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Master's Thesis

Nitrogen removal from municipal wastewater by Partial Nitritation/Annamox process

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Abstract

The removal of nutrients, such as nitrogen, from municipal wastewater is fundamental for a sustainable urban development since it prevents a well-known phenomenon named as eutrophication. Mainstream Partial Nitritation/Anammox, also known as Mainstream Deammonification, is a promising technology for future water purification that aims to remove nitrogen from wastewater in order to prevent the eutrophication. It is less costly than the traditional nitrification/denitrification process and it heads towards the direction of converting the WWTPs from energy consuming into energy producing facilities.

This Master's thesis consists in a study project regarding the nitrogen removal from mainstream wastewater. It was conducted at Hammarby Sjöstadsverk that is a research facility in the area of the Henriksdal Waste Wastewater Treatment Plant in Stockholm. Three studies were developed. The main one had the purpose to evaluate the process performances of a biological pilot reactor used for Mainstream Deammonification. This evaluation was addressed to comprehend how the pilot reactor works at different operational conditions. The remaining studies analysed the progress of the pilot reactor in relation to different factors and to the settling properties of the activated sludge used in the process.

It was found that the process performances improved by changing the aeration pattern from 40 to 50 minutes for non-aeration time and from 20 to 10 minutes for aeration time and by increasing the dissolved oxygen set-point from 0.6 to 1.0 mg/L. The enhancement of the performances consisted in an inhibition of nitrite oxidizing bacteria and rise of the total nitrogen removal efficiency. In addition, anammox biofilm was observed to grow on the carriers and it was discovered that the activated sludge had very low settling properties.

Keywords

Mainstream; Deammonification; IFAS; UASB; Anammox, Anoxia, Aeration, Online; Inhibition.

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It is a real pleasure to thank Professor Elzbieta Plaza that gave me the opportunity to work on such an interesting argument as the Mainstream Partial Nitritation/Anammox is. This Master's thesis project allowed me to become aware of a hot topic that is the cornerstone of several ongoing researches worldwide and I am honoured that my work will contribute in broadening the knowledge around it. I also need to thank Professor Barbara Ruffino that was always willing to give me useful advices and clarify my doubts.

I also want to thank Dr. Jozef Trela, that devoted his time for teaching and supporting me on the field, and all my colleagues at Hammarby Sjöstadsverk that have always been very kind whenever I asked for help. In particular, I would like to express my gratitude to Gabriel Persson and Jingjing Yang, for sharing with me their experience concerning the ananmmox, and Mayumi Narongin, Niclas Bornold, Jesper Karlsson and Mila Harding, for helping to solve any practical problem that I came across on site.

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Summary in English

Water is an essential element for all the life forms and it needs to be preserved. The urban development constitutes a threat for its quality and usability if the correct measures of protection are not put in place. The wastewater treatment plants represent the public facilities that have the function of treating the municipal and industrial wastewater by removing organic matter, nutrients and hazardous pollutants in general before discharging it into the waterbodies. A High concentration of nitrogen in the water is dangerous for the life of the aquatic ecosystems because it causes eutrophication. Nowadays the most commonly used technologies to remove nitrogen from the wastewater are based on the nitrification/denitrification process. It is a two-stage biological process where nitrogen is removed by specific groups of bacteria through biochemical reactions. It is a successful technology that allows to reach very low concentrations of nitrogen in the outflow, but it is not exempt from costs. Therefore, other processes are under study in order to achieve similar, or higher, nitrogen removal efficiencies but in a less costly way.

Mainstream Partial Nitritation/Anammox is now gaining the interest of researchers and scientists worldwide because of its economic advantages. The feasibility of the deammonification, as sidestream process, has already been demonstrated, but it does not still meet that economic efficiency that would allow to revolutionise the concept of wastewater treatment plant into an energy generating facility. There are still costs to cut, especially related to the high temperature involved in process. The Mainstream Deammonification at low temperature might do the trick instead and different processes based on it are nowadays investigated, but many challenges have still to be overcome. This Master's thesis starts to examine what has been discovered so far about the Mainstream Partial Nitritation/Anammox, from the bacterial groups that perform the chemical reactions to the reactor types, process factors and operational strategies that condition the process.

Successively, an individual study project is introduced. It was carried out on a two-stage pilot system composed of a UASB reactor for carbon removal and of an IFAS reactor for nitrogen removal. The project was conducted from October 16th to February 2nd and it was divided into three studies, but first the initial performances of the reactor were checked (Initial state analysis).

Study 1 analysed the progress of the process performances by applying different operational strategies. First the aeration pattern was changed. Non-aeration time was increased from 40 to 50 minutes and the aeration time was decreased from 20 to 10 minutes. These caused the inhibition of the nitrite oxidizing bacteria. Consequently, the ammonium conversion to nitrate stopped and the ammonium removal efficiency and the total nitrogen removal efficiency overlapped. This meant that all the ammonium, that was removed, actually left the system as gaseous nitrogen. The process performances increased when the dissolved oxygen set-point was increased from 0.6 to 1.0 mg/L and the total nitrogen removal reached an average value of 34.7%. When the dissolved oxygen was increased to 1.4, the total nitrogen removal efficiency increased even more, with a peak of 41%, but the NOB reactivated.

Study 2 was focused on the correlation between performances and parameters and factors (pH, alkalinity, $sCOD_{in}/NH_4\text{-}N_{in}$ ratio, TSS, SRT) were analysed. The $sCOD_{in}/NH_4\text{-}N_{in}$ ratio revealed that the UASB did not work effectively. Study 3 concerned the settling properties of the activated sludge that was used in the process. The settling tests showed that the sludge had very low sedimentation properties because the Sludge and the Stirred Sludge Volume Index have always been much higher than the thresholds for good settleability . This means that it was a poorly settling sludge.

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Abbreviations

Alk	Alkalinity
Alk cons	Alk consumption
ANAMMOX	ANaerobic AMMonium OXidation
AnAOB	Anammox Bacteria
AOB	Ammonium Oxidizing Bacteria
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FA	Free Ammonia (HN_3)
FNA	Free Nitrous Acid (HNO_2)
HB	Heterotrophic Bacteria
HRAS	High Rate Activated Sludge
HRT	Hydraulic Retention Time
IFAS	Integrated Fixed-Film Activated Sludge
MBBR	Moving Bed Biofilm Reactor
MD	Mainstream Deammonification
NH_4-N	Nitrogen in Ammonium form
NH_4-N_{rem}	Ammonium removed
NH_4-$N_{rem,eff}$	Ammonium removal Efficiency
NO_2-N	Nitrogen in Nitrite form
NO_3-N	Nitrogen in Nitrate form
NOB	Nitrite Oxidizing Bacteria
NO	Nitric Oxide
N_2O	Nitrous Oxide
PN/A	Partial Nitritation/Anammox
Q	Flow Rate
SBR	Sequencing Batch Reactor
SD	Sidestream Deammonification
SRT	Sludge Retention Time
SS	Suspended solids
TN	Total Nitrogen
TN_{rem}	Total Nitrogen removed
$TN_{rem,eff}$	Total Nitrogen removal Efficiency
$TN_{load,rate}$	Total Nitrogen loading rate
$TN_{rem,rate}$	Total Nitrogen removal rate
TSS	Total Suspended Solids
UASB	Upflow Anaerobic Sludge Blanket Reactor

V	Volume
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WWTP	Waste Water Treatment Plant

1. Introduction

Nitrogen removal from domestic and industrial wastewater performed at municipal wastewater treatment plants (WWTPs) permits to reduce the nitrogen loads discharged in waterbodies that are by now acknowledged to be a serious threat for the life of the water ecosystems. Most of full-scale plants are based on the traditional nitrification-denitrification process that is relatively costly. This process implies a few expenses related to large basin volumes, high capacity pumps, operation and maintenance, energy use and external sources of alkalinity and carbon. In addition, since the denitrification process needs organic matter as carbon source, the production of biogas, that is based on organic matter removal, cannot be maximized through the regular sludge handling processes (Malovanyy et al., 2015).

This is why new cost-effective techniques are currently investigated in order to convert the existing WWTPs from energy-consuming into energy-producing facilities (Wang et al., 2016 see Feng et al., 2017). However, it is hard to find the most suitable method for nitrogen removal since there are many factors that need to be considered such as nitrogen discharge concentration limits, existing infrastructures, characteristics of the influent wastewater and others (Han at al., 2016).

A special attention is given to the Partial Nitritation/Anammox (PN/A) process. This is considered as a new promising technology that is already successfully implemented as sidestream process (Sidestream Deammonification, SD), but it is also studied in order to be applied directly as mainstream process (Mainstream Deammonification, MD) (Feng et al., 2017). The PN/A process is an autotrophic nitrogen removal method and combines two biochemical reactions that are partial nitritation and anammox. It has several important advantages compared to the conventional nitrification/denitrification:

- The partial oxidation of ammonium to nitrite reduces the oxygen demand up to 60%(Cao et al., 2017);
- It does not require external carbon sources (Cao et al., 2017);
- It decreases the amount of excess sludge up to 80% (Cao et al., 2017);
- It does not require additional alkalinity for maintaining stable buffering conditions and pH (Sandino et al., 2016);
- It reduces the emissions of greenhouse gases such as carbon dioxide

(CO_2) by 90% and nitrous oxide (N_2O) that is produced very little (Feng at al., 2017).

The purpose of this Master's thesis is to study the process performances of a biological pilot reactor for nitrogen removal based on the Mainstream Deammonification. This reactor was located at Hammarby Sjöstadsverk that is a research facility in Stockholm near the Henriksdal Wastewater Treatment Plant. This thesis starts from analysing the initial state of the reactor. Afterwards, different studies are described and their results are discussed.

2. Background

High concentrations of nitrogen in aquatic and terrestrial environments represent a hot topic in the natural resources management worldwide. The presence of nitrogen is due to natural sources, like forests and moist, but especially to human activities and anthropic sources such as usage of nitrogen-based fertilizers and farmland run off, emissions from residential and industrial buildings, wastes from slaughterhouses, livestock sludge treatments, factories for yeast, meat processing, fish canning and dairies (Zekker at al., 2011).

Excess of nitrogen and other nutrients, such as phosphorus, is responsible of a very serious problem known as eutrophication. This is a phenomenon that occurs in both fresh and sea water and it consists in a massive algae blossom on the water surface because of the great availability of these nutrients in the water. When algae die, an aerobic decomposition process takes place and it deprives the water of oxygen. Therefore, animals and fish either die or abandon the area.

Eutrophication has also other consequences like the worsening of water quality and the increase of potential risk for humans' life. The water in fact becomes unsuitable for recreational purposes and human consumption. In addition, nitrate-rich drinking water makes the conversion from nitrate to nitrite occur rapidly in the stomach that can cause a reduction in the oxygen-carrying capacity of the blood (European Environmental Agency, 2016). Furthermore, nitrogen has a high solubility which makes it easily transportable by both ground and surface waters (Du et al., 2016).

These are the main reasons why several countries have been applying stricter and stricter regulations in order to reduce the discharge of nitrogen and have been putting great efforts in the optimization of nitrogen and organic matter removal technologies.

3. Aims and Objectives

The main aim of the Master's thesis is to evaluate the process performances of a biological pilot reactor that was used to perform a MD process on actual municipal wastewater. This evaluation is divided into three studies that focus on different aspects. The first study investigates how the system reacts at different operational conditions and estimates the process performances in relation to a few process parameters related to the aeration and temperature in the reactor. The second study concerns the influence of different process parameters and physical and chemical factors on the process performances. The third study evaluates the settling properties of the activated sludge used in the process and try to assess their weight on the process performances.

This goal will be pursued through the following intermediate objectives:

- Enhance the author's personal knowledge about Mainstream Partial Nitritation/Anammox through an accurate literature review, that will focus on several studies and projects that were carried out worldwide with different process technologies and having different goals (implementation of different strategies to improve the process performances, analysis of the bacterial groups taking part in the process, evaluation of the physical and chemical factors that play a role in process and more);
- Study what have been achieved so far through the pilot reactor under consideration by reading the previous reports and thesis of students and professionals that already worked on it;
- Draw up an operational program that will cover all the aspects that need to be considered while working with the reactor (daily checks, chemical analyses, calibration and cleaning of the and more);
- Check the process performances of the pilot reactor at the initial state of the study ("Initial state analysis");
- Select three studies in order to evaluate the process performances of the pilot reactor;
- Analyse how the process performances will vary by applying different operational strategies ("Study 1");

- Analyse how the chemical and physical factors will affect the process performances ("Study 2");
- Perform sedimentation tests in order to comprehend the settling properties of the activated sludge used in the process, since they also influence the performances of the process ("Study 3").

4. Thesis Outline

The first part of the thesis presents a literature review. This is described in chapters 5th and 6th. First of all, the biochemical reactions that take place in the PN/A process are described and compared with the ones that are performed in a traditional nitrification/denitrification process. The bacterial groups, that are responsible of these biochemical reactions as part of their metabolic activity, are then discussed. Their description is focused on their physical and chemical characteristics and especially on the multifactorial competition that exists between these bacteria. Successively, the deammonification technologies are described. First the Sidestream Deammonification is briefly introduced and then the Mainstream Deammonification is thoroughly explained. The MD description starts to analyse different processes and reactor types, then it introduces the physical and chemical factors that play a role in the mainstream processes and it ends with exposing a list of different operational strategies that are applied to improve the process performances of reactors performing Mainstream PN/A.

The second part of the thesis opens with the description of the pilot reactor that was used to conduct an individual project on the MD process. From this point forward the thesis focuses on the project only. The pilot reactor and the detailed methodology followed during the whole project are exposed in chapter 7th. The project was carried out in two phases. The first phase considered the initial state of the pilot reactor. It was important to know the current situation and the process performances at the beginning of the study. The second phase is divided into three studies. Study 1 analyses how the process performances improved after the application of some operational strategies and on the growth of the anammox bacteria involved in the process. Study 2 focuses on how chemical and physical factors and process parameters influenced the process performances. Study 3 evaluated the settling properties of the activated sludge used in the process. In chapter 8th, all the results achieved during the project are discussed and compared with the literature review. Chapter 9th exposes the conclusions related to each study and the general ones. Further calculations, data and operational schedules are reported in the appendixes at the end of this report.

5. Partial Nitritation/Anammox - Deammonification

Partial nitritation and anammox are two consecutive biochemical reactions that take place in the deammonification process. In the first reaction (Eq. (1)) a part of ammonium, as electron donor, is aerobically oxidized to nitrite by chemo-litho-autotrophic ammonium oxidizing bacteria (AOB) using carbon dioxide (CO_2) and bicarbonates (HCO_3^-) as both inorganic carbon and energy sources. Successively, the nitrite, produced in the previous reaction, as electron acceptor (Gustavsson, 2010), and the rest of ammonium, as electron donor, are transformed into nitrogen gas by anammox bacteria (AnAOB) under anoxic conditions (Eq. (2)). The following equations represent the reactions previously described (Cao et al., 2017):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (1)$$

$$NH_4^+ + 1.32NO_2^- + H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O \quad (2)$$

Eq. (3) represent the overall chemical reaction:

$$NH_4^+ + 0.88O_2 \rightarrow 0.11NO_3^- + 0.44N_2 + 1.14H^+ + 1.43H_2O \quad (3)$$

Commonly, nitrogen is removed from wastewater through another two-step biological process that consists in nitrification followed by denitrification. Nitrification is also composed of two reactions: nitritation, in which ammonium is converted to nitrite, as for PN/A, by AOB, usually Nitrosomonas, and nitratation, where nitrite is oxidized to nitrate by nitrite-oxidizing bacteria (NOB), which are mainly Nitrobacter (Cao et al., 2017).

In the second reaction nitrate (or nitrite directly), as electron acceptor, is reduced to gaseous nitrogen under anoxic conditions by heterotrophic bacteria, usually Pseudomonas, using organic matter as carbon and energy source. Secondary gaseous products, like nitric oxide (NO) and nitrous oxide (N_2O), are usually produced as well (Feng at al., 2017).

By converting the nitrogen removal process from nitrification/denitrification to partial nitritation/anammox, important advantages are obtained: there is no need to add external organic matter to achieve nitrogen removal and alkalinity to maintain stable pH (Sandino et al., 2016), less aeration is required and sludge production is reduced. These advantages would make the operational costs decrease of 60% (figure 1). In addition, nitrogen removal through deammonification reduces greenhouse gasses emissions by 90% because there is very little formation of N_2O (Hu et al., 2010 see Feng et al., 2017).

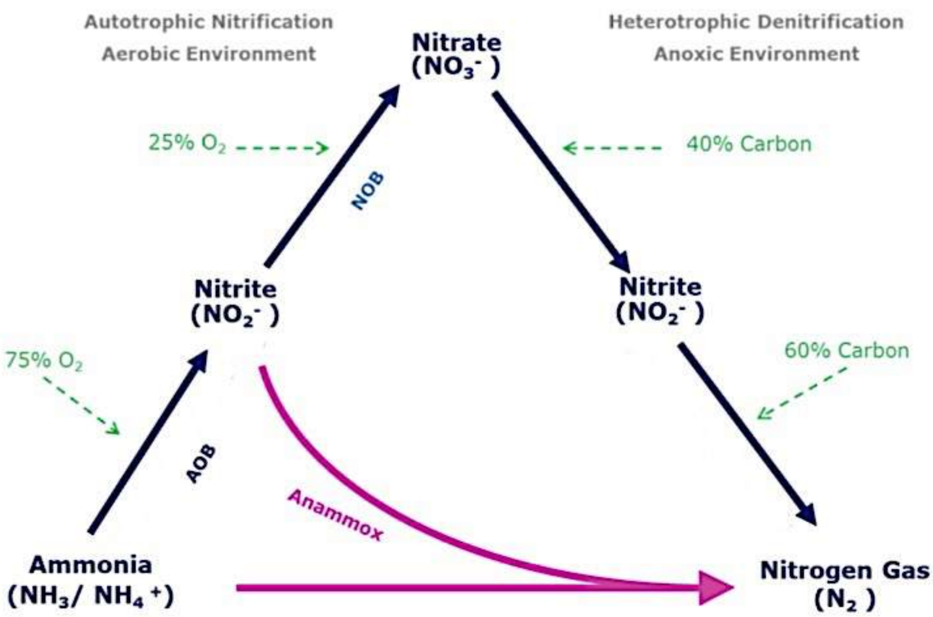

Figure 1: Nitrogen removal pathways: conventional nitrification/denitrification (blue) and partial nitritation/anammox (purple) (modified after Watson et al., 2016)

5.1 Microbial Communities Involved

There are four main groups of bacteria that are involved in the PN/A process:
Ammonium oxidizing bacteria (AOB);
Nitrite oxidizing bacteria (NOB);
Anammox bacteria (AnAOB);
Heterotrophic bacteria (HB).

As figure 2 shows, these groups compete for different substrates: AOB, NOB and HB compete for oxygen, AOB and AnAOB for ammonium. NOB, HB and AnAOB also compete for nitrite that is usually a process limiting factor, therefore its absence has a great impact on the whole process (Cao et al., 2017). However, the predominance of one group over the other depends on several factors (Feng et al., 2017).

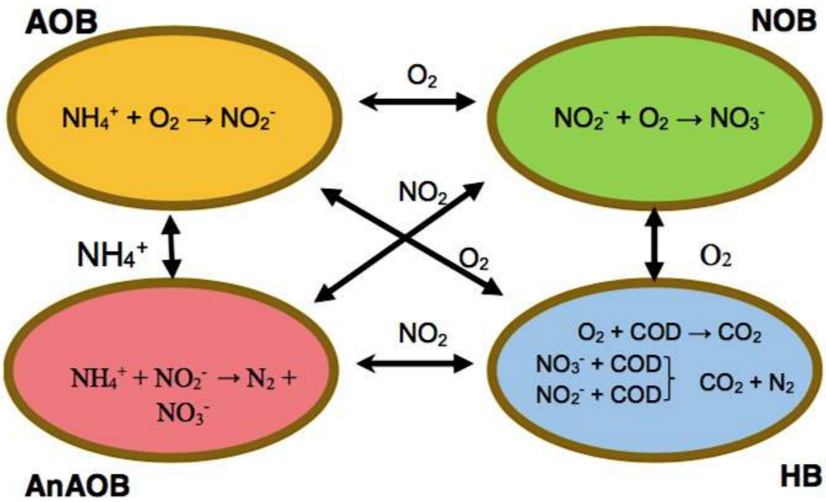

Figure 2: Competition among the bacteria involved in the PN/A process (Cao et al., 2017)

5.1.1 Ammonium Oxidizing Bacteria

AOB belong to the phylum of Proteobacteria and they are divided into five genera: Nitrosomonas, Nitrosospira, Nitrosovibrio, Nitrosolobus and Nitrosococcus (Feng et al., 2017), among which Nitrosomonas and Nitrospira are the most abundant in municipal wastewater (Ge et al., 2015).

AOB are aerobic chemo-litho-authotrophic bacteria that use ammonium and oxygen as substrates (NH_4-N is the electron donor and O_2 the electron acceptor) and carbon dioxide as carbon source in order to produce nitrite. The optimal temperature range for growing is 25-30 °C and the optimal pH range is 7-8.5 (Jaroszynski et al., 2011 see Feng et al., 2017). AOB activity can be influenced by different factors: temperature (see section 6.2.3), free ammonia and free nitrous acid concentrations (see sections 6.2.5 and 6.3.2), reactor types, sludge retention time (see sections 6.2.7 and 6.3.3) and others.

5.1.2 Nitrite Oxidizing Bacteria

NOB are also aerobic chemo-litho-authotrophic bacteria that belong to seven genera divided into four phyla: Proteobacteria (Nitrobacter, Nitrotoga, Nitrococcus), Nitrospirae (Nitrospira), Nitrospinae (Nitrospina, ‘Candidatus Nitromaritima’) and Chloroflexi (Nitrolancea) (Feng at al., 2017). They have a lower optimal pH range compared to AOB: 6 - 7.5 (Yin et al., 2016 see Feng et al., 2017). They use nitrite as electron donor and oxygen as electron acceptor to produce nitrate. Nitrospira and Nitrobacter have always been considered the dominant genera in wastewater, but recent studies state that Nitrospira and Nitrotoga are the ones that actually predominate (Saunders et al., 2016). However, the kinetic characteristics of Nitrobacter are still used to represent NOB in general. Nitrospira are K-strategist bacteria, while Nitrobacter are r-strategists. This means that the former has a lower growth rate and higher affinity with the substrate, whereas the latter has a higher growth rate and a lower affinity with the substrate. Therefore, Nitrospira predominate under condition of substrate scarcity, while Nitrobacter dominate at high concentrations of substrate (Cao et al., 2017).

The competition between AOB and NOB depends on several factors and the temperature is one of the most important ones (see section 6.2.3). NOB usually predominate at cold temperature, while AOB dominate in warm waters. This is problematic because the purpose of using MD is to remove nitrogen at low temperature in order to save costs. That is why several strategies (see section 6.3) have been investigated in order to achieve NOB suppression and wash-out at low temperature without compromising AOB's activity.

5.1.3 Anammox Bacteria

AnAOB are strictly anaerobic chemo-authotrophic bacteria that belong to the Planctomycetales phylum. They are divided into five genera and thirteen species (Feng et al., 2017), but while NOB usually co-exist, AnAOB tend to compete each other since one species of them only is usually found in the biological reactors (Vilpanen, 2017).

Anammox bacteria have an optimal temperature and pH range of 30-35 °C (Jetten et al. 2001 see Cao et al., 2017) and 7.5 – 8.0 (Magrì et al. 2013 see Cao et al., 2017) respectively. Despite this temperature range, previous studies demonstrated a relevant activity at 12 and 10 °C in anammox (Hendrickx et al., 2014 see Cao et al., 2017) and deammonification reactors (Lotti et al. 2014). However, as the temperature decreases, the growth rate becomes slower too and consequently nitrite accumulates. This was observed by Dosta et al. (2008) (see Cao et al., 2017) at 15°C and by Lackner et al. (2015) (see Cao et al., 2017) while studying anammox activity from 13 to 10°C.

