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Assessment of the design and operational conditions on the performance of sponge-bed trickling filters for autotrophic nitrogen removal

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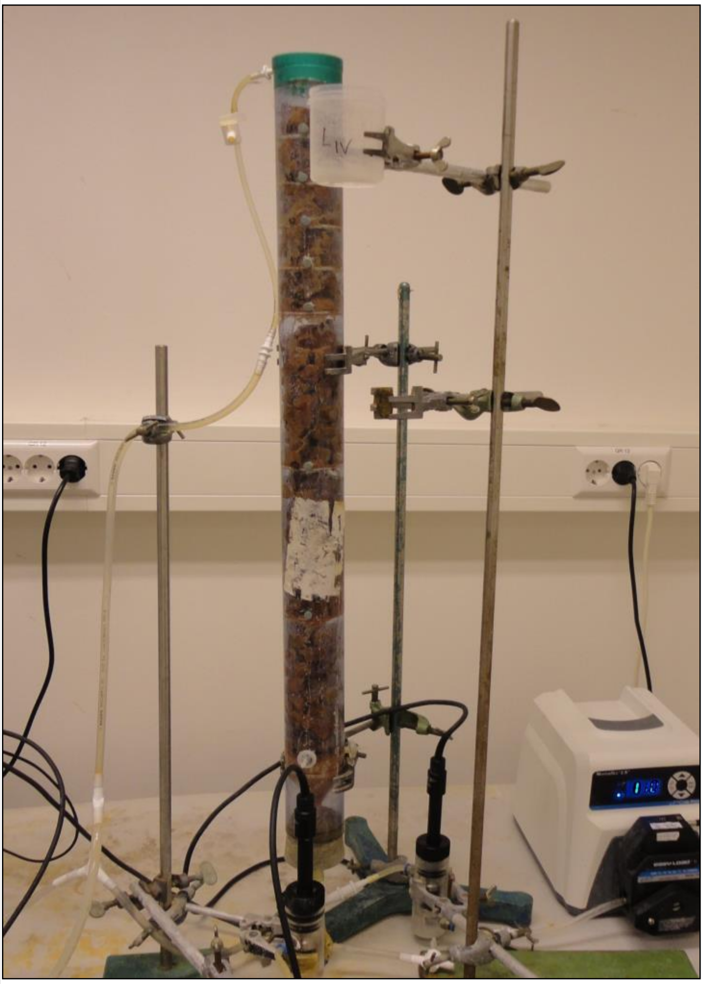

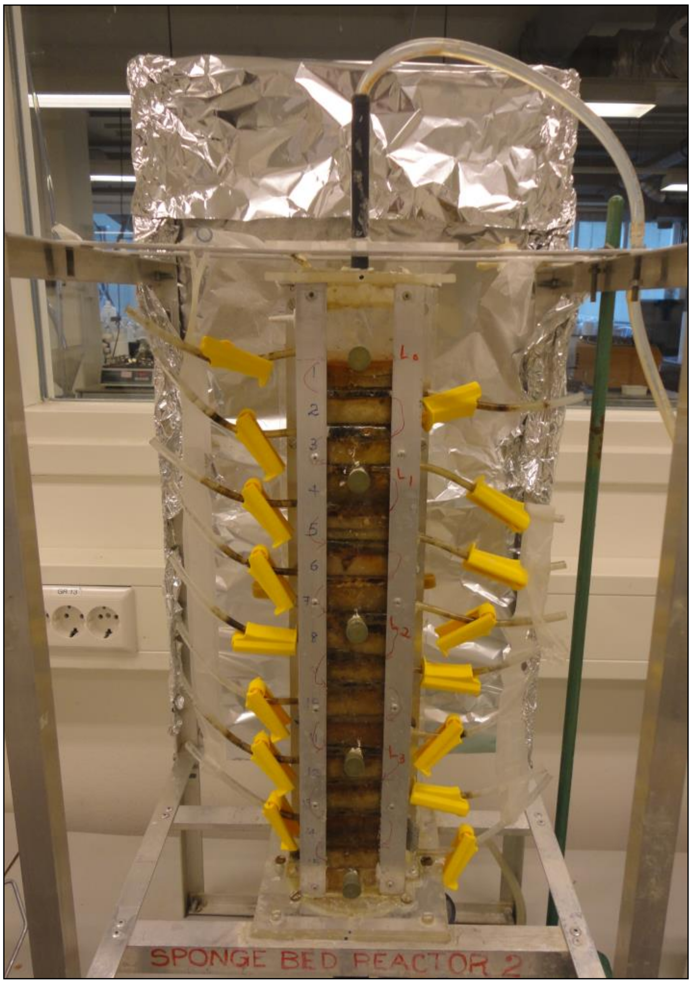

Assessment of the design and operational conditions on the performance of sponge-bed trickling filters for autotrophic nitrogen removal

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Abstract

Anammox processes have been strongly recommended as alternative for nutrient removal due their efficiency and reduction in cost-energy during the wastewater treatment. In this study, maximum removal efficiency was assessed through Anammox bacteria in sponge trickling filter operating without effluent recirculation, to test the potencial removal in terms of load that the system can achieve. In addition, the feasibility of using a new arrangement with sponge-based trickling filter for autotrophic nitrogen removal was also tested to assess the improviment in terms of removal efficience and reduce mainteinace issues detected in previous studies.

Two lab-scale sponge-bed trickling filters ($\text{SBTF}_{\text{ANAMMOX}}$ and $\text{SBTF}_{\text{CANON}}$) were run at same temperature (30℃) and different environment and operational conditions. The $\text{SBTF}_{\text{ANAMMOX}}$ reactor was filled with polyurethane cubes and operated under totally closed environment (anoxic condition) while the $\text{SBTF}_{\text{CANON}}$ was filled with polyurethane sponge with horizontal layers (zig-zag) operating in an open environment (aerobic condition) with a provision of open air inlets points around the reactor. Synthetic substrate was composed, respectively, by NH_4^+-N and NO_2^-- N with a concentration of 50 mg NH_4Cl/L and 50 mg$NaNO_3$/L, was used to feed the $\text{SBTF}_{\text{ANAMMOX}}$ reactors while the $\text{SBTF}_{\text{CANON}}$ was feed with synthetic substrate containing 100 mg NH_4Cl/L, mixed with micronutrient soluction.

The experiments were started with the acclimatization of both reactors during a period of 15 days, in that period, the Nitrogen Loading Rate (NLR) applied to the reactors were 2.7 ± 0.3 kg-N/m^3.d for the $\text{SBTF}_{\text{ANAMMOX}}$ and 1.6 kg-N/m^3.d for the $\text{SBTF}_{\text{CANON}}$ and it remained the same for both reactors in the phase I of the experiment. The conversion rates reached by the two systems were 2.0 ± 0.3 for $\text{SBTF}_{\text{ANAMMOX}}$ and 1.0-1.21 ± 0.2 kg-N/m^3.d. Between 103 to 175 days of operation , the $\text{SBTF}_{\text{ANAMMOX}}$ reactor showed a trend of stabilization, with removal efficiencies between 63 to 100% for NH_4^+-N, 60 to 70% for NO_2^--N and 64 to 75% for TN, corresponding to 77 ± 13.74 %, 65 ± 8.04 and 67.8 ± 3.60 % for NH_4^+-N, NO_2^- and TN removal, respectively. On the other hand, the $\text{SBTF}_{\text{CANON}}$ had a total nitrogen removal efficiency of 37.7 ± 7.9 %, in which the species in the system reached: an ammonium removal of 73.9 ± 8.5 %, nitrite production in 15.3 ± 6.3% and nitrate production of 25.9 ± 5.6%.

In phase II, the NLR for $\text{SBTF}_{\text{ANAMMOX}}$ was lowered to half of that one previously applied in the system, meaning 1.2 kg-N/m^3.d. The $\text{SBTF}_{\text{CANON}}$ did not undergo through phase II, as a result of malfunction over the phase I of the experiment. Due the favourable substrate concentration and consequently lower NLR, the Anammox bacteria was capable to increase the ammonium conversion by increasing the total removal efficiency in the $\text{SBTF}_{\text{ANAMMOX}}$ system. High removal efficiency was attained in this phase, in $\text{SBTF}_{\text{ANAMMOX}}$ with an average of 84.1 ± 5.3 and maximum nitrogen removal observed of 90%. The removal rate attained by the system in that phase was 1.1 ± 0.3 kg-N/m^3.d.

From this research can be concluded that the $\text{STBF}_{\text{ANAMMOX}}$ and $\text{SBTF}_{\text{CANON}}$ are promising technologies that could be applied to treat diluted anaerobically wastewater pre-treated in UASB system in development coutries.

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Abbreviations

AOB	Ammonia Oxidizing Bacteria
Anammox	ANerobic AMMonia OXidation
NOB	Nitrite Oxidizing Bacteria
TSS	Total Suspends Solids
VSS	Volatile Suspends Solids
BOD	Biochemical Oxygen Demand
DO	Dissolved Oxygen
CANON	Completely autotrophic nitrogen removal over nitrite
COD	Chemical Oxygen Demand
FA	Free Ammonia
SBTF	Sponge-Bed Trickling filter
SRT	Sludge Retention Time
HRT	Hydraulic Retention Time
NLR	Nitrogen Load Rate
UASB	Up-flow Anaerobic Sludge Blanket
NLR	Nitrogen Loading Rate
DHS	Down-flow Hanging Sponge
pH	Hydrogen-ion concentration
TN	Total nitrogen
DHS	Down Flow Having Sponge
HRT	Hydraulic retention time
NLR	Nitrogen Loading Rate
NOB	Nitrite Oxidazing Bacteria
N	Nitrogen
N_2	Dinitrogen
TF	Trickling filter
UASB	Up-flow Anaerobic Sludge Blanket

List of Symbols

℃	Degree Celsius
%	Percentage
±	Standard Deviation

CHAPTER 1

Introduction

This Chapter present an overview of Anammox process and brief step wide that the technology has reach. The comparison in terms of economic advantages between the conventional treatment process and Anammox technology are presented. The importance to remove nitrogen in the mainstream also is discussed.

1.1. Background

Nitrogen is the most abundant gas, with estimation of 78% in the atmosphere. Although it represents the majority, it is not available in large proportion for the living organisms, because they do not assimilate it as presented (dinitrogen gas (N_2)) without a conversion to ammonia. In water bodies we can find many forms of nitrogen: such as organic nitrogen (amino, acids, proteins and urea), ammonia-nitrogen (NH_4^+ and NH_3), nitrite-nitrogen (NO_2^-) nitrate-nitrogen (NO_3^-) and dissolved nitrogen gas (N_2) (Chin 2013).

In terms of nutrients removal, nitrogen is the most critical nutrient to remove from the wastewater in view of fact that can affect public health, contribute for eutrophication also has toxic impact (Reed et. al, 2014). Nitrogen compounds has a negative effect in public health, it can seed that water containing high concentration of nitrate lead to cause methamoglobinaemia in infants (Winkler, 1981).

Is possible to remove nitrogen from wastewater by e combination of nitrification and denitrification process. During nitrification ammonia is assimilated by Ammonia Oxidising Bacteria (AOB) and oxidised to Nitrite and later, Nitrite is oxidised to nitrate by Nitrite Oxidising Bacteria (NOB). By denitrification the nitrite and nitrate already produced are reduced in to nitrogen gas and water under anoxic condition (Winkler, 1981).

Actually the wastewater treatment technology has been focus of discussion in worldwide in such way to find the best and environmentally solution to treat it. The selection of appropriate technology also became a challenge in treatment pathway, because of cost involved with development and implementation of certain technology to remove nutrients. The biological process is common used in most of developed countries to treat wastewater with propose to achieve the environmental standard for discharge in water bodies.

Wastewater treatment via anaerobic process up-flow anaerobic sludge blanket (UASB) is an alternative to treat sewage, but that process has limitation to remove nutrients like phosphorus and nitrogen .The effluent coming from the UASB needs to be followed by a post-treatment process in order to comply with the required standard for discharge in the water body. However, the UASB is a great benefit and powerful technology in terms of energy production, it can produce biogas with high methane (CH_4) concentration that can be used to generate electricity.

To cover that limitation from the UASB reactor in such way to remove nutrients from the wastewater, simple and cheaper technology for side stream treatment was developed such, such as: SHARON® - Single High Rate Ammonium Removal over Nitrite, BABE® - Bio- Augmentation Batch Enhancement, CANON® - Completely Autotropic Nitrogen Removal Over Nitrate, Anammox® - ANerobic AMMonia Oxidation (Henze et. al., 2008).

Anammox process is a prosperous alternative for post-treatment system of anaerobic effluents. In the Anammox process is not necessary to achieve a full nitrification, the biological nitrogen removal takes in place in short-cut way over the nitrogen cycle (Henze et. al., 2008). The process converts ammonium directly into dinitrogen gas under anaerobic conditions with nitrite as an electron acceptor. In that conversion the Anammox bacteria use CO_2 as a carbon source like normal nitrifying bacteria (Henze et. al., 2008).

In terms of economic assessment the Anammox process can save up to 90% of operation cost, the reason of that is the the possibility to exclude external organic carbon source and aeration (Jetten et al., 2001; Chamchoi and Nitisoravut, 2007).

Since the discovery of Anammox bacteria, nowadays many full-scale treatment plant in worldwide are operating using Anammox technologiy as alternative to wastewater treatment attaining high conversion as showed in table bellow:

Table 1. 1 Diffent full-scale treatment plants using Anammox processes

Location	Plant volume (m^3)	Maximum conversion (kg-N/ day)
Rotterdan, NL	70	750
Lichtenvoorde, NL	100	150
Olburgen, NL	600	720
Pitsea, GB	240	408
Strass, AT	500	350

[Source: Van der Star, et al. (2007)]

To cover and boost the Anammox technology, cost-effective technologies have been deeply used to cultivate Anammox bacteria as cost-effective technologies to remove organic matter and nutrients from sewage in developing countries. One of those technologies are trickling filters, which essentially consist of a fixed-film aerobic treatment system that utilizes microorganisms attached to a medium such as: rocks, sand and plastic (Stediger, 2005) to remove organic matter from wastewater. In developing countries, it has been observed a trend of using trickling filters as a post-treatment of effluent from UASB reactors (Chernicharo et al., 2006; Kassab et al., 2010; Khanet et al., 2011).

Over the time, new and improved alternatives for packing media for wastewater treatment have been developed with the aim to decrease and enhance the efficiency of wastewater treatment. Recently research around the feasibility to use sponge-bed as packing medium in trickling filters has been studied in order to assess its contribuition on nutrients removal. A couple of years ago, lab-scale studies, to access the cultivation of Anammox bacteria in sponge-bed trickling filter to remove nitrogen from wastewater (Guardado,2013) and to test the nitrification under natural air conversion using a sponge-bed trickling filter us a support medium to govern the air diffusion in the system (Jayawardana, 2014) were done and shown good results. High biomass concentration was verified ensuring the biological reaction (as the Anammox bacteria and nitrifiers growh slowly) in the systems and good removal efficiency was attained in both systems, (83% and 54%) respectively.

The high biomass retion and simple operation are the main benefits given by the sponge-bed trickling filter (Chuang, et al 2006).

Studies made in Brasil using two trickling filters filled with also two differents packing medium *Rotopack* (plastic-based medium) and *Rotosponge* (sponge-based packing medium) to acess the performance of trickiling filters in the treatment of wastewater shown excellent performance in the average between 80-95% in which TF-*Rontosponge* was the kind of medium how contribute to attain high nitrogen removal (Almeida, et al., 2013).

In Japan organic removal and ammonium removal as well was tested using a (DHS) feed by synthetic wastewaters containing amminiun and COD. The reactor was operating at room temperature (25°C) without aeration; the system was able to attain a good removal efficiency, at the level of 90% for both ammonium and COD (Uemura, et al., 2012).

1.2. Problem Statement

The anammox process is a prosperous alternative for post-treatment system of anaerobic effluents. In the Anammox process is not necessary to achieve a full nitrification, the biological nitrogen removal takes in place in short-cut way over the nitrogen cycle (Henze et. al., 2008). The process converts ammonium directly into dinitrogen gas under anaerobic conditions with nitrite as an electron acceptor. In that conversion the Anammox bacteria use CO_2 as a carbon source like normal nitrifying bacteria (Henze et. al., 2008).

In terms of economic assessment the Anammox process can be save up to 90% of operation cost, the reason of that is the possibility to exclude external organic carbon source and aeration (Jetten et al., 2001; Chamchoi and Nitisoravut, 2007).

Anaerobic processes represent a good alternative for sludge reduction and saving of energy consumption. When the process is coupled with post-treatment has showed better results regarding removal of organic and inorganic matter as well, according Draai et al., (1992). Nutrient removal and organic matter removal in wastewater was tested in India using a down-flow hanging sponge (DHS); it show a good result in terms of efficiency removal for particulate (94%), organic matter (96%) and inorganic matter (93%) (Okudo et. al., 2015).

Nowadays (DHS) systems have been chosen as an alternative for post-treatment of anaerobic pre-treated sewage of municipal wastewater treatment because several advantages that it offer. DHS play an effective roll in assessing potential trends for nitrogen via Anammox process to achieve nitrogen removal giving advantages such as:

- High porosity allowing the entrapment of microorganisms and increasing the Sludge Retention Time (SRT);
- Lower Hydraulic Retention Time (HRT);
- High biomass concentration on the sponge medium;
- Small footprint required;
- No aeration is needed supply in reactor, therefore, lot energy is saved reducing the operation cost;
- Do not require a sophisticated operation and maintenance technology to handle the system.
- Good efficiency to remove organic and nitrogen, when coupled a post treatment of Up-flow Anaerobic Sludge Blanket (UASB) (Mahmoud et. al., 2011).

Recently, a study carried out by Jayawardana (2014), 54% of nitrogen removal was attained under aerobic conditions in a DHS under coexistence with nitrifies and Anammox bacteria as a result of environmental conditions provided in reactors. The large amount of biomass growing within the layers created clogging problems in the reactor and affected the removal efficiency.

The study taken over by Guardado (2013) was be done using sponge-bed trickling filters in closed system by recirculating the effluent and showed promising results, but a problem related with this system is the practical application in the field. For running a system with recirculation imply energy expenses for running the pumps.

In general, this research has aim to assess the higher capacity that the DHS working without effluent recirculation (previously study by Guardado (2013)) can reach at fixed flow rate and diffent NLR under anoxic conditions.
In addition, this research also has aim to assess the feasibility to improve maintainance issues and nitrogen removal via authotropic nitrogen removal using diferent configuration ‘zig-zag’of DHS (previously study by Jayawardana (2014)) in aerobic condition. At this stage of the research, enhancements in the design and operational strategies will be considered to further application of the technology as a post-treatment step of sewage treatment plants in developing countries.

1.3. Research questions

- What is the maximum nitrogen removal efficiency that an $\text{SBTF}_{\text{ANAMMOX}}$ system can reach without considering any internal recirculation compared to first generation SBTF system when treating typical diluted anaerobically pre-treated wastewater at similar NLR?
- What will be the impact of the 'zig-zag' configuration on the total nitrogen removal efficiency observed and to reduce previous operation and maintenance issues previously in CANON horizontal layers configuration?

1.4. Hypotheses

- In trickling filters filled with random sponge-based medium, significant nitrogen removal via Anammox metabolism can be achieved even without recirculation of the final effluent. The choice of a proper hydraulic loading rate is a sufficient operational strategy providing good wetting efficiency of the packing media.
- In trickling filters filled with horizontally layered sponge-based packing medium, the arrangement of the polyurethane sponge slabs in 'zig-zag' configuration provides operational enhancements and improvements on autotrophic nitrogen removal process over nitrite and decrease the operation and maintenance issues.

1.5. Research Objectives

1.5.1. Main Objective

The main objective of this research is to assess the performance of sponge-bed trickling filters (SBTF) reactors with differents configurations and operational conditions for autotrophic nitrogen removal, by using a synthetic substrate to simulating a realy wastewater at 30°C.

1.5.2. Specific objectives

- To assess the maximum efficiency for nitrogen removal via Anammox process in a SBTF system operating without recirculation of the final effluent;
- To assess the feasibility of using a new arrangement of the packing media (zig-zag) in a horizontally layered sponge-based trickling filter for autotrophic nitrogen removal over nitrite.
- To evaluate the performance of nitrogen removal via Anammox and CANON reactors at similar flow rate and different NLR, compared with the previous NLR applied; NLR of 2.09 ± 0.19 kg-N/m^3.d in $\text{SBTF}_{\text{ANAMMOX}}$ and 1.6 ± 0.1 kg-N/m^3.d in $\text{SBTF}_{\text{CANON}}$.

CHAPTER 2

Literature Review

This chapter support the relevant knowledge to develop the present research. A general review information about nitrogen removal is presented in with enfaces in Anammox as key microorganism responsible on removal process. The factors affecting Anammox bacteria growth, key factor and conditions to enhance the performance of CANON process, hydraulic operational parameters to affect the reactor removal efficiency over the operation are briefly explained in this chapter.

2.1. Trickling filters

Trickling filter is the oldest biofilm system developed to treat wastewater through biological process of both domestic and industrial wastewater. Typically tickling filter consist of kind of fixed-film in which microorganism's grow under substrates, specifically, rocks, sand and plastic (Stediger et. al., 2005). In general, the technology was developed to organic matter and ammonium removal. The reactor was conventionally filled with different packing material, such as rock and plastic on which the wastewater is evenly distributed (Metcalf & Eddy, 2003). Its ability to retain microorganisms in the biofilm for longer period is one of the most aspect the cultivation of slow-growers within the system.

Stenquist et al., (1974) cited in Metcalf & Eddy (2003) concluded that the full nitrification is reached inside of the trickiling filter if it work at lower organic loading and it occur only when BOD concentration decrease at least less than 15 mg/l.

The dosing rate of the influent inside of the trickiling filter also represent an important factor in the efficiency of the filter, it can decrease if the retention time is less at high dosing rate. In the trickling filter is fundamentaly to avoid the growth of snail within the nitrifying filter, because it can affect the nitrifying bacteria and the nitrification performance (Metcalf & Eddy, 2003).

2.2. Sponge-Bed Trickling filter and its application for nitrogen removal

Treatment of domestic and industrial waste water aerobically through activated sludge system have shown a good results in terms of nutrient removal, organic matter and nutrients as well. Treating waste water anaerobically via up-flow anaerobic sludge blanket (UASB) also can give a good performance and represent a reliable treatment option at temperatures above 20˚C (Joseph, 1992).

Over the past years different post treatments configuration coupled with UASB have been investigated, one of them is Down-flow Hanging Sponge (DHS). DHS was created between 1995 to 1997. Since that time many modifications was done in your own configuration. The DHS cube type was the first generation of DHS (DHS G1) with shaped cubes of 1.5 cm for each side and freely hung in the air. When texted the efficiency in terms of organics and nitrogenous compounds the system show good result whenever applied as a post treatment unit for UASB treating sewage (Mahmoud et. al., 2011).

But the (DHS G1) system show incompatibility on application in real-scale, because of your own configuration. Later, same different types of DHS were created to couple the limitation of DHS G1 and modified as well. Examples of same generations are: second generation (DHS G2) or curtain type DHS, third generation (DHS G3) or trickling filter DHS, fourth generation DHS, actually the fifth generation (DHS G5) created in 2004 and sixth generation (DHS G6) created in 2007 (Nurmiyanto, 2003). The figure below show the sequence evolution of the DHG generation.

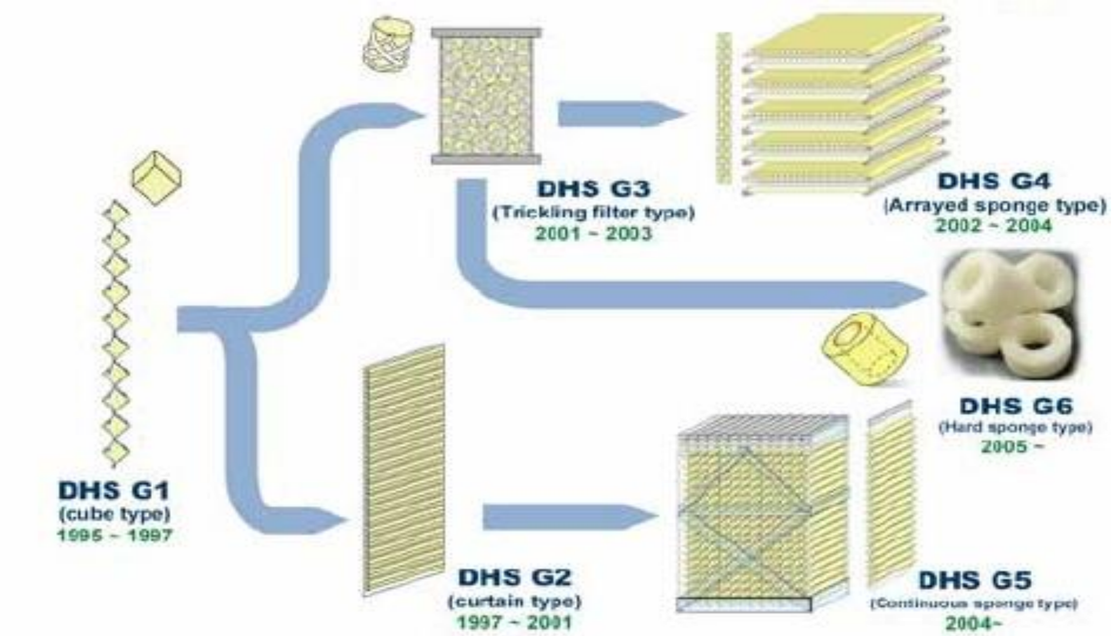


Figure 2. 1 The process evolution of DHS sponge.

Source:
https://www.academia.edu/7050396/Downflow_Hanging_Sponge_DHS_Reactor_for_treating_domestic_wastewater

Okudoa et. al,(2015) during their study in India, reported to achieve a 95% of nutrient removal good efficiency removal the achievement of 95% of nutrient when the UASB system was combined with DHS reactor applying a high HRT (13h) for the UASB and lower HRT (1.5 h) for the DHS reactor. Looking for the performance of DHS based on comparison of DHS and Trickling filter (TF) as reported by Chernicharo and Nascimento, (2001), they identified that the DHS operating with recirculation can give a good effluent quality in terms of nutrient, when operate at high.

