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Bachelor of Science in Chemical and Biochemical Engineering

Anaerobic Nitrogen Removal: The start-up of an Anammox Reactor with pre-treated Real wastewater from the Anaerobic Digestion of municipal solid waste.

Dissertation to obtain the degree of Masters in Chemical and Biochemical Engineering

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Science is about knowing; engineering about doing
Henry Petroski

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Abstract

In this study, wastewater produced by the Anaerobic Digestion of municipal solid waste and pre-treated by partial-nitrification was fed to a granular Anammox reactor. A conservative exponential Law was used to slowly replace the synthetic wastewater for the pre-treated wastewater, starting at 10%.

This strategy was adopted in order to avoid stressful conditions to bacteria, which could thus acclimate to the reactor conditions.

The experiment was divided in two phases, the first one with increasing real wastewater ratio and a second phase totally fed with real wastewater.

The influent is characterized by a nitrogen loading rate of 1.5gNL^{-1}d^{-1}, with a NO2-N/NH4-N ratio of 1.20.

Removal efficiencies were stable during all experiment, around 90%.

The Anammox activity also increased during the experimentation. The Volatile suspended solids grown during phase 1 of experiment, with an initial value of 4,2 gVSSL^{-1}.

The kinetics assay results also helped checking out the removal rates for ammonium and nitrite, and production rates for nitrate. On the average the specific nitrate and ammonium removal rate, with continues NLR was 0,37±0,07 g NO_2-N gVSS^{-1}d^{-1} and 0,27±0,07 gNH_4-N/gVSS^{-1}d^{-1}, respectively. For nitrate production was 0,055±0,030 gNO_3-NgVSS^{-1}d^{-1},

The organic carbon removal was also tested. The effluent values were variable, and real wastewater had no direct effect on the capability of the Anammox process to remove organic carbon. This is due to a perfect partial-nitrification, who allowed the real effluent to have low organic carbon, which not developed inhibition effect to the Anammox bacteria.

During phase 2, the reactor was fed with real wastewater without any pre-treatment.

This experiment wasn’t very successful, and Anammox system failed.

After, was again fed with synthetic wastewater and the bacteria could recover.

Results showed that the process can work with this type of wastewater, if a conservative start-up strategy is adopted and a partial-nitrification step is used to remove organic carbon.

Keywords: Anaerobic digestion, Anammox, partial-nitrification, ammonium, nitrite, real wastewater, Nitrogen removal

Resumo

Durante este estudo, água residual proveniente da Digestão Anaeróbica de resíduo solido municipal foi pré-tratada e posteriormente alimentada a um reator Anammox granular. O reator foi iniciado com um rácio sintético:real de 90%:10%

Uma estratégia de alimentação foi adotada de maneira a substituir gradualmente o influente sintético pelo real, de maneira a não colocar a bactéria sobre condições de stress químico.

A experiencia foi dividida em duas fases, uma primeira com um crescimento gradual do rácio de influente real e uma segunda fase com 100% influente real.

O influente é caracterizado por um *Nitrogen Loading Rate* de 1. 5gN L^{-1} d^{-1}, com um rácio NO2-N/NH4-N de 1.20 (818 mg/L e 682 mg/L, respetivamente.

.

As eficiências de remoção foram estáveis durante toda a experiencia, por volta dos 90%

A atividade da bactéria aumentou durante o período experimental. Foi observado um crescimento de sólidos suspensos voláteis durante a primeira fase. O valor inicial foi de 4,2 g VSS L^{-1}.

De maneira a avaliar a atividade do processo, ensaios cinéticos foram realizados. Foi então possível observar e calcular as velocidades de remoção de amónia e nitrito e produção de nitrato. Em media, taxa de remoção especifica de amónia e nitrito foram 0,37±0,07 g NO_2-N gVSS^{-1}d^{-1} e 0,27±0,07 g NH_4-N /gVSS^{-1}d^{-1}, respetivamente, com NRL continuo.

Já a taxa de produção de nitrato foi 0,055±0,030 g NO_3-N gVSS^{-1}d^{-1}.

A capacidade de remoção do processo foi também testada. A concentração de TOC no efluente foi variável durante todo o processo, as dentro de valores que não causaram inibição do processo.

Por trás disto, estará a elevada eficácia do processo de pré-tratamento em remover, permitindo o influente do processo Anammox ter uma concentração baixa de TOC.

Durante a segunda fase, o reator foi alimentado com agua residual não tratada. A experiencia não foi bem-sucedida, e o Sistema falhou durante uns dias.

De maneira a resolver este problema, foi novamente alimentado a 100% com influente sintético e o Sistema voltou ao normal.

Os resultados obtidos mostram que o sistema e capaz de trabalhar com este tipo de agua residual, se uma estratégia de alimentação conservativa for adotada e um pré-tratamento for usado para remover carbono orgânico.

Keywords: Anaerobic digestion, Anammox, partial-nitrification, ammonium, nitrite, real wastewater, Nitrogen removal.

Abbreviations

HyMeCA – Hydrogen-Methane-Compost-Ammonia

Anammox – Anaerobic ammonium oxidation

SHARON – Single reactor

COD – Chemical oxygen demand

NOB – Nitrite oxidizing bacteria

AOB – Anammox oxidizing bacteria

HRT – Hydraulic Retention Time

SRT – Sludge Retention Time

FISH – Fluorescence in situ Hybridization

SNAD - Simultaneous partial-nitrification, ANAMMOX and denitrification

NLR – Nitrogen Loading Rate

NLRmáx – Nitrogen Loading Rate maximum

Ntot- Total Nitrogen

TOC – Total Organic Carbon

NRR – Nitrogen Removal Rate

NitDR – Nitrite Discharge Rate

NRE – Nitrogen Removal Efficiency

TC – Total Carbon

IC – Inorganic Carbon

TSS – Total Suspended Solids

VSS – Volatile Suspended Solids

SBR – Sequencing Batch reactor

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1. Introduction

1.1 Overview

Ammonia is one of the most important components in wastewater which must be removed before effluents can be discharged, otherwise it can cause eutrophication and consequent oxygen depletion, originating profound changes in the ecosystem. This N-removal is most of the times achieved by complete ammonia oxidation to nitrite and subsequent reduction of the nitrate to dinitrogen gas under anoxic conditions.

This type of removal has been done for many years, but the need for new and sustainable systems for nitrogen removal has increased in last decades since the conventional systems cannot longer deal with the increasing nitrogen loads in a cost-effectiveness way. An alternative way was with the implementation of a partial-nitrification/Annamox processes for biological N-removal.

1.2 Main goals of this thesis

First of all, assess the main problem: after the anaerobic digestion of municipal solid waste and the many benefits we can take from that (the energy recovery as H_2 and CH_4) we need to make sure that the liquid residues, ammonium-rich liquid streams, are treated before being discharged in the environment.

To do such, is necessary to evaluate the applicability of the partial-nitrification/Anammox process in the treatment of real wastewater.
Inside the HyMeCA project, this thesis was developed during the operation objective 3.4, the evaluation of the SHARON-Anammox process behaviour when fed with increasing amounts of real wastewater produced by anaerobic digestion in a semi-continuous mode, up to the complete substitution of the synthetic influent. The performance was evaluated in terms of N-removal.

In short, starting with low ratio between real and synthetic ammonium rich influents, increasing this ratio up to the influent is totally real wastewater and then optimize.

The results of this thesis were achieved by controlling the Anammox reactor within certain parameters such as pH, temperature, nitrogen loading rate.

1.3 Structure of the thesis

In chapter 2 an overview about the nitrification/denitrification process is provided, with a special interest in the Anaerobic nitrogen removal: partial-nitrification/Anammox, with close-up on the process parameters and applications.

Materials and methods used to carry out the experiment are presented in chapter 3.

Chapter 4, the results and respective discussion.
The main conclusions of the experimental activity are given in chapter 5.

1.3 HyMeCA project

One of the main problems concerning the environment are associated with the municipal solid waste and its correct management. Despite the significant reduction in production, proper management of organic waste is mandatory by legislation. The most applied treatment technologies are composting and anaerobic digestion, which are characterized by important weak points as stand-alone processes (i.e operative costs are not counter-balanced by product income and instability due to intrinsic heterogeneity of organic residues are the main problems).
Aiming the correct management of organic residues and looking forward to the objectives expressed in HORIZON 2020, which considers the maximum energy recovery as an important valorisation of process residues and minimum environmental impact as the true eco-sustainable approach, the HyMeCA project appears as a novel research proposal, which intends the development of an integrated system for the biological treatment, combining the production of H_2 and CH_4 from the organic fraction of MSW, the valorisation of solid residues by anaerobic bio-oxidation (also known as composting) and the biological treatment of ammonium rich liquid streams, based on a double-stage partial-nitritation/anaerobic ammonium oxidation process (SHARON-Annamox).

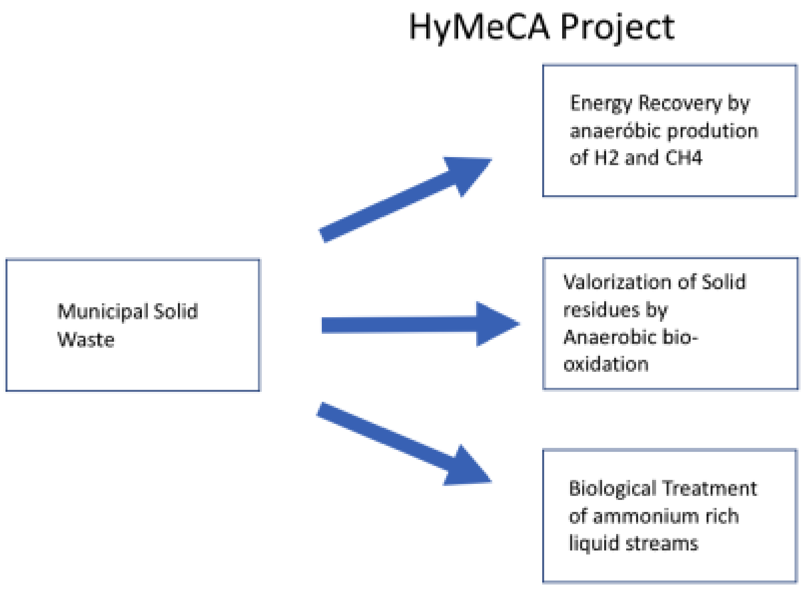


Figure 1 - HyMeCa Project block diagram

2. Article review

In the past four decades, much focus has been placed on solving the harmful environmental effects of discharging high concentration nitrogen (in form of ammonia or organic nitrogen) effluents into lakes and rivers. These effluents have been shown to be toxic for most of the fish and other aquatic life, causing depleting dissolved oxygen levels and eutrophication [1].

The most recognizable manifestation of eutrophication are algal blooms that occur during summer. This growth can increase chlorine in the drinking water, which leads to higher levels of disinfection by-products that can increase the risk of cancer [2].

The World Health Organization (WHO) has developed *WHO Guidelines for Drinking-water Quality* due to ill effects of nitrogen compounds in water, especially those waters with which the human being has more contact with, such as drinking water.

Is known that the toxicity of nitrate to humans is mainly attributable to it's reduction to nitrite. The major biological effect of nitrite in humans is it involvement in the oxidation of normal haemoglobin (Hb) to methaemoglobin (metHb) which is unable to transport oxygen. This condition, called methaemoglobinaemia, causes cyanosis, and at higher concentrations, asphyxia. This problem is even more critical when found in infants under 3 months of age.

Nitrite has also shown to be reactive in the presence of N-nitroso compounds, which is carcinogenic.

Some guideline values for nitrate concentration stated a maximum of 50mg/L, based in epidemiological evidence for methaemoglobinaemia. For nitrite, the guideline value is 3mg/L based [3].

Also, ammonia concentration can be an issue, even though there is no maximum contaminant level for it. It is known that ammonia is toxic to fish and that toxicity increases with increasing pH and temperature (due to NH_4^+/NH_3 equilibrium favors the un-ionized form). US EPA, an environmental agency, has recommended a water quality or aquatic life expressed as total ammonia nitrogen of 17mg/L for acute exposure (1h) and 1,9mg/L for chronic exposure (30 days) (pH=7 and temperature= 20ºC) [4]

Is legislated by the European Union, the nitrates directive (1991). This directive aims to protect water quality across Europe by preventing nitrates from agriculture or industry sources polluting ground or surface waters. Was stated that nitrate levels in groundwater should not rise above 50mg/L [5].

In order to treat effluents with an excess of total nitrogen, various treatments can be applied, from chemical-physical procedures such as ammonia stripping to biological nitrogen removal processes, like conventional nitrification/denitrification.

Typical wastewaters with high ammonia concentrations are reject waters, piggery manure, landfill leachate and some industrial wastewaters (e.g from pharmaceutical and petrochemical industries).

2.1 Biological nutrient removal

It's an established technology, being a typically used process for nitrogen and phosphorus removal from wastewater before being discharged into surface or ground waters.

A high concentration of such nutrients can cause harmful and irreversible changes in the ecosystems, causing cultural eutrophication.

Althought, conventional biological processes are not suited to remove total nitrogen (TN) or total phosphorus (TP) due to is high concentration effluents.

A Biological nutrient removal plant can be built to suit perfectly the type of effluent to be treated, including oxidation ditches membrane bioreactors (MBR's) and sequencing batch reactors (SBR's). This different BNR designs are widely use in wastewater treatment plants, and very successfully. The secret is to combine excellent modeling and design, high quality instruments and controlling with highly trained operators monitoring and testing every aspect of the operation. Although, it's a biological process, and we should not treat the bacteria as a chemical catalyst, some unpredictability is expected [6].

Biological nutrient removal is can outcompete physicochemical processes since its capable of removing nitrogen from such effluents transforming it into harmless dinitrogen gas (N_2). This has shown to be a more economical and effective way to reduce nitrogen loads in wastewater.

The most commonly used approach for nitrogen removal is a combination of aerobic autotrophic nitrification of NH_4^+ to nitrite NO_2^- and nitrate NO_3^-, followed by an anoxic heterotrophic denitrification producing N_2 [7].The first step, called nitrification, is done by different bacterial genera which use ammonia or nitrite as energy source and oxygen as electron acceptor and inorganic carbon as carbon source. As the second step, denitrification is performed by heterotrophic bioconversion process. The oxidized nitrogen compounds are reduced to dinitrogen gas by heterotrophic bacteria (denitrifiers) that uses nitrite/nitrate instead of oxygen as electron acceptors and organic matter as carbon and energy source [8]. This conventional wastewater nitrogen removal system requires a lot of energy for nitrification and an external carbon source for denitrification.

A sustainable alternative is the nitrification/denitrification over nitrite. These processes require only 25-60% less oxygen consumed and 40% of the added COD [9].
Another alternative includes a partial nitrification followed by an anaerobic (in reality anoxic) ammonia oxidation(Anammox). As main advantage, the doesn't need any external carbon source to convert nitrogen to gas.

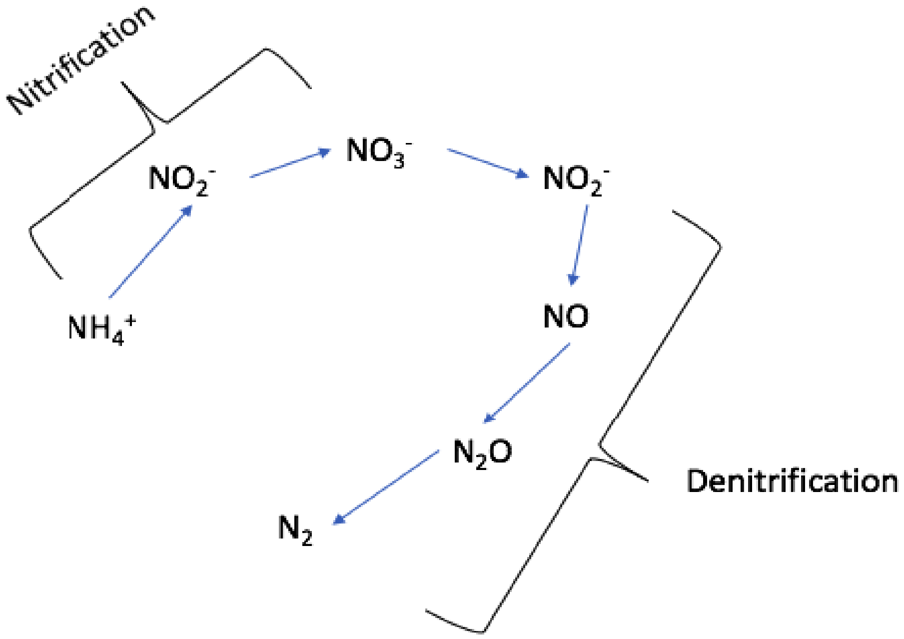


Figure 2 - Conventional nitrification/denitrification process

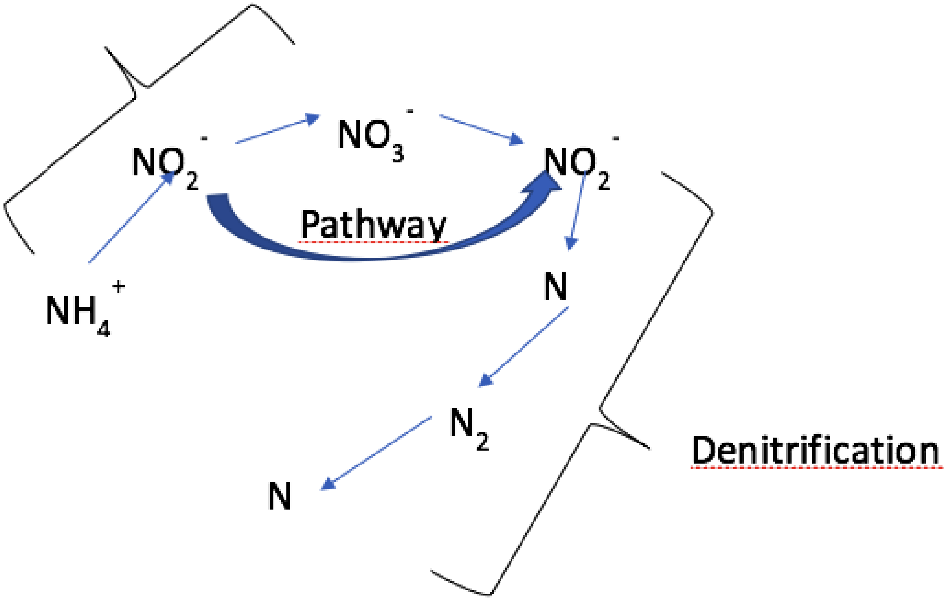


Figure 3 - Autotrophic nitrogen removal : nitrification/denitrification over nitrite

In the ANaerobic AMMonia OXidation (ANAMMOX) process, the ammonia is oxidized (in anoxic conditions) with nitrite as an electron acceptor. Both ammonia and nitrite are consumed on an almost equimolar basis. This process must be always combined with a partial nitrification process, such as the SHARON, where half of the ammonia is oxidized to nitrite. The combined process was named autotrophic nitrogen removal.

