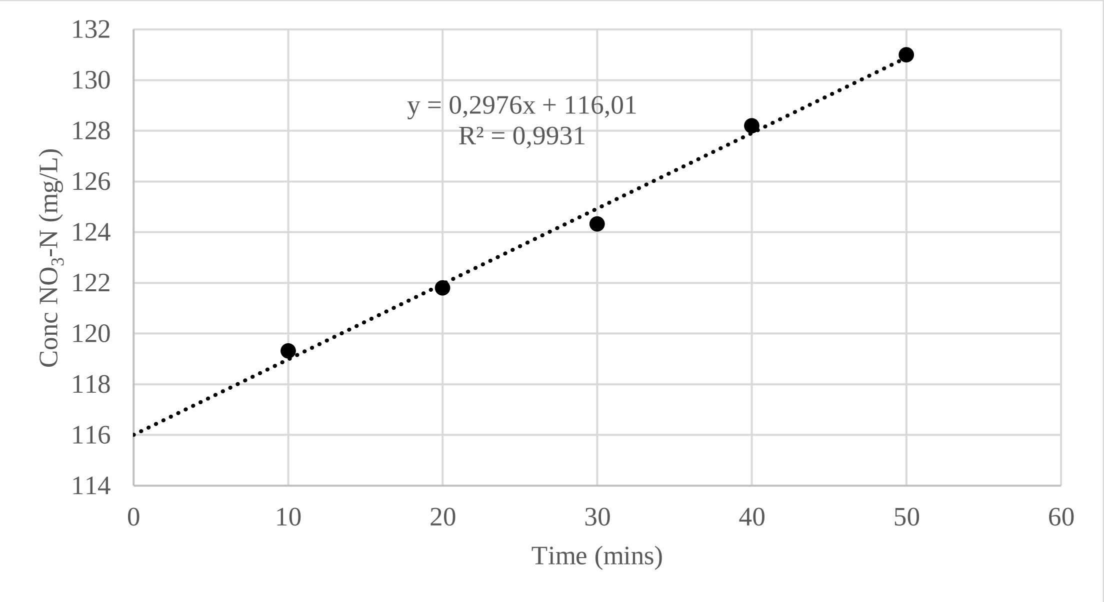


Figure 30- Volumetric nitrate production rate (day 212).

According to Figure 31 there is an increase of the production in Phase 3, coherently with ammonium and nitrite removal rates.