The DHS system offer more advantage when compared with the activated sludge system, as Mahmoud et, al., (2010) and El-Gohary (2010) supported, the DHS reactor :

- Can concentrate high biomass.
- Provide high SRT.
- Lower HRT as high footprint.
- Large amount of organic matter is dissolved and provide a good efficiency in terms of nutrient removal.
- The reactor has ability to resist high shock load, do not need external air supply.

Combination between DHS technology and Anammox process can provide good results and provide high removal efficiency. A work done by Chuang et al., (2006) show a good efficiency removal (95%) for nitrogen in the range of 1.85 kgN/m3 when a closed DHS reactor was fed with synthetic wastewater via Anammox process.

2.3. Anaerobic Ammonia Oxidation (Anammox)

Anaerobic Ammonia Oxidation (Anammox) is a fully autotropic method for N-removal discovered in the 1980’s, but the studies for use Anammox bacteria with proposal to apply in wastewater treatment started fully in 1990’s (Mulder et al.,1995). The Anammox conversion is a kind of short-cut in nitrification cycle. The process convert ammonium directly into dinitrogen gas under anaerobic conditions with nitrite as an electron acceptor. In that conversion the Anammox bacteria use CO_2 as a carbon source like normal nitrifying bacteria (Henze, et. al., 2008).

Anammox bacteria is capable to form biofilms and granules as well, creating higher biomass concentration in reactor. The figure below show proper granule formed by Anammox bacteria.

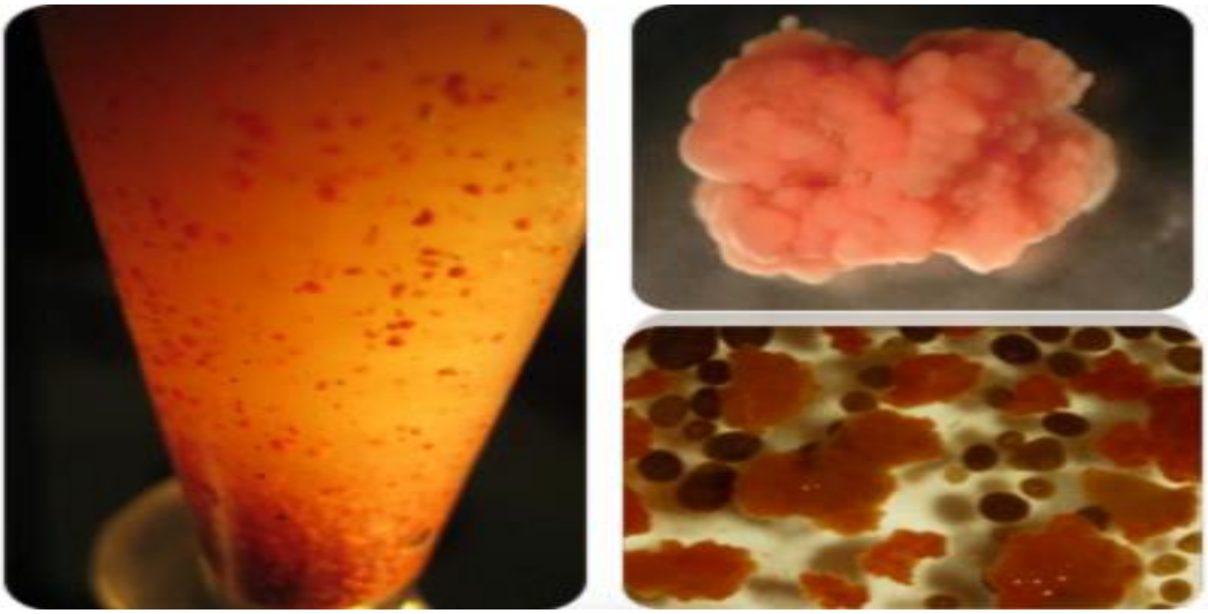


Figure 2. 2 Anammox bacteria granule

Source:http://www.aaees.org/images/e3swinners/e3competition-winners-2013gp-researchfigure05.jpg

One of the reason for use Anammox process in nitrogen removal is because the Anammox no need for external organic carbon source, only 50% of the ammonium has to be oxidized to nitrite and left part has is used by Anammox to convert the NO_2^- in dinitrogen gas, in this process there is a low biomass yield.
The main problem of the Anammox process is regarding with their organism, the Anammox bacteria has low growth rate (0.069 day^{-1}, Van de Graaf et al., 1996) citted on Henze, et. al., (2008). But their slow growth rate is no limitation towards high reactor capacity because they easily can achieve 5-10 $kgN/m^3.d$ allowing high biomass content in the reactor.

The Stoichiometry equation of Anammox process conversion of NH_4^+ and NO_2^- - to N_2 in Anammox reaction is show below (Strous et al., 1998):

$$\boldsymbol{NH_4^+ + 1,32\ NO_2 + 0,066\ HCO_3 + 0,13\ H^+ \ \rightarrow 1,02\ N_2 + 0,26\ NO_3^- + 2,03\ H_2O + 0,066\ CH_2O_{0,5}N_{0,15}} \qquad (1)$$

Since the conversion of ammonium take over HCO_3^- instead of carbon source under anoxic condition, the Anammox bacteria use disposable ammonium as an electron donor to convert nitrite to nitrogen gas (Van Dongen et, 2001).

The main function that make difference which the Anammox bacteria and the rest of microorganisms groups is the fact that the catabolic reactions in Anammox take place on an internal membrane, whereas for the other microorganisms it is happen out of their membranes (Henze, et al., 2008). The other is strong advantage in Anammox bacteria is the ability to product hydroxylamine has intermediate product over the reaction process, instead of N_20 (strong greenhouse gas) as intermediate.

According with (Henze, et al., 2008), Anammox as efficient process and effective cost compared with the traditional biological nitrogen removal process because it reduces around 60% of oxygen and alkalinity demands for nitrification, and it does not need external organic carbon source for denitrification. That figure meaning that the production greenhouse gas and biomass decrease a lot, therefore the process is environmentally safety.

The figure bellow show the kind of conversion that happens within nitrogen cycle metabolism.

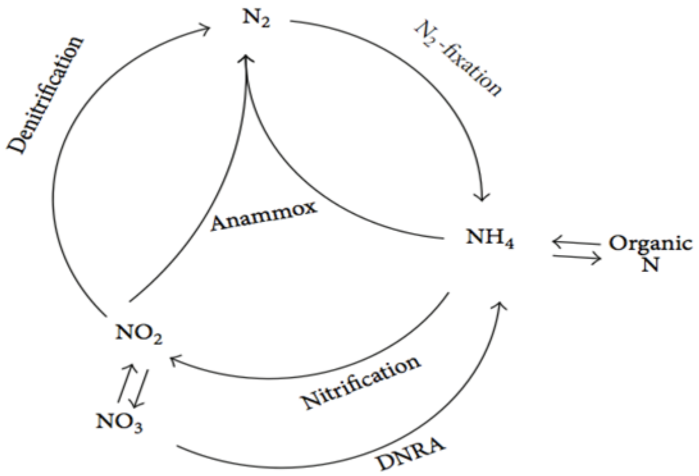


Figure 2. 3 The biological N-cycle combining nitrification, denitrification and Anammox process.
Source: https://The_nitrogen_cycle_Arrigo.png

Summary with examples of lac-scale and full-escale studies based in Anammox process for nitrogen removal from wastewater and different applications are showed in the figures below.

Table 1 Different examples of application of Anammox process and N-removal attained

Type of reactor	Innoculum	Total N-removal (%)	Reference
CSTF-1	Anammox granules and activated sludge	74 ± 5	Sánchez-Guillén et al.,(2015a)
CSTF-2	Anammox granules and activated sludge	78 ±4	Sánchez-Guillén et al.,(2015a)
Closed DHS	Anammox granules and activated sludge	60-95	Chuang et al 2008
Sequency batch reactor	Anammox granules and activated sludge	77	Zang et al., 2010

[Sánchez-Guillén et al.,(2015a)]

2.4 Partial nitrification (nitritation)

Nitrification is a biological process in which ammonium is oxidased to nitrite and then into nitrate in the presence of oxygen. The partial nitrification is the first step of nitrification process.

Partial nitrification is a promising process, where ammonia is converted to nitrite with presence of oxygen. The success of partial nitrification lay in to prevent the nitrate formation by oxidation of nitrite previously formed. The AOB is the bacteria responsible to take care of partial nitrification.
The stoichiometric equations for this process is given below:

$$NH_4^+ + 2HCO_3^- + 1.5O_2 \rightarrow NO_2^- + 3H_2O + 2\,CO_2 \qquad (2.1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad (2.2)$$

2.5 Completely Autotrophic Nitrogen Removal Over Nitrite (CANON)

CANON is an autotropic process used for treatment of wastewater that has a low carbon and nitrogen ratio (Zhang et. al., 2012) discovered recently. This process involve two group of autotropic bacteria, respectively: the AOB and Anammox bacteria. The AOB take advantage of oxygen to oxidize ammonium to nitrite. After the depletion of oxygen, nitrite with the remaining ammonium is converted to gaseous nitrogen by Anammox bacteria. The overall reaction is as follows:

$$NH_4^+ + 0.85O_2 \rightarrow 0.435N_2 + 0.13NO_3^- + 1.4H^+ + 1.3H_2O \qquad (3.1)$$

The CANON process is more economic when compared with nitrification and denitrification process, because it save 100% carbon source and 63% of oxygen (Third et al., 2001).
In other words the CANON process can be classified has a kind of partial nitritation-ANAMMOX compacted in one single reactor.

As Sánchez Guillén *et al.*, (2014a,b) has supported, to achieve partial nitritation-Anammox by CANON process a role of conditions has to be created, such as :

a) Temperature in the reactor has to be between 30-40˚C,
b) The influent reactor have to have high inorganic content and salinity concentration,
c) NOB has to be inhibited via Free Ammonia (FA),
d) DO concentration in the reactor should be monitored.

Okubo, et. al., (2015) identified that the Anammox conversion happen in lower section of sponge together with nitrification and denitrification and Jayawardana, (2014) was found out from your study that most the same approach.

2.5.1 Key conditions to enhance CANON process (DO/Ammonium)

The Dissolved oxygen (DO) and ammonium loading rate in the influent and inside of reactor has an important role in performance of CANON process, has a directly consequence on microbial activity inside of the CANON reactor. If indoor of reactor the DO is supplied excessively and ammonium limited or too low, the Anammox bacteria can be inhibited by drop down their activity in favour of AOB activity, they rapidly use the DO to oxidase the amount of ammonium available (Third et al. 2001).

The accumulated nitrite and relatively excess oxygen in bulk liquid stimulate the outgrowth of NOB over the ANAMMOX bacteria. Third et al., (2001) reported that lower limit of ammonium loading rate of 0.12 kg N/(d·m3) and 0.24 mg/L can stabilise the nitrogen removal.

The CANON process is a promising process for wastewater treatment, since now Zhang et.all (2012) has supported that the system has reached the highest range on Nitrogen Removal Rate (NRR) 1.44 kg-N/ m^3.d One of the conditions to maximize the efficiency of CANON in such way to get advantage of the Anammox activity inside of reactor is to control the ammonium concentration (Third et al. 2001).

Although Jayawardana (2014) record in your study the same deficit of N removal in a DHS reactor regarding to the coexistence with nitrifies and Anammox bacteria as a result of environmental conditions provided in reactor, the same found was not detected in Zhang et. al., (2012) study, it can conclude that the CANON process is efficient technology to remove nutrients from the waste water at environmental temperature and fed of inorganic carbon source.

Table 2. 1 Comparison of nitrogen loading rate in some CANON processes

Process	Sludge type	NLR (kg-N/ m^3.d)	NRR (kg-N/ m^3.d)	Reference
CANON	Floc	0.262	0.064	Sliekers et al., 2002
CANON	Floc	3.7	1.4	Sliekers et al., 2003
CANON	Biofilm	0.87	0.77	Gong et al., 2007
CANON	Granules	0.46	0.36	Vazques-Padin et al., 2009

[Source: Zheng, et. al. (2012)]

2.6 Factors affecting the Anammox growth

The table below show the main factors that influence the growth of Anammox bacteria as the respective implication of each factors in their metabolic activity. The data provided in the table was extracted from the respective publishers.

Table 2. 2 Factors affecting Anammox growth

Factor	Implication
Temperature	• The optimal temperature for Anammox growth is 37 ˚C. (Wang et al.,2004)
Dissolved oxygen inside of reactor	• In a DHS under the oxygen concentration below 0, 5 mg/l in the synthetic wastewater do not affect ammonia oxidation and is suitable to carry out partial nitritation. • As below is the dissolved oxygen in the reactor as fast Anammox bacteria can growth. Ammonium oxidisers take over higher nitrogen conversions at DO concentration below 1 mg/l and rapidly growth. (Hellinga, 1999),
pH	• The optimum pH for Anammox bacteria is in range between 7 to 8, without this range the activity of Anammox can be inhabited by or affecting you own growth or activity (Wang et al., 2004). • Inside of reactor the pH has to be alkaline instead of acidic, other ways the conversion of NH_4^+/NH_3^+ will be strongly affected .
Influent alkalinity/ammonium	• For achievement of stoichiometry proportion between ammonium 1 mol alkalinity per mol ammonium and nitrite is suitable for partial nitritation in Anammox process. (Strous et al.,998) • Under high concentration of ammonium the Anammox inhibit and decrease the metabolic activity. • Alkalinity, ammonia and dissolved oxygen (DO) concentrations is necessary for inhibiting nitratation and enhance partial nitritation and Anammox process (Van Dongen et al., 2001).
Nitrite	• Less nitrite concentration at less can affect the Anammox activity by stopping their conversion process. (Arroyo et al.,M 1998)

CHAPTER 3

Research approach and Methodology

This chapter present the methodology applied for the entire experiment. A full description regarding to the research approach is described, respectively: experimental apparatus, experimental phases and operation conditions, substrate supplied in the reactors, parameters monitored and measured, samples collection and storage. All analytical methods applied are also described in this chapter.

3.1 Research approach

Two lab-scale sponge-bed trickling filters were run at same temperature (30°C) and different environment and operational conditions. The $SBTF_{ANAMMOX}$ reactor was filled by sponge cubes and operated in totally closed environment (anoxic condition) while the $SBTF_{CANON}$ was filled by sponge with horizontal layers (zig-zag) operating in an open environment (aerobic condition) with a provision of open air inlets points around the reactor.

The experiments were started with acclimatization of both reactors during a period of 15 days, over this period a couple of samples were collected and analysed in order to control de substrate concentration. In that period, the Nitrogen Loading Rate (NLR) applied in the reactors were 2.7 kg-N/m^3.d for the $SBTF_{ANAMMOX}$ and 1.6 kg-N/m^3.d for the $SBTF_{CANON}$ and it remained the same for both reactors in the phase I of the experiment.

In the phase II, the NLR for $SBTF_{ANAMMOX}$ was lowered to half of previously applied in the system, meaning 1.2 kg-N/m^3.d, since the $SBTF_{CANON}$ did not experience the phase II, as result of malfunction observed over the phase I of the experiment. This aspect will be futher discussed in chapter 6.

To assess the maximum removal efficiency of $SBTF_{ANAMMOX}$ reactor, samples for each reactor compartment including influent and effluent were taken and analysed its concentrations of NH_4^+-N, NO_2^--N and NO_3^--N 2 -3 times a week. The TSS and VSS were measured periodically. In addition, the pH in each reactor compartments ($SBTF_{ANAMMOX}$), DO (from influent Demi water) and alkalinity (from the effluent of reactor) were monitored.

To assess the feasibility of using a new arrangement of the packing media in a horizontally layered sponge-based trickling filter (zig-zag) for nitrogen removal in $SBTF_{CANON}$, samples from the influent and effluent were collected and analysed theirs concentrations of NH_4^+-N, NO_2^--N and NO_3^--N in the same frequency as

SBTFANAMMOX reactor. The TSS and VSS and DO were monitored as in the SBTFANAMMOX with exception of pH, in which was monitored just in the influent and effluent of reactor.

After finish the period of sample analysed and reactor monitoring in both reactor, the results were analysed and discussed with aim to make conclusion and address recommendation for the present research.

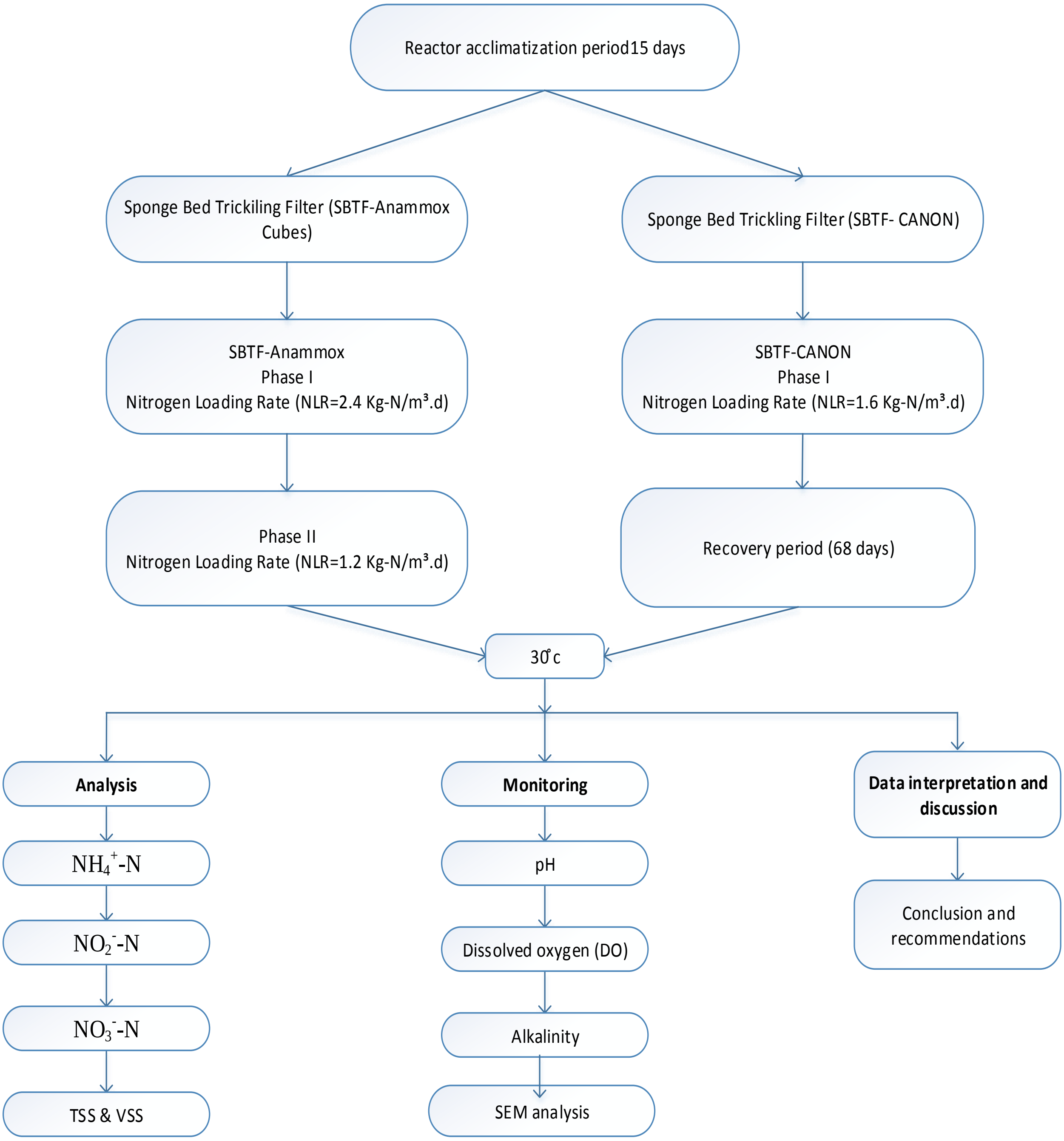


Figure 3. 1 General research overview

3.2 Experimental apparatus

For this study**,** two previously constructed reactors and run during 2 years (SBTF$_{\text{ANAMMOX}}$ and SBTF$_{\text{CANON}}$) were used to do the laboratory experiments. The SBTF$_{\text{ANAMMOX}}$ with 82.8 cm height, a total sponge volume of 1.3 cm^3, was previously filled by sponge randomly distributed in four compartments; each compartment was composed of 95 polyurethane sponges. The compartment 1 was divided in 5 sub-compartments. Each sponge had a cubic configuration, with cubes size of (1.5×1.5×1.5 cm).

The SBTF$_{\text{CANON}}$ with total sponge volume of 873 cm^3, total reactor height of 46 cm, number of sponge layers of 15 with thickness of 1.5 cm and sponge sheet size of (6.75×5.75), representing an occupation volume faction of sponge medium of 58% .The thickness of sponge was fixed as 1.5 cm, in with a separation space between sponge layers was fixed in 1 cm.

In terms of oxygen availability, the SBTF$_{\text{ANAMMOX}}$ was operating in anoxic conditions. To ensure the anoxic condition and prevent oxygen intrusion inside of system, a nitrogen gas was periodically supplied in the influent demineralised water and influent substrates. The SBTF$_{\text{CANON}}$ was set in order to operate in aerobic conditions, in which the provided opening air layers was remained open during the acclimatization and phase I of their experiment period, with aim to provide oxygen in the reactor in such way to attain nitritation by Ammonia oxidizing organisms (AOBs).

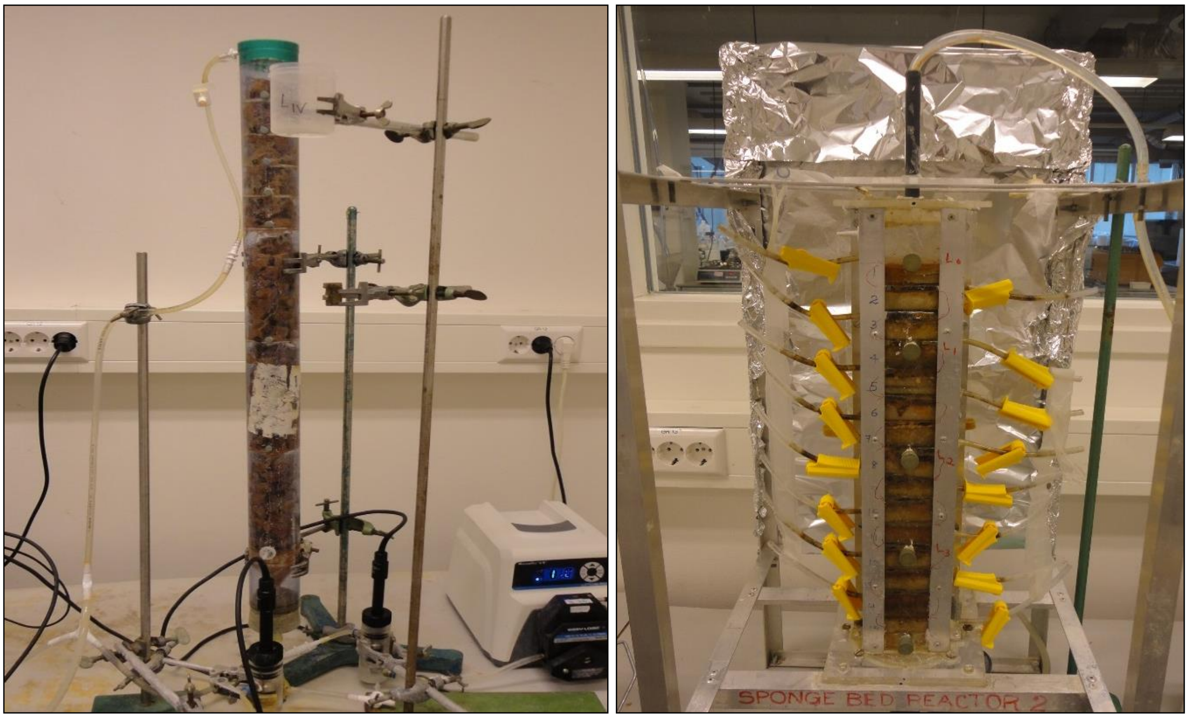


Figure 3. 2 Experimental set up : SBTF$_{\text{ANAMMOX}}$ reactor (left side) ; SBTF$_{\text{CANON}}$ reactor (right side).

3.3 Synthetic Substrate

Both reactors were fed from the top with synthetic substrate to simulate the wastewater composition. For $SBTF_{ANAMMOX}$ two separate solutions containing NH_4^+-N and NO_2^-- N, respectively were prepared with a concentration of 50 mg/l and fed in reactor while for $SBTF_{CANON}$ the solution feed in reactor was only composed of NH_4^+-N with a concentration of 100 mg/l coupled with a micronutrient solution (trace element).

In addition $KHCO_3$ and $CaCl_2$ were supplied in substrate of both reactor in order to ensure provide a proper alkalinity in the systems and enhance growth of nitrifies.

Table 3. 1 Composition of synthetic wastewater substrate for $SBTF_{ANAMMOX}$ reactor

Reactor	**Chemical Compounds**	**Conc.(mg/l)**
($SBTF_{ANAMMOX}$)	NH_4Cl (Ammonium Chloride)	2.9828
	KH_2PO_4 (Monopotassium Phosphate)	0.3906
	$CaCl_2 . 2 H_2O$ (Calcium Chloride Dihydrate)	4.6875
	$MgSO_4 . 7 H_2O$ (Magnesium Sulphate Heptahydrate)	0.7700
For NO_2^- (trace element solution)	EDTA Na salt (Ethylenediaminetetraacetic acid dipotassium magnesium salt)	0.1786
	$FeSO_4 . 7 H_2O$ (Iron Sulphate Heptahydrate)	0.1786
	Trace element solution *	19.53 ml/l
	$KHCO_3$ (Potassium Hydrogen Carbonate)	19.5313
	$NaNO_2$ (Sodium Nitrite)	3.8505

[Source: *Van de Graaf, et al.1996*]

Table 3. 2 Composition of synthetic wastewater substrate for SBTF $_{CANON}$ reactor

Reactor	Chemical Compounds	Conc.(mg/l)
($SBTF_{CANON}$)	NH_4Cl (Ammonium Chloride)	5.9656
	KH_2PO_4 (Monopotassium Phosphate)	0.3906
	$CaCl_2 . 2 H_2O$ (Calcium Chloride Dihydrate)	4.6875
	$MgSO_4 . 7 H_2O$ (Magnesium Sulphate Heptahydrate)	0.7700
Trace element without NO_2	EDTA Na salt (Ethylenediaminetetraacetic acid dipotassium magnesium salt)	0.1786
	$FeSO_4 . 7 H_2O$ (Iron sulphate Heptahydrate)	0.1786
	Trace element solution*	19.53
	$KHCO_3$ (Potassium Hydrogen Carbonate)	19.5313

[Source: *Van de Graaf, et al.1996*]

Table 3. 3 Composition of trace element solution (micronutrients).