Such a sustainable approach is now intensely studied around the world, and would be a matter of time until the pollutants are no longer seen as a problem, but a source or renewable energy.

2.2 Autotrophic Nitrogen removal

2.2.1 The partial nitrification process

Nitrification, as stated before, is the ammonium oxidation to nitrate. It is a double-step reaction operated by two completely different genera of bacteria. First, ammonium is oxidized to nitrite by ammonia-oxidizing bacteria (AOB). For every mol of ammonium oxidized, 2 mol of protons are produced. We can then conclude that ammonia oxidation is an acidifying process. In the second step, the nitrite-oxidizing bacteria oxidizes nitrite to nitrate. There are no single bacteria which can perform this all process in one step, so we are in continuous presence of both genera inside the reactor.

The reactions are given by the following:

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad Eq.1$$

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \quad Eq.2$$

Since the partial-nitrification effluent should be suitable to the ANAMMOX process, nitrite oxidizing activity should be suppressed and therefore ammonium should only be oxidized by 50%-55% to nitrite. Basically, we should allow AOB to grow and suppress NOB activity washing out and preventing nitrate production.

To achieve this, some parameters should be considered and monitored, such as the free ammonia (NH_3) and the free nitrous acid (HNO_2) concentrations, temperature, pH and dissolved oxygen concentration. Managing this considering the difference in sensitivity of ammonium and nitrite oxidizers determines the accumulation of each bacteria in the reactor.

2.2.1.2 Free ammonia (NH3) and Free nitrous acid (HNO2) concentration

Free ammonia and free nitrous acid concentration have a large influence since they are the actual substrate/inhibitor for ammonium and nitrate oxidation [10] .The concentration of these unionized forms can be calculated, using the Monod curve and taking in to account the total ammonia (TAN) and total nitrous (TNO_2) concentration. From,

$$TAN = NH_4^+ + NH_3 \quad Eq.3$$

and

$$Ke,_{NH_4^+} = \frac{NH_3.H^+}{NH_4^+} = e^{\frac{6344}{T+273}} \quad Eq.4$$

the concentration of uncharged ammonia (NH_3) is given by:

$$NH_3 = \frac{TAN}{1 + \frac{10^{-pH}}{Ke,_{NH_4^+}}} \quad Eq.5$$

For nitrous acid concentration,

$$TNO_2 = NO_2^- + HNO_2 \quad Eq.6$$

and

$$Ke,_{HNO_2} = e^{\frac{-2300}{T+273}} \quad Eq.7$$

the Monod curve for the HNO_2 concentration is given by

$$HNO_2 = \frac{TNO_2}{1 + \frac{Ke,_{HNO_2}}{10^{-pH}}} \quad Eq.8$$

the ratio between both unionized and ionized forms is determined by the pH and temperature values inside the reactor.

So, in way to make Anammox-suited effluent we should suppress nitrite oxidizers by increasing the pH. Was stated that in a pH environment between 7,5-8 nitrite oxidizers are outcompeted. Even though this is a good approach to somehow wash-out the nitrite oxidizers, NOB adaptation has been reported [9]. Therefore, to achieve a good Anammox influent, we should also regulate other factors.

2.2.1.2 pH

pH has a great influence in the good performance of partial nitrification. The main reason is the influence on the NH_3/NH_4^+ and HNO_2/NO_2^- equilibrium. Due to this equilibrium, the preference of ammonium oxidizers for slightly alkaline environments is the fact that these organisms use NH_3 as substrate [10] while at certain pH values both NH_3 and HNO_2 can inhibit the oxidizers. Also, other inhibitions can show, as **Heillinga, et al.** [11] observed, a decrease in growth rate of nitrite oxidizers at pH 7 compared with pH 8. The growth rate difference for ammonium oxidizers were negligible for these values. For pH values below 7, nitrification performance decrease due to carbon limitation, owing to CO2 stripping.

2.2.1.3 Other substrates

Nitrification, as stated above, is an acidifying process. To neutralize, bicarbonate is added in equimolar basis. Although, since we only want 50% of ammonia oxidized (partial-nitrification), only half is required. Some reduction on ammonia oxidizing activity was reported due to bicarbonate limitation [12].

Moreover, **Ganigue, et al.** [13] showed that bicarbonate is a key parameter for controlling the molar ratio of ammonia and nitrate in the effluent. Sludge reject water is a good influent for partial nitrification since a proper ammonium:alkalinity ration of 1 is found in these streams. Also, the presence of phosphate has an impact in oxidizing performance, specially the nitrite oxidizing bacteria is unable to oxidize nitrite to nitrate in its absence, the so-called phosphate block [9].

2.2.1.4 Temperature

Temperature is also a very important parameter in partial-nitrification process performance. Although, the real influence is hard to determine because of its interaction with mass transfer, chemical equilibria and growth rate [9].

The temperature rise increases the NH_3 inhibition and also increases the organisms activity due to the Arrhenius principle (until a certain level, and then obvious decrease), which can be seen has two opposite effects. Experiments showed an optimal temperature of 35ºC for the ammonia oxidizers and 38ºC for nitrite oxidizers. And so, a temperature between 35-45ºC is optimal for partial nitrification [10] .It's also expected that long-term exposure to temperatures above 40ºC can cause deactivation **Hellinga, et al.** [11] concluded that at temperatures above 25ºC the specific growth of AOB was highter that NOB.

This principal is based in a relation between Sludge retention time and temperature. The process is established in a chemostat by working at high temperature (above 25ºC) and maintaining an appropriate SRT of 1-1.5 days, so the AOB are maintained in the reactor and the NOB are wash-out, preventing NOB accumulation in the reactor.

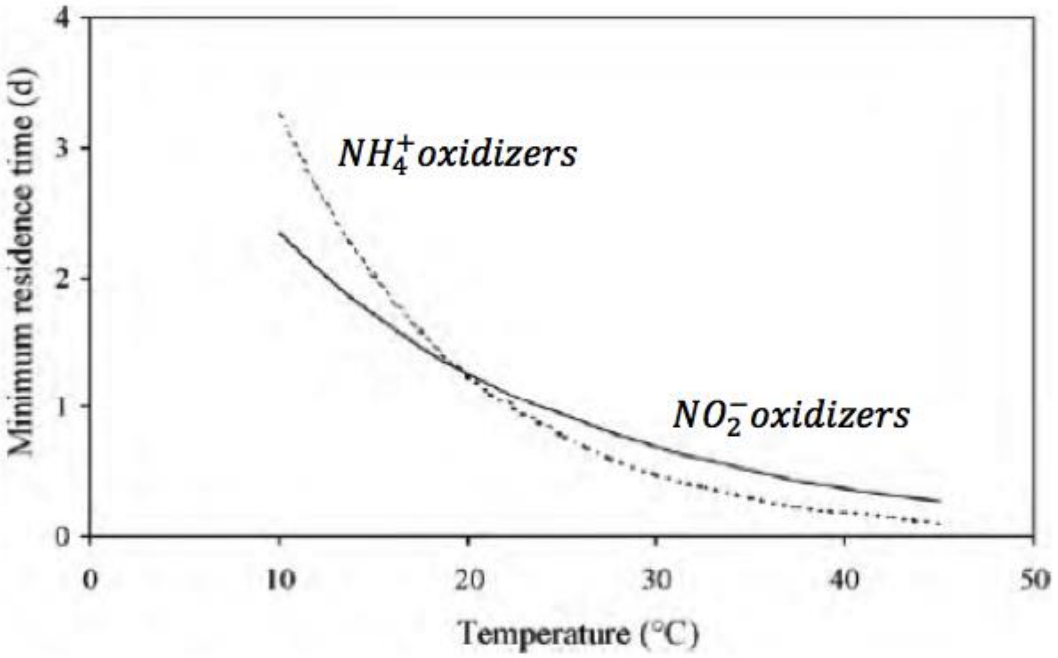


Figure 4 - Relation between residence time and temperature.

The partial-nitrification process was also successfully started up at lower temperature (between 15-30ºC) [14] . This means that partial nitrification is not restricted to streams with high temperatures, but can be applied to multiple industrial wastewaters. Is important to refer that below 15ºC, the process performance decreases dramatically.

2.2.1.5 Dissolved oxygen concentration

Since the process is aerobic, the dissolved oxygen concentration Is of most importance when we talk about nitrification, for both AOB and NOB. The ammonium oxidizers though seem to be stronger against low dissolved O_2 than nitrite oxydizers, which means that low dissolved O_2 concentrations influence more the NOB activity. This can be explained by the difference in oxygen half saturation constant (ko) for both bacteria. According to **Hunik, et al.** [15] the half saturation constant for dissolved oxygen is 0,16mgO2.L-1 and 0,54 mgO2.L-1 for AOB and NOB respectively. The constant is also influenced by mass transfer inside the reactor, biomass density, floc size, the mixing intensity and the oxygen diffusion in the floc [9].

2.2.1.6 Sludge age, SRT and HRT

As explained before, ammonium oxidizers retention and nitrite oxidizers wash-out can be accomplished by choosing the appropriate SRT since they required different minimum sludge ages, depending on temperature. The minimum doubling time for AOB is 7-8h and for NOB is 10-13h. Was found that a SRT between 1-2,5 days result in a good performance [16]. So, for the SHARON process, HRT (which is equal to SRT) is 1 day under high temperature and high oxygen concentration to favor AOB growth above NOB. Other reports showed that partial nitrification can be accomplished in different conditions, such as low temperature (<13ºC), with higher SRT [17].

2.2.1.7 Organic carbon and salts

Is known that the partial-nitrification process is suitable to treat wastewater streams with low organic to carbon (C/N) ratio. **Mosquera-Corral, et al** [18] observed a stimulation in ammonium oxidation in the Sharon process when acetate was fed as carbon source (0,2gCgN-1), leading to a higher nitrate to ammonia molar ratios in the effluent (stoichiometricly speaking). It also reported inhibitory effect when 0,3gCgN-1 was fed to the reactor. This was explained by **Hanaki, et al** [19] as decreasing affinity of ammonia oxidizers for ammonia. It was also found that for the same SRT, the ammonia oxidation efficiency decreased at higher COD concentrations, but at constant COD concentration efficiency was restored by increasing the SRT.

In some industrial wastewaters, the presence of high concentration of salts can inhibit ammonia concentration. However, the biomass can adapt to saline environments [9].

2.2.1.8 Other influencing parameters

Other influencing factors to the partial nitrification performance were reported. **Zepeda, et al.** [20] showed that BTX (benzene, toluene and xylene) decrease the nitrification specific rates, mainly the ammonia oxidation pathway. Also, many metals such as chromium, nickel, copper, zinc, lead and cadmium might inhibit both steps of nitrification. Some organic acids, such as formic, acetic, propionic and n-butyric acid all exhibit inhibition to nitrite oxidation, with no effect in ammonia oxidation.

Also, chloride, cyanide and azide showed inhibitory effects, affecting more the nitrite oxidation than ammonia.

Light is also an inhibiting factor for both bacteria since cytochrome C is present and is oxidized by light in the presence of oxygen [9].

2.2.2 The ANAMMOX process

The ANAMMOX process has a lot of history. It was first discovered almost 3 decades ago, but was already predicted 10 years before, based on thermodynamic calculations [21]. **Broda** pointed out anaerobic ammonium oxidizers as “missing in nature” based on his considerations.

Also, **Van de Graaf, et al.** [22] showed by inhibition experiments that anammox is a microbially mediated process and not a chemical reaction [9].

The first evidence of anaerobic ammonium oxidation to dinitrogen gas was obtained from denitrifying fluidized-bed reactor system [23].

The Anammox reaction takes place in an anoxic environment, where the ammonia is oxidized to dinitrogen gas, using nitrite as an electron acceptor. The nitrite is also oxidized to nitrate, but in a much fewer quantity. This oxidation doesn’t need the addition of a carbon source (organic matter) since process is autotrophic.

The overall reaction 9, half reactions 10 and 11 [24] and cell synthesis reaction proposed by **Strous, et al.** [25] are presented below:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad Eq.9$$

$$NO_2^- + 2H^+ \rightarrow NO + H_2O \quad Eq.10$$

$$NO + NH_4^+ + 2H^+ + 3e^- \rightarrow N_2H_4 + H_2O \quad Eq.11$$

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+$$
$$\rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad Eq.12$$

Since it was discovered, the ANAMMOX process is reported in different wastewater treatment plants with different stream compositions. It is also present in nature and largely contributes to world nitrogen cycle.

2.2.2.1 Bacteria Background

Strous, et al. [26] showed that the Anammox bacteria belongs to the Planctomycetes family. In figure 5 is showed Anammox bacteria phylogeny.

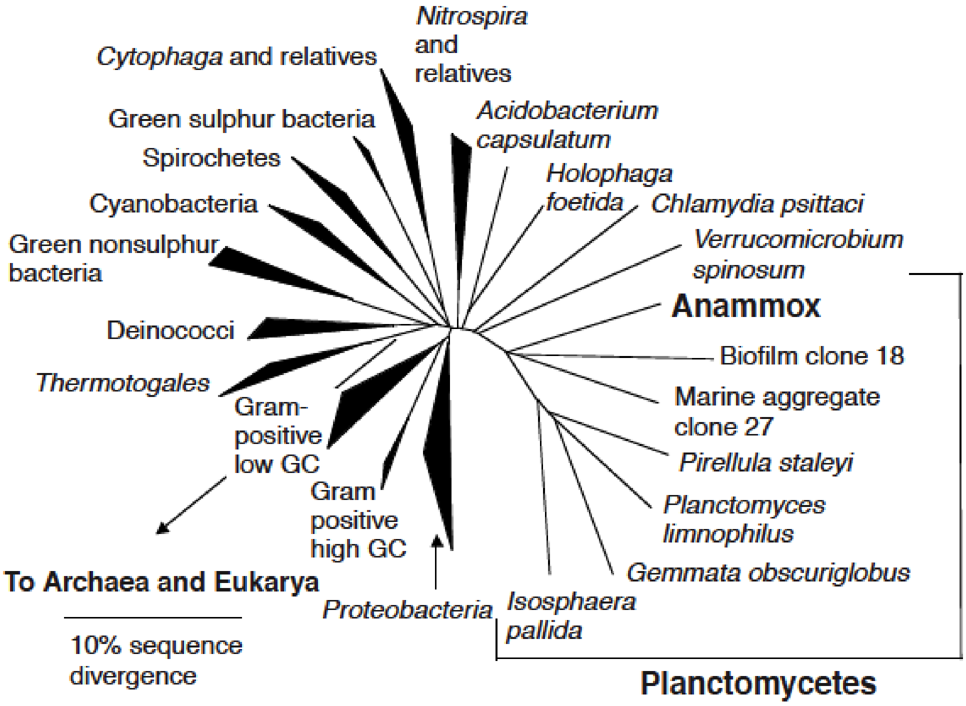


Figure 5 - Anammox bacteria phylogeny.

Fluorescent in situ hybridization (FISH) probes were developed for the different Anammox bacteria, and was found by **Schmid, et al.** [27] that rarely two or more types of Anammox bacteria could grow in the same environment. This proves that each one genera of bacteria occupy their own typical environment.

The Anammox bacteria has a brown-reddish color and doubling time of 5.5-7.5 days in a membrane bioreactor [28] .The doubling time depends the method used to proceed the counting [9] .

Moreover, these microrganisms have several unique features, including the use of hydrazine (N_2H_4) as free catabolic intermediate.

The discovery of Anammox bacteria changed completely the scientific view of the nitrogen cycle, and turn out to be a fundamental step on the evolution of wastewater treatments around the globe.

So far 10 Anammox bacteria species have been identified. The known species are divided in 5 genera:

1. *Kuenenia – Kuenenia stuttgartiensis*;
2. *Brocadia – B. Anammoxidans, B. fulgida and B. sinica*
3. *Anammoxoglobus – A. propionicus*
4. *Jettenia – J. asiatica*
5. *Scalindua – S.brodae, S.sorokinii, S.wagneri and S. profunda*

All 5 genera of Anammox bacteria share unique physiological and morphological traits, with the key being the presence of anammoxsome [29]**.**

2.2.2.2 Anammox in natural environments

The Anammox was firstly found in lab-scale, in different reactor configurations, followed by its detection in the natural environments such as marine sediments.

There the Anammox bacteria are found in low oxygen zones, the major source of nitrogen release into the atmosphere from the oceans [30] . It was also detected in deeper marine hypersaline systems [31] . Moreover, it was found in terrestrial ecosystems, such as lakeshores, agricultural soil, permafrost soil and in samples associated with nitrophilic or nitrogen-fixing plants [32] [29].