Lotti at al. (2015) observed that the history of the sludge, where the AnAOB grow, matters too: anammox sludge increases its growth rate once it has adapted to low temperature. According to Hendrickx et al. (2014) (see Cao et al., 2017), biomass concentration is another parameter to consider.

AnAOB showed to be less affected by temperature drops if growing as biofilm. Gilbert et al. (2015) (see Cao et al., 2017) observed a stable activity of anammox bacteria in biofilm when temperature decreased from 20 to 10°C, whereas a significant decrement in biomass activity occurred in a suspended sludge when temperature dropped from 25 to 12°C (Hu et al., 2013). Furthermore, Laureni et al. (2016) showed that when temperature raised from 10 to 13 °C, anammox bacteria recovered much faster in the biofilm than in the sludge. These results clearly demonstrated the benefits of biomass immobilization.

The main problem with anammox bacteria is that they have a slow growth rate. This causes a process start-up time of several months that is usually reduced by inoculation of activated sludge already stabilized at the process conditions. Anammox bacteria grow much slower than the nitrifiers: it was observed that they have a maximum growth rate which is ten times lower than AOB and NOB (Gustavsson, 2010). Furthermore, too high nitrite concentrations are toxic for the anammox bacteria (Zekker et al., 2016; Gustavsson, 2010). Zekker et al. (2016) found out that 50% of the biomass activity decreased at different nitrite concentrations depending on the reactor type. The lowest inhibiting concentration was equal to 85 mg/L and was obtained in a batch test conducted in a Moving Bed Biofilm reactor (MBBR, see section 6.1.6). The level of toxicity also depends on sizes of the flocs and acclimation periods. In addition, anammox bacteria are also inhibited by methanol (Gustavsson, 2010).

Interestingly, some AnAOB species can accomplish oxidation of volatile fatty acids (VFA). They can oxidize acetate and propionate by using NO_3 as electron acceptor. The advantage of this mechanism is that in case of NO_2 unavailability, anammox bacteria can remove nitrate. Anammox bacteria also produce a lower quantity of sludge compared to heterotrophic denitrifiers (Vilpanen, 2017).

5.1.4 Heterotrophic Bacteria

Most of heterotrophic bacteria living in the wastewater belong to the Proteobacteria phylum (Kraft et al., 2011). They are usually facultative aerobic bacteria, which means that they consume oxygen for respiration if this is available, but under anoxic conditions other substrates such as sulphate, nitrate and sulphur, are used (Vilpanen, 2017).

HB correspond to 50% of bacterial population in a biological reactor and their abundancy is difficult to reduce. They are active even in total absence of external carbon dosage because they can take carbon from products released by bacterial endogenous decay (Kindaichi et al., 2004 see Cao et al., 2017). It is important to inhibit their activity since they out-compete with AnAOB for nitrite in anoxic environment (Sandino et al., 2016). The ratio influent COD/N is the key factor to control the HB growth (Han et al. 2016). For suspended sludge systems, a value lower than 3 g sCOD/g NH_4-N is usually considered acceptable and it can be achieved through a carbon removal pre-treatment process. Biofilm and hybrid systems can tolerate higher values since heterotrophic bacteria can be washed out together with NOB through short sludge retention times (SRT, see section 6.2.6) while the anammox bacteria, that are growing on carriers, remain inside the reactor (Cao et al., 2017)

Some HB have a positive effect on process performances. They can use part of the nitrate produced by NOB, as electron acceptor to degrade fermentation products, and reduce it to nitrite that can be used later by AnAOB (Speth et al., 2016 see Cao et al., 2017).

5.2 Sidestream Partial Nitritation/Anammox

Deammonification is currently applied as sidestream treatment to remove nitrogen from ammonium-rich wastewater that has a low COD/NH_4-N influent ratio (Cao et al., 2017). Figure 3 shows an example of a wastewater treatment plant operating with a conventional nitrogen removal that is coupled with Sidestream Deammonification. Raw and waste activated sludge (RAS, WAS) are pumped to an anaerobic digester for sludge stabilization and biogas production. Afterwards, the digested sludge is dewatered in a thickener and the reject water, containing ammonium-rich supernatant, is supplied to another reactor where Sidestream PN/A is then performed.

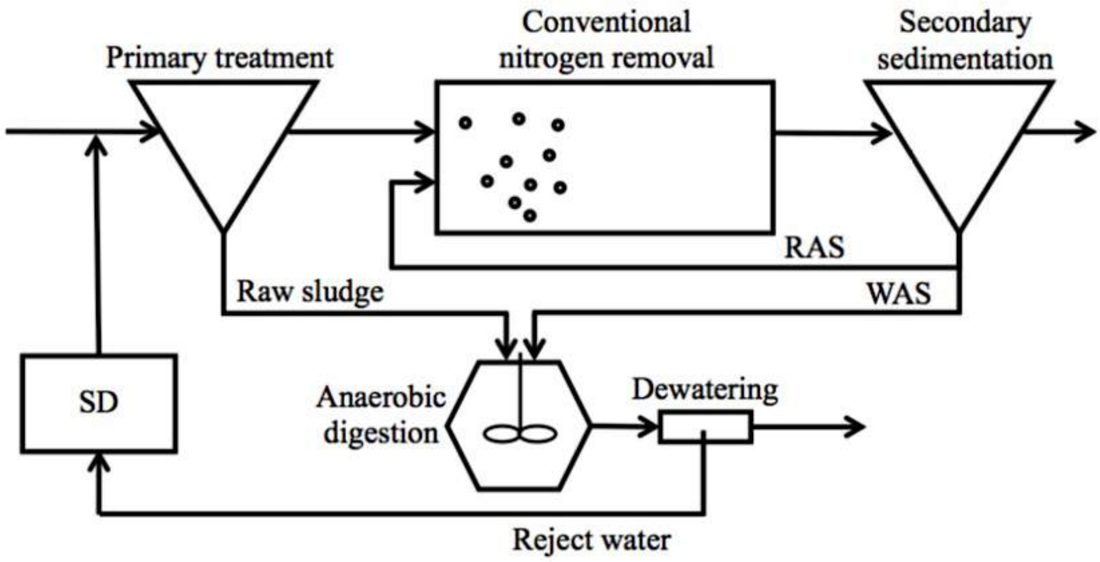

Figure 3: Scheme flow of an ordinary sidestream process (Vilpanen, 2017)

5.3 From Sidestream to Mainstream

Sidestream Deammonification is a successful technology for removing nitrogen from reject water. Since 1990 more than 100 wastewater treatment plants were operated with this process (Watson et al., 2016). However, because of a new economic strategy that addressed to convert WWTPs into energy recovery facilities, Sidestream PN/A did not represent any longer the best available option for nitrogen removal. New studies aim now to make Mainstream Deammonification a competitive and inexpensive strategy. In SD, wastewater was pumped back to the primary clarifier at the end of the process. This was done because carbon, still present in this water, was needed for denitrification, therefore biogas production was not maximized in order to limit the supplement of new carbon sources in the biological reactor.

In mainstream PN/A process nitrogen and carbon removal are completely decoupled (Cao et al., 2017). This allows to obtain high nitrogen removal efficiency, profits from biogas production and sale and reduction in operational costs because the supplement of carbon is not any longer required. Full scale mainstream PN/A implementation is not a reality yet since there are still several challenges to overcome. The first one concerns the influent ration of COD/NH_4-N. In SD processes this ratio is less than 1 gCOD/gNH_4-N, while it is around 7-12 for mainstream. These higher values lead to a higher population of heterotrophic bacteria that compete against AOB and AnAOB; and consequently, nitrogen removal efficiency decreases (Cao et al., 2017). Another problem concerns the concentrations of free ammonia (FA) and free nitrous acid (FNA). Previous studies (Anthonisen et al., 1976; Lackner et al., 2014) show that there are threshold values that inhibit the NOB's growth (see section 6.3.2). These values were reached in reject water where the ammonium concentration varied between 500 and 1500 mg/L, but not anymore in mainstream wastewater, that has a concentration range of 30-100 mg NH_4-N/L; therefore, it is more difficult to achieve a sufficient NOB's suppression (Cao et al., 2017).

The main challenge is about obtaining a high removal efficiency at a temperature that is lower compared to the one in sidestream treatments, which operate at around 30°C. Mesophilic temperature is important for the activation energies of the PN/A reactions. When temperature decreases to 15 °C and ammonium concentration in the inflow is low, anammox bacteria are particularly affected: their specific activity can drop to 10 times (Cao et al., 2017) and, consequently, anammox biomass production decreases, which causes a reduction of nitrogen transformation rates of 70–80% (Trela et al., 2014). Anammox biomass retention is also problematic: they need to be retained in the reactor longer than nitrifiers since they have a slower growth rate (Plaza et al., 2016). NOB suppression is also more challenging at low temperature because of a higher dominance of NOB over AOB in cold wastewater that also leads to nitrate accumulation.

Furthermore, another issue is the uncertainty about the time needed to start up the process. Mesophilic bacteria, such as AOB, need to be acclimatized to colder waters and this process can require years (Feng et al., 2017). In the end, meeting the discharge requirements is also problematic. If NOB suppression is not successful, high nitrate concentration will degrade the quality of the effluent (Cao et al., 2017; Feng at al., 2017).

6. Mainstream Partial Nitritation/Anammox

Deammonification can be performed with different reactor types and process configurations. However, the PN/A process does not stand on its own, but it is a part of a whole wastewater treatment plant. Therefore, the wastewater undergoes the ordinary pre- and primary treatments before entering the biological compartment where MD is carried out.

6.1 Carbon Pre-Treatment Process

Carbon and nitrogen removal are usually coupled in an A/B process where carbon removal represents the A stage and nitrogen removal the B one. The A stage is a pre-treatment process through which the COD content is reduced so that, when the wastewater reaches the B stage, HB are prevented from performing denitrification and therefore the anammox bacteria are not deprived of NO_2. From a wider point of view, carbon pre-treatment also meets the worldwide tendency of converting the WWTPs into self-sufficient facilities by maximizing energy, which organic carbon actually is, recovery. COD can be lowered by biological methods, such as high-rate activated sludge (HRAS) process and up-flow anaerobic sludge blanket (UASB) reactor or by chemical methods (Cao et al., 2017). Chemical methods are more effective in removing particulate COD than soluble, whereas the opposite occurs for biological treatments (Vilpanen, 2017).

HRAS uses a high loaded aerobic process with low sludge retention time and hydraulic retention time (HRT, see section 6.3.4). A period between half a day and four days is usually applied for SRT, while between half an hour and four hours for HRT instead. For example, in Strauss WWTP, SRT is around 0.5 d and HRT is 0.5 h resulting in a COD removal efficiency of 60% (Wett et al., 2013). However, these settings are temperature-dependent (Jimenez et al., 2015 see Cao et al., 2017). HRAS is the most applied technology worldwide to remove COD from wastewater, because HB are effectively inhibited by the low resulting COD content (Cao et al., 2017). Jimenez et al. (2015) (see Cao et al., 2017) obtained a soluble COD removal efficiency of 80% at the following parameters: DO (dissolved oxygen) > 0.3 mg O_2/L, SRT > 0.5 days and HRT > 15 min. HRAS is less effective with particulate COD: in this case its efficiency is around 50% (Regmi et al., 2015). This is also due to the short retention times applied. However, particulate COD removal can be enhanced through flocculation by addition of chemicals. Another disadvantage with HRAS is that the energy recovery is difficult to maximize because carbon mineralization can occur and prevent up to 20-30% of influent carbon from being converted into biogas (Cao et al., 2017).

UASB process is applied for maximizing energy recovery (Cao et al., 2017). It is a single reactor process working in continuous mode that removes organic matter from wastewater. Water flows upwards and enters the tank from the bottom. The tank contains a suspended sludge blanket that filters the wastewater when this passes through it and bacteria anaerobically digest the organic matter, present in the water, and convert it into biogas (Yang et al., 2015).

Compared to aerobic processes, such as HRAS, UASB does not need aeration, produces less sludge and generate biogas (Wan et al., 2016). Full scale UASB reactors, that treat mainstream domestic wastewater, have already been implemented with total COD removal efficiency of 45-75% at HRT of 5-19 h (Seghezzo et al., 1998). Laboratory experiments achieved even higher efficiency: 65–90% (Foresti, 2002). Malovanyy et al. (2015) using an UASB reactor coupled to an IFAS reactor (see section 6.1.7) which is the same pilot reactor used in this study, obtained a total COD removal efficiency of 90%. In addition, 65% of that removed COD was converted into biogas, which would result in an amount of recovered electrical energy of 3.55 kJ/gCOD. This value is more than the energy consumed for removing 1 g of COD through an aerobic process, which would cost 3.2 kJ/gCOD (Malovanyy et al., 2015).

UASB reactor has the disadvantage that a large fraction of biogas remains in the liquid phase and cannot be exploited. Furthermore, it can happen that high organic matter loads from UASB reactor are discharged into the deammonification reactor. This can drastically affect the process performances by favouring HB’s growth (Cao et al., 2017).

6.2 Process Alternatives

6.2.1 Single or Two-Stage Process

The definition of “single” or “two-stage” relies on how many biological reactors are used in the process: one in the first case and two in the second. Therefore, in a single-stage process both partial nitritation and anammox occur in the same reactor, whereas in a two-stage process they are separated. Initially the single-stage process was considered to be less stable due to AnAOB inhibition in aerobic conditions (Siegrist et al. 2008 see Cao et al., 2017), but it became more popular later because it has more advantages than the two-stage one. In fact, it needs less encumbrance and less costs for infrastructure and operation (Wett et al. 2013), it requires less control (Gustavsson, 2010) and it also causes less nitric and nitrous oxide emissions because the process is performed under low dissolved oxygen concentration and nitrite limitation (Cao et al., 2017).

The main advantage of a two-stage process is its stability. In addition, it offers a wider range of process conditions (Gustavsson, 2010). It is possible to have aerobic and anoxic environments separated in order to avoid the competitions between bacteria (Cao et al., 2017). In a single-stage process a suitable DO concentration for all bacteria is still challenging to find. AnAOB need anoxic conditions and they are inhibited by high DO levels (Vilpanen, 2017). However, even in case of a two-stage process, the anammox bacteria can be still inhibited by high DO levels coming from the PN reactor as effluent (Gao et al., 2014). Therefore, the DO cannot be controlled actually. The real advantage is that it is possible to contrast better the activity of NOB without compromising the AnAOB (Piculell et al., 2016b). PN effluent can also contain high nitrite levels which are toxic for the anammox bacteria. A solution in this case would be to by-pass the anammox reactor partially or dilute the PN effluent with the anammox effluent by recirculation of the last one (Gustavsson, 2010).

Since the pilot reactor used in this project is part of a single-stage process, the following sections will focus only on single stage processes that have characteristics in common with the pilot reactor.

6.2.3 Batch-Mode or Continuous Mode

MD processes can also be operated in either batch or continuous mode. In a batch process, a certain volume of water enters the reactor, is treated and is finally discharged. In a continuous mode, the water continuously enters and exits the reactor while the treatment takes place.

The sequencing batch reactor (SBR) is one of the most popular reactor types used for PN/A processes. 50% of the all deammonification processes implemented worldwide at full scale till 2014, for sidestream treatment with reject water, were SBRs (Vilpanen, 2017). A SBR is a batch reactor in which an operational cycle is applied (figure 4). This cycle consists in a cyclical sequence of the following phases: filling, reaction, sedimentation, decantation and idling. Initially the filling phase was intermittent, like all the other phases, but later it was observed that a continuous feed is beneficial for the stability of the process and for speeding up the reaction rate (Gustavsson, 2010). This process is usually operated as a suspended growth system (see section 6.1.4) (Vilpanen, 2017) and it is suitable for mainstream processes because it allows to obtain an adequate biomass retention. It also facilitates NOB suppression thanks to high concentrations of substrates at the start of the sequence (Lotti et al., 2014). However, a lot of storage space is needed to store the water while waiting for the end of the sequence before entering the reactor. This makes this system less compact compared to others.

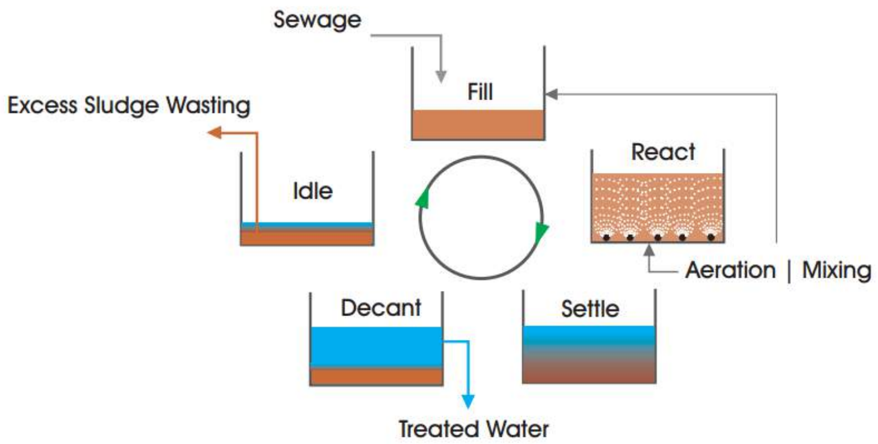

Figure 4: Scheme flow of a SBR (Ethich Infinity PVT LTD)

Nowadays they are more batch mode reactors implemented at full scale, because they proved to meet better the expectations of the users. In a batch system the operator can wait for discharging until he is totally satisfied with the treatment, whereas in a continuous system the discharge cannot be interrupted (Cao et al., 2016 see Cao et al., 2017). However, this also implies that batch mode works better with low flow rates and continuous mode is more flexible with inflow fluctuations (Vilpanen, 2017). Furthermore, batch systems are more suitable when the sludge, resulting from secondary sedimentation, has a variable volume, because in a continuous system the sludge needs to be estimated in advanced and wrong estimations can affect both the effluent and the sludge quality (Cao et al., 2017).

6.3 Process Types

6.3.1 Suspended Growth Systems

Suspended growth systems work with flocculent or granular biomass. The microorganisms are free to move and float in the liquid phase and they gather into flocs and granules (Hendrickx et al., 2014 see Cao et al., 2017). AOB and NOB usually form larger aggregates compared to Anammox bacteria due to a higher predisposition of the former to grow as suspended sludge (Vilpanen, 2017). In fact suspended sludge systems are used to enhance the population of nitrifiers and increase the nitritation rate (Lemaire et al., 2013).

The process mechanism is based on this difference in size: larger aggregates are separated from smaller ones in order to wash-out NOB and keep Anammox bacteria inside the reactor. In floc-type sludge systems AOB and NOB form large flocs that settle down when they reach a sufficient diameter, while smaller Anammox flocs are kept in suspension. Separation between the biomass flocs is done by using hydrocyclones, sieves, mechanisms based on different size and density or by operating different SRTs (Vilpanen, 2017).

In granular sludge system, AOB tend to form very large granules with good settling properties. Therefore, Anammox bacteria can be easily held back, while the rest of the biomass is washed out. Even though the separation between granular biomass is easier to achieve, large granules imply higher mass transfer resistance and slower biomass activity. A smaller size entails higher biotical activity, but on the other hand, a faster oxygen transfer can inhibit Anammox bacteria if the process is carried out in a single stage reactor. However, granular sludge systems are more popular because large granules are easier to separate and they reduce the Anammox inhibition. (Vilpanen, 2017).

Geilvoet et al. (2015) obtained 70% of total nitrogen (TN) removal efficiency working on a granular sludge system at a temperature between 17 °C and 19 °C. They observed that 80% of the nitrifiers' population was contained in the granules that were retained while the rest of the flocculent biomass was washed-out. That efficiency dropped when conditions in the inflow changed because of a period of heavy rain that caused nitrifiers to be washed out too. This confirms the importance of nitrifying biomass retention.

6.3.2 Biofilm Systems

Biofilm systems are attached growth systems which have been less implemented at full-scale compared to suspended growth systems, but they are gaining in popularity because they are more efficient in biomass retention (Vilpanen, 2017). In these systems the biomass grows on supports and carrying material forming a biofilm. The most commonly used attached growth system in MD is the moving bed biofilm reactor (MBBR).

6.3.2.1 Moving Bed Biofilm Reactor

A Moving Bed Biofilm Reactor (MBBR) is a biological reactor in which carriers, are free to move in the bulk liquid. Carriers are circular supports for biomass with a diameter of few millimetres. There is no return sludge and the biomass grows mainly attached to carriers instead of forming flocs and granules as in a suspended growth system. Carriers are contained inside the reactor by an outlet sieve (Vilpanen, 2017) and they are made of plastic material that makes them float. The variety of the biotical community on the carriers depends mostly on the biofilm thickness. Gilbert et al. (2014) observed that the biotical community tends to be stable even with decreasing temperature. Aerobic bacteria are located on outer layers, while anammox bacteria on the inner ones. Therefore, thick biofilms provide a better protection to anammox bacteria in case that aeration is operated.

However, thick biofilm implies high mass transfer resistance and therefore higher DO concentrations are required. This determines higher operational costs for aeration (Lemaire et al., 2013) and inhibition of anammox bacteria due to a deeper oxygen propagation into the biofilm. Therefore, lower DO concentration is better for AnAOB, but on the other hand, it prevents AOB from performing partial nitritation. Another problem that can occur is the detachment and wash-out of AOB from the carriers (Malovanyy et al., 2015). In addition, it is also important to reduce the shear stress caused by the stirring device that can intensify the detachment (Gustavsson, 2010). Even though the anammox bacteria are more willing to grow on carriers, their slow growth rate does not change. Start-up time are still very long (several months) if inoculation of already conditioned sludge is not operated. Sometimes the anammox bacteria are not able to form a biofilm structure at all and they need an existing one to enrich (Gustvasson, 2010).

Finally, MBBR reactors are less sensitive to variation of suspended solids since the solids leave the reactor through sieves and screens, while carriers are retained.

6.3.2.2 Hybrid Reactor

A hybrid reactor combines suspended and attached growth systems together. A typical example is the integrated fixed-film activated sludge (IFAS) reactor that consists in a MBBR with the addition of activated sludge and sludge recirculation. Therefore, an IFAS reactor has a higher suspended sludge concentration and biomass grows both attached to the carriers and as suspended flocs in the liquid phase. Veuillet et al. (2015) observed that when a MBBR reactor was converted into an IFAS reactor a change in the biofilm composition and thickness occurred. In an IFAS reactor AOB, NOB and HB are found mainly in the liquid phase, while the anammox bacteria are distributed on the carriers. Therefore, the biofilm becomes thinner (Cao et al., 2017). This distribution occurs because in the bulk liquid there is a higher availability of substrates, such as ammonium and oxygen, for aerobic bacteria than in the suspended sludge (Veuillet et al., 2014).

The IFAS reactor improves the biomass retention, and AOB detachment from biofilm, as mentioned in the previous section, is not a problem anymore. This was confirmed by Malovanyy et al. (2015) who observed that 60% of the aerobic activity occurred in the suspended sludge, while almost the total anammox activity was found in the biofilm. On the other hand, IFAS reactor is much more sensible to fluctuating levels of suspended solids since the nitritation process depends mainly on it (Zhang et al., 2015).

IFAS reactors usually have higher process performances than MBBR reactors: Plaza et al. (2016) stated that nitrite production was always very little in a MBBR relying on biofilm only and resulting in a total nitrogen removal efficiency of 38% on average. When this reactor was converted to an IFAS one, that removal efficiency doubled.

