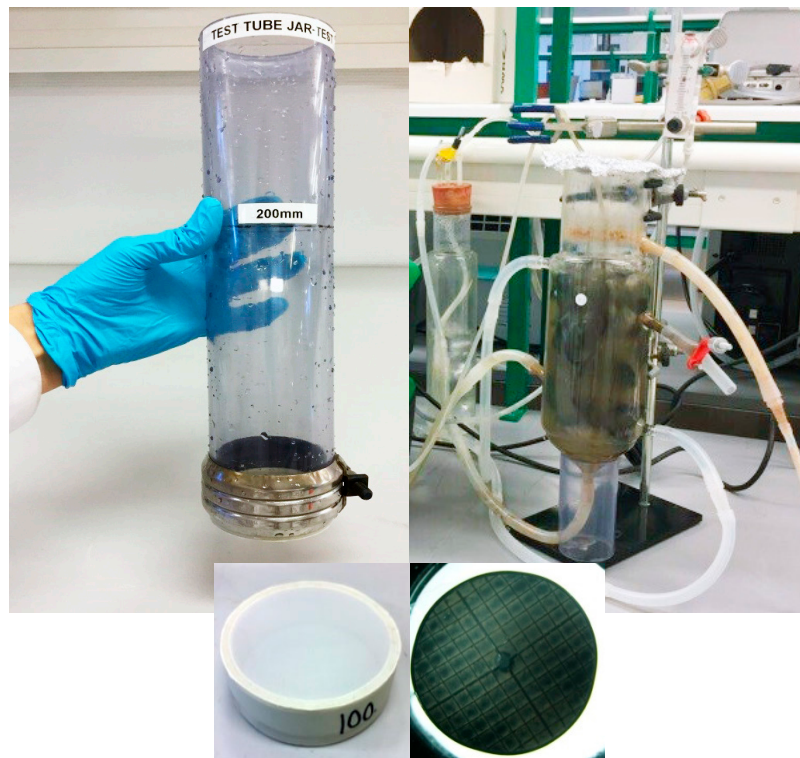


Evaluation of the effect of different SCOD/TN ratios in a mainstream nitrification process



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Water and Environmental Engineering
Department of Chemical Engineering
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Picture on front page: Few moments of the experiment setup by Doaa El Halabi

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Preface

By this master thesis I will fulfil the final part of five years education at Lund University, a master's degree in biotechnology. The master thesis was performed at Water and Environmental Engineering, Department of Chemical Engineering, Lund University in cooperation with Veolia Water Technologies AB, AnoxKaldnes.

I want to express my grateful thanks to my supervisors and examiner. Thanks for sharing your knowledge and support. I want to thank all the employees at Veolia Water Technologies AB for being helpful and welcoming. Thanks for giving me the chance to be one of your big family during my master thesis. It has been very instructive and a great pleasure. Special thanks to Maria Piculell and Dora Stefansdottir for their support through the work and valuable instructions. Thanks to Pia Welander for the support with the laboratory setup. Thanks to Henrique Sánchez and Stig Stork for all help regarding delivering wastewater from Sjölanda.

Thanks for Michael Sjölin and Petter Olsson from Hydrotech for being helpful and for their valuable instructions for the filtration part. Last but not least, I want to thank the service group at Veolia for all help regarding the analysis.

Many thanks to my family for their support and patience, special thanks to my husband, Hassan, and kids, Samir and Joseph, your existence have been my inspiration and motivation.

Lund March 2017
Doaa EL Halabi

Summary

The organic matter which the wastewater is rich in has been advantageously used for biogas production. Since, the traditional biological nitrogen wastewater treatment by nitrification denitrification requires organic matter; an external organic matter needs to be added. Recently, researches have been focusing on alternative processes for wastewater treatment where increased amount of carbon can be separated from the wastewater with primary treatment and utilized for biogas production. Hence, new approaches for nitrogen removal without requirements of carbon are needed. One of the nitrogen removal processes that is studied intensively is the nitrification-anammox combination. The nitrification-anammox nitrogen removal treatment for wastewater is ideal in saving organic matter, in addition to less energy requirements, due to lower oxygen demand.

This master thesis has been studying the nitrification step in a moving bed biofilm reactor (MBBR), which is meant to be preceded by a microscreen for chemical oxygen demand (COD) removal. The main focus was to evaluate the effect of higher COD in the influent on the nitrification step. Since the achievement of the nitrification step is mainly based on the suppression of the nitrite oxidizing bacteria, the nitrite oxidizing bacteria was meant to be inhibited by performing a reject phase, 2 days a week. Three different soluble chemical oxygen demand (SCOD)/total nitrogen (TN) ratios were studied.

During the first phase SCOD/TN ratio 1 (using high rate activated sludge effluent) was studied where successful nitrification (ammonium ($\text{NH}_4\text{-N}$) load rates were 0.8 ± 0.1 gN/L,d and $\text{NH}_4\text{-N}$ reduction rates were 0.4 ± 0.1 gN/L,d) was obtained in two MBBR lab reactors operated in parallel. Two of the studied SCOD/TN ratios were decided after evaluating microscreen filtration step in laboratory scale. The results from the filtration laboratory experiment indicated a low SCOD reduction rate. Thereafter, SCOD/TN ratios to be studied were decided to be 5 and 7.

After 31 days of continuous operation with high rate activated sludge effluent with successful nitrification, the operation was switched to test the effect of higher SCOD/TN in the influents (5 and 7) for further 45 days. Unfortunately, no successful nitrification operation was achieved for neither 5 or 7 SCOD/TN, even though $\text{NH}_4\text{-N}$ load rates were decreased to 0.2-0.3 gN/L,d. It was believed that the higher COD results in obtaining more heterotrophic bacteria in the biofilm which results in difficulties in directing the process. The development of the heterotrophs was believed to end in limiting the activity of ammonia oxidizing bacteria, due to the change in the biofilm structure which results in diffusion limitation.

Activity batch trials were also performed on a biweekly basis to obtain the maximum capacity of the bacteria during the study. The results from the activity trials indicated a lower nitrite-production rate when operated at a higher SCOD/TN ratio, which furthermore indicated the AOB activity limitation due to heterotrophic development.

Keywords: Partial nitrification, nitrification, microscreen, filtration, Chemical oxygen demand, moving bed biofilm reactor, Partial nitrification anammox, PNA.

Sammanfattning

Avloppsvatten är rikt på organiskt material, det organiska materialet har med fördel använts för biogasproduktion. Den traditionella kvävereningsprocessen kräver organiskt material, därför har ett extern organiskt material använts. Flera studier har fokuserat på att finna en alternativ process för kväverening av avloppsvatten, där inget organiskt material behövs. En alternativ process som har studerats brett är nitritation-anammox processen. Den här alternativa processen för kväverening möjliggör sparandet av organiskt material samt energi.

Examensarbetet har studerat nitritation steget i en MBBR, som är tänkt att föregås av ett filtreringssteg. Huvudfokus är att utvärdera effekten av utflödet från filtreringssteget på nitritation steget. Den största utmaningen med nitritation-anammox för behandling av huvudströmmen är att undvika etablering av nitritoxiderande bakterier. Nitritoxiderande bakterier var tänkt att inhiberas genom utförandet av en rejektfas.

Två parallella MBBR laborationsreaktorer användes för att studera tre olika SCOD/N. Under den första fasen studeras ett SCOD/N kvot som är ungefär lika med 1 (med ett hög aktiverat slam utflöde) i både reaktorerna. Två av de studerade SCOD/N-förhållanden bestämdes efter att ha utvärderat filtreringssteget i laboratorieskala. Resultaten från filtreringslaboratorieexperiment indikerade en låg SCOD reduktion. De två SCOD/N som skall studeras bestämdes att vara 5 och 7. I den andra fasen studeras SCOD/N lika med 5 i den ena reaktorn respektive 7 i den andra.

Efter 31 dagar av kontinuerlig drift med utflöde från hög aktiverat slam, kunde en framgångsrik nitritation ($\text{NH}_4\text{-N}$ belastnings hastighet på $0,8\pm 0,1$ gN/L,d och $\text{NH}_4\text{-N}$ reduktions hastighet på $0,4\pm 0,1$ gN/L,d) kunde uppnås. Därefter växlad operationen för att testa effekten av högre COD i inflödet (5 och 7) under ytterligare 45 dagar. I den här fasen kunde inte en nitritation uppehållas för varken 5 eller 7 kvoten, trots att $\text{NH}_4\text{-N}$ belastning hastigheten sänktes så småningom till 0,2-0,3 gN/L,d. Den högre COD anses resultera i att erhålla fler heterotrofa bakterier i biofilmen som resulterar i svårigheter att styra processen. Tillväxten av heterotrofa bakterier skapar en förändring i biofilm strukturen. Diffusions begränsningar antas vara ett faktum som förklarar begränsningen i aktiviteten på ammoniakoxiderande bakterie.

Aktivitetsförsök utfördes varannan vecka för att erhålla den maximala kapaciteten av bakterier under de olika faserna. Resultaten från aktivitetsförsöken tydde på en lägre nitritproduktions hastighet under MPB, som reflekterade en begränsad AOB aktivitet under fasen. Detta kunde förklaras med diffusions begränsningar som uppstod på grund av heterotrofas utveckling.

Nyckelord: Partiell nitrifikation, filtrering, COD, MBBR, PNA, nitritation.

Acronyms and symbols

Anammox	Anaerobic Ammonium Oxidation
AnAOB	Anaerobic Ammonium Oxidation Bacteria
AOB	Ammonia Oxidizing Bacteria
BOD	Biological Oxygen Demand
BOD₇	7-day Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FA	Free Ammonia
FNA	Free Nitrous Acid
HB	Heterotrophic bacteria
HRAS	High Rate Activated Sludge
HRT	Hydraulic Retention Time
MBBR	Moving Bed Biofilm Reactor
MPA	Mainstream phase A- Low COD
MPB	Mainstream phase B- High COD
NOB	Nitrite Oxidizing Bacteria
TP	Total phosphorus
PNA	Partial Nitrification Anammox
RP	Reject Phase
SCOD	Soluble Chemical Oxygen Demand
SMP	Soluble Microbial Products
SS	Suspended Solids
TN	Total Nitrogen
TOC	Total Organic Carbon
WWTP	Wastewater Treatment Plant

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1 Introduction

The introduction section is divided into three minor parts, background, aim and limitations. The background part gives an overall overview of the study and why it is needed. Furthermore, the aim part focuses on the main purpose of the study and identifies the insights that the study provides and includes. The limitations distinguish the boundaries of the investigation.

1.1 Background

Veolia Water Technologies AB (VWT AB) is taking part in a European research project, POWERSTEP. The objective of the project is to demonstrate an energy positive wastewater treatment process, while maintaining a high water quality treatment. One of the demonstration sites of the project is located at VA SYD Sjölanda, a municipal wastewater treatment plant (WWTP) in Malmö Sweden. VWT AB, AnoxKaldnes (here after referred to as AnoxKaldnes) demonstrate an energy efficient nitrogen removal process in a two-stage concept. In the first reactor (50 m^3) partial nitrification (nitritation) occurs, followed by a second reactor (50 m^3) where an anammox reaction takes place. Partial nitritation anammox (PNA) is believed to be an energy positive process solution specially when compared to the traditional nitrification-denitrification process. This is due to the lower aeration needed as well as no required additional carbon source. In POWERSTEP the organic carbon in wastewater is meant to be separated to be advantageously used to produce biogas. Currently, the partial nitrification reactor is fed by the effluent from a high rate activated sludge (HRAS). VWT AB, Hydrotech (here after referred to as Hydrotech), a sister company of AnoxKaldnes, is responsible for filtration of the influent to the partial nitrification reactor. They are aiming to design a microscreen which can achieve a high carbon extraction with minimum load of chemicals and lowest possible effluent COD. The plan is to connect the microscreen with the MBBR in April 2017. A more clarifying picture is displayed below in Figure 1.1.

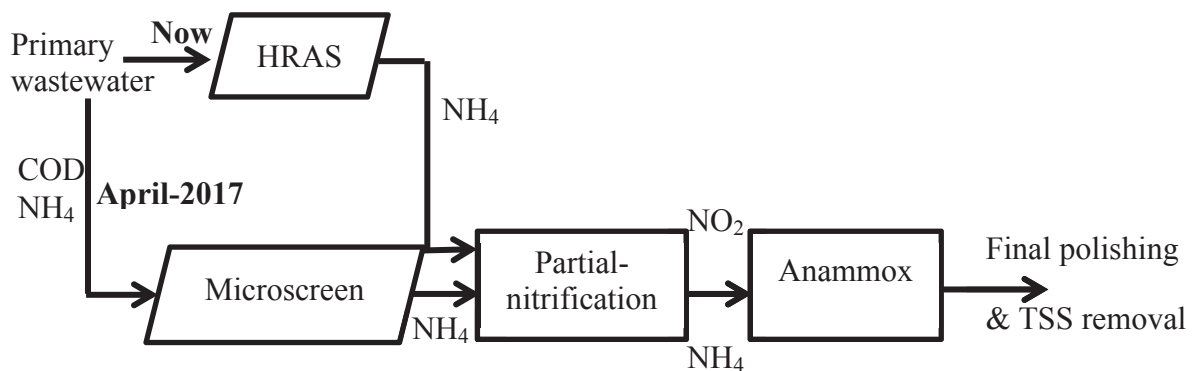


Figure 1.1. The different steps that preceded and followed the nitritation step. Microscreen and anammox respectively. A schematic of the pilot process at Sjölanda.

The composition of the effluent from the Hydrotech microscreen is expected to differ from the HRAS-effluent. The organic matter concentration after filtration is expected to be higher compared to the concentration in the HRAS effluent. The availability of organic matter is believed to affect the competition between microorganisms in the PNA system.

The competition between nitrifying bacteria and heterotrophs is a known phenomenon within wastewater treatment, due to the common optimal conditions (such as pH, temperature and oxygen) for both types of microorganisms. Due to the higher growth rate of the heterotrophs compared to nitrifying bacteria, it is a challenge to prevent the development of heterotrophic bacteria. The availability of the organic matter (COD) is critical for the growth of the heterotrophic bacteria. Higher COD will result in higher amounts of heterotrophs and vice versa.

The critical value organic carbon/total nitrogen ratio (COD/TN) in wastewater links the availability of COD, and in turn the existence of the heterotrophs. However, it can be difficult to define the critical limit of the COD/TN ratio in which the heterotrophs can be developed. This ratio varies among systems and is directly related to the features of the accessible organic carbon (Hanaki *et al.*, 1990; Henze *et al.*, 2000; Ruiz *et al.*, 2006 and Van Loosdrecht *et al.*, 2016).

Believing that the nitritation step is the limiting step in this nitrogen removal process, in addition to considering that this step will be affected with the change in the composition of the influent, AnoxKaldnes requested a master thesis for investigating the effect of the carbon influent on the nitritation step. Analyzing and studying the nitritation step in lab will avoid experimenting in large scale and will make the team aware of the future challenges in the project.

1.2 Aim

The aim of the study is to analyze and evaluate the effect of 3 different SCOD/TN ratios on the nitritation process in lab scale. Two of the studied SCOD/TN ratios will be determined based on studying the reduction ratio of the filtration step in lab scale. The effect of SCOD/TN ratios on the nitrifying activity will be evaluated, by studying the competition of ammonium oxidizing bacteria, nitrite oxidizing bacteria and heterotrophic bacteria, performing continuous operation and activity trials.

1.3 Limitations

No optimization of the filtration trials will be performed; the filtration trials will be only used to obtain the expected reduction of COD and TN. The study will be limited in studying three SCOD/TN ratios in a continuous mode, in addition to conducting few batch trials to maintain more knowledge about the bacteria activity. The results will be based on a short-term study.

2 Theory

The theory part below summarizes the main knowledge that is needed in order to get an understandable overview of the study. Furthermore, the theory will be used in order to understand the results, in addition to develop a realistic discussion and conclusion.

2.1 Wastewater treatment

H₂O is the colorless chemical substance; water. “Looking for life? Look for water”. This expression may be the best way to describe the importance of water for all organisms. However, water can cause danger for organisms and life in some situations, for example when water is polluted; such as wastewater (also called sewage). The best description of what happens when spilling sewage directly in nature is the citation by Joseph Heller: “You don’t find fish in lakes and rivers anymore. You have to catch them in cans. Towns die. Oil spills. Money talks”, in *Something happened* (Henze *et al.*, 2000). Therefore, there is virtually a need of treating sewage, to get rid of the substances harmful for the environment (more specifically, organic matter, nitrogen and phosphorous).

The problem with sewage has been observed for a long time. One of the earliest wastewater treatment plants was built in Rome, about 400 years BC (Gillberg *et al.*, 2003). With time sewage treatment has advanced and a need of making the process more effective and environmental friendly has developed. Sewage water treatment has been done mechanically, biologically and chemically for more than 100 years. However, the mechanisms of the different methods and how they can be combined need to be further understood in order to optimize the treatment for energy and efficiency.

Wastewater treatment consists of several steps. The primary treatment step is to remove the suspended solids (SS), which is important for the following treatment steps. Organic compounds are substances containing carbon. The biodegradable organic compounds consists carbon and nitrogen and can be removed through the biological path (Templeton *et al.*, 2011). This is achieved by having different kinds of bacteria in the process, consuming large quantities of nitrogen, phosphorus, carbon and sulfur. Microorganisms consume these substances in order to grow and reproduce; and as a result, these compounds will be effectively treated from wastewater.

2.1.1 Nitrogen

Nitrogen is one of the substances that needs to be removed from wastewater in order to avoid problem when released to the environment. Nitrogen is one of the critical nutrients for all living organisms. Nitrogen can be consumed by different types of microorganisms, producing other types of nitrogen molecules. This will result in removing nitrogen biologically. In Figure 2.1 (modified from Plaza *et al.*, 2015, p. 9) some paths of nitrogen can be seen. Four different paths for nitrogen are shown in the figure. Ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB) and anaerobic ammonium-oxidizing (anammox) bacteria (AnAOB) has the ability to convert substances containing nitrogen using CO_2 . This is unlike heterotrophic bacteria which have the ability to use organic matter converting it into CO_2 .

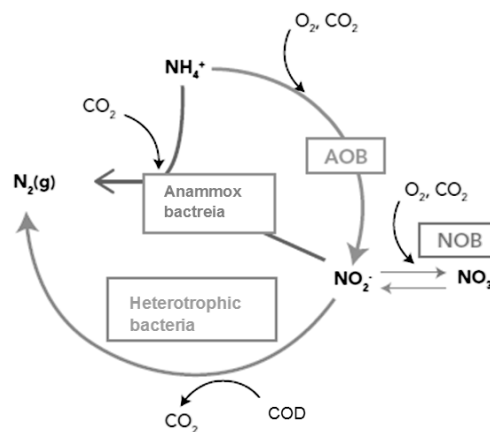


Figure 2.1. The nitrogen cycle (Plaza *et al.*, 2015, p.9). Image used with permission.

2.1.2 Organic matter

The removal of organic matter from wastewater is also needed just like nitrogen removal. Organic matter can be removed mechanically, chemically and biologically. The energy in organic matter can be advantageously used to produce biogas. This is why the techniques for removing the carbon mechanically or chemically are preferred. These techniques will be ideal in combination with the biological processes where organic matter is not needed. The organic compounds in wastewater are quantified by Chemical Oxygen Demand (COD). COD is defined by the oxygen needed to oxidize organic matter chemically. Sometimes, Total Organic Carbon (TOC) is used to estimate organic matter in wastewater.