Anammox was also reported to occur in low temperatures (-2,5ºC) in sea ice and high temperatures (70ºC) in hot springs and hydrothermal vent areas. [29].

2.2.2.3 Metabolism inhibition and process performance

Like the partial-nitrification process, the Anammox is also inhibited but much more sensitive to the environment changes.

Some parameters must be controlled and managed so we can establish high performance to the process.

2.2.2.3.1 Temperature and pH

Is known that the optimum temperature for the Anammox process is around 30-40ºC [9]. **Dosta, et al** [33] found a maximum activity for Anammox biomass in a temperature ranged between 35-45ºC, while higher temperatures can cause irreversible Anammox

bacteria activity inhibition. It was also proved by **Cema, et al.** [34] and **Isaka, et al.** [35]that the Anammox process can be achieved successfully at 20ºC, being the biomass slow adaptation the key factor in order to operate at low temperature.
The optimal pH value is between 6.7-8.3

2.2.2.3.2 Oxygen

As Anammox bacteria favors anaerobic conditions, the presence of dissolved oxygen inhibits the process, reversibly. Particularly, oxygen is very significant in single stage reactors where both partial-nitrification and Anammox occur simultaneously. Normally, the reactor configuration of such systems (Biofilm) allows the process by protecting the Anammox bacteria in inner layer from nitrifiers in the outer layer.

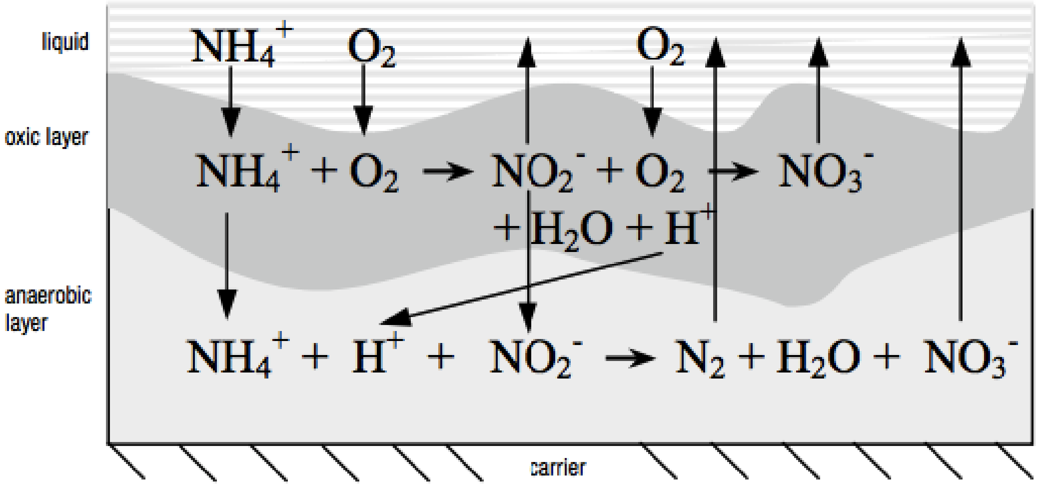


Figure 6 - Anammox bacteria protection from nitrifiers, Biofilm configuration (Szatkowska, 2014)

2.2.2.3.3 Organic Carbon

High organic carbon to nitrogen concentrated streams are usually used as Anammox influent. In such streams is can found landfill leachate and wastewater from digested animal waste. During Anaerobic digestion, fast biodegradable organic content is converted to biogas. And so, only biodegradable organic matter will be present in these wastewaters [9].In the partial-nitrification, this organic matter is oxidized and the denitrifiers present cannot outcompete the Anammox bacteria inside the reactor. This is true until a certain level, since the growth rate of denitrifiers is significantly higher than the Anammox. Also, the denitrification reaction is thermodynamically favorable than Anammox reaction (-427 kJ/mol and -355kJ/mol, respectively) [9].

The Anammox only removes 90% of incoming nitrogen (in form of nitrite/ammonia) and leaves the remaining 10% inside the reactor. The presence of both denitrification and Anammox reaction in the same reactor could help to reduce the nitrate concentration, since it can be reduced by denitrifiers to nitrite and used by ammonium for oxidation [9]. Some organic carbon such as methanol an ethanol showed total and irreversible inhibition at low concentrations [27].

2.2.2.3.4 Biomass concentration

Biomass concentration is key parameter, that plays an important role in the Anammox activity. [26] reported that Anammox is only active when the cell concentration is above 10^{10}-10^{11} cells/ml. This could be explained by the need of intercellular communication for activity. Can also be explained by the fact that hydrazine diffuses easily to the outside of the cell and minimum internal concentration is needed for activity [9].

The presence of contaminating cells is also requested, since these can supply vitamins and remove toxic components. If the activity suffers inhibition, the addition of intermediates such as hydroxylamine and hydrazine is necessary to restart Anammox activity.

2.2.2.3.5 Suspended solids

Flocculants are usually used to remove colloidal organic and inorganic substances from wastewater before the Anammox reaction. By using flocculants settling ability of the influent suspended solids can be improved and prevent their accumulation inside the reactor. In other hand, it can also be attached to the Anammox bacteria hence reducing their activity [9].

2.2.2.3.6 Salts

In natural saline environments only one type of Anammox bacteria was found, the *Scalindua genus*.

[36] found that NaCl concentrations below 150mM did not affect Anammox activity while KCl and Na_2SO_4 affected it at concentrations higher than 100mM and 50mM respectively. This difference was attributed to the concentration of Na^+ ions in the medium.

The nitrogen removal efficiency and maximum Anammox activity of salt adapted sludge were similar to the reference freshwater sludge [9].

2.2.2.3.7 Inhibition of substrates and products

Nitrite presence inside the Anammox reactor cause inhibition. Hence, its concentration should be an important parameter to control. The decreasing activity due to its presence can be restored by adding trace amounts of Anammox intermediates hydroxylamine and hydrazine, even after long term exposure.

It was also reported different tolerance for nitrite inhibition between different Anammox bacteria genera.

Also, high concentration of nitrite can change the stoichiometry of ammonium to nitrite, which means that the bacteria did not use ammonium as electron donor, but actually, have generated an internal electron donor to reduce nitrite. This change in stoichiometry was also found at higher temperatures.

The process is not inhibited by ammonium concentration up to 1g N/L [25].

Is known that chemolithoautothrophs use inorganic carbon as carbon source, therefore bicarbonate concentration is an important factor which can affect the Anammox activity. Low concentration of bicarbonate means low activity of Anammox and high concentration of bicarbonate can lead to inhibition, this is due to the increase of pH inside the reactor which means the production of high amount free ammonia.

2.2.2.3.8 Other influence factors

Like partial-nitrification, Anammox is also very sensible to light. It was observed a decrease in activity between 30-50% [22].To prevent this to happen, the reactor is usually covered by black plastic or aluminum paper to eliminate light effects in the reactor. The stirring can also have a negative effect over the Anammox. Was stated by **Arrojo, et al.** [37] that at stirring speeds up to 180 rpm no negative effect was showed but at 250 rpm some nitrite accumulation and 40% activity reduction was found.

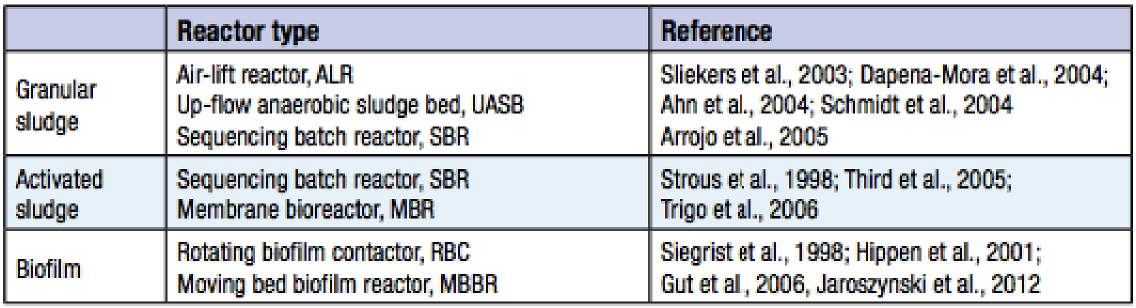
2.2.2.4 Process configurations

Lots of systems can be successfully applied with Anammox bacteria. The major difference between them is whether the complete process is run in two separate steps

(Partial-nitrification in one reactor and Anammox in another), or in the same step (one single reactor). Other difference is the bacteria growth type (granular sludge, activated sludge or biofilm).

In table 1 are showed the different reactor configurations.

Table 1 - Anammox reactor configurations [29]



	Reactor type	**Reference**
Granular sludge	Air-lift reactor, ALR Up-flow anaerobic sludge bed, UASB Sequencing batch reactor, SBR	Sliekers et al., 2003; Dapena-Mora et al., 2004; Ahn et al., 2004; Schmidt et al., 2004 Arrojo et al., 2005
Activated sludge	Sequencing batch reactor, SBR Membrane bioreactor, MBR	Strous et al., 1998; Third et al., 2005; Trigo et al., 2006
Biofilm	Rotating biofilm contactor, RBC Moving bed biofilm reactor, MBBR	Siegrist et al., 1998; Hippen et al., 2001; Gut et al., 2006, Jaroszynski et al., 2012

2.2.2.5 Anammox applications

The first full-scale reactor (for reject water treatment at Dokhaven, Rotterdam, Netherlands) was established in 2002. Since then, a total of 114 full-scale Anammox installations were reported around the world (data from 2015) being 88 of these plants constructed in Europe.

The first reactor had a total capacity of 72m^3, which is considerably low considereding that, nowadays, there are full-scale plants with more than 142.000 m^3 and treat 134 tons per day of nitrogen load.

The main target are industrial wastewaters and up to now full-scale Anammox has not been applied to mainstream waters treatment for domestic sewage.

High C/N ratio, low temperature and poor effluent quality are still the main reasons why Anammox treatment is still not applied everywhere.

Even though the presence of organic matter at low concentrations does not affect Anammox bacteria activity, it improves the total nitrogen removal via heterotrophic denitrification, the so called SNAD process [38]

It is reported that in 23 full-scale Anammox installations that NH_4^+ and NO_3^- concentrations are 100 and 50 mg N L^{-1}, respectively, which indicates the need of further treatment. This post-treatment results in an increase in cost and energy consumption, and therefore, further development of integrated systems, like the SNAD, is necessary.

3. Materials and Methods

3.1 Reactor system and equipment control

During the experiment a glass sequencing batch reactor (SBR) with a working volume of 2,13 L was used.

It was operated in a fed-batch mode with a 6 h cycle (267 minutes continues feeding, 83 minutes reaction, 5 minutes settling, 5 minutes withdrawal).

In every cycle, 533 ml of influent were pumped inside the reactor with a flowrate of 2 ml/min. 4 cycles a day, making the HRT = 1 day.

Mechanical mixing was provided by a stirrer (100±5rpm).

To assure anaerobic conditions inside the reactor, N2 gas was flushed into the reactor in the beginning of every cycle for 5 minutes.

The reactor worked at temperature of 35ºC and pH between 7-7,10 and was covered by aluminum paper to assure no light presence inside.

The temperature was maintained by using a water jacked and the pH by using 1M HCl.

Process timing and control and monitoring were performed using LabVIEW (v10.0).

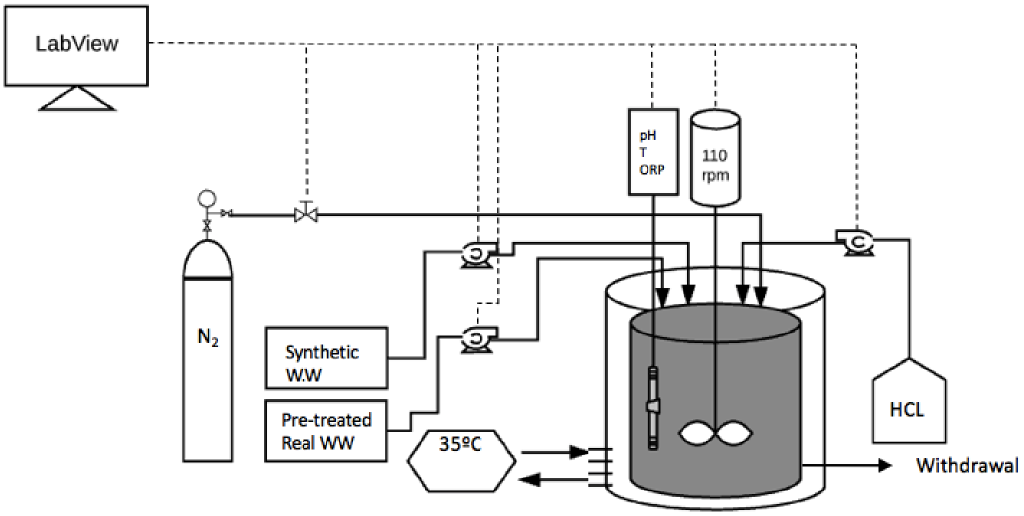


Figure 7 - Schematic representation of the Anammox SBR system (Lucidchart software)

Figure 8 - The Anammox reactor – Experimental set-up

3.2 Biomass origin

The reactor was originally inoculated with Anammox granular biomass from the Dokhaven-Sluisjesdijkwastewater treatment plant in Rotterdam (The Netherlands), and gently provided by Paques, B.V.

At the start of this experiment, the biomass in the reactor came from a previous experiment and was running under stable conditions, fed with a synthetic medium.

The synthetic influents were prepared in lab with the following compositions:

Table 2 - Synthetic Bag composition

Compound	***Concentration***
NO_2-N (NaNO2)	818 mgN/L
NH_4-N (NH_4HCO_3)	682 mgN/L
$MgSO_4.7H_2O$	200 mg/L
KH_2PO_4	6,25 mg/L
$CaCL_2.H_2O$	226 mg/L
Trace Elements	1,25 mL/L
Iron solution	2,50 mL/L

The molar ratio NO_2/NH_4 used was 1.20 (the stoichiometric value for Anammox is 1.30) to avoid nitrite accumulation inside the reactor. The total nitrogen fed was about 1500mgN/L per cycle.

The real wastewater coming from the anaerobic digestion of the municipal solid waste, integrated in the HyMEca project. was pre-treated in partial-nitrification process (in this case, the SHARON)

The reactor was started-up with 90% synthetic wastewater (Ntot=1500mgN/L and NRLmáx=1.5KgN/m^3d and 10 %real wastewater (same conditions)

It was progressively changed to the pre-treated real wastewater, until 100% of influent was real pre-treated wastewater.

For better acclimation of Anammox biomass, a feeding strategy was implemented using an exponential law, increasing the real wastewater share in the Anammox influent.

In table 3 are shown the average characteristics for the Real and Synthetic Influents.

Table 3 - Synthetic and real wastewater characteristics.

TYPE	*NH_4-N mg/L*	*$N0_2$-N mg/L*	*$N0_2/NH_4$*	*TN mgN/L*
Synthetic WW	682	818	1.20	1500
*Real WW**	676±72	828±62	1.24±0,16	1505±100

*average characteristics

3.3 Feeding strategy (exponential law)

As stated before, the feeding strategy used in this experiment was based on an exponential law [39] given by:

$$NLR(t) = A * e^{\mu PA * t} \quad eq. 13$$

The A constant corresponds to the NLR value in the beginning of the experiment, which is 0,15 (10% share in NLRmáx=1,5KgN/Ld). μPA is the μmáx (maximum specific growth rate for the Anammox bacteria) 0.065 times a safety factor (0,3). t is time in days (d).

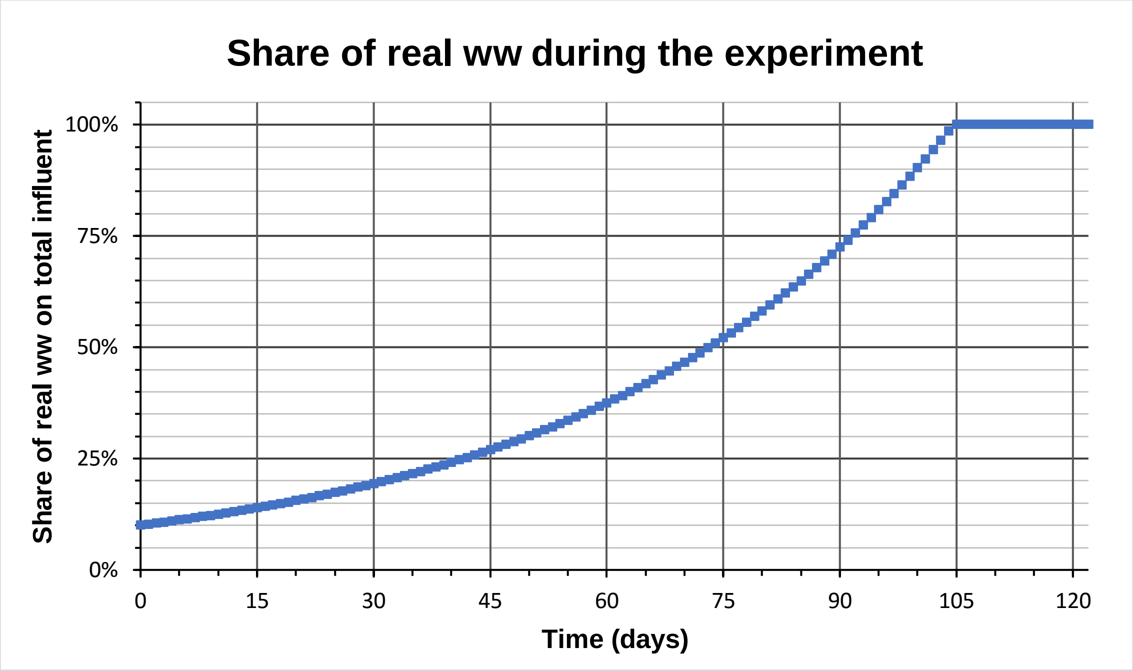


Figure 9 - Exponencial Low plotted - Feeding strategy

Was predicted the achieve of 100% on the day 105 of the experiment. The accumulated real wastewater volume was 89,2 L

3.4 Analytic methods

In order to study the reactor some parameters needed to be calculated. And for so, some measurements were made.