Reagents	Conc. (g/l)
EDTA Mg salt (Ethylenediaminetetraacetic acid dipotassium magnesium salt)	15.000
$ZnSO_4 . 7 H_2O$ (Zinc sulphate heptahydrate)	0.4300
$CoCl_2 . 6 H_2O$ (Cobalt Chloride Hexahydrate)	0.2400
$MnCl_2 . 4 H_2O$ (Manganese chloride tetrahydrate)	0.9900
$CuSO_4 . 5 H_2O$ (Copper sulphate pentahydrate)	0.2500
$Na_2MoO_4 . 2 H_2O$ (Sodium molybdate(VI) dehydrate)	0.2200
$NiCl_2 . 6 H_2O$ (Nickelous Chloride Hexahydrate)	0.1900
Na_2SeO_4 (Sodium Selenate)	0.1076
H_3BO_3 (Boric Acid)	0.0140
$Na_2WO_4 . 2 H_2O$ (Sodium Tungstate dehydrate)	0.0500

* Composition of Trace element solution presented in table 3

3.4 Experimental phase and operational conditions

3.4.1 Experimental phase I for SBTF$_{ANAMMOX}$

The two reactors that were used to do the experiments, were already operated for more than 2 years, which means was not need to do start-up of both reactors. A period of 15 days was observed for reactors acclimatization, before start the phase I of its experiments. In the phase I, both reactors were operating at full time (24 hours per day) and run at temperature of 30 ˚C.

After reactor acclimatization, the reactor was run and tested for a period of 90 days. During the experiment period the reactor was fed by synthetic wastewater from the influent composed by a mixture of ammonia and nitrite and mixture then with demineralised water and measurement of nitrogen species were made 2 -3 times a week in order to achieve the stability of reactor in terms of removal efficiency, in order to change to phase II of the experiment.

As a result of a precipitate formation in the reactor, in the middle of this experiment phase the alkalinity was changed in the system from around 650 mg/L$CaCO_3$ to around 200 mg/L $CaCO_3$.

3.4.2 Experimental phase II for SBTF$_{ANAMMOX}$

During this phase, the NLR was lowered by half, meaning from 2.4 kg-N/m3.d to 1.2 kg-N/m3.d, to simulate the diluted wastewater and assess the removal efficiency in terms of nitrogen removal. The substrates, respectively NH_4^+-N and NO_2^--N were diluted and decreased their concentrations from 50 mg/l to 25 mg/l. The Hydraulic Loading Rate (HLR) and remain other parameter like: flow rate, Hidraulic Retention Time (HRT), and temperature were kept the same as in the phase I. The entire operation period in this phase (phase II) was 26 days.

A period of one week was stablished as acclimatization of reactor. After the acclimatization period, the reactor was showing a trend of stabilization and a removal of almost 90 % for NH_4^+-N and 87% for NO_2^--N. Up to the end of phase II experiment a maximum performance in terms of NH_4^+ -N and NO_2^--N were reached as 100% and 97% respectively.

In the entire experiment, the reactor was operated as a closed reactor (anoxic condition) without effluent wastewater recirculation according to the operation conditions showed in table 3.4, followed by the reactor scheme displayed in the figure 3.4.

Table 3. 4 Operation conditions of ($\text{SBTF}_{\text{ANAMMOX}}$).

Parameter	Unit	($\text{SBTF}_{\text{ANAMMOX}}$)	($\text{SBTF}_{\text{ANAMMOX}}$)
		Phase I	Phase II
Influent flow rate	L/d	24	24
Influent NH_4^+	mg-N/L	50	25
Influent NO_2^-	mg-N/L	50	25
Nitrogen Loading Rate (NLR)	kg-N/m^3.d	2.7	1.2
Nominal Hydraulic Retention Time (HRT)	h	1.0	1.0
Hydraulic Loading Rate (HLR)	m^3/m^2.d	10.1	10.1
Temperature	˚ C	30	30
Duration	days	90	26

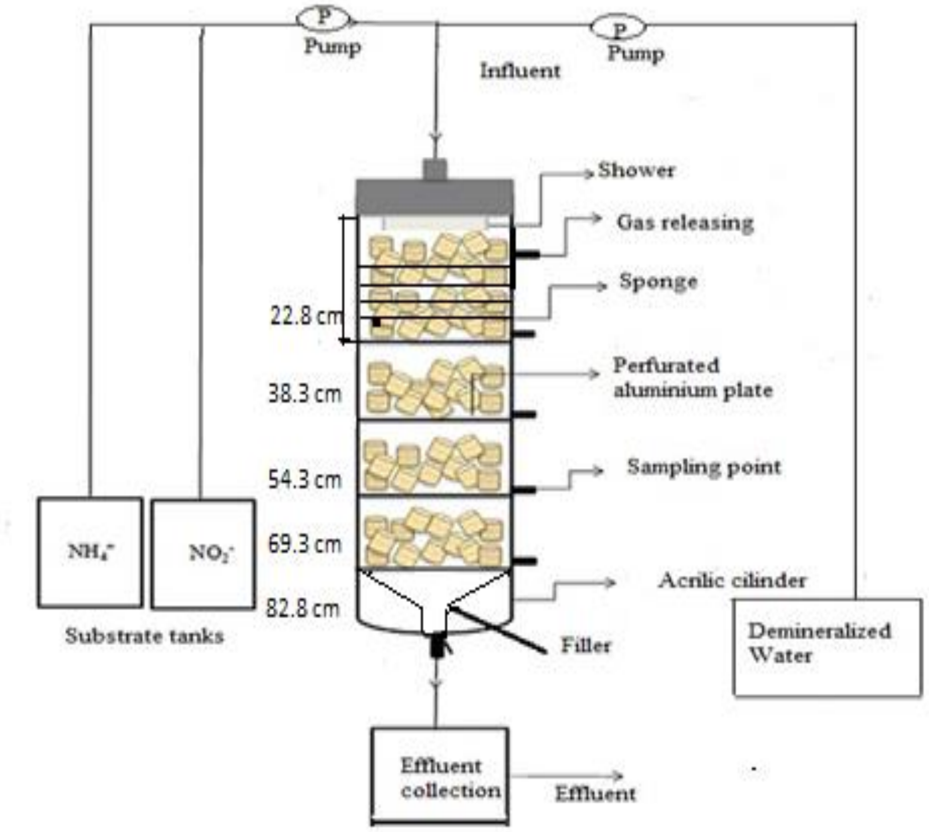


Figure 3.4. 1 $\text{SBTF}_{\text{ANAMMOX}}$ reactor scheme

[Source:Sánchez-Guillén et al.,(2015a)]

3.4.3 Experiment phases I for ($SBTF_{CANON}$)

The acclimatization period applied in $SBTF_{CANON}$ was the same as $SBTF_{ANAMMOX}$. After reactor acclimatization, the reactor was run and tested for a period of 90 days. During the experiment period the reactor was feed wth synthetic wastewater from the influent composed by a mixture of ammonia and trace element (micronutrient) and mixed them with demineralised water.

The nitrogen species were measured 2 -3 times a week in order to achieve the stability of reactor in terms of removal efficiency and test the feasibility of using a new arrangement of the packing media in a horizontally layered sponge-based trickling filter (zig-zag), aim to avoid clogging and precipitation in the upper part of sponges. In order to ensure nitritation in the system all the air points were kept opened, since the acclimatization period up to the end of the experiment period. After 22 days of operation, the reactor showed a reduction in terms of performance. A period of 69 days was provided to see if recovery would be achieved.Since the not significant recovery was observed the reactor was switched off.

The $SBTF_{CANOIN}$ was operated in a completely open environment (aerobic condition) with air inlet points located in the sides of reactor. The air inlet points were full opened in the entire experiment phase. The table 3.5 and figure 3.5 bellow, show the operation conditions imposed in the system, followed by their scheme, respectively.

Table 3.5 1 Operational conditions of ($SBTF_{CANON}$).

Parameter	Unit	($SBTF_{CANON}$)
		Phase I
Influent flow rate	L/d	16
Air inputs to sponge layers		Totally opened
Influent NH_4^+	mg-N/L	100
NLR	kg-N/m^3.d	1.6
Nominal Hydraulic Retention Time (HRT)	h	1.5
Hydraulic Loading Rate (HLR)	m^3/m^2.d	3.2
Temperature	˚ C	30
Duration	days	90

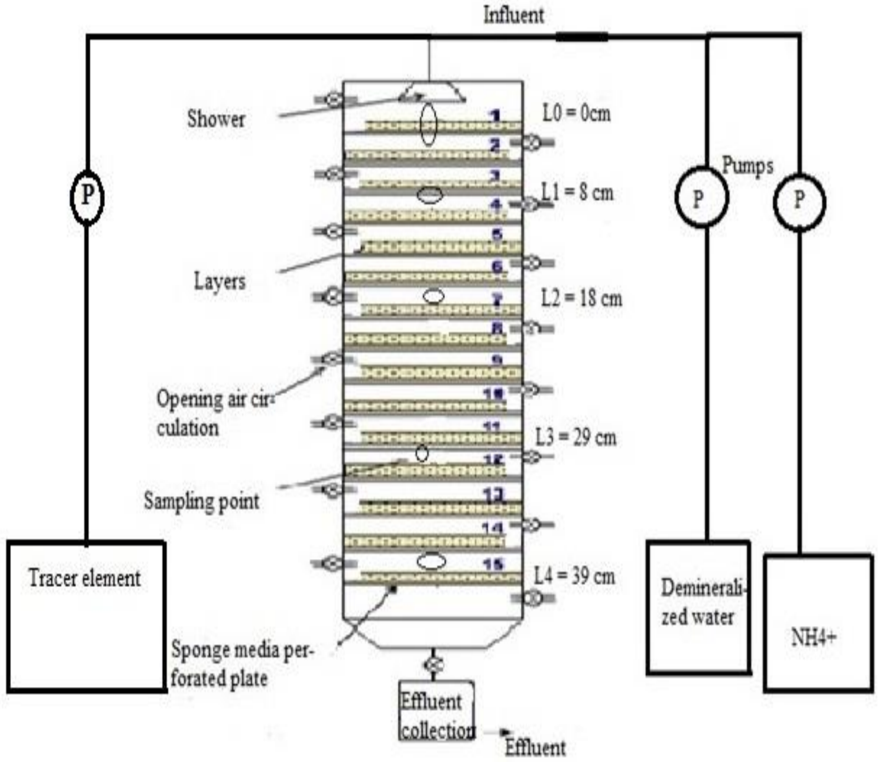


Figure 3.4. 2 SBTF$_{CANON}$ reactor scheme

[Source:Sánchez-Guillén et al.,(2015ac)]

3.5 Scanning Electron Microscopy (SEM) analysis

SEM image analyse were carried out in some sponges taken from SBTF$_{CANON}$ reactor to identify sponges' morphology and its respective composition. For this analysis, a piece of sponge sample with approximately 5 mm was cut (from the top, middle and bottom of reactor) after previously stored for 1 month at 5oC in NO_3-N solution (concentration of 50 mg-N/lL). To select the samples ensure a representative sponge composition, all the sponges were previously observed in with the use of optical microscopy at an image resolution of 50 µm. Before optical microscopically observation. To improve the quality of the visualization, the sponge samples were slightly dried, using an absorbent clean tissue to decrease the presence of water within the pores and increase the image visibility.

3.6 Sampling

The samples were collected during 4 months, (starting from November, 03, 2015 to February 29, 2016) and were analysed two times a week. At the beginning a total of twelve (12) samples were collected in every sample days. For SBTF$_{ANAMMOX}$ reactor an amount of ten (10) samples were collected, eight (8) for their correspondent layers, remain two (2) from influent and effluent of reactor. In the (SBTF$_{CANON}$) number of two (2) samples were collected, from the influent and effluent, respectively.

The samples for NH_4^+-N and NO_2^-- N, $NO3^-$-N and pH measurement, were collected by using a syringe with a respectively needle. An amount of 10 ml of sample was used to measure and monitor those parameters. All the samples were previously filtered using cronus 25mm cellulose acetate sterile syringe filter 0.2µm, before preparation. For the alkalinity and TSS and VSS measurements, 50 and 250 ml were collected and used to measure those parameters, respectively.

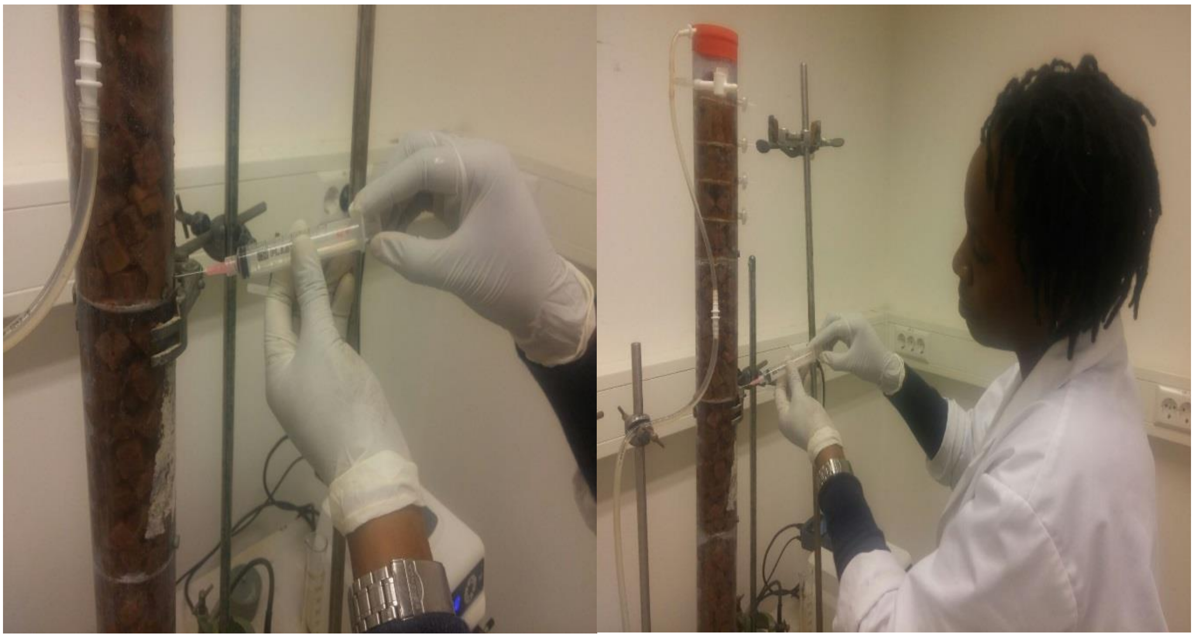


Figure 3.6. 1 Sample collection in SBTF$_{ANAMMOX}$ reactor compartments

(a)

Figure 3.7.2 a) and b) Samples filtration

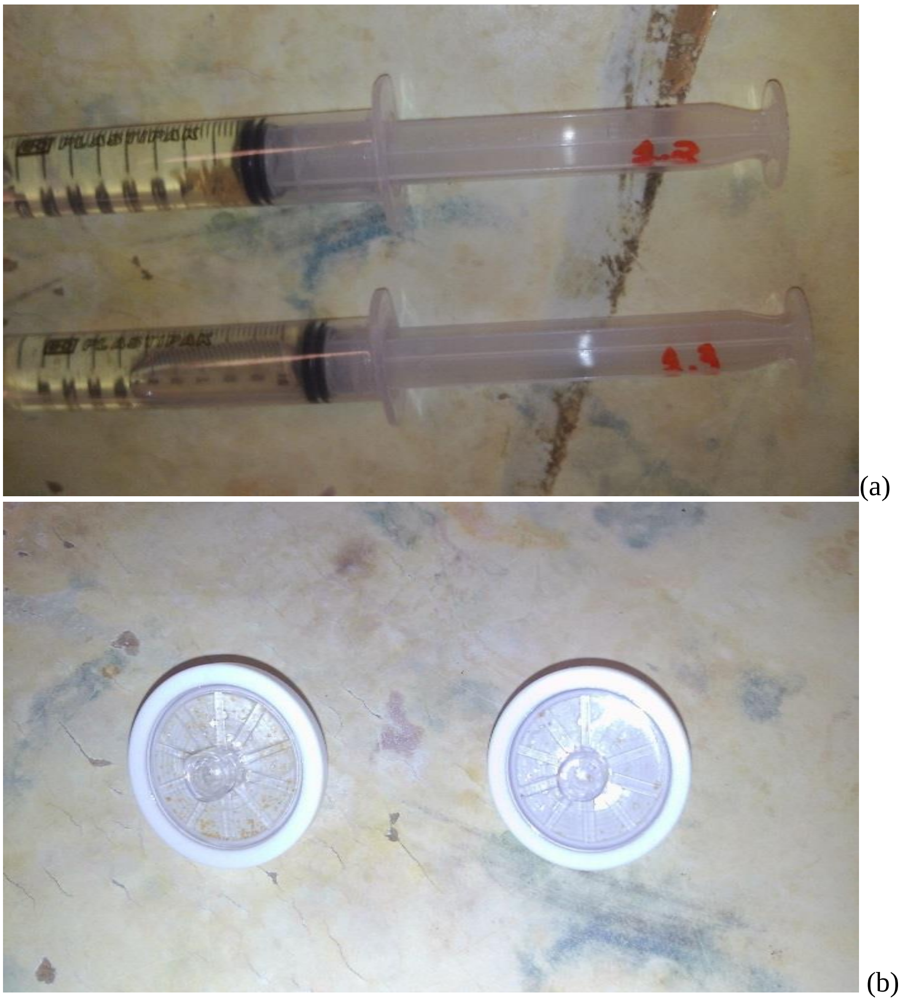


Figure 3.8 1 Biomass collected in $\mathrm{SBTF_{ANAMMOX}}$ reactor during sampling (syringe). b) Biomass retained in the filters after filtration (left side) $\mathrm{SBTF_{ANAMMOX}}$ and $\mathrm{SBTF_{CANON}}$ (right side).

3.7 Analytical methods

3.7.1 Nitrogen measurements

For the experiment the total inorganic nitrogen was fixed as sum of NH4$^+$-N, NO_2^--N, NO_3^--N, in which their removal efficiency was computed based on the influent and effluent wastewater concentration of reactors. The same analytical methods were applied in both reactors ($SBTF_{ANAMMOX}$ and $SBTF_{CANON}$). The method for NH_4^+-N measurement are described in Dutch Standard NEN 6472. The remaining nitrogen species (NO_2^- -N and NO_3^--N) were measured using Ion Chromatography, as described in their respective manual. For nitrogen analysis in the $SBTF_{ANAMMOX}$ reactor, 10 ml of samples were collected through a syringe with a needle, in each reactor compartment while for the $SBTF_{CANON}$, the samples were collected directly from the taps installed in the influent and effluent of reactor.

3.7.1.1 Ammonium

Ammonium was determined using ultraviolet Spectrophotometry as described in Dutch Standard NEN 6472. Plastic cuvettes were used to read the absorbance, with a Waveland fixed in 655nm. The samples concentration was varying in the range between 0-40 mg NH_4^+-N/20 ml according to the calibration curve. A small amount of 0.1 of sample was pipetted and diluted with a 16.7 ml of demineralized water followed by addition of 1.6 ml of salicylate and 1.6 ml of dichloroisocyanurate reagents. Before samples reading in the Spectrophotometer, a waiting period between 1 to 3 hours was observed to enhance the reagents mixing. (**figure 3.9**)

3.7.1.2 Nitrite and Nitrate

For nitrite (NO_2^-- N) and nitrate (NO_3^-- N) an Ion Chromatography machine was used. Plastic cuvettes were used to store and read samples after preparation. A amount of 1 ml of samples were diluted in 9ml end volume of MilliQ water with a concentration range between 0.5 to 10 mg NO_2^-- N/10ml as in the calibration curve provided for NO_2^--N. Since the Ion Chromatography machine was not automatically calibrated for NO_2^--N measurement, a series standard solutions of 0.2, 0.4, 1.0, 1.6, 2.0 and 3.2 mg/l were provided to determine the calibration curve and read the sample concentration. A period of 13 minutes was the reading period within samples inside of machine.(**figure 3.10 a and b**)

3.7.2 pH and DO

For the $SBTF_{ANAMMOX}$ reactor, the pH was measured in the different reactor compartments including influent and effluent. In the $SBTF_{CANON}$ reactor the pH was measured in the influent and effluent of reactor; while the DO was measured only in influent Demineralised water for both reactors. For these measurement a pH meter and a DO meter (WTW, pH 323), (WTW, LDO 340) was used. At the beginning of experiment (acclimatization period), the pH was measured in the influent and effluent of reactors. After reactor stabilization ($SBTF_{ANAMMOX}$), since day 152 the pH was measured along the reactor profile including influent and effluent. To prevent oxygen intrusion in the systems, a scavenger solution was provided and nitrogen gas was often supplied in the Demineralised water as well in the reactors substrates.

3.7.3 Alkalinity

A titrimetry machine was used to measure the alkalinity. Fixed amount of 50 ml of sample was collected from reactors in every measurement and neutralised with concentrated HCl, at molarity in the range between 0.0195 to 0.0206. At the beginning of experiment, the alkalinity in $SBTF_{ANAMMOX}$ reactor was fixed as 650 mg $CaCO_3$/L and $SBTF_{CANON}$ around 700 mg $CaCO_3$/L. This value was changed in the middle of experiment to 200 mg $CaCO_3$ /L for $SBTF_{ANAMMOX}$ and 300 mg $CaCO_3$/L for $SBTF_{CANON}$.

3.7.4 Total Suspended Solids (TSS and VSS)

The TSS and VSS were measured based on dry method as described in Kruis (2015) Laboratory manual. An amount of 250 ml of effluent wastewater was taken as sample for both reactors to measure the total concentration of suspended solids and volatile suspended solids in the effluent reactors. Periodically, the effluent wastewater from the reactors were well mixed and collected in order to have a representative sample for analysis. (**figure 3.11 a and b**)

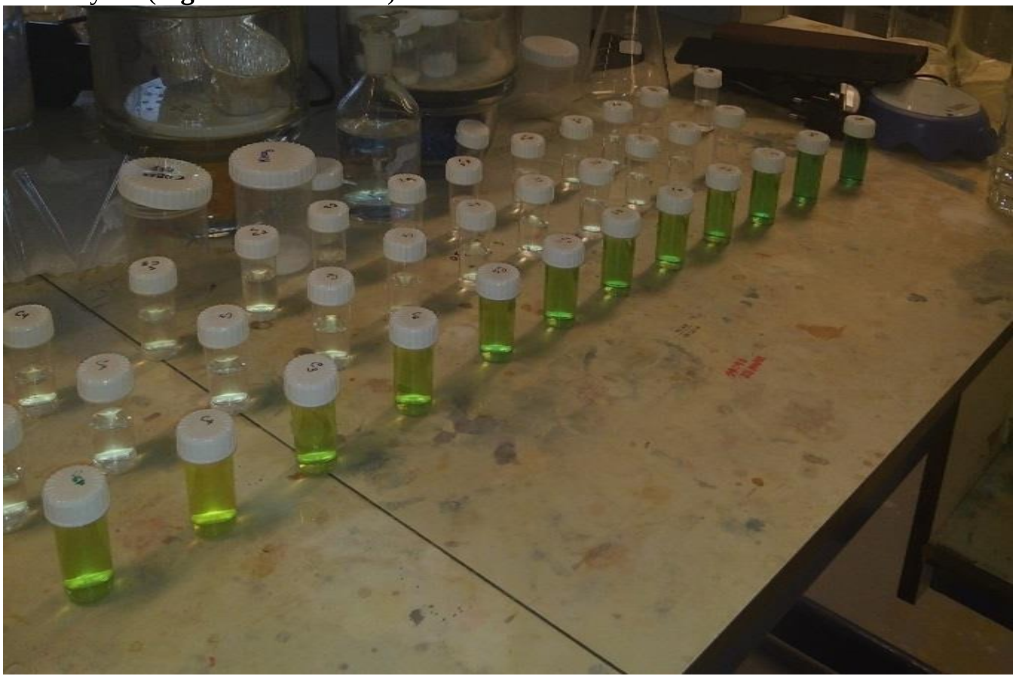


Figure 3.9. 1 Ammonia measurement based in Spectrophotometric method

(a)

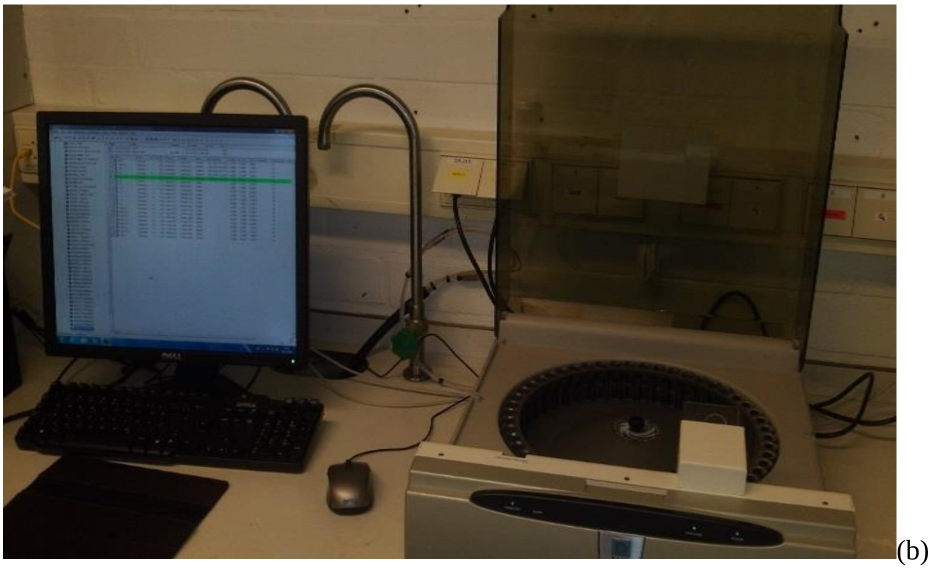


Figure 10. 1 Sample for nitrite and nitrate measurement. b) Ion Chromatography machine.