However, this approximation is believed not to be as good as the COD (Silva *et al.*, 2011). The biochemical oxygen demand (BOD) is the amount of oxygen the microorganisms need to break down the organic matter (Silva *et al.*, 2011).

COD can be divided into particulate COD (PCOD) and soluble COD (SCOD) (Figure 2.2). PCOD contains organic suspended solids (SSCOD) and organic colloids (CCOD). However, The SCOD only includes truly soluble organic matter, which in turn is the most available for the microorganisms. PCOD may convert to SCOD in some situations (Jimenez *et al.*, 2005). Both PCOD and SCOD are available, but SCOD is more accessible. Analyzing the amount of organic matter in wastewater can be done in different ways, where analyzing COD is the most time efficient (AnoxKaldnes, 2015).

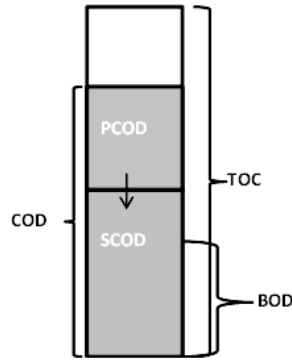


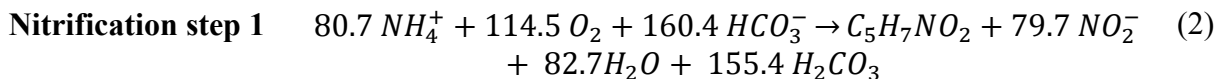
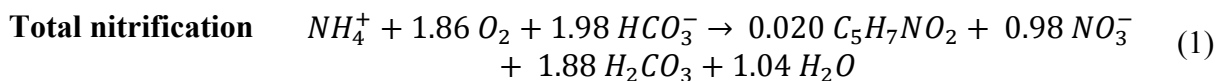
Figure 2.2 The relation between COD, BOD and TOC.

2.2 Processes in biological wastewater treatment

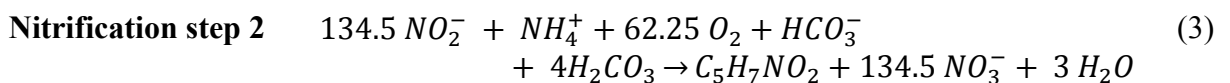
This chapter gives an overall description of some microbiological processes that are important for biological wastewater treatment. Some processes for removing nitrogen and carbon are described below.

2.2.1 Nitrification

Nitrification is the oxidation of ammonium, NH_4^+ , into nitrate, NO_3^- , (Equation 1). The reaction can be divided into two steps which take place through two different types of nitrifying bacteria. The first step is the ammonia oxidation, where ammonia (NH_3) is oxidized to nitrite (NO_2^-) (Equation 2) (Henze *et al.*, 2000). The bacteria that are responsible for this reaction are called ammonia-oxidizing bacteria (AOB), for example *Nitrosomonas* (Wang *et al.*, 2011). The second step is known as nitrite oxidation (Equation 3). This reaction is done by nitrite-oxidizing bacteria (NOB) for example *Nitrobacter* which oxidize nitrite (NO_2^-) into nitrate (NO_3^-) (Wang *et al.*, 2011). Both AOBs and NOBs are aerobes (use oxygen as electron acceptor), chemotrophs (obtain chemical energy through oxidation), autotrophs (use inorganic chemicals as carbon source (substrate)) and lithotrophs (reduce inorganic molecules as ammonia as an electron source) (Paredes *et al.*, 2007).



$$\Delta G^\circ(W) = -270 \text{ KJ/mol } NH_4^+ - N$$



$$\Delta G^\circ(W) = -80 \text{ KJ/mol } NO_2^- - N$$

Both AOBs and NOBs grow slowly compared to other bacteria, with a generation time as long as 7-10 hours in laboratory under optimal conditions (pH, temperature and substrate availability) (Hermansson *et al.*, 2006), as opposed to *Escherichia coli* (*E.coli*), the most studied bacteria, with a generation time of 20 minutes. The low growth rate is because of the low energy yield. Nitrifying bacteria produce low energy compared to heterotrophic bacteria.

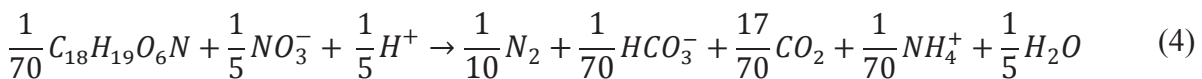
To favor the nitrifying bacteria over heterotrophic bacteria in WWTPs a low concentration of organic material (energy and carbon source for heterotrophs) is required in the nitrogen removal steps (Hermansson *et al.*, 2006).

There is a large variety of growth rate values for nitrifying bacteria in literature depending on differences in evaluated systems, operational conditions applied as well as the biomass growth type and the techniques used. Hellinga *et al.* (1999) obtained a maximum specific growth rate of 2.1 d⁻¹ for AOB at 35°C and pH 7. However, it is identified that the growth rates decrease when the temperature decreases. The growth rate of AOB declined from 1.5 to 0.2 d⁻¹ with decreasing temperature 27 to 8.3°C, in a study by Knowles *et al.* (1965). The temperature dependency was not as clear for the NOB in this study. The growth rate of NOB exceeded the AOB at lower temperatures; the limit reported according to Knowles *et al.* (1965) was 23.2°C. It was reported that temperatures between 15-20°C will end in having approximately the same growth rates for both NOB and AOB (Knowles *et al.*, 1965).

Bae *et al.* (2002) observed an increased oxidation rate of both ammonia and nitrite with the increase of the temperature from 10 to 30°C. It was clearly detected that the increase in the ammonia oxidation rate was faster than the nitrite oxidation rate in the study (Bae *et al.*, 2002). Downing and Hopwood (1965) indicated doubling in the rate of the ammonium oxidation when increasing the temperature by 10°C within the range 5-30°C. Similar identifications were obtained by different studies here mentioned Groeneweg *et al.* (1994) and Wang and Yang (2004).

2.2.2 Denitrification

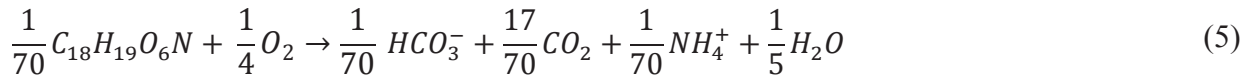
The process where nitrate is converted into atmospheric nitrogen is called denitrification. It is an anoxic (absence of free oxygen) process where nitrate is the electron acceptor (Equation 4). The reaction needs organic matter in order to occur. This process is performed by heterotrophic microorganisms using organic chemicals as carbon source. Most heterotrophs can use both nitrate and oxygen as an electron acceptor, although, they prefer oxygen if present (Henze *et al.*, 2000). The maximum specific growth rate for the denitrification process was stated to be between 3-6 d⁻¹ according to Henze *et al.*, 2000.



$$\Delta G^\circ(W) = -103 \text{ KJ}/e\text{-}eqv$$

2.2.3 Aerobic COD reduction

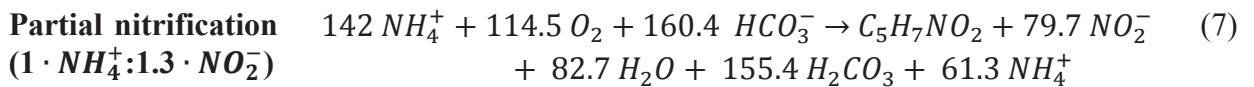
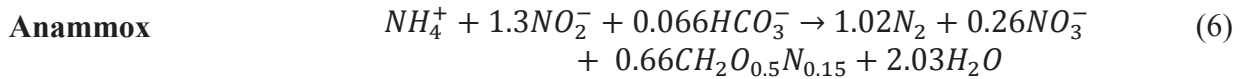
The heterotrophic microorganisms have the ability to convert organic matter in wastewater into carbon dioxide using oxygen under aerobic conditions (Equation 5). This will end in the aerobic heterotrophic conversion of organic matter. The maximum growth rate is between 4-8h⁻¹ (Henze *et al.*, 2000).



$$\Delta G^\circ(W) = -110 \text{ KJ}/e\text{-}eqv$$

2.2.4 Anaerobic ammonia oxidation - Anammox

In 1977, Broda predicted the existence of a microorganism that nobody had identified earlier. It was what nowadays is called the anaerobic ammonia oxidizing bacteria (AnAOB). However it was first in 1990 the process description was presented by Strous *et al.* (1999a) (Mulder *et al.*, 1995). The process, usually known by the short name anammox, oxidizes ammonium by using nitrite as electron acceptor, with no need of organic matter (Equation 6) (Paredes *et al.*, 2007). Since anammox requires nitrite as electron acceptor the ideal previous step of an anammox is a partial nitrification step. For the ideal stoichiometry of 1:1.3 $NH_4^+ : NO_2^-$, only 57% of ammonium needs to be converted to nitrite, where 43% of ammonium will remain (Equation 7). The maximum specific growth rate of *Brocadia anammoxidans* a type of anammox bacteria was reported as 0.10 d^{-1} (Oshiki *et al.*, 2011).



2.2.5 Competition of microorganisms

Achieving a better understanding of the complicity of the community of the microorganisms in the biofilm, a figure summarizing the competition between conceivable microorganisms in biofilm was developed (Figure 2.3). Studies that have been analyzing the distribution between AOB and NOB in biofilm have reported that AOB was distributed throughout the biofilm independently on the depth, where NOB population was increased with depth (Lydmark *et al.*, 2006). Ni *et al.* (2012) reported a higher heterotrophic growth in nitrifying biofilm compared to anammox biofilm. This was related to the high amount of soluble microbial products (SMP) in nitrification unlike anammox reaction. Kindaichi *et al.* (2004) indicated that nitrifying biofilm producing soluble microbial products supported heterotrophs. The biofilm distribution was 50% nitrifiers and 50% heterotrophs during limited carbon conditions. Okabe *et al.* (2005) and Nogueira *et al.* (2005) obtained 70% nitrifiers and 30% heterotrophs when analyzing the distribution in a nitrifying biofilm.

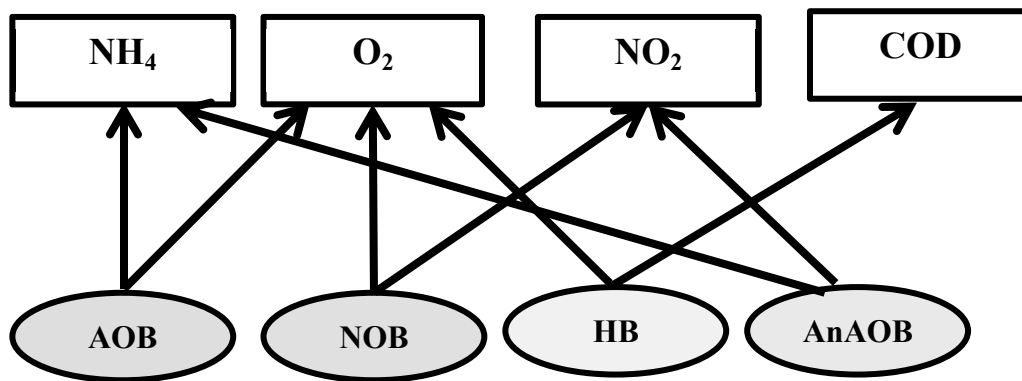


Figure 2.3 The elements of competition between the organisms (AOB, NOB, HB and AnAOB).

Several studies have indicated that high organic loading results in lower nitrification rate. Hanaki *et al.* (1990) reported inhibition of the ammonia oxidation due to the presence of heterotrophs. The maximum specific growth rate for the aerobic heterotrophic bacteria at 20°C was defined between 4-8 d⁻¹ according to Henze *et al.* (2000). This high growth rate, in addition to higher energy generation, will make it more difficult to prevent them from developing especially when high COD is available. All microorganisms will compete with each other for space. The heterotrophs will also compete with AOB for oxygen. The NOB will compete with AnAOB for nitrite and with AOB for oxygen.

Henze *et al.* (2000) has summarized the COD/TN ratios in domestic wastewater and divided them into three parts (Figure 2.4 a). Figure 2.4 a, describes the limits for low, typical and high COD/TN ratio. High COD/TN ratio favors denitrification. Ruiz *et al.* (2006) identified COD/TN of 5 or lower where denitrification was not observed. Van Loosdrecht *et al.* (2016) distinguish ranges for COD/TN ratio in which the different nitrogen removal processes are achieved. For ratios over 20 only heterotrophic organisms were obtained. Ratios between 5 and 20 both nitrifying bacteria and heterotrophic were applied. For ratios lower than 5 only nitrifying bacteria were functional, due to the lack of organic matter (Figure 2.4 b). For inflows with low COD/TN ratios the PNA process is suitable.

Hem *et al.* (1994) demonstrated the effect of total BOD on nitrification in MBBR. Consequently, insignificant nitrification was reported when the organic load exceeded 5 g BOD₇/m².d. All in all, researchers identified an increment in heterotrophic activity linked to increasing COD availability.

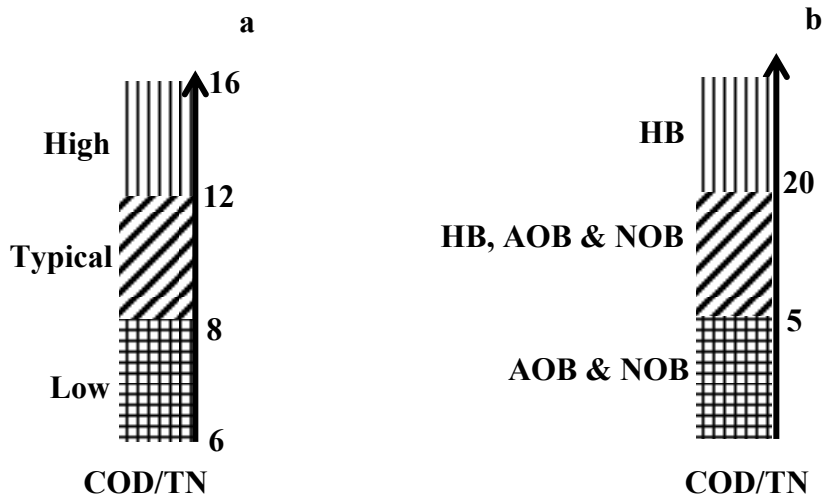
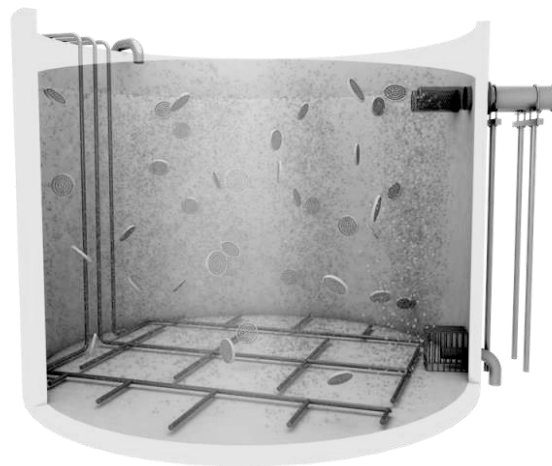


Figure 2.4 a) The different limits (low, typical and high) of COD/TN ratio (Henze *et al.*, 2000). b) The favored organisms depending on the COD/N ratio (van Loosdrecht *et al.*, 2016).

2.3 The Moving Bed Biofilm Reactor - MBBR

One of the techniques used for biological wastewater treatment is moving bed biofilm reactor (MBBR). The principle of the MBBR is to have moving carriers in a continuous reactor. This biofilm technology was invented in the late 1980s (AnoxKaldnes, 2015). The carriers (the place where the bacteria are supposed to grow and reproduce) are prevented from following the effluent by a sieve that is placed over the outlet (McQuarrie and Boltz, 2011). This will ensure that the bacteria growing on the carriers will be able to remain in the reactor, while free-swimming bacteria will be washed out with the effluent (Figure 2.5). Maintaining the bacteria is more important in the cases where the slowly growing bacteria are used, losing these bacteria with the effluent results in several months of reproducing them. Growing the bacteria on carriers ensures the continuation of the process in the reactor, even at high hydraulic rates and short retention time (Henze *et al.*, 2000). This can be compared to the traditional activated sludge process where using high hydraulic rates is not possible because of the risk of losing the capacity of the process.

As a result, the opportunity of using high hydraulic rate will make a more compact design of reactors (less space demand) possible with MBBR technique. On the other hand, having the bacteria attached to solids has some limitations. The substances needed to be transported through the biofilm in order to be removed by the bacteria. The transportation takes place by molecular diffusion. In the cases where the diffusion is slower than the reaction rate, the process will be substrate limited. This is where the design of the carrier plays a vital role for the process. The design of the carrier takes into account the bacteria's needs and tries to favor them. Most carriers are designed to have a large protected surface, with a density slightly lower than water (Piculell, 2016). There are different carriers, with different characteristics and design. Different processes have different needs, which carrier to use depends on the needs and the aim of the process.



*Figure 2.5. Image of a MBBR (not to scale).
Image by Veolia Water Technologies. Image
used with permission.*

One example of the carriers is the Z-200, see Figure 2.6. The Z carrier is the carrier used in processes where controlling the biofilm thickness is important (Piculell, 2016). The thickness can be controlled by the scrubbing action because of the collision between the carriers in the MBBR (Piculell, 2016). The maximum biofilm thickness on Z-200 is 200 μm .



*Figure 2.6. Z-200 carrier. Image used with
permission (Piculell, 2016).*

2.4 Filtration by microscreening

Removing substances from wastewater mechanically can be done by filtration. One example of filtration is microscreening. The microscreen is a fine mesh filter which is able to remove nutrients in particulate form (Lekang, 2013). Discfilter is the kind of microscreen filter used in this project. Discfilters usually consist of between 1-20 discs. On each side of the discs there are filter panels surrounding a central drum. Due to gravity the treated water flows from the central drum into the disc. The solids end up on the inner side of the filter panels (Figure 2.7) (Persson *et al.*, 2006). When enough amounts of solids attach to the filter panels, the filter will be clogged. This will increase the water level inside the discs and the level sensor will be triggered to start the backwash cycle for which the discs will rotate and backwash nozzles spray filtered water from the outside of the discs, resulting in washing off the solids. The solids end up in the solid collection channel. The duration of the cleaning process can be regulated and the filtration occurs continuously and is not paused during the backwash cycle (Väänänen *et al.*, 2016).

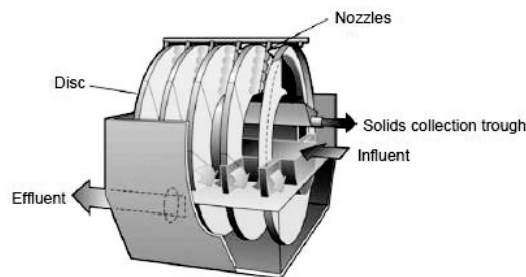


Figure 2.7 The components of a discfilter (Persson et al., 2006). Image used with permission.

Disc filtration has different types of cloths. The standard for Hydrotech is the twill weave monofilament polyester. This kind of cloth has shown to be mechanically very strong. Furthermore, the resistance of polyester against chemicals is a great advantage (Persson *et al.*, 2006).