There were two kinds of measurements performed, Chemical measurements, which included the NH_4-N, NO_2-N and $N0_3$-N concentration values in the influent and effluent and Total organic carbon (TOC).

Also, biomass concentration and granules density inside the reactor were periodically assessed, as well as solids concentration in the effluent. Specific Anammox activity was also determined once a week

Before analysis, samples were centrifuged (10.000 rpm for 20 minutes) and filtered (pore size 0,45 μm).

3.4.1 Chemical Measurements

Ammonium

For the NH_4-N measurement the Nessler's method was performed using the spectrophotometer **(HITACHI, mod U-2000)** (Fig.11) in a 420nm wavelength
The samples were prepared using 50 ml volumetric flasks, with proper dilution (with NH4-N concentration between 1-5mgNH4-N/L) using distilled water. To these flasks were added 3-4 drops of potassium sodium tartrate and 1 ml of Nessler's reagent. The reaction between the Nessler's and the ammonia makes the color transition from transparent to yellow-brownish depending on the concentration of ammonia in the sample.
15-30 minutes after the addition of the Nessler's reagent, the samples were analyzed in the spectrophotometer
Each sample were made in triplicates, to assure a more precise measurement.

Figure 10 - Laboratory Spectophotometer (HITACHI, mod U-2000)

Nitrite and Nitrate

The NO_2-N and $N0_3$-N concentrations, together with other anions of interest, were measure in an ion chromatographer, using a **DIONEX ion-chromatograph (mod IC-90)** (Fig.12) equipped with a DIONEX ION PAC AS14A 4x250 mm column and a DIONEX ION PAC AG14A 4x50 mm) guard column.

The samples were prepared in volumetric flasks (with 10 and 20 ml) properly diluted using de-ionized water.

1 ml of sample was manually pumped to the ion-chromatograph with a syringe, and the analysis took approximately 15minutes.

Figure 11 - Laboratory ion chromatographer DIONEX ion-chromatograph (mod IC-90)

3.4.2 Mass balances

After measurements, it is possible to do mass balances to the Anammox reactor, using the follow equations:

For Nitrogen removal:

$$Total\ Ammonium\ removal = NH4 - N \inf - NH4 - N\ eff \quad eq. 14$$

$$Total\ Nitrite\ removal = N02 - N \inf - N02 - N\ eff \quad eq. 15$$

$$Total\ Nitrate\ removal = N03 - N \inf - N03 - N\ eff \quad eq. 16$$

$$NH4 - N\ removal\ efficiency(\%) = \frac{Total\ removed\ NH4 - N}{NH4 - N\ iff} * 100 \quad eq. 17$$

$$NO2-N\ removal\ efficiency(\%)=\frac{Total\ removed\ NO2-N}{NO2-N\ iff}*100\quad eq.18$$

And Nitrate production (using theoretical equation 13)

$$Theoretical\ prodution\ NH4-N\ consumption(mgNO3-N)=\frac{NH4-N\ removal*Vfed}{Reactor\ Volume}*0{,}26\quad eq.19$$

$$Theoretical\ prodution\ NO2-N\ consumption(mgNO3-N)=\frac{NO2-N\ removal*Vfed}{Reactor\ Volume}*\frac{0{,}26}{1.32}\quad eq.20$$

$$Measured\ prodution(mgNO3-N)=\frac{Total\ prodution\ NO3-N*Vfed}{Reactor\ Volume}\quad eq.21$$

$$NRE=\frac{(NO_2-N+NH_4-N+NO_3-N)\ inf-(NO_2-N*NH_4-N*NO_3-N)eff}{(NO_2-N+NH_4-N+NO_3-N)\ inf}*100\quad eq.22$$

Nitrogen loading rate (NLR), Nitrogen removal rate (NRR) and Nitrite discharge rate (NitDR), i.e. the mass of nitrogen loaded, removed and withdrawn as nitrite from reactor (respectively) per day and per unit of volume, and expressed as mgN $L^{-1}d^{-1}$, were also calculated as follows:

$$NLR=\frac{(NO_2-\mathrm{N})_{inf}+(NH_4-\mathrm{N})_{inf}}{1000}*\frac{Vfed*NC}{Vmin+Vfed}\quad eq.23$$

$$NitDR=\frac{(NO_2-\mathrm{N})\mathrm{eff}}{1000}*\frac{Vfed*NC}{Vmin+Vfed}\quad eq.24$$

$$NRR=\frac{(NO_2-N+NH_4-N)inf+(NO_2-N+NH_4-N)eff}{1000}*\frac{Vfed*NC}{Vmin+Vfed}\quad eq.25$$

- $N0_2$-N_{inf}, NH_4-N_{inf} and NO_3-N_{inf} = influent concentrations of NO_2-N and NH_4-N in mg/L.

- $N0_2$-N_{eff}, NH_4-N_{eff} and NO_3-N_{eff}= effluent concentrations of NO_2-N, NH_4-N and NH_3-N in mg/L.
- V_{fed} = volume fed in each cycle, in ml.
- V_{min} = the minimum remaining volume immediately after withdrawing.
- NC= number of cycles per day.

3.4.3 TOC – Total organic carbon

Total organic carbon was analyzed using **TOC-V CSN (Shimadzu corp.)** liquid module analyzer (Fig.13)

Samples from influent and effluent were prepared using 20 ml and 50 ml volumetric flasks, with proper dilution (calibration curve ranged between 10 and 200 mgC L^{-1}). The instrument and separately determined total carbon (TC, i.e. the sum of inorganic and organic carbon) and inorganic carbon (IC) in each sample;. total organic carbon was then calculated by the difference between TC and IC:

$$TOC = TC - IC \quad eq. 26$$

- TOC= Total organic carbon, mg/L
- TC= Total Carbon, mg/l
- IC = Inorganic Carbon, mg/L

Figure 12 - Laboratory Total organic carbon instrument

3.5 Activity measurements

3.5.1 Solids Analysis

For the solids analysis, samples were taken from the reactor and from the effluent. VSS analysis was performed every 2-3 weeks, in order not to remove too much biomass from the reactor, given the low growth rate of Anammox bacteria.

The samples were filtered using a glass filter at vacuum pressure and then placed in a cup. The cup (previously weighed + empty filter) was after placed in the 105ºC oven, for at least 1 night.

It was again weighed (dry weight) and placed in the 570ºC oven for 3-4 Hours.

The remaining ashes were then weighed (ashes weight).

The VSS and TSS were determined based on the following relations:

$$TSS = \frac{DW - EW}{Vsample} * 1000, in\ g/L \quad eq. 27$$

$$VSS = \frac{DW - AW}{Vsample} * 1000, in\ g/L \quad eq. 28$$

- DW = dry weight, g
- EW = empty weight, g
- AW= ashes weight, g
- Vsample = sample volume, ml

The relation VSS/TSS was also calculated.

For the analysis of the reactor, granules volume measurement was also performed, using a small volumetric cylinder.

Specific Volume and granules density were determinate by:

$$Specific\ volume = \frac{Granules\ Volume}{Sample\ volume}, \text{ Lgranules/L} \quad eq. 29$$

$$Granules\ density = \frac{TSS}{Specific\ volume}, \text{ gTSS/Lgranules} \quad eq. 30$$

3.5.2 Specific Anammox Activity

SAA was assessed according to the chemical tracking method described by Test were performed every week directly inside the operating reactor, during the reaction phase of the working cycle (after the end of the feeding phase).

For the dosage of the anammox substrates, a stock solution containing nitrite and ammonium at a molar ratio of 1:1 was used. Composition is given in table 3 addition of supplementary inorganic carbon and micronutrients wasn't needed since they were provided in excess by both synthetic and real wastewater used during the experiment.

The ammonium+nitrite stock solution was spiked into the reactor to achieve a neither limiting nor inhibiting nitrite concentration in the reactor of 40-60 mgN/L. Then the stopwatch was started just after the addition, in order to track the sampling times. Mixed liquor samples were collected at regular time intervals (usually, 5 or 10 minutes) throughout the duration of the test (30-60 minutes).

The samples were immediately centrifuged, filtered and stored at 4°C for analysis of nitrite, nitrate and ammonium. The values are shown in table 4.

Table 4 - Kinetics assay solution concentration

Kinetic Assay solution	**Concentration (mgN/L)**
NO_2-N (as $NaNO_2$)	2000
NH_4-N (as NH_4CL)	2000

The linear regression over these data can be used to determine the ammonium and nitrite removal rates (rAnmx,NO_2 and rAnmx,NH_4) and nitrite production (rAnmx,NO_3).

The typical N output of this test is shown in figure 13

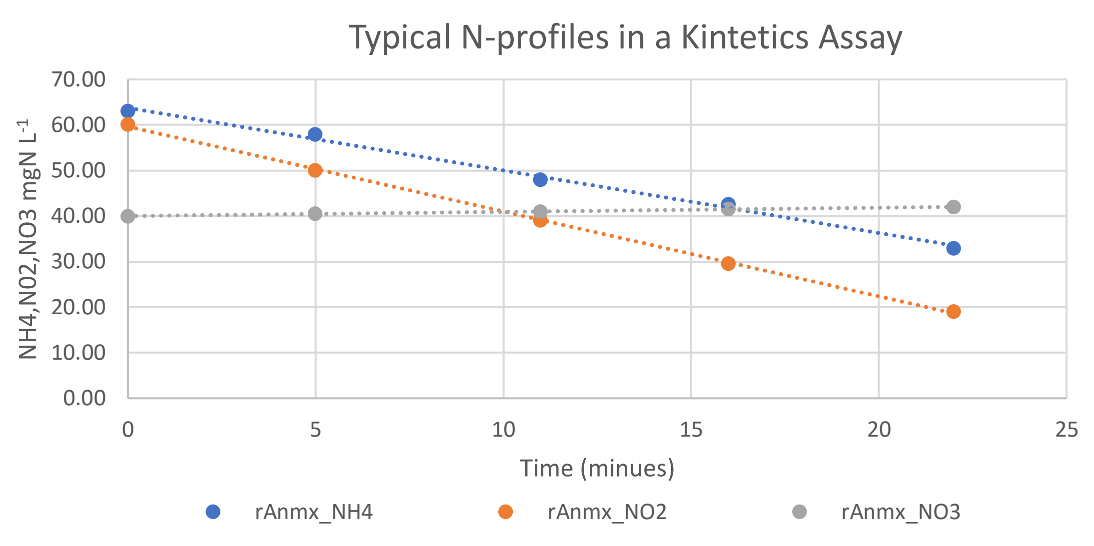


Figure 13 - Typical N-profiles in a kinetics assay

The Anammox rate is usually expressed as the dinitrogen gas produced, which is equivalent to the nitrogen removed from the wastewater.

The maximum specific Anammox rate can be calculated by:

$$q_{Anmx,N2}=\frac{rAnmx,NH4+rAnmx,N02-rAnmx,NH4\_NH3}{X_{VSS}} \qquad eq.31$$

the Xvss value was determined in the solids analysis.

Other stoichiometric coefficient ratios of interest, such as $Y_{NH4\_NO2,ANMX}$ and $Y_{NH4\_NO3,ANMX}$ can be easily calculated from the removal/production rates using the following expressions:

$$Y_{\mathrm{NH4\_NO2,ANMX}} = \frac{rAnmx, NO2}{rAnmx, NH4} \qquad eq.32$$

$$Y_{\mathrm{NH4\_NO3,ANMX}} = \frac{rAnmx, NH4\_NH3}{rAnmx, NH4} \qquad eq.33$$

4. Results and discussion

4.1 General Aspects

The experimental activity lasted for 132 days, divided in two phases. During the first phase share of real wastewater fed to the reactor was continuously increased, according to NRL exponential law described by equation 14, and a second phase with continues feeding of 100 % real wastewater. The progressive replacement of synthetic influent started with 10% real:90% synthetic feeding, 100% of real wastewater feeding was reached on the day 105.

Then a second phase with continuous feeding of 100% real wastewater followed (days 105-132):

- Nitrogen Loading Rate (NLR), Nitrite Discharge Rate (NitDR) and Nitrogen Removal Rate (NRR);
- NH_4-N removal efficiency, NO2-N removal efficiency and Nitrogen Removal Efficiency (NRE)
- Stoichiometry and Kinetics
- Suspended Solids
- NH_4-N and NO_2-N removal rates and NO_3-N production rates
- Removal of organic matter

4.2 Process Performance

4.2.1 Nitrogen loading rate, Nitrogen removal rate and Nitrite discharge rate

During the experiment, when necessary, real wastewater influent was integrated with nitrite or ammonium salts in order to maintain a nitrogen loading rate of 1.5 kgN/m^3 with a NO_2-N/NH_4-N ratio between 1.20-1.30. We can see in figure 14 that, in both phases, NRR and NLR curves are generally coincidental, with an average NRR/NLR ratio of 97,0±2,6%. This difference is explained by ammonium or nitrite accumulation in the reactor. The NitDR was negligible and mostly zero throughout the whole experiment, indicating a good process performance. On the day 99, 113, 114 and 131 a nitrite accumulation was observed: this was due to a too high nitrite/ammonium molar ratio in the influent. It is noticeable that this accumulation didn't affect the process, and nitrite was immediately removed after, when a lower nitrite/ammonium ratio was fed into the reactor. During phase 2, in fact, chemical correction of influent was not applied, in order to assess the process response to fluctuating nitrogen load and NO_2-N/NH_4-N ratio.

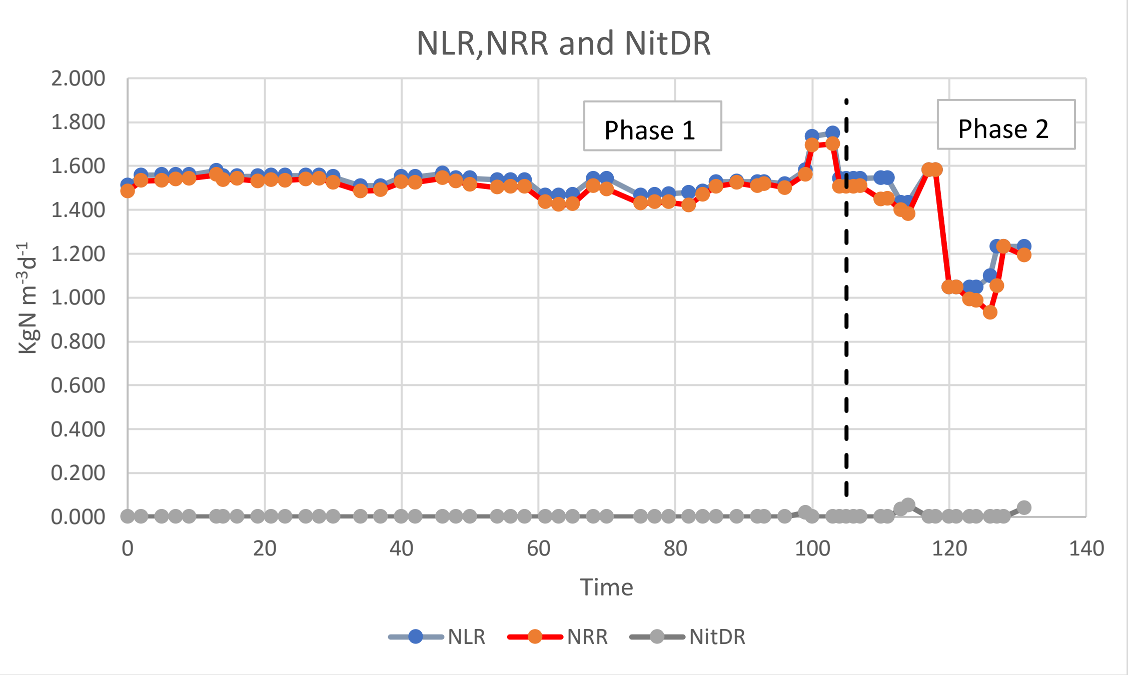


Figure 14 - Time course of NLR, NRR and NitDR during the experimental activity

4.2.2 Removal efficiencies

In the figure 15 both nitrite and ammonium removal efficiency throughout the experiment are depicted, together with overall nitrogen removal efficiency.