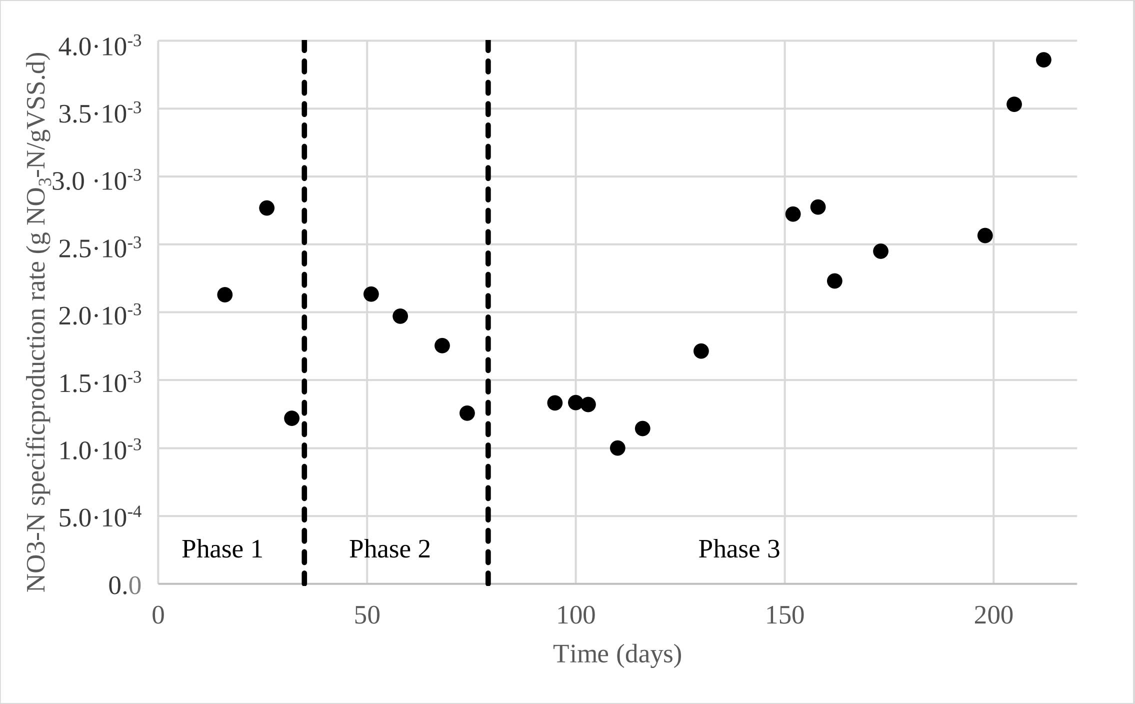


Figure 31- The specific nitrate production rates during all the experiment.

Table 21 shows that the increase is actually very small for both the volumetric and specific production rate, with a small variation.

Table 21- Volumetric and specific production rates of nitrate for each Phase.

NLR kg N/m^3·d	**Volumetric NO$_3$-N production rate** mg NO$_3$-N/l·h	**Specific NO$_3$-N production rate** gNO$_3$-N/gVSS·d
1	11.40± 4.33	$2.04 \cdot 10^{-3} \pm 7.79 \cdot 10^{-4}$
1.2	8.96± 1.31	$1.78 \cdot 10^{-3} \pm 3.81 \cdot 10^{-4}$
1.5	10.35± 3.77	$2.15 \cdot 10^{-3} \pm 9.30 \cdot 10^{-4}$

4.3.5.4 Day 193 system failure and biomass inhibition

During the experiment there were few problems that caused nitrite accumulation in the reactor: most of them had a very little impact, except for the system failure that occurred on day 193.

As said in Paragraph 4.3.2, Concentrations of 409 mg NO_2-N/L and 369.77 mg NH_4-N/L were measured in the effluent. The concentration of nitrite obtained was higher than the inhibiting concentration reported (180 mg NO_2-N/L) by Van Hulle et al. (2010). It can be assumed that the inhibition was provoked by this high concentration of nitrite and not from the ammonium, since according to the author the inhibiting concentration of ammonium is 1 g NH_4-N/L. The reactor feeding was then immediately stopped and samples were taken at different times in order to evaluate nitrite and ammonium removal rates. Since no significant nitrogen removal activity was detected, biomass metabolism appeared to be almost completely inhibited. The reactor was flushed 3 times with 1.2 L of warmed (35°C) tap water in order to progressively dilute the nitrite and ammonium concentrations down to 31.02 mg NO_2-N/L and 36.21 NH_4-N/L, respectively. After that, samples were taken in order to evaluate if nitrite and ammonium removal occurred again (Figure 32 and Figure 33).