2.4.1 Flocculation and coagulation

Trying to make the filtration step more efficient, chemical substances such as coagulants and flocculants can be added to the water before filtration. This is referred to as chemical treatment of water. There are some substances that are too small to be removed by filtration. When adding coagulant and flocculant to the wastewater, small particles will come together to form larger particles. This will make it possible to eliminate these particles by filtration. The function of the coagulant is to neutralize the colloidal particles existing in the water from the electrical charges (Gomes *et al.*, 2015). Poly aluminum chloride (PAX) is an example of a coagulation agent used in wastewater treatment (Gillberg *et al.*, 2003). Almost all particles in natural water are negatively charged. Aluminum is a positive ion, and therefore will be able to reduce the repulsion forces between the negatively charged molecules in the water. In addition, the coagulant will facilitate coagulation because of the appearance of opposite

charges. The flocculation process is when the particles aggregate thanks to the addition of a polymer (Gillberg *et al.*, 2003).

Charged polymers can be divided into two different sections: cationic polymers and anionic polymers. These polymers will connect charged molecules resulting in formation of flocs. Combining an anionic polymer with coagulant will result in much larger flocs compared to the flocs formed when only a coagulant is used. After filtration a rapid analysis of the amount of suspended particles in water can be estimated, by measuring the turbidity (Rosenblum *et al.*, 2016).

2.5 The process

Wastewater treatment can be designed in several ways. The combination of different techniques and processes has many varieties. Studies have been focusing on trying to make the process more efficient and utilize the organic matter in wastewater for biogas production, without a need of adding external organic matter in the further treatment. Applying partial nitrification-anammox process (PNA), for mainstream wastewater treatment, the energy requirements for aeration can be reduced, in addition to independency of organic matter availability (Diagger, 2014; Gao *et al.*, 2014).

PNA was developed by combining nitrification and anammox reactions which can be performed using the MBBR technique. The partial nitrification step will allow lower oxygen demand and the anammox reaction in turn has been believed to be a very useful way to save organic material (which is supposed to be separated by a microscreen in this case), which then can be maintained for biogas production (Hellings *et al.*, 1998; Siegrist *et al.*, 2008). Therefore, this nitrogen removal process has been seen as an approach for a low-cost process compared to the traditional nitrification-denitrification process. For a more detailed description of the process see Figure 2.8.

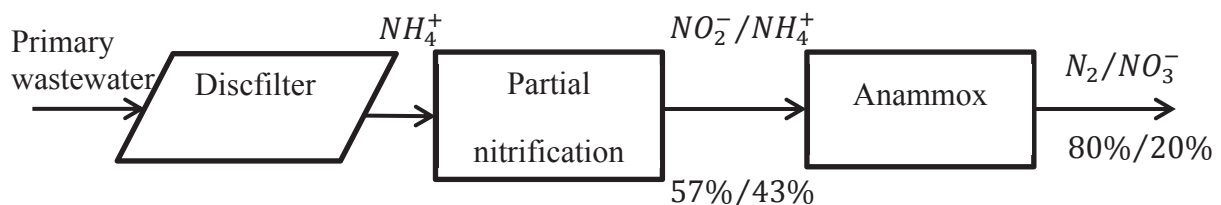


Figure 2.8 The ideal nitrogen removal process using Partial Nitrification and Anammox, preceded by a discfilter.

In theory the partial nitrification ($1 \cdot NH_4^+ : 1.3 \cdot NO_2^-$) has the advantage to save up to 57% of the oxygen consumption for every mole oxidized ammonium. Appendix A shows the oxygen consumption for reactions described in 2.2.1. However, achieving the advantages with this process solution, some challenges need to be overcome. The saving in oxygen will depend on the efficiency of the nitrification by the AOB and an efficient NOB inhibition, preventing the second step of nitrification to take place. Regarding the pre-treatment for COD removal, a major challenge is to develop a suitable COD/TN ratio in the effluent from the discfilter where the AOB will be favored and heterotrophs unfavored. Väänänen (2017) reported that the filtration step could achieve COD concentrations between 50-300 mg/L and TN concentrations between 20-40 mg/L, which would correspond to COD/TN ratios of 1.3-15.

2.5.1 Strategies for inhibition of NOB in nitrification

Obtaining a successful PNA process depends on achieving nitrification, which is obtained by optimization of the growth conditions for the AOB, in addition to inhibiting the NOB. Many studies, even though using the same approaches for inhibition of NOB, have defined different ranges for the inhibition factors (Anthonisen *et al.*, 1976; Blackburne *et al.*, 2007; Chung *et al.*, 2006; Vadivelu *et al.*, 2006a, 2006b, 2007). The most common methods for obtaining a successful partial nitrification are oxygen limitation, inhibition by free ammonia (FA) and free nitrous acid (FNA) (Wu *et al.*, 2016). AOB have better oxygen affinity, higher growth rate at low DO and/or high temperatures and they are more resistant toward FA and FNA. Both AOB and NOB are strictly aerobic, this is why it is believed that using oxygen as inhibiting factor will limit the nitrification rate while inhibiting NOB (Bao *et al.*, 2016). However, recent studies showed that some specific NOB species have higher DO affinity than AOB (Wu *et al.*, 2016). This means that limiting the DO will cause more inhibition of the AOB than the NOB.

There are several inhibitors which have been identified in different research by their toxicity on nitrification. However, this part will focus in the inhibitors FA and FNA. The ammonium equilibrium is adjusted when pH is decreased, resulting in decreasing FA concentration. FA inhibition will hence be obtained when pH is increased. On the other hand, FNA inhibition is obtained when pH is decreased, as the decline in pH results in high FNA concentration due to the equilibrium between FNA and NO_2^- (Figure 2.9).

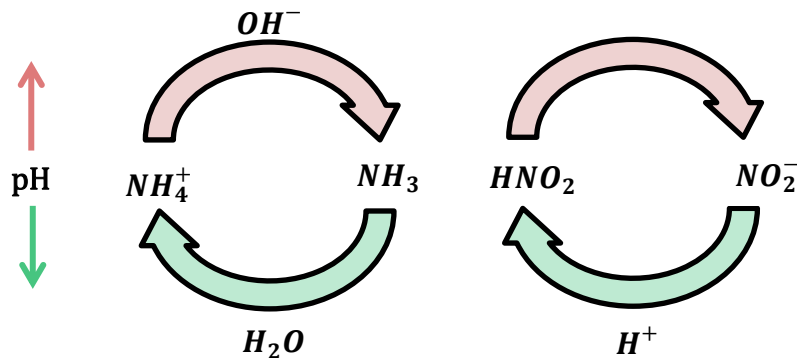


Figure 2.9 The effect of pH change on the equilibrium of FA (NH_3) and FNA (HNO_2).

Inhibition of nitrification due to reduced pH was postulated, according to observations in both treatment plants and laboratory experiments (Anthonisen *et al.*, 1976). Anthonisen *et al.* (1976) studied the inhibiting effect of FA and FNA on nitrification. The research identified the FA values of 10 to 150 mg/L for AOB, *Nitrosomonas* inhibition and 0.1 to 1.0 mg/L for NOB, *Nitrobacter* inhibition. The inhibition values of FNA recognized to be between 0.22 and 2.8 mg/L for nitrifying bacteria. The values where inhibition occurs mainly depended on temperature according to Anthonisen *et al.* (1976) for example the FA concentration at 30°C was noticed to be about twice that at 20°C for the same pH and total ammonia nitrogen concentration.

Zhou *et al.* (2011) on the other hand, defined that colder conditions favors FNA inhibition. It was stated that FNA concentration increased by 20-25% when decreasing the temperature from 20°C to 10°C. Kim *et al.* (2008) obtained no inhibition effect on ammonium oxidation

for FA concentrations 4.3-90 mg/L, pH was set to 8.3 and the temperatures tested were 10, 15, 20, 25 and 30°C. Wang *et al.* (2014) studied the FNA effect on AOB and NOB, at pH 7.2-7.5 and at temperature 22±2°C. The study obtained lost NOB activity at FNA concentration of 0.24 mg/L, whereas, AOB maintained 50% of their activity at as high FNA concentration as 1.35 mg/L. The activities were tested after 24 h FNA treatment.

Vadivelu *et al.* (2006b) accomplished FNA inhibition to the anabolic processes of *Nitrobacter* at 0.011 mg/L, 0.023 mg/L was observed to result in completely biomass synthesis inhibition. However, the same study could not achieve any inhibition of the catabolism of the *Nitrobacter*, the highest FNA concentration tested was 0.05 mg/L. Vadivelu *et al.* (2007) studied the inhibitory effect of FA and FNA on *Nitrosomonas* and *Nitrobacter*, finding that no inhibition of catabolic or anabolic processes of *Nitrosomonas* obtained for FA concentrations up to 16 mg/L. The same study achieved inhibition effect of both processes with FNA, where FNA levels of 0.4-0.63 mg/L inhibited the energy production capability of *Nitrosomonas* by 50%, while a concentration of 0.40 mg/L resulted in complete inhibition of the growth process. The inhibitory effect of both catabolism and anabolism processes for *Nitrobacter* was declared by Vadivelu *et al.* (2007), however, the effect on the catabolism was stated to be limited. FA levels of 6.0 mg/L or FNA levels of 0.02 mg/L was resulting in totally inhibiting the biosynthesis of the *Nitrobacter*.

All in all, an obvious huge variety in the inhibition ranges of FA and FNA on AOB and NOB is observed in literature. However, most studies reported that both AOB and NOB can be inhibited by FA and/or FNA. It was also reported that NOB are more sensitive whereas higher values are needed in order to inhibit the AOB.

The FNA and FA concentrations are correlated to both temperature and pH, see equations (8, 9, 10 and 11) (Anthonisen *et al.*, 1976).

$$FNA \text{ as } NO_2^- - N(mg/L) = \frac{46}{14} \cdot \frac{\text{Total nitrite ion as } N}{K_a \cdot 10^{pH}} \quad (8)$$

$$FA \text{ as } NH_4^+ - N(mg/L) = \frac{17}{14} \cdot \frac{\text{Total ammonia as } N \cdot 10^{pH}}{K_a/K_b + 10^{pH}} \quad (9)$$

$$K_a = e^{-\frac{2300}{(273+T)}} \quad (10)$$

$$\frac{K_b}{K_w} = e^{\frac{6344}{(273+T)}} \quad (11)$$

3 Materials and Methods

The materials and methods section includes some main parts where the reactor design and operation are described, where the start-up, reject phase and different mainstream operations also being mentioned. The analysis, measurements and the activity batch trials are declared.

3.1 Reactor design and operation

Two 1 L lab-scale reactors (R1 and R2) were operated in continuous mode at atmospheric pressure (Figure 3.1). Each reactor contained 200 pieces of carriers, type Z-200, giving approximately 25% volumetric filling degree. When starting up the laboratory trials, the carriers were already colonized with biofilm from the 50 m³ nitrifying MBBR pilot from the POWERSTEP site at Sjölanda WWTP (Powerstep, 2017). The operation of the lab reactors was divided into different feeding phases; 1) a start-up phase with reject feed, 2) a reject phase (RP) where reject water was fed to the reactors to inhibit the NOB, 3) a mainstream phase A (MPA) where the influent to the reactors was the effluent from the high rate activated sludge (HRAS) at Sjölanda WWTP and 4) a mainstream phase B (MPB) where the influent to the reactors was a synthetic discfilter effluent, which was created by adding an external carbon source to the HRAS water in order to increase the SCOD in the influent (see Figure 3.2).

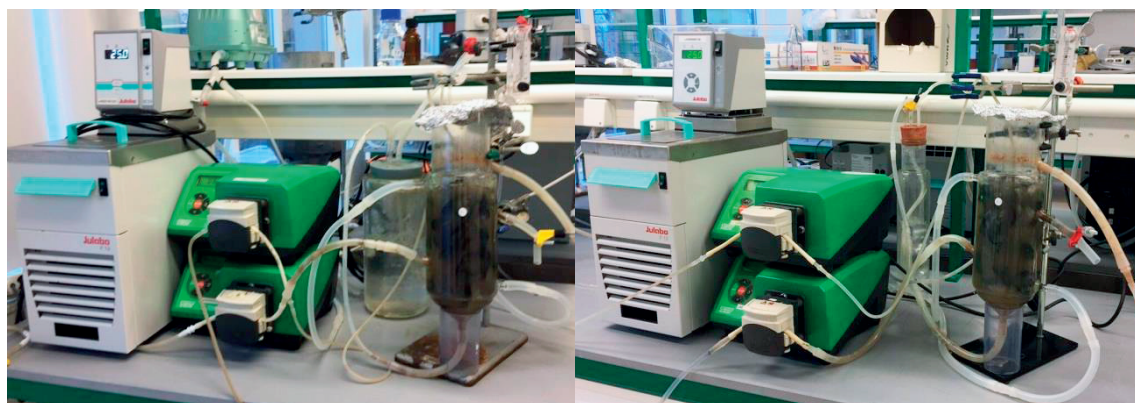


Figure 3.1 The lab-scale set-up. R1 to the left and R2 to the right.

For both reactor set-ups, the temperature was controlled using a thermostat bath (Julabo F12). The flowrates of all feeds were controlled using Watson Marlow 520 S pumps. Aeration was used to mix the reactors as well as to provide the required DO. Compressed air was supplied through the bottom of the reactors using a Hiblow HP-80 septic air pump. The HRAS effluent from Sjölanda WWTP was used during both MPA and MPB (see Figure 3.2). New batches of HRAS water was collected at Sjölanda WWTP on a biweekly basis and was stored in a 1000 L tank, provided with cooling and mixing. Reject water was fed during RP. During MPB the SCOD was increased in the feed by adding a synthetic COD substrate consisting of acetate (Figure 3.2).

The reject water and the synthetic COD source were both stored in a small refrigerator placed under the reactors and supplied to the reactors from the top (see Figure 3.2).

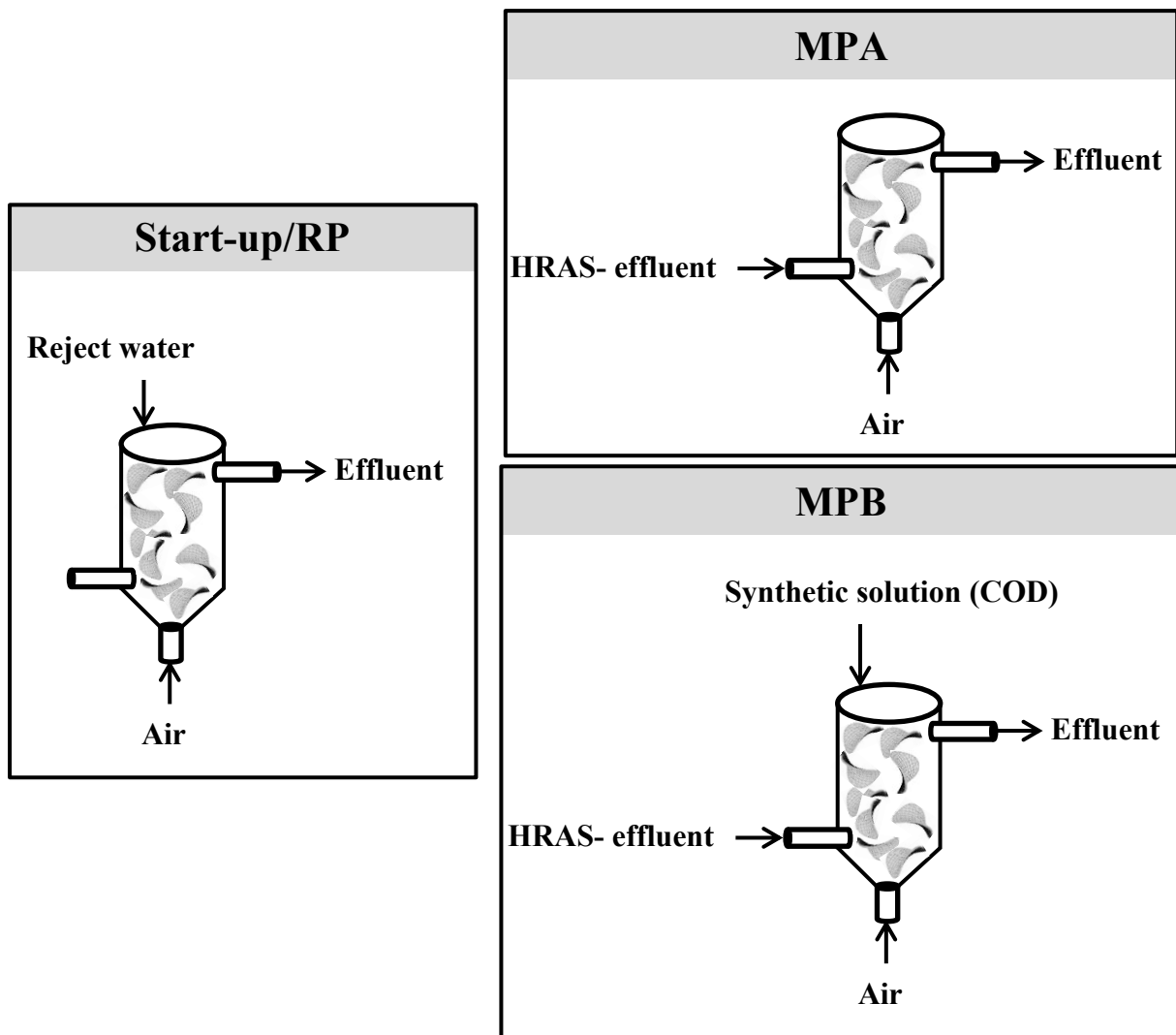


Figure 3.2 The reactor set-up during the different phases, start-up/RP, MPA and MPB (not to scale).

3.2 Start-up and reject phase (RP)

The start-up solution for both reactors was generated in the same way, by adding 225 ml reject water with 675 ml tap water to the reactors, after which the reactors were fed with reject water for 2 weeks.

Reject water is the outflow from dewatering of sludge after the sludge thickening process. This wastewater is characterized by a high ammonium concentration and high temperature. This can be advantageously used in NOB inhibition as observed in different studies (Lackner *et al.*, 2014 and Piculell, 2016). During start-up and RP, reject water was used at different loading rates in order to achieve a change in pH, ammonia, nitrite, FA and FNA

concentrations, with the aim to inhibit NOB. In addition to the varying load of reject feed, the temperature was adjusted to 25°C, which is believed to favor the AOB over the NOB. The conditions during reject operation were based on different studies about AOB and NOB (see chapter 2.5.1). The concentrations of FA and FNA were calculated during RP (see 2.5.1 for the formulas used).

3.3 MPA and MPB continuous operation

During MPA, the reactors were fed by already treated wastewater with HRAS. Therefore, this water is characterized by the low TN and COD concentrations. During mainstream operation the temperature was adjusted to 17±0.3°C, for both MPA and MPB. The aeration was adjusted to 1±0.5 L/min, while the feed flow rate was varying from 10-40 L/d. The aim was to try to determine the suitable load in order to ensure high substrate availability (ammonium) for AOB as well as finding a suitable retention time. The ideal effluent from the reactors should contain approximately 50% ammonium 50% nitrite. The mainstream operation was switched to RP during two days a week aiming to inhibit the NOB activity.

During MPB, the reactors were fed artificial water. The results from the filtration trials were used to mimic two different wastewater compositions. This was illustrated by adding an external carbon source (acetate) which was fed separately in addition to the HRAS effluent. As a result, the COD/TN ratio was increased into both MBBRs. The COD value that was attained to calculate the COD/TN ratio in the reactors was the value of COD measured for the filtered samples, here after referred to as SCOD.

Sodium acetate trihydrate (CH₃COONa·3H₂O, M=136.08 g/mole) was used in this study as an external source for increasing SCOD in the influent. The prepared solution consisted of 6.37 g NaCH₃COO·3H₂O for each liter tap water used, resulting in the concentration of approximately 3 gSCOD/L. Total nitrogen (TN) and SCOD was analyzed in the HRAS effluent and the flow of the acetate solution was adjusted in order to obtain the desired SCOD/TN ratios in the inlet. The amount of SCOD in HRAS effluent was subtracted in order to attain an accurate SCOD/TN ratio.