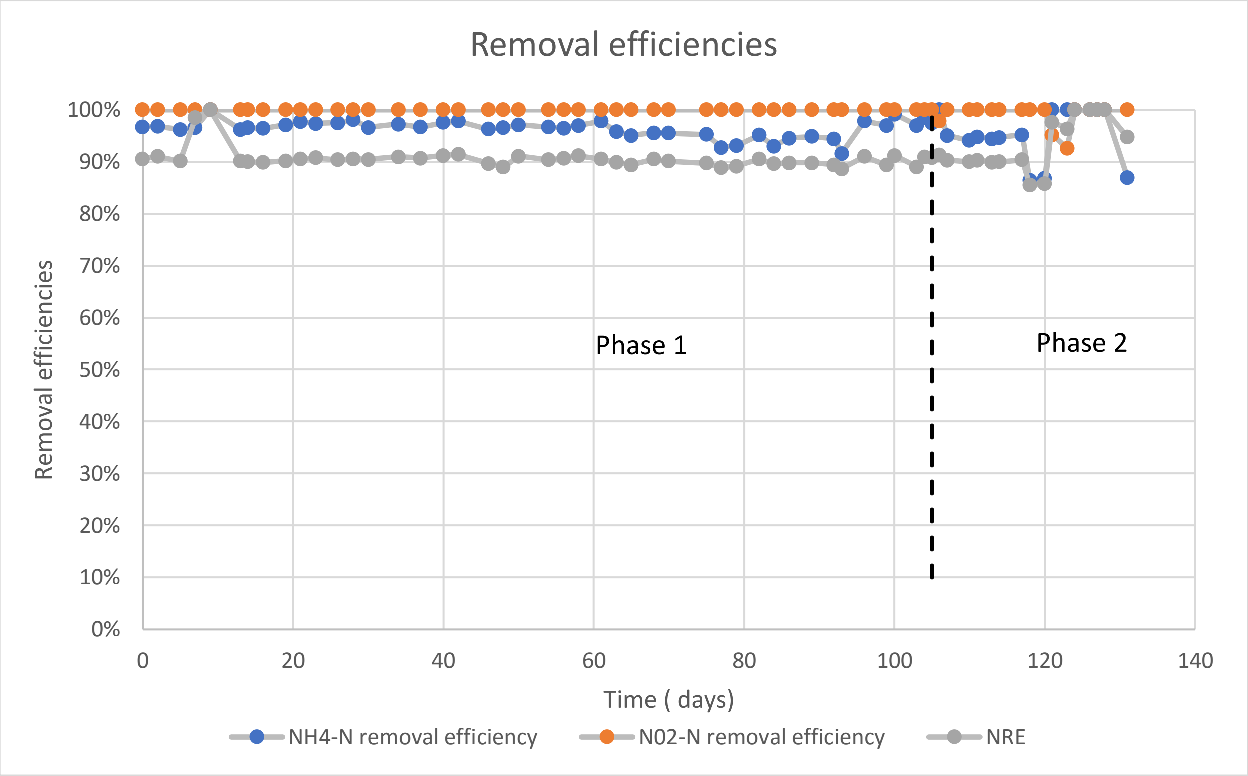


Figure 15 - Removal efficiencies

The observed NO_2-N removal efficiency was always almost 100% during phase 1, except in the day 99. During phase 2, the average value was 98,8±0,3%. The observed ammonium removal efficiency was also calculated, with values of 96,2±1,7% and 92,2±10,1%, for phase 1 and phase 2, respectively.

The observed ammonium removal is higher than expected

The overall nitrogen removal efficiency value was 90,2±3,7%.

In other studies, using SBR's, **Millia, et al**. [40] treated a mix of synthetic and pre-treated PN refinery wastewater with a NLR=0,35±0,01 gN $L^{-1}d^{-1}$ achieved a NRE of 84±4 %. Also, **Tang, et al.** [41] with synthetic influent obtained a nitrogen removal efficiency of 86% with a NLR= 0,71 gN.$L^{-1}d^{-1}$. **Dosta, et al.** [33], also using a SBR ,achieved NRE = 88 % (with observed NH_4-N removal rate of 92 %) when treating pre-treated wastewater from anaerobic digestion of municipal sewage sludge.

This experiment shows a better performance, this was achieved with a bigger NRL,soft and progressive share of real wastewater.

4.3 Stoichiometry and Kinetics

The Anammox reaction is the anaerobic oxidation of ammonium to dinitrogen gas, using nitrite as electron donor.

As stated before, the Anammox reaction proposed by **Strous, et al.** [25] was:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_2^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad Eq.12$$

Base on this stoichiometry, the influent NO_2-N/NH_4-N ratio which made it perfect for Anammox reaction was 1.32.

Other studies proposed different ratios, like **Lotti** [42] proposed a different stoichiometry.

$$NH_4^+ + 1.15NO_2^- + 0.071HCO_3^- + 0.13H^+ \rightarrow 0.99N_2 + 0.16NO_2^- + 0.071CH_{1.74}O_{0.31}N_{0.2} + 2.0H_2O \quad Eq.34$$

The molar ratio proposed by **Lotti** [42] was 1.15.

During the experiment a stable NO_2-N/NH_4-N molar ratio of 1,22±0,12 was applied (in both phase 1 and 2), but some fluctuation in the influent was found in terms of mg NO_2-N removed.L^{-1}:mg NH_4-N removed.L^{-1}, mostly during phase 2.

During phase 1, there is a small variation on the influent ratio values, but it's almost stable between both curves, as we can see in figure 16, which means that nitrite is being more consumed that was expected in the **Strous's** stoichiometry, but less that in **Lotti's**. We can therefore conclude that value is acceptable.

Also, the nitrate production assimilates more to the **Lotti's** experiment in phase 1.

This result indicated a pretty good performance and stability of the process during the replacement of synthetic influent with the real wastewater.

However, in phase 2 accumulation of nitrate (firstly) and ammonium due to the malfunction provoke high fluctuation in mg NO_2-N removed.L^{-1}:mg NH_4-N removed.L^{-1}.

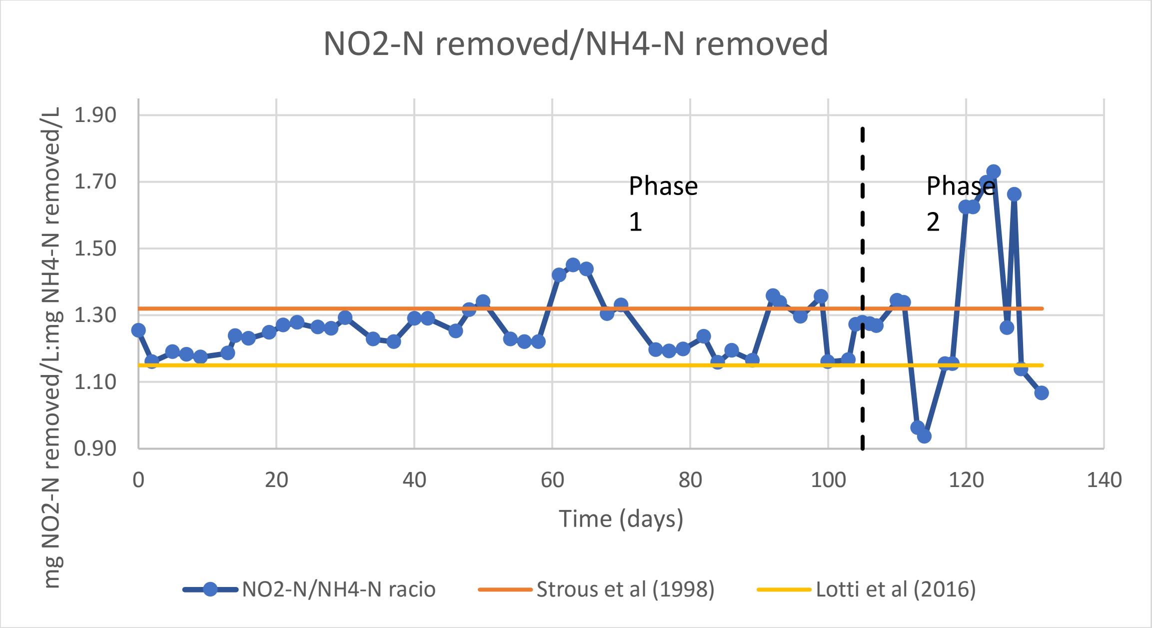


Figure 16 - NO2-N/NH4 removal ratio compared to literature

As to nitrate production, as stated before, the value is much more like **Lotti's** stoichiometric value (figure 17) the average NO_3-N/ NH_4-N value is 0,18±0,03 in phase 1.

During second phase, uncontrolled influent nitrite/ammonium ratio led to higher fluctuation in both removed nitrite and produced nitrate over ammonium ratios.

When influent had an excessive content in nitrite, it accumulated in the reactor; subsequently, when excess ammonium was fed, biomass showed to be able to remove also the accumulated nitrite, thus leading to altered measure in removal stoichiometry.

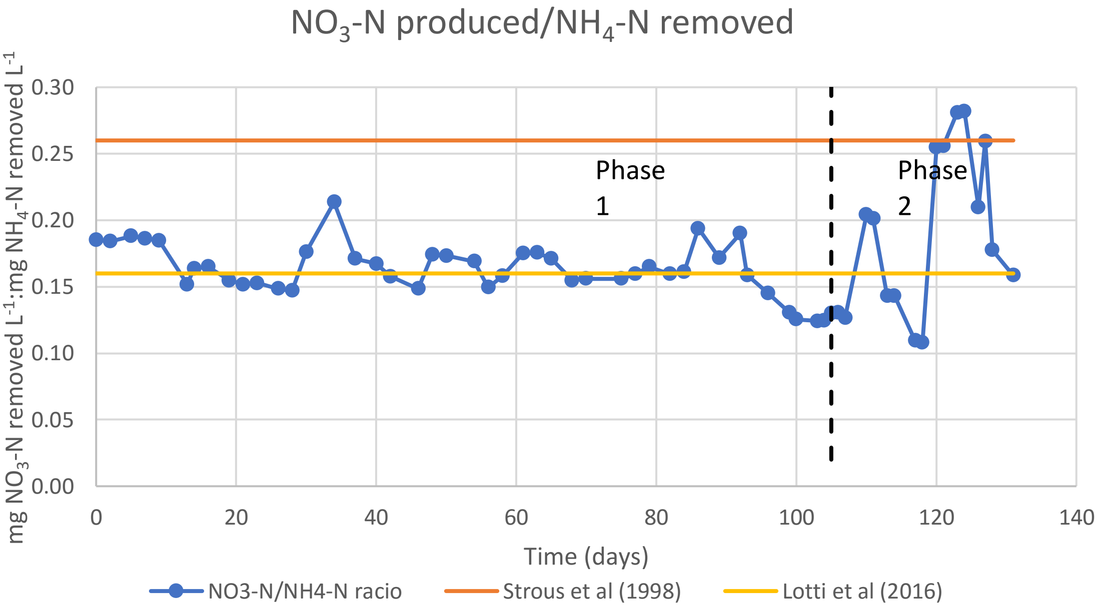


Figure 17 - NO3-N produced/NH4 removal ratio compared to literature

In table 5 are displayed literature values

Table 5 - Other literature values

References	**NLR (KgN m3 d-1)**	**NO2-N removed/NH4-N removed**	**NO3-N produced/NH4-N removed**	**Reactor configuration**
Dapena-mora et al. [43]	0,7	1,11	0,2	SBR
Lopéz et al. [39]	0,02-1,6	1,32	0,23	SBR
Millia et al. [40]	0,35+-0,01	1,30	0,22	SBR
Sousa [44]	1,5	1,22+-0,04	0,18+-0,02	SBR

Comparing to the literature values, we can directly see that values are very similar to Sousa's values from last year. She also performed the experiment in the Laboratory, in Cagliari, but the reactor was only fed with synthetic medium.

We can conclude that, even thought there was a replacement with real wastewater, the reactor was capable of obtaining the same performance.

4.4 TSS and VSS concentration; density and granular sludge

The solids analysis was performed for both reactor and effluent. The measurements were made every 3-4 weeks. In figure 18 are showed the total suspended solids (TSS), Volatile suspended solids (VSS) and ratio VSS/TSS

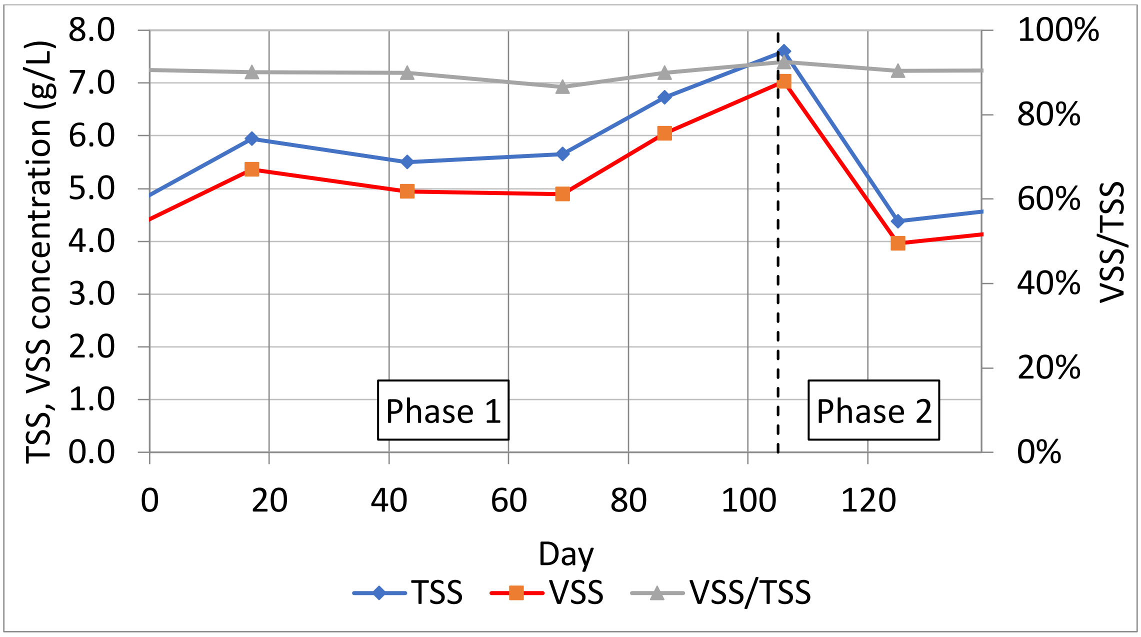


Figure 18 - Reactor's VSS, TSS and Ratio

As to the reactor, during phase 1 both VSS e TSS showed an increasing tendency with consistent VSS/TSS ratio of 90%±0,5 on average, which means consistent NVSS and no loss of activity. This is an important result, since we can establish that no inhibition affected the reactor, overall.

Since the Anammox bacteria are slow growing, was expected a slow grow on TSS and VSS, which is not observed. During phase 1, TSS and VSS grow 64% and 67%, respectively. This can be explained by the optimal growing conditions, with zero inhibition. Also, the discharged biomass was always recirculated to the reactor after some few withdrawals.

On day 105 reactor activity was temporarily suspended in order to perform the periodic cleaning of the inner part of the vessel. This activity resulted in the detachment of a remarkable quantity of biofilm from the walls and the stirring apparatus. It is likely that the biofilm provided a certain quantity of suspended solids, just because of the continuous detachment of its outer layers. As the biofilm grew inside the reactor in time, accordingly the TSS and VSS content increased.

After the reactor cleaning, a great part of that biofilm (which had poor settlement capacity) was washed out of the reactor, thus leading to lower measured values of TSS and VSS concentration. Accordingly, of the same parameters in the effluent increased, as depicted in figure 18.

The density of the granular sludge was also measured. In the figure 19, its observed that the granules density was almost always constant, throughout the experiment.

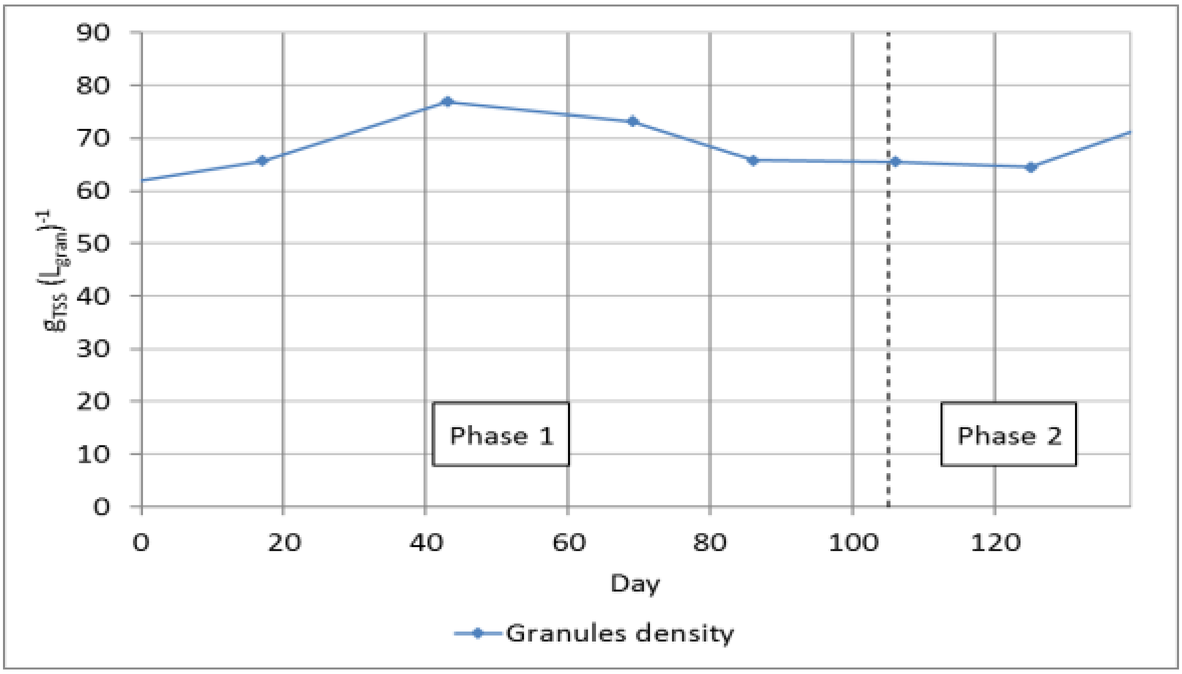


Figure 19 - Reactor's granules density

In table 6, the average values for reactor.