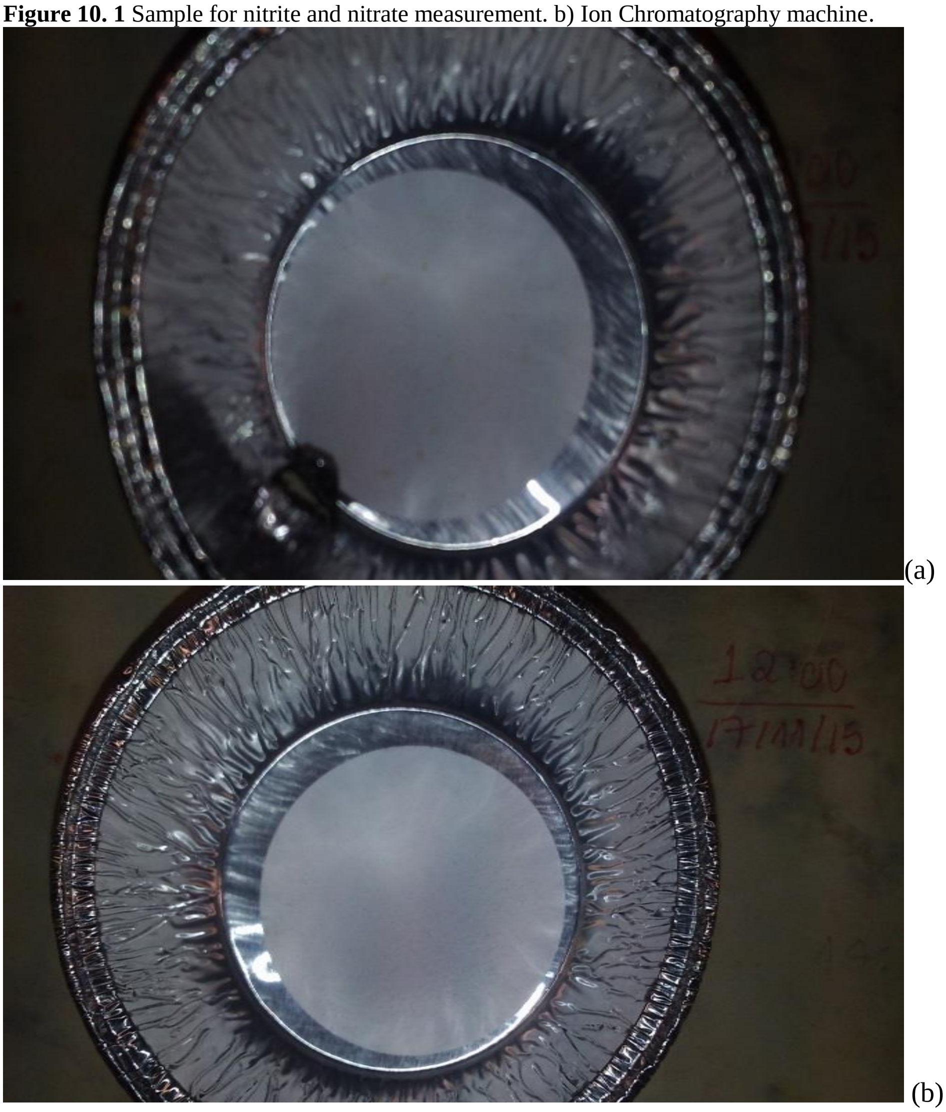


Figure 3.11. 1 Biomass retained in the filters before TSS and VSS measurement. a) Sample from $\mathrm{SBTF_{ANAMMOX}}$ reactor. b) Sample from $\mathrm{SBTF_{CANON}}$ reactor.

CHAPTER 4

Results

This chapter will provide a description of the performance and results of both reactors (SBTF-Anammox and SBTF- CANON) in terms of Anammox bacteria stoichiometry including the ratios of species analysed (NH_4^+, NO_2^-, NO_3^-), and nitrogen balance. The results of microbiology analysis (SEM analyse) will be presented as a support of the $SBTF_{CANON}$ reactor performance.

4.1 Nitrogen conversions

In this study, the performance of both reactors were assessed based on the results from the nitrogen measurements. For $SBTF_{ANAMMOX}$ reactor the results regarding the nitrogen conversion over the long-term and vertical profile as well are shown in the following graphs (**figure 4.1** and **figure 4.2),** respectively. The nitrogen conversion for $SBTF_{CANON}$ reactor was analysed based on the results from long term profile (influent and effluent of reactor), since the reactor do not experience completely the phase I of the experiment.

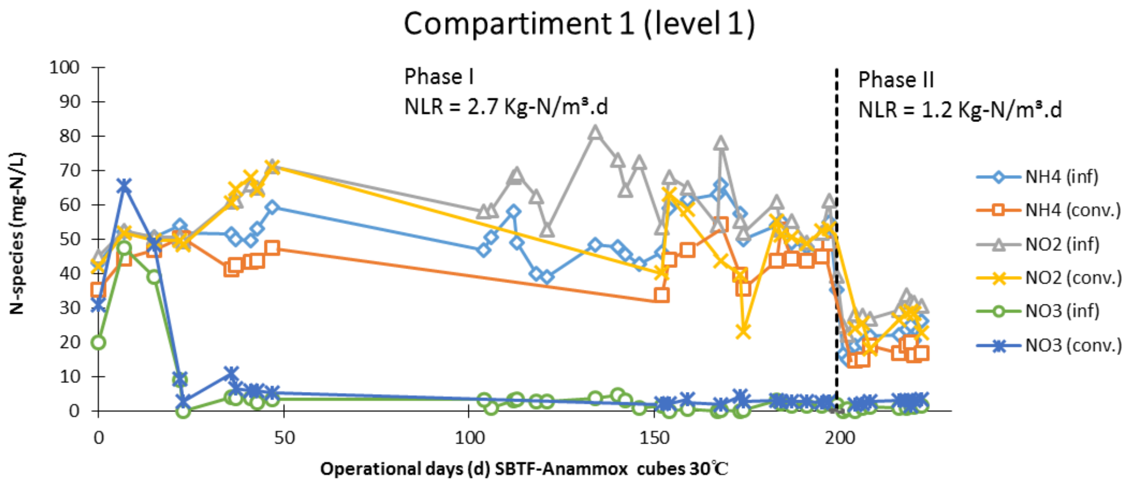
4.1.1 Nitrogen conversion long-term profile in $SBTF_{ANAMMOX}$

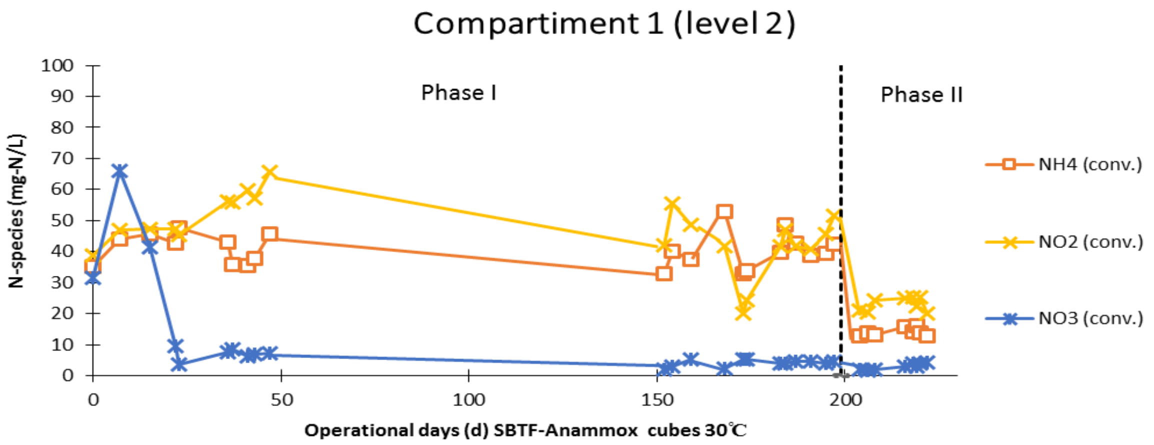
Phase I and II

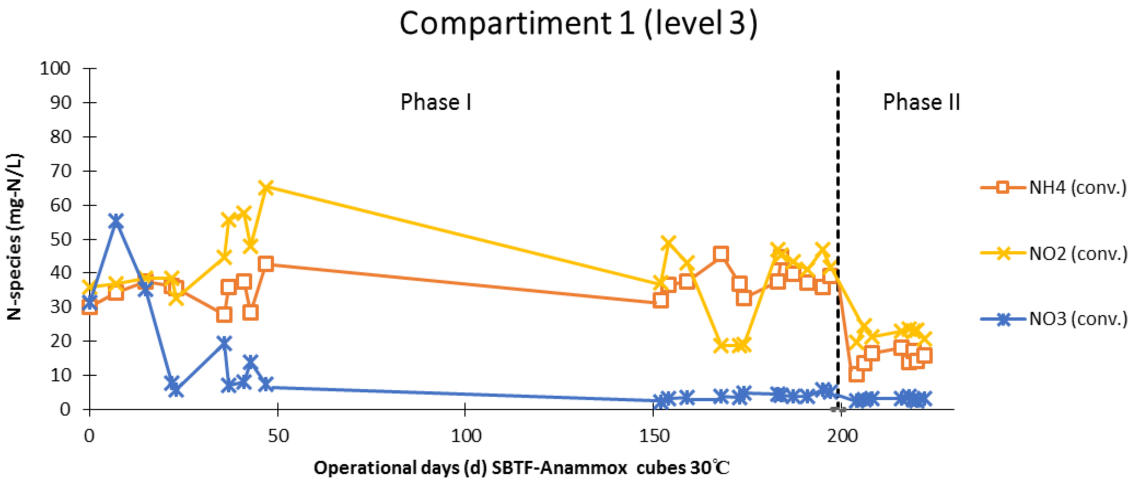
The graphics below (figure 4.1) show the concentration of ammonium and nitrite along the long term profile of SBTF-Anammox along the two experimental phases. From the profiles a decrease of ammonium and nitrite concentration in each reactor compartments, was seen in which the conversion was higher in the compartment 1. From compartment 2 to compartment 4 during the time, a reduction in terms of conversion is clearly verified in both phases, which emphasise in the compartment 4, in this compartment almost there is no conversion process taking place.

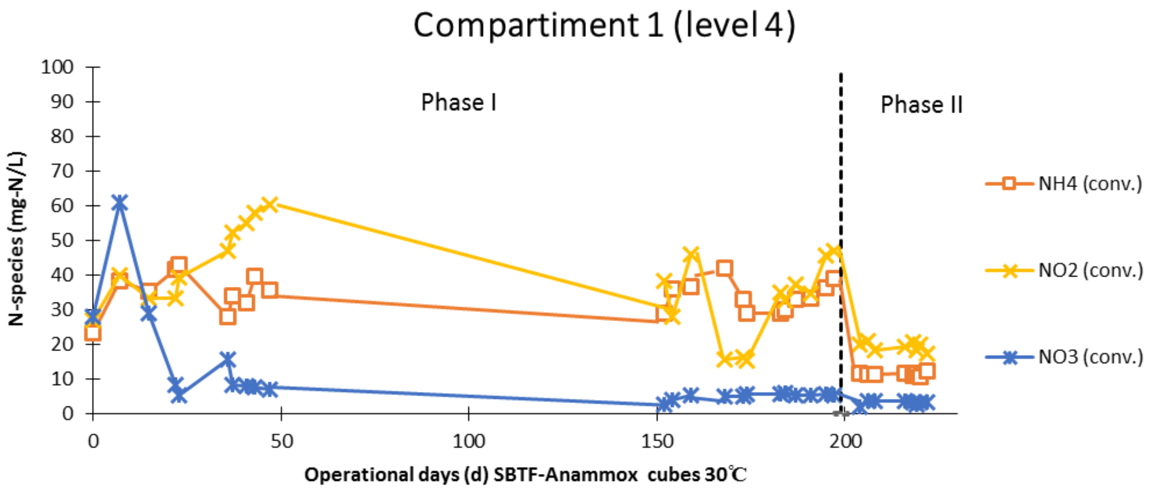
During phase I, at the beginning the ammonium conversion per compartment was lower, but over the experimental period it increased up to reach a point in which all the ammonium fed in the system was used up, probable as a result of bacteria activity in the reactor; Ammonia Oxidasing Bacteria (AOB) and Anammox. But, from day 140 to 175 the effluent ammonium concentration start increase and get a certain stabilization with efluent concentration iqual to 12.4 ± 7.8 mg/l, leading a decrease in the conversion process and also in the total nitrogen (TN) removal eficiency.

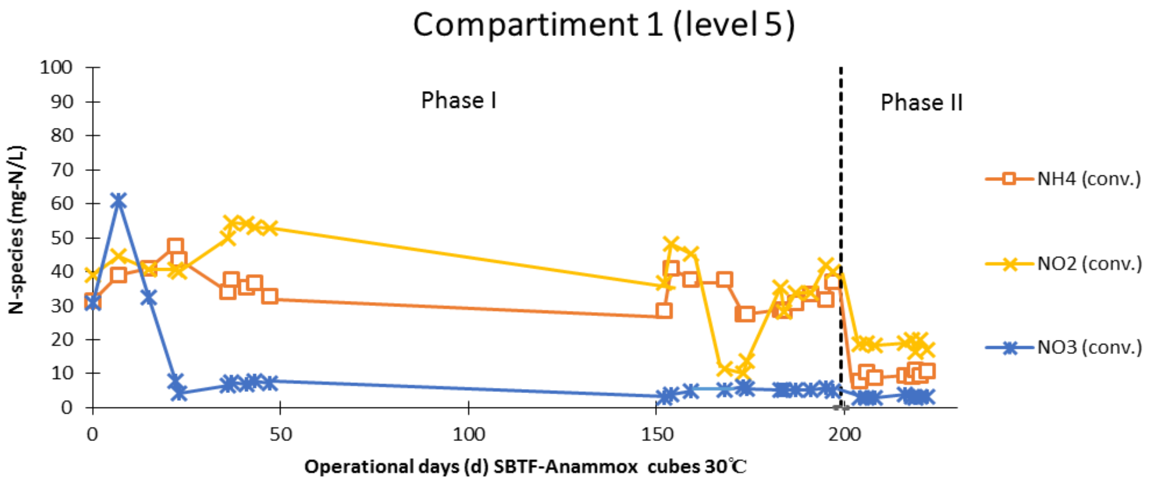
In the phase II the system was capable to increase significantly the rate of conversion process in the system, with a decrease of ammonium effluent almost iqual to zero (0,1 mg/l), after 8 day of reactor operation.

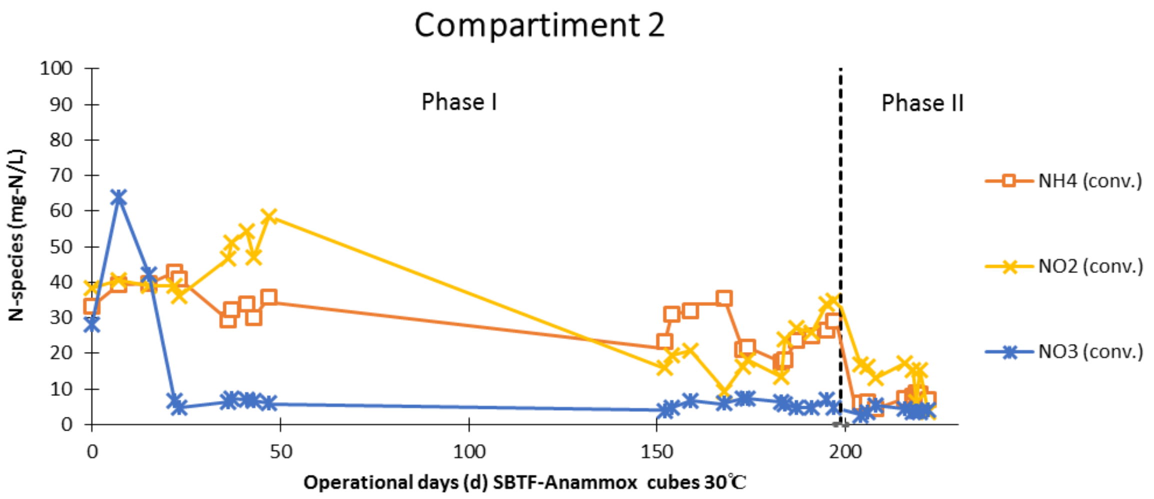

Compartiment 1 (level 1)
Phase I
NLR = 2.7 Kg-N/m³.d
Phase II
NLR = 1.2 Kg-N/m³.d
N-species (mg-N/L)
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
Operational days (d) SBTF-Anammox cubes 30℃
NH4 (inf)
NH4 (conv.)
NO2 (inf)
NO2 (conv.)
NO3 (inf)
NO3 (conv.)


Compartiment 1 (level 2)
Phase I
Phase II
N-species (mg-N/L)
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
Operational days (d) SBTF-Anammox cubes 30℃
NH4 (conv.)
NO2 (conv.)
NO3 (conv.)


Compartiment 1 (level 3)
Phase I
Phase II
N-species (mg-N/L)
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
Operational days (d) SBTF-Anammox cubes 30℃
NH4 (conv.)
NO2 (conv.)
NO3 (conv.)


Compartiment 1 (level 4)


Compartiment 1 (level 5)


Compartiment 2

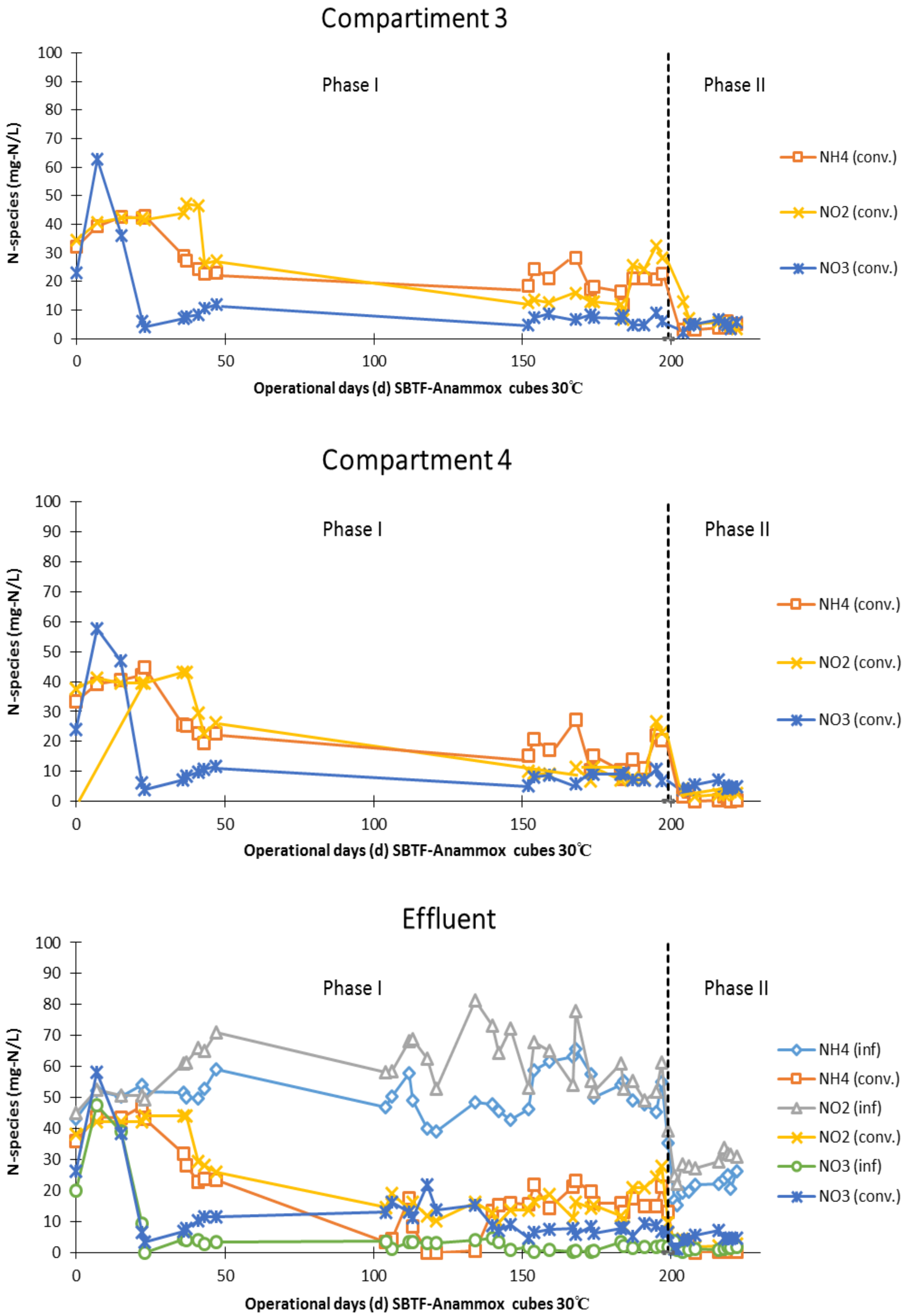


Figure 4.1. 1 Nitrogen conversion in long term conversion from the top to bottom in $\mathrm{SBTF_{ANAMMOX}}$ reactor.

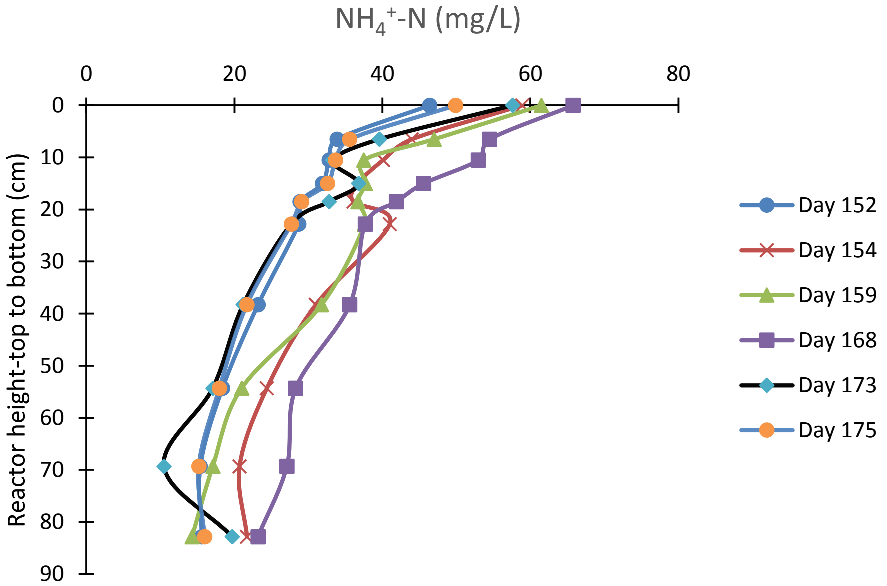
4.1.2 Nitrogen conversion profile vertical profile ($SBTF_{ANAMMOX}$)

Phase I and II

As ilustrated in the long-term profile, the following graphics (**figure 4.2.1.1 to 4.2.1.2**) showed the nitrogen spicies (ammonium, nitrite and nitrate) along the vertical profile of $SBTF_{ANAMMOX}$ reactor, during the last 6 days of experimental phase I. From the graph it can be seen that during phase I the ammonium and nitrite in the reactor are mostly removed in the first upper compartment of reactor, decreasing the influent ammonia concentration from an average of 58 mg/l to an effluent concentration of around 19 mg/L and also lowering the nitrite concentration from an average of 61 mg/L to 15 mg/L. The other way around, nitrate concentration is increasing from influent to effluent with an average concentration of 0.58 mg/l to 6.6 mg/L.

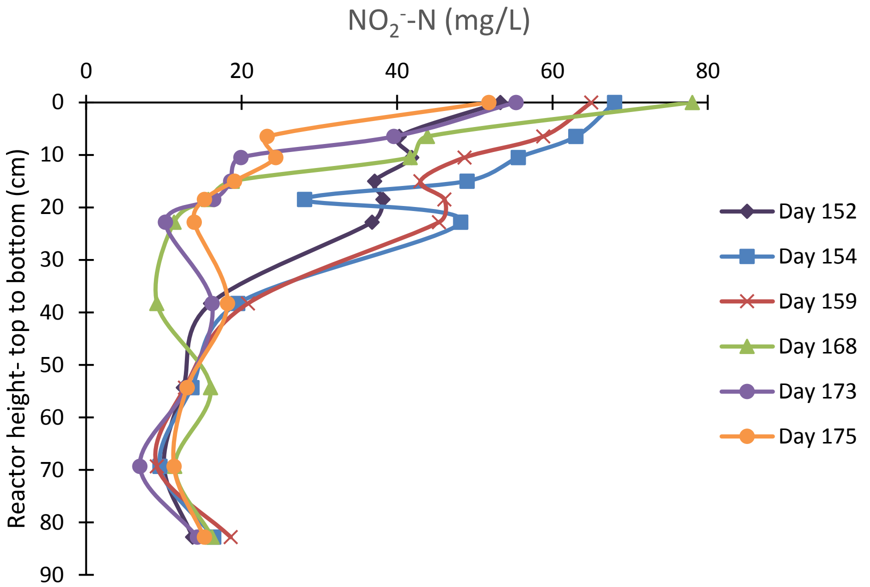
In phase II the system decreased the nitrite and ammonium concentrations from around 21.6 to 0.1 mg/L for ammonium and 33.8-1.0 mg/l for nitrite and ammonium in effluent, starting from a concentration below 26.3 mg-N/L to 0.3 mg/l. In terms of nitrate concentration, the profiles show a significant reduction when compared with phase I of the experiment, meaning in the average influent concentration 0.9 mg/L and effluent concentration of 4.2 mg/L.

Based on the profile, is possible verify that the reactor had reached better stable conditions in the phase II of their experiment than in phase I, but the removal efficiency was higher in phase I.