Equation 12 describes how the flowrate for the SCOD solution (Q_S L/d) was determined using the mainstream flow rate (Q_M L/d) and mainstream SCOD concentration (SCOD_M mg/L) and the total SCOD concentration (SCOD_T mg/L) and total flow rate (Q_T L/d). The real SCOD/TN ratios were calculated when the flow measurements and the analysis of the SCOD (mg/L) were performed.

$$Q_S = \frac{Q_T \cdot SCOD_T - Q_M \cdot SCOD_M}{SCOD_S} \quad (12)$$

The COD load was calculated in g/m²,d according to equation 13. The area is calculated by multiplying the total number of carriers used with the surface area of 1 carrier (0.001277 m²).

$$COD \text{ Load} = \frac{SCOD \cdot Q}{Area \cdot 1000} \quad (13)$$

When operating on mainstream water, the load, hydraulic retention time (HRT), NH₄-N reduction rate and NO₃-N and NO₂-N production rates were calculated as shown in equations 14-18, where C_{in} (mg/L) refers to the inlet concentration, C_{out} (mg/L) refers to the concentration in the outlet and V (L) is the volume of the reactor. All calculations were performed assuming steady state conditions during mainstream operations.

$$\text{Load NH}_4\text{-N} \quad \frac{Q \cdot C_{NH_4-in}}{V} \quad (14)$$

$$\text{HRT} \quad \frac{V}{Q} \quad (15)$$

$$\text{NH}_4\text{-N reduction rate} \quad \frac{Q \cdot C_{NH_4-in} - Q \cdot C_{NH_4-out}}{V} \quad (16)$$

$$\text{NO}_2\text{-N production rate} \quad \frac{Q \cdot C_{NO_2-out} - Q \cdot C_{NO_2-in}}{V} \quad (17)$$

$$\text{NO}_3\text{-N production rate} \quad \frac{Q \cdot C_{NO_3-out} - Q \cdot C_{NO_3-in}}{V} \quad (18)$$

3.4 Filtration trials with jar-test

The microscreen step was evaluated in the lab. The main purpose of the filtration trials was to obtain an approximation of the possible reduction of COD and N by the Hydrotech filters. The filtration trials were done on primary wastewater brought from WWTP Sjölanda. More precisely, the water was collected after the very beginning of the aerated grit chamber, where most of the sand is expected to be removed. The filtration trials were divided into two parts, conducted on two different days. As a result, different influent water was used for the filtration trials. However, the tested water was initially analyzed in order to take into account the differences and how it could affect the results.

In Table 3.1, the different filtration experiments are summarized. Experiments 2-10 were performed with water A (WA) collected on 23/11/2016 and 12-16 with water B (WB) collected on 08/12/2016. The trials performed on WA and WB were carried out the same day as the collection date.

Four different polymers were tested in different doses, three of them were cationic polymers (Hydrex 6356, 6456 and 6346) and one was an anionic polymer (Hydrex 6161). Aluminium (PAX XL-100, Kemira) was used as a coagulant in the tests where the effect of coagulant additions was evaluated. The coagulant was also tested in different doses.

Table 3.1 An overview of the filtration trials performed in lab-scale.

		Polymer - Hydrex	Polymer-dose 0.1% (mL)	Coagulant-dose (mg Al/L)
1	Water A (WA)	-	-	-
2	Filtration (WA)	-	-	-
3	WA	6356	1	-
4	WA	6356	2	-
5	WA	6356	3	-
6	WA	6456	1	-
7	WA	6456	2	-
8	WA	6456	3	-
9	WA	6356	2	5
10	WA	6356	2	10
11	Water B (WB)	-	-	-
12	Filtration (WB)	-	-	-
13	WB	6346	2	-
14	WB	6346	4	-
15	WB	6161	2	10
16	WB	6161	4	12

Coagulation and flocculation

Before starting the flocculation process all the polymers were diluted to 0.1 weight%, by weighing 0.1 g powder of the specific polymer and adding 100 ml distilled water.

Carrying out the flocculation experiments in the lab was done by using the Flocculator 2000 (Figure 3.3 a) as a batch test. The installation was programmed as a fast mode mixing (10 s) and slow mode mixing (4 min), see Figure 3.3 b. One liter of wastewater was added to the beaker. The polymer was added under the fast mode mixing. In the experiments where the polymer was combined with the coagulant the same program was used, the coagulant addition was preceding the polymer addition, the coagulant was also added in the fast mode mixing (Figure 3.3 b). Thereafter, the filtration was completed. However, the water level of 200 mm was not retained in the experiments where polymer was added due to the limits in filtered wastewater as a result of the beaker volume which was only 1 L (Sjölin and Olsson, 2016).

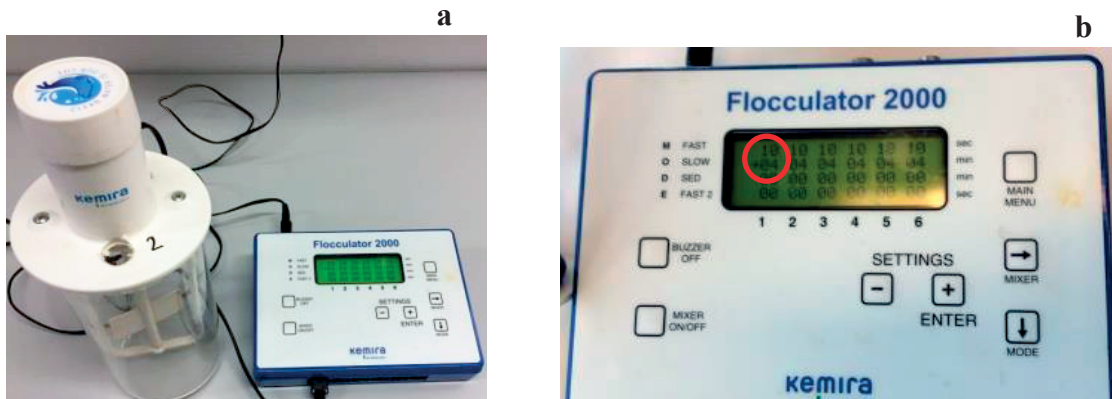


Figure 3.3 a) The instruments used for the flocculation and coagulation and b) Flocculator 2000, programmed for 10 s fast mixing (400 rpm) and 4 min slow mixing (50 rpm).

Filtration

The aim of the filtration was to mimic the full scale filters, in bench scale. The filtration was performed using the filter discs with pore openings of 100 μm (Figure 3.4 a). The filter discs were attached to the bottom of a test tube by a clamp with a rubber joint (Figure 3.4 b). Before initiating the filtration, some tap water was added to the filter to make it wet. The wastewater was poured from a jug to another in order to get good mixed water. The filtration was then carried out pouring the wastewater as quickly as possible into the tube, until reaching the mark of 200 mm. The water level was kept to 200 mm above the filter throughout the filtration. This is done to maintain constant hydraulic pressure which is believed to result in best full scale imitation, since the differential pressure inside the filters are 200 mm between the inlet side and the effluent side.

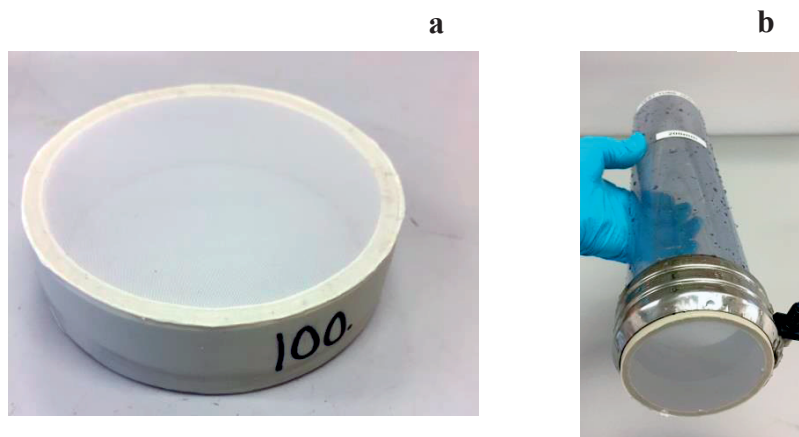


Figure 3.4 a) The filter disc used for filtration trials and b) The test tube used for the laboratory-scale filtration.

The filtration time set to be constant (10 s) under all the experiments, which corresponds to the filtration time a specific part of the filter cloth is in direct contact with water and filtering during a backwash (drum rotation) (Sjölin and Olsson, 2016). The filtered volumes under 10 s were measured in order to calculate the capacity of the filtration step after each condition, see Table 3.2.

3.5 Analysis & measurements

Daily analysis was performed two to three times a week during mainstream operation. During RP analysis were done twice a day. The routine analysis included the ammonium, nitrate and nitrite concentrations. Suspended solids (SS) was analyzed only under mainstream operation. COD (filtered and unfiltered) was measured mainly during mainstream operation in addition to some extra occasions when it was considered interesting.

LCK 114 COD was used for all the COD analysis. The COD concentration was measured as filtered (corresponded to SCOD) and unfiltered (corresponded to COD). Before reading the 114 COD cuvettes a zero was used (a cuvette where distilled water is the sample). Total nitrogen (TN) was done by LCK 238, unfiltered samples were used.

The results for total phosphorus (TP) were obtained by LCK 349; the analysis was done with unfiltered samples. The COD cuvettes were boiled for 160 min at 140°C. TP and TN cuvettes/tubes were boiled for 60 min at 140°C. HT 200S, cuvette boiler, Hach Lange was used for boiling the cuvettes. LCK303, LCK339 LCK342 were used for ammonium, nitrate and nitrite, respectively (Hach Lange, Germany). However, some of the samples except nitrate (always conducted by LCK339) were analyzed using Gallery™ Plus Automated Photometric Analyzer.

Table 3.2 The steps preceeded filtration where only polymer and both coagulant and polymer were used.

Filtration with polymer addition	Filtration with coagulant and polymer addition
10 sec fast mode mixing - Polymer dose addition	10 sec fast mode mixing - Coagulant dose addition
4 minutes slow mode mixing	4 minutes slow mode mixing
Filtration	10 sec fast mode mixing - Polymer dose addition
	4 minutes slow mode mixing
	Filtration

The spectrophotometer used for reading the results of the cuvettes was (DR 2900, Hach). Hach HQ40d multi DO probe was used for measuring the DO. The temperature and pH were measured using HANNA H1991001 pH-meter (Hach, USA). Dwyer rotameters (0-5 ranges) were used for controlling the aeration. The filtered samples were filtered through 1.6 µm pore size glass fiber filters (Whatman/GE Healthcare).

Glass fiber filters with 1.6 μm (Whatman/GE Healthcare) were used for performing suspended solids (SS), using filtration analysis. Dry-baking was performed at 105°C for at least 1 h. The filters were placed in desiccator until cooling down before weighting. The SS value was calculated according to equation 19.

$$SS\left(\frac{\text{mg}}{\text{L}}\right) = \frac{(\text{filter weight before (mg)} - \text{filter weight after (mg)}) \cdot 1000}{\text{Volume of the sample (mL)}} \quad (19)$$

The samples for SS analysis for R1 and R2 were gathered from the effluent. SS samples for the effluent from HRAS were taken as a grab sample from the tube where the inlet into R1 was attached. Samples for analyzing the SCOD, COD, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations were taken directly from the reactors as grab samples. The flow rates during all operations were regularly measured, by gathering the outflow from the reactors.

Analysis filtration trials

All wastewater samples from 1-16 in Table 3.1 have been analyzed in several ways to recognize the effect of filtration as well as the dose of polymer and coagulant. The concentration of TN, TP and COD (filtered and unfiltered) was conducted as described above. The turbidity was measured by Hach 2100 Turbidimeter. The capacity was calculated after measuring the achieved filtered volume of wastewater in 10 s for each experiment. SS analysis was done to identify the reduction of the suspended solids, the method mentioned above.

3.6 Activity batch trials

In order to determine the maximum capacity of the nitrifying bacteria in the laboratory reactors, activity trials were conducted. Mainly, when operating in continuous mode it is believed that the real microbial capacity is higher than what is observed (Piculell, 2016). This is due to the limitation in substrate which will be more important in the cases where the different groups of bacteria compete for a common substrate. During this study, batch trials were mainly performed to determine the AOB and NOB capacity in the biofilms, although two tests were also performed for the anoxic endogenous activity of HB during MPB. AOB and NOB batch trials were conducted during both MPA and MPB. Two 1 L reactors were set up for conducting the activity trials in batch mode (Figure 3.5).



Figure 3.5 The activity test set-up.

The recipes for the start solutions when testing the AOB, NOB and HB activity can be seen in Table 3.3.

The temperature was controlled at 20°C during the batch trials using a thermostat bath. The activity tests were performed using all 200 carriers from the continuous trials. Samples were taken at time 0 and every 15 minutes under 90 minutes. The pH, temperature and DO were recorded when taking the samples, and pH was adjusted to 7 ± 0.3 using 0.5 M sulfuric acid (H_2SO_4) and 0.5 M sodium hydroxide (NaOH).

During AOB and NOB activity trials, air and nitrogen gas were supplied continuously through the bottom of the reactor. The DO was controlled to 2.0 ± 0.3 mg/L, adjusted by changing the flowrate of nitrogen gas and air but keeping the sum of both flows at 3 L/min. During the endogenous HB activity trials, only nitrogen gas (3 L/min) was supplied.

The NH₄-N, NO₂-N and NO₃-N concentrations were measured when performing AOB activity trials. When performing the NOB activity trials, NO₂-N and NO₃-N concentrations were measured, while only NO₃-N was measured when conducting HB activity trials. The measured concentrations were calculated to g/m² (equation 20), and the figures illustrating the values in g/m² over time were used to calculate the activity. The slope for NH₄-N reduction and NO₂-N production were used to obtain the AOB activity, while the NOB activity was obtained through the slope for reduction of NO₂-N. The anoxic endogenous HB activity was obtained through the slope of the NO₃-N reduction.

$$\text{Concentration (g/m}^2\text{)} = \frac{\text{Concentration (mg/L)} \cdot \text{Volume (L)}}{\text{Area (m}^2\text{)} \cdot 1000} \quad (20)$$

Table 3.3 The recipes for the AOB, NOB and HB activity trials used in the study.

AOB	NOB	HB
750 mL tap water	750 mL tap water	750 mL tap water
1 g Sodium bicarbonate (NaHCO ₃ , M=84.01 g/mole)	1 g Sodium bicarbonate (NaHCO ₃ , M=84.01 g/mole)	1 g Sodium bicarbonate (NaHCO ₃ , M=84.01 g/mole)
1.5 mL NH ₄ -N solution (NH ₄ Cl, 95.5 g/L)	1.5 mL NO ₂ -N solution (NaNO ₂ , 123.2 g/L)	1.5 mL NO ₃ -N solution (NaNO ₃ , 151.5 g/L)
0.2 mL PO ₄ -P (KH ₂ PO ₄ , 56 g/L)	0.2 mL PO ₄ -P (KH ₂ PO ₄ , 56 g/L)	0.2 mL PO ₄ -P (KH ₂ PO ₄ , 56 g/L)

3.7 Microscopy

The carriers were analyzed by stereo microscope (Olympus SZ-ET) to follow up the change in biofilm through time and phase change, and pictures were taken to document the biofilm structure, using a Nikon DS-U3 microscope camera.

4 Results & Discussion

The results and discussion part starts by presenting the results from filtrations trials which were performed to decide the different SCOD/N ratios tested in MPB. Thereafter, the results from the continuous operation of both start-up, MPA and MPB are presented followed by the outcomes from the activity batch trials and some microscope pictures of the carriers during the different phases. The discussion is performed continuously in connection to the presentation of the results.

4.1 Filtration trials

Several filtration trials were conducted in laboratory scale, trying to achieve a better understanding of how the dose of polymer and coagulant affected the reduction rate of COD. Figure 4.1 presents the COD reduction rate (%) variation in relation to polymer dosage (mg/g influent SS). The COD reduction was generally increased regularly with the polymer dose. The highest COD reduction was obtained when combining the anionic polymer Hydrex 6161 (2 mL/ppm) with the coagulant (10 mg Al/L), which indicated an effective collaboration between the polymer and the coagulant. On the other hand, the lowest reduction rate (10% for WA and 20% for WB) was obtained when filtration was performed without any chemical addition. The higher reduction rate with WB was believed to be linked to the higher COD in WB (514 mg/L) compared to WA (469 mg/L) (Appendix III). Conversely, combining the polymer Hydrex 6356 (2 mL/ppm) with the coagulant (10 mg Al/L \diamond), resulted in low reduction, which indicated a non-functioning combination.

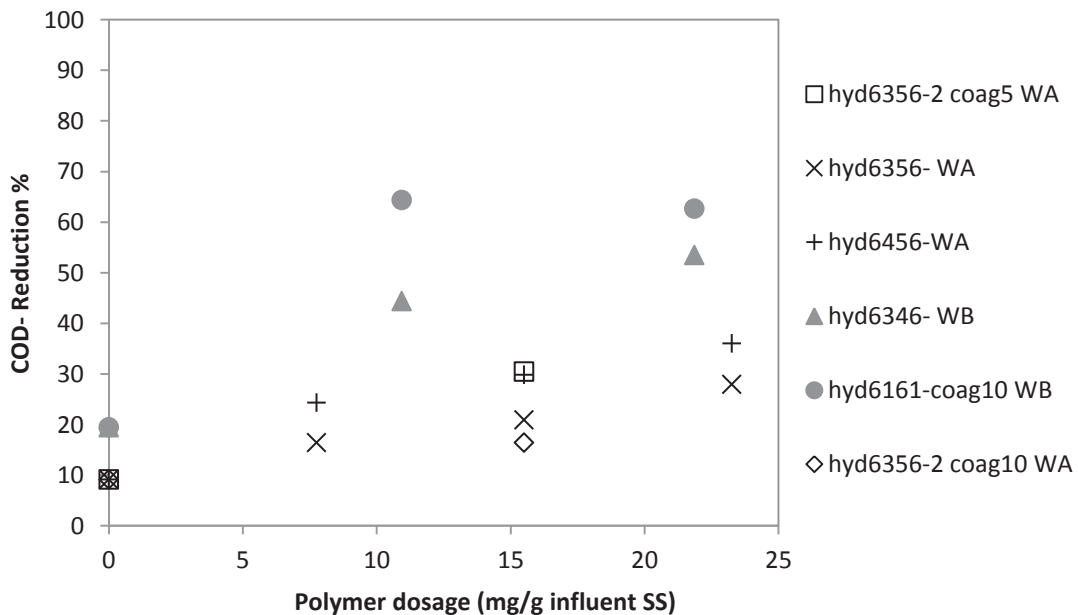


Figure 4.1 The COD reduction in % of the different trials performed.

The reduction rate of SCOD was also generally increased with increasing the polymer dose (Figure 4.2). It was observed that the highest reduction rate of SCOD was obtained when the anionic polymer (Hydrex 6161; 2 mL/ppm) was combined with the coagulant (10 mg Al/L) (Figure 4.2). As described in 2.1.2, the SCOD is the most accessible COD for the bacteria, making the SCOD reduction important.

However, since SCOD consists of very small substances, mechanical separation of SCOD is impossible with no chemical addition. Figure 4.1 and Figure 4.2 indicates a higher reduction rate of COD compared to SCOD. Figure 4.2 shows that the SCOD reduction rate could be increased using the coagulant, but the highest reduction rate obtained was 30%. The highest maintained COD reduction rate (almost 60%) was also obtained as a result of the coagulant addition. The increment in the reduction rate for filtration when adding chemicals designated the capacity of the polymer and the coagulant in streamlining filtration.