Table 6 - Experimental values for VSS, TSS, VSS/TSS and Granules density

VSS Concentration g L^{-1}	*TSS Concentration g L^{-1}*	*VSS/TSS ratio %*	*Granules Density gTSS $Lgranules^{-1}$*
5,24±1,05	5,82±1,12	90±1	65,99±3,60

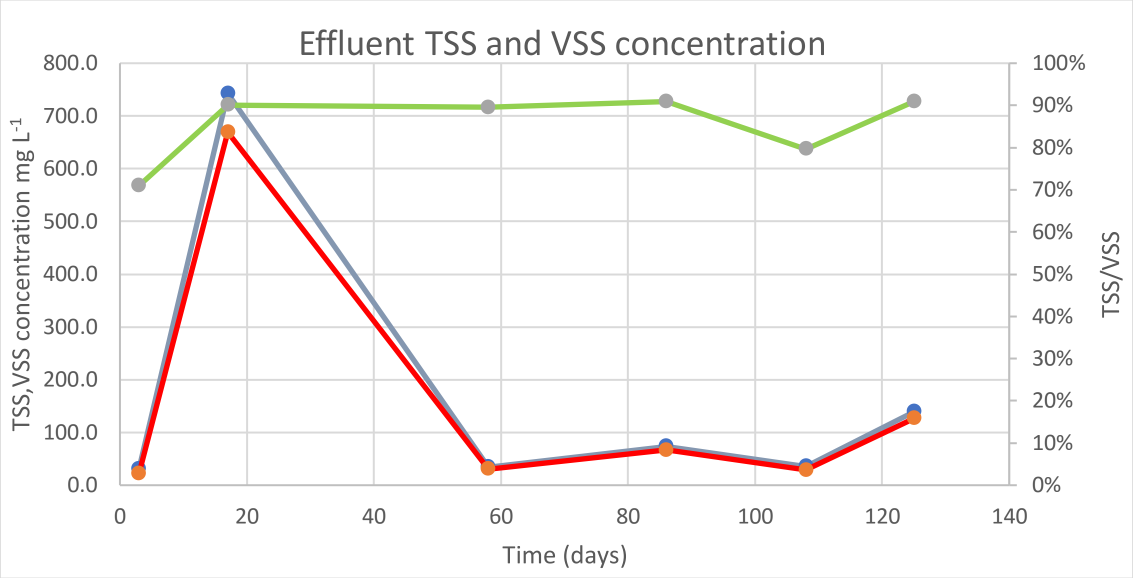


Figure 20 - Effluent's TSS, VSS and VSS/TSS ration

The reactor withdrawal is done in the end each cycle, after settling. Even thought is supposed to withdraw only the effluent without any biomass, some is washed out.

Looking to the figure 20, we can see a slow increasing (almost constant) in TSS and VSS. The only value outside this tendency is relative to the day 17. This can be explained by a wrong analysis

Ignoring the previous value, the average values for effluent are shown in the following table:

Table 7 - Experimental VSS, TSS and VSS/TSS ration

VSS Concentration g L^{-1}	**TSS Concentration g L^{-1}**	**VSS/TSS ratio %**
49,4±16,71	56,5±17,11	85±7

4.5 Specific Anammox Activity: NH_4-N and NO_2-N removal rates and NO_3-N production rate

In order to determine the volumetric removal rates for NH_4-N and NO_2-N and NO_3-N production rates, a kinetic assay was performed. This combined with the corresponding volatile suspended solids (VSS), was possible to calculate the specific removal rates for NH_4-N and NO_2-N, as well as the specific NO_3-N production rate, in gN g VSS^{-1} d^{-1}.

4.5.1 NH4-N and NO2-N removal rates

The following figure shows a typical successful kinetics result for NO_2-N and NH_4-N removal.

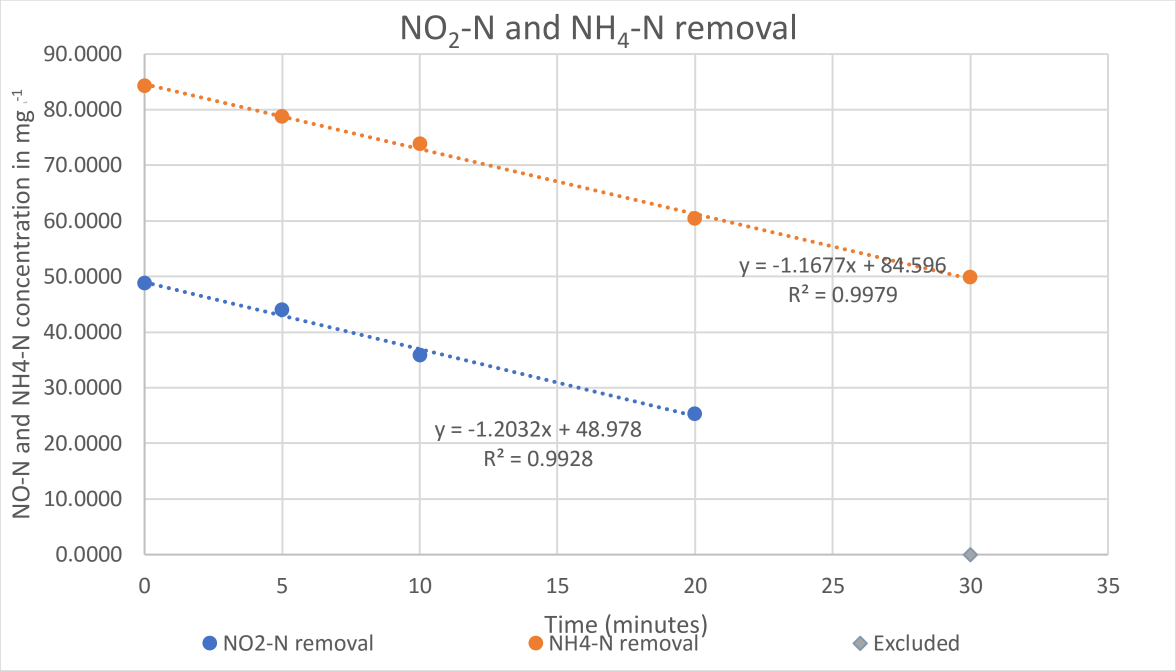


Figure 21 - NO2-N and NH4-N removal velocity

After the kinetics solution was added, samples were taken 5-10 minutes, with a total of 5 samples. The kinetics showed is relative to day 107, with 100% real wastewater.

The R2 value for both curves are >0,99 which indicates a good kinetics. Also, the slope is bigger for NO_2-N removal, meaning that NO2-N is consumed faster than NH_4-N. This is proven by the molar ratio expected according to **Strous** equation.

4.5.1.1 Volumetric NO2-N removal rate

The Volumetric NO_2-N removal rates are displayed in the figure 22.

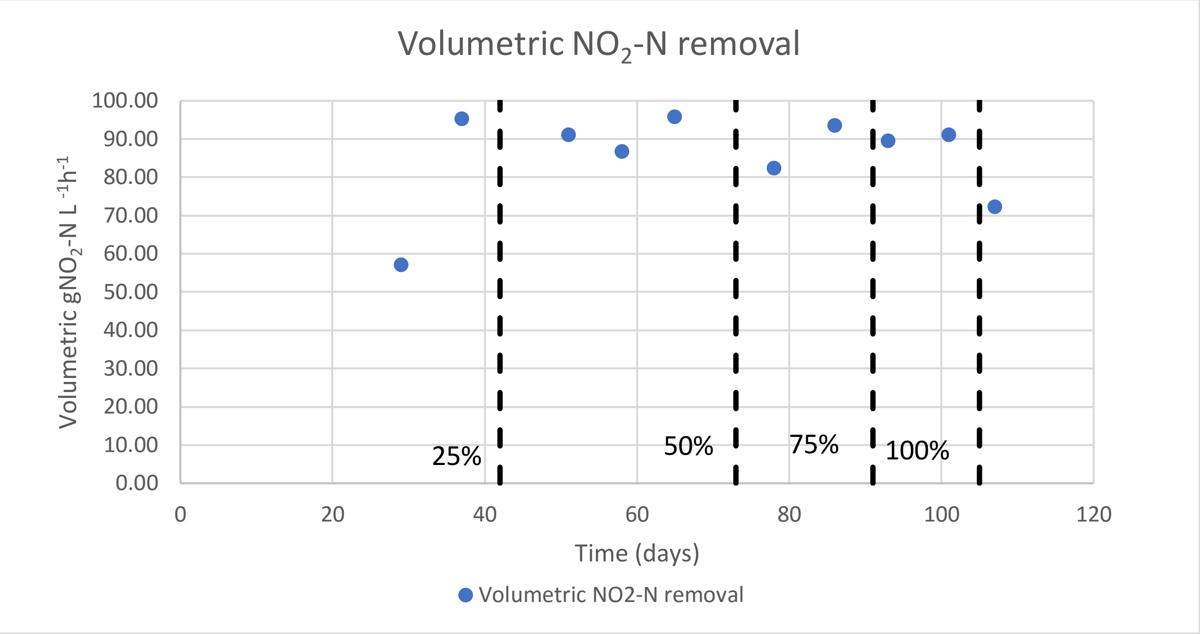


Figure 22 - Volumetric NO2-N removal

For better analysis, the phase 1 of the experiment was divided in 4 sub-phases, when the share of real wastewater achieved 25%,50%,75% and 100% of the total influent.

It can be observed that the volumetric nitrite removal rates are constant throughout the experiment, between 80-95 g NO_2-N L^{-1} h^{-1}, with an average value of 86,80±11,6 g NO_2-N L^{-1} h^{-1}.

Since bacteria grow inside the reactor, proven by the VSS increase in figure 20, was expected an increasing trend of nitrite removal, which is not observed. This can be explained due to inactive cells (i.e. dead biofilm detached from the walls).

4.5.1.2 Specific NO2-N removal

The specific removal rate is a different and better method to measure the removal rates for NO_2-N and NH_4-N, and production rates for NO_3-N. Also, is perfect to correlate the volumetric removal with the biomass activity inside the reactor

In the figure below, the specific NO_2-N removal rates are showed.

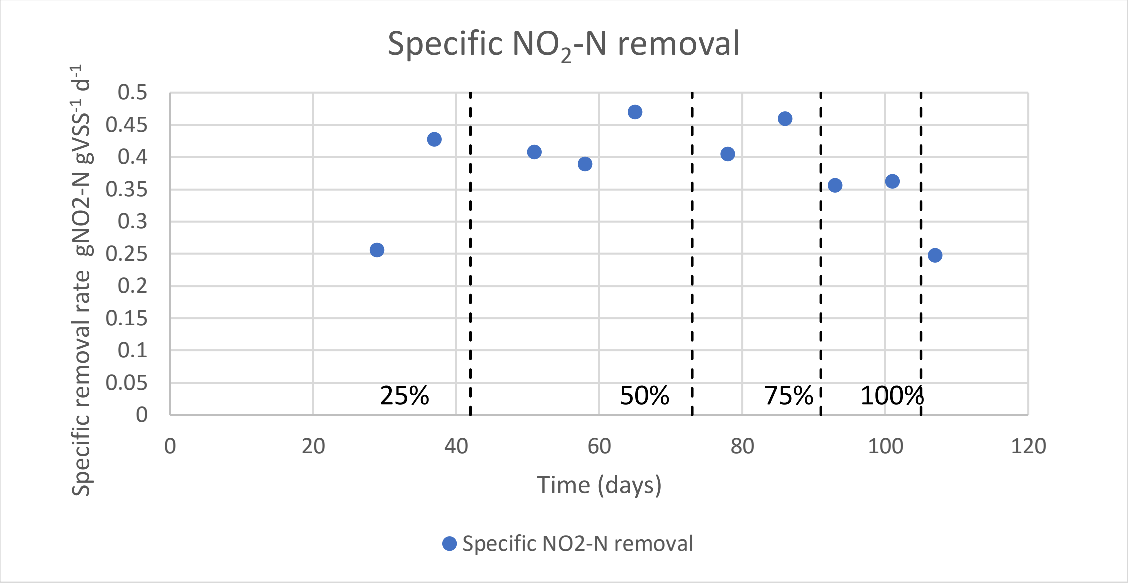


Figure 23 - Specific NO2-N removal

The removal rates are much more variable than the volumetric removal rates, as demonstrated in figure 23, but still, a constant trend is still observed. The increasing share of real wastewater doesn't seem to affect the specific nitrite removal rate, even thought, there is an increase in VSS inside the reactor.

4.5.1.3 Volumetric NH4-N removal rate

In the figure 24, the Volumetric NH_4-N removal rate is presented

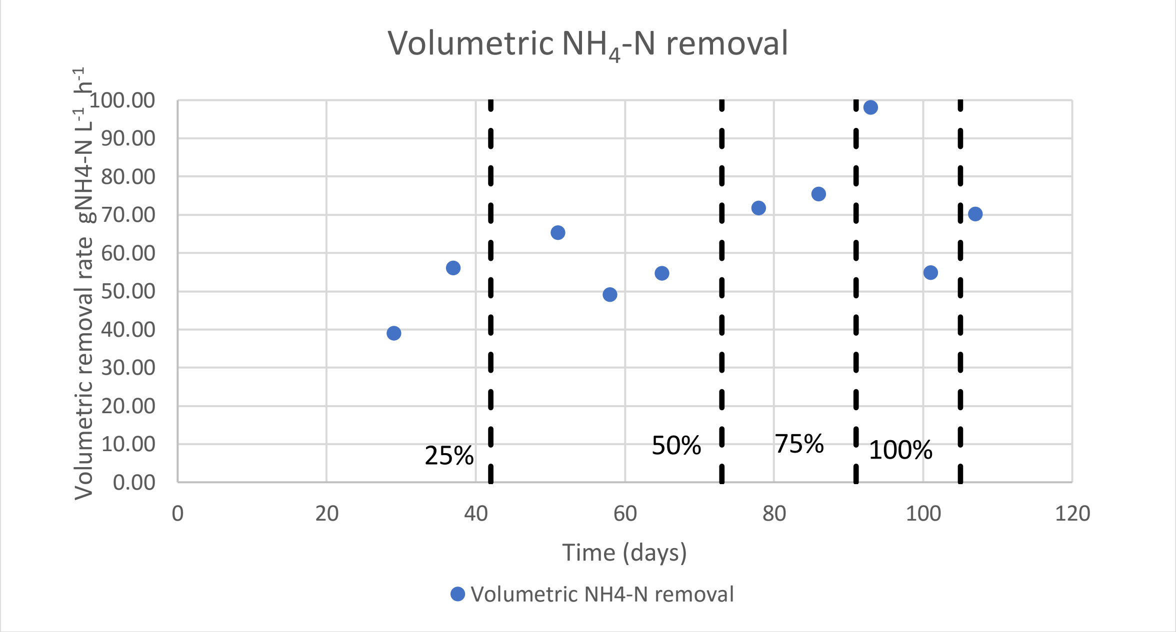


Figure 24 - Volumetric NH4-N removal

In this figure, we can observe an increasing trend in ammonium removal rate, which was expected, since with continues NLR the bacteria were perfect acclimated to the reactor conditions, and grown during experiment.

4.5.1.4 Specific NH4-N removal

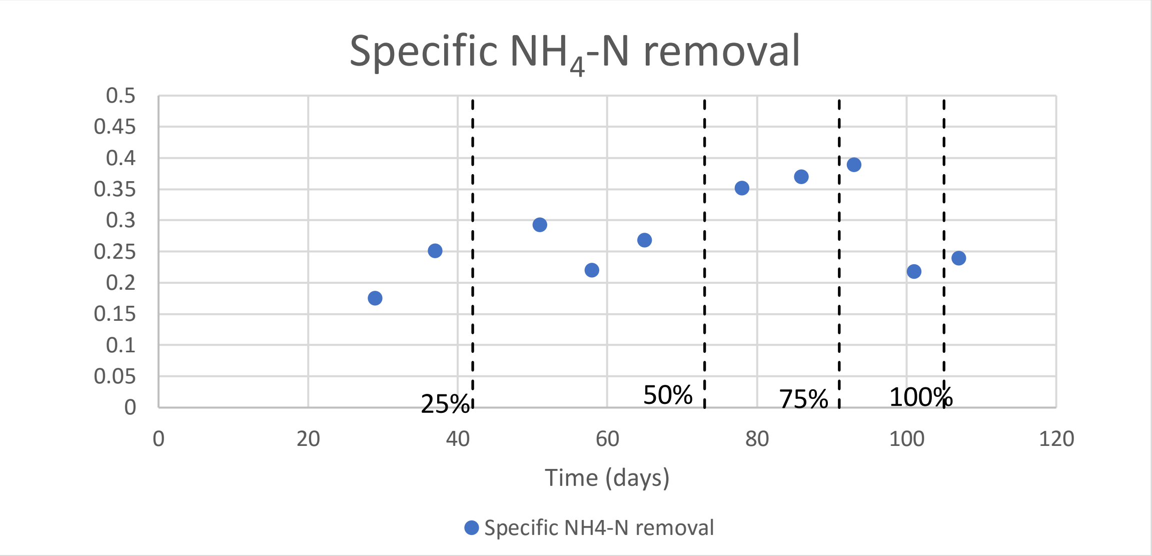


Figure 25 - Specific NH4-N removal

Similar to nitrite, specific NH$_4$-N removal was calculated and displayed in figure 25.
Is observed an increasing trend but at slower pace compared to the volumetric removal. The stoichiometric ratio $Y_{NH4\_NO2,ANMX}$ was also calculated for each kinetics, using equation 32, with an average value of 1,38±0,28 closed to the stoichiometric value proposed by **Strous**,1,32.
The ammonium removal rates are also lower than the nitrite overall, which was expected since there is only one metabolic process.

In the table 8 are summarized the average values for nitrite and ammonium removal ratios.