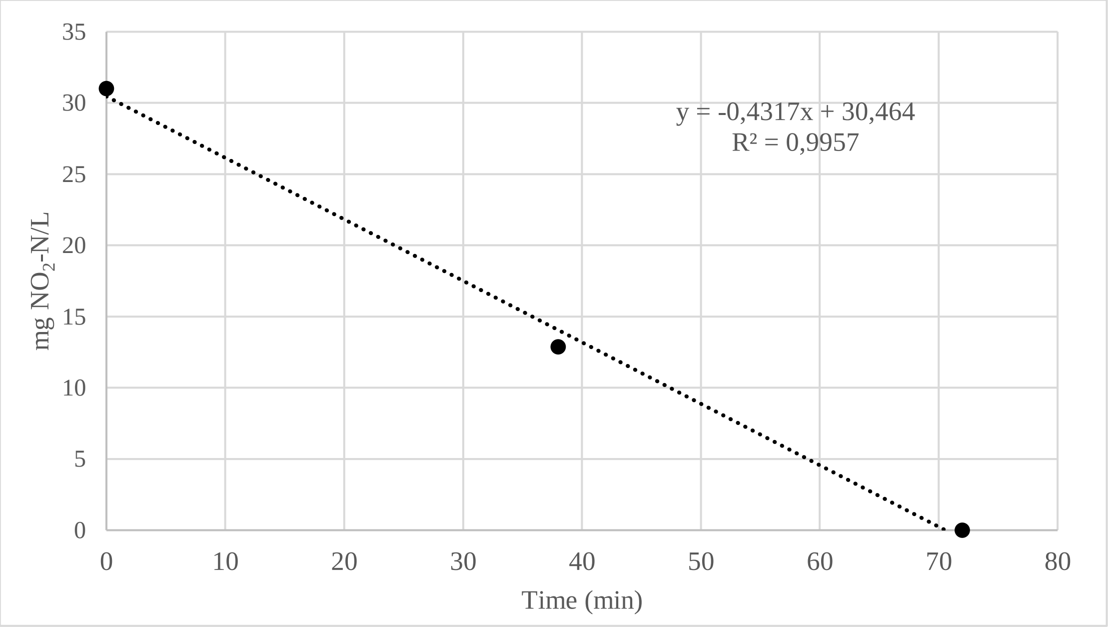


Figure 32- Volumetric nitrite removal rate (day 193).

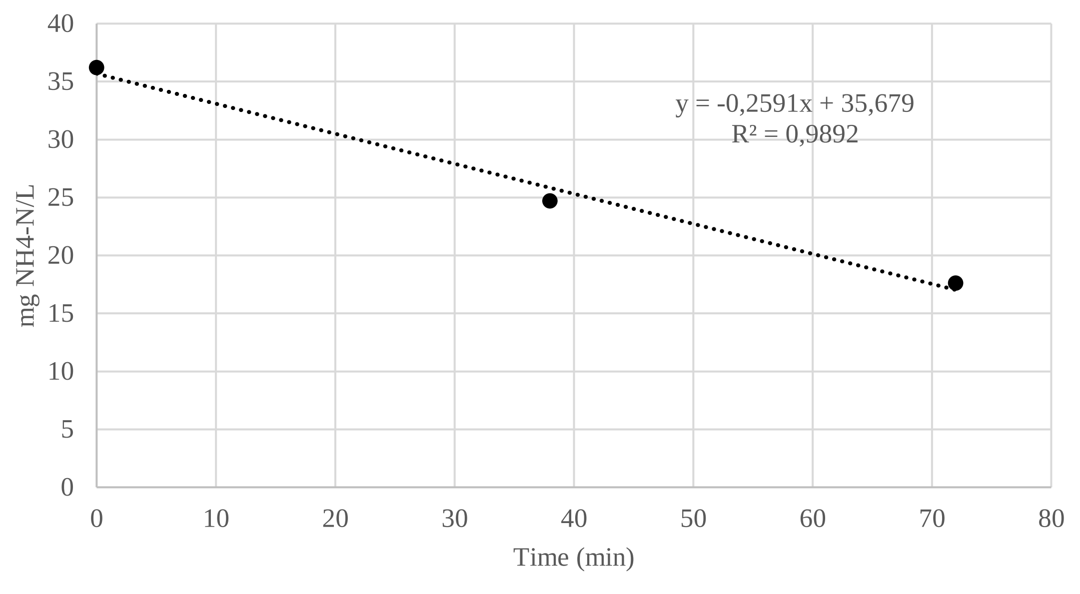


Figure 33- Volumetric ammonium removal rate (day 193).