It was observed that when the coagulant was combined with Hydrex 6356 (\diamond and \square) this ended in a low reduction rate of COD (15-30%), even though a relatively high reduction rate of SCOD (20-25%) was still obtained (Figure 4.1 and Figure 4.2). This could be explained by the conversion of SCOD into PCOD (2.1.2) as a result of coagulant addition, but the size of the particles was still not big enough to be removed by the filter, resulting in high COD remaining in the effluent from the filter. Here again, the unsuitable combination between the coagulant and the Hydrex 6356 was indicated.

Generally, the reduction rate of SCOD was believed to be low, no lower SCOD values than 150 mg/L could be obtained. Effluents from the filtration step had SCOD values between 150-250 mg/L (Appendix III, Figure III.2), these values were significantly higher than values maintained with the effluent of HRAS, which had an average SCOD of approximately 50 mg/L during the study (Appendix III, Figure III.2).

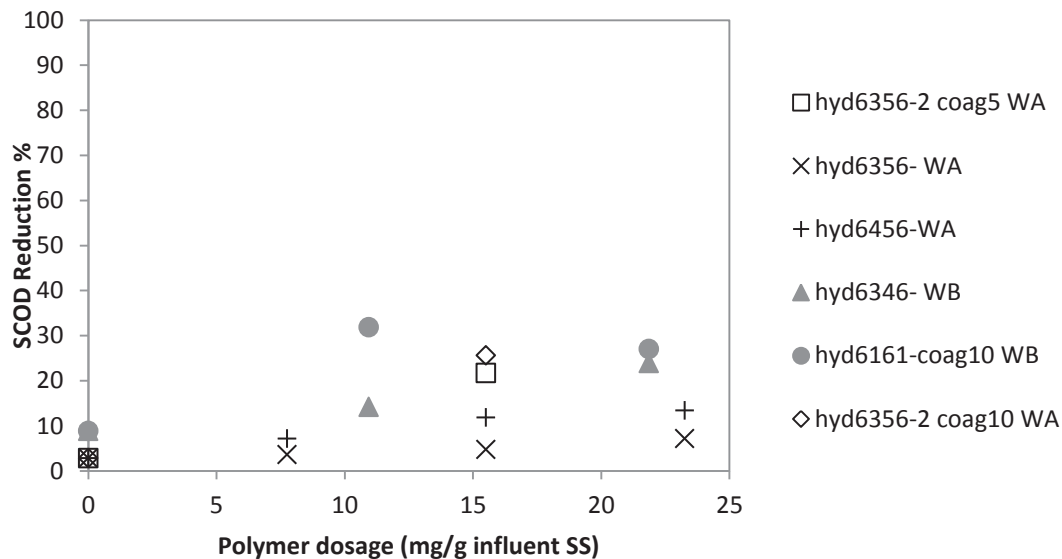


Figure 4.2 The SCOD reduction in % of the different trials performed.

TN was analyzed before and after filtration for all the trials, thereafter the reduction rates of nitrogen were calculated. The results for nitrogen reduction are presented in Figure 4.3. The reduction rate of nitrogen was significantly increased when using the anionic polymer in combination with the coagulant (see Figure 4.3). The reduction of soluble nitrogen is assumed to be impossible chemically or mechanically, the increasing of the reduction in Figure 4.3 was believed to be a result of the increasing in the particulate reduction. The particulate nitrogen is the only nitrogen that could be reduced chemically and/or mechanically (Sjölin and Olsson, 2016). The TN for the effluent from the filter for the point with highest nitrogen reduction was 23.53 mgN/L (Appendix II). An ammonium concentration of 23.53 mgN/L was considered to be a realistic NH₄-N concentration at WWTP Sjölanda, and the results were believed to be reasonable. The average of NH₄-N of the batches used during the study was calculated, the results are presented in Appendix IV, Figure IV.1.

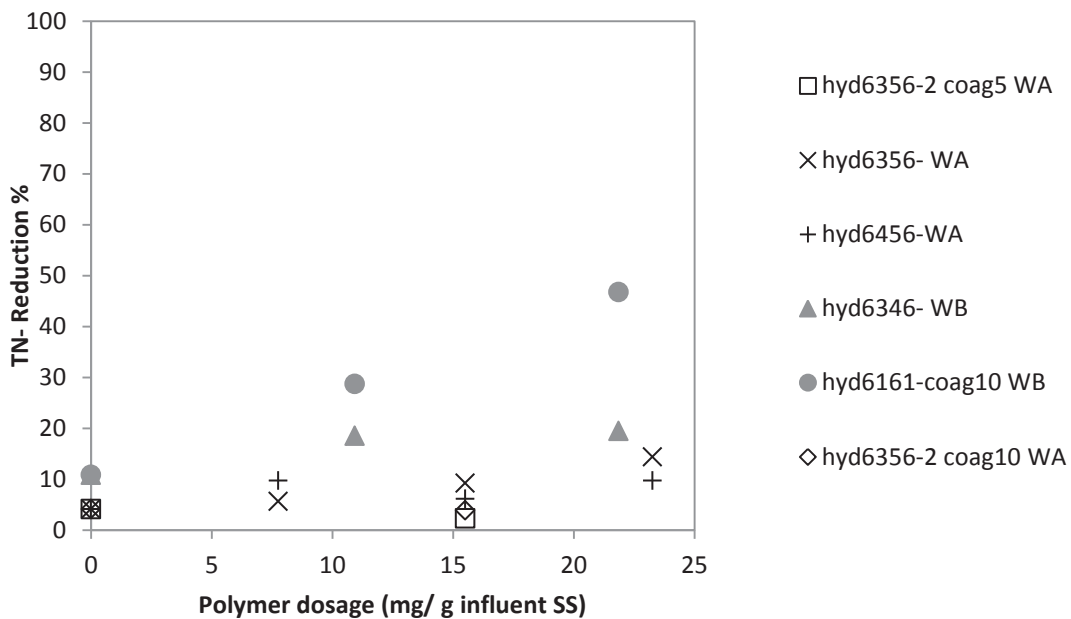


Figure 4.3 The nitrogen reduction of TN in % for the different trials performed.

Figure 4.4 presents the results for the turbidity measurements. The reduction of turbidity is linked to the reduction of the particulates in the water (2.4.1). The highest turbidity reduction rate (nearly 98%) was obtained when combining the anionic polymer with the coagulant, which here again indicated the effective particulate removal with this combination. The lowest reduction rate (20%) of turbidity was obtained when combining the coagulant (5 mg Al/L) with Hydrex 6356 (2 mL/ppm) (\diamond), this reduction was the same as the reduction obtained with no chemical addition (see Figure 4.4). These results could be linked to the results obtained by COD and SCOD reduction, signifying the conversion of soluble elements into particulates (the effect of the coagulant addition). However, this indicated that Hydrex 6356 was unsuitable to be combined with the coagulant. Specifically, it was believed that the coagulant was increasing SS but the Hydrex 6356 (2 mL/ppm) was not able to increase the particle size to be able to be removed by the filter discs with pore openings of 100 μ m.

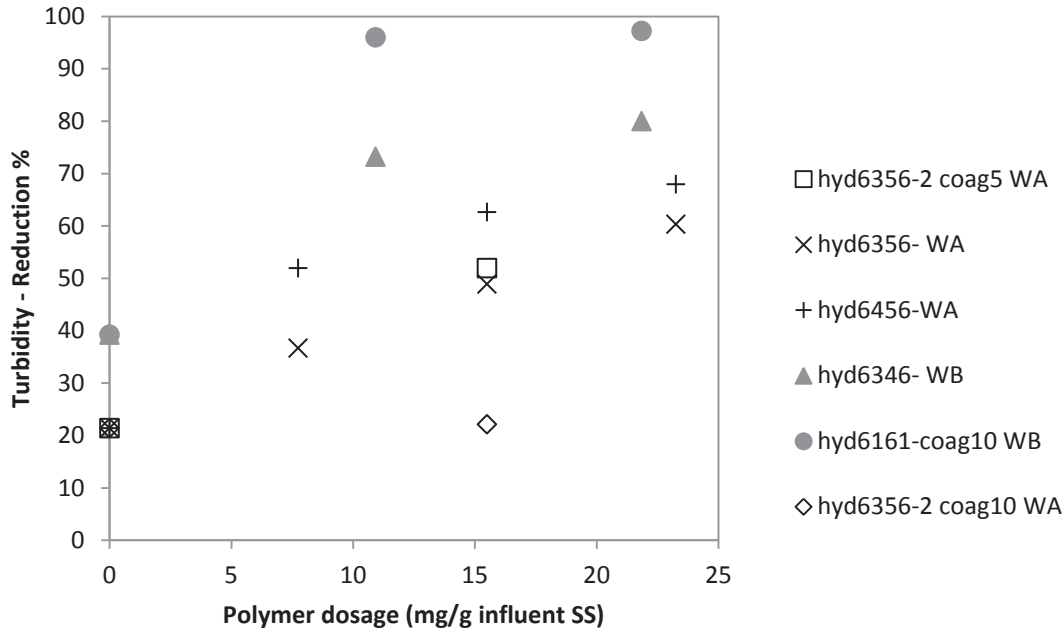


Figure 4.4 The turbidity reduction in % for the different trials performed.

Figure 4.5 presents the different SCOD/TN ratios obtained from the different filtration trials. The results show that even though the reduction rate of SCOD varied, the SCOD/TN ratio was not much affected due to a similar reduction rate of both SCOD and TN. The SCOD/TN ratios in the filtered water varied between approximately 5 and 7 in the different trials (see Figure 4.5). These ratios were relatively high compared to the ratio of the HRAS-effluent which had an average of approximately 1 (the ratio was calculated as an average of all HRAS effluent batches used in this study).

Based on the results from the filtration trials, the lowest SCOD/TN ratio (5) was obtained when anionic polymer (Hydrex 6161; 2 mL/ppm) was combined with coagulant (10 mg Al/L) and when the polymer Hydrex 6346 (4 mL/ppm) was used, Figure 4.5. The highest SCOD/TN ratio (7) was obtained when Hydrex 6356 (3mL/ppm) was added and when Hydrex 6161 (4 mL/ppm) was combined with 12 mg Al/L coagulant, Figure 4.5.

The combination of the anionic polymer with the coagulant maintained optimal combination for SCOD reduction, though the SCOD/TN ratio did not decrease, due to the corresponding drop in TN. There is a significant demand to optimize the filtration step in order to obtain higher SCOD reduction, in addition of obtaining lower SCOD/TN which is a critical value for the process as described in 2.2.5.

A higher reduction of TN when using the anionic polymer compared to the cationic was observed as shown in Figure 4.3. This might imply that it could be better to try to optimize the combination with a cationic polymer in order to obtain a lower SCOD/TN ratio, which in turn favor the process; see the discussion about the low COD/TN ratio in chapter 2.3. Hence, it is recommended to analyze the concentration of $\text{NH}_4\text{-N}$ (before and after filtration) to estimate the effect of the dose of (the polymer and the coagulant) and try to achieve a more understandable image of the filtration step.

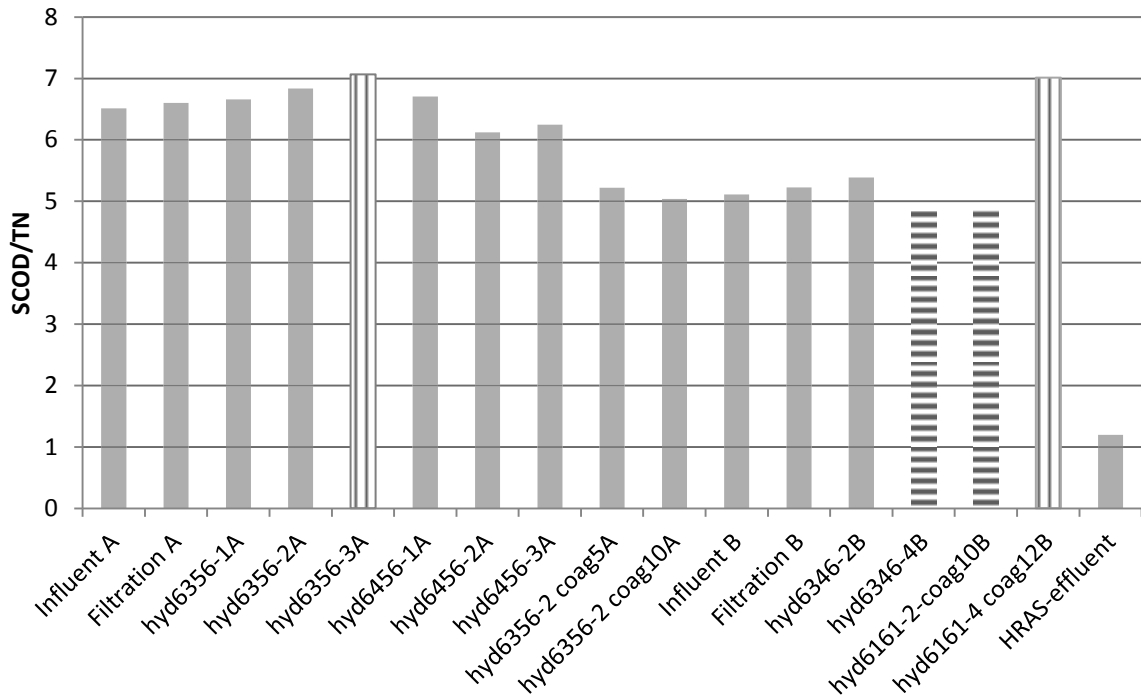


Figure 4.5. The different SCOD/TN ratios obtained from the filtration trials. The horizontal patterned staples are the lowest ratios obtained. The vertical patterned staples are the highest ratios obtained.

Pettersson, (2004) has reported a COD reduction of 30% when using drum filtration on municipal wastewater, without chemical addition. These results were a bit higher than the results obtained when performing the current filtration trials (20% reduction of COD is obtained with only filtration, see Figure 4.1), most likely as a result of using larger pore openings in the current study. The reduction was also believed to be correlated to the high concentration in the influent to the filter. Higher concentrations in the influent could be the reason for the high reduction, as seen in the difference in reduction between WA and WB, where WB contained higher concentrations of SS, COD and TN, Appendix III. On the other hand this higher reduction could also be an indication of suitable polymers, used with WB.

Research performed by Ljungren *et al.* (2007) attained higher COD reduction than obtained in this thesis. Ljungren *et al.* (2007) reported a reduction rate of 60% of both BOD and COD when combining Fe (10 mg/L) with a cationic polymer (4 ppm) and 40 micron filter openings. This indicates the ability of optimizing the filtration step, which could include smaller filter openings and maybe another polymer and/or coagulant. However, using a smaller filter opening will result in lower capacity of the filtration step.

Väänänen *et al.* (2016) reported COD concentration of 200 mg/L and a COD/NH₄-N ratio 7 for Sjölund WWTP. The results were obtained independently of the polymer and coagulant dose in the range of (5-30 mg polymer/mg influent SS). These results are similar to results obtained in the current study.

Based on the results from the filtration trials, the SCOD/TN ratios to be studied during the continuous MPB operation were determined to be 5 in R1 respectively 7 in R2, to cover the full range of potential Hydrotech filter effluent composition.

4.2 Start-up of MBBR

The study was started by two weeks of reject feed, ensuring optimal conditions for the AOB regarding temperature and high substrate availability (ammonium), while high FA and FNA concentrations were expected to disfavor the NOB. The effect of reject operation was studied by performing activity trials at operation day 0 and 9 (Table 4.1 for both R1 and R2).

As seen in Table 4.1 the NOB activity was approximately the same as the AOB activity when the continuous operation was started. However, after reject operation for 9 days the NOB activity was inhibited to a certain extent and the AOB activity was favored. The NH₄-N consumption rate increased from 0.49 to 0.74 g/m²,d and the NO₂-N production rate was increased from 0.25 to 0.68 g/m²,d, indicating increased AOB activity, while the NOB activity decreased as observed by a descending NO₂-N consumption rate from 0.40 to 0.13 g/m²,d (Table 4.1). The same values were observed in both reactors, which were expected as a result of the similar operation conditions during start-up.

Table 4.1 The results from AOB and NOB activity trials during start-up: NH₄-N consumption rate and NO₂-N production rate linked to the AOB activity, and NO₂-N consumption rate linked to the NOB activity. Observe that the results were the same for R1 and R2.

	Day of operation (d)	AOB		NOB
		NH ₄ -N consumption rate (g/m ² ,d)	NO ₂ -N production rate (g/m ² ,d)	NO ₂ -N consumption rate (g/m ² ,d)
R1	0	0.49	0.25	0.40
	9	0.74	0.68	0.13
R2	0	0.49	0.25	0.40
	9	0.74	0.68	0.13

After the second activity trial, the reject operation continued for an additional 6 days before switching to mainstream operation. Although the observed NOB activity dropped in the activity trials, the nitrate concentration never decreased to 0 mg/L during the start-up phase. This indicated that the NOB was never fully washed out of the system during start-up. Kouba *et al.* (2014); Isanta *et al.* (2015) and Trojanowicz *et al.* (2016) had reported the difficulties of NOB elimination when established in the biofilm. Istana *et al.*, (2015) stated that the achievement of NOB elimination from the biofilm required long term operation under non-favorable conditions.

Hellinga *et al.* (1999), Knowles *et al.* (1965), Groeneweg *et al.* (1994) and Wang and Yang (2004) described in chapter 2.3, have reported higher growth rate of the AOB for higher temperature which was also detected in this study where switching to reject water (higher temperature) was favoring the AOB activity over the NOB.

4.3 MPA and MPB

The MPA operation was initiated at day 15 of the operation and ended day 48. The operation was carried out by feeding both reactors with HRAS effluent, and switching to reject temporarily to suppress NOB. After finding a suitable MPA load, the reject phase was performed once a week for two days. The results from MPA (Figure 4.6 and Figure 4.7) show that the NH₄-N load was needed to be increased in order to obtain the same concentrations of NO₂-N and NH₄-N in the effluent. The high NH₄-N load (approximately 1g/L,d) used indicated a high capacity of the process. Operating on lower load was resulting in obtaining NOB problems after 3-4 days of mainstream operation, due to high nitrite production rate. The NH₄-N load where mainstream nitrification was successfully achieved during MPA was found to be around 0.8±0.1 g/L,d, for which almost the same concentrations of NH₄-N and NO₂-N were obtained in the effluents (Figure 4.6 and Figure 4.7). The NH₄-N reduction rate was 0.4±0.1 g/L,d, NO₃-N production rate was 0.1±0.05 g/L,d and the NO₂-N production rate was 0.3±0.1 g/L,d. No significant difference in performance was observed between R1 and R2 during MPA (Figure 4.6 and Figure 4.7). Poot *et al.* (2016) reported a successful nitrification process with nitrogen loading rates of 0.8 g/L,d, when treating synthetic wastewater with 50 mgN-NH₄/L and no COD at 20°C. The results obtained by Poot *et al.* (2016) are similar to the results obtained during MPA. Similar results were also obtained by Piculell *et al.* (2016a).

On day 48, the MPA was ended by a reject phase before switching to MPB. In the beginning of the MPB operation no nitrite was observed when operating with almost the same NH₄-load as in MPA. Therefore, the load was gradually decreased until nitrite was observed. However, no successful mainstream nitrification was observed during MPB (Figure 4.6 and Figure 4.7). Even though NH₄-load between 0.2-0.3 g/L,d was tested during MPB nitrite observation was still negligible. No 50% NO₂-N 50% NH₄-N could be obtained in the effluent during MPB. Nevertheless, the results were based on a short term study, and it is likely that the bacteria require longer time to adapt to the new conditions.