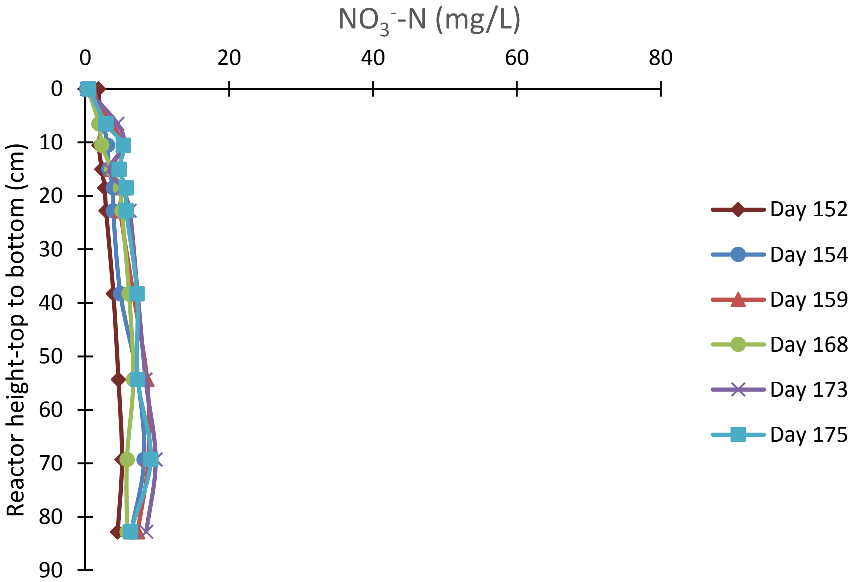
(1)

Figure 4.1. 2.1 NH_4^+-N concentration in the $SBTF_{ANAMMOX}$ reactor – PHASE I



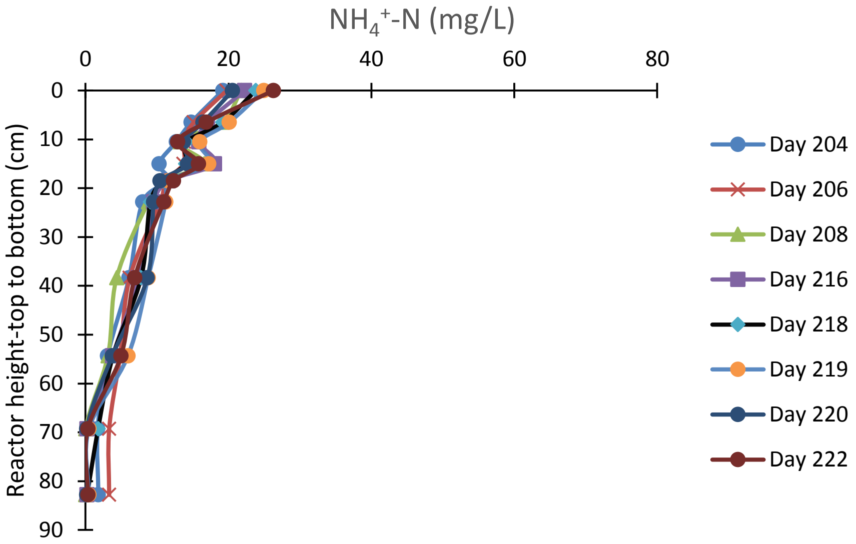
(2)

Figure 4.1. 2.2 NO$_2^-$-N concentration in the SBTF$_{ANAMMOX}$ reactor – PHASE I.



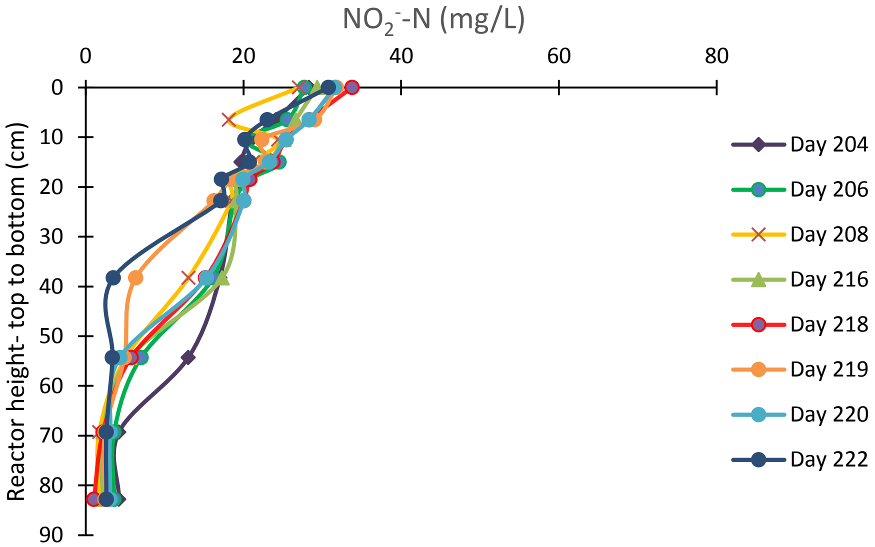
(3)

Figure 4.1. 2.3 NO$_3^-$-N concentration in the SBTF$_{ANAMMOX}$ reactor – PHASE I.



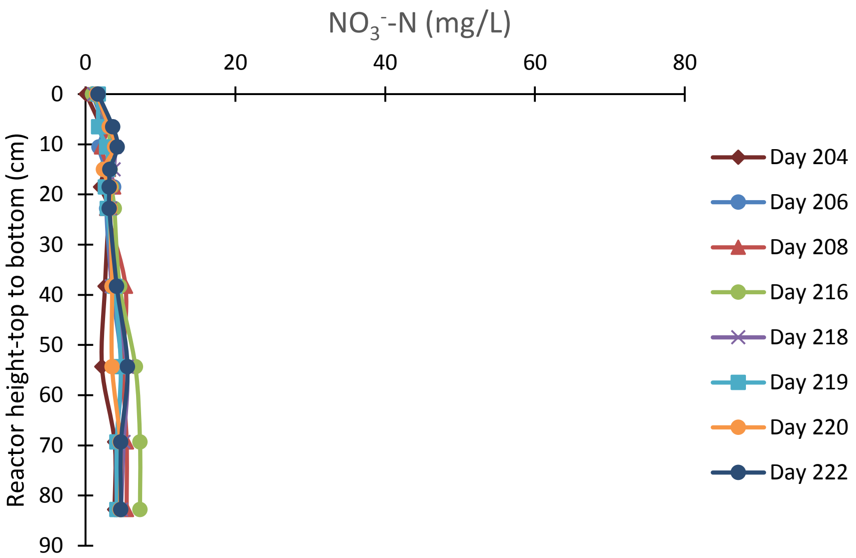
(4)

Figure 4.1. 2.4 NH_4^+-N concentration in the SBTF_{ANAMMOX} reactor – PHASE II.



(5)

Figure 4.1. 2.5 NO_2^--N concentration in the SBTF_{ANAMMOX} reactor – PHASE II.



(6)

Figure 4.1. 2.6 NO_3^--N concentration in the SBTF_{ANAMMOX} reactor – PHASE II.

4.1.3 Nitrogen conversion ratios

Based on Strous et al, 1998, the Stoichiometry ratio value for Anammox bacteria is 1.32 for NO_2^--N/NH_4^+-N and 0.26 for NO_3^--N/NH_4^+-N.

Phase I of the experiment started with a ratio a bit lower, 1.05 for NO_2^--N/NH_4^+-N and a bit higher 0.46 for NO_3^--N/NH_4^+-N. But during the time, between days 38 to 121 and 152 to 222, the reactor showed a good ratio, in average of 1.3 for NO_2^--N/NH_4^+-N . This ratio was reduced a bit to 1.2 in period II between day 152 to 222. In the same period days reported, the reactor was shown a reduction in terms of NO_3^--N/NH_4^+-N ratios, varying from 0.2 to 0.1, respectively. Despite of that, the overall ratios during the phase was fixed in the average of 1.3 ± 0.3. at the and Anammox Stoichiometry ratio was observed, varying from average value of 1.3 ± 0.3 g-NO_2^--N/g-NH_4^+-N and 0.1 ± 0.2 g-NO_3^--$N_{produced}$/g-NH_4^+-$N_{consumed}$, suggesting the Anammox metabolism as the dominant nitrogen removal pathway.

In phase II, a continue effluent concentration was observed and a increase of NO_2^--N/NH_4^+-N ratio was verified, that reduction could be probably related with the increase of Anammox activity in the system; if compared this figure with the stoichiometric ratios (see annex: 2). This phase showed average ratios of 1.4 ± 0.1 g–NO_2^--N/g-NH_4^+-N and 0.1 ± 0.1g-NO_3^--$N_{produced}$/g-NH_4^+-$N_{consumed}$, suggesting again the Anammox metabolism as the dominant nitrogen removal patway, when relating this value with a considerable amount of gas produced by the reactor in the last days of this experimental phase (**Appendix B 1-4**).

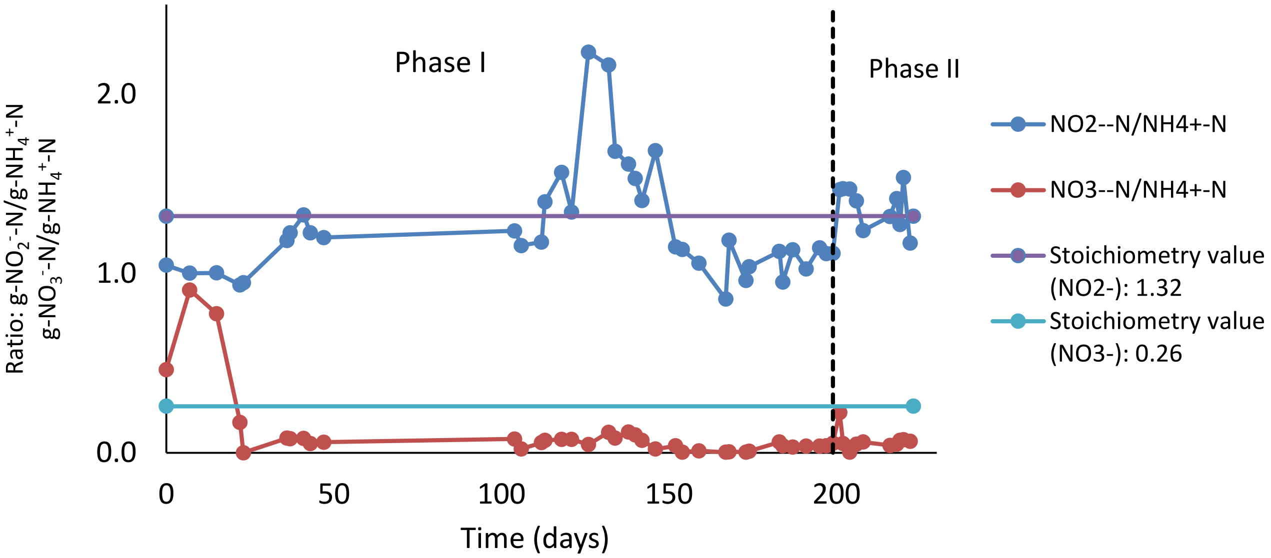


Figure 4.1. 3 Stoichiometry ratios variation in the SBTF_{ANAMMOX} reactor.

4.1.4 Nitrogen removal efficiency

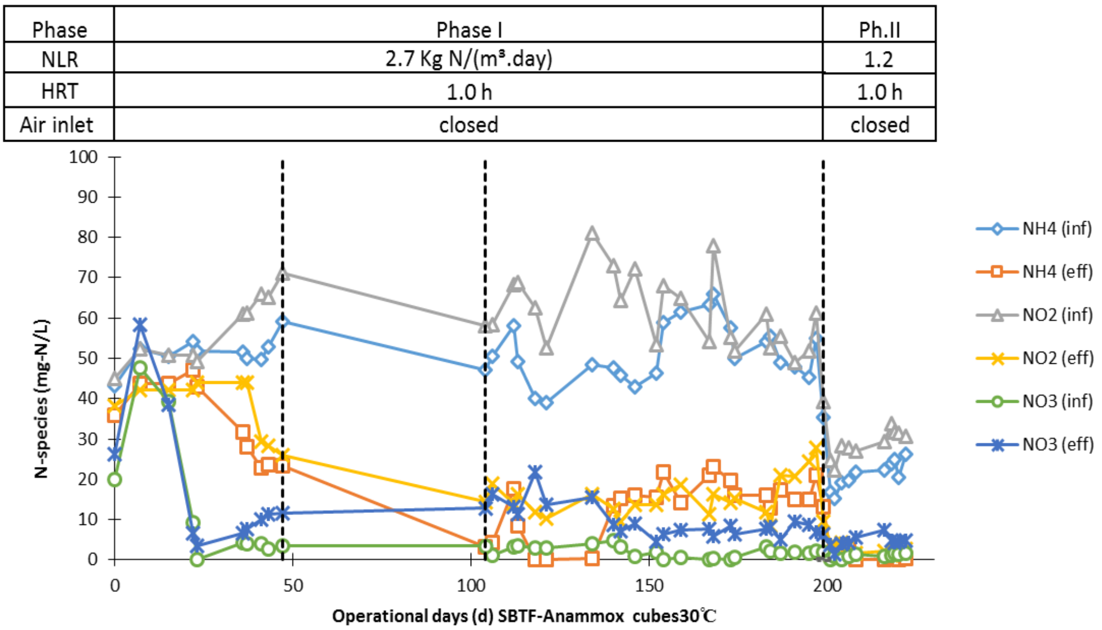
Figure shows the concentration of N-species in the influent and effluent for the SBTF-Anammox in both phases. In the phase I, during day 47 to 103 no sampling activity was taken place and between 103 to 175 days of operation, the reactor showed a trend of stabilization, with a range between 63 to 100% for NH_4^+-N, 60 to 70% for NO_2^--N and 64 to 75% for TN, corresponding to 77 ± 13.74 %, 65 ± 8.04 and 67.8 ± 3.60 % for NH_4^+-N, NO_2^--N and TN removal, respectively (**Figures 4.1.4a, b, c, d and e**).

In the same phase, concretely after 112 days of operation the reactor showed a trend of stability with a TN removal efficiency of 65.6 ± 2.06 %. From the graphics, it can be seen that the ammonium and nitrite in the reactor are mostly removed in the first upper compartment of reactor, reducing the influent ammonia concentration from an average around 50 ± 7.6 mg/l to effluent concentration around 18.3 ± 12.8mg/L, also lowering the nitrite concentration from an average of 61.2 ± 10.5 mg/L to 22 ± 11.2 mg/L.

From day 140 to 199 a reduction of NO_3^+-N effluent was observed, when compared with concentration expected according with Anammox Stoichiometric, that reduction could be probably by occurrence of denitrification (even without the carbon source) in the system and consequently nitrogen gas released. The same behaviour happen in the last days of phase II, concretely between days 218 to 222. (see **figure 4.0 in annex**)

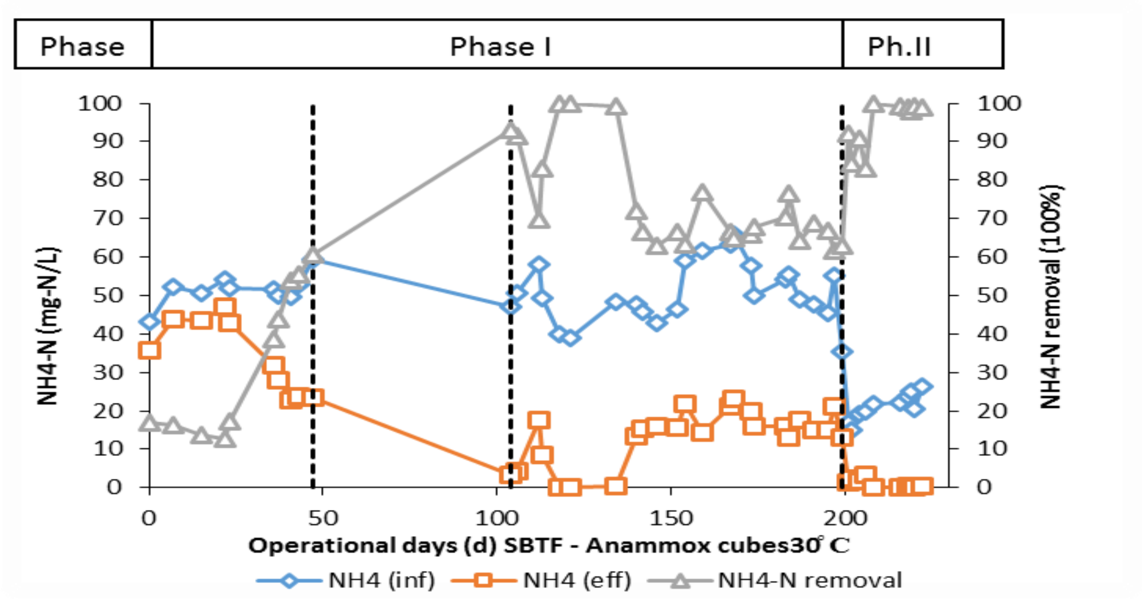
In phase II the a continuously reduction of ammonium and nitrite also was verified with an average of 22.3 ± 5.2 mg/L for influent ammonium concentration and 2.1 ± 3.6 mg/L of effluent nitrite concentration meaning that the nitrite removal is higher than ammonium, which is expected according Anammox Stoichiometry that establish an amount of 1.32 mg NO_2^--N per gram of NH_4^+-N removed. In addition nitrate production in the system also resisted a reduction in terms of concentration, varying from average influent concentration of 1.4 ± 0.9 mg/L and effluent concentration of 4.4 ± 1.7 mg/L.

In terms of TN removal, the maximum TN removal attained resisted in the phase II was 90% and the removal rate that the system achieve was 1.1 ± 0.3 kg-N/m^3.d from the 1.2 K kg-N/m^3.d of NLR applied in the system.



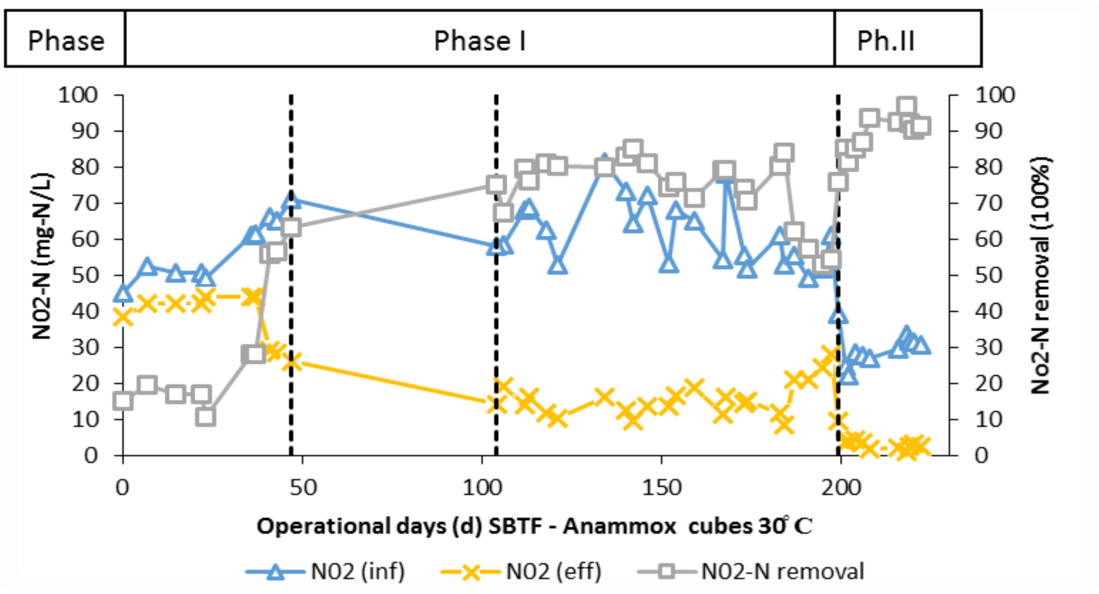
(a)

Figure 4.1. 4 a) N-species performance in SBTF_{ANAMMOX} reactor.



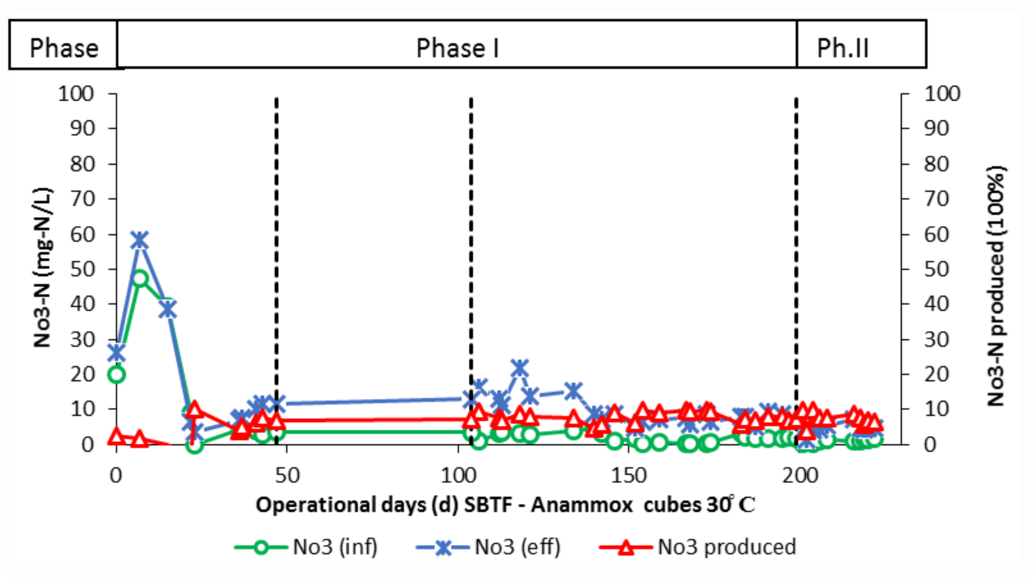
(b)

Figure 4.1. 4 b) NH_4^+-N removal efficiency along the time in SBTF_{ANAMMOX} reactor .



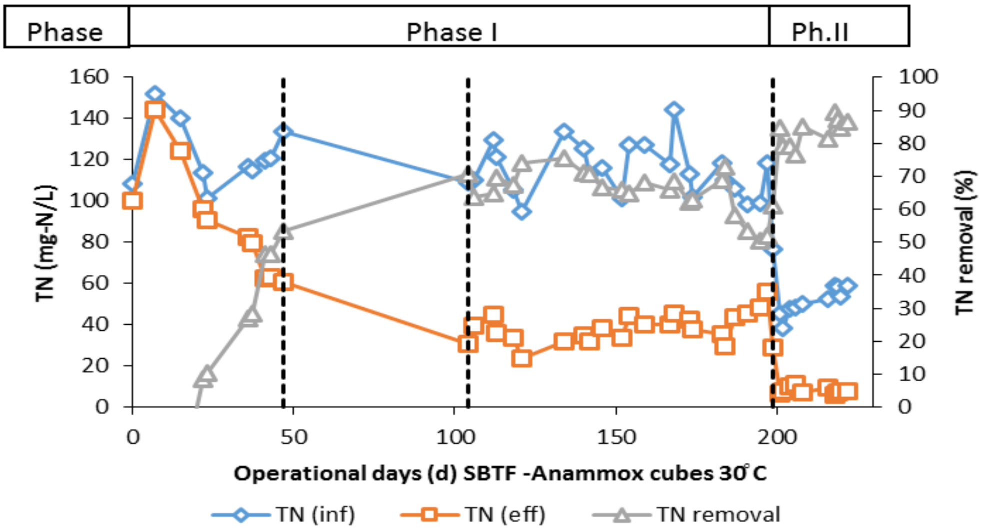
(c)

Figure 4.1. 4 c) NO$_2^-$-N removal efficiency along the time in SBTF$_{\text{ANAMMOX}}$ reactor .



(d)

Figure 4.1. 4 NO$_3^-$-N removal efficiency along the time in SBTF$_{\text{ANAMMOX}}$ reactor.



(e)

Figure 4.1. 4 e) TN removal efficiency along the time in SBTF$_{\text{ANAMMOX}}$ reactor .

4.2 Dissolved Oxygen (DO)

At the beginning of phase I dissolved oxygen were a bit higher in the influent of Demineralised water, reaching a maximum concentration of 2.10 mg/L. After D.O. meter calibration and change in scavenger solution, dissolved oxygen in the influent Demineralised water, the system was able to achieve a certain stability along the time. Between days 60 to 100 the dissolved oxygen was not recorded in the system. The figure 4 gives a picture of the DO behavior during phase I. In phase II of the experiment the DO concentration in the influent demineralised water concentration was fixed below 1.0 mg/l. This variation can be compared with efficiency removal of the system in term of total nitrogen removal (**Figure 4.1.4 e**), it can be seen that there is a strongly inversely proportional correlation between dissolved oxygen and performance of reactor, as the DO was increasing in the system the removal efficiency was decreasing.

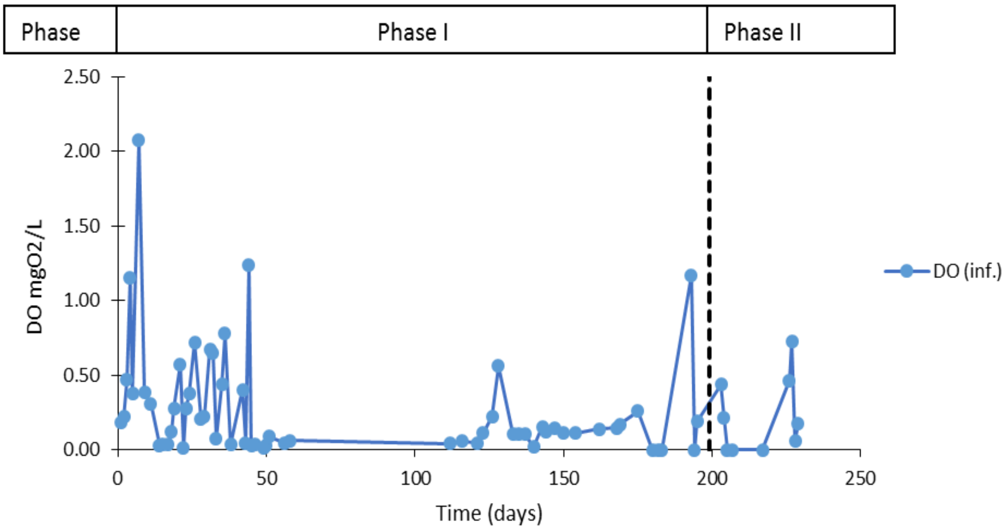


Figure 4.2. Influent Dissolved oxygen variation.