Volumetric removal rates of 25.9 mg NO_2-N/L·h and 15.5 mg NH_4-N/L·h, were measured, corresponding to 40% and 31% of the previous (day 179) measured values, respectively. Since activity showed to recover quickly, normal working operations were then started again after just one cycle of suspension. As previously reported, the day after removal efficiencies showed to be restored to usual values. A complete kinetic assay was performed 5 days after the system failure occurred (day 198), showing that the anammox activity was completely recovered since volumetric nitrite and ammonium removal rates of 61.50 mg NO_2-N/L·h and 44.54 mg NH_4-N/L·h were measured. The specific removal rates were also obtained (0.28 gNO_2-N/gVSS·d and 0.21 gNH_4-N/gVSS·d) and interestingly were higher than the average values calculated for Phase 3.

Anammox biomass showed a quick and complete recovery after a complete inhibition due to a prolonged exposition to high nitrite and ammonium concentrations. Inhibition was shown to be reversible. Comparing this study with different literature values (Table 21) only Lotti et al. (2012) has a similar performance recovery (with very high NO_2-N concentrations). Their study concluded that the anammox process can be stable and not prone to temporarily adverse effects of nitrite in the reactor. A similar conclusion can be drawn in this study, based on the data analysed.

Table 22- Literature values for nitrite and ammonium inhibition and corresponding activity recovery.

	NO_2-N mg/L	**NH_4-N mg/L**	**Time recovery**
Strous et al. (1999)	100	1000	Completely inhibited
López et al. (2008)	101.8	-	5 days
Ni et al. (2010)	310	310	26 days
Lotti et al. (2012)	1000	-	2 days
This study	409	396.38	5 days

5. GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

5.1 General conclusions

The present study was approached in order to provide a preliminary evaluation of the applicability of the SHARON-anammox process to the treatment of the ammonium-rich liquid fraction of the AD digestate. This preliminary test had a duration of 275 days for the SHARON reactor and 214 days for the anammox reactor.

The results were positive starting with the SHARON process which showed a stable behaviour for all the different HRT tested. However the lowest HRT (1 d) can be considered as the optimal one: a lower HRT implies a highest treatable wastewater flow (if the volume is given), or a lower reactor volume (if the wastewater flow is given). The obtained values for the effluent NO_2-N/NH_4-N ratio were slightly lower (0.86-1.04) than those used in the anammox reactor (1.15), however such ratios are suitable for the anammox process. So a change to real wastewater in the SHARON reactor can be applied.

An extensive study was realized for the anammox reactor, with promising results: the NRR/NLR ratio was 96.4± 4.8% for all the experiment, indicating that process remained stable despite the change in operating conditions. The NitDR was always extremely low (< 0.049 kg N/$m^3 \cdot$d), and temporary nitrite accumulation inside the reactor occurred very few times due to system malfunctions.

In terms of efficiency the process showed a very good nitrogen removal, with NRE values of 88.4± 2.3%, 88.7± 0.6% and 89.0±0.9% for Phase 1, 2 and 3, respectively. The nitrite removal efficiencies were always 100%, except during Phase 3 (99%), while the ammonium removal efficiencies were stable at 93-94% throughout the experimental campaign. A comparison with the literature was made, concluding that removal efficiencies are very close to what had been previously reported. In conclusion the anammox process is very efficient and stable in the nitrogen removal, independently of NLR applied.

Also a comparison between the anammox stoichiometry obtained in this study and the ones reported in the literature has been carried out. The obtained values for all the experiment were 1.22 ± 0.08 and 0.18± 0.02 for both NO_2-$N_{removed}$/NH_4-$N_{removed}$ and NO_3-$N_{produced}$/NH_4-$N_{removed}$ respectively. Both values appear to be between those proposed by Lotti (1.15 and 0.16, respectively) and Strous (1.32 and 0.26, respectively), however other values taken from different literature seem to be also close despite the different reactors employed in each study.

The solids analysis showed that biomass behaved in agreement with the slow growth of autotrophic and anaerobic organisms with a VSS/TSS ratio of 85.2 ± 3.7% and a density of 111.94 ± 23.95g TSS/$L_{gran.}$

The specific removal rates showed interesting results, the values followed an increasing trend despite the increase of NLR. The average values for both NO_2-N and NH_4-N specific removal rates when the NLR was 1.5 kg N/m^3·d were 0.26± 0.03 gNO_2-N/gVSS·d and 0.20± 0.02 gNH_4-N/gVSS·d, respectively. This suggests that the process can be capable of withstanding nitrogen loads higher than the ones already applied.