Table 8 - Experimental Values for Volumetric and specific removal rates

Volumetric NO_2-N ($mg\ NO_2$-$N\ L^{-1}\ h^{-1}$)	**Volumetric NH_4-N ($mg\ NH_4$-$N\ L^{-1}\ h^{-1}$)**	**Specific NO_2-N ($g\ NO_2$-$N\ gVSS^{-1}d^{-1}$)**	**Specific NH_4-N ($g\ NH_4$-$N\ /gVSS^{-1}d^{-1}$)**
86,80±11,28	62,56±5,37	0,37±0,07	0,27±0,07

In table 9 are showed some literature values for the specific Anammox removal

Table 9 - Specific removal literature values

Reference	**Specific NO2-N (g NO2-N gVSS-1d-1)**	**Specific NH4-N (g NH4-N /gVSS-1d-1)**	**Reactor configuration**
Millia et al. [40]	0,102±0,002	0,078±0,002	SBR
Tang et al.[45]	0,1	0,08	Fluidized bed

By looking to table 9, and comparing with the our results, we can conclude that values obtained were considerably higher than the ones found in literature, which indicates an outstading performance of the reactor in terms of specific removal.

4.5.2 Specific Anammox Activity: NO3-N production rates

Can be observed in the figure below a typical nitrate production rate.

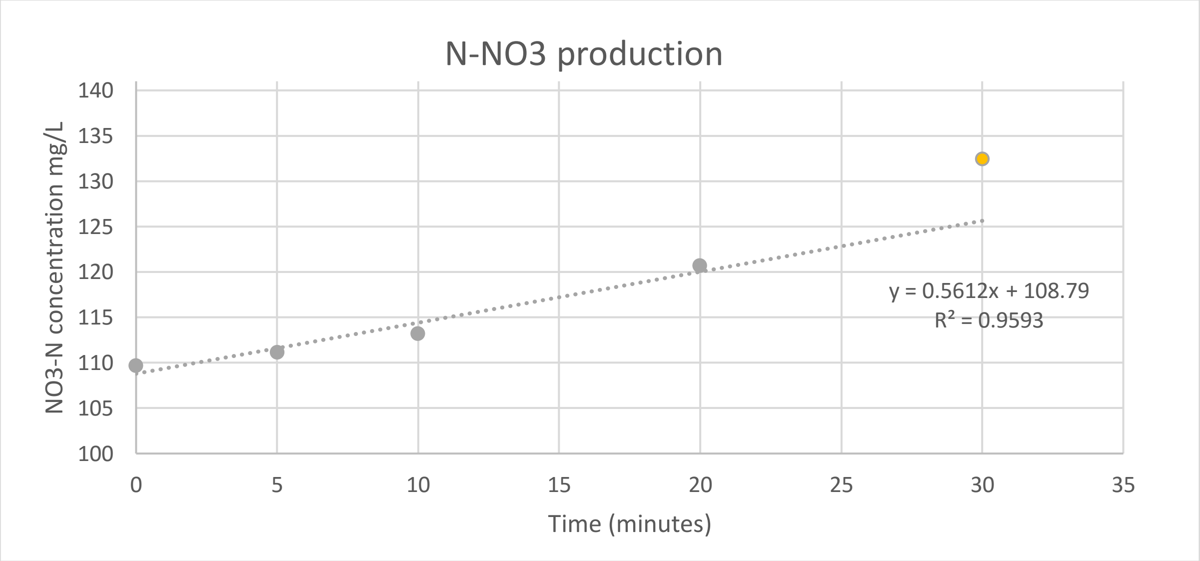


Figure 26 - NO3-N production velocity

The nitrate production measurements throughout the experiment were unstable, and so, the curves were not as perfect as the curves from the removal rates, <0,99. Although, is possible to see an increasing tendency as expected and coherent to the nitrite and ammonium removal rates.

4.5.2.1 Volumetric and Specific NO3-N production rate

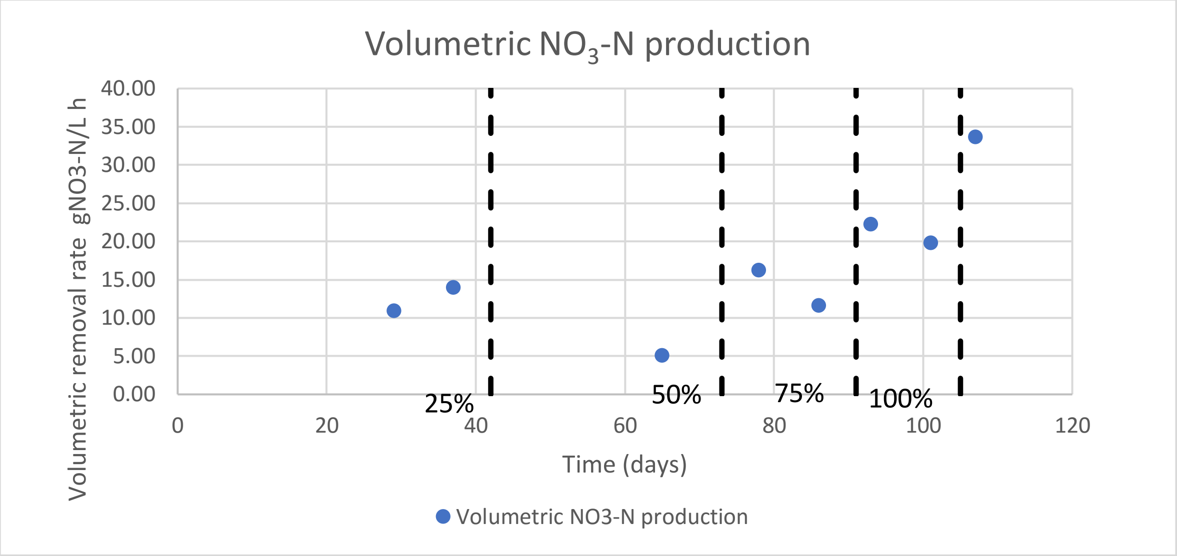


Figure 27 - Volumetric NO3-N production rate

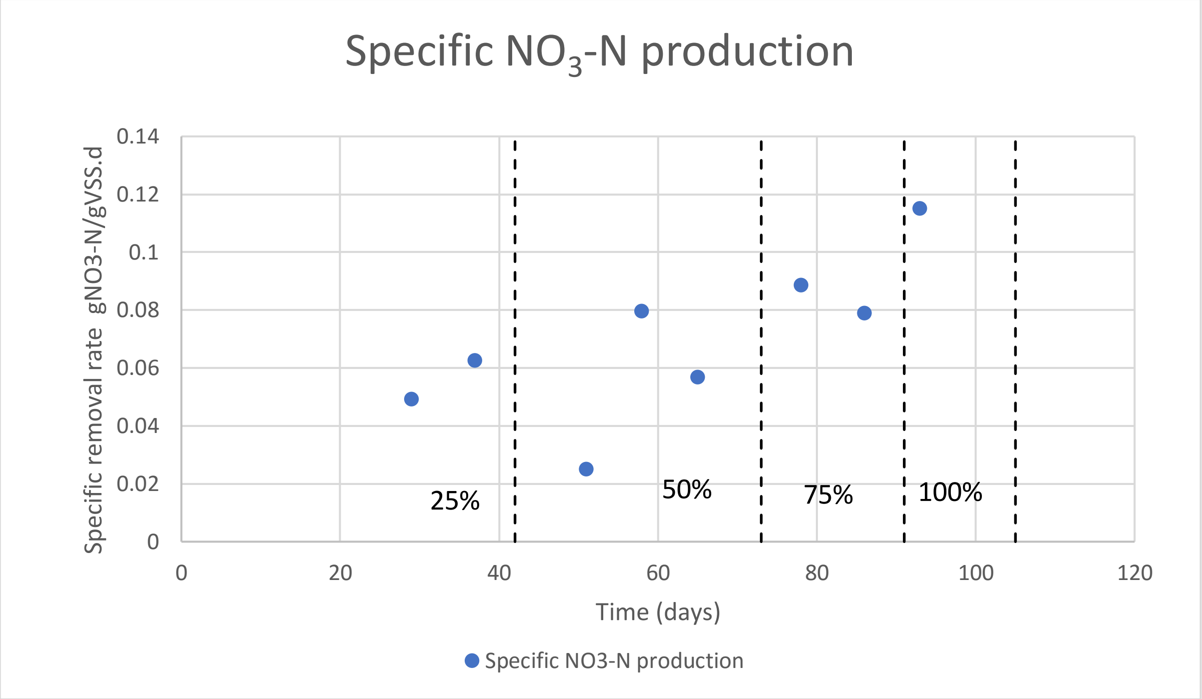


Figure 28 - Specific NO3-N production rate

As stated before, the nitrate measurements weren't accurate, and so, some variability is expected and observed in figure 27 and 28.
Is also observed an increasing tendency in the production rates, volumetric and specific, which is expected.

The stoichiometric ratio, $Y_{NH4\_NO3,ANMX}$, was also calculated with a resulting value of 0,22±0,08, which stays between both Strous and Lotti values (0,26 and 0,16, respectively).

In the table 10 is represented the average values for the experiment

Table 10 - Experimental Values for Volumetric and Specific production rate

Volumetric NO_3-N production (mg NO_3-N L^{-1} h^{-1})	*Specific NO_3-N production (g NO_3-N $gVSS^{-1}d^{-1}$)*
14,26±5,37	0,055±0,030

4.5.3 The Specific Anammox Activity

Finally, the Specific Anammox Activity (SAA) is expressed as dinitrogen gas produced per day per unit of biomass equivalent to nitrogen removed from wastewater. The maximum specific Anammox rate, $q_{Anmx,N2,}$ given by equation 31, was on average, 0,0248±0,0050 gN_2-N $gVSS^{-1}$ h^{-1}

4.6 Removal of organic matter

In order to assess the removing organic matter efficiency inside the Anammox reactor, total organic carbon was calculated by measuring the total carbon and inorganic carbon for both influent (synthetic and real bags) and effluent.
For the real wastewater is a little bit different, since it depends on the water and also the removal efficiency from the partial-nitrification step.
Is known that partial-nitrification has a crucial role in removing organic matter, which can be used in the Anammox reactor by denitrifiers, hindering the process.

The average values for the bags are showed in table 11

Table 11 - Total organic values for Synthetic and real influent

Synthetic Bag (mg L^{-1})	*Pre-treated real wastewater bag(mg L^{-1})*
48,15±12,25	*44,15±12,84*

Relatively to the effluent, the organic matter concentration was almost constant during the experiment, as observed in figure 29.

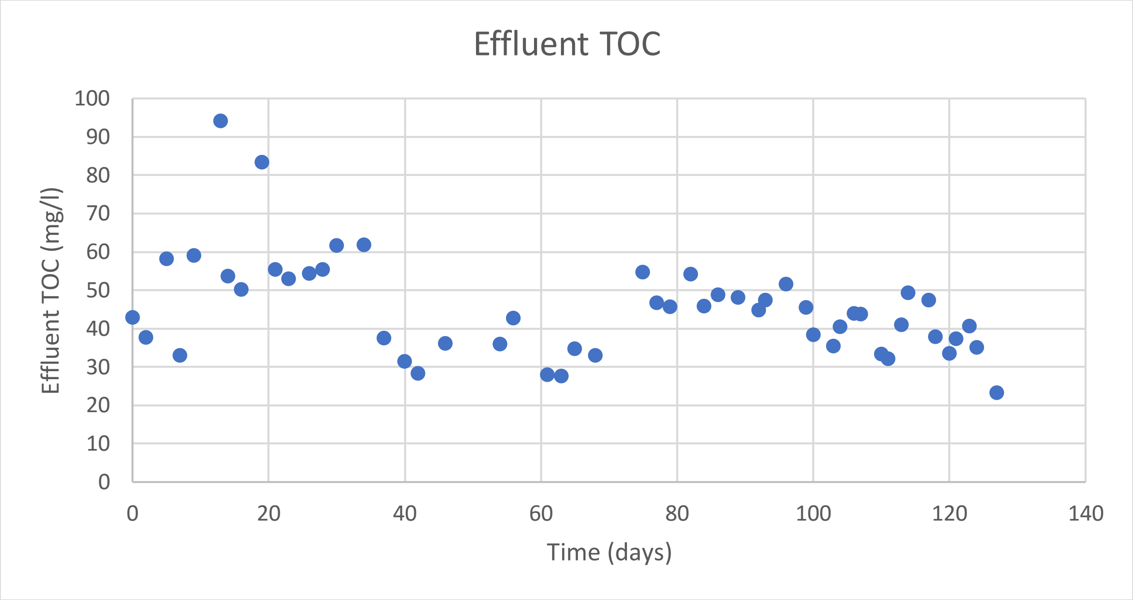


Figure 29 - Effluent total organic carbon concentrations during experience.

Except for a few days, the effluent values were stable, which indicates that there was no significant heterotrophic bacteria growth inside the reactor.

Finally, the total organic carbon removal efficiency was calculated, based in the equation 34.

$$TOC\ removal\ Efficiency = 100 * (1 - \left(\frac{TOCeff}{TOC\ Inf}\right)), \quad \text{eq.34}$$

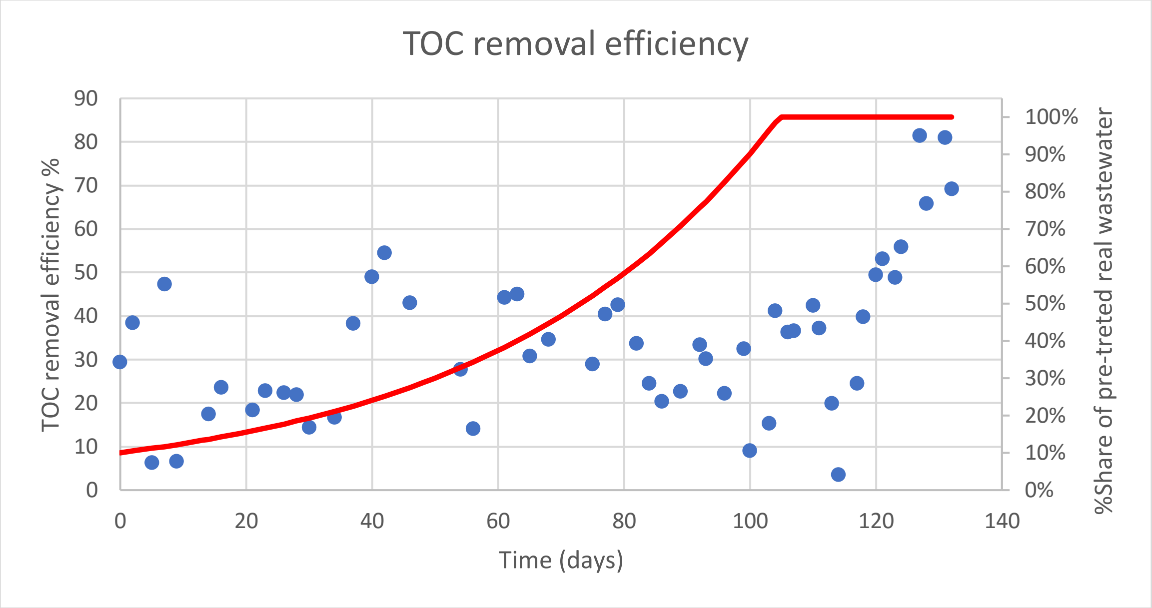


Figure 30 - Total organic carbon removal efficiency during experience

Can be observed looking to figure 30, that the removal efficiency is very variable with increasing share of real wastewater.

Was expected a decreasing in efficiency, since real wastewater was supposed to have more organic matter.

If we look closer to table 11, the pre-treated wastewater has a higher organic carbon concentration than the synthetic, on average, and this can explain the figure above.

The lower concentration of organic carbon on the real influent is due to outstanding performance of the partial-nitrification reactor, which was very successful in making perfectly suitable influent for the Anammox.

5. Conclusions

The main purpose of this thesis was to assess if the Anammox reactor was capable to transit from synthetic wastewater to pre-treated real wastewater from anaerobic digestion of municipal solid waste, with a gradually increased share based on conservative exponential law, proposed by **Lopez e al**.

The results showed that the reactor start-up was successful achieved using this feeding strategy. The gradual increase of share avoided stressful conditions for the granular Anammox biomass.

The reactor performance was very good overall, throughout all experiment, with removal efficiencies around 90%, which were outstanding comparing with the ones found in literature.

The perfect acclimation of the bacteria is clearly seen in the slightly increase in VSS and TSS, which allowed the Anammox bacteria to grow during the activity.

The partial-nitrification process had a huge impact on the Anammox performance, since it was capable of removing the organic matter from the Anammox influent, which allowed the bacteria to grow and have an exceptional performance.

Even when facing malfunctions, the reactor was capable to recovering relatively easy, during phase 1. The phase 2 malfunction really affected the system, and the re-start with 100% synthetic influent was demanded. We can conclude once more that partial-nitrification has an important role beside converting ammonium to nitrite.

In conclusion, the experiment was successful, the main objective was achieved.