4.3 pH

In phase I of the experiment the pH in the influent, effluent and over the profile of the system were recorded in the range of 7.90 to 8.75, corresponding an average of 8.3 ± 0.2/ 8.3± 0.2 as showed in the profile below. In the phase II a slight reduction in pH was observed with an average of 8.2 ± 0.2 / 8.2 ± 0.2 (see table.).

pH along the profile phase I

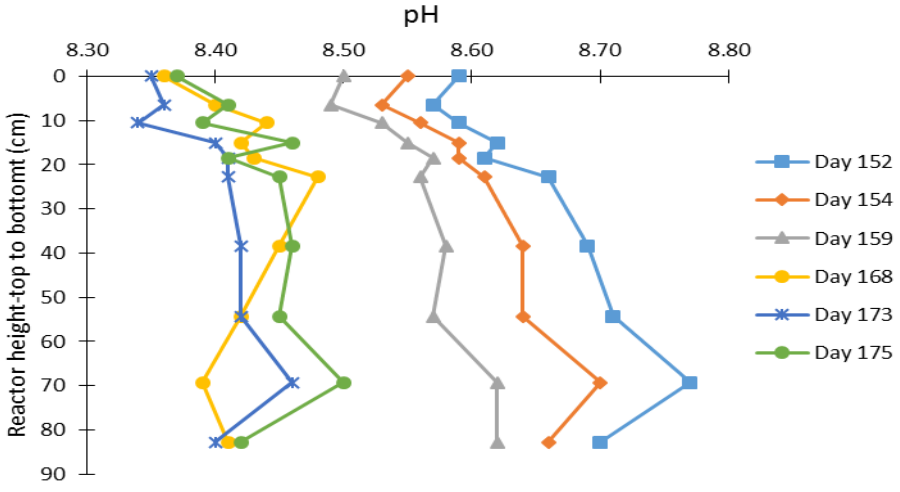


Figure 4.3. 1 pH over the profile of SBTF_{ANAMMOX} reactor - PHASE I.

pH along the profile phase II

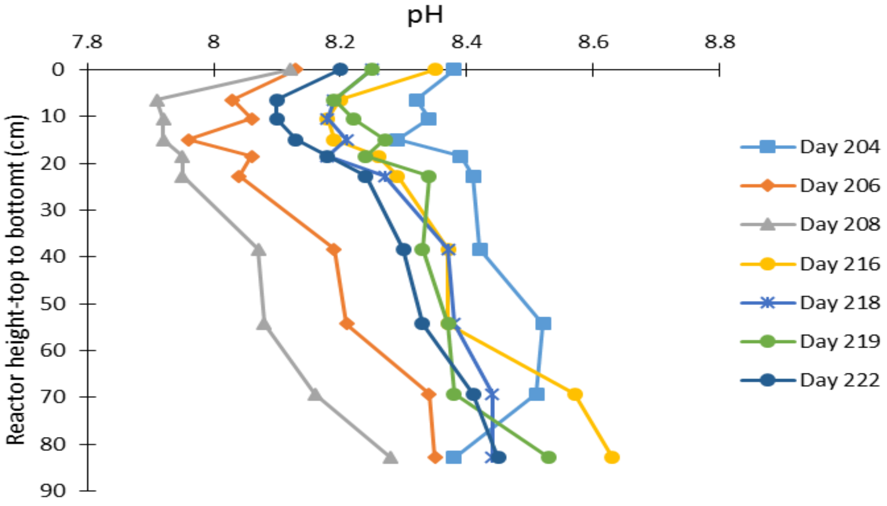


Figure. 4.3.2 pH over the profile of SBTF_{ANAMMOX} reactor - PHASE II.

4.4 Alkalinity

From the figure 4.7 it can be observed that same time a little bit of alkalinity, is consumed, that could be a reason of same inhibition of the Anammox activity in the system, creating environment for nitrification in the system. This probably happen because of the oxygen intrusion via demineralised water and natural oxygen intrusion when during the times in which the reactor was opened for maintenance purpose.

Most of the time the alkalinity is produced in the system, favouring the Anammox process in the system, as the Anammox process is coupled with alkalinity production. From the figure we can see that the alkalinity in the system is almost constant from the last days of the phase I of the experiment and still constant in phase II.

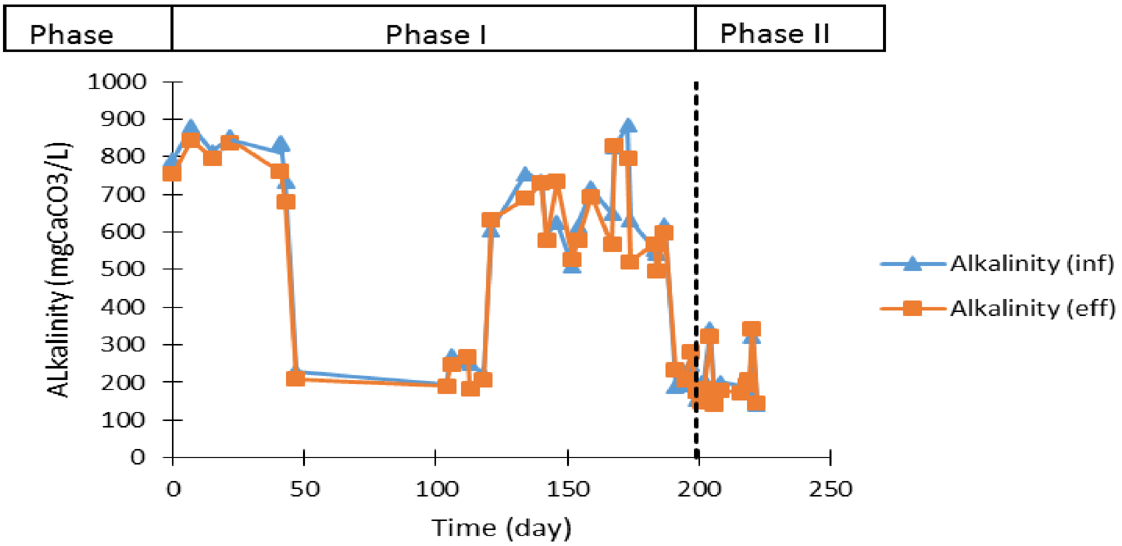


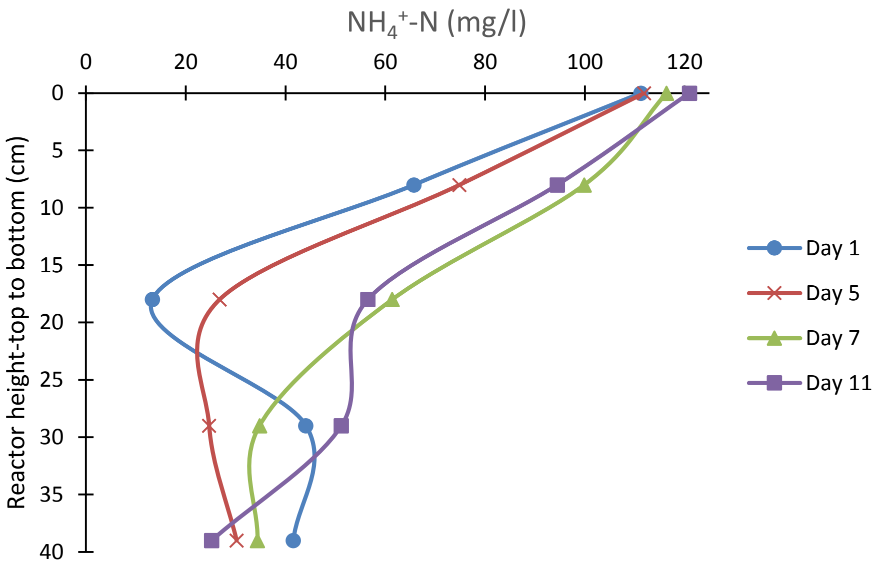
Figure.4.4 Effluent and influent alkalinity concentration in SBTFANAMMOX reactor.

4.5.1 Nitrogen Nitrogen conversion vertical profile in SBTF$_{CANON}$

As a result of the short time that tise reactor was operated, a long-term profile over the reactor was not possible to acquire. To assess the nitrogen conversion of previous reactor configuration (horizontal layer from 2014/2015), the profiles are also presented below.

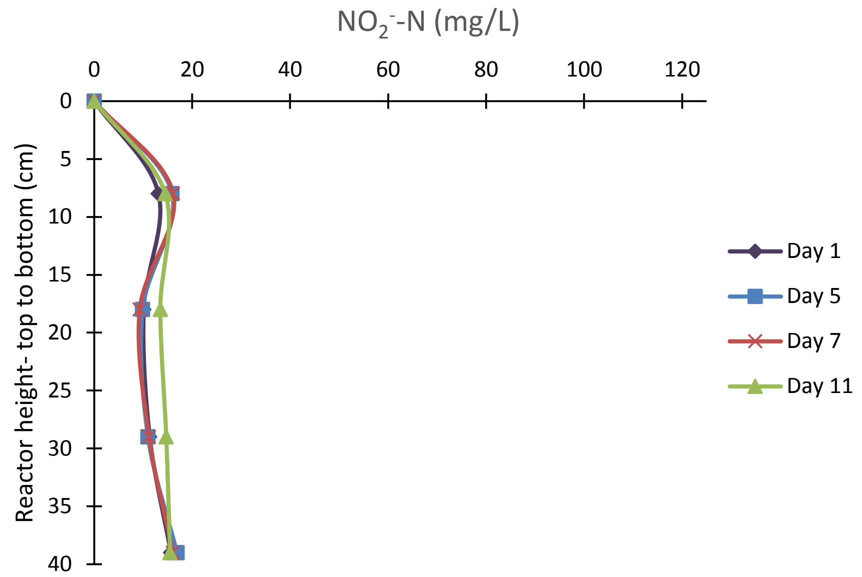
Phase I (SBTF$_{CANON}$)

The figures below (**figure 4.5.1 a,b and c**) present the vertical profile of reactor performance during the first day up to day 11 of their experiment illustrated in graphics. It can be observed a reduction of substrate concentration (NH_4^+-N) in each reactor compartment due the conversion process. A significant amount of ammonium is converted in compartment 1 of the reactor, followed by a rapid conversion in the compartment 2, meaning that the compartment 2 was converting more than the compartment 1. Through the profile below it can be seen a decrease in ammonium concentration from an average influent concentration of 111 mg/l to almost 29 mg/l effluent concentration. Meanwhile, as a result, of conversion performed by nitrifiers (AOB and Anammox) in each compartment the nitrite and nitrate were produced in an average of 15 mg/l and 25 mg/l, respectively.



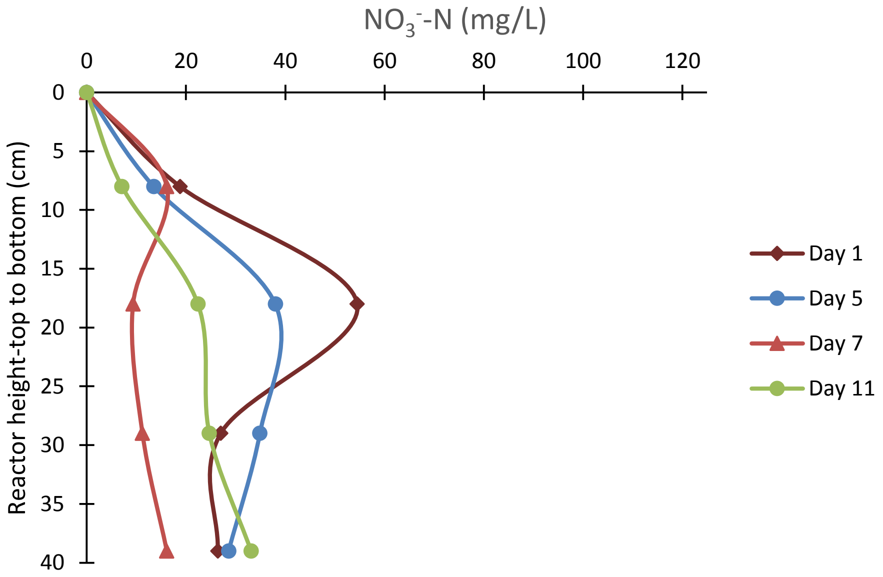
(a)

Figure 4.5.1 a) NH_4^+-N concentration in the SBTF$_{CANON}$ reactor – PHASE I.



(b)

Figure 4.5.1 b) NO_2^--N concentration in the SBTF_{CANON} reactor – PHASE I.



(c)

Figure 4.5.1 c) NO_3^--N concentration in the $SBTF_{CANON}$ reactor – PHASE I.

4.5.2 Nitrogen conversion ratios in the $SBTF_{CANON}$

Figures 4.1.3.1 present the conversion ratios for NO_2^--N/NH_4^+-N and NO_3^--N/NH_4^+-N in $SBTF_{CANON}$ reactor during the entire experimental period. During the entire operation period the reactor showed a lower ratio for NO_2^--N/NH_4^+-N and higher for NO_3^--N/NH_4^+-N, in the average of 0.2 ± 0.1 g –NO_2^--$N_{produced}$/g-NH_4^+-$N_{consumed}$ and 0.2 ± 0.0 g-NO_3^--Nproduced/g-NH_4^+-$N_{consumed}$ in accordance with Strous, et al,(1998), who established the Stoichiometry ratio value for Anammox bacteria as 1.146 for NO_2^--N/NH_4^+-N and 0.161 for NO_3^--N/NH_4^+-N.

Although the influent ammonium concentration was converted in an average of 73.9 ± 8.5% and TN in 37.7 ± 7.9 %, the amount of nitrate production was not balanced, the system was producing more nitrate than expected by an Anammox reaction (24.9 ± 5.6 %) instead of nitrite (15.3 ± 6.3 %) (**annex G table G.2 and G.3**), leading to limitation in the Anammox process and favouring the nitrification in the system.

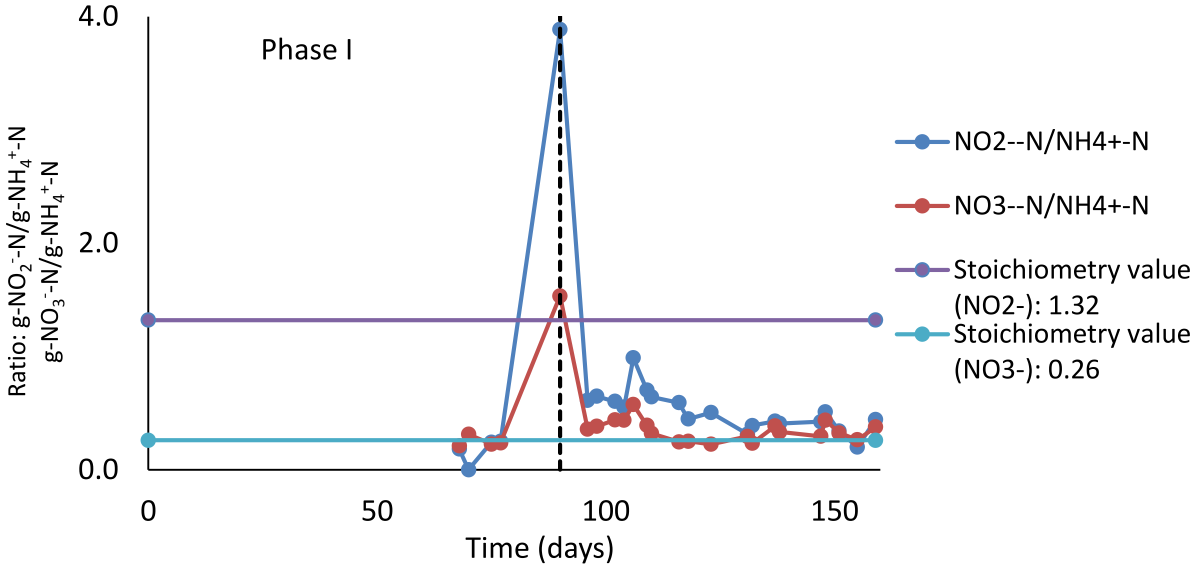
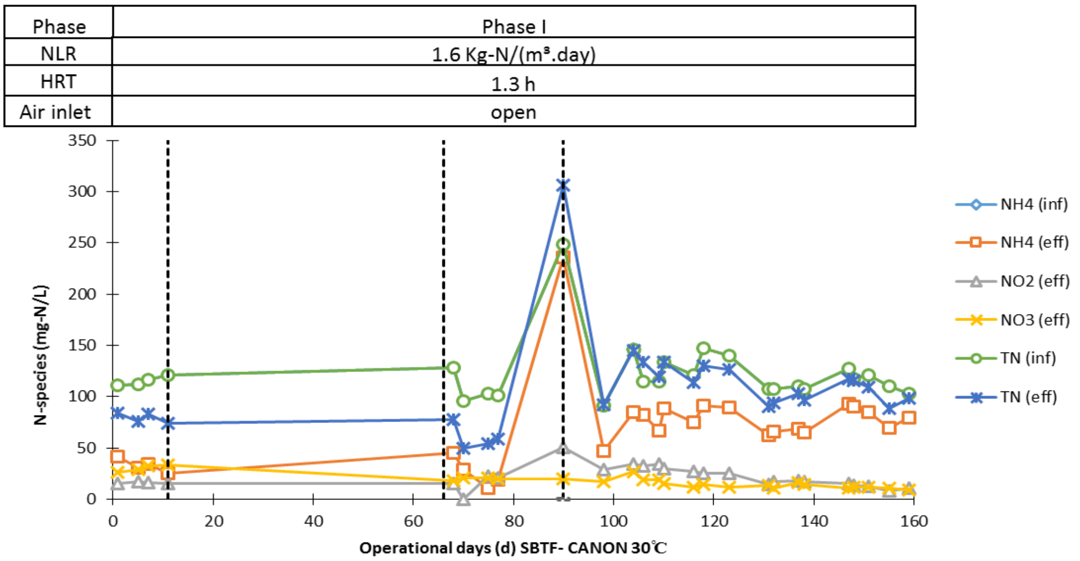


Figure 4.5.2 Stoichiometry ratios variation in the SBTF$_{\mathrm{CANON}}$ reactor.

4.5.3 Nitrogen removal efficiency (SBTF$_{\mathrm{CANON}}$)

Figure 4.5.3a to e shows the concentration of N-species in the influent and effluent for the SBTF$_{\mathrm{CANON}}$ during the experimental period. From the charts it can be observed an almost constant removal efficiency since the beginning of the experiment (day 0) up to the day that the reactor started to decrease its performance on day 90. In that period an average TN removal efficiency was 37.7 ± 7.9 %, in which the species in the system reached an average ammonium removal of 73.9 ± 8.5 %, nitrite production in 15.3 ± 6.3% and nitrate of 25.9 ± 5.6%.

For the entire operation period, the system was reached a maximum TN removal of 48% and minimum of 25%. Due to process upsets of the system, from day 90 forward no removal efficiency was assessed.



(a)

Figure 4.5.3 a) N-species performance in SBTF$_{\text{CANON}}$ reactor.

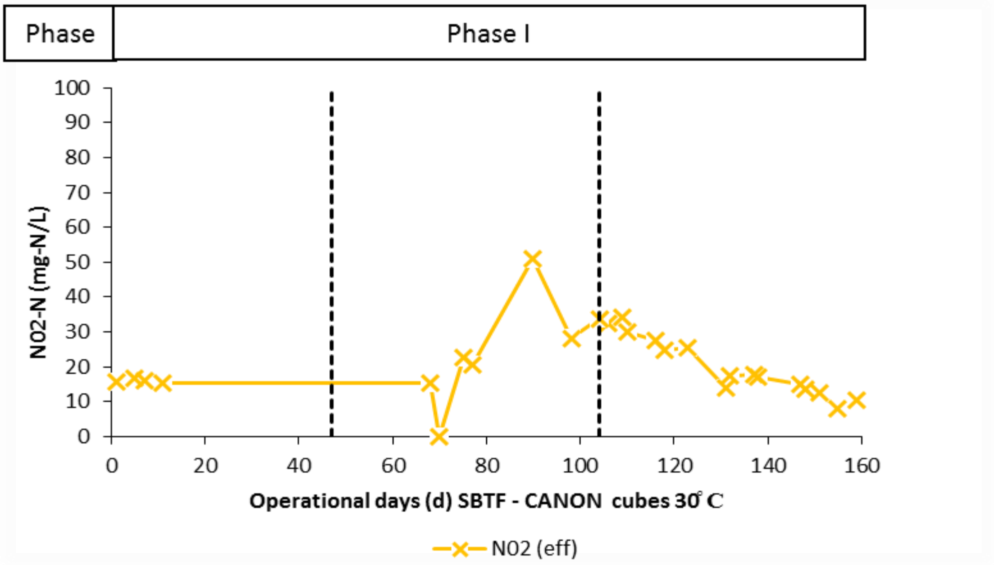


Figure 4.5.3 c) NO_2^--N production along the time in SBTF$_{\text{CANON}}$ reactor.

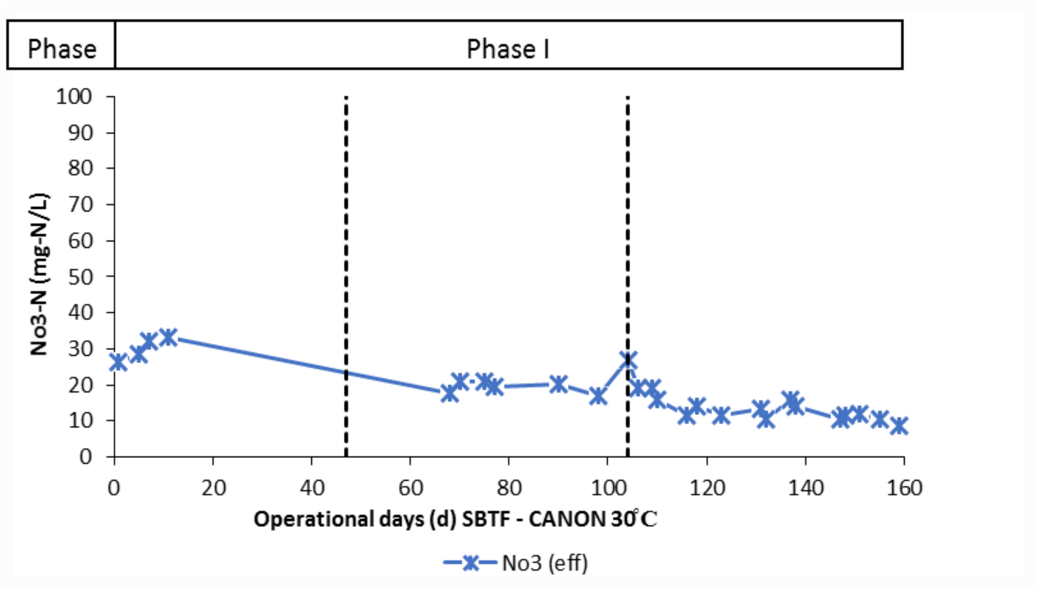
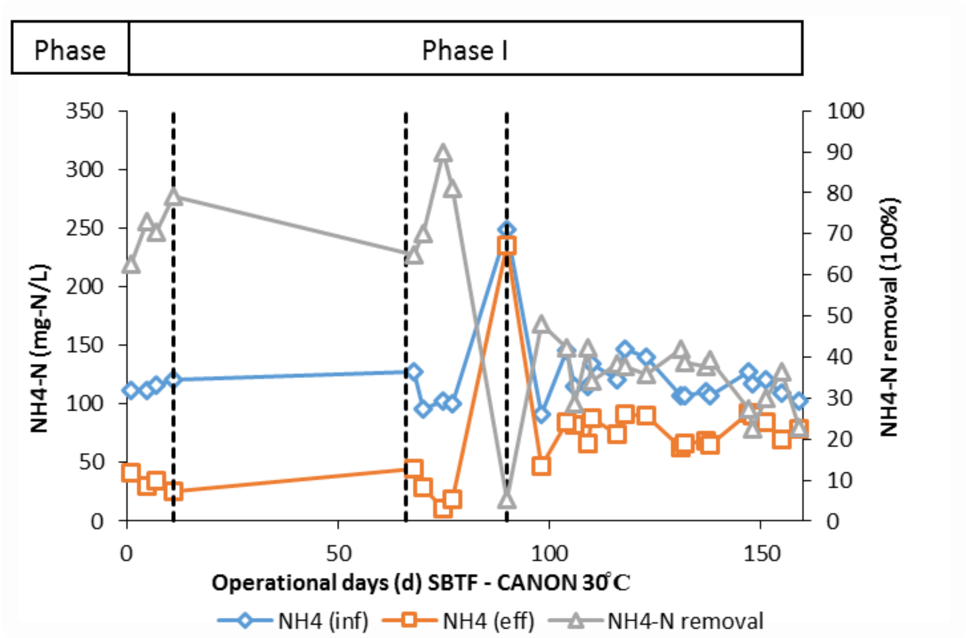
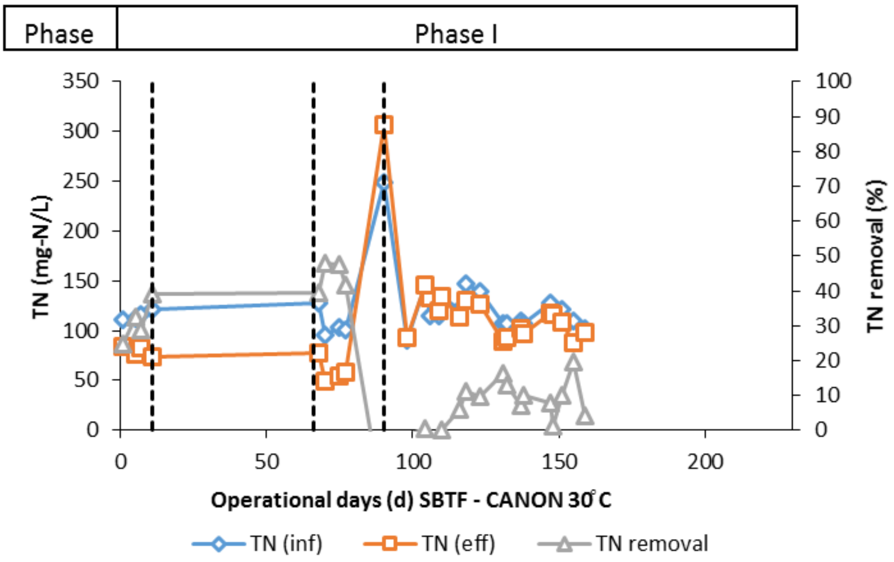


Figure 4.5.3 d) NO_3^--N production along the time in SBTF_{CANON} reactor.



(b)

Figure 4.5.3 b) NH_4^+-N removal efficiency along the time in SBTF_{CANON} reactor.



(e)

Figure 4.5.3 e) TN removal efficiency along the time in $\mathrm{SBTF_{CANON}}$ reactor.