In the end of the experiment (day 193) a malfunction occurred in the reactor provoking an inhibition of the anammox bacteria. After analysing and applying corrective measures it was observed a quick recovery of the activity. The specific nitrite removal rate was recovered in few days showing the strength of this process even when compared to literature.

This process seems ready to the next phase of the experiment, the gradual change of the synthetic influent to real wastewater. It has been proven to be very stable in the removal efficiencies, and in case of malfunction of the system it recovers in a few days.

5.2 Recommendations for further research

As mentioned before, this Thesis focuses on a preliminary evaluation of both the SHARON and anammox reactors before they can be fed with real liquid fraction of the AD digestate.

Some key operating parameters in the SHARON reactor should be changed in order to determine the best conditions. After HRT (object of this Thesis), oxygen level is going to be changed, and the corresponding process performance evaluated, also considering the evolution of N_2O concentration in the effluent gas. The synthetic influent will be then replaced by the real wastewater.

As for the anammox reactor, the progressive replacement of the synthetic influent with real wastewater (pre-treated in the SHARON reactor) will be carried out. It is critical, due the anammox sensitivity and low growth nature, to perform preliminary batch tests before replacing the synthetic influent: such tests could predict the inhibition that the real wastewater or some major compounds in it may have on anammox activity.

For both the SHARON and anammox process fed with real wastewater, the operating parameters will be further optimized in order to maintain stable process performance.

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APPENDICES

APPENDIX I

Table 23- Examples of SHARON-anammox full scale applications.

Plant	Influent composition			Effluent composition				
	NH_4^+-N mg l^{-1}	COD/ NH_4^+-N	PO_4^{3-}-P mg l^{-1}	NH_4^+-N mg l^{-1}	NO_3^--N mg l^{-1}	NO_2^--N mg l^{-1}	COD mg l^{-1}	PO_4^{3-}-P mg l^{-1}
Olburgen	<500	1.1	<20	<25	<25	<25	<200	<15
Bergen op Zoom	<500	3.7	≈50	<5	<10	-	-	-
Lichtenvoorde	≈ 500	2	< 5	≈25	≈25	<10	<250	<5
Zürich	700	0.9	<50	<50	<20	<1	-	-
Balingen	>500	1.6	-	<100	<50	<1	-	-
Plettenberg	>500	-	-	<100	<50	<5	-	-
Amersfort	>500	-	-	≈150	<25	<5	-	-
Heidelberg	≈1000	-	-	<50	≈50	<5	-	-
Malmö	≈1000	0.7	-	<10	<100	<5	-	-
Ingolstadt	≈1000	0.7	-	≈150	<100	<1	≈ 250	<25
Nieuwegein	≈1000	0.6	≈200	≈200	<100	<20	-	-
Rotterdam	≈1000	15	<50	≈500/ <50	-/<100	≈500/<5	-	-
Apeldoorn	>1000	1.8	≈200	≈50	≈ 50	<5	<1000	≈ 150
Landshut	>1500	0.3	<20	≈10/≈50	≈10/≈50	>500/ <1	-	-

APPENDIX II

Table 24- The operating conditions on anammox for each phase.

Phase	Total Nitrogen mgN/L	Initial NLR $kgN/m^3 \cdot d$	NLR target $kgN/m^3 \cdot d$	HRT target d	Flow rate mL/min	Feed duration (min) min	Feed duration (min) max	Volume fed per cycle (mL) min	Volume fed per cycle (mL) max	Volumetric exchange ratio	Duration (days) Whole step	Duration (days) NLR increasing	Duration (days) NLR constant
1	1500	0,8	1,0	1,5	1,2	208	267	250	320	0,17	35	10	25
2	1500	1,0	1,2	1,25	1,5	213	267	320	400	0,20	43	9	34
3	1500	1,2	1,5	1,0	2,0	200	267	400	533	0,25	107	11	96