Furthermore, a thinner biofilm layer allows to lower DO concentration and aeration time and makes possible to control AOB/NOB competition by adjusting the DO (Cao et al., 2017). Finally, it is possible to operate different SRTs: a short one is applied to wash-out NOB that leave the reactor through a sieve, while anammox bacteria, that are attached to the carriers, remain inside. This is because they need a longer SRT in order to have an impact on the overall process (Sandino et al., 2016).

6.4 Factors and Parameters affecting the Process Performances

In this chapter chemical and physical factors and process parameters are described. The chemical and physical factors introduced here are chemical oxygen demand, pH, alkalinity, free ammonia and free nitrous acid. The process parameters are temperature, dissolved oxygen, hydraulic retention time, sludge retention time, and total suspended solids.

6.4.1 Chemical Oxygen Demand

The COD corresponds to the amount of oxygen consumed for oxidizing soluble and particulate organic material in the wastewater (Regmi et al., 2014 see Cao et al., 2017) and it is usually expressed as milligrams of oxygen per litre of solution. The COD is a fundamental water quality factor because it permits to estimate the pollutant load in the wastewater (Khayi, 2017) and how it might affect the receiving environment. High COD concentrations indicate a consistent amount of organic material that needs oxygen to be consumed. This causes a reduction of the oxygen level and low DO level can lead to anaerobic conditions which are hazardous for aquatic ecosystems (Regmi et al., 2014 see Cao et al., 2017).

Regarding the deammonification process, COD must be maintained low in order to prevent heterotrophic bacteria from growing and, consequently, competing with AOB for oxygen and with AnAOB for nitrite. The factor that is usually considered is not only the COD, but the influent ratio COD/NH_4-N, because it allows to assess the competition between authotrophic and heretotrophic bacteria involved in the process. COD can also play a positive role. Nitrate can be partially reduced to nitrite by HB before being totally converted to gaseous nitrogen through denitrification. This nitrite can be used by anammox bacteria before that the reaction of denitrification is concluded (Malovanyy et al., 2015).

6.4.2 pH and Alkanity

The pH has an important influence on the PN/A process performances: it affects the reaction rates and inhibits the bacteria activity. According to Jaroszynski et al. (2011) (see Feng et al., 2017) the optimal pH range for AOB is 7.0-8.5 and Lu et al. (2017) observed that these bacteria reached their activity maximum at pH 7.4. For AnAOB the optimal interval was found to be 7.5 – 8.0 (Magrì et al. 2013 see Cao et al., 2017) with a maximum growth rate at 7.6 (Lu et al., 2017). On the contrary NOB have a lower optimal range of 6.0 – 7.5 and their highest production rate is at 7 (Yin et al. 2016 see Feng et al., 2017). These differences in optimal ranges represent a potential strategy to inhibit NOB without compromising AOB and AnAOB's growth (Feng at al., 2017).

Alkalinity, free ammonia concentration (FA) and free nitrous acid concentration (FNA) are pH-dependent. Alkalinity is related to the presence of carbonates in the water and it plays a role of pH buffering (Zhang et al., 2013 see Feng et al., 2017). During nitritation the decrease of pH, due to the release of H^+ ions, is contrasted by the consumption of alkalinity. This decrease in pH is due to the oxidation of NH_4-N and for each mole of NH_4-N two moles of HCO_3^- are used (Khayi, 2017). The contrary occurs during anammox: pH increases and alkalinity is produced (Kouba et al., 2017). In case of suspended growth systems, the increase in pH is due to the anammox reaction and to the continuous filling of return sludge (Gustavsson, 2010).

6.4.3 FA and FNA

FA and FNA are the actual substrates for nitrification. FA concentration increases when the pH is also increasing, whereas FNA is high when the pH is low. FA and FNA can inhibit the AOB, NOB and anammox bacteria, but their concentrations are usually very low in mainstream wastewater (Zhou et al., 2011).

AOB and NOB are both affected by FA and FNA, but NOB are more sensitive than AOB to high FA (Vilpanen, 2017). Several researchers studied FA and FNA inhibitions under various conditions and in different processes. Different values and ranges are reported. However, in all the cases analysed inhibition of NOB was achieved with FA and FNA concentrations much lower than the ones for AOB's inhibition. This strategy was studied for the first time by Anthonisen et al (1976) (see Cao et al., 2017). They indicated an inhibition range of 0–150 mg FA/L for AOB and 1.0 - 10 mg FA/L for NOB, but it was observed that NOB began to be inhibited at 0.1 mg FA/L. In a granular sludge system Wang et al. (2016) (see Feng et al., 2017) reported that AOB managed to adapt to 5-10 mg FA/L.

Regarding FNA inhibition, Vadivelu et al. (2006) (see Feng et al., 2017) found a concentration range of 0.1–0.4 mg FNA/L for AOB and a range of 0.011–0.023 mg FAN/L for NOB.

6.4.4 Temperature

Temperature is a key parameter in PN/A process since it is proportional to the bacteria's growth and it influences the competition between the bacteria involved (Feng et al., 2017). AOB are mesophilic bacteria, which means that thrive at warm temperature (Cao et al. 2017). They have a wide range of temperature in which they are active, but they are more affected at psychrophilic temperature than NOB. In fact, most of NOB species are also mesophilic, but some have their optimum ranges at low temperature. Nitrotoga, for example, are psychrophilic bacteria and they have a maximal biotical activity at 10-17°C (Saunders et al. 2016). This also explains how temperature influences the competition between AOB and NOB: high temperature is a successful and low-cost nitritation strategy for PN/A processes in warm climates. When the temperature is lower than 12 °C, NOB activity is higher than the AOB's one, but at higher temperature the opposite occurs. Dominance of AOB over NOB occurs especially above 25 °C (Hunik et al., 2003; Lotti et al., 2014). The predominance of AOB over NOB at high temperature was also confirmed by Regmi et al. (2014) (see Cao et al., 2017) even in case of low dissolved oxygen (DO) concentration. However, this competition depends on the species involved. Gilbert at al. (2015) (see Cao et al., 2017) found that certain genera of NOB were very affected by cold temperature, therefore AOB's predominance was easy to achieve even at low temperature.

Anammox bacteria are also mesophilic bacteria (Laureni et al., 2016), but they adapt more easily than nitrifiers to low temperature and this results in stable conversion rates (Lotti et al., 2015b) and they can recover faster than all other bacteria from a sudden drop of temperature (Laureni et al., 2016). Sobotka et al. (2016) managed to operate an anammox biomass system at 15°C without any difficulties and only when the temperature dropped to 11°C nitrite accumulation occurred. Isaka et al. (2008) (see Feng et al., 2017) and Hu et al. (2013) successfully grew an enriched AnAOB biomass fed with synthetic wastewater at 6°C and 12°C, respectively, and obtained a high nitrogen removal, which was up to 90% for the latter authors.

According to Lotti et al. (2015b), AOB are more active than anammox bacteria with decreasing temperature even if the latter are subjected to a more stable acclimatization. This can become an issue when implementing a single stage PN/A process, because the AOB and AnAOB conversion rates are coupled. Therefore, a proper balance needs to be maintained between the nitrite production by the former and the nitrite consumption by the latter. If this balance is not met, nitrite accumulation might occur and lead to nitrate production by NOB. DO level and reactor type matter too. Lotti et al. (2015b) found that anammox bacteria were more influenced by a temperature decrease if they were growing under total anoxic conditions than in an aerated partial nitritation/ anammox. Similar results were found in case of biofilm and suspended sludge.

Most of the studies, that have been carried out so far, demonstrate the feasibility of high temperature as operation strategy to enhance the nitrogen removal in PN/A processes. Nowadays the attention is focused on the deammonification process treating mild/cold municipal mainstream wastewater. Stable PN/A was demonstrated to be possible at low temperature in several studies (Laureni et al., 2015; Gao et al., 2014; De Clippeleir et al., 2013). However, the main challenge to face in case of cold water is still to support AOB and AnAOB's activity while inhibiting the NOB's one.

6.4.5 Dissolved Oxygen

Most of the MD processes have been run so far at low DO since AOB have a lower affinity with oxygen than NOB. In fact, under low oxygen conditions AOB have a higher growth rate than NOB (Feng et al., 2017). Low DO represents, therefore, a good strategy to out-select NOB. Low DO concentrations are usually between 0.15 and 1.0 mg/L (Fernandes et al., 2013 see Feng et al., 2017), but it is also possible to achieve a successful nitritation at higher concentrations: Cao et al (2013) observed high nitritation in the full-scale PN/A plant in Singapore at DO between 1.4 and 1.8 mg/L, at high temperature though. Concentrations lower than 0.15 mg/L are not usually recommended since NOB have shown to adapt at as low concentrations as 0.06 mg/L (Wett et al., 2013).

DO is not good for anammox bacteria since they are strictly anaerobic organisms. High DO has a greater effect on AnAOB than low DO. Magrì et al. (2013) (see Cao et al., 2017), in fact, observed that the anammox bacteria were inhibited by both low and high dissolved oxygen, but this inhibition was irreversible in the second case.

6.4.6 Hydraulic Retention Time

The hydraulic retention time (HRT) is the average amount of time that the influent wastewater is contained in the system. It is a parameter that can influence the efficiency of the process, because it is an important agent in balancing the activity of AnAOB and AOB (Feng et al., 2017). HRT is usually adjusted in order to have a stable nitrogen loading rate. Therefore, if the influent ammonium concentration is high, the HRT will be reduced, but in suspended sludge systems too short HRTs can wash out AOB and AnAOB, if no biomass retention mechanisms are applied. Consequently, the nitrogen removal efficiency will be affected. However, since NOB will be also wash-out a solution to this problem could be the immobilization of an AOB (and AnAOB)-rich culture without the presence of NOB (Kouba et al., 2017).

6.4.7 Sludge Retention Time

The sludge retention time (SRT), also called sludge age, is the average amount of time that the activated sludge remains in the process. It is normally expressed in days and it is a critical operating parameter because of the diversity of the bacterial species that the sludge contains (Feng et al., 2017). SRT usually varies between 10-30 days. Furthermore, the sludge age has to be set depending on the growth rate of the bacteria involved. Slower growth rate requires a longer SRT. Nitrifying bacteria grow much faster than the anammox ones, therefore different SRTs should be applied. This is crucial because if the SRT is too long for the nitrifiers, then their decay and the COD, consequently, will increase (Gustavsson, 2010). A short SRT is also useful to remove from the biological reactor biomass that has low settling properties (Sandino et al., 2016).

6.4.8 Total Suspended Solids

The concentration of total suspended solids (TSS) is a water quality parameter that gives a measure of the turbidity of the wastewater. It corresponds to the dry-weight of particles, having a diameter bigger than 2 microns, that are blocked by a filter (Gustavsson, 2010). These particles can be of organic or inorganic nature.

Leimare et al. (2013) observed a correlation between the nitrogen removal efficiency in an IFAS reactor and TSS. When the suspended solids reached a concentration of 3 g/L, the nitritation reaction was effectively achieved and the total nitrogen removal efficiency went up to 70%, but when an accidental loss of sludge occurred the nitritation was very affected and the TN removal efficiency dropped down. Malovanyy et al. (2015) and Khayi (2017), who both worked on the same pilot IFAS reactor of this project, obtained similar results. The former found that when the TSS concentration increased to 800 mg/L the total nitrogen removal efficiency rose from 37% to 70%, whereas the latter observed a maximal removal efficiency for TSS between 900 and 1200 mg/L.

However, it is not important the value of TSS on its own, but the amount of biomass related to it. In case of IFAS reactor, AOB grow as in the suspended sludge, therefore, high TSS stands for a thriving population of AOB (Geilvoet et al., 2015). AOB provide nitrite that constitutes the substrate for anammox bacteria. This mean that TSS is directly connected to the process performances of IFAS reactors (Veuillet et al., 2014).

6.3 Operational Strategies for improving the Process Performances

6.3.1 Intermittent Aeration

Intermittent aeration is applied in order to create a condition of transient anoxia inside the reactor that is a cyclical transition between aerobic and anoxic environment. This is obtained by applying aeration on/off intervals. First of all, this is a strategy to save operational costs related to aeration. Yang et al. (2015) proved that it did not cause any loss in nitrogen removal efficiency when compared to continuous aeration; this was observed in a sidestream process, but it is also promising for MD. Furthermore, transient anoxia proved to be very successful in out-selecting NOB in favour of AOB and AnAOB (Feng et al., 2017). Several mechanisms are hypothesized in order to explain this success: deprivation of oxygen as substrate, inactivation of NOB metabolism under anoxic conditions and slower reactivation of NOB compared to AOB after the anoxic phase (Cao et al., 2017).

Initially this strategy was applied to restore nitritation after a break down (Feng et al., 2017), but after Katsogiannis et al. (2003) (see Feng et al., 2017), who was the first to understand its real potential, several researchers studied the intermittent aeration as an approach for NOB suppression. In order to maximize NOB inhibition long intervals are chosen for non-aeration (15-20 min or longer), whereas aeration periods are shorter. Han et al. (2014) observed that it was easier to out-select NOB by applying a shorter aeration frequency (1 min of air over 15 min) rather than a longer one (7 min over 45 min).

However, the effectiveness of intermittent aeration also depends on the reactor type. Malovanyy et al. (2015) conducted an experiment on a MBBR and on an IFAS reactor. He obtained a TN removal efficiency that was up to 70% with the IFAS reactor and three times lower with the MBBR. The aeration pattern was 15 min of aeration and 15-45 min of non-aeration and DO was equal to 1 mg/L. Similar results were found by Trojanowicz et al. (2016). The reason is that when using a system relying on the biofilm only, long non-aerated periods are necessary so that the oxygen is consumed even in the inner part of the biofilm. On the contrary, in case of hybrid reactors, most of AOB are found in the biomass flocs, therefore it is possible to achieve an adequate nitritation even for shorter aeration times. Khayi (2017), working on the same IFAS reactor of Malovanyy et al. (2015) and Trojanowicz et al. (2016), experimented intermittent aeration with different aeration patterns and he found an average total nitrogen removal efficiency of 52% when setting the DO level at 1.3 mg/L, the non-aeration period at 40 min and the aeration one at 20 min.

Laureni et al. (2016) tested a different aeration strategy on a hybrid MBBR with flocculent biomass. Instead of setting fixed aeration and non-aeration intervals, they chose an ammonium concentration of 2 mg/L as threshold to interrupt the aeration time. They achieved an average ammonium and total nitrogen removal efficiencies of 90% and 63% respectively.

Long non-aerated periods are usually combined with low dissolved oxygen in order to enhance the efficacy of this strategy by depriving NOB of their substrate. On the contrary, Kornaros et al. (2010) (see Cao et al., 2017) observed that the activity of Nitrospira, that are usually found to be the most abundant in mainstream wastewater, were successfully inhibited even for short anoxic period (5-15 min) but with high DO. To conclude, this strategy is not always effective. Nitrospira in fact were found to be able to adapt at low DO and not to be very affected by anoxic conditions. That is why, in case of Nitrospita, high DO is the main solution that is applied with the purpose of supporting AOB over NOB instead of actually inhibiting NOB (Regmi et al., 2014 see Cao et al., 2017).

6.3.2 Inhibition by FA and FNA

Dosing FA and FNA in combination with other factors, represents another strategy for NOB out-selection (Feng et al, 2017). The potential of this strategy is still not totally certain since Piculell et al. (2016a) observed that NOB were able to adapt at high FA concentrations. However, the effectiveness of this strategy also depends on the mass transfer resistance (Piculell et al.,2016b). Kouba et al. (2017) observed that in suspended growth systems NOB were inhibited by a FA concentration range that was lower than the one in a biofilm system. This is probably due to a greater contact with FA for AOB when growing as suspended sludge than as biofilm. In attached growth systems, this strategy is usually less effective because thicker biofilms are more difficult to penetrate (Veuillet et al. 2015).

In order to make this strategy more successful, FA and FNA concentrations in the mainstream wastewater need to be enhanced. This can be achieved by using intermittent reject water from sludge digestion as temporary feeding into the biological reactor (see section 6.3.5) (Vilpanen, 2017).

6.3.3 Short Hydraulic Retention Time

According to Zekker et al. (2011) short HRT is a potential strategy that can be used to enhance the NOB suppression. Short HRT in fact may increase the nitrogen removal rates in wastewater with low nitrogen concentration (Feng et al., 2017). This strategy would be more effective if short HRT is combined with low DO in order also to enrich AnAOB's population. However, an approach based on HRT as control parameter requires further studies (Zhang et al., 2013 see Feng et al., 2017). Shortening HRT is a promising solution, but, on the other hand, it also leads to a reduction in biomass aggregation and size of the flocs (Khayi, 2017).

6.3.4 Short Sludge Retention Time

According to Zhou et al. (2011) (see Feng et al., 2017), low SRT combined with other strategies (low DO, intermittent aeration, high temperature, FA and FNA inhibition) is effective to suppress NOB bacteria without compromising the AOB's activity because of different growth rates. This is confirmed by Han et al. (2016) who found that short SRT and high aeration frequency facilitate AOB over NOB. Joss et al. (2009) (see Feng et al., 2017) found that a short sludge age is also effective if it is coupled with high DO (1-1.5 mg/L). However, SRT has to be short enough to wash out NOB, but at the same time long enough to support AOB's growth (Regmi et al. 2014 see Cao et al., 2017).

There are cases in which a high ammonium removal efficiency has been achieved coupling high temperature and very short SRT (3-3.5 days). The Water Reclamation Plant at Changi WRP, Singapore (Cao et al., 2016 see Cao et al., 2017) and the wastewater treatment plant in St. Petersburg, USA (Jimenez et al. 2014 see Cao et al., 2017) are two examples of this. Nowadays there is the tendency to test aggressive (very short) SRT even in cold and moderate water, but still very little experimental information is available. One example of such a tendency is reported by Veuillet et al. (2015). By shortening SRT from 12 to 3 days they manage to recover an IFAS reactor at 23°C that was unstable because of excessive aeration.

6.3.5 Transfer between Sidestream and Mainstream

This term is used to indicate a strategy to enhance AOB and AnAOB's population and suppress the NOB one by switching the feed between mainstream and sidestream wastewater (Lemaire et al., 2013; Picullel et al., 2016b) or by moving anammox bacteria from sidestream to mainstream processes (Wett et al., 2013). The bacteria can be easily transferred in case of attached growth systems: carriers with biofilm can be moved from mainstream to sidestream and vice versa. When they are moved back to the sidestream process they regenerate because of the better conditions in the SD reactor (Veulleit et al., 2015). Little experimental information about augmentation is available. However, the results achieved so far (Veulleit et al., 2015), prove its feasibility. This strategy may be applied even only temporally to enhance the efficiency of the mainstream process during the periods in which the system struggles more, like the winter time for example (Cao et al., 2017).

Piculell et al. (2016b) studied how to inhibit the NOB's growth by switching periodically from mainstream to sidestream feed. It was shown that NOB were actually affected by a sudden switch from ammonium rich reject water at high temperature to cold mainstream wastewater with low ammonium content. This experiment was carried out by using a two-stage MBBR reactor (figure 5). The first stage corresponded to a multi-celled reactor for partial nitritation: one cell received the reject water for a certain period, while the rest received the mainstream wastewater. Then the cell fed with reject water changed. Carriers were used in both stages. In this case, a two-stage configuration was considered more suitable to achieve a successful NOB's inhibition in the first stage by flooding the PN reactor with reject water and preventing NOB from entering the second tank. In this way, the water, leaving the nitritation reactor, had better characteristics for the anammox bacteria.

The success of this strategy depended on load exposure time of the reject water that were both not constant. In particular, the frequency of the reject water feed is still under study; in this experiment it was 1-2 days/week. However, when the nitritation was performed efficiently, the total nitrogen concentration in the effluent was below 10 mg/L, while the influent ammonium concentrations were 15-40 mgNH_4-N/L in the mainstream and 1000-1500 mgNH_4-N/L in the sidestream.

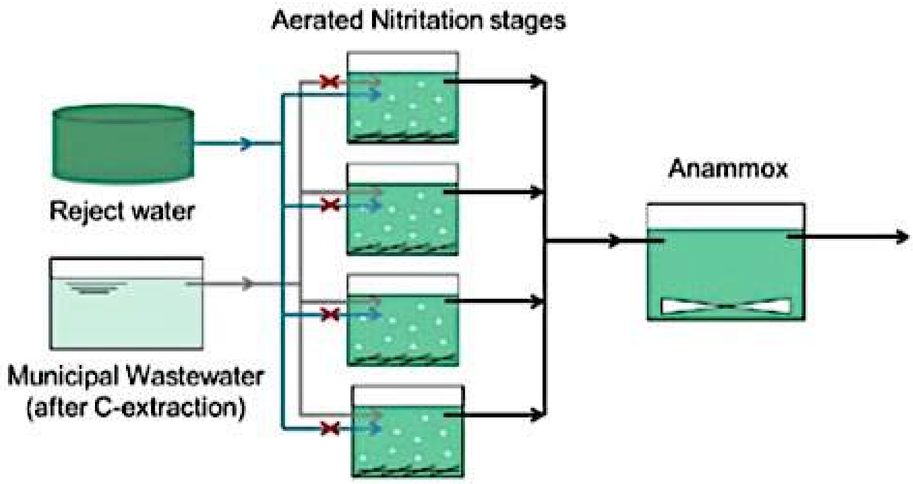

Figure 5: Scheme flow of a double stage MBBR reactor (Piculell et al, 2016b)

6.4 Comparison of Results from Previous Studies

Table 1 summarized some of the articles and thesis that have been examined during the project. These reports were found particularly important for their achievements and they are cited several times in the thesis text. They helped the author of this research to find new ideas to carry out his individual work.

Table 1 summarizes all the relevant aspects of their research: influent and effluent concentrations, process factors and process performance indicators.

Table 1: Example of previous studies on Mainstream Deammonification

		Articles and Master's Thesis				
		Khayi (2017)	*Laureni et al. (2016)*		*Trojanowicz et al. (2016)*	*Lotti et al. (2014)*
	Reactor type	**IFAS**	**MBBR**	**IFAS**	**IFAS**	**SBAR (air lift)**
Input:	**TN (mg/L)**	not given	21.8	21.8	not given	not given
	NH4 - N in (mg/L)	44,1	21.2	21.2	47,1	162
	NO2 -N in (mg/L)	not given	<0.2	<0.2	not given	not given
	N03 - N in (mg/L)	not given	0.4	0.4	not given	not given
	sCOD (mg/L)	83,4	46	46	not given	not given
	COD (mg/L)	not given	69	69	71	not given
	sCOD/NH4 - N in	1,9	3,3	3,3	1,5	not given
	TN load rate (g/m3d)	0.39	40	38	0,11 (gN/m2d)	530
	Alk in (mmol/L)	5,9	not given	not given	not given	not given
Results:	**TN rem,eff (%)**	48,7	73	63	43,9	73
	NH4-N rem, eff. (%)	65,6	91	89	85,7	89
	TN rem rate	0,15 (g/m3d)	30 (g/m3d)	26 (g/m3d)	0,05 (gN/m2d)	400 (g/m3d)
	NO3 prod / NH4 rem	0,2	0,2	0,3	not given	0,1
Output:	**TN out (mg/L)**	not given	5.7	8	25,9	not given
	NH4-N out (mg/L)	15,2	1.8	2.1	6,5	18,1
	NO2 - N out (mg/L)	1,4	<0.2	<0.2	0,3	15
	NO3-N out (mg/L)	4,9	3.6	5.7	19,1	15
	sCOD (mg/L)	40,2	18	20	not given	not given
	COD (mg/L)	not given	40	33	53	not given
	Alk out (mmol/L)	3,1	not given	not given	2,6	not given
Parameters:	**T (°C)**	15	15	15	17	15
	DO (mg/L)	1.3	0.18	0.15	1.5	2.1
	air flow (mL/min)	230	350	100	not given	not given
	Aeration strategy (ar/non-ar)	20 min /40 min	air till NH4 out = 0.2 mg/l	air till NH4 out = 0.2 mg/l	15 min /60 min	1h / 2h
	pH	7	not given	not given	7,2	7,3
	HRT (h)	14 and 49 min	14	14	38,4	7,2
	SRT (d)	not given	uncontrolled	uncontrolled	not given	150
	TSS (mg/L)	900 - 1200	not given	not given	not given	not given
	Q in (L/h)	13.8	not given	not given	not given	not given
	Q sludge (L/h)	20.4	not given	not given	not given	not given

7. Methodology

7.1 Pilot Reactor

The first part of the thesis regarding the literature review has just terminated. The second part, that now begins, describes the development of a project regarding the Mainstream Deammonification. The project was conducted in Hammarby Sjöstadsverk which is a research facility built in 2003 and located in the area of the Henriksdal Waste Wastewater Treatment Plant in Nacka, Stockholm. The Royal Institute of Technology (KTH) and the Swedish Environmental Research Institute (IVL) run this facility and they make it available to students and researchers for their studies and experiments. The research plant also contains a pilot PN/A reactor on which this Master's thesis project was developed.