The change of NH₄-N, NO₂-N and NO₃-N concentrations in the both R1 and R2 can be seen in Appendix IV Figure IV.2 and IV.3.

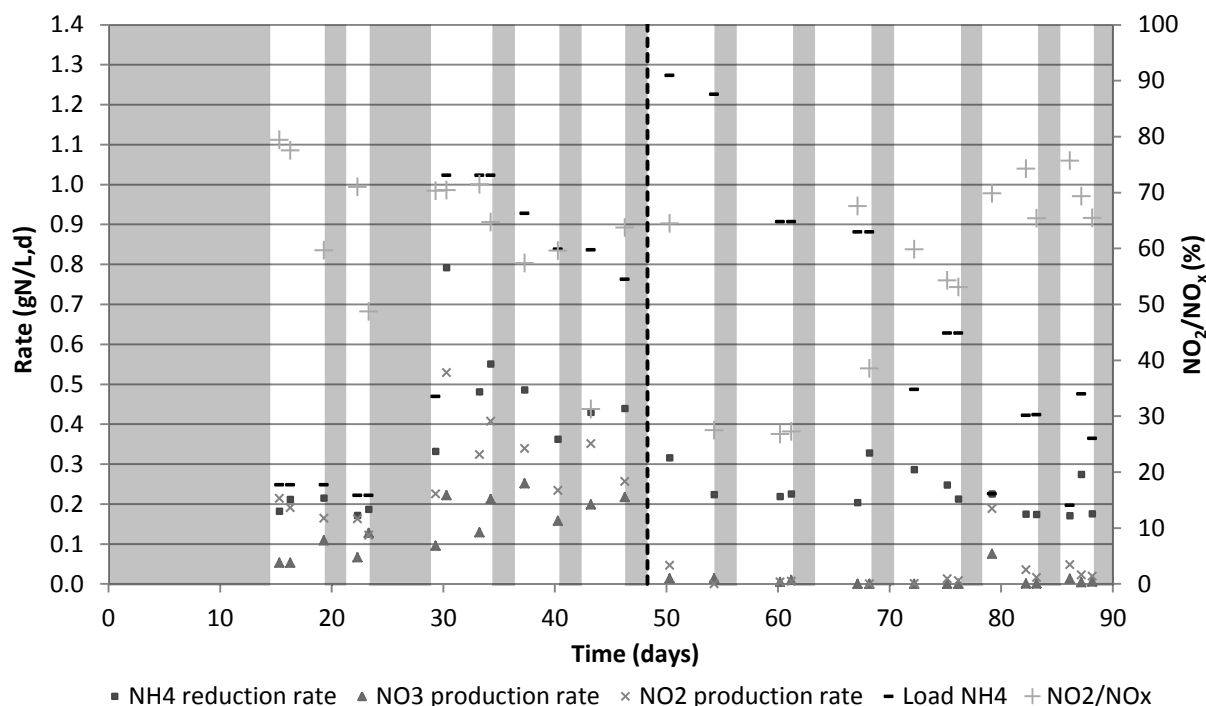


Figure 4.6 The results from the continuous operation for R1. NH₄ reduction rate, NO₂ and NO₃ production rate are presented, in addition to the percentage of the produced NO₂ relative to the NO_x (NO₂+NO₃) produced. The marked areas in the figure illustrate RP operations. The dashed line illustrates the time when switching to MPB occurred.

After switching to MPB, the production of nitrite and nitrate fell to zero as shown in Figure 4.6 and Figure 4.7. However, there was still a substantial ammonia removal. Since the mass balance of nitrogen no longer added up during MPB, there were indications that simultaneous denitrification occurred in the reactors, as a result of the elevated COD. An inlet SCOD/TN ratio of 5-7 could probably enable full denitrification (discussed in chapter 2.5). Both Aspegren *et al.* (1992) and Kristensen *et al.* (1992) have reported that a COD/TN ratio of 4.7-8.7 was sufficiently for a full scale nitrification/denitrification process. This indicated that lower SCOD/TN is desired in order to use this process combination for PNA nitrogen removal treatment.

According to Gerardi (2002) the dry weight of the bacteria contains approximately 14% nitrogen. This could be another explanation of the unbalance of nitrogen between the influent and effluent in the reactors. The consumed nitrogen could have been used for building up the heterotrophic biomass. On the other hand, nitrate consumption could have been occurred due to denitrification (2.2.2 for detailed reaction) because of the diffusion limitation in the biofilm, obtaining anoxic areas. Classifying the reactions occurred in the reactors was difficult, and it was believed that a better assessment could be performed if the nitrogen gas emissions had been measured.

In general, the decrease in the process capacity was believed to be directly correlated to the extensive development of heterotrophs. The higher COD availability in the reactors was believed to result in a change in the biofilm structure and dynamics. Heterotrophs considered to be growing on all available surfaces on the carriers resulting in increased biofilm thickness and abundance, which resulted in diffusion limitation, which in turn could explain the limited AOB and NOB activity during MPB.

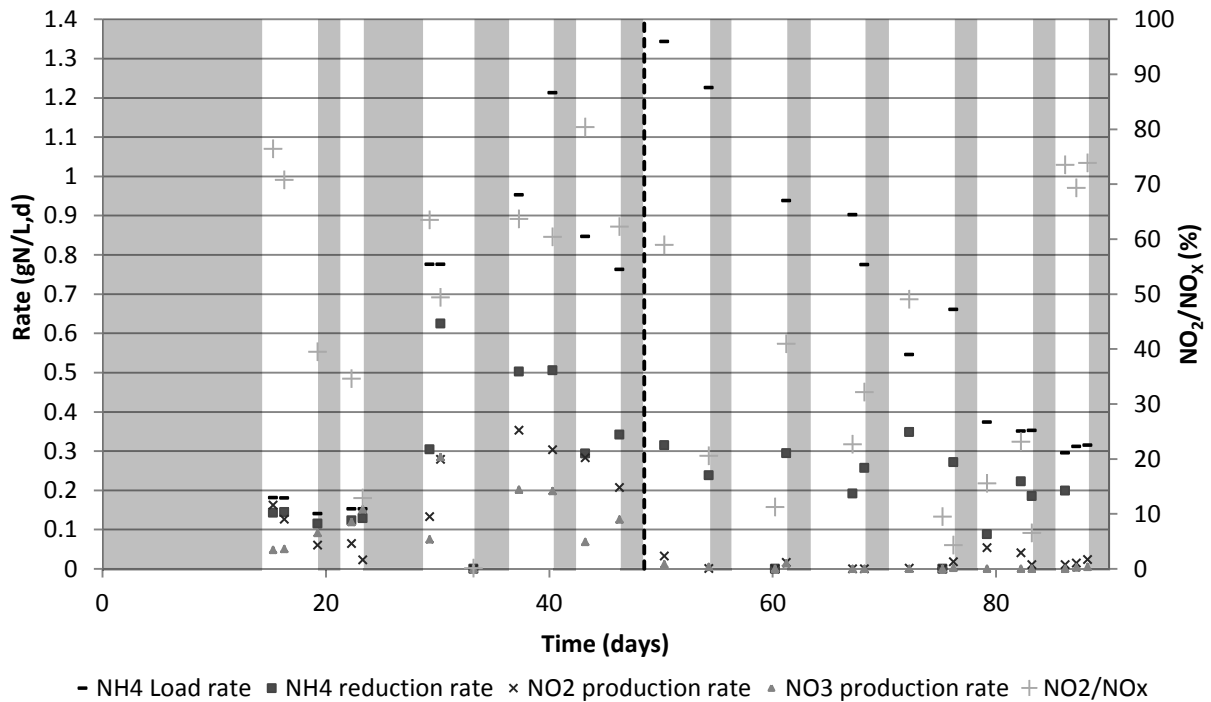


Figure 4.7 The results from the continuous operation for R2. NH_4 reduction rates, NO_2 and NO_3 production rates are presented, in addition to the percentage of the produced NO_2 relative to the NO_x ($\text{NO}_2 + \text{NO}_3$) produced. The marked areas in the figure illustrate RP operations. The dashed line illustrates the time when switching to MPB occurred.

The flow rate of the acetate solution was adjusted depending on the TN and SCOD in the HRAS effluent. Due to a small variation in the flow rate of the different influents, a variation in SCOD/TN value was expected. Therefore, the SCOD/TN values were calculated after conducting the analysis and different measurements. In Table 4.2 the calculated SCOD/TN ratios obtained in influents to R1 and R2 are presented. Due to slightly varying composition of the HRAS batches, it was difficult to obtain a stable SCOD/TN value. For every new batch of HRAS-effluent used, the pumps had to be adjusted, ending in a test period which could imply in higher or lower ratio. There were also a few occasions where the pumps feeding the HRAS effluent accidentally dropped, due to growth in the tubes, which resulted in much higher ratios. The SCOD/TN ratio in the influent to R1 varied between 3-12, and between 6-9 in R2.

Another important value was presented in chapter 2.3, this value is the BOD load rate $\text{g/m}^2, \text{d}$. BOD was not measured during the study, therefore, the BOD load could not be calculated. Instead, the SCOD load has been calculated in $\text{g/m}^2, \text{d}$. The BOD and SCOD correlation is described in 2.1.2.

Table 4.2 presents the SCOD load rate in $\text{g/m}^2, \text{d}$ for both R1 and R2. The initial SCOD load rate into the reactors was high during MPB, $27 \text{ g/m}^2, \text{d}$ in R1 and $37 \text{ g/m}^2, \text{d}$ in R2. By decreasing the overall load rate ($\text{g/L}, \text{d}$) into the reactors, the load rate of SCOD was consequently decreased. Nevertheless, no lower SCOD load rate than $9 \text{ g/m}^2, \text{d}$ was obtained even though the dramatical reduction of $\text{NH}_4\text{-N}$ load rate $\text{g/L}, \text{d}$ from 1 to $0.3 \text{ g/L}, \text{d}$.

The initiation of MPB operation with high $\text{NH}_4\text{-N}$ load and consequently a high SCOD load was believed to end in a worst case scenario. The high SCOD load to the reactors was believed to result in high heterotrophic development. Most likely, it would have been better if the operation had been initiated at a lower load (approximately $\text{NH}_4\text{-N}$ load of $0.5 \text{ g/L}, \text{d}$, which gives a SCOD load of approximately $10\text{-}13 \text{ g/m}^2, \text{d}$) which potentially could have been increased gradually.

In chapter 2.3, it was referred to a study (Hem *et al.*, 1994) which could not achieve nitrification at a load of $5 \text{ gBOD}_7/\text{m}^2, \text{d}$. For this study, the lowest SCOD load studied was $9 \text{ g/m}^2, \text{d}$ where no significant nitritation was detected as well. Henze *et al.* (2000) presented typical ratio of COD/BOD, 2-2.5 for domestic wastewater. The COD source used to increase the COD in HRAS effluent during the study was acetate. Acetate is assumed to deliver more accessible COD than the effluent from the filter. Therefore, the SCOD/BOD ratio of 2 was believed to be a relatively good assumption. This resulted in a BOD load of approximately $5 \text{ g/m}^2, \text{d}$ which ended up to be the same value tested by Hem *et al.* (1994).

The most crucial significance for achieving partial nitrification is the changes in the biofilm community and abundance. Generally, both Ahn (2006) and Ge *et al.* (2015) defined the partial nitrification as an unbalanced overall activity of the nitrifying bacteria ($\text{AOB} > \text{NOB}$). However, results from the current study shows that maintaining partial nitrification in the presence of COD will be more challenging. The process operation will most likely be threatened by an additional bacteria type (HB).

Table 4.2 The calculated SCOD load in $g/m^2,d$, in addition to calculated SCOD/N ratios in the influents during MPB.

Day of operation (d)	R1		R2	
	gSCOD/ m^2,d	gSCOD/gN	gSCOD/ m^2,d	gSCOD/gN
50	27	5	37	7
54	25	5	36	7
60	20	5	36	8
61	20	5	36	8
67	19	3	39	7
68	19	3	39	7
72	16	8	22	9
75	19	5	28	7
76	19	5	28	7
79	9	12	12	6
82	11	4	12	6
86	10	8	12	7
87	11	4	13	7

Figure 4.8 presents changes in HRT in R1 and R2. During MPA the HRT was needed to be decreased from approximately 0.1-0.15 d to approximately 0.025 d in order to obtain a successful nitrification process. The ability of operating with low HRT identified a high capacity for the process. Contrary, HRT needed to be dramatically increased during MPB which indicated a declining nitrification capacity. The HRT was increased to approximately 0.1 d, but still no nitrification was observed during MPB.

The change in SS in the effluent from the reactors was also analyzed during the different phases and the results are presented in Figure 4.8. During MPA, SS was varying between 20-40 g/L. On the other hand, during MPB SS was varying between 40-160 g/L. Obviously, SS was dramatically increased when switching to MPB. The increase in SS could be explained by an increased HB growth. The growth rate of HB is much higher than nitrifying bacteria (2.2.2 and 2.2.3) which resulted in higher suspended solids during MPB. Higher SS values were observed at the start of MPB and this was believed to be a result of the higher SCOD load rate (more HB development), Table 4.2.

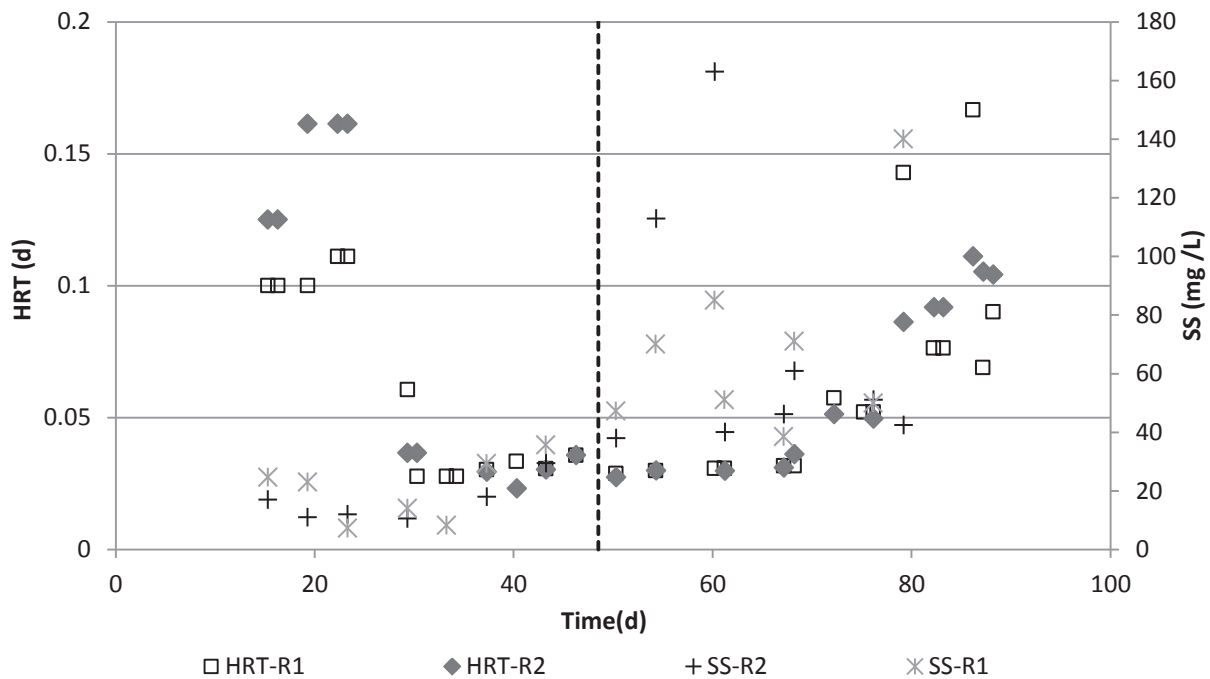


Figure 4.8 The HRT and SS in the effluent, for both R1 and R2 during MPA and MPB. The dashed line illustrates the time when switching to MPB occurred.

Table 4.3 presents the load rates of SCOD in g/L,d during the different phases. During MPA, R1 and R2 had the same influent however the minor differences in flow rates resulted in different SCOD load rates. The SCOD load rates varied between 0.40-1.66 g/L,d in R1 and between 0.25-1.90 g/L,d in R2 during MPA. On the other hand, the SCOD load rates during MPB were significantly higher. The highest SCOD load rates were obtained in the initial phase of MPB, as a result of high flow rates in both reactors. R1 had a SCOD load rate of 6.86 g/L,d. The SCOD load rates in R2 was 9.84 g/L,d as peak. However, the dramatically decreasing in flow rates resulted in lower SCOD load. The SCOD load rates were decreased to 2.25 g/L,d in R1 and 3.16 g/L,d in R2 respectively.

Table 4.3 The SCOD load (g/L,d) in R1 and R2 respectively, during MPA and MPB.

	Day of operation (d)	SCOD load in R1 (g/L,d)	SCOD load in R2 (g/L,d)
MPA	15	0.45	0.36
	19	0.40	0.25
	29	1.12	1.09
	37	1.66	1.71
	40	1.31	1.90
	46	1.30	1.30
MPB	50	6.86	9.46
	54	6.47	9.27
	60	5.01	9.27
	61	5.01	9.27
	67	4.95	9.84
	68	4.95	9.84
	72	4.09	5.56
	75	4.82	7.04
	76	4.82	7.04
	79	2.25	3.19
	82	2.70	3.16
	83	2.70	3.16
	86	2.43	3.19
87	2.87	3.22	

4.4 Activity batch trials

During the continuous operation the maximum capacity of the bacteria was studied by performing batch trials. The batch trials were performed to get a better assessment of the change in the biofilm during the different phases. Figure 4.9 and Figure 4.10 present the results from the different activity trials performed during the study for both R1 and R2. In general, almost the same results were obtained in R1 and R2. However, a relative higher NOB activity was indicated in R2, during MPA. NH₄-N consumption rate varied between 0.75-1.00 gN/L,d and NO₂-N production rate between 0.75-0.80 in gN/L,d in both reactors. These values appeared to be in an acceptable rate relative to the NO₂-N consumption rate (0.2-0.6 gN/L,d). An obviously ascending trend in the NO₂-N consumption rate was considered to be linked to rising NOB activity with time. This was suspected to be a result of NOB acclimatization that has been even reported in several studies (Svehla *et al.*, 2014; Turk and Mavinic, 1989).

The low nitrite production rate at day 30 of operation in R2 (considered as an outlier) was believed to be affected by the low pH obtained during the trials; this was avoided in the other trials by adding NaOH. Therefore, the AOB activity at this point was believed to be almost the same as in R1.

Observe that the difference in the operation phase time when activity trials were performed affect the results. Performing activity trials directly after reject operation believed to end in low NOB activity but when performing activity trials after few days of mainstream operation, the NOB activity believed to be a bit higher. Therefore, when comparing the results from the activity trials performed, the moment of operation should be taken into account. To avoid this complication activity trials should be done at the same moment of operation (for example 1 day after RP termination).