4.6 pH

The pH in the system do not varied that much, even when the system registed malfunction. The average pH was fixed in the range influent of 8.2 and effluent of 8.3. (**figure 4.6)**

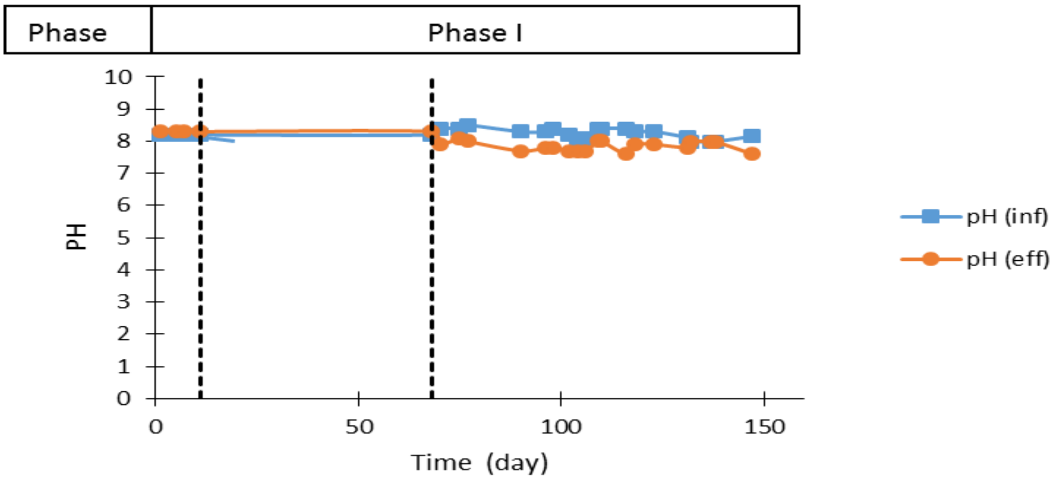


Figure 4.6 pH over the system.

4.7 DO

At the early stage of experiment, up to the end the influent DO was kept below 1.0 mg/l by flushed the demineralised water with nitrogen gas two or three times a week and remaining opened all the air inlet (**figure 4.7**).

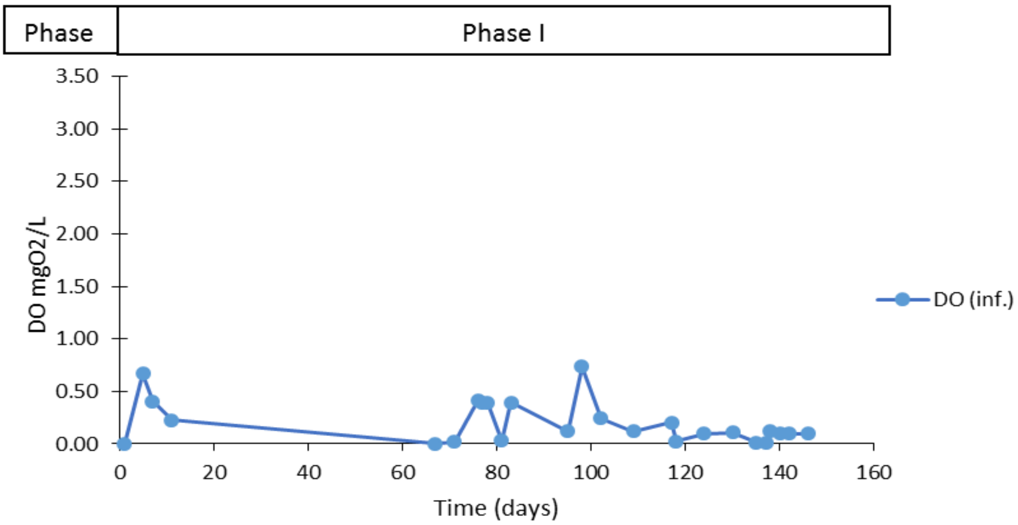


Figure 4.7 Influent DO concentration.

4.8 Alkalinity

The system started it operation with an average alkalinity of 700 mg CaCO3/L, since was facing with formation of same precipitate in the upper part of reactor sponges layer and part of substrate was not percolating through sponge forming a water colum in same sponge layers, the alkaninity was reduced from almost 700 mg CaCO3/L to 300 mgCaCO3/L. After alkalinity reduction, the reactor precipitation reduced satisfactory and percolation as well. The **figure (4.8)** below shows the proportion in alkaninity reduction in the system.

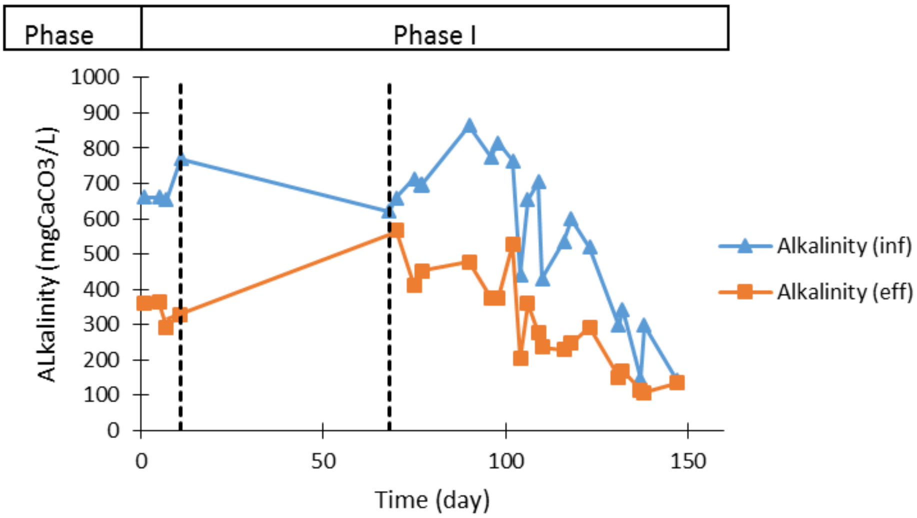


Figure 4.8 Alkalinity variation in the system

4.9 Total suspended solids (TSS)

In general the TSS observed in both reactors were relative low, and most of them, not visually detected.
In the previous 20 days, the amount was lower than 15 mg/l in both reactors. But after day 25 an increase in the TSS in the $SBTF_{ANAMMOX}$ reactor was evident. It is possible that higher effluent TSS concentration were related with the amount of biomass growing in the reactor. In the last 10 day of experiment (between day 212 to 222), when the reactor was close to reach the stable condition, the amount of TSS was reduced again. For the $SBTF_{CANON}$ the increase of TSS could be related with same precipitated particles coming from the reactor during the malfunction period. That variation can be shown by the graph below.

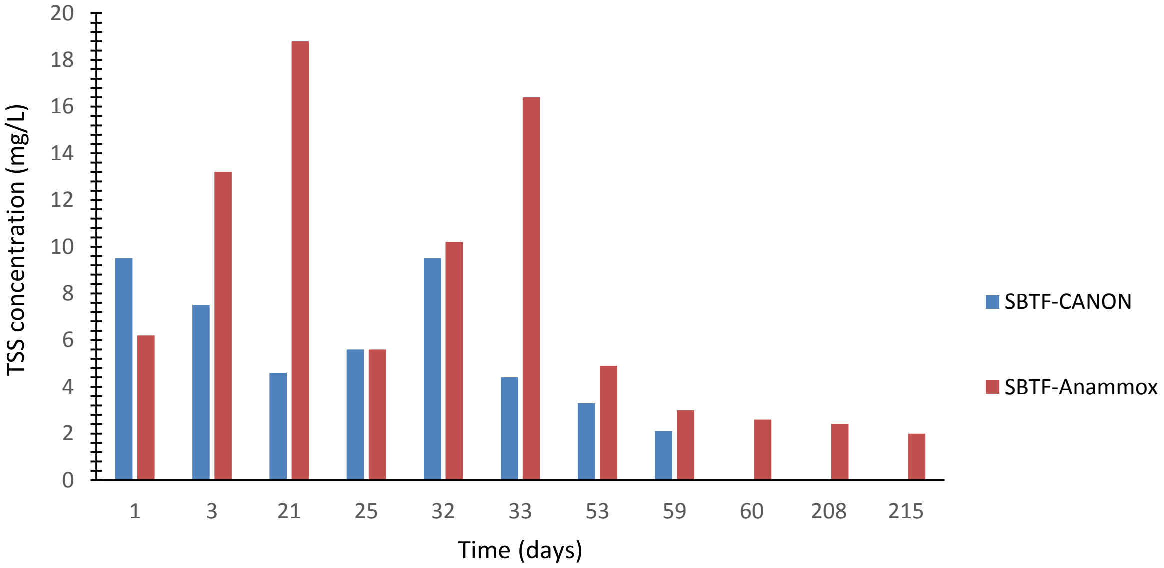


Figure 4.9 Effluent TSS concentration in $SBTF_{ANAMMOX}$ reactor and $SBTF_{CANON}$ reactor.

SBTF$_{CANON}$ (PREVIOUS STUDY)

The table below provide a summary of previous studies made in SBTF$_{CANON}$, from Jayawardana (2014) and Sanchez-Guillen (2015a,b). From the table it can be observed that:

The pH in the system was registed a bit variation from 8.2 ± 0.2 in influent to 7.3 ± 0.3 in the effluent wastewater.

At the beginning of the experiment the alkalinity was fixed in average of 500 mg CaCO3/L but suddenly this amount had change to an average of 800 mg CaCO3/L. A remain in this change in alkalinity was the efficiency of reactor that registered a decrease in efficiency, probably because of precipitation forming in the system.

In terms of removal efficiency, when comparing with the performance observed by Sanchez-Guillen et al. (2015a, b), who applied the same NLR as the SBTF$_{CANON,}$ it can be seen that the system performed almost at the same performance with the previously system and some times even higher in terms of effluent ammonium removal.

Table 5. 1 Performance of SBTF$_{CANON}$ previously studies

Parameter	Phase I (day 68-77)	Sanchez-Guillen et al. (2015a) (phase IID)	Sanchez-Guillen et al. (2015b)
Influent NH_4^+-N (mgN/L)	111.0 ± 10.1	118.5 ± 4.6	104.6 ± 6.3
Effluent NH_4^+-N (mgN/L)	29.3 ± 10.6	21.7 ± 3.6	34.9 ± 8.7
Effluent NO_2^--N (mgN/L)	15.3 ± 6.3	0.4 ± 0.1	3.3 ± 4.4
NH_4^+-N removal (mgN/L)	81.7 ± 9.2	64.1(70.9)	-
NO_2^--N produced (mgN/L)	15.3 ± 6.3	-	-
TN removal (mgN/L)	41.5 ± 7.7	54.4	-
Effluent NO_3^- N (mg N/L)	24.9 ± 5.6	32.4 ± 1.1	23.5 ± 9.7
NO_3^- N produced (mg N/L)	24.9 ± 5.6	-	-
NO_2^-—N / NH_4^+-N	0.2 ± 0.1	-	0.1 ± 0.1
NO_3^-—N / NH_4^+-N	0.2 ± 0.0	-	0.3 ± 0.2
NH_4^+-N removal (%)	41.5 ± 7.7	81.6 (86.7)	66.6 ± 8.0
TN removal (%)	37.7 ± 7.9	54 (61.9)	43.0 ± 14.8
pH (influent / effluent)	8.2 ± 0.0/ 8.3± 0.0	-	8.2 ± 0.2/7.3 ± 0.3
Alkalinity Influent (mgCaCO$_3$/L)	686.0 ± 48.1	-	584.7 ± 155
Alkalinity Effluent (mgCaCO$_3$/L)	334.8 ± 28.8	-	258.7 ± 82.8
NLR (kg-N/m^3.d) applied in the system	1.7 ± 0.2	0.95	1.6 ± 0.1
TN removal (KgN/m^3.d)	0.6 ± 0.1	0.51 (0.57)	0.7 ± 0.3

CHAPTER 5

Discussion

In this chapter a specific interpretation of results and performance given by both reactors ($SBTF_{ANAMMOX}$ and $SBTF_{CANON}$) will be discussed, with emphasise in the factors that were influenced the reactors performance (NLR, DO and pH). In addition, same operations problems faced during the experiment period will be described.

5.1Nitrogen removal in the SBTF reactors

5.1.1 Nitrogen removal in $SBTF_{ANAMMOX}$ reactor

Making a comparison and discussing behind the performance of the two phases that the $SBTF_{ANAMMOX}$ was run, it can be supported by the datas provided by **table 5.1.** From the nitrogen balance in the system in both reactor experimental phases (table **B.3 and D.3**) it can seen that after day 104 to 138 a slight variation of NO_3^-N (deficit) was observed, but that decific become continuous from day 140 to day 197 (end of phase I experiment) a gradualy reduction in NO_3-$^-$N amount was registed in the system supported by gas production in reactor (f**igure A.1-4).** In phase II, N-deficif was noticed after 5 days of operation in which gas production was verified in almost every day of experimental phase.

From day 140 to 199 a reduction of NO_3^+-N effluent was observed, when compared with concentration expected according with Anammox Stoichiometric, that reduction could be probably by occurrence of denitrification (even without the carbon source) in the system and consequently nitrogen gas released. The same behaviour happened in the last days of phase II, concretely between days 218 to 222. (**appendix B figure B.1-4).**

In phase II a continuous decrease of ammonium and nitrite also was verified at an average of 22.3 ± 5.2 mg/L for influent ammonium concentration and 2.1 ± 3.6 mg/L of effluent nitrite concentration. Meaning that the nitrite removal was higher than that of ammonium, which is expected according to Anammox Stoichiometry that establishes an amount of 1.32 mg NO_2^--N per gram of NH_4^+-N removed and 1.146 mg NO_2^--N per gram of NH_4^+-N (Strous et al., 1998; Lotti et al., 2014). In addition nitrate production in the system also decrease in terms of concentration, varying from average influent concentration of 1.4 ± 0.9 mg/L and effluent concentration of 4.4 ± 1.7 mg/L.

From the data (**table 5.1**) it can be seen that the SBTF-Anammox had an increase in nitrogen removal in phase II of experiment but phase I was more efficient in terms of NLR. The table below shows a summary performance of different parameters in $SBTF_{ANAMMOX}$ reactor.

Table 5. 2 Performance of SBTF $_{ANAMMOX}$

Parameter	Phase I	Phase II
Influent NH_4^+-N (mgN/L)	50.0 ± 7.6	22.3 ± 5.2
Effluent NH_4^+-N (mgN/L)	18.2 ± 12.8	2.1 ± 3.6
Influent NO_2^--N (mgN/L)	61.2 ± 10.5	22.0 ± 11.2
Effluent NO_2^--N (mgN/L)	29.7 ± 4.4	3.5 ± 2.1
NH_4^+-N removal (mgN/L)	31.8 ± 11.8	21.5 ± 4.0
NO_2^--N removal (mgN/L)	39.2 ± 17.5	27.4 ± 4.0
TN removal (mgN/L)	63.8 ± 26.3	45.2 ± 6.7
Influent NO_3^- N (mg N/L)	5.3 ± 9.9	1.4 ± 0.9
Effluent NO_3^- N (mg N/L)	12.6 ± 10.3	4.4 ± 1.7
NO_3^- N produced (mg N/L)	7.3 ± 4.4	3.7 ± 2.2
NO_2^-–N / NH_4^+-N	1.3 ± 0.3	1.4 ± 0.1
NO_3^-–N / NH_4^+-N	0.1 ± 0.2	0.1 ± 0.1
TN removal (%)	55.1 ± 20.7	81.9 ± 7.3
pH (influent / effluent)	8.4 ± 0.2/ 8.4± 0.2	8.2 ± 0.2 / 8.3 ± 0.2
Alkalinity Influent ($mgCaCO_3/L$)	532.3 ± 255.6	208.1 ± 47.2
Alkalinity Effluent ($mgCaCO_3/L$)	513.6 ± 245.4	210.9 ± 44
NLR ($kg\text{-}N/m^3.d$) applied in the system	2.7 ± 0.3	1.2 ± 0.2
TN removal ($KgN/m^3.d$)	2.0 ± 0.7	1.1 ± 0.3

5.1.2 Effect of operational parameters for nitrogen removal in the SBTF$_{ANAMMOX}$ reactor

5.1.2.1 DO

At the beginning of phase I the dissolved oxygen were a bit higher in the influent of Demineralised water, it reached a maximum concentration of 2.10 mg/L. This increase in DO concentration could influence the removal efficiency at the first days of the experiment, since the DO concentration above 1 mg/L can inhibit Anammox bacteria. After DO meter calibration and change in scavenger solution the dissolved oxygen in the influent Demineralised water, the system was able to achieve a certain stability along the time. Between days 60 to 100 the dissolved oxygen was not recorded in the system.

5.3.2 NLR

The NLR in this study was one of the key parameters controlled to assess the nitrogen removal in both reactors (SBTF$_{ANAMMOX}$ and SBTF$_{CANON}$). In the acclimatization period and phase I of SBTF$_{ANAMMOX}$ experiment the NLR was fixed to 2.7 kg-N/m^3.d and lowered to 1.2 kg-N/m^3.d in phase II; in the same period the influent substrate wastewater was lowered from 50 mgN/L for NH_4^+-N and 50 mgN/L for NO_2^--N to 25 mgN/L for NH_4^+-N and 25 mgN/L for NO_2^--N while the influent flow rate was kept the same in both experimental phases in the average of 24L/d**.** The reduction in the NLR in the system was made in order to assess whether the system increase the removal rate and consequently increase its removal efficiency.

As Chuang et al. (2008) found an increase in nitrogen removal one of the phases of your experiment using DHS reactor by lowering the influent loading from 5.96 kg-N/m^3.d to 2.98 kg-N/m^3.d and archieved a removal rate of 2.06 kg-N/m^3.d, and reduced the total influent wastewater substrate concentration from 160 mgN/L to 80 mg N/L, the system showed an increase in efficiency from 40% to 68%. Later the system experience a decrease in NLR and substrates concentration as well; from 1.94 N/m^3.d and 18 L/d, respectively, obtaining a good nitrogen removal efficiency of 95%. At this experimental period the system was operating with an effluent recirculation of 300%.

The other way around Sánchez-Guillén et al. (2015) working with two lab-scale CSTF, in which NLR was fixed as 2.15 ± 0.21 kg-N/m^3.d and 2.09 ± 0.9 kg-N/m^3.d, a total influent substrate concentration (NH_4^+-N and NO_2^-- N) of 102 mgN/L, reached a nitrogen removal rate within of 1.60 kg-N/m^3.d and total nitrogen removal efficiency of 78 ± 4% , respectively **(table.2).**

From the present study the SBTF$_{ANAMMOX}$ achieved a average total nitrogen removal in phase I of 68.6 ± 5.3 % and the removal rate attained by the system in both phases was 2.0 ± 0.7 kg-N/m^3.d and 1.1 ± 0.3 kg-N/m^3.d, respectively. Even with that average removal, the system reached a maximum removal of 77% in phase I.

In phase II after lower the NLR to half the reactor was shown an increase in removal efficiency; in day 218 the system reached the maximum removal efficiency (90%) for the entire operational phase. The system was operating without recirculation in both phases, then from this reason it can be suggested this system reached a higher removal rate and removal efficiency than those system described above when comparing the removal rates achieved in phase I of this experiment.

5.3.3 pH

As reported by Wang and Yang (2004), the optimum pH for Anammox bacteria is in a range between 7 to 8, without this range the activity of Anammox can be inhibited by or affecting its own growth or activity. In the phase I of the experiment the pH in the influent, effluent and over the profile of the system was recorded in the range of 7.90 to 8.75, corresponding to an average of 8.3 ± 0.2/ 8.3± 0.2. But, even with this range of pH the system was performing well in terms of removal efficiency. In the phase II a slight reduction in pH was noticed with an average of 8.2 ± 0.2 / 8.2 ± 0.2 (see table5.1). That reduction in pH could be one of the factors that enhanced the good removal performance (NH_4^+-N and NO_2^--N were 100% and 97%, respectively) in this phase.

5.3.4 Alkalinity

Since the conversion of ammonium take over HCO_3^- instead of carbon source under anoxic condition, the Anammox bacteria use disposable ammonium as an electron donor to convert nitrite to nitrogen gas (Van Dongen et al., 2001). In the system, the alkalinity does not have a significant variation. The system had at the beginning of phase I, an alkalinity concentration of around 800 mg CaCO3/L and at the end of phase I a reduction to an average concentration of 200 mgCaCO3/L, but this variation did not affect the removal efficiency. Most of time was the produced as expected according to the Anammox reaction.

From **figure 4.7**, it can be seen that same time a little bit of alkalinity, is consumed, that could be a reason of same inhibition of the Anammox activity in the system, creating an environment for nitrification in the system. This probably happen because of the oxygen intrusion via demineralised water and natural oxygen intrusion when during the times in which the reactor was opened for maintenance purpose.

5.2.3 Nitrogen removal in SBTF$_{CANON}$ reactor

During the operational period, the system was apparently stable with a slight increase in terms of NH_4^+-N and TN removal. At the beginning (starting from beginning of experiment, day 68) the reactor was showing a similar performance as reported in Sanchez-Guillen et al. (2015b)'s study, with average of $NH4^+$-N and TN removal of about 60-90%, and 40-50 %, respectively. But since day 90 the reactor showed a poor performance, as a result of a sudden change in flow rate.

As reported in table below, the system was operated with all the air points opened to enhance the oxygen diffusion indoor of the sponge layer; a large amount of ammonium was converted leading to high ammonium oxidation (nitrification) and also high Anammox activity. The effluent nitrate produced via Anammox bacteria was almost 3 times higher that the expected to be produced (**appendix G.2 and G.3**).

Apart from the reactor failure, the reactor exhibited a satisfactory removal rate if compared with a previously $SBTF_{CANON}$ reactor, which reached 54% of removal efficiency and a removal rate of 0.51 kg-N/m^3.d at applied NLR of 0.91 kg-N/m^3.d after almost 120 days of operation (Sanchez-Guillen et al, 2015a,b). The zig-zag configurantion was able to reach a average removal rate of 0.6 ± 0.1 kg-N/m^3.d after 90 days of operation and it reached a maximum removal rate of 1.21 kg-N/m^3.d at the applied NLR of 1.6 kg-N/m^3.d on day 68 (table 5.2).

5.2.4 Probable reason for the $SBTF_{CANON}$ failure

5.2.4.1 Operation conditions imposed in the system (decrease in NLR)

The $SBTF_{CANON}$ was operated for almost 3 years with diffents operational conditions and same configuration (horizontal layer). In this study the reactor changed from horizontal configuration to zig-zag and same parameters were changed, like: reactor superfice area and reactor volume, as a result of cutting the side of the sponge layer. That change had an impact in the HLR and HRT in the system. The theoretical HRT got likely shorter. Jayawardana.(2014) made tracer test in his study using the same reactor and found on that time an actual HRT of 2.96 h, later new operation conditions was imposed in the system as shown in table below.

Which reduction in HRT affect directly in the time do biologicaly reaction take in place, the time for bacteria to metabolise the substrate, as the HRT can directly affect the microbial community and consequently affecting the partial nitritation in the reactor (Rodriguez-Sanchez[1] et al., 2014).

From nitrogen balance data (**appendix G table G.2 ang G.3**), we can see that the reactor was producing less nitrite and the system experience a production of lot amount of nitrate.

Table 5. 3 $SBTF_{Previous\ CANON}$ and $SBTF_{CANON}$ operation conditions

Parameter	**Unit**	**(SBTF Previous CANON)**	**(SBTFCCANON)**
		Sanchez-Guillen et al. (2015b)	
Influent flow rate	L/d	16	16
Air inputs to sponge layers		Totally opened	Totally opened
Influent NH_4^+	mg-N/L	100	100
NLR	kg-N/m^3.d	(1.3-1.6)	1.6
Reactor surfice area	m^2	0.005	0.00388
Reactor volume	m^3	0.001025	0.000873
Nominal Hydraulic Retention Time (HRT)	h	1.5	1.3

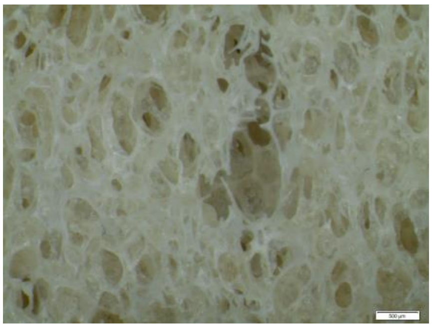
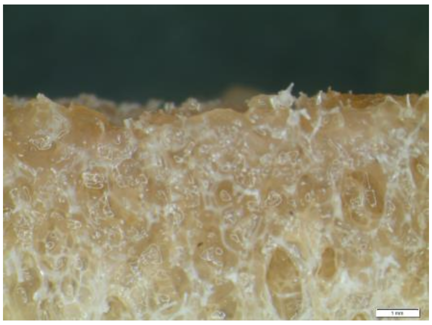
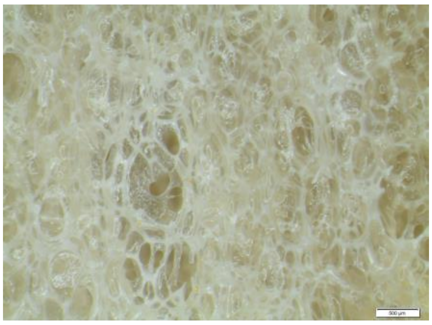
Parameter	**Unit**	**(SBTF Previous $_{\text{CANON}}$)**	**(SBTFC$_{\text{CANON}}$)**
		Sanchez-Guillen et al. (2015b)	
Hydraulic Loading Rate (HLR)	$m^3/m^2.d$	3.2	3.5
Temperature	˚ C	30	30
Duration	days	-	90

5.2.5 Precipitate formation and biomass removal

Apart from the change in operation conditions, the higher rate of biomass growth in the first layers of reactor, because of higher substrate availability in the upper part of layers in the system (Sanchez-Guillén et al., 2015ac) could be the reason for precipitation formation by accumulation of chemical (e.g. $CaCO_3$) and consequently originated the clogging followed by short-circuits in the system.

During the operation of the SBTF$_{\text{CANON}}$, an accumulation of white precipitants was observed at the upper part of the sponge layers. This finding could explain the substantial decrease in TN removal observed from day 90 **(Figure 4.5.3.e).** In this case, the accumulation of minerals tended to act as a physical barrier to the liquid percolation, and, consequently, to the microbial colonization of the inner part of the sponges. In addition, considering the sistematic clogging at the upper portions, the hydraulic retention time within the system was probably reduced, which contributes to the decrease in performance for N-removal. **Figure 5.2.5** shows the dense entrappment of precipitants at the upper part of the sponges and the weak colonization of the inner sponge.