The pilot reactor consisted in a single stage IFAS reactor working in continuous mode with a capacity of 200 L and it was coupled with a pilot

UASB reactor for carbon pre-treatment process. The reactor contained Kaldnes K1 carriers having a specific surface of 500 cm^2/m^3 and it was filled with them up to 48.5 % of its volume. The whole system is shown in figure 6.

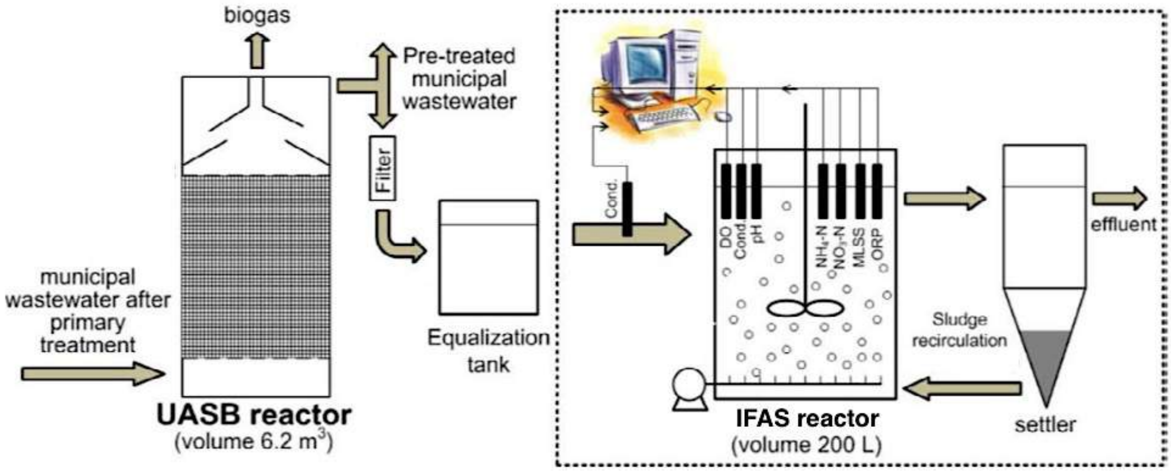

Figure 6: Scheme flow of the pilot reactor (modified after Plaza et al., 2016)

The wastewater used in this project was actual municipal wastewater that comes regularly from the city of Stockholm and it was pumped from the Henriksdal WWTP to Hammarby Sjöstadsverk. At the Henriksdal WWTP the wastewater underwent some ordinary pre-treatment steps that were screening, grit removal and primary sedimentation and afterwards it was pumped to the research facility. The organic matter was then removed in the UASB reactor. Successively, the effluent was filtrated through four filters arranged in series. Filters had different structure and pore size. The first was a pierced vessel with large holes, the second and the third ones were nylon filters with a pore size of 80 µm and the forth was a nylon filter with 20 µm as pore size. After the filtration, the wastewater was pumped into an equalization tank of 2.0 m^3 before being supplied to the IFAS reactor.

This reactor was equipped with a mechanical stirrer, for mixing its content, a cooler for temperature control and several sensors for online measurements. There were two sensors for monitoring the ammonium concentration and the conductivity in the inflow, and there were other sensors inside the reactor for measuring pH, DO concentration, oxidation-reduction potential (ORP), TSS concentration, conductivity and ammonium and nitrate concentrations in the outflow. Connected to the reactor there was a 200 L sedimentation tank for solid-liquid separation and recirculation of the settled activated sludge that was collected at the bottom of tank and sent back to the IFAS reactor. Photographs of the pilot IFAS reactor are shown in figure 7.

Figure 7: Photographs of the pilot reactor (photos by A. Robiglio)

The DO worked as a control parameter for the aeration in the reactor. The online measuring program allowed to choose a DO set-point. This corresponded to the DO concentration that does not have to be exceeded in the reactor. Air was pumped at a constant rate and entered the reactor through a valve that was opened when the aeration period began. Valve opening and DO sensor were correlated. Therefore, as long as the DO concentration was lower than the set-point the valve stayed open and it was closed when the set-point was reached.

The project was focused on evaluating the process performances of the PN/A pilot reactor. First the current state was analysed (Initial State Analysis) and afterwards three studies (Study 1, Study 2 and Study 3) were performed. A general operational program was followed for the whole study (from October 16th to February 2nd). This program contained several daily tasks that were scheduled according to a weekly timetable. Results were obtained from an online-measurement system and from chemical analyses performed on samples. The online system constantly kept track of process parameters and nitrogen concentrations. Results from the chemical analyses on the samples were used for calibration of the sensors, for further calculations and as term of comparison with the online data. Part of the project was also dedicated to evaluate the growth of biofilm on the carriers and the settling properties of the activated sludge used in the process.

7.2 Operational Program and Weekly Schedule

The operation program was divided into four sections: control and measuring program, analysis program, calibration program and cleaning program. Each part contained different of tasks and each of them had a specific frequency that established how often it needed to be accomplished. The overall operational program and the weekly schedule are reported in Appendix A.

7.2.1 Control and Measuring Program

The control and measuring program concerned daily routine checks and few simple measurements. It was important to control constantly that there was no floating sludge in the sedimentation tank in order to avoid return sludge with low TSS. Flow rate into the IFAS reactor was measured every day. The return sludge pump was very accurate, whereas the inflow pump was not. Therefore, it was crucial to measure the inflow rate manually because it was an important parameter that was needed for further calculations.

7.2.2 Analysis Program

The analysis program regarded the chemical analyses that were run on samples taken from inflow and outflow of the IFAS reactor during the non-aeration period. Inflow samples were taken from the pipe that connected the equalization tank to the pilot reactor, while outflow samples were taken directly inside the reactor. These analyses were performed in a chemical analytical laboratory inside the facility. Frequency was once or twice a week depending on the importance and on the changeability of the chemical species to analyse. Laboratory chemical results were particularly important because they were used as reference values for calibration, as matching points for online measurements and they were also used to calculate some indicators of process performance that are described in section 7.3.

The liquid was taken by means of pipettes and it was filtered with a 0,45 μm fiber filters in order to remove particles that might have interfered with the analyses. This filtration was not done in case of total COD. The analyses (COD, NH_4-N, NO_2-N, NO_3-N, TN and Alk) were then performed by injecting the filtered fluid in pre-prepared cuvettes together with other reagents that were specific for the chemical to trace. Afterwards, the cuvettes were inserted in a photometer that measured the corresponding concentration. Finally, total suspended solids and Volatile Suspended solids (VSS) were measured by following the standard procedure approved by the Standard Methods Committee (1997) and the pH was also measured.

Total nitrogen (both in the inflow and in the outflow) was measured once a week. This frequency was considered suitable because most of the influent nitrogen was assumed to be in the form of ammonium. This was also the reason why nitrite and nitrate were measured only in the outflow. Two ways were used to calculate TN in the outflow: preforming a chemical analysis as all the other chemical species and calculating it from the balance with all the nitrogen forms. The correlation between these two methods was also evaluated.

7.2.3 Calibration Program

Results from the analysis program were used in the calibration program. Calibration meant to adjust the online-measurement system with the results obtained from the laboratory analyses. All sensors were calibrated regularly with a frequency that was dependent on the importance and variability of the parameter/concentration to measure. Each sensor used to monitor the nitrogen concentrations worked with two electrodes in combination. One sensor was for the relative nitrogen species and the other one for a chemical element used as reference. The sensors for ammonium had also one electrode for Potassium and the sensor for nitrate had one electrode for Chlorine.

7.2.4 Cleaning Program

Filters were washed once a week, preferably on Monday. It was also important to check if the inflow into the equalization tank was restored after cleaning. Filters should be replaced with new ones after a couple of months. All sensors were also cleaned regularly. Cleaning was performed twice a week, but taking into account that calibration already included cleaning. Therefore, cleaning and calibration were done together once a week and only cleaning was performed one day more.

7.3 Calculations

Some of the acronyms, that will be used from this point forward, are here introduced:

- Alk_{in}: Alkalinity concentration in the inflow;
- Alk_{out}: Alkalinity concentration in the outflow;
- COD_{in}: Total COD concentration in the inflow;
- COD_{out}: Total COD concentration in the outflow;
- $sCOD_{in}$: Soluble COD in the inflow;
- $NH_4\text{-}N_{in}$: Ammonium concentration in the inflow;
- $NH_4\text{-}N_{out}$: Ammonium concentration in the outflow;
- $NO_2\text{-}N_{out}$: Nitrite concentration in the outflow;
- $NO_3\text{-}N_{out}$: Nitrate concentration in the outflow;
- pH_{in}: pH value in the inflow;
- pH_{out}: pH value in the outflow;
- TN_{in}: Total Nitrogen concentration in the inflow;
- TN_{out}: Total Nitrogen concentration in the outflow;
- TSS: Total suspended solids concentration in the reactor;
- VSS: Volatile suspended solids concentration in the reactor.
- V: Volume of the reactor;
- Q_{in}: Inflow rate to the reactor;
- Q_e: Overflow from the sedimentation tank;
- SS_e: Suspended solids in the overflow.

Basing on the results from the analysis program and the control and measuring program a few indicators were calculated and were used to evaluate the process performances of the pilot reactor in the Initial State Analysis, in Study 1 and in Study 2. These indicators were:

- $sCOD_{in}$ / $NH_4\text{-}N_{in}$ ratio in the inflow;
- Ammonium removed concentration (mg/L), determined as:
  $NH_4\text{-}N_{rem} = NH_4\text{-}N_{in} - NH_4\text{-}N_{out}$

- Ammonium removal efficiency (%), determined as:
  $NH_4\text{-}N_{rem,eff} = (NH_4\text{-}N_{rem} / NH_4\text{-}N_{in})*100$

- Total nitrogen removed concentration (mg/L), determined as:
  $TN_{rem,1} = NH_4\text{-}N_{in} - (NH_4\text{-}N_{out} + NO_2\text{-}N_{out} + NO_3\text{-}N_{out})$
  $TN_{rem,2} = TN_{in} - TN_{out}$

- Total nitrogen removal efficiency (%), determined as:
  $TN_{rem,eff\,1} = (TN_{rem,1} / NH_4\text{-}N_{in})*100$
  $TN_{rem,eff\,2} = (TN_{rem,2} / TN_{in})*100$

- Total nitrogen loading rate (g/m^2d), determined as:
  $TN_{load,rate} = \frac{NH4-Nin*Qin}{A_{bio}}$
  Where:
  A_{bio} is the total biofilm surface on all the carriers and it was determined as the volume occupied by all the carriers (97 L) multiplied by the specific surface of one carrier (500 cm^2/m^3) (A_{bio} = 48.5 m^2).

- Total nitrogen removal rate (g/m^2d), determined as:
  $TN_{rem,rate} = \frac{TNrem,1 *Qin}{A_{bio}}$

- The ratio between the nitrate produced and the ammonium removed, determined as:
  $NO_3\text{-}N_{prod} / NH_4\text{-}N_{rem}$ ratio

- The alkalinity consumption (%), determined as:
  $\text{Alk cons} = \frac{Alk_{in}-Alk_{out}}{Alk_{in}} * 100$

- The hydraulic retention time, determined as:
  $HRT = V / Q_{in}$

- The sludge retention time, determined as:
  $SRT = \frac{V*TSS}{Q_e*SS_e}$

7.4 Initial State Analysis

Initially the pilot reactor was operated as it follows. Intermittent aeration was applied with 20 minutes as aeration time and 40 min as non-aeration time and the DO concentration was set to 0.8 mg/L. The temperature inside the reactor was maintained at 20°C. The average inflow rate was 144 ml/min and the average HRT was 23.15 h. The average return sludge flow rate was 130 ml/min. The flow rate of the return sludge was often adjusted, between 80 and 150 ml/min, in order to keep the TSS concentration stable in the reactor and inside a range of 900-1200 mg/L. The inflow rate was kept constant for the whole project.

This initial study started on October 16th which is indicated as day 0. All other days were also indicated with cardinal numbers from this starting date. As the study started, the operational program (see section 8.1) was applied regularly as previously described and the initial state was from day 0 to day 42, which corresponds to more than one month. Theoretically, this first phase was supposed to require less time, but two important breakdowns occurred and after each of them it was necessary to plan some days for the system to recover. The Initial State Analysis will be discussed together with the results of Study 1 and Study 2 in chapter 8th.

7.5 Study 1: Operational Strategies for improving the Performances

Study 1 consisted in applying seven strategies in order to evaluate the process performances of the pilot reactor under different operational conditions. This study lasted from day 42 to day 109. The strategies are summarized in table 2 and table 3.

Table 2: Operational strategies (Part 1)

Strategy	Period	Strategy type	Aim	From	To
1	day 42 – day 109	Change of aeration pattern	Suppress NOB	20 min air/40 min non-air	10 min air/50 min non-air
2	day 42 – day 109	Increase of Temperature	Support AnAOB	20 °C	23 °C
3	day 50 – day 57	Increase of DO set-point	Support AOB over NOB	0.6 mg/L	1.0 mg/L
4	day 57 – day 64	Increase of DO set-point	Support AOB over NOB	1.0 mg/L	1.4 mg/L

On day 64 Study 1 was interrupted because of the Christmas holidays. The DO was decreased to 0.6 mg/L in order to keep the reactor as more stable as possible during the break. Study 1 started again on day 85.

Table 3: Operational strategies (Part 2)

Strategy	Period	Strategy type	Aim	From	To
5	day 86 – day 92	Initial State Analysis	Monitor after Christmas break	\	\
6	day 92 – day 109	Increase of DO set-point	Support AOB over NOB	0.6 mg/L	0.1 mg/L

Strategy 1

The first strategy was applied on day 42 and concerned the transient anoxia. The duration of the aeration period was shortened from 20 min to 10 min and the length of the non-aeration one was increased from 40 min to 50 min. The reason why this change was chosen was to support AOB's growth over NOB. The hope was that, by extending the non-aerated phase, the lag phase of NOB would have increased too. Therefore, while NOB would have remained inactive, the AOB, which are supposed to reactivate faster than NOB after an anoxic period, would have reduced ammonium to nitrite without any nitrate production. This new aeration pattern was maintained for the rest of the study period till day 109.

Strategy 2

On day 42 a change in temperature was also operated. The temperature was increased from 20°C to 23°C in order to support AnAOB's growth on the carriers. When this study started, little biomass was present on the carriers (figure 8), but thicker biofilms were reported by previous authors (see Malovanyy et al., 2017) that worked on the same reactor. Therefore, detachment of the biomass was thought to have occurred.

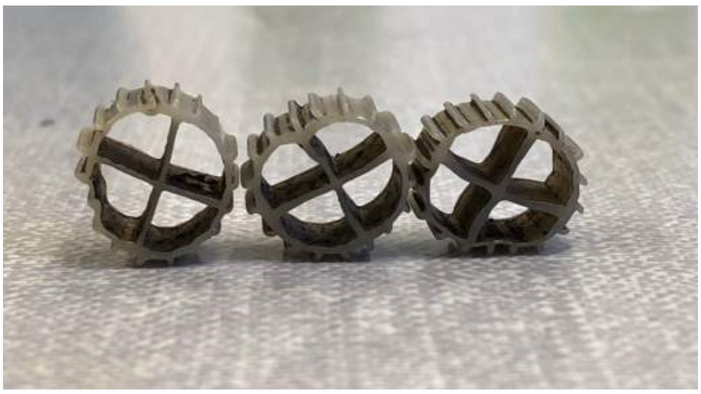

Figure 8: Carriers picked on day 8

This strategy was applied with the purpose of having a population of anammox bacteria as more flourishing as possible. Therefore, a temperature increment of few degrees was considered appropriate in order to make the environment more suitable for AnAOB's growth without affecting the results achieved by applying Strategy 1. Afterwards, this temperature was maintained constant at 23°C for the whole study. The results of Strategy 2 were investigated by performing tests on the growth of biofilm.

The growth of the biofilm was analysed by measuring the dry-weight of the biomass after removing it from the carriers. This procedure is not standard: to evaluate the biofilm growth the thickness of the biofilm layer is usually used. However, this method only wants to give a general idea of the phenomenon.

A number of 10 carriers was chosen. Every time ten new carriers were picked from the reactor and placed in an aluminium pan. The pan was then heated up to 105°C in a pre-heated oven in order to remove the water adsorbed by the biofilm and present on the surface of the carriers. The pan was left inside the oven overnight. Successively, the dish was inserted in a desiccator to stabilize its temperature. The dish and carriers were weighted together. After that, the carriers were immersed in a 33% HCl solution overnight in order to remove all the biomass attached. The empty carriers were then placed in the same aluminium plate that was inserted again in the oven at 105°C overnight and in the desiccator after. Successively, it was weighted again. Finally, the weight of the biomass on carriers was calculated as the dry-weight of the carriers with the biomass attached minus the dry-weight of the empty carriers.

This test was performed on day 29 and 36 before implementing Strategy 2 and on day 92. The test on day 36 was done in order to check the consistency of the methodology. In fact on day 29 and on day 36 similar results were supposed to be obtained since no increment of biomass should have been found after one week only.

A second phenomenon was also studied. The empty carriers that had already been used on day 29 were put in a pierced plastic vessel that was immersed in the liquor of the reactor. These carriers were analysed on day 92 as well. The purpose was to evaluate the AnAOB's growth from the beginning and to observe if the biofilm was growing faster on these cleaned carriers than on the others that had biomass already attached. In a such a case, partial or total substitution of the old carriers with new ones would have been considered.

Strategy 3
The third strategy was about the DO concentration in the reactor. The hypothesis was that while the NOB were inactive because of the longer non-aerated phase, AOB's activity could have been sped up by increasing the DO level in the reactor. This strategy started on day 50. On this date the DO set-point was increased from 0,6 mg/L to 1,0 mg/L.

Strategy 4
On day 57 the DO set-point was increased to 1,4 mg/L. The process performances were monitored till day 64 that is when the Study 1 stopped. On day 64 the DO set-point was reduced again to 0.6 mg/L.

Strategy 5
After the Christmas holidays Study 1 restarted and from day 86 the pilot reactor was monitored again. The DO set-point was maintained at 0.6 mg/L and an initial state analysis was run again from day 86 to day 92 in order to check the process performances after the break.

Strategy 6
On day 92 the DO level was increased again to 1.0 mg/L and the process performances were evaluated till day 109 (February 2th), which represent the end of Study 1.

7.6 Study 2: Influence of Different Parameters and Factors

This study was focused on investigating the correlation between the process performances and the physical and chemical process factors and few other parameters that play a role in the process. This correlation was studied from day 2 to day 64. Therefore, this study also included the Initial State Analysis. The parameters that are discussed in chapter 9th are: sCOD/NH_4-N_{in} ratio, Alkalinity, pH, TSS and SRT. The trend of these parameters/factors over time depended on a several features that were the chemical reactions of the process, the collapses during the Initial State Analysis, the strategies of Study 1 and more.

7.7 Study 3: Settling Properties

Sedimentation tests were necessary to evaluate the settling properties of the activated sludge in the clarifier. In this tank, the sludge was observed several times to float instead of settling. Two properties were therefore investigated. The first one was the sludge settleability that was evaluated by calculating two quantitative measures that are sludge volume index (SVI) and the stirred sludge volume index $SSVI_{3.5}$. The second property under study was the initial sedimentation velocity (ISV). Two different vessels were used: a 1 L regular sedimentation cylinder, for SVI and a 3.5 L sedimentation cylinder with slowly stirring (1 r.p.m.), for determining $SSVI_{3.5}$ and ISV.

7.7.1 Sludge Volume Index

SVI is the most commonly test to evaluate the ability of the activated sludge to sediment and compact. It can also be used to investigate if there are any changes occurring in the sludge by trending the SVI values over time.

To determine SVI the following formula was applied:

$$SVI(ml/g) = \frac{SV_{30}}{TSS_{sludge}}$$

Where:

SV_{30} is the sludge volume at 30 min. The sludge is let to thicken and the height of the interface is measured in the graduated cylinder after 30 min;

TSS_{sludge} is the value of total suspended solids in the solution.

Both SV_{30} and TSS_{sludge} were determined every time.

Several guidelines values exist for SVI. Table 4 reports some general guidelines that were used to assess the settling properties of the activated sludge used under question. For each SVI range the related sludge characteristics are described.

Table 4: Sludge settling properties in relation to SVI (Trygar, 2010; Yousuf, 2013)

SVI (ml/g)	Sludge characteristics
< 80	It is a dense, old and over-oxidized sludge with fast settling properties. This sludge begins to sediment quickly right after that the settling test has started. Before settling it does not usually create large aggregates. The water above the settled sludge blanket may appear cloudy. It can cause a relatively high effluent turbidity.
80 – 250	It is a sludge with good settling properties because it settles more slowly and forms a uniform blanket that traps particles better. This blanket floats at the beginning of the test and then it starts to settle after few minutes when larger particles come together. As the sludge compacts, channels through the sludge are created by the liquid that is squeezed. Most of the plants have a good-quality effluent when their SVI is within this range.
> 250	It is a sludge that settles slowly and with poor settling properties. High values of SVI are usually of a young sludge in which flocs have just started to form. This usually occurs in the process start-up. The surface above the sludge blanket is cloudy with flocs left behind which settle more slowly than the blanket or that do not settle at all. It can produce an effluent with very high effluent turbidity.

SVI has some weaknesses. First of all, SVI variations depend on the concentration of suspended solids. Poorly settling sludges have a TSS critical concentration of 2 g/L. Once this concentration is reached, it is not possible to perform the test because the sedimentation process becomes very long. For sludges with good sedimentation properties this concentration limit goes up to 6 g/L (Stypka, 1998).

In addition, it is possible that two sludges have different settling properties even if they have the same SVI. Therefore, it is hard to compare SVI results between diverse wastewater treatment plants. Every plant obtains specific results and this is why there are several SVI guidelines. Finally, SVI is influenced by the dimensions of the sedimentation cylinder. The sludge settles more slowly in smaller vessels because of the friction on the walls (Stypka, 1998).

7.7.2 Stirred Sludge Volume Index

SSVI is a more accurate test because it is used in order to recreate the non-ideal conditions in the sedimention tanks, its results are not affected by the dimensions of the sedimentation column and it produces less variable values compared to SVI (Stypka, 1998; Yousuf, 2013).

To determine $SSVI_{3.5}$ the following formula was applied:

$$SSVI_{3.5}(ml/g) = \frac{SSV_{30}}{TSS_{sludge}}$$

Where:

SSV_{30} is determined as:

$SSV_{30} = \frac{\text{height of the sludge at 30 min}}{\text{initial height}} * 1000\, ml/L;$

TSS_{sludge} is the value of total suspended solids in the solution.