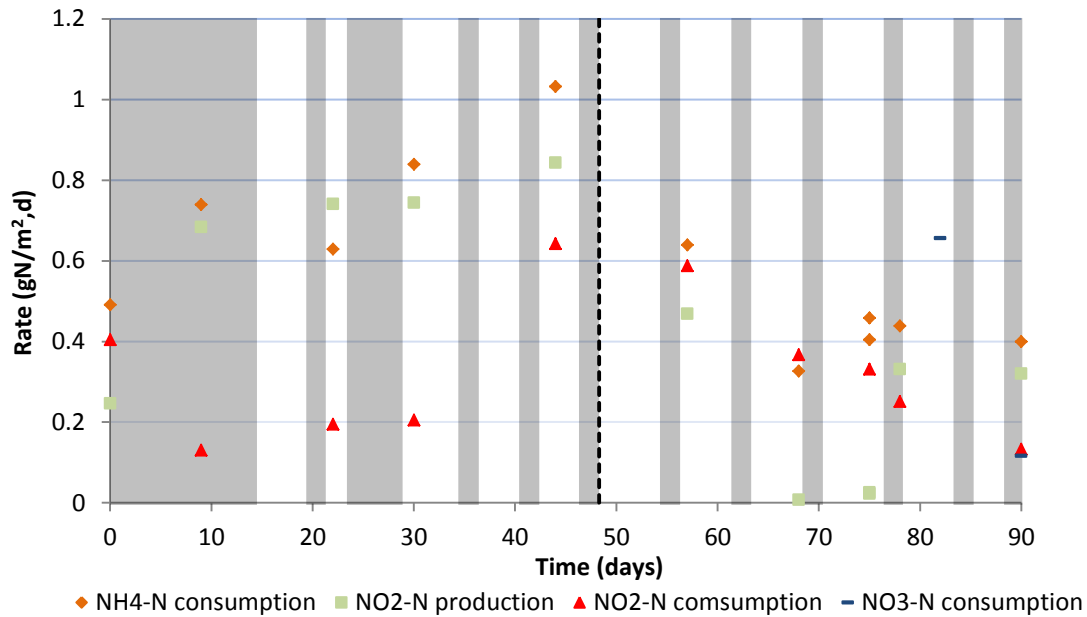


Figure 4.9 The results from the activity batch trials for R1. NH₄-N consumption rate and NO₂-N rate production is linked to the AOB activity, the NO₂-N consumption rate is linked to the NOB activity. The denitrification rate is obtained from the NO₃-N consumption rate. The marked areas in the figure illustrate RP operations. The dashed line illustrates the time when switching to MPB occurred.

At operation day 57, the activity trials were conducted for the first time after switching to MPB. The results show descending rates in general, these results were believed to be related to HB development. The biofilm growth was believed to end in higher DO demand, due to diffusion limitation. Diffusion limitation was believed to limit the AOB activity.

When AOB activity trials were performed at operation day 68 and 75 during MPB, no nitrite production was observed. However, ammonium reduction was still noticed (Figure 4.9 and Figure 4.10). This was unexpected, and indicated that other bacteria were also active in the biofilm during the activity trails. The suspected bacteria activity was believed to consume the ammonium directly or any nitrite/nitrate being produced by potential AOB. However, this would require that HB were active in the batch trials although no COD was added. Two theories were tested to explain this activity.

Theory A was that some SCOD remained inside the biofilms when the carriers were removed from the reactors, which then supplied the HB with SCOD during the activity trials. This theory was strengthened by the nitrogen mass balance adding up better in the NOB trials, which were generally performed directly after the AOB trials. It was hence assumed that any remaining COD in the biofilms would have been consumed by HB during the initial AOB trials. This theory was tested by performing an additional AOB activity trial directly after the NOB batch trial. However, since the similar results obtained at both AOB activity trials (before and after the NOB batch trial) the theory was not valid.

Hence, a new theory was reasoned (theory B), in which SCOD was not contained but rather produced continuously inside the biofilms by the degradation of the bacteria during the tests. The degradation of the bacteria was believed to result in SCOD secretion (i.e. endogenous respiration). Furthermore, HB activity believed to disturb the results although no external COD was added during the tests.

It was expected that an extensive biofilm layer was developed during mainstream operation at high COD, by a substantial build-up of HB in the biofilms, which could then degrade during activity trials and result in denitrification. This theory was confirmed by: 1) performing activity trials directly after an RP and 2) performing endogenous denitrification trials at the end of a MPB.

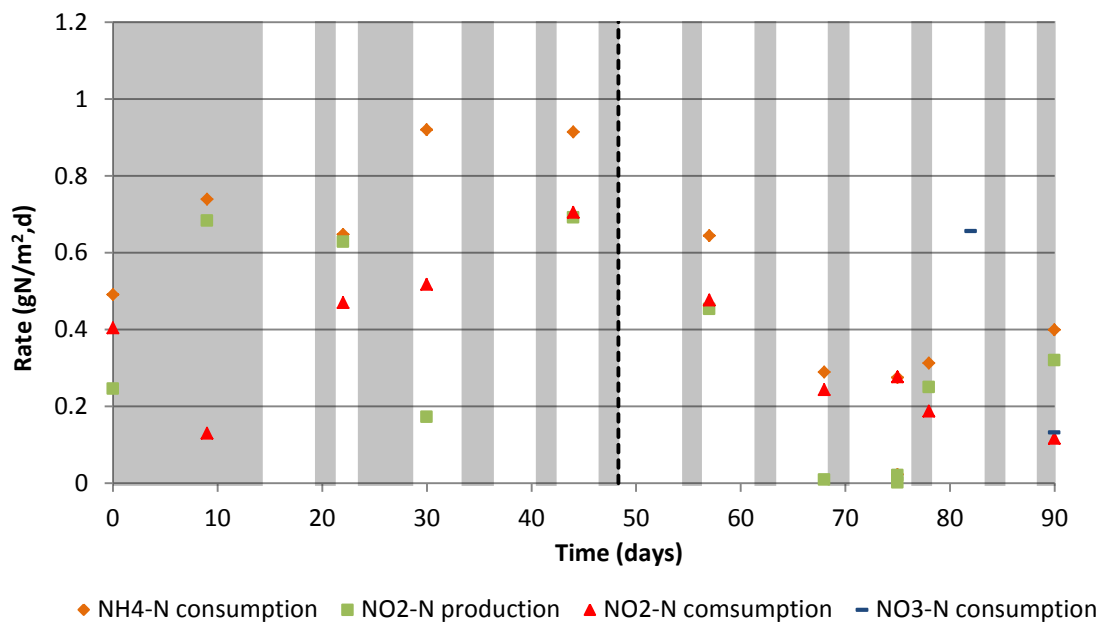


Figure 4.10 The results from the activity batch trials for R2. $\text{NH}_4\text{-N}$ consumption rate and $\text{NO}_2\text{-N}$ rate production is linked to the AOB activity, the $\text{NO}_2\text{-N}$ consumption rate is linked to the NOB activity. The denitrification rate is obtained from the $\text{NO}_3\text{-N}$ consumption. The marked areas in the figure illustrate RP operations. The dashed line illustrates the time when switching to MPB occurred.

As seen in Figure 4.9 and Figure 4.10, the ammonia removal and nitrite production rates matched better in the AOB trial on day 78, directly after a RP. During the RP the reactors were not fed any COD, why it was expected that any significant HB build up in the biofilm during mainstream feed would have degraded during the RP.

Performing endogenous denitrification trials at the end of MPB (operation day 82) indicated that in both reactors, denitrification could be achieved without any external COD source. The $\text{NO}_3\text{-N}$ consumption rate was $0.66 \text{ g/m}^2\text{,d}$ in both R1 and R2. On the other hand, the $\text{NO}_3\text{-N}$ consumption rate decreased to $0.12 \text{ g/m}^2\text{,d}$ in R1 and $0.13 \text{ g/m}^2\text{,d}$ in R2, when performing the same activity trials at the end of a RP (operation day 90), these results confirmed the biofilm degradation theory.

Friedrich and Takács (2013) have studied the endogenous decay rate of heterotrophic bacteria in activated sludge. The study reported the ability of heterotrophic bacteria in degradation in two different phases. Both phases included degradation of active biomass. However, the first phase which was assumed to be the fastest involved degradation of additionally stored material. This degradation was assumed to be occurring in the reactors when no COD was added, during RP and activity trials.

Ma *et al.* (2009) presented a study where the inhibition effect of the FNA on the HB was reported. The nitrate reduction activity was totally inhibited for FNA greater than 0.2 mg/L . This could explain why the AOB and NOB activity were only observed during the RP when operating on MPB. This could indicate that denitrification was taking place in the reactors during MPB. Therefore, the AOB and NOB activities were not observed due to denitrification. On the other hand, when RP was started and high FNA values were obtained the HB bacteria were perhaps inhibited and then the nitrite and nitrate concentration started to increase. The effect of FA and FNA was also clearly shown to limit the NOB activity more than the AOB, which also is similar to different studies mentioned in the theory part 2.5.

In Figure 4.11 and Figure 4.12 the tracked changes in COD and SCOD during the last 5 days of the study are presented. It was obvious that when switching to RP the COD concentrations were interestingly increasing. Both COD and SCOD concentrations were exceeding the values observed during the mainstream feed. Since reject water did not contain any COD and no external COD source was added during RP, endogenous respiration was further confirmed.

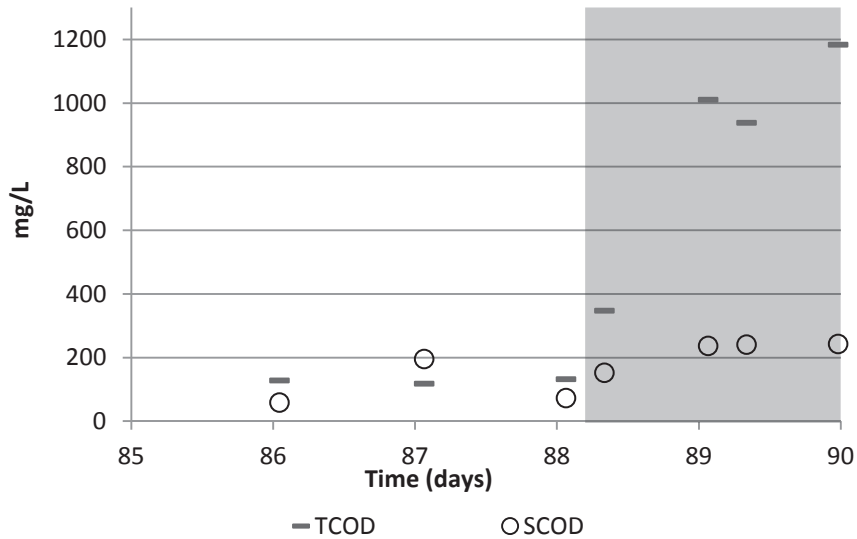


Figure 4.11 The change in the COD and SCOD concentration when switching to reject in R1. The marked area in the figure illustrates RP operation.

During MPB, COD values were varying between 70-200 mg/L in both reactors. However, when switching to RP, COD was gradually increased from 200 mg/L till nearly 1200 mg/L, additionally SCOD was also increased to nearly 300 mg/L in both reactors (Figure 4.11 and Figure 4.12). This indicated that the biofilms were gradually degrading during RP. Furthermore, this clarified why activity trails were better reflecting AOB activity after RP. Observe that the effect of $\text{NO}_2\text{-N}$ on the COD analysis was rectified in order to avoid excessive high COD concentrations.

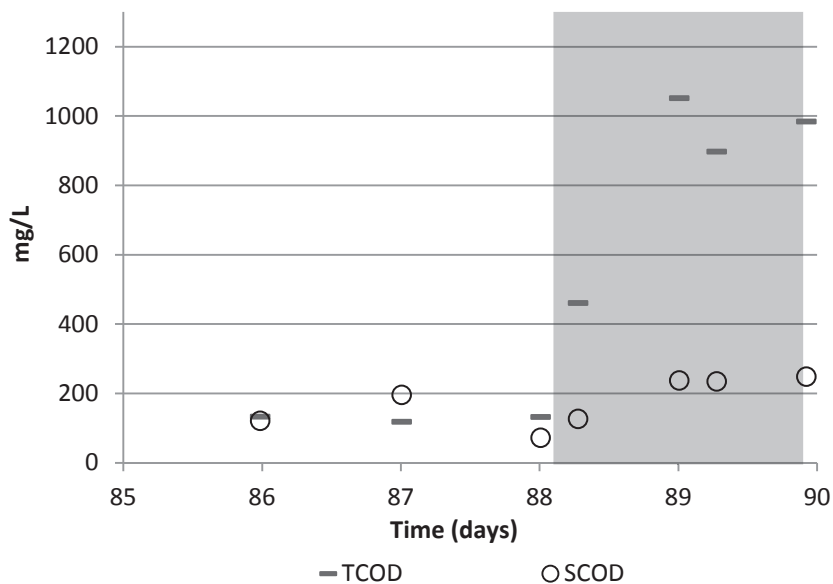


Figure 4.12 The change in the COD and SCOD concentration when switching to reject in R2. The marked area in the figure illustrates RP operation.

4.5 Results obtained from reject phase (RP)

Different results were maintained during RP operation during the different mainstream phases, probably as a result of varying nitrifying activity. Even though, approximately the same RP operation conditions were applied in the two phases (MPA and MPB) there was a difference in the FA and FNA concentrations obtained during RP. Depending on load, the FA and FNA varied between 30-46 mg/L and 795-1373 µg/L respectively, during MPA in R1. In R2, FA values varied between 29-47 mg/L while FNA values ranged between 740-1308 µg/L.

During MPB, significantly higher variation in the concentrations was observed. Depending on load, FA and FNA varied between 36-106 mg/L respectively 25-911 µg/L, during MPB in R1. In R2, FA and FNA varied between 14-106 mg/L respectively 57-1195 µg/L during MPB. The variety of the values obtained during MPB was believed to be linked to the variation in bacteria activity during this phase, correlated to HB activity. Here again another indication of the change in the biofilm structure occurred when operating on reject during MPB was obtained.

4.6 Results from Microscopy

To observe the biofilm visually, microscopic images were taken during MPA, MPB and after RP operation during MPB. The images can be seen Figure 4.13-Figure 4.15. Since the microscopic images were same for R1 and R2, only the images of carriers from R1 are presented here. The images of carriers from R2 can be seen in Appendix V.

Figure 4.13 indicates that the cells of the carriers are still not full with biofilm during MPA. In the middle of each cell, the biofilm was clearly thinner which was explained by Piculell *et al.* (2016b) to be correlated to the scraping effect between the carriers.



Figure 4.13 Microscopic images of the carriers in R1 during the MPA. The date of the picture is 22/12/2016 (operation day 49).

Figure 4.14 shows an obvious development of the biofilm in thickness and darkness. The biofilm change was believed to be corresponded to high development of HB during MPB. HB was available to be developed much faster than the nitrifying bacteria due to higher growth rate (2.2.2 and 2.2.3).

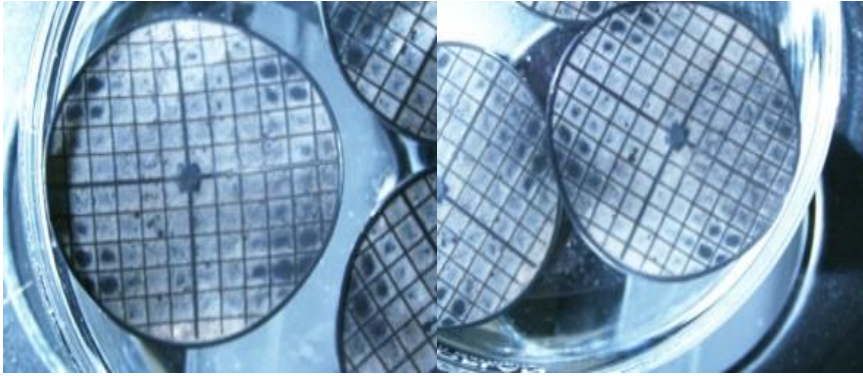


Figure 4.14 Microscopic images of the carriers in R1 during MPB. The date of the picture is 17/01/2017 (operation day 76).

Though, a remarkable change in the abundance of the biofilm after RP operation was detected in both reactors after RP during MPB (Figure 4.15). The loss of a substantial part of the biofilm during RP further confirms the theory about the change of the biofilm structure.

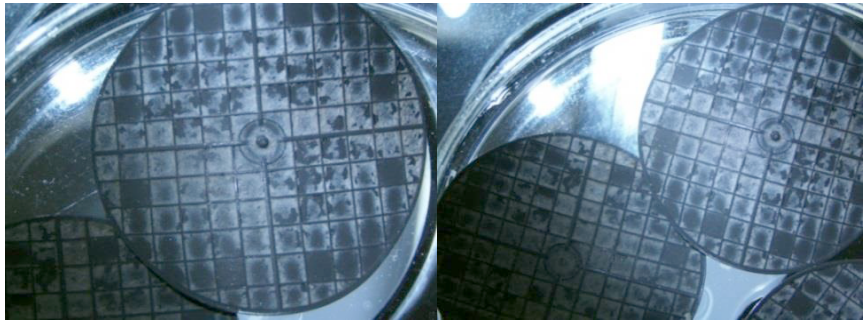


Figure 4.15 Microscopic images of the carriers in R1 after the RP during MPB. The date of the picture is 19/01/2017 (operation day 78).

5 Conclusions

The study has achieved pointing out different challenges in the mainstream nitrification process. The few filtration trials performed indicated a low SCOD reduction overall and difficulties in obtaining a low SCOD/TN. The lowest SCOD/TN obtained was 5 while the highest ratio was 7. It was decided to test SCOD/TN ratio 5 and 7 in two parallel lab reactors during the second mainstream phase.

During the first mainstream phase ($\text{SCOD/TN} \approx 1$) of the operation a successful nitrification was accomplished in both reactors. The nitrification process was successfully achieved with a $\text{NH}_4\text{-N}$ load of approximately 1 g/L,d. However, when switching to the second mainstream phase insignificant nitrification was observed in both reactors. The $\text{NH}_4\text{-N}$ load was gradually decreased to 0.3 g/L,d, but still insignificant nitrifying activity was observed.

A dramatically decreased capacity was obtained in both reactors when the second mainstream phase was started. The capacity decline seemed to be directly correlated to the COD presence in the influent, which was believed to result in the development of the heterotrophic bacteria and diffusion limitation.

A difference in the condition obtained when operating on reject during the first and the second mainstream phase was detected. Different values of FA and FNA were obtained during reject phase when switching to the second phase. The difference in FA and FNA was believed to be correlated to drastic changes in bacterial activity during the second phase. Difficulties in achieving the same inhibition conditions during the second phase as the first phase are to be expected.

The batch activity trials performed assumed to be a good method in order to obtain the capacity of the different bacteria species during the first phase. On the other hand, activity trials were considered to be less reflecting the real capacity of the bacteria during the second phase which designated a need of developing suitable activity batch trials during the second phase.

6 Future Work

Little has been done and there is absolutely more to do. Some of the interesting areas that still need to be addressed are mentioned below.

The importance of optimizing the filtration step is obvious after performing this study due to the sensitivity of the nitrification step. It is also crucial for the future of the process combination. If the filtration step is not able to remove more SCOD than observed in these few trials, this step is needed to be combined and/or replaced with other processes/methods in order to achieve a higher SCOD reduction and in turn a successful nitrification step.

Operating on reject should be further studied. It is important to study the long-term effect of the reject inhibition on NOB. The adaptation ability of the bacteria to new conditions is a known phenomenon. Therefore, it is critical to study the long-term effect of this inhibition type. A long-term study is also needed in order to investigate the ability of AOB adaptation to HB development.

More understanding is needed in order to develop an activity test in which the real capacity is determined. Performing activity trials with higher DO concentration (limiting diffusion boundary) and measuring nitrogen gas emissions can be some options for the development of the activity trials.