After the significant decrease on N-removal (from 50 to 0%) a large amount of precipitates (and probably biomass) was removed from the upper part of the sponge layers (appendix**: figure B 1-2**) and the influent alkalinity concentration was reduced (from 600 to 250 mg$CaCO_3$/L) in order to provide a proper condition to the colonization of the inner sponge. However, the system had just a slight and unstable improvement on N-removal varying from 7 to 15% in approximately 25 days.

Position	**Upper sponge**	**Inner sponge**
1st layer (top)		
8th layer (middle)		

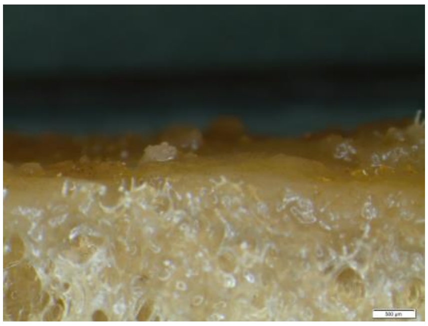
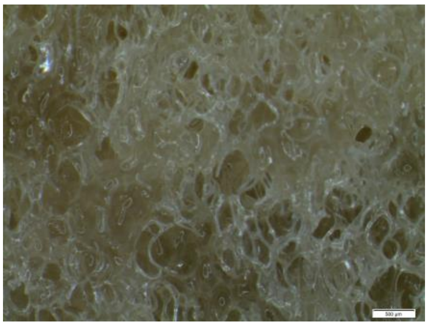
Position	**Upper sponge**	**Inner sponge**
15th layer (bottom)		

Figure 5.2.5 1 Visualization of upper and inner portions of the sponge layers (scale bars: 500µm).

5.2.6 Excess of oxygen and algae growth

The reactor was operated with opened inlet air points and some algae growth was visible in the system. Since the AOB and Anammox need relative lower amount of oxygen to do the convertion process, the oxygen was kept below 0.7 mg/L to carry out partial nitritation to enhance the Anammox bacteria transform in dinitrogen gas the nitrite converted AOB. Since the reactor was operating at fully opened environment without the control the air point in the system, probably the amount of oxygen that was entering in the system was realtively high and was created inhibition for AOB and Anammox bacteria, in favour of NOB. This can explain the high amount of nitrite produced in the system.

5.2.7 Life span of sponge

The system was operating continuously for more than 3 years, during the operation period the system was feced some clogging problems and short-circuits deriving by the precipitation and chemical accumulation in the sponge layers. Probably the sponge in the system already had reached the maximum retention capacity and had collapsed.

5.2.8 Lack of substrate

From the vertical reactor profile (**figure 4.5.1.1, 4.5.1.2 and 4.5.1.3**) it can be seen that lower part of reactor (L_3-L_4) was receiving lower substrate, thus creating dead zones in the system; almost 50-80 % of wastewater substrate was converted in the firt two layers; probably that situation was affecting and limiting the amount of substrate needed to the Anammox bacteria convert the NO_2^--N produced by AOB into N_2.

CHAPTER 6

Conclusions and Recommendations

Conclusions

The autotropic nitrogen removal was tested in this study and showed good results in terms of nitrogen removal using $SBTF_{ANAMMOX}$ reactor.

From this research is possible to conclude that:

$SBTF_{ANAMMOX}$

1. The $STBF_{ANAMMOX}$ reactor operated without effluent recirculation showed more efficiency in terms of amount of load removed at concentrated wastewaster than the diluted wastewater if compared the NLR applied in the system and removal rate attained. In the phase I, when the system was operating with concentrated wastewater, the NLR applied in the system was 2.7 ± 0.3 kg-N/m^3 and the removal rate attained in the system reached in an average of 2.0 ± 0.7 kg-N/m^3.d, in phase II the system was operated with diluted wastewater with an average NLR of 1.2 ± 0.2 kg-N/m^3.d and achieved a removal rate of 1.1 ± 0.3.Thus it suggests that this system is promising, more economical and efficient for nitrogen removal .

2. The higher removal rate conversion (1.1 kg-N/m^3.d) and efficiency (90%) in the $SBTF_{ANAMMOX}$ was observed at lower NLR (1.2 kg-N/m^3.d), probably meaning that the Anammox bacteria has more affinity to convert nitrogen in nitrogen gas at more diluted wastewater.The choice of a proper hydraulic loading rate is a sufficient operational strategy providing proper environment for bacteria growth by interaction between substrate supplied and the distribution of packing media.

3. Comparing the previously $SBTF_{ANAMMOX}$ reactor, who was operating in phase I with effluent recirculation at NLR of 2.09 ± 0.19 kg-N/m^3.d and attained a nitrogen removal rate of 1.60 kg-N/m^3.d it can be concluded that the $SBTF_{ANAMMOX}$ operating without effluent recirculation is more efficient and ecomonically viable.

SBTF$_{CANON}$

1. Based on data provided from the previously SBTF$_{CANON}$ (Sanchez-Guillen et al., 2015b), it can be concluded that the SBTF$_{CANON}$ reactor filled with sponge-bed medium, the arrangement of the polyurethane sponge slabs in 'zig-zag' configuration provides almost the same improvement in terms of nitrogen removal efficiency as the previously SBTF$_{CANON}$ filled with horizontal layers. The system was capable to remove 0.6 ± 0.1 kg-N/m^3.d when applied a NLR of 1.7 ± 0.2 1 kg-N/m^3.d during the 77 days that was operated against the 0.7 ± 0.3 kg-N/m^3.d removal rate reached in the previous study when applied a NLR of 1.6 ± 0.1 kg-N/m^3.d.

2. The probable reason for the SBTF$_{CANON}$ failure could be the life span of the sponge and reduction of the HRT due to the clogging problem registed at some times during the experiment. Since the sponge was receiving and accumulating continuously chemicals from the wastewater substrate, the sponge might have achieved its maximum retention capacity and collapsed.

Recommendations

1. $STBF_{ANAMMOX}$ is prommissing technology that could be recommended to apply for treatment anaerobically wastewater pre-treated in UASB system in development coutries.

2. In order to continue test the reactor performance in terms of removal efficiency in the $SBTF_{ANAMMOX}$ new phase has to be experienced by kepping the same substrate concentration and flow rate, lower a bit the HRT, and increase HLR and NLR.

3. Partial aerobic condition can be tested in the $SBTF_{ANAMMOX}$ system, to provide nitritation via AOB oxidation by provide air poros on top of reactor and stoping sparging nitrogen gas in demineralised water and influent substrates

4. Deep research has to be done to explain the limitation in ammonium conversion or increase in the ammonium in the effluent after reach 100% of conversion in $SBTF_{ANAMMOX}$.

5. To test in deph the performance of $SBTF_{CANON}$ in zig-zag configurantion for nitrogen removal efficiency, new experiment has to be run at similar operation conditions as applied in this sudy.

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Appendices

Appendix A : Nitrogen gas production

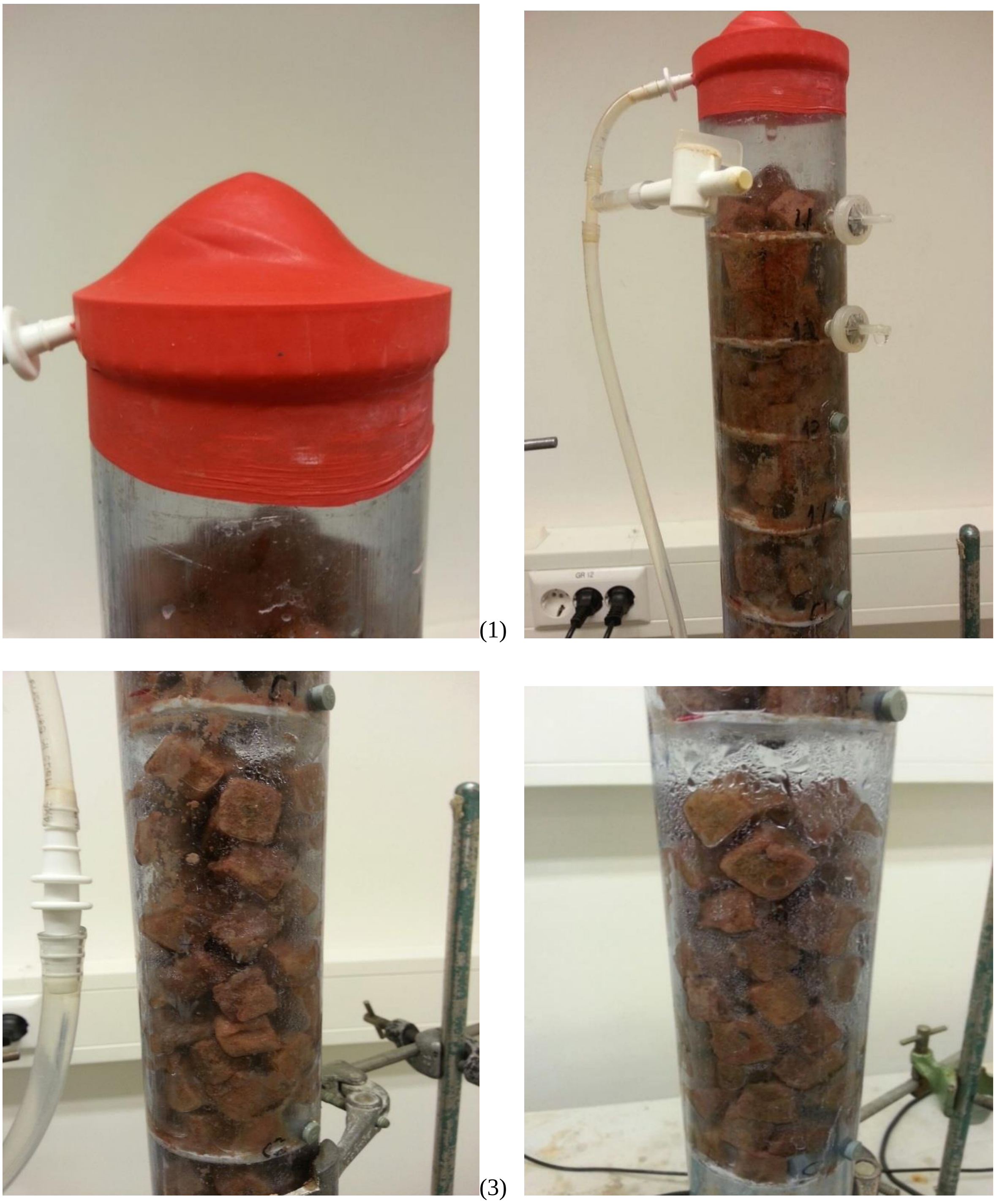
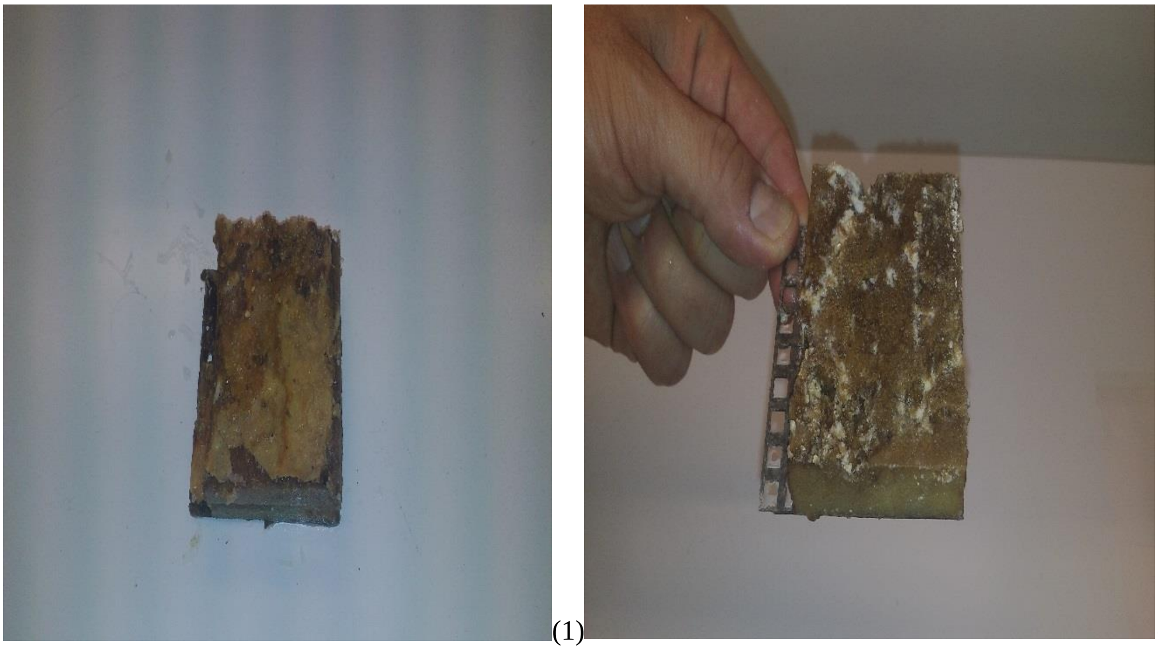

(1) (2) (3) (4)

Figure A.1-4 SBTF$_{ANAMMOX}$ gas production

Appendix B: Nitrogen balance in SBTF$_{ANAMMOX}$ reactor (phaseI)



(1) (2)

Figure B.1-2 Layer precipitate and biomass cleaning in SBTF$_{CANON}$. (1) before cleaning, (2) after cleaning gas production

(3)

Figure B.3 Growth of algae in SBTF$_{CANON}$ reactor around sponges plate

Appendice C: Nitrogen balance in SBTF$_{ANAMMOX}$ reactor (phaseI)

Table C.1 Daily NH4+-N removal

Days	Amount influent (mg/l)	Amount effluent (mg/l)	NH_4^+-N removal (mg/L)	NH_4^+-N removal (%)
0	43.1	35.8	7.3	22.7
7	52.3	43.8	8.6	25.1
15	50.5	43.6	6.9	20.0
22	54.1	47.2	6.9	21.9
23	51.9	43.0	8.9	14.0
36	51.6	31.7	19.8	50.1
37	49.9	28.1	21.8	49.6
41	49.8	22.9	26.9	54.4
43	53.0	23.6	29.4	63.5
47	59.2	23.3	36.0	61.9
104	47.0	3.3	43.7	82.9
106	50.5	4.3	46.2	100
112	58.0	17.6	40.4	100
113	49.2	8.4	40.8	100
118	40.0	0.0	40.0	100
121	39.2	0.0	39.2	100
126	43.5	0.0	43.5	100
132	29.4	0.0	29.4	100
134	48.3	0.4	47.9	99.2
138	33.7	7.0	26.7	79.1
140	47.8	13.4	34.3	71.9
142	45.8	15.3	30.5	66.6
146	42.8	15.9	26.9	62.8
152	46.4	15.6	30.8	66.9
154	59.9	19.1	40.8	70.9
159	61.5	14.3	47.1	72.2
167	63.2	21.1	42.1	68
168	65.8	23.2	42.6	58.7
173	57.6	19.7	37.9	81.8
174	49.9	16.0	33.8	69.4
183	54.2	16.0	38.2	80.9
184	55.4	12.9	42.4	86.6
187	49.0	17.5	31.5	71.3
191	47.8	15.0	32.8	76.6
195	45.4	15.1	30.3	51.5
197	55.0	21.1	33.9	63.1
Average	**50.0**	**18.2**	**31.8**	**70.8**
St.deviation	**7.6**	**12.8**	**11.8**	**9.2**

Table C.2 Daily NO_2^-- N removal (Anammox reactor)

Days	**Amount influent (mg/l)**	**Amount effluent (mg/l)**	**NO_2^--N removal (mg/L)**	**NO_2^--N removal (%)**
0	45.1	38.3	6.8	16.8
7	52.4	42.2	10.2	21.3
15	50.7	42.2	8.5	21.7
22	50.7	42.2	8.5	21.7
23	49.3	44.1	5.2	19.5
36	61.1	44.0	17.1	29.2
37	61.2	44.0	17.2	29.4
41	66.0	29.2	36.8	55.4
43	65.0	28.2	36.8	65.3
47	71.1	26.1	45.0	62.9
104	58.2	14.5	43.7	75
106	58.4	19.0	39.4	67.4
112	68.2	13.9	54.3	79.6
113	68.9	16.3	52.6	76.4
118	62.6	11.9	50.7	76.4
121	52.7	10.3	42.4	81
126	97.2	19.4	77.8	80.4
132	63.5	16.8	46.7	80
134	81.2	16.2	65.0	73.6
138	54.3	16.9	37.4	80
140	73.1	12.6	60.4	68.9
142	64.4	9.6	54.8	82.7
146	72.2	13.7	58.5	85
152	53.3	13.7	39.6	81
154	68.0	16.4	51.5	74
159	65.0	18.6	46.4	76
167	54.2	11.2	43.0	71
168	78.0	16.2	61.8	85.4
173	55.3	14.3	41.1	87.6
174	51.8	15.2	36.7	78.2
183	60.9	11.9	49.0	88.1
184	52.7	8.5	44.3	84.9
187	55.4	21.0	34.5	86.0
191	49.0	20.9	28.2	85.0
195	51.9	24.5	27.4	49.0
197	61.2	27.9	33.3	62.3
Average	**61.2**	**22.0**	**39.2**	**80**
St. deviation	**10.5**	**11.2**	**17.5**	**11**

Table C.3 Daily NO_3^--N production

Days	Amount expected to be produced (mg/l)	Amount produced by reactor (mg/l)	Gap in NO_3^-N concentration (mg/l)	NO_3^-N produced (%)
0	1.9	6.2	-4.3	2.4
7	2.2	10.7	-8.5	1.8
15	1.8	-0.7	2.5	-0.2
22	1.8	-2.7	4.5	-4.2
23	2.3	3.5	-1.2	10.0
36	5.2	2.5	2.7	3.7
37	5.7	3.7	2.0	4.9
41	7.0	6.1	0.9	6.0
43	7.6	8.7	-1.1	7.6
47	9.3	8.1	1.2	7.0
104	11.4	9.4	2.0	7.2
106	12.0	15.1	-3.1	9.3
112	10.5	9.8	0.7	7.5
113	10.6	7.9	2.7	7.0
118	10.4	18.7	-8.3	8.6
121	10.2	10.7	-0.5	7.8
126	11.3	12.6	-1.3	8.6
132	7.6	16.1	-8.5	8.3
134	12.5	11.5	1.0	7.5
138	6.9	13.4	-6.5	7.8
140	8.9	4.1	4.8	4.6
142	7.9	4.0	3.9	5.6
146	7.0	7.9	-0.9	9.0
152	8.0	2.7	5.4	6.0
154	10.6	6.2	4.4	9.6
159	12.3	6.6	5.6	9.1
167	10.9	7.5	3.4	9.7
168	11.1	5.4	5.7	9.3
173	9.9	8.3	1.6	9.7
174	8.8	5.8	3.0	9.3
183	9.9	4.5	5.4	5.8
184	11.0	5.9	5.1	7.3
187	8.2	3.6	4.6	6.9
191	8.5	7.6	0.9	8.1
195	7.9	6.9	0.9	8.1
197	8.8	4.6	4.2	6.8
Average	**8.3**	**7.3**	**1.0**	**6.8**
S. deviation	**3.1**	**4.4**	**4.0**	**3.0**

Appendice D: Stoichiometric ratios in $SBTF_{ANAMMOX}$ reactor (phase I)

Table D.1 Stoichiometric ratios

Days	**NO_2^--N/NH_4^+-N**	**NO_3^--N/NH_4^+-N**
0	1.05	0.46
7	1.00	0.91
15	1.00	0.78
22	0.94	0.17
23	0.95	0.00
36	1.18	0.08
37	1.23	0.08
41	1.33	0.08
43	1.23	0.05
47	1.20	0.06
104	1.24	0.08
106	1.16	0.02
112	1.18	0.06
113	1.40	0.07
118	1.57	0.08
121	1.34	0.08
126	2.23	0.05
132	2.16	0.11
134	1.68	0.08
138	1.61	0.11
140	1.53	0.10
142	1.41	0.07
146	1.69	0.02
152	1.15	0.04
154	1.13	0.00
159	1.06	0.01
167	0.86	0.00
168	1.19	0.01
173	0.96	0.00
174	1.04	0.01
183	1.12	0.06
184	0.95	0.04
187	1.13	0.03
191	1.03	0.04
195	1.14	0.04
197	1.11	0.04
Average	**1.3**	**0.1**
St. deviation	**0.3**	**0.2**

Appendice E: Nitrogen balance in SBTF$_{ANAMMOX}$ reactor (phaseII)

Table E.1 Daily NH_4^+-N removal

Days	Amount influent (mg/l)	Amount effluent (mg/l)	NH_4^+-N removal (mg/L)	NH_4^+-N removal (%)
199	35.3	13.1	22.2	-
201	16.8	1.3	15.5	-
202	15.1	2.4	12.8	-
204	19.2	1.8	17.4	91.0
206	19.7	3.3	16.4	83.2
208	21.8	0.1	21.7	99.8
216	22.3	0.2	22.0	99.0
218	23.8	0.2	23.6	92.5
219	24.9	0.5	24.4	98.0
220	20.6	0.2	20.4	99.2
222	26.3	0.3	26.0	98.7
Average	**22.3**	**2.1**	**21.5**	**95.2**
St. deviation	**5.2**	**3.6**	**4.0**	**5.5**

Table E.2: Daily NO_2^--N removal

Days	Amount influent (mg/l)	Amount effluent (mg/l)	NO_2^- -N removal (mg/L)	NO_2^--N removal (%)
199	39.3	9.5	29.7	-
201	24.6	3.7	21.0	-
202	22.3	4.1	18.2	-
204	28.3	4.2	24.0	85.0
206	27.7	3.6	24.1	87.0
208	27.0	1.7	25.3	93.6
216	29.3	2.2	27.2	92.5
218	33.8	1.0	32.7	93.5
219	31.8	2.5	29.3	92.3
220	31.6	3.1	28.5	90.2
222	30.8	2.6	28.1	91.5
Average	**29.7**	**3.5**	**27.4**	**90.7**
St.deviation	**4.4**	**2.1**	**4.0**	**2.9**

Table E.3 Daily NO_3^--N produced

Days	Amount expected to be produced (mg/l)	Amount produced by reactor (mg/l)	Gap in NO_3^- -N concentration (mg/L)	NO_3^--N produced (%)
199	5.8	4.5	1.3	7.0
201	4.0	-2.5	6.5	-19.4
202	3.3	0.5	2.8	4.0
204	4.5	3.9	0.7	9.9

206	4.3	3.2	1.1	7.7
208	5.7	4.2	1.5	7.6
216	5.7	6.3	-0.6	8.7
218	6.1	3.6	2.6	7.5
219	6.4	2.5	3.9	5.9
220	5.3	3.1	2.2	6.7
222	6.8	3.1	3.7	6.5
Average	**5.6**	**3.7**	**1.9**	**7.6**
St.deviation	**1.0**	**2.2**	**1.8**	**7.8**

Appendice F: Stoichiometric ratios in SBTF$_{ANAMMOX}$ reactor (phase II)

Table F.1 Daily Stoichiometric ratios

Days	NO_2^--N/NH_4^+-N	NO_3^--N/NH_4^+-N
199	1.11	0.05
201	1.47	0.22
202	1.47	0.05
204	1.47	0.00
206	1.41	0.05
208	1.24	0.06
216	1.32	0.04
218	1.42	0.05
219	1.27	0.07
220	1.53	0.07
222	1.17	0.06
Average	**1.4**	**0.1**
St. deviation	**0.1**	**0.1**

Appendice G: Nitrogen balance in SBTF$_{CANON}$ reactor (phase I)

Table G.1 Daily NH_4^+-N removal

Days	Amount influent (mg/l)	Amount effluent (mg/l)	NH_4^+-N removal (mg/L)	NH_4^+-N removal (%)
1	111.3	41.55	69.7	27.7
5	111.9	30.2	81.7	36.2
7	116.4	34.4	82.0	33.7
11	121.0	25.21	95.8	47.2
68	128.0	44.8	83.2	50.4
70	95.9	28.8	67.1	46.1
75	102.9	10.3	92.6	49.1
77	100.7	19.0	81.7	41.9
Average	**111.0**	**29.3**	**81.7**	**41.5**
St.deviation	**10.1**	**10.6**	**9.2**	**7.7**

Table G.2 Daily NO_2^--N production

Days	Amount produced by reactor (mg/l)	NO_2^--N expected to be produced (mg/L)	Gap in NO_2^--N concentration (mg/L)	NO_2^--N produced (%)
1	15.62	36.6	20.9	15.6
5	16.9	47.8	30.9	16.9
7	16.1	44.5	28.4	16.1
11	15.5	62.3	46.8	15.5
68	15.2	66.5	51.3	15.2
70	0.0	60.9	60.9	0.0
75	22.5	64.8	42.3	22.5
77	20.4	55.3	34.9	20.4
Average	**15.3**	**54.8**	**39.5**	**15.3**
St.deviation	**6.3**	**10.1**	**12.3**	**6.3**

Table G.3 Daily NO_3^--N production

Days	Amount produced by reactor (mg/l)	NO_3^--N expected to be produced (mg/L)	Gap in NO_3^--N concentration (mg/L)	NO_3^--N produced (%)
1	26.4	7.2	10.2	26.4
5	28.6	9.4	19.2	28.6
7	32.2	8.8	12.3	32.2
11	33.1	12.3	29.2	33.1
68	17.6	13.1	48.9	17.6
70	21.0	12.0	39.9	21.0
75	21	12.8	43.8	21.0
77	19.4	10.9	35.9	19.4
Average	**24.9**	**10.8**	**29.9**	**24.9**
St.deviation	**5.6**	**2.0**	**13.7**	**5.6**

Appendice H: Stoichiometric ratios in SBTF$_{CANON}$ reactor (phase I)

Table H.1 Stoichiometric ratios

Days	NO_2^--N/NH_4^+-N	NO_3^--N/NH_4^+-N
1	-	-
5	-	-
7	-	-
11	-	-
68	0.2	0.2
70	0.0	0.3
75	0.2	0.2
77	0.2	0.2
Average	**0.2**	**0.2**
St.deviation	**0.1**	**0.0**