Activated sludges with good settling properties have a $SSVI_{3.5}$ lower than 120 ml/g, whereas poorly settling sludges have a $SSVI_{3.5}$ higher than 200 mg/L (Stypka, 1998).

7.7.3 Initial Sedimentation Velocity

ISV is parameter used in designing and projecting sedimentation tanks (Vanderhasselt and Vanrolleghem, 2000). For determining ISV the sludge was poured in 3.5 L sedimentation cylinder with slowly stirring and the sludge level was measured according to the following time intervals for one hour:

- 0 – 10 min: the sludge level was measured every minute;
- 10 – 30 min: the sludge level was measured every two minutes;
- 30 – 60 min: the sludge level was measured every 10 minutes.

The sedimentation curve was then drawn by plotting the sludge level (h) versus the time (t) and a tangent was drawn to the first part of the sedimentation curve. Successively two points of intersection, A and B, between the tangent and the curve were chosen and the following formula was applied:

$$\text{ISV} = -\frac{dh}{dt}$$

Sedimentation tests for ISV and SVI were performed according to the scheduled exposed in Appendix B.

8. Results and Discussion

The following section presents and discusses the results achieved through the whole project. All the data come from the chemical analyses and from the online measurement system. Appendix C reports the results of chemical analyses, the online data, the results of the calculations and the operational conditions of the pilot reactor.

8.1 Nitrogen Conversions

The process performances of the pilot reactor at the initial state (day 2 – day 42) and during Study 1 (day 42 – day 109) are here described. Figure 9 reports the influent ammonium concentration (NH_4-N_{in}) and ammonium (NH_4-N_{out}), nitrite (NO_2-N_{out}) and nitrate (NO_3-N_{out}) concentrations in the outflow in relation to the time. Figure 10 shows the removal efficiencies of ammonium (NH_4-$N_{rem,eff}$) and total nitrogen ($TN_{rem,eff\ 1}$ and $TN_{rem,eff\ 2}$). Figure 11 reports the total nitrogen loading rate ($TN_{load,rate}$) and the total nitrogen removal rate ($TN_{rem,rate}$). Breakdowns with recovery periods and strategies are also depicted in the figures.

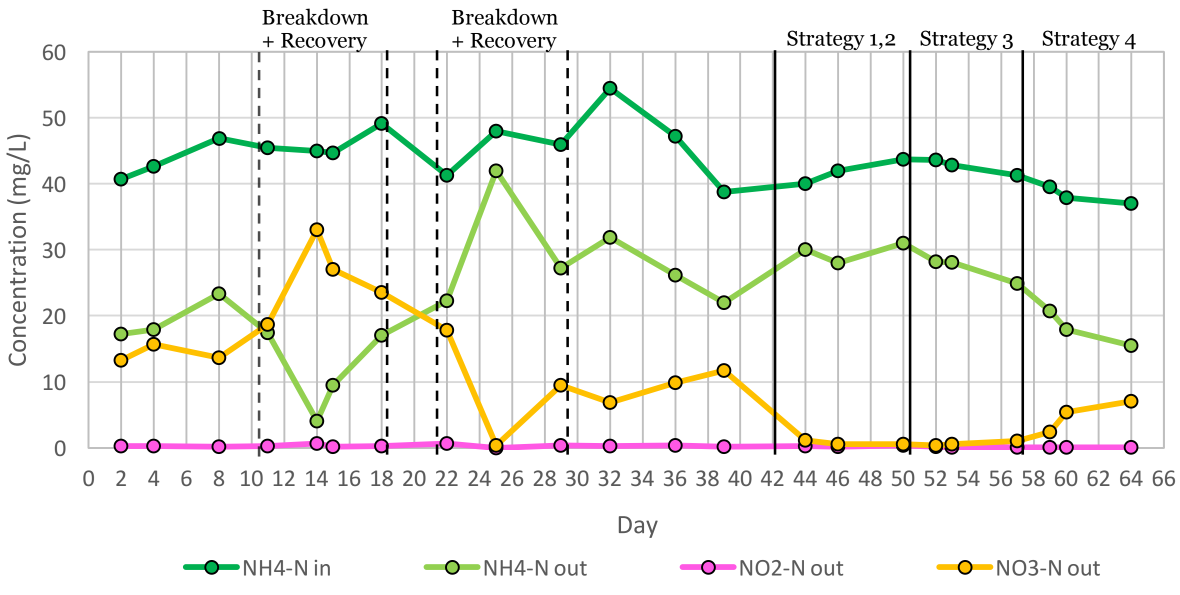

Figure 9: Process performances, inflow and outflow concentrations (Initial State Analysis and Study 1)

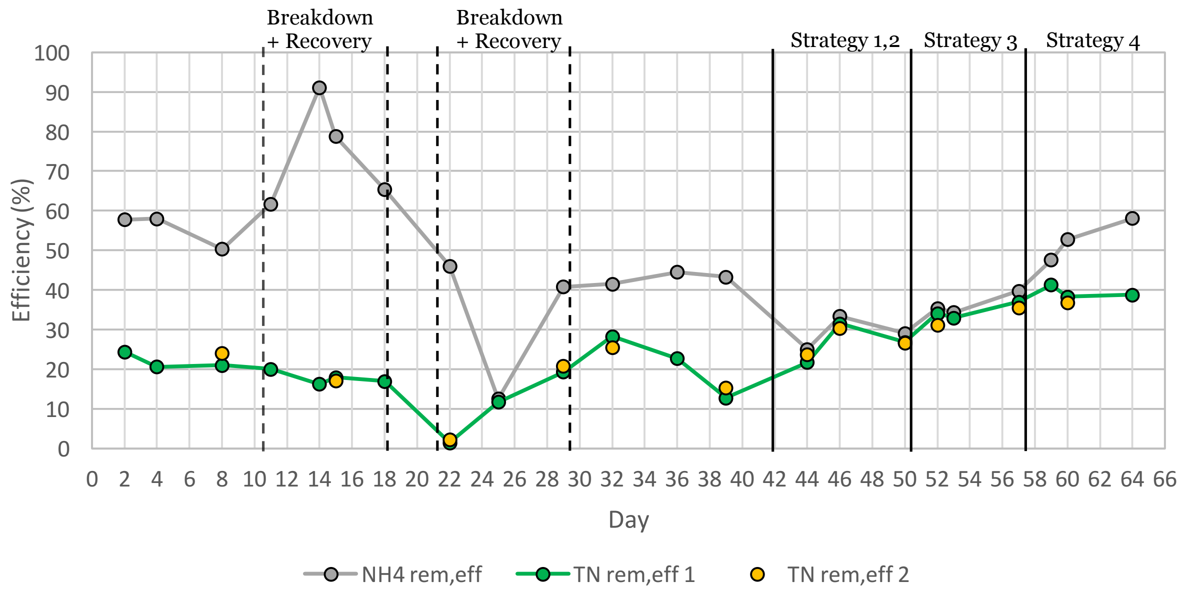

Figure 10: Process performances, nitrogen removal efficiencies (Initial State Analysis and Study 1)

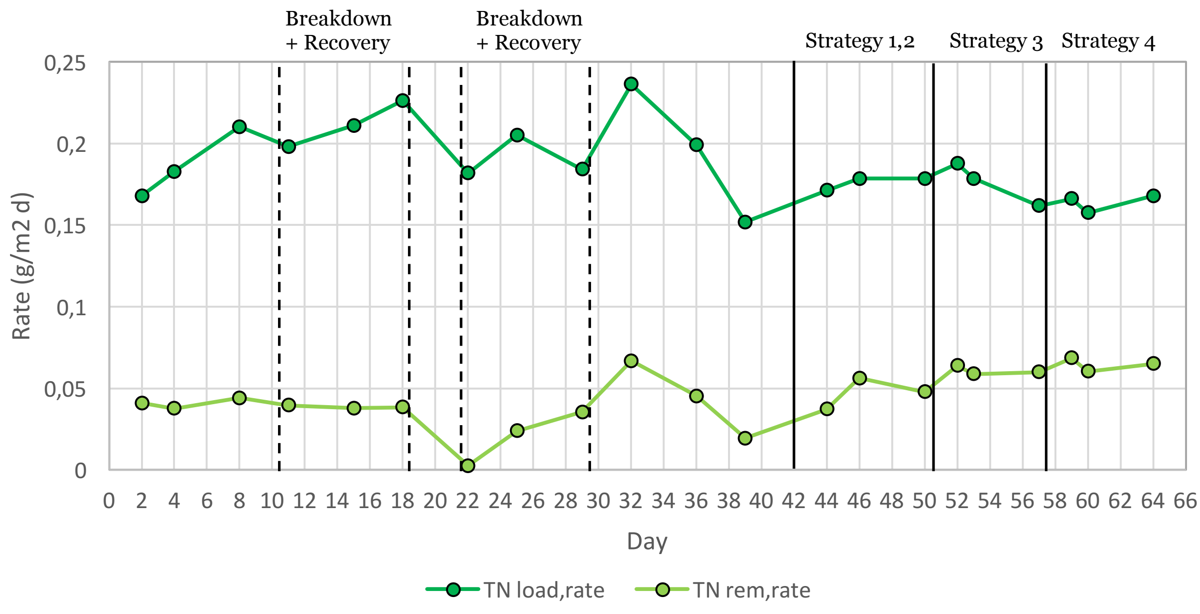

Figure 11: Process performances, nitrogen loading and removal rates (Initial State Analysis and Study 1)

Figure 10 shows two different total nitrogen removal efficiencies: $TN_{rem,eff\ 1}$ and $TN_{rem,eff\ 2}$. The former corresponds to a balance on all nitrogen forms (ammonium, organic nitrogen, nitrate and nitrite), whose concentrations are measured twice a week. Organic nitrogen is ignored since it is considered to have a very low concentration in the inflow. The latter is calculated as difference between TN_{in} and TN_{out} that are both measured only once a week.

The procedure to estimate $TN_{rem,eff\ 2}$ is more accurate, but $TN_{rem,eff\ 1}$ and $TN_{rem,eff\ 2}$ overlap each other in figure 10. This means that the assumptions made to calculate $TN_{rem,eff\ 1}$ were correct. Only $TN_{rem,eff\ 1}$ will be discussed from this point forward.

8.2 Initial State Analysis

8.2.1 Analytical Results

At the beginning of the initial state analysis the process was working with stability. Overall, the inflow and outflow concentrations were constant. Between day 2 and day 11, NH_4-N_{in} was equal to 44.0 mg/L on average (standard deviation, σ=2.8), $TN_{load,rate}$ was also stable and it was 0.2 g/m²d on average (σ=0.02). NH_4-N_{out}, NO_2-N_{out} and NO_3-N_{out} were respectively 19.0 (σ=2.9), 0.2 (σ=0.04) and 15.3 (σ=2.5) mg/L on average and $TN_{rem,rate}$ was equal to 0.04 g/m²d (σ=0.003) (figure 9 and figure 11). However not good performances were achieved, since average NH_4-$N_{rem,eff}$ was equal to 56.9% (σ=4.7), but $TN_{rem,eff\ 1}$ was only 21.5% (σ=2) on average (Figure 10). From these initial results it was possible to deduce that full nitrification was taking place more than actual nitrogen removal, since the removal efficiencies, NH_4-$N_{rem,eff}$ and $TN_{rem,eff\ 1}$, did not match, there was a wide gap between them (figure 10) and nitrate concentration in the outflow was high (figure 9).

The very low effluent nitrite concentration and the high nitrate production in the effluent suggest that partial nitritation was performed by AOB, but most of the nitrite produced was consumed by NOB. Therefore, there was either a predominance of NOB over AnAOB in competing for nitrite and an inadequate anammox activity. Figure 8, in fact, showed that biofilm on the carriers was very thin.

Breakdown 1
The stability was lost when the first breakdown occurred on day 11: the inflow pump to the IFAS reactor clogged first and then totally blocked on day 14. The filters, between the equalization tank and the UASB reactor, were the cause of this failure: they were not working properly because of overuse. Therefore, the particles were not filtered out, but entered the equalization tank and caused a blockage in the inflow pump. This problem was solved by placing new filters and applying a recovery time of four days (till day 18) and

decreasing the DO set-point to 0.6 mg/L. This was actually done because in the beginning this collapse was thought to be due to an excess of dissolved oxygen.

Because of this breakdown NH_4-N_{out} decreased to zero since no ammonium entered the reactor anymore and nitrate started to accumulate. This means that most of the ammonium left was converted into nitrate by NOB. Nitrite production also increased to 0,7 mg/L on day 14 (figure 9). However, this NO_3 accumulation suggests that HB's activity was low, otherwise nitrate would have been converted into gaseous nitrogen through denitrification. The aspect can also be observed in figure 10: NH_4-$N_{rem,eff}$ was extremely high, whereas $TN_{rem,eff\ 1}$ slightly decreased and dropped down after (figure 10).

Breakdown 2
The second collapse occurred on day 22: the aeration valve stopped and no oxygen was supplied to the IFAS reactor for three days (till day 25). The problem was fixed and a 4 days-recovery period was applied (till day 29).

A 3 days long non-aeration period made the nitrate dropped to zero nearly and the ammonium in the outflow reach almost the concentration in the inflow. This means that the reactions were performed very little. It is also visible from figure 10: NH_4-$N_{rem,eff}$ and $TN_{rem,eff\ 1}$ overlapped on a value of 19% on day 25. Therefore, nitrogen removal was still operated even if very limited. Anammox bacteria were the only responsible of this removal. NOB were inhibited by the lack of oxygen and, therefore, they did not produce nitrate that could have been consumed by HB for denitrification. It was observed that the system recovered much faster in this occasion than after the first problem. This is probably because in this case the anammox activity was not inhibited since AnAOB thrive under anoxic conditions. However, NOB seemed to have been affected permanently because after the recovery period the situation went back as it was at the beginning of this study apart from the nitrate production that remained lower. This means that the second collapse had the positive effect of limiting the NOB's activity.

Between day 30 and day 39 the process was stable and the initial state was studied again: NH_4-N_{out}, NO_2-N_{out} and NO_3-N_{out} were respectively 26.9 (σ=2.1), 0.3 (σ=0.1) and 9.5 (σ=2) mg/L on average. Average $TN_{load,rate}$ and average $TN_{rem,rate}$ were respectively 0.2 (σ=0.04) and 0.04 (σ=0.02) g/m^{2}d and NH_4-$N_{rem,eff}$ and $TN_{rem,eff\ 1}$ were 42.5 % (σ=1.7) and 20.7% (σ=6.5) respectively on average.

8.2.2 Online Monitoring: Process Cycle Analysis

Figure 12 shows the changes in the concentrations of dissolved oxygen and ammonium and nitrate in the outflow during three aeration cycles of one hour. It is possible to observe that nitrate production was only taking place when the aeration started and NO_3-N_{out} decreased when the aeration was interrupted. NH_4-N_{out} was steadier instead because the consumption of ammonium was counterbalanced by the continuous incoming of new ammonium in the inflow.

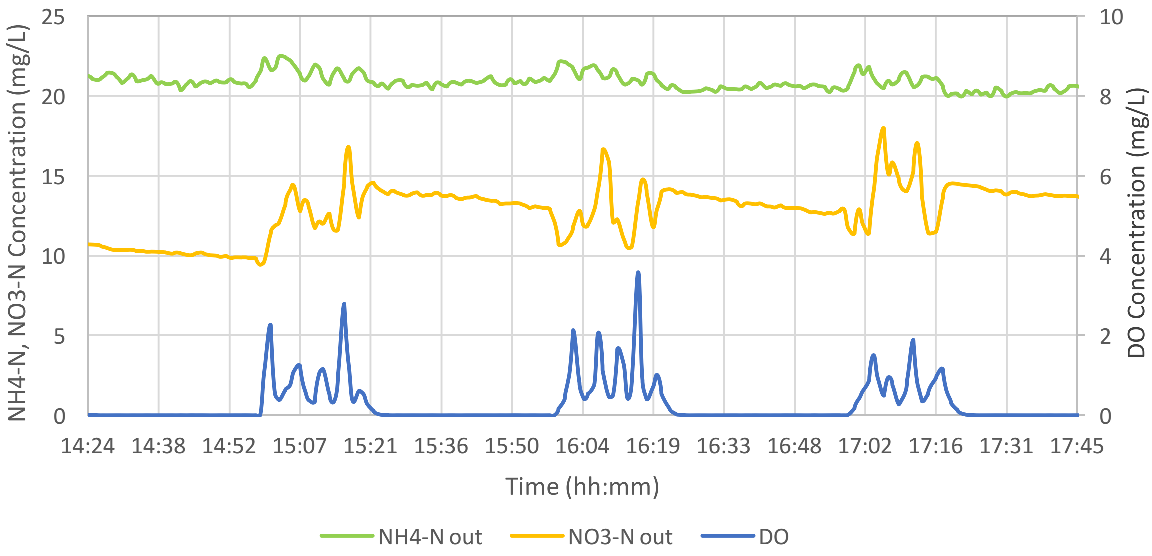

Figure 12: Three process cycles of one hour each on day 39

8.3 Study 1: Enhancement of the Process Performances

During study 1 the influent ammonium concentration was stable till the end of the study period, and equal to 40.9 mg/L (σ=1.4) on average. The other concentrations (NH_4-N_{out}, NO_2-N_{out} and NO_3-N_{out}) were more variable instead because of the strategies that were applied.

8.3.1 Strategy 1

8.3.1.1 Analytical Results

The first strategy consisted on changing the aeration pattern by increasing the non-aeration time from 40 to 50 minutes and decreasing the aeration time from 20 to 10 minutes. The main effect, that was hypothesized, was that, by extending the anoxic period, aerobic bacteria, such as AOB and NOB, would have been inhibited. As figure 9 shows, on day 44 the nitrate concentration dropped of 91% (from 11.7 mg/L on day 38 to 1.1 mg/L on day 44) and remained at an average concentration of 0.8 mg/L (σ=0.2) in the following days (till day 50), while nitrite concentration, even if it was still very limited, increased of 30% (from 0.17 mg/L on day 38 to 0.29 mg/L on day 44). This suggested that the transient anoxia was efficient in out-selecting NOB, while AOB were less affected.

The achievement of NOB inhibition is also visible in figure 10: on day 44 the total nitrogen and the ammonium removal efficiencies started to be identical. This means that all the ammonium that was removed, leaved actually the system in the form of gaseous nitrogen instead of being converted into nitrate.

The reactor was operated for one week with the aeration pattern changed only. At this early stage the NH_4-N_{out} was still high, 29.6 mg/L (σ=1.5) on average between day 44 and day 50 (figure 9). Average NH_4-$N_{rem,eff}$ and $TN_{rem,eff\ 1}$ were equal to 29.1% (σ=4.2) and 26.7% (σ=3.9) respectively. A lower NH_4-$N_{rem,eff}$ in this period, compared to the initial state, indicates that even AOB were affected by a longer anoxic period. However, as mention in section 7.5, NOB were thought to have a longer inhibition lag phase. Therefore, AOB were supposed to reactivate faster in case they were stimulated by applying other strategies, such as an increase of the DO set-point.

8.3.1.2 Online Monitoring: Transition between Initial State and Strategy 1

Effects of Strategy 1 are also visible in figure 13. Online data show the exact moment when the aeration pattern was changed in a time interval between two days before and after that implementation of Strategy 1. The main visible effect is the reduction of nitrate production while the concentration of ammonium in the outflow remained stable, which means that Strategy 1 was very successful in inhibiting especially NOB.

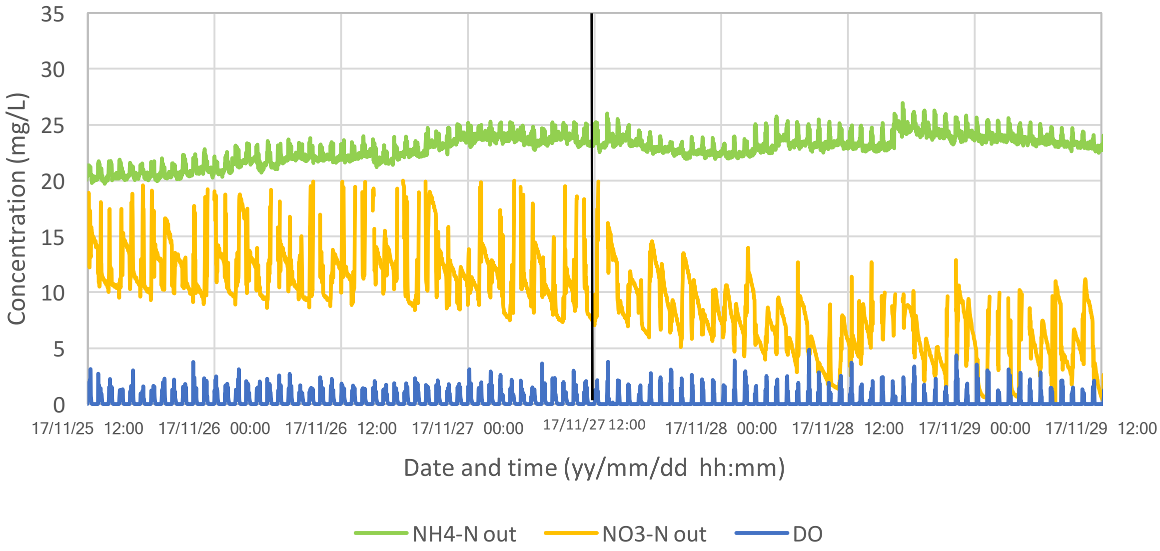

Figure 13: Transition phase between Initial State Analysis and Strategy 1 on day 42

9.3.1.3 Online Monitoring: Process cycle Analysis

NOB inhibition is also visible in figure 14 where three process cycles of one hour each on day 49 are shown. The NO_3-N_{out} trend is very different compared to the one in figure 12, which represents an example of process cycles during the Initial state analysis. In fact, after applying Strategy 1 the nitrate production dropped and the nitrate concentration in the outflow was often close to zero during the anoxic periods.

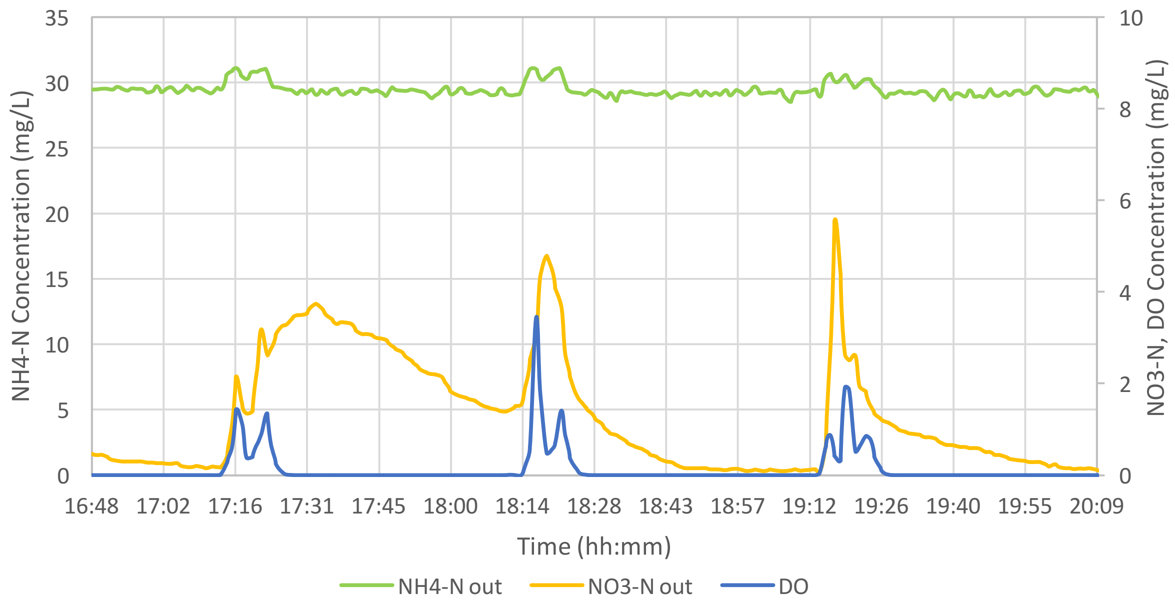

Figure 14: Three process cycles of one hour each on day 49

8.3.2 Strategy 2

On day 42 an increment of temperature was also applied together with the change of aeration pattern in order to support the AnAOB's growth on the carriers. The purpose was to enhance the process performances by having a more flourishing anammox bacterial community. The influence of this strategy was assessed by a few laboratory tests which results are summarized in table 5.