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7.Appendices

7.1 Appendix I – Influent Characteristics

Table 12 - Real Wastewater influent Loads

Date	Day	Fed volume mL	Feeding duration min	since sec	for sec	NLR [kgN/m^{3}d]	Share of real ww % v/v	Volumes	
								Per day (L)	Cumulated volume (L)
14/03/2017 ter	0	53	27	14400	1600	0,150	10%	0,21	0,2
15/03/2017 qua	1	55	27	14364	1636	0,153	10%	0,22	0,4
16/03/2017 qui	2	56	28	14328	1672	0,157	10%	0,22	0,7
17/03/2017 sex	3	57	28	14291	1709	0,160	11%	0,23	0,9
18/03/2017 sáb	4	58	29	14253	1747	0,164	11%	0,23	1,1
19/03/2017 dom	5	60	30	14214	1786	0,167	11%	0,24	1,4
20/03/2017 seg	6	61	30	14174	1826	0,171	11%	0,24	1,6
21/03/2017 ter	7	62	31	14134	1866	0,175	12%	0,25	1,8
22/03/2017 qua	8	64	32	14092	1908	0,179	12%	0,25	2,1
23/03/2017 qui	9	65	33	14050	1950	0,183	12%	0,26	2,4
24/03/2017 sex	10	66	33	14006	1994	0,187	12%	0,27	2,6
25/03/2017 sáb	11	68	34	13962	2038	0,191	13%	0,27	2,9
26/03/2017 dom	12	69	35	13917	2083	0,195	13%	0,28	3,2
27/03/2017 seg	13	71	35	13870	2130	0,200	13%	0,28	3,5
28/03/2017 ter	14	73	36	13823	2177	0,204	14%	0,29	3,7
29/03/2017 qua	15	74	37	13774	2226	0,209	14%	0,30	4,0
30/03/2017 qui	16	76	38	13725	2275	0,213	14%	0,30	4,3
31/03/2017 sex	17	78	39	13674	2326	0,218	15%	0,31	4,7
01/04/2017 sáb	18	79	40	13623	2377	0,223	15%	0,32	5,0
02/04/2017 dom	19	81	41	13570	2430	0,228	15%	0,32	5,3
03/04/2017 seg	20	83	41	13516	2484	0,233	16%	0,33	5,6
04/04/2017 ter	21	85	42	13460	2540	0,238	16%	0,34	6,0
05/04/2017 qua	22	87	43	13404	2596	0,243	16%	0,35	6,3
06/04/2017 qui	23	88	44	13346	2654	0,249	17%	0,35	6,7

07/04/2017 sex	24	90	45	13287	2713	0,254	17%	0,36	7,0
08/04/2017 sáb	25	92	46	13227	2773	0,260	17%	0,37	7,4
09/04/2017 dom	26	94	47	13165	2835	0,266	18%	0,38	7,8
10/04/2017 seg	27	97	48	13102	2898	0,272	18%	0,39	8,2
11/04/2017 ter	28	99	49	13038	2962	0,278	19%	0,39	8,6
12/04/2017 qua	29	101	50	12972	3028	0,284	19%	0,40	9,0
13/04/2017 qui	30	103	52	12904	3096	0,290	19%	0,41	9,4
14/04/2017 sex	31	105	53	12835	3165	0,297	20%	0,42	9,8
15/04/2017 sáb	32	108	54	12765	3235	0,303	20%	0,43	10,2
16/04/2017 dom	33	110	55	12693	3307	0,310	21%	0,44	10,7
17/04/2017 seg	34	113	56	12620	3380	0,317	21%	0,45	11,1
18/04/2017 ter	35	115	58	12544	3456	0,324	22%	0,46	11,6
19/04/2017 qua	36	118	59	12468	3532	0,331	22%	0,47	12,1
20/04/2017 qui	37	120	60	12389	3611	0,339	23%	0,48	12,5
21/04/2017 sex	38	123	62	12309	3691	0,346	23%	0,49	13,0
22/04/2017 sáb	39	126	63	12226	3774	0,354	24%	0,50	13,5
23/04/2017 dom	40	129	64	12143	3857	0,362	24%	0,51	14,0
24/04/2017 seg	41	131	66	12057	3943	0,370	25%	0,53	14,6
25/04/2017 ter	42	134	67	11969	4031	0,378	25%	0,54	15,1
26/04/2017 qua	43	137	69	11879	4121	0,386	26%	0,55	15,7
27/04/2017 qui	44	140	70	11788	4212	0,395	26%	0,56	16,2
28/04/2017 sex	45	144	72	11694	4306	0,404	27%	0,57	16,8
29/04/2017 sáb	46	147	73	11598	4402	0,413	28%	0,59	17,4
30/04/2017 dom	47	150	75	11500	4500	0,422	28%	0,60	18,0
01/05/2017 seg	48	153	77	11400	4600	0,431	29%	0,61	18,6
02/05/2017 ter	49	157	78	11298	4702	0,441	29%	0,63	19,2
03/05/2017 qua	50	160	80	11193	4807	0,451	30%	0,64	19,9
04/05/2017 qui	51	164	82	11086	4914	0,461	31%	0,66	20,5
05/05/2017 sex	52	167	84	10977	5023	0,471	31%	0,67	21,2
06/05/2017 sáb	53	171	86	10865	5135	0,481	32%	0,68	21,9
07/05/2017 dom	54	175	87	10751	5249	0,492	33%	0,70	22,6
08/05/2017 seg	55	179	89	10634	5366	0,503	34%	0,72	23,3
09/05/2017 ter	56	183	91	10515	5485	0,514	34%	0,73	24,0
10/05/2017 qua	57	187	93	10393	5607	0,526	35%	0,75	24,8

11/05/2017 qui	58	191	96	10268	5732	0,537	36%	0,76	25,5
12/05/2017 sex	59	195	98	10141	5859	0,549	37%	0,78	26,3
13/05/2017 sáb	60	200	100	10011	5989	0,562	37%	0,80	27,1
14/05/2017 dom	61	204	102	9877	6123	0,574	38%	0,82	27,9
15/05/2017 seg	62	209	104	9741	6259	0,587	39%	0,83	28,8
16/05/2017 ter	63	213	107	9602	6398	0,600	40%	0,85	29,6
17/05/2017 qua	64	218	109	9460	6540	0,613	41%	0,87	30,5
18/05/2017 qui	65	223	111	9314	6686	0,627	42%	0,89	31,4
19/05/2017 sex	66	228	114	9165	6835	0,641	43%	0,91	32,3
20/05/2017 sáb	67	233	116	9013	6987	0,655	44%	0,93	33,2
21/05/2017 dom	68	238	119	8858	7142	0,670	45%	0,95	34,2
22/05/2017 seg	69	243	122	8699	7301	0,684	46%	0,97	35,1
23/05/2017 ter	70	249	124	8537	7463	0,700	47%	1,00	36,1
24/05/2017 qua	71	254	127	8371	7629	0,715	48%	1,02	37,2
25/05/2017 qui	72	260	130	8201	7799	0,731	49%	1,04	38,2
26/05/2017 sex	73	266	133	8027	7973	0,747	50%	1,06	39,3
27/05/2017 sáb	74	272	136	7850	8150	0,764	51%	1,09	40,3
28/05/2017 dom	75	278	139	7669	8331	0,781	52%	1,11	41,5
29/05/2017 seg	76	284	142	7484	8516	0,798	53%	1,14	42,6
30/05/2017 ter	77	290	145	7294	8706	0,816	54%	1,16	43,8
31/05/2017 qua	78	297	148	7100	8900	0,834	56%	1,19	44,9
01/06/2017 qui	79	303	152	6902	9098	0,853	57%	1,21	46,2
02/06/2017 sex	80	310	155	6700	9300	0,872	58%	1,24	47,4
03/06/2017 sáb	81	317	158	6493	9507	0,891	59%	1,27	48,7
04/06/2017 dom	82	324	162	6282	9718	0,911	61%	1,30	50,0
05/06/2017 seg	83	331	166	6066	9934	0,931	62%	1,32	51,3
06/06/2017 ter	84	339	169	5845	10155	0,952	63%	1,35	52,6
07/06/2017 qua	85	346	173	5619	10381	0,973	65%	1,38	54,0
08/06/2017 qui	86	354	177	5388	10612	0,995	66%	1,41	55,4
09/06/2017 sex	87	362	181	5152	10848	1,017	68%	1,45	56,9
10/06/2017 sáb	88	370	185	4910	11090	1,040	69%	1,48	58,4
11/06/2017 dom	89	378	189	4664	11336	1,063	71%	1,51	59,9
12/06/2017 seg	90	386	193	4412	11588	1,086	72%	1,55	61,4
13/06/2017 ter	91	395	197	4154	11846	1,111	74%	1,58	63,0

14/06/2017 qua	92	404	202	3890	12110	1,135	76%	1,61	64,6
15/06/2017 qui	93	413	206	3621	12379	1,161	77%	1,65	66,3
16/06/2017 sex	94	422	211	3346	12654	1,186	79%	1,69	67,9
17/06/2017 sáb	95	431	216	3064	12936	1,213	81%	1,72	69,7
18/06/2017 dom	96	441	220	2776	13224	1,240	83%	1,76	71,4
19/06/2017 seg	97	451	225	2482	13518	1,267	84%	1,80	73,2
20/06/2017 ter	98	461	230	2182	13818	1,295	86%	1,84	75,1
21/06/2017 qua	99	471	235	1874	14126	1,324	88%	1,88	77,0
22/06/2017 qui	100	481	241	1560	14440	1,354	90%	1,93	78,9
23/06/2017 sex	101	492	246	1239	14761	1,384	92%	1,97	80,9
24/06/2017 sáb	102	503	251	910	15090	1,415	94%	2,01	82,9
25/06/2017 dom	103	514	257	575	15425	1,446	96%	2,06	84,9
26/06/2017 seg	104	526	263	232	15768	1,478	99%	2,10	87,0
27/06/2017 ter	105	533	267	0	16000	1,500	100%	2,13	89,2
28/06/2017 qua	106	533	267	0	16000	1,500	100%	2,13	91,3
29/06/2017 qui	107	533	267	0	16000	1,500	100%	2,13	93,4
30/06/2017 sex	108	533	267	0	16000	1,500	100%	2,13	95,6
01/07/2017 sáb	109	533	267	0	16000	1,500	100%	2,13	97,7
02/07/2017 dom	110	533	267	0	16000	1,500	100%	2,13	99,8
03/07/2017 seg	111	533	267	0	16000	1,500	100%	2,13	102,0
04/07/2017 ter	112	533	267	0	16000	1,500	100%	2,13	104,1
05/07/2017 qua	113	533	267	0	16000	1,500	100%	2,13	106,2
06/07/2017 qui	114	533	267	0	16000	1,500	100%	2,13	108,4
07/07/2017 sex	115	533	267	0	16000	1,500	100%	2,13	110,5
08/07/2017 sáb	116	533	267	0	16000	1,500	100%	2,13	112,6
09/07/2017 dom	117	533	267	0	16000	1,500	100%	2,13	114,8
10/07/2017 seg	118	533	267	0	16000	1,500	100%	2,13	116,9
11/07/2017 ter	119	533	267	0	16000	1,500	100%	2,13	119,0
12/07/2017 qua	120	533	267	0	16000	1,500	100%	2,13	121,2
13/07/2017 qui	121	533	267	0	16000	1,500	100%	2,13	123,3
14/07/2017 sex	122	533	267	0	16000	1,500	100%	2,13	125,4
15/07/2017 sáb	123	533	267	0	16000	1,500	100%	2,13	127,6
16/07/2017 dom	124	533	267	0	16000	1,500	100%	2,13	129,7
17/07/2017 seg	125	533	267	0	16000	1,500	100%	2,13	131,8

18/07/2017 ter	126	533	267	0	16000	1,500	100%	2,13	134,0
19/07/2017 qua	127	533	267	0	16000	1,500	100%	2,13	136,1
20/07/2017 qui	128	533	267	0	16000	1,500	100%	2,13	138,2
21/07/2017 sex	129	533	267	0	16000	1,500	100%	2,13	140,4
22/07/2017 sáb	130	533	267	0	16000	1,500	100%	2,13	142,5
23/07/2017 dom	131	533	267	0	16000	1,500	100%	2,13	144,6
24/07/2017 seg	132	533	267	0	16000	1,500	100%	2,13	146,8
25/07/2017 ter	133	533	267	0	16000	1,500	100%	2,13	148,9
26/07/2017 qua	134	533	267	0	16000	1,500	100%	2,13	151,0
27/07/2017 qui	135	533	267	0	16000	1,500	100%	2,13	153,2
28/07/2017 sex	136	533	267	0	16000	1,500	100%	2,13	155,3

Table 13 - Real influent characteristics

TANK	**R**	**Date of connection**	**N-NO2 (mgN/L)**	**N-NH4 ((mgN/L)**	**N-N02/N-NH4**	**N tot (mgN/L)**	**N-N03 (mgN/L)**
1	R1	14/03/17	829	725,23	1,143085642	1554,23	38,82
	R2	29/03/17	802,945	694,84	1,15558258	1497,78	24,08
	R3	12/04/17	811,415	676,23	1,199909794	1487,64	34,98
2	R4	20/04/17	909,65	681,72	1,334345479	1591,37	26,35
	R5	02/05/17	802,54	702,57	1,14	1505,11	23,92
	R6	15/05/17	801,675	576,09	1,39	1377,76	27,85
3	R7	22/05/17	823,46	720,13	1,14	1543,59	30,82
	R8		855,08	642,21	1,33	1497,29	32,24
4	R9	06/06/17	870,23	642,2	1,36	1512,43	29,41
	10	12/06/17	883,65	652,08	1,36	1535,73	30,12
5	11	16/06/17	852,70	668,55	1,28	1521,25	31,05
	12	21/06/17	931,65	659,77	1,41	1591,42	34,46
	12UP	22/06/17	919,27	839,76	1,09	1759,03	32,20
Remanings	13	26/06/17	843,51	699,28	1,21	1542,79	33,68
5	14	30/06/17	830	715,09	1,16	1545,09	
5+ remanings	15	-----	720,02	713	1,01	1433,02	27,99
6	16		847	734,4	1,15	1581,4	49,02
6	17	11/07/17	810	499	1,62	1309	,,,,,
Not pre-treat	18	13/07/17	780	528,35	1,48	1308,35	,,,,,
Not pre-treat	19	17/07/17	650	721,14	0,90	1371,14	,,,,,.
Not pre-treat	19up	18/07/17	820	721,14	1,14	1541,14	,,,,,,

7.2 Appendix II – TSS and VSS concentration; density and granular sludge

Table 14 - Reactor Solids analysis

				Weights			**Actual measure**		**Measured referred to fixed vol of 2 L**					
Date	**Day**	**Fed volume**	**Sample volume**	**Empty**	**Dry**	**Ashes**	**TSS**	**VSS**	**TSS**	**VSS**	**TSS / VSS**	**Granules volume**	**Specific volume**	**Granules density**
		mL	mL	g	g	g	g / L	g / L	g / L	g / L	%	mL	Lgran/L	gTSS / Lgran
10/03/17	-4	0,0	39,0	1,3938	1,6194	1,4148	5,78	5,25	4,63	4,20	91%	2,95	0,076	61,18
31/03/17	17	0,1	42,0	1,3940	1,7060	1,4249	7,43	6,69	5,94	5,35	90%	3,8	0,090	65,69
26/04/17	43	0,0	23,8	1,3847	1,5481	1,4012	6,88	6,19	5,50	4,95	90%	1,7	0,072	76,89
22/05/17	69	0,0	35,0	1,3828	1,6299	1,4160	7,06	6,11	5,65	4,89	87%	2,7	0,077	73,21
08/06/17	86	0,0	47,0	1,3874	1,7822	1,4275	8,40	7,55	6,72	6,04	90%	4,8	0,102	65,80
28/06/17	106	0,0	50,0	1,3858	1,8607	1,4214	9,50	8,79	7,60	7,03	93%	5,8	0,116	65,50
17/07/17	125	0,0	28,0	1,4725	1,6258	1,4872	5,48	4,95	4,38	3,96	90%	1,9	0,068	64,55
14/08/17	153	0,0	41,0	1,3854	1,6283	1,4083	5,92	5,37	4,74	4,29	91%	2,5	0,061	77,73

Table 15 - Effluent Solids analysis

Date	Day	Sample volume mL	Empty g	Dry g	Ashes g	TSS mg/L	VSS mg/L	TSS / VSS %
08/02/17	-34	540,0	1,3769	1,3904	1,3786	25,0	21,9	87%
17/03/17	3	400,0	1,3906	1,4030	1,3942	31,0	22,0	71%
31/03/17	17	420,0	1,3940	1,7060	1,4249	742,9	669,3	90%
11/05/17	58	250,0	1,3859	1,3945	1,3868	34,4	30,8	90%
08/06/17	86	150,0	1,3755	1,3865	1,3765	73,3	66,7	91%
30/06/17	108	150,0	1,3746	1,3800	1,3757	36,0	28,7	80%
17/07/17	125	150,0	1,3791	1,4000	1,3810	139,3	126,7	91%

7.3 Appendix III - Specific Anammox Activity

Date	Day	NO2-N mg/Lh	NH4-N mg/Lh	NO3-N mg/Lh	**YNO2_NH4**	**YNO3_NH4**	Specific gNO2-N/gVSS.d	Specific gNH4-N/gVSS.d	Specific gNO3-N/gVSS.d	qMAX
12/04/17	29	56,91	38,92	10,94	1,46	0,28	0,26	0,17	0,05	15,85
20/04/17	37	95,20	55,92	13,95	1,70	0,25	0,43	0,25	0,06	25,62
04/05/17	51	90,89	65,21		1,39		0,41	0,29	0,00	29,15
11/05/17	58	86,66	48,95		1,77		0,39	0,22	0,00	25,33
18/05/17	65	95,61	54,62	5,05	1,75	0,09	0,47	0,27	0,02	29,69
31/05/17	78	82,30	71,58	16,19	1,15	0,23	0,40	0,35	0,08	28,16
08/06/17	86	93,41	75,21	11,57	1,24	0,15	0,46	0,37	0,06	32,12
15/06/17	93	89,36	97,86	22,26	0,91	0,23	0,36	0,39	0,09	23,47
23/06/17	101	90,89	54,76	19,83	1,66	0,36	0,36	0,22	0,08	20,84
29/06/17	107	72,19	70,06	33,67	1,03	0,48	0,25	0,24	0,11	17,98
05/07/17	113	68,84	61,68	15,86	1,12	0,26	0,24	0,21	0,05	18,99
13/07/17	121			34,57			0,00	0,00	0,12	
14/07/17	122	22,80	39,05	4,44	0,58	0,11	0,08	0,13	0,02	
18/07/17	126		101,33				0,00	0,61	0,00	