Table 5: Results of biofilm tests

Day	Biomass weight (mg/10 carriers)
29	40.3
36	43.4
92	60.2

Before implementing Strategy 2, on day 29 the weight of biofilm on ten random carriers was equal to 40.1 mg/10 carriers and on day 36 it was equal to 43.4 mg/10 carriers. This demonstrates that the procedure used was correct. In fact, since the biofilm usually grows very slowly, an increment of biomass was not expected after one week only.

After implementing Strategy 2, on day 92 the mass of the anammox biofilm was equal to 60.2 mg/ten carriers. Therefore, the biomass growth had an increment of 50.12 % in nine weeks from day 29. This increment is also visible to the naked eye in figure 15.

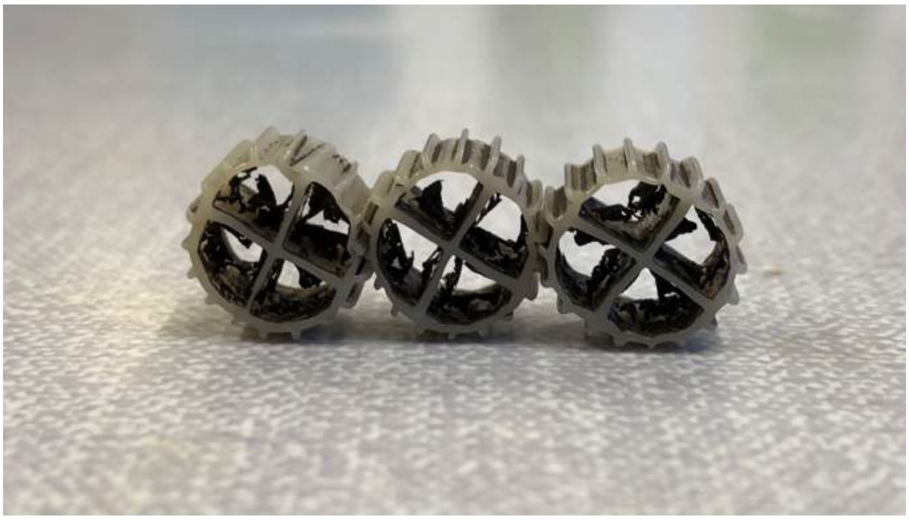

Figure 15: Carriers picked on day 92

The second aspect that was investigated was the AnAOB's growth on clean carriers from day 29. On day 92 the weight of biomass on these carriers was equal to 11.6 mg/10 carriers. Therefore, the biomass increment was equal to 0.54% in nine weeks as well.

Therefore, the anammox bacteria grow faster on carriers that had biomass already attached. Colonizing new carriers requires much longer time. As Gustvasson (2010) already anticipated, it can also happen that the anammox bacteria are not able to form a biofilm structure at all and they need an existing one to enrich (Gustvasson, 2010). Therefore, the suggestion to replace the old carriers with new ones does not have a basis in fact. If this replacement will take place, the time required to have a sufficient anammox activity might be very long.

8.3.3 Strategy 3

8.3.3.1 Analytical Results

On the day 50 the DO set-point was increased from 0.6 to 1.0 mg/L in order to foster the AOB's activity while the NOB were supposed to be still inhibited. Between day 52 and day 57 the removal efficiency increased: 36.5% (σ=2.4) for ammonium and 34.7% (σ=0.6) for total nitrogen (figure 10). Meanwhile nitrate production further decreased: 0.65 mg/L (σ=0.3) on average, which means that NOB were still inactive. The average nitrite concentration was 0.11 mg/L (σ=0.06) (figure 9). The total nitrogen removal rate increased and stabilized too; its average value was 0.06 g/m^{2}d (σ=0.003) between day 52 and day 57 (figure 11).

The increase of DO concentration had no effect on NOB that remained inhibited, but the increase of NH_4-$N_{rem,eff}$ and $TN_{rem,eff}$ 1 suggests that both AOB and AnAOB were more productive. Once again, this enhancement is not due to a denitrification process because of the lack of nitrate. Therefore, the only possible explanation concerns AnAOB and AOB. Anammox bacteria probably needed more time to adapt to the new aeration pattern in order to have an impact on the process performances. In addition, there was more nitrite available because either AOB's activity was higher and NOB's inhibition was still occurring.

9.3.3.2 Online Monitoring: Transition between Strategy 1 and Strategy 3

Figure 16 shows the transition phase when Strategy 3 was applied in a time window of 4 days with the implementation of the strategy in the middle of it. Even if DO was increased, the nitrate production remained low. As a matter of fact, the average NO_3-N_{out} concentration calculated from 2 days before increasing the DO set-point was equal to 1.55 mg/L and it was 1.68 mg/L after. This proves that the NOB's activity was still inhibited. The NH_4-N_{out} profile assumed a descending trend. Consequently, the total nitrogen removal efficiency increased.

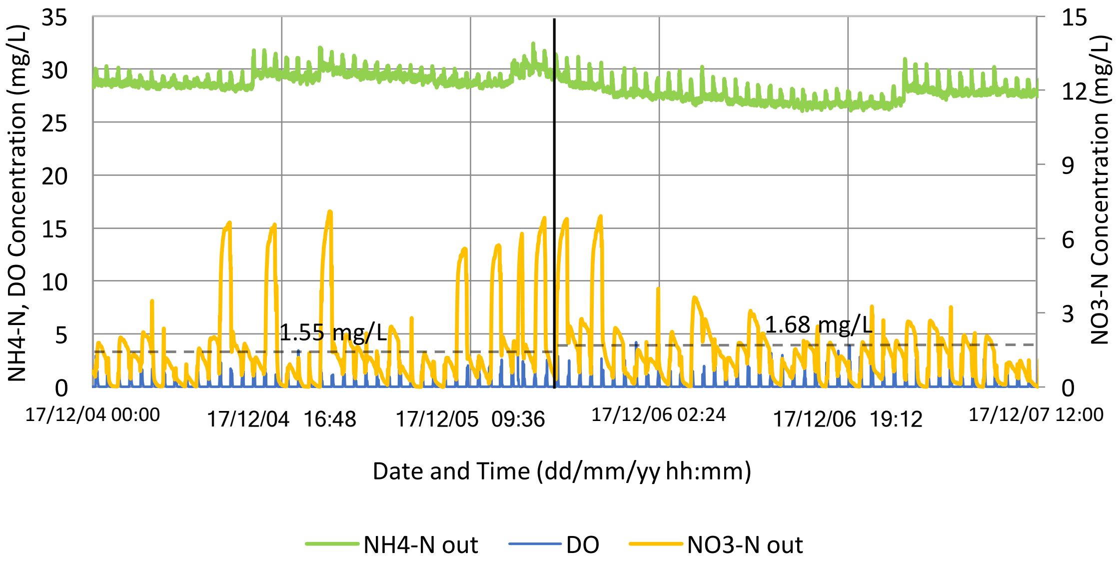

Figure 16: Transition phase between Strategy 1 and Strategy 3 on day 50

8.3.4 Strategy 4

8.3.4.1 Analytical Results

On day 57 the DO set-point was increased further to 1.4 mg/L. NOB reactivated and nitrate reached an average concentration of 5.0 mg/L (σ=0.01) between day 59 and day 64 (figure 9). Because of this reactivation the NH_4-$N_{rem,eff}$ and $TN_{rem,eff\ 1}$ differentiated. Therefore, part of the incoming ammonium was again converted to nitrate instead of leaving the reactor as N_2. However, between day 59 and day 64 the total nitrogen removal efficiency still increased to 39.4% (σ=1.6) with a peak of 41,26 % on day 59 (figure 10).

$TN_{rem,rate}$ remained stable around 0.06 g/m²d (σ=0.04) on average with a peak, on day 59, equal to 0.07 g/m²d that represents the highest value on the whole study (figure 11). However, this removal rate is very low compared to the ones achieved by other authors that worked on the same pilot plant. Plaza et al. (2016) obtained a maximal $TN_{rem,rate}$ equal to 0.2 g/m²d together with a maximal total nitrogen removal efficiency of 74.8%. Trojanowicz et al. (2016) achieved a nitrogen removal of 0.5 g/m²d at its maximum and an average $TN_{rem,eff}$ equal to 55%.

8.3.4.2 Online Monitoring: Transition between Strategy 3 and Strategy 4

Figure 17 shows the transition phase when the DO was increased from 1.0 mg/L to 1.4 mg/L in a time window the goes from 2 days before and after the implementation of Strategy 4. It is visible from figure 17 that after increasing the dissolved oxygen the NO_3-N_{out} trend changed with higher and more frequent peaks. In the two days before applying Strategy 4, NO_3-N_{out} was equal to 2.5 mg/L and it was 4.1 mg/L in the two days after. At the same time the NH_4-N_{out} profile assumed a descending trend. This occurred because either the total nitrogen removal efficiency enhanced and the NOB reactivated oxidizing ammonium to nitrate again.

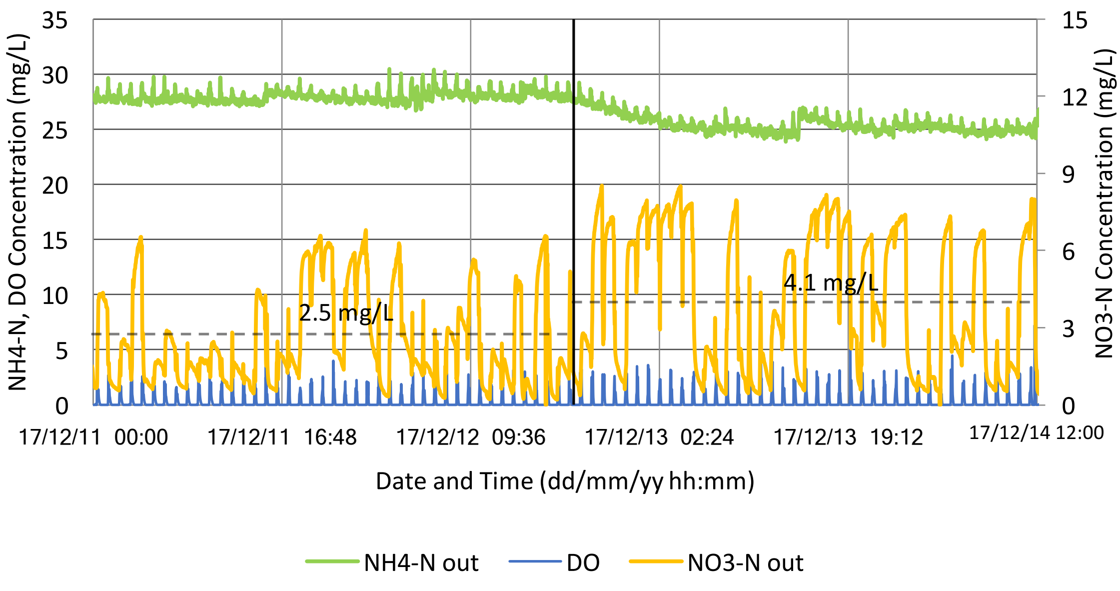

Figure 17: Transition phase between Strategy 3 and Strategy 4 on day 57

8.3.5 Strategy 5 and Strategy 6

On day 64 Study 1 was interrupted and the DO set-point was decreased to 0.6 mg/L. Study 1 restarted on day 86 and the current state was monitored again for one week (Strategy 5). Figure 18 shows the nitrogen conversions between day 86 and day 102. Influent and effluent ammonium had similar profiles and the concentration values were close each other. In addition, both nitrite and nitrate concentrations in the outflow were low and very close to zero. This means that the chemical reactions were performed very little.

The principle cause of this collapse event was due to the inhibition of AOB that were not preforming the reaction of nitritation, as the very low ammonium removal efficiency suggests: NH_4-$N_{rem,eff}$ was equal to 17.0 % (σ=2.2) between day 86 and day 92. This unexpected inhibition happened probably because of a negative combination of several factors that were the low DO level, the long anoxic period and the lack of daily monitoring for more than two weeks.

Strategy 6 was applied on day 92, but the situation did not improve despite the fact that the higher DO level should have stimulated the AOB's activity. Both NH_4-$N_{rem,eff}$ and $TN_{rem,eff\ 1}$ had a variable trend but their values were very inferior to the ones before the Christmas break. A similar behaviour was also assumed by the total nitrogen removal rate.

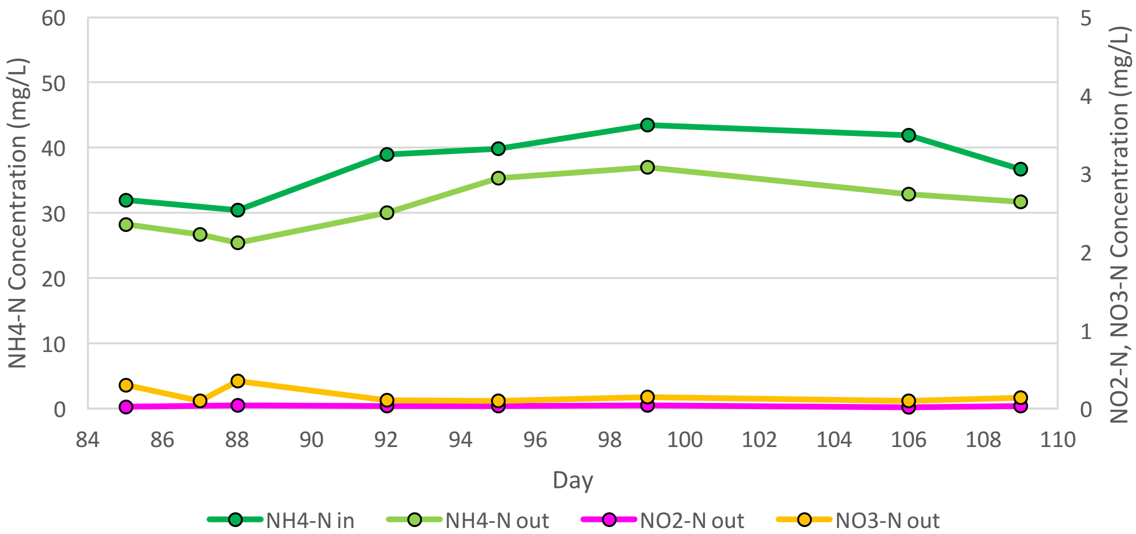

Figure 18: Process performances after Christmas break, inflow and outflow concentrations.

One of the most probable reasons of this total collapse is the high $sCOD_{in}$/NH_4-N_{in} ratio. The ratio was measured as soon as Study 1 restarted and it was 3.3 on day 86. Therefore, a competition between HB, that thrive when there is abundance of carbon sources, and AOB and AnAOB, might have been the cause of the breakdown. The ratio decreased in the days that followed, but the process performances did not improve. It is not possible to know for how long the ratio stayed at an unacceptable level. However, since the process did not recover, it is possible that the UASB reactor did not work well for few days that were enough to cause an irreversible failure of the process.

8.3.6 NOB Inhibition

NOB inhibition was achieved during this study as already anticipated in the previous sections. This phenomenon can be observed from several points of view. Figure 21 represents the ratio between the NO_3-N produced (NO_3-N_{prod}) over the NH_4-N removed (NH_4-N_{rem}). The concentration of NO_3-N produced corresponds to NO_3-N_{out}. This ratio gives an indication on how much ammonium was actually removed instead of being converted into nitrate by NOB. The lower this ratio is, the lower the NOB activity is. Consequently, low values of this ratio indicate that the conversion from ammonium to nitrate was limited and the total nitrogen removal was actually taking place.

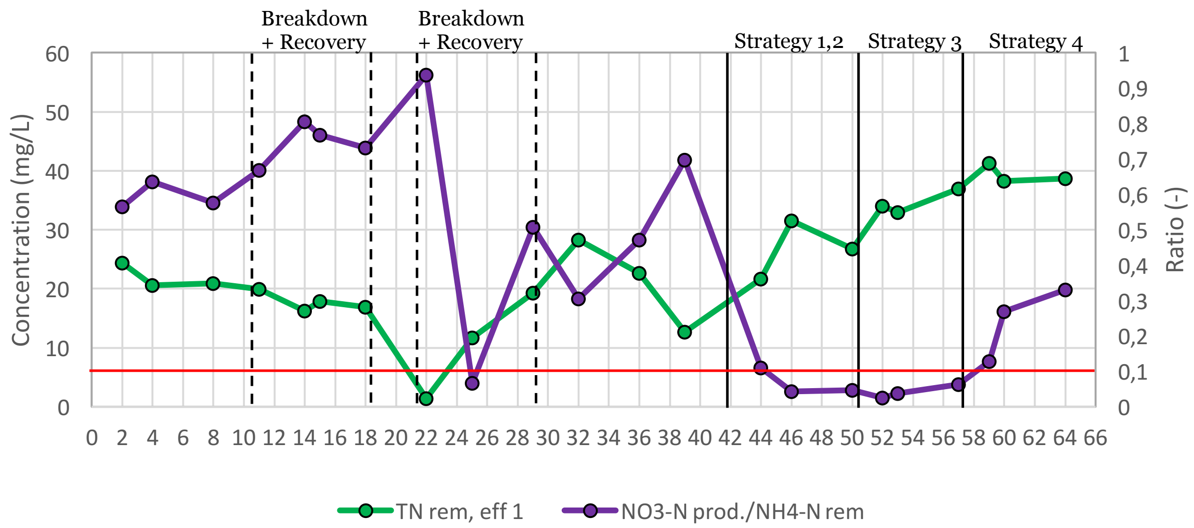

Figure 19: NOB inhibition (Initial state analysis and Study 1)

At the beginning of Study 1 the profile of the NO_3-N_{prod}/NH_4-N_{rem} ratio assumed a constant increasing trend from 0.5 on day 2 to 0.9 on day 22. This peak was due to the nitrate accumulation that occurred because of the first break down. This trend indicates a very high NOB's activity since as Veuillet et al. (2015) stated, good NOB's repression corresponds to values lower than 0.11 (red line in figure 19).

When the aeration stopped working the ratio dropped to 0,07 on day 25 because after three days of non-aeration all aerobic bacteria were totally inhibited. When the recovery period started on day 26 the NO_3-N_{prod}/NH_4-N_{rem} ratio increased again since the aerobic activity was restored.

Between day 29 and day 39 the ratio was equal to 0.5 (σ=2) on average, but it rapidly decreased to a value of 0.11 on day 44 when the aeration pattern was changed. This is the starting point of the NOB's inhibition. From this moment the nitrate production is very little compared to the nitrogen removed because the NOB's activity is very limited. The ratio decreased further to an average of 0.07 (σ=0.03) between day 44 and day 50. When the DO set-point was increased to 1.0 mg/L, the NO_3-N_{prod}/NH_4-N_{rem} ratio was still low and equal to equal to 0.04 (σ=0.02) on average between day 52 and day 57. This means that a higher DO concentration was not enough to reactivate the NOB.

The inhibition stopped when the DO level was increased to 1.4 mg/L. NOB reactivated and the ratio increased to an average of 0.234 (σ=0.1) between day 57 and day 64, with a peak of 0.33 on day 64.

8.3.7 Summary of Operational Conditions

Table 6 summarizes the operational conditions with which the IFAS reactor was operated during the whole study and the process performances. The data reported in table 6 are average values on each strategy period. The first row corresponds to the Initial State Analysis between day 30 and day 42. Table 6 does not report Strategy 5 and Strategy 6.

Table 6: Summary of operational conditions and process performances during study 1

Strategy	Period	Q in (ml/min)	Q return (ml/min)	Temp (°C)	HRT (h)	DO (mg/L)	ar/non-ar (min)	TSS (mg/L)	N-NH4 rem,eff (%)	TN rem,eff 1 (N forms) (%)
\	day 30 – day 42	138.7	130	20	24.1	0.6	20/40	576.0	42.5	20.7
1,2	day 42 – day 50	141.7	140	23	23.5	0.6	10/50	705.8	29.1	26.7
3	day 50 – day 57	139.1	150	23	24.0	1	10/50	700.8	36.5	34.7
4	day 57 – day 64	141.9	146	23	23.5	1.4	10/50	579.7	52.8	39.4

8.4 Study 2: Influence of Different Parameters and Factors

8.4.1 $sCOD_{in}$ / $NH4\text{-}N_{in}$ ratio

Figures 20 shows the inflow concentrations in relation to the $sCOD_{in}/NH4\text{-}N_{in}$ ratio. Between day 2 and day 15 the $sCOD_{in}/NH4\text{-}N_{in}$ ratio was stable and always lower than 2.6. This was because both $NH4\text{-}N_{in}$ and sCOD were stable.

The ratio was higher on day 22 and on day 29 and almost equal to 3. This value represents a threshold that must not be exceeded in case of suspended growth systems in order to prevent heterotrophic bacteria from performing denitrification reactions. Hybrid reactors can usually tolerate higher values because heterotrophic bacteria can be washed out through short sludge retention times (Cao et al., 2017). However, in this project the SRT is very high (section 9.3.5), therefore, a value equal or higher than 3 is not acceptable. This was also shared by Veuillet et al. (2015) that, working on a

IFAS reactor similar to the one used in this project, always had 1.5-2.0 as a target even if short SRT (12 days) was applied.

A correlation can be observed between the total nitrogen removal efficiency and the $sCOD_{in}/NH4\text{-}N_{in}$ ratio from day 39. When the ratio goes down, the efficiency goes up. This is perfectly visible on day 46, 50, 57 and 64.

In conclusion, overall the UASB reactor did not work well. The $sCOD_{in}/NH4\text{-}N_{in}$ ratio did not always have acceptable values and it showed high fluctuations that are not favourable to the process performances.

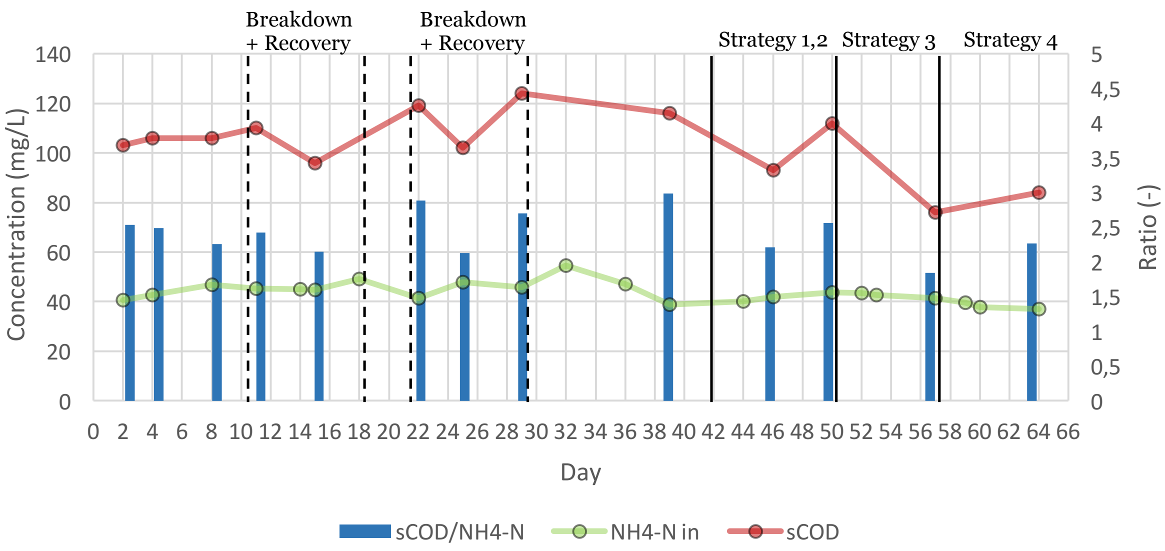

Figure 20: sCODin / NH4-N ratio and inflow concentrations (Initial State Analysis and Study 2)

8.4.2 Alkalinity

Figures 21 correlate the alkalinity consumption and the nitrate concentration. The consumption of alkalinity variates along the study period. From day 2 to day 8, there was a consumption of alkalinity equal to 46% (σ=5.1) on average. From day 11, when the first collapse occurred, to day 18 Alk *cons* increased to an average of 52.6 % (σ=3.2). This was due to the nitrate accumulation that occurred because of the collapse event. In fact, when the nitritation predominates over anammox, as in this case, the consumption of alkalinity is not balanced by its production that occurs during the anammox reaction.

Between day 22 and day 29 the alkalinity consumption decreased to an average of 20.8 with its lowest value equal to 8.7 on day 25. This happened because no aeration was applied for three days. Therefore, both nitritation and alkalinity consumption stopped. Alk *cons* rose again from day 29 as a consequence of restoring the aeration. Between day 32 and day 39, in which the initial state was analysed again, Alk *cons* was very variable with an average of 32.2%.

When Study 1 started, Alk *cons* maintained a variable trend, but with lower peaks. Between day 42 and day 50, in which the aeration pattern was changed, the average consumption of alkalinity was equal to 23.3 % and it was equal to 21.2% between day 50 and day 57 when the DO set-point was increased. The reduction of Alk *cons* can be seen as a consequence of NOB's suppression. In fact, when DO set-point was increased to 1.4 mg/L, the average alkalinity consumption increased too because of NOB's reactivation and it was equal to 27.3% between day 57 and day 64.

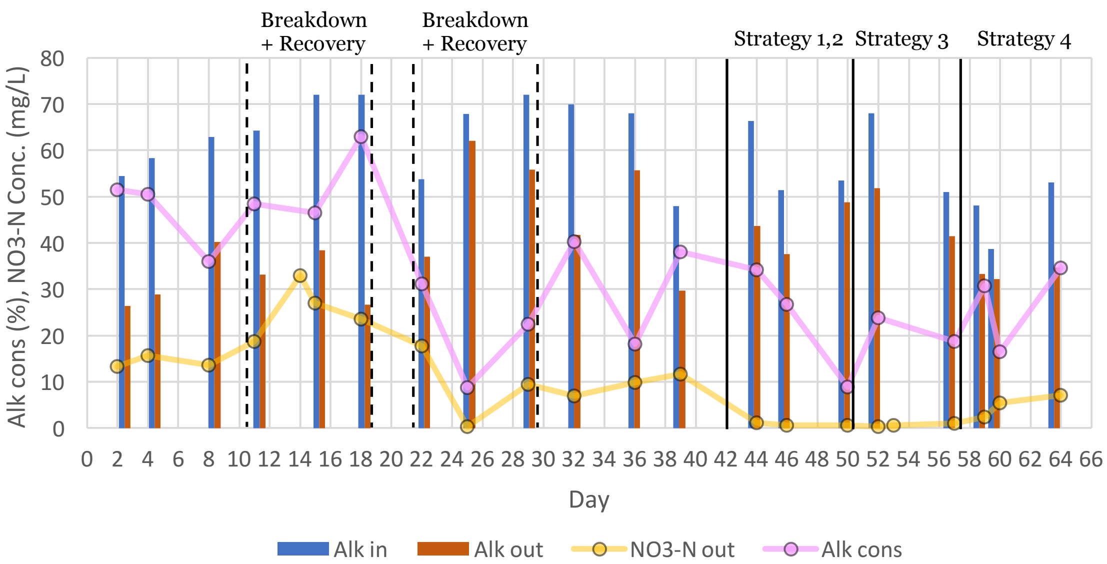

Figure 21 Alkalinity consumption and nitrate production (Initial State Analysis and Study 2)

8.4.3 pH

Figure 22 correlates the pH data with the nitrogen conversions. Both pH_{in} and pH_{out} were always in a range that was between 7 and 8. At the beginning of the study between day 2 and day 18 pH_{in} was always higher than pH_{out}. On day 15, during the first collapse event, there was a wide difference between pH_{in} and pH_{out} because the nitrification of ammonium occurred without the presence of the influent alkalinity that, working as a pH buffer, would have contrasted the decrease of pH.

From day 22 to day 32 pH_{out} started to increase and be higher than pH_{in}. This can be explained as a consequence of the second collapse. Nitritation was not performed because of the lack of oxygen and the increase of pH_{out} was probably due to the anammox activity that was the only one to be accomplished in an anoxic environment. From day 44 the situation was stable with pH_{out} that was always higher than pH_{in} for the rest of the study period.

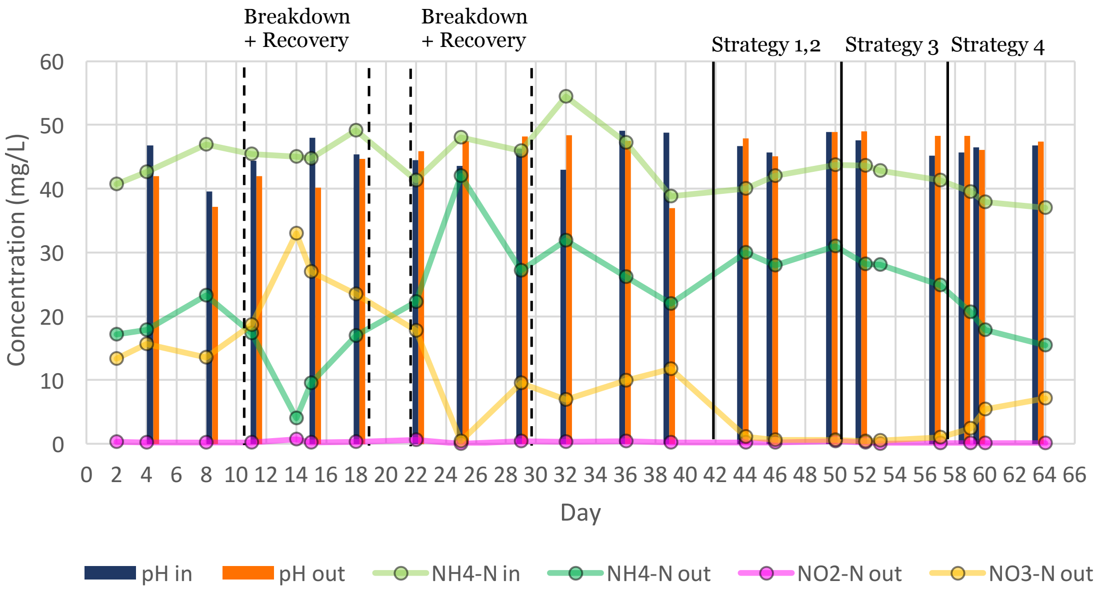

Figure 22: pH and nitrogen concentrations (Initial State Analysis and Study 2)

8.4.4 Total Suspended Solids

During the whole study it was very difficult to maintain a stable value of TSS in the reactor. The main reason was that the activated sludge had very low settling properties, as described further in section 8.1.5.

The sludge was often observed to float on the surface of the sedimentation tank, especially during the Initial State Analysis. This probably caused a periodic wash-out of activated sludge and a return sludge with a low content of suspended solids, which had a negative impact on the process performances. In fact, a relatively high TSS value (around 900-1200 mg/L according to Khayi (2017)) is always needed to maintain a flourishing population of AOB which live in the suspended sludge mostly. In addition, it often occurred that clear water was pumped from the bottom of the sedimentation tank. This happened because the sludge was not settling at the bottom of the clarifier. This problem was always solved by stirring the liquor inside the tank and opening the valve at the bottom of the tank in order to summon the sludge with more pressure. Unfortunately, the situation could not be fixed during the week-ends and during these days the TSS level in the reactor was used to decreasing a lot and reaching even values lower than 300 mg/L. Therefore, the trend of TSS concentration in the reactor is very variable as figure 23 shows.

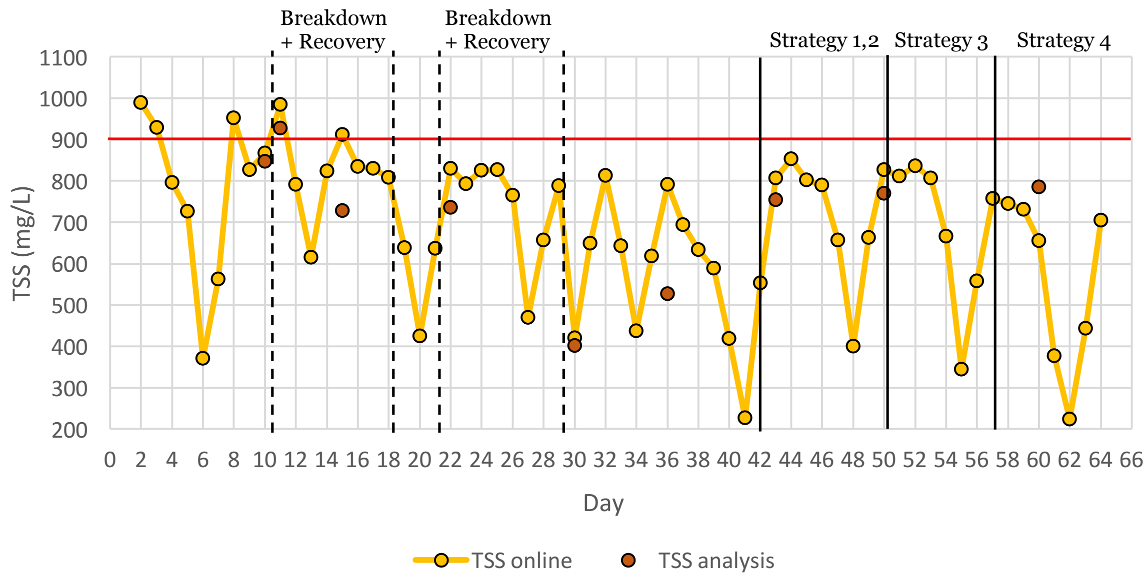

Figure 23: TSS results (Initial State Analysis and Study 2)

The online system constantly kept track of the TSS trend and a daily average was calculated. The TSS analysis was usually done once a week. Since the TSS level in the reactor was very changeable, even daily, analytical results and online data do not always match. However, overall a correspondence is visible, therefore the trend of online data is considered accurate.

Variable TSS, as such, is not beneficial to the process performances, since the process needs a stable and relatively high TSS concentration in order to have a flourishing population of AOB in the sludge performing the reaction of partial nitritation that correspond to first fundamental step of the deammonification process. Unfortunately, in this project the TSS level was either unstable and low. Higher values than 900 mg/L (red line in figure 23), that is the minimum to maintain according to Khayi (2017), were measured only at the beginning of the Initial State Analysis. It is author’s opinion that both the instability and low concentration were the main causes of the relatively low performances of the pilot reactor in the whole project.

8.4.5 Sludge Retention Time

SRT was calculated only once at the end of the process and it was on day 121 (February 14th) in a situation of process stability. Therefore, there was not floating sludge on the surface of the equalization tank and, consequently, the suspended solids (SS_e) in the overflow of the clarifier were very low.

Table 7 reports the values of all the data that were determined on day 121 and were used to calculate the SRT by applying the formula already exposed in section 7.3.

Table 7: Data for the calculation of SRT

V (L)	TSS (mg/L)	SS_e (mg/L)	Q_e (ml/min)
200	921	11,9	130

SRT was calculated to be equal to 82,24 days, which corresponds to an extremely high sludge age. Too long SRT is not beneficial to the process performances because of two main reasons. It impedes that sludge with low settling properties is washed out of the system (Sandino et al., 2016) and it also forces already decayed nitrifiers to remain in the system causing an increase of COD inside the reactor, which can lead to denitrification. Long SRT is suitable for AnAOB, but not for AOB and NOB, because the latter have a faster growth rate (Gustavsson, 2010). Therefore, it is author's suggestion to shorten the SRT down to 30-40 days at least. This reduction will not affect the anammox bacteria because their sludge age does not depend on the retention time of the suspended sludge. AnAOB, in fact, are attached to the carriers that remain in the reactor until they are picked or substituted, therefore they are subjected to a different SRT.

8.5 Study 3: Settling Properties

8.5.1 Sludge Volume Index

The results of the settling tests to determine SVI are shown in table 8. These tests were performed on a solution of activated sludge. This solution consisted in diluted sludge from the well-mixed sedimentation tank and return sludge in order to reach an adequate thickness. In other cases the return sludge was directly mixed with tap water. However, it was not possible to keep track of the partitioning between the different sludge types and water. The sludge from the sedimentation tank, and the return sludge as well, had a different thickness every time and different adjustments were always necessary to reach a sufficient level of TSS in order to perform the test. However, when the TSS level exceeded 2 g/L the sedimentation was incredibly slow and it was possible to observe the settled sludge blanket only after several minutes. According to Stypka (1998) a TSS value of 2 g/L represents a critical concentration for pootly settling activated sludges.

Table 8: SVI results

Day	**Sludge from:**	**SV_{30}** (ml/L)	**TSS** (g/L)	**SVI** (ml/g)
35	well mixed sed. Tank + return sludge	910	1,916	475,0
38	well mixed sed. tank + return sludge	865	1,819	475,5
39	well mixed sed. tank + return sludge	745	1,860	400,6
42	well mixed sed. tank + return sludge	917	2,177	421,3
43	well mixed sed. tank + return sludge	754	1,786	422,2
44	well mixed sed. tank + return sludge	625	1,661	376,4
46	return sludge + tap water	935	1,813	515,7
49	return sludge + tap water	925	1,796	515,1
50	return sludge + tap water	470	1,250	376,0
53	well mixed sed. tank + return sludge	475	1,323	358,9
60	well mixed sed. tank + return sludge	470	1,388	338,7

All the tests show a SVI above 250 ml/g and this confirms that it is a sludge with low settling properties. The sludge was often seen to float on the water surface of the sedimentation tank as already described previously and the design of this tank was considered to be the cause of such a phenomenon. However, it is possible to clarify now by observing the results in table 8 that the problem is in the sludge itself.

In order to improve the situation the activated sludge used in this process should be replaced or mixed with another one having better settling properties. This operation is not usually recommended though. Adding sludge taken from another plant can introduce new bacteria that might compete with the ones already present. In addition, it will be necessary some time to restore the whole process since the new sludge and the bacteria that it contains need time to adapt to the new operational conditions.

There are two main disadvantages of an activated sludge with low settling properties. The first one is the risk of having a return sludge with a low content of suspended solids. This will decrease the TSS level in the reactor and consequently the amount of AOB, whose presence is fundamental to perform the reaction of partial nitritation. The second one is that a low settling sludge can also extend the sludge retention time. This will force NOB and already decayed nitrifiers to remain inside the reactor causing a worsening of the process performances.

8.5.2 Stirred Sludge Volume Index

Table 9: SSVI Results

Day	Sludge from:	SSV_{30} (ml/L)	TSS (g/L)	$SSVI_{3.5}$ (ml/g)
42	well mixed sed. tank + return sludge	456	2,177	209,5
43	well mixed sed. tank + return sludge	358	1,786	200,5
44	well mixed sed. tank + return sludge	340	1,661	204,7
53	well mixed sed. tank + return sludge	280	1,323	211,6
60	well mixed sed. tank + return sludge	284	1,388	204,6

$SSVI_{3.5}$ results confirm further that the activated sludge used in the process has low settling properties, because $SSVI_{3.5}$ was always higher than 200 ml/g. However, compared to SVI results, $SSVI_{3.5}$ showed to produce less variable values as already anticipated by Yousuf (2013).

8.5.3 Initial Sedimentation Velocity

Figure 24 shows an example of sedimentation curve, obtained on day 44, and the graphic procedure to determine ISV as described in section 7.7.3. The rest of the sedimentation curves are shown in figures 25 and 26. In all figures, the orange colour represents the part of the curve that was followed to draw the tangent. Table 9 summarized all the ISV results and correlated them to the concentration of suspended solids.

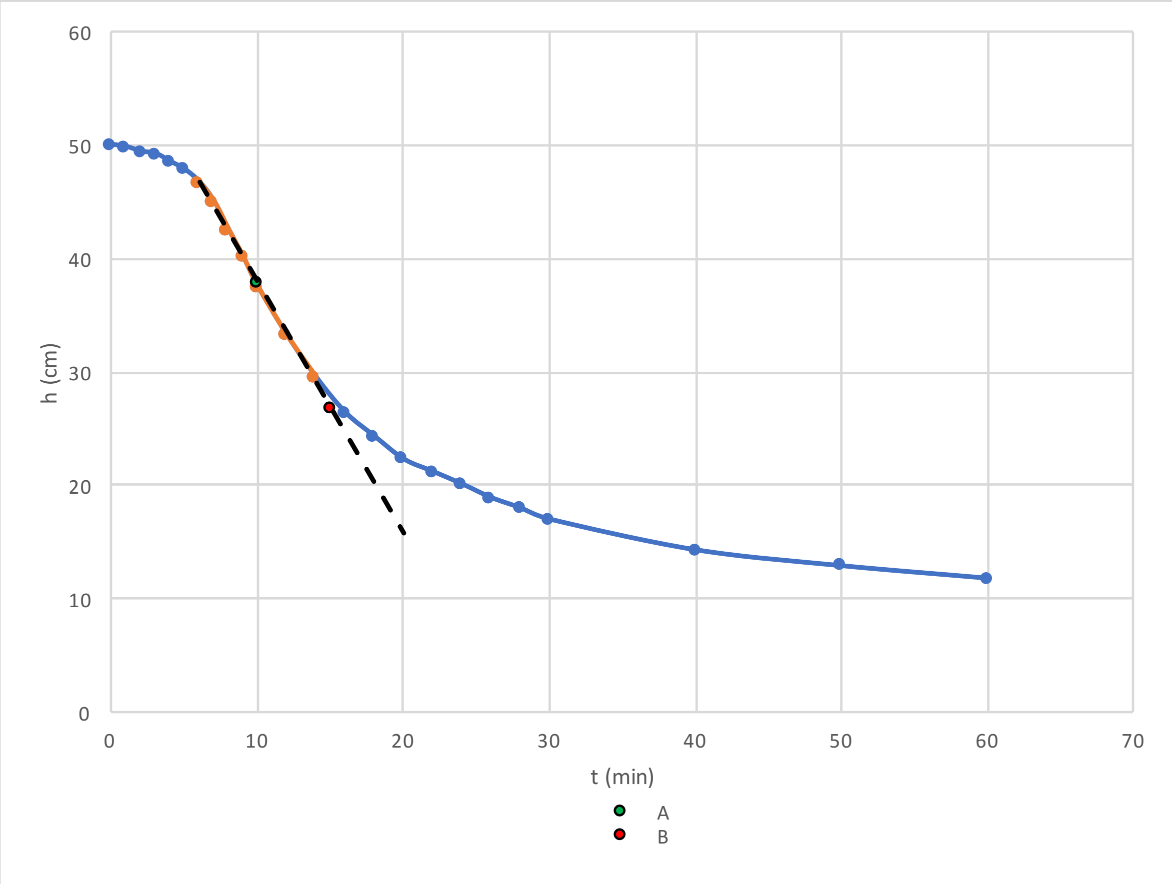

Figure 24: Sedimentation curve with tangent and points of intersection on day 44

Table 10: ISV results

Day	TSS (mg/L)	ISV (cm/min)	ISV (m/h)
42	2,177	1,42	0.85
43	1,786	1,96	1.17
44	1,661	2,21	1.33
53	1,323	2,49	1.5
60	1,388	2,31	1.38

All the sedimentation curves present a typical profile: parabolic in the beginning with slow sedimentation because flocs have not formed yet, then it becomes linear, with flocs that increase their sizes causing a faster sedimentation, and finally asymptotic when the sedimentation of the remaining flocs is slowed by the presence of the ones below that have already settled.

These profiles demonstrate that the settling process occurs as it should. Therefore, SVI and $SSVI_{3.5}$ revealed that it is an activated sludge with low settling properties, but the sedimentation curves show that the sedimentation process do not present other faults.

Table 9 shows that ISV increases when TSS gets lower. This is because the higher the TSS is, the more the friction is between flocs and other particles, which causes a slowdown of the whole settling process.

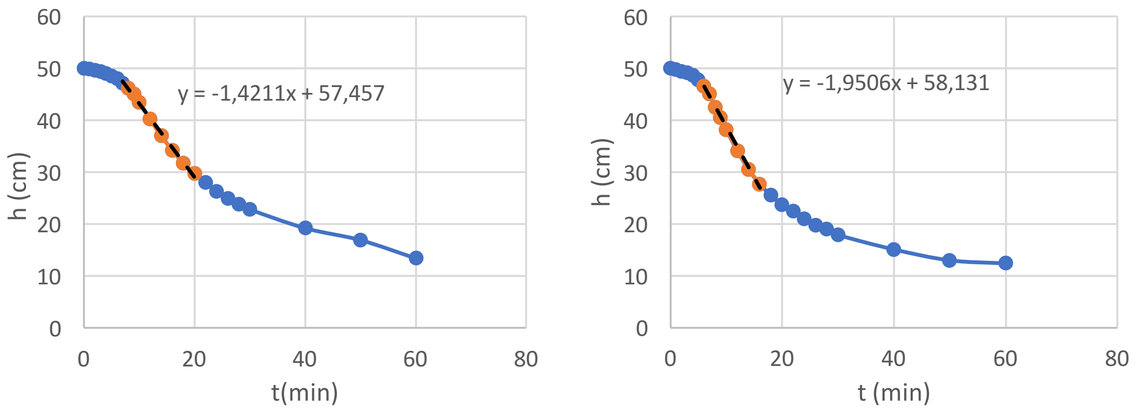

Figure 25: Sedimentation curves on day 42 (left) and 43 (right)

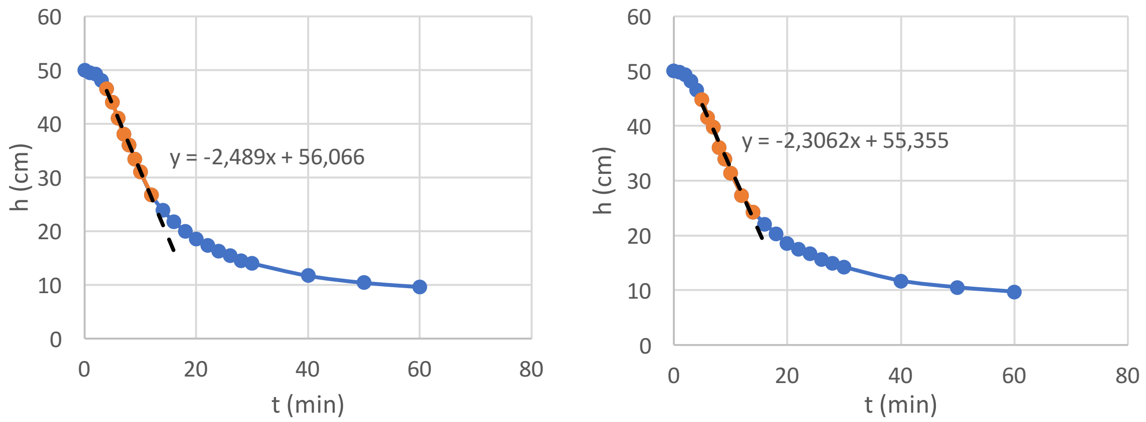

Figure 26: Sedimentation curves on day 53 (left) and on day 60 (right)

Figure 25 and 26 show that by means of Excel it is possible to the determine the initial sedimentation velocity directly as the inverse of the angular coefficient of the trendline. Therefore the points of intersection are needed only in the case in which this graphic procedure is carried out manually.

9. Conclusions

9.1 Study 1

The evaluation of the process parameters in relation to the process parameters was the goal of Study 1. The parameters evaluated were the ones related to the aeration inside the reactor and the evaluation consisted in applying a few operational strategies that aimed to interfere on the competition among the bacterial groups involved. The hypothesis was that if the strategy applied had been successful, the process performances would have improved.

During the initial state the process performances were not good overall. Both the removal efficiencies were much lower compared to the ones determined by Khayi (2017) when working on the same pilot reactor and operating the same aeration pattern 20/40. He obtained 65,6% for NH_4-$N_{rem,eff}$ and 42.5% for $TN_{rem,eff\ 1}$. The main causes of this worsening of the process performances were the low TSS level, that implies a reduced AOB's activity, and the limited AnAOB's activity especially in the beginning of Study 1. On the contrary, the wide gap between the removal efficiencies suggests a high NOB's activity in this initial period.

When Strategy 1 was applied the gap reduced, which indicates a successful NOB's suppression. Consequently, $TN_{rem,eff\ 1}$ increased since AnAOB had more nitrite available, but NH_4-$N_{rem,eff}$ dropped because AOB were also affected by a longer anoxic period and the amount of AOB was not adequate, which is visible from the low TSS value, to provide enough nitrite before being inhibited by the non-aeration period.

The period that goes from day 50 to day 57 is considered the best since it is the period in which the reactor worked with more stability. NOB suppression was still achieved and the process performances increased. NH_4-$N_{rem,eff}$ enhanced because of a higher oxygen availability for AOB, while NOB remained inactive. In addition, AOB were more productive and provided more nitrite to AnAOB. That is why $TN_{rem,eff\ 1}$ increased too. However, the removal efficiencies were still low, compared to the previous studies, and the main reason is due to the TSS level that was still below the limit of 900 mg/L suggested by Khayi (2017).

After implementing Strategy 4 the process performances enhanced further and they reached their maximum in this study, but the process lost its stability. NOB reactivated and the removal efficiencies differentiated. Therefore, ammonium was again partially converted to nitrate instead of leaving the system in the form of gaseous nitrogen. However, it is interesting to notice that the NH_4-$N_{rem,eff}$ went up to 52.8% even if the TSS concentration was very low. This suggests that even if NOB reactivated there was still a predominance of AOB over NOB, since AOB consumed most of the oxygen available.

9.2 Study 2

Regarding the physical and chemical factors, Study 2 showed that the UASB reactor did not maintain a stable and suitable value of the sCOD/NH4-N_{in} ratio. It also showed that the pH always remained in a range between 7 and 8 and that the alkalinity consumption can be used as a further term to evaluate the balance between partial nitritation and anammox. Unfortunately, it was not possible to find any correlation between TSS and the process performances therefore it is author's suggestion to maintain what Khayi (2017) had already found: his recommended TSS range was between 900-1200 mg/L. Finally, SRT was extremely high and therefore it needs to be shortened.

9.3 Study 3

Study 3 showed that the activated sludge used in this IFAS reactor had very low settling properties. Of course, this phenomenon had an effect on the process performances, since slow settling sludge can produce a return sludge with a low content of suspended solids that reduce the TSS level in the reactor. In addition, the settling properties are related to the sludge retention time. Very long retention time might keep in the reactor bacteria, like NOB and already decayed nitrifiers, that it would be better to wash-out instead.

9.4 General Conclusions

Mainstream Partial Nitritation/Anammox process achieved through IFAS reactors can become a competitive solution in nitrogen removal, but this Master's thesis revealed that there are still several challenges to overcome.

- NO_2-N represents the limiting factor of all the process. The nitrite production is the fundamental step because it corresponds to the substrate that the AnAOB need in order to perform the anammox reaction. Partial nitritation needs to be fostered, but full nitrification must be avoided. Therefore, more studies are needed to address further how to support AOB over NOB. In this project the concentration of nitrite was always very low because of the low amount of AOB's population. A preliminary solution to support AOB's activity is to maintain a constant TSS level inside the reactor, at a concentration not lower than 900 mg/L. After that, different strategies can be experimented. Study 1 proved that intermittent aeration, with the aeration pattern 10/50, was successful to suppress NOB. AOB were also inhibited though, but the new aeration pattern together with an increase of the DO set-point made the AOB be more active while keeping NOB inhibited. However, the low TSS level did not allow to have a flourishing AOB's population and this affected the process performances.

- The anammox activity also needs to be fostered. Results of Strategy 2 revealed that the biofilm growth is a long and delicate phenomenon, but it is actually possible. The environment inside the reactor needs to be as more suitable as possible for this growth, otherwise the biofilm will detach. Temperature adjustments helped to support the biofilm growth. Substitution of old carriers with new empty ones did not work in this project. However, this last phenomenon needs to be studied during a longer period of time. It is author's suggestion for future studies to substitute old carriers with others having already a flourishing biofilm. A periodic transfer from sidestream to mainstream can be the solution to actually improve the process performances.

- Study 2 revealed that the sludge age needs to be shortened. A solution to this problem can be the addition of the quota of the excess sludge by modifying the sludge recirculation system. This would also allow to test the effects of aggressive SRT on the NOB's out-selection. Therefore, further studies need to address how to improve and stabilize the process performances of UASB reactors

- Study 2 also revealed that the UASB reactor did not work effectively to maintain the $sCOD_{in}/NH4\text{-}N_{in}$ ratio at an acceptable level. The function of the UASB reactor is of extreme importance, because carbon removal is the stage that is essential to out-select HB. Otherwise, HB will compete against AOB and AnAOB and the whole PN/A process will be compromised. This might be the reason of the total collapse that occurred during the Christmas break.

- Finally, the activated sludge had very low settling properties. In this case the situation can be improved by inoculating sludge with better properties. However, it is important that this new sludge comes from a similar process, otherwise it will introduce other bacterial species that might compete with the ones already present and lead to a total collapse of the process.

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Appendix A

Table 11: General operational program

Program		What to do	Frequency
Control and measuring		**Measure**: - Inflow rate into IFAS reactor; - Return sludge flow rate into IFAS reactor.	Daily
		Check: - Inflow into equalization tank; - Filters between UASB and equalization tank; - TSS sensor and floating sludge in the sedimentation tank.	Daily
Analysis	**Inflow**	**Analyse concentration:** - Alkalinity (Alk_{in}); - Ammonium (NH_4-N_{in}); - pH (pH_{in}).	Twice a week
		Analyse concentration: - Total Nitrogen (TN_{in}); - Total COD (COD_{in}); - Soluble COD ($sCOD_{in}$).	Once a week
	Outflow	**Analyse concentration:** - Alkalinity (Alk_{out}); - Ammonium (NH_4-N_{out}); - Nitrite (NO_2-N_{out}); - Nitrate (NO_3-N_{out}). **In addition**: - pH (pH_{out}).	Twice a week
		Analyse concentration: - Total Nitrogen (TN_{out}); - Soluble COD ($sCOD_{out}$); - TSS and VSS.	Once a week
Calibration		**Calibrate:** - DO sensor; - NH_4-N_{in} sensor; - NH_4-N_{out} sensor; - NO_3-N_{out} sensor; - pH_{out} sensor.	Once a week
		Calibrate: - Conductivity sensor; - Redox sensor.	Once a month
		Calibrate: - TSS sensors.	Every second week
Cleaning		**Clean:** - Filters.	Once a week
		Clean: - All sensors.	Twice a week

Table 12: Weekly schedule for operational program

	Control and measuring	Calibration	Cleaning	Analysis
Monday	X		X (only filters)	
Tuesday	X	X	X (sensors)	X
Wednesday	X			
Thursday	X			
Friday	X		X (sensors)	X

Appendix B

Table 13: Schedule for sedimentation tests. A - 3.5L sed. cylinder with slowly stirring. B - 1L regular sed. cylinder

Day	Instrument	ISV	SVI
35	B		X
38	B		X
39	B		X
42	A/B	X	X
43	A/B	X	X
44	A/B	X	X
46	B		X
49	B		X
50	B		X
53	A/B	X	X
60	A/B	X	X

Appendix C

Table 14: Results of the chemical laboratory analyses (part 1)

		ANALYSIS														
		Influent							Effluent							
Day	Phase	COD (mg/L)	sCOD (mg/L)	pH	Alk (mmol/L)	TN (mg/L)	NH4-N (mg/L)	sCOD/NH4-N	SCOD (mg/L)	pH	Alk (mmol/L)	Tot N (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
0															
1															
2		111,0	103,0		6,8		40,7	2,5	68,0		3,3		17,2	0,3	13,3
3															
4		113,0	106,0	7,9	7,3		42,6	2,5	68,0	7,5	3,6		17,9	0,2	15,7
5															
6															
7															
8		119,0	106,0	7,3	7,9	50,0	46,9	2,3	80,0	7,1	5,0	38,0	23,3	0,2	13,6
9															
10															
11	Breakdown 1	122,0	110,0	7,7	8,0		45,4	2,4	72,0	7,5	4,2		17,4	0,2	18,7
12	Breakdown 1														
13	Breakdown 1														
14	Breakdown 1						45,0						4,0	0,7	33,0
15	Recovery	106,0	96,0	8,0	9,0	47,0	44,7	2,1	72,0	7,4	4,8	39,0	9,5	0,2	27,0
16	Recovery														
17	Recovery														
18	Recovery			7,8	9,0	50,0	49,1			7,7	3,3		17,0	0,3	23,5
19															
20															
21															
22	Breakdown 2	124,0	119,0	7,7	6,7	45,0	41,3	2,9	102,0	7,8	4,6	44,0	22,3	0,6	17,8
23	Breakdown 2														
24	Breakdown 2														
25	Breakdown 2	171,0	102,0	7,6	8,5		48,0	2,1	102,0	8,0	7,8		42,0	0,0	0,4
26	Recovery														
27	Recovery														
28	Recovery														
29	Recovery	131,0	124,0	7,9	9,0	48,0	45,9	2,7	94,0	8,0	7,0	38,0	27,2	0,4	9,5
30															
31															
32				7,6	8,8	55,0	54,5			8,0	5,2	41,0	31,9	0,3	6,9
33															
34															
35															
36				8,1	8,5		47,2			8,0	7,0		26,2	0,4	9,9
37															
38															
39		129,0	116,0	8,1	6,0	46,0	38,8	3,0	84,0	7,1	3,7	39,0	22,0	0,2	11,7
40															
41															
42	Strategy 1 and 2														
43															
44				7,9	8,3	55,0	40,0			8,0	5,5	42,0	30,0	0,2	1,1
45															
46		104,0	93,0	7,8	6,4	43,0	42,0	2,2	75,0	7,8	4,7	30,0	28,0	0,2	0,6
47															
48															
49															
50		132,0	112,0	8,1	6,7	45,0	43,7	2,6	80,0	8,1	6,1	33,0	31,0	0,4	0,6
50	Strategy 3														
51															
52				8,0	8,5	45,0	43,6			8,1	6,5	31,0	28,2	0,2	0,4
53							42,8						28,1	0,1	0,6
54															
55															
56															
57		121,0	76,0	7,8	6,4	45,0	41,3	1,8	40,0	8,0	5,2	29,0	24,9	0,1	1,0
57	Strategy 4														
58															
59				7,8	6,0		39,5			8,0	4,2		20,7	0,1	2,4
60				7,9	4,8	38,0	37,9			7,8	4,0	24,0	17,9	0,1	5,4
61															
62															
63															
64		116,0	84,0	7,9	6,6		37,0	2,3	30,0	7,9	4,3		15,5	0,1	7,1

Table 15: Results of the chemical laboratory analyses (part 2)

		ANALYSIS													
		Influent							Effluent						
Day	Phase	COD (mg/L)	sCOD (mg/L)	pH	Alk (mmol/L)	TN (mg/L)	NH4-N (mg/L)	sCOD/NH4-N	SCOD (mg/L)	pH	Alk (mmol/L)	Tot N (mg/L)	NH4-N (mg/L)	NO2-N (mg/L)	NO3-N (mg/L)
65-85	Christmas break														
86	Strategy 5		104	7,96	>8		32	3,3	83,0	8,0	7,9		28,3	0,024	0,3
87															
88				7,9	>8		30,4			8,0	8,7		25,4	0,0	0,4
89															
90															
91															
92		130,0	79,0	7,8	>8		39,0	2,0	31,0	8,0	8,1		30,0	0,0	0,1
92	Strategy 6														
93															
94															
95				7,7			39,8			8,0			35,3	0,0	0,1
96															
97															
98															
99		127,0	91,0	7,7	7,6		43,5	2,1	52,0	7,9	8,2		37,0	0,0	0,2
100															
101															
102															
103															
104															
105															
106			114,0	8,2	7,6		41,9	2,7	63,0	8,3	6,5		32,9	0,0	0,1
107															
108															
109				7,8	6,4		36,7	0,0		8,1	5,4		31,7	0,0	0,1

Table 16: Measurements and results of calculations (Part 1)

		MEASUREMENTS and PROCESS PARAMETERS						CALCULATIONS									
		IFAS Reactor															
Day	Phase	Q in (ml/min)	Q sludge (ml/min)	HRT (h)	T (°C)	DO (mg/L)	Ar/non-ar (min)	NH4 rem (mg/L)	NH4 rem eff (%)	TN rem (mg/L)	TN rem eff (%)	TN rem (N forms) (mg/L)	TN rem eff (N forms) (%)	TN load rate (g/m2d)	TN rem rate (g/m2d)	NO3 prod/NH4 rem	Alk cons (%)
0																	
1																	
2		139,0	129,0		20,0	0,8	20 40	23,5	57,7			9,9	24,4	5,4	1,3	0,6	51,5
3																	
4		144,5	129,7	23,1	20,0	0,8	20 40	24,7	58,0			8,8	20,6	5,5	1,1	0,6	50,5
5																	
6																	
7																	
8		151,0	130,0	22,1	20,0	0,8	20 40	23,6	50,3	12,0	24,0	9,8	20,9	5,8	1,2	0,6	36,0
9																	
10																	
11	Breakdown 1	147,0	129,7	22,7	20,0	0,8	20 40	28,0	61,7			9,1	20,0	5,7	1,1	0,7	48,4
12	Breakdown 1																
13	Breakdown 1																
14	Breakdown 1							41,0	91,1			7,3	16,2			0,8	
15	Recovery	159,0	130,0	21,0	20,0	0,8	20 40	35,2	78,7	8,0	17,0	8,0	17,9	5,2	0,9	0,8	46,6
16	Recovery																
17	Recovery																
18	Recovery	53,0	129,0	62,9	20,0	0,8	20 40	32,1	65,4			8,3	17,0	5,9	1,0	0,7	62,9
19																	
20																	
21																	
22	Breakdown 2	148,2	130,0	22,5	20,0	0,6	20 40	19,0	46,0	1,0	2,2	0,6	1,4	5,2	0,1	0,9	31,1
23	Breakdown 2																
24	Breakdown 2																
25	Breakdown 2	144,0	130,0	23,1	20,0	0,6	20 40	6,0	12,5			5,6	11,7	6,2	0,7	0,1	8,7
26	Recovery																
27	Recovery																
28	Recovery																
29	Recovery	135,1	130,5	24,7	20,0	0,6	20 40	18,7	40,7	10,0	20,8	8,8	19,2	6,3	1,2	0,5	22,4
30																	
31																	
32		146,0	130,0	22,8	20,0	0,6	20 40	22,6	41,5	14,0	25,5	15,4	28,2	6,9	2,0	0,3	40,3
33																	
34																	
35																	
36		142,0	129,8	23,5	20,0	0,6	20 40	21,0	44,5			10,7	22,7	6,2	1,4	0,5	18,1
37																	
38																	
39		131,8	129,8	25,3	20,0	0,6	20 40	16,8	43,3	7,0	15,2	4,9	12,7	5,5	0,7	0,7	38,1
40																	
41																	
42	Strategy 1 and 2																
43																	
44		144,3	130,0	23,1	23,0	0,6	10 50	10,0	25,0	13,0	23,6	8,7	21,7	5,1	1,1	0,1	34,2
45																	
46		143,2	130,0	23,3	23,0	0,6	10 50	14,0	33,3	13,0	30,2	13,2	31,5	5,4	1,7	0,0	26,8
47																	
48																	
49																	
50		137,5	150,0	24,2	23,0	0,6	10 50	12,7	29,1	12,0	26,7	11,7	26,8	5,9	1,6	0,0	9,0
50	Strategy 3																
51																	
52		145,0	150,0	23,0	23,0	1,0	10 50	15,4	35,3	14,0	31,1	14,8	34,0	5,6	1,9	0,0	23,8
53		140,4	150,0	23,7	23,0	1,0	10 50	14,7	34,3			14,1	32,9	5,6	1,9	0,0	
54																	
55																	
56																	
57		132,0	150,0	25,3	23,0	1,0	10 50	16,4	39,7	16,0	35,6	15,3	37,0	5,8	2,1	0,1	18,7
57	Strategy 4																
58																	
59		141,6	150,0	23,5	23,0	1,4	10 50	18,8	47,6	14,0	36,8	16,3	41,3	5,2	2,1	0,1	30,8
60		140,0	150,0	23,8	23,0	1,4	10 50	20,0	52,8			14,5	38,3	5,0	1,9	0,3	16,6
61																	
62																	
63																	
64		152,8	149,0	21,8	23,0	1,4	10 50	21,5	58,1			14,3	38,7	4,5	1,7	0,3	34,6

Table 17: Measurements and results of calculations (Part 2)

		MEASUREMENTS and PROCESS PARAMETERS						CALCULATIONS									
		IFAS Reactor															
Day	Phase	Q in (ml/min)	Q sludge (ml/min)	HRT (h)	T (°C)	DO (mg/L)	Ar/non-ar (min)	NH4 rem (mg/L)	NH4 rem eff (%)	TN rem (mg/L)	TN rem eff (%)	TN rem (N forms) (mg/L)	TN rem eff (N forms) (%)	TN load rate (g/m2d)	TN rem rate (g/m2d)	NO3 prod/NH4 rem	Alk cons (%)
65-85	Christmas break																
86	Strategy 5	146	80	22,8	23,0	0,6	10 50	3,7	11,6			3,38	10,6	0,1	0,01	0,1	
87																	
88		150,4	100,0	22,2	23,0	0,6	10 50	5,0	16,4			4,6	15,2	0,1	0,02	0,07	
89																	
90																	
91																	
92		135,5	100,0	24,6	23,0	0,6	10 50	9,0	23,1			8,9	22,7	0,2	0,04	0,01	
92	Strategy 6																
93																	
94																	
95		136,0	110,0	24,5	23,0	1,0	10 50	4,5	11,3			4,4	11,0	0,2	0,02	0,02	
96																	
97																	
98																	
99		137,0	110,0	24,3	23,0	1,0	10 50	6,5	14,9			6,3	14,5	0,2	0,03	0,02	-7,1
100																	
101																	
102																	
103																	
104																	
105																	
106		124,0	120,0	26,9	23,0	1,0	10 50	9,0	21,5			8,9	21,2	0,2	0,03	0,01	15,2
107																	
108																	
109		131,0	100,0	25,4	23,0	1,0	10 50	5,0	13,6			4,8	13,2	0,1	0,02	0,03	16,6

Table 18: TSS online values

Day	TSS (mg/L)	Day	TSS (mg/L)	Day	TSS (mg/L)	Day	TSS (mg/L)
0	-	17	829,57	34	436,48	51	811,52
1	-	18	807,95	35	618,15	52	835,75
2	988,80	19	638,65	36	791,15	53	806,05
3	928,86	20	425,00	37	694,10	54	666,35
4	796,15	21	636,15	38	633,90	55	344,81
5	725,55	22	830,30	39	589,20	56	557,60
6	370,43	23	793,35	40	419,10	57	757,55
7	563,05	24	824,86	41	227,19	58	745,05
8	951,20	25	826,95	42	553,60	59	731,20
9	826,10	26	764,70	43	807,15	60	655,70
10	867,00	27	469,00	44	853,33	61	376,81
11	985,10	28	656,85	45	801,45	62	223,65
12	791,35	29	788,10	46	789,85	63	443,20
13	615,48	30	420,81	47	656,85	64	704,15
14	823,80	31	648,30	48	400,57	65	-
15	911,70	32	813,40	49	663,00	66	-
16	835,00	33	642,50	50	826,50		

Table 19: Results of TSS analyses

Day	Filter weight (g)		Filter weight + dried sample (g)		Sample volume (ml)		TSS (mg/L)		
	A1	A2	B1	B2	V1	V2	TSS 1	TSS 2	Average TSS
10	0,0906	0,09	0,1115	0,1123	25	26	836,00	857,69	846,85
11	0,0911	0,0915	0,1142	0,1157	25	26	924,00	930,77	927,38
15	0,09	0,0904	0,108	0,1106	26	26,5	692,31	762,26	727,29
22	0,0914	0,0914	0,1098	0,1116	25,5	27	721,57	748,15	734,86
30	0,0912	0,0907	0,1036	0,1012	30	27	413,33	388,89	401,11
36	0,0911	0,0907	0,1109	0,1057	37	29	535,14	517,24	526,19
43	0,091	0,0903	0,109	0,1112	25	26,5	720,00	788,68	754,34
50	0,0913	0,0913	0,1099	0,1116	24,5	26	759,18	780,77	769,98
60	0,0904	0,0908	0,1109	0,1099	25	25,5	820,00	749,02	784,51

Table 20: Complete SVI results

Day	Sludge from..	Volume (ml) at t=30 min	Filter weight (g)	V sample (ml)	Dried sample + filter (g)	TSS (g/L)	TSS average (g/L)	SVI
35	well mixed sed. tank	910	0,0907	29	0,1465	1,924	1,916	475,037
			0,091	28	0,1444	1,907		
38	well mixed sed. tank + return sludge	865	0,0905	28	0,1442	1,918	1,819	475,456
			0,0896	26,5	0,1352	1,721		
39	well mixed sed. tank + return sludge	745	0,0911	25,9	0,1405	1,907	1,860	400,634
			0,0901	25,5	0,1363	1,812		
42	well mixed sed. tank + return sludge	917	0,0904	26	0,1483	2,227	2,177	421,318
			0,0905	23	0,1394	2,126		
43	well mixed sed. tank + return sludge	754	0,0915	24,5	0,1354	1,792	1,786	422,240
			0,0917	24,5	0,1353	1,780		
44	well mixed sed. tank + return sludge	625	0,0925	25,5	0,1355	1,686	1,661	376,386
			0,0918	23	0,1294	1,635		
46	return sludge + tap water	935	0,0914	27	0,1408	1,830	1,813	515,751
			0,0905	26	0,1372	1,796		
49	return sludge + tap water	925	0,0899	24	0,1334	1,813	1,796	515,145
			0,0918	23,5	0,1336	1,779		
50	return sludge + tap water	470	0,0893	25,5	0,1217	1,271	1,250	376,037
			0,0898	24	0,1193	1,229		
53	well mixed sed. tank + return sludge	475	0,0899	23	0,1195	1,287	1,323	358,903
			0,0907	25	0,1247	1,360		
60	well mixed sed. tank + return sludge	470	0,0904	24,5	0,1244	1,388	1,388	338,676
			0,0908	25	0,1234			