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8 Appendices

Appendix I: Calculation of the oxygen consumption.

Appendix II: Different options for nitrogen removal treatment.

Appendix III: Experimental data for the filtration trials.

Appendix IV: Experimental data for the continuous operation.

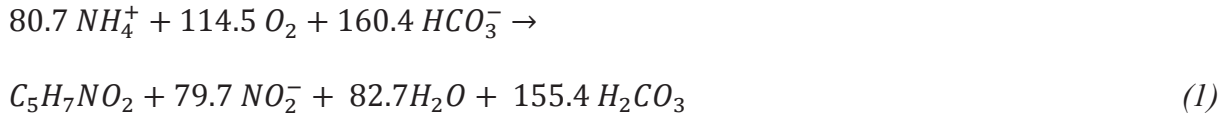
Appendix V: Microscopic images for R2

Appendix VI: Popular science summary (Swedish)

Appendix I

The oxygen consumption in theory

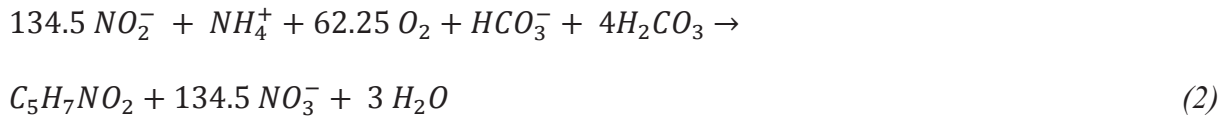
1. Step 1



Oxygen consumption step 1

$$\text{Oxygen}_{\text{consumed-step 1}} = \frac{114.5 \text{ mole O}_2 \cdot 32 \text{ g O}_2/\text{mole O}_2}{80.7 \text{ mole NH}_4^+ \cdot 14 \text{ g N/mole NH}_4^+} = 3.24 \text{ g O}_2/\text{g N}$$

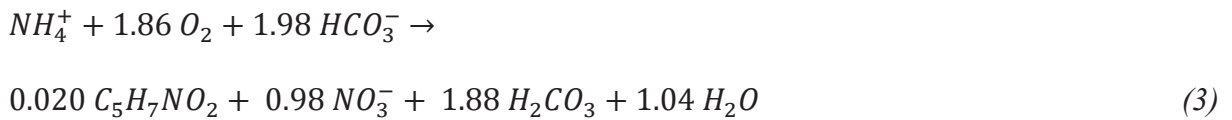
2. Step 2



Oxygen consumption step 2

$$\text{Oxygen}_{\text{consumed-step 2}} = \frac{62.25 \text{ mole O}_2 \cdot 32 \text{ g O}_2/\text{mole O}_2}{134.5 \text{ mole NO}_2^- \cdot 14 \text{ g N/mole NO}_2^-} = 1.062 \text{ g O}_2/\text{g N}$$

Total



Oxygen consumption nitrification

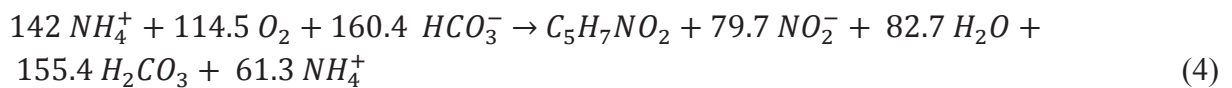
$$\text{Oxygen}_{\text{consumed-total}} = \frac{1.86 \text{ mole O}_2 \cdot 32 \text{ g O}_2/\text{mole O}_2}{1 \text{ mole NH}_4^+ \cdot 14 \text{ g N/mole NH}_4^+} = 4.30 \text{ g O}_2/\text{g N}$$

Alternative

$$\text{Oxygen}_{\text{consumed-total}} = \text{Oxygen}_{\text{consumed-step 1}} + \text{Oxygen}_{\text{consumed-step 2}}$$

$$= 3.24 \text{ g O}_2/\text{g N} + 1.062 \text{ g O}_2/\text{g N} = 4.30 \text{ g O}_2/\text{g N}$$

Partial nitrification (1 · NH₄⁺:1.3 · NO₂⁻)



$$\begin{aligned}
 \text{Oxygen}_{\text{consumed-PN (56.5\% conversion)}} &= \frac{114.5 \text{ mole } O_2 \cdot 32 \text{ g } O_2/\text{mole } O_2}{142 \text{ mole } NH_4^+ \cdot 14 \text{ g } N/\text{mole } NH_4^+} \\
 &= 1.84 \text{ g } O_2/\text{g } N
 \end{aligned}$$

Oxygen saved comparing to the total nitrification

$$\frac{\text{Oxygen}_{\text{consumed-total}} - \text{Oxygen}_{\text{consumed-PN (56.5\% conversion)}}}{\text{Oxygen}_{\text{consumed-total}}} = \frac{4.30 - 1.84}{4.30} = 0.57$$

Appendix II

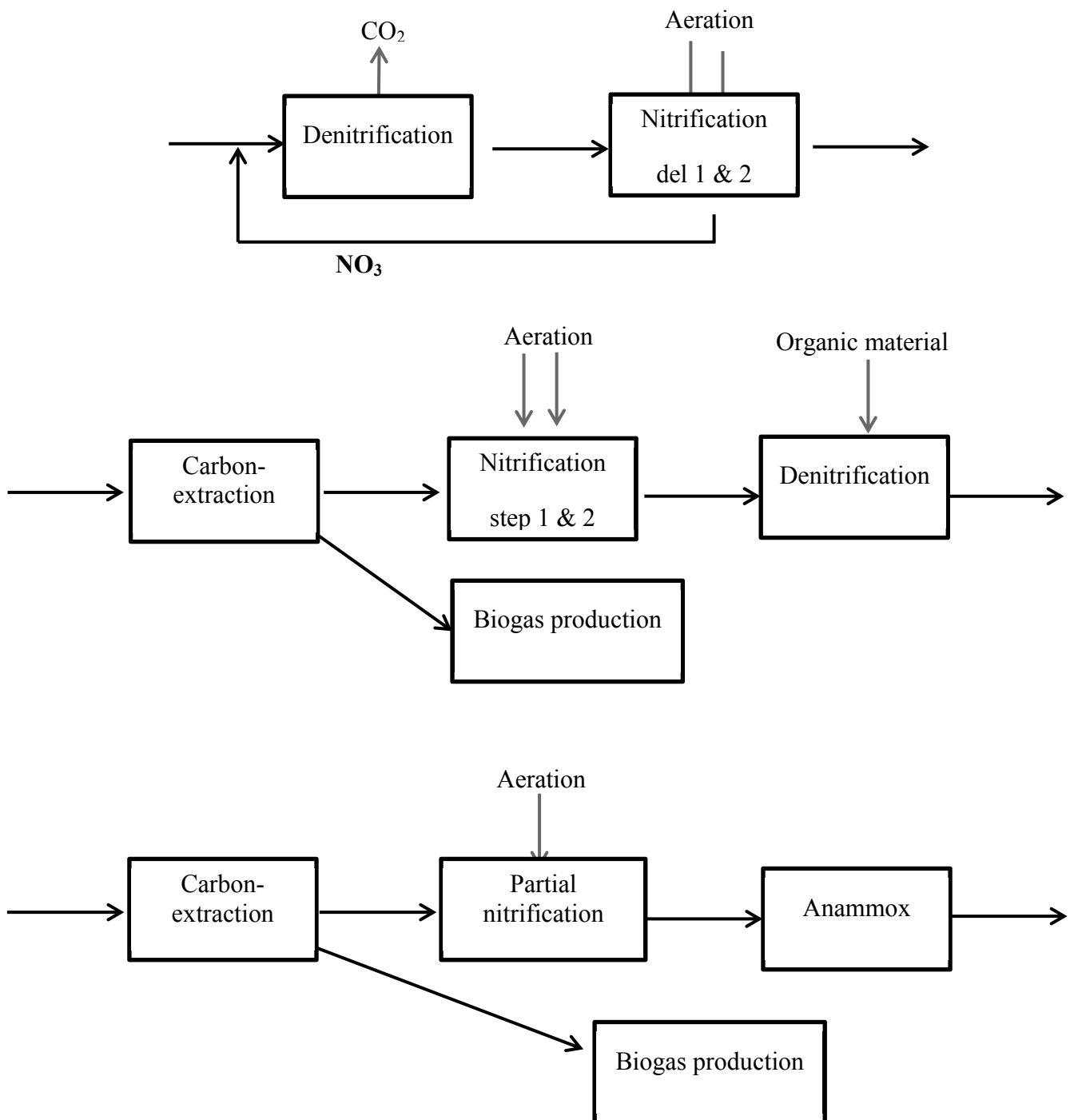


Figure II.1 Different process options for nitrogen removal treatment

Appendix III

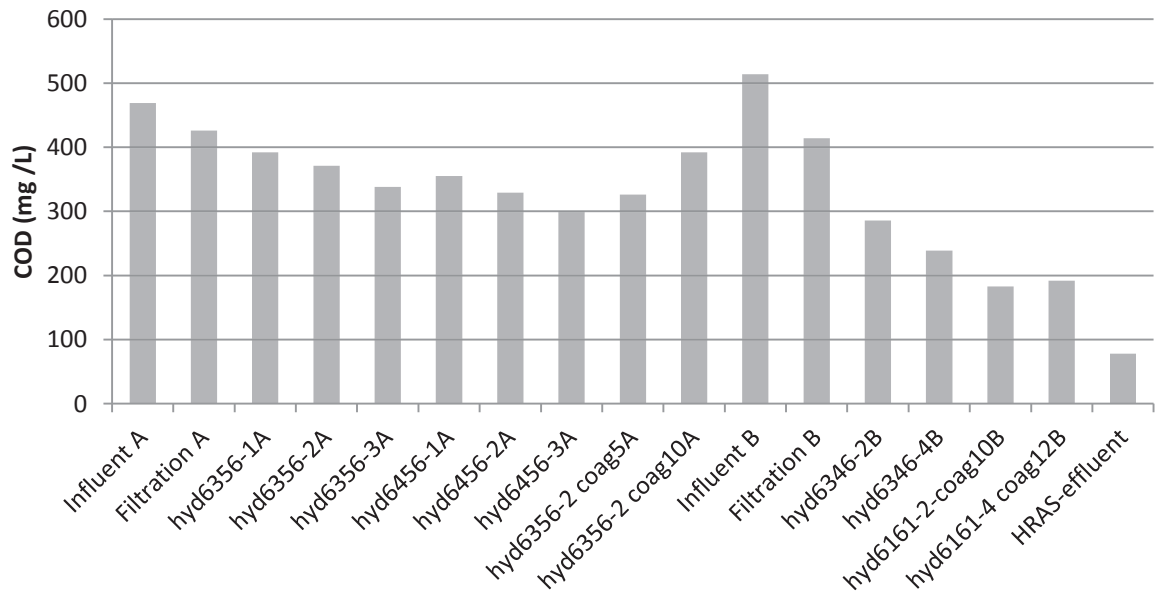


Figure III.1. The different COD values in mg/L obtained after performing the different filtration trials.

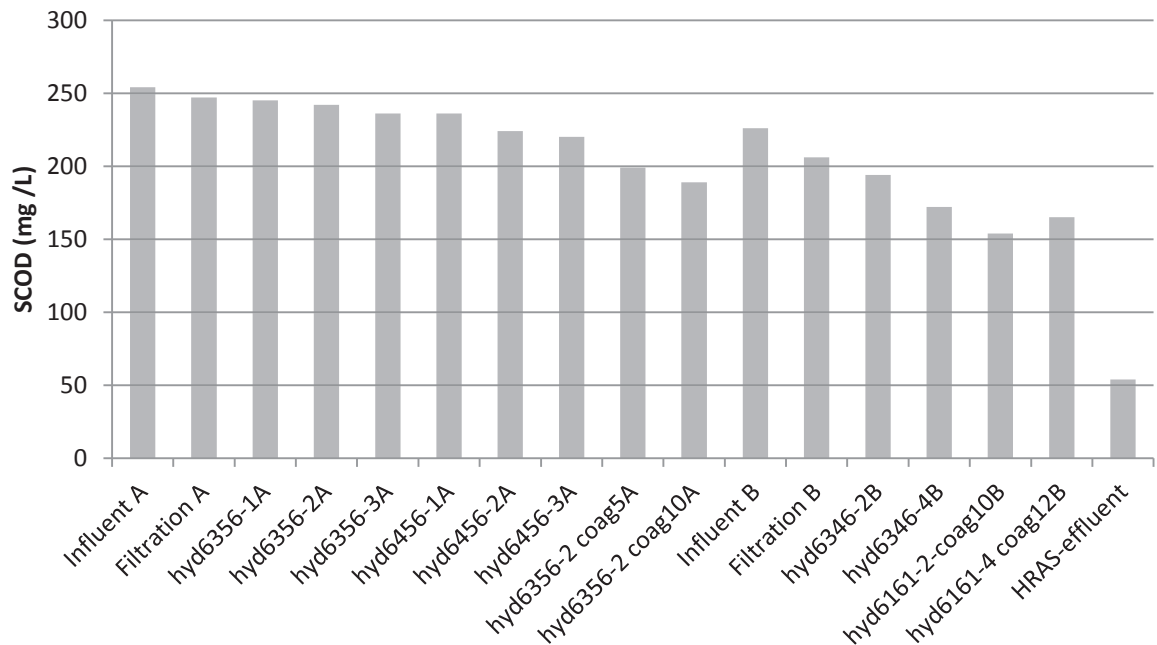


Figure III.2. The different SCOD values in mg/L obtained after performing the different filtration trials.

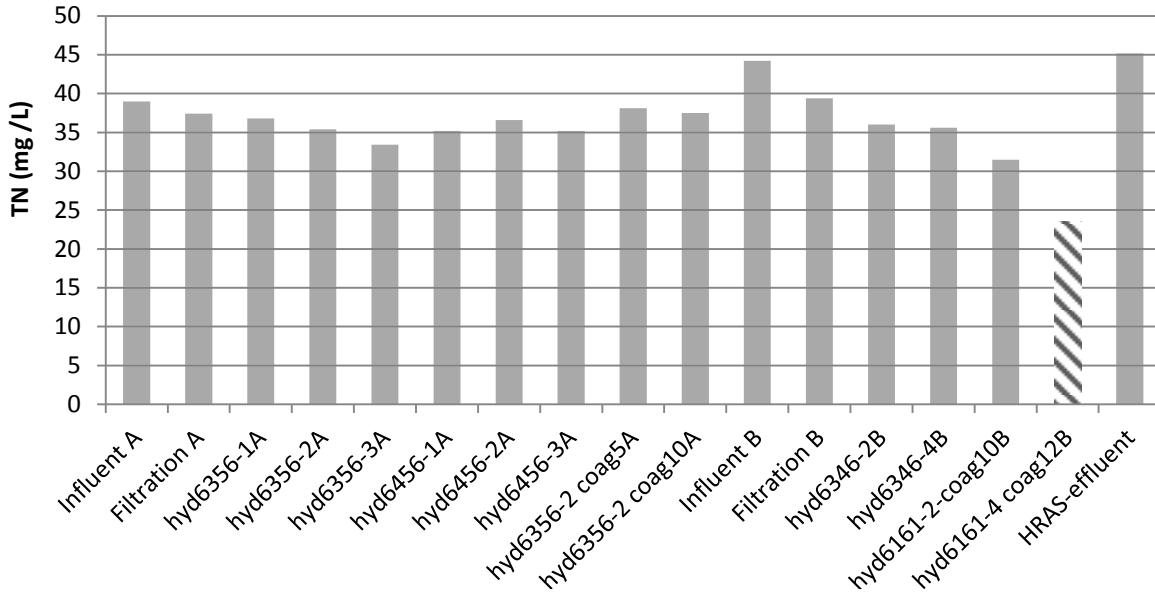


Figure III.3 The different TN values in mg/L obtained after performing the different filtration trials.

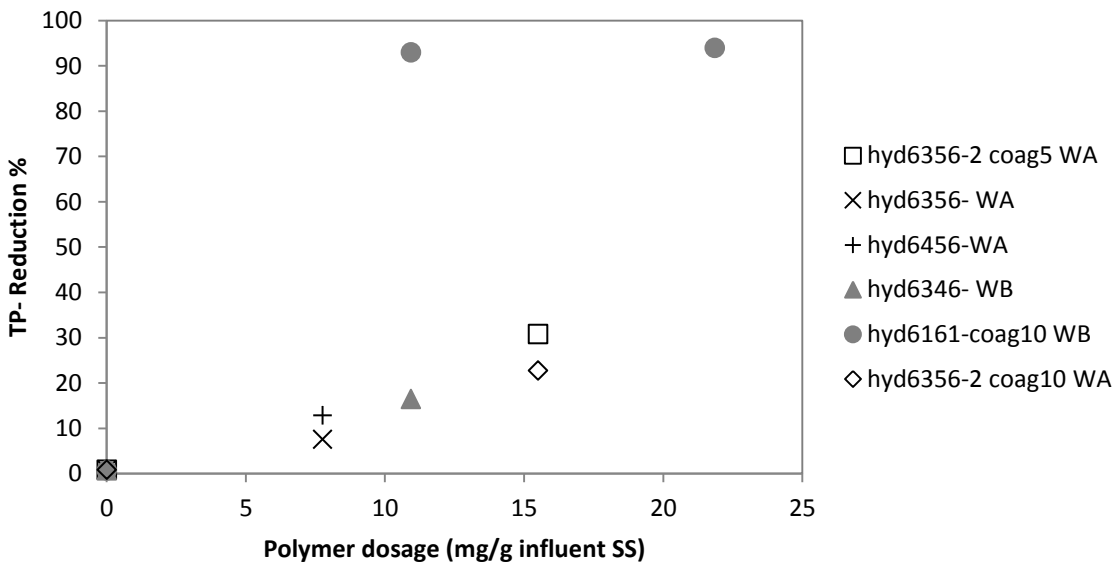


Figure III.4 The reduction of total phosphorus in % presented in a relationship to the polymer dosage in (mg/g influent SS).

Appendix IV

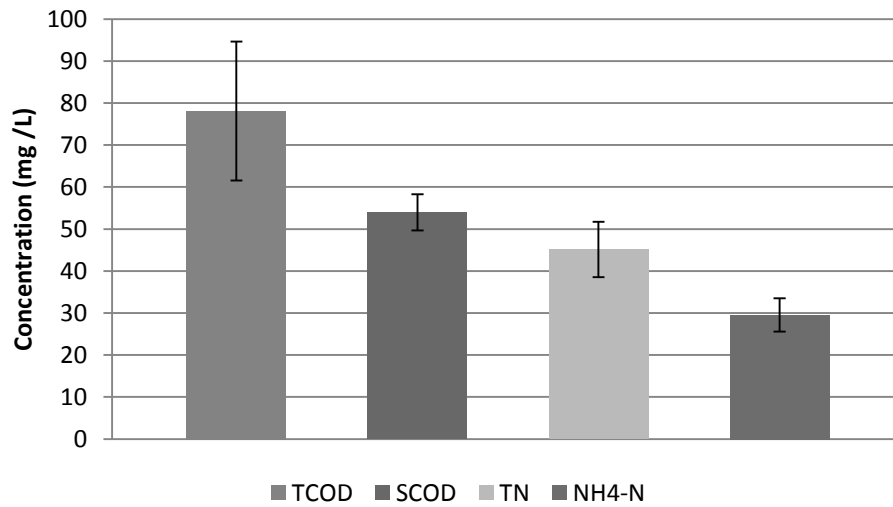


Figure IV.1 The average of the different batches of HRAS effluent used in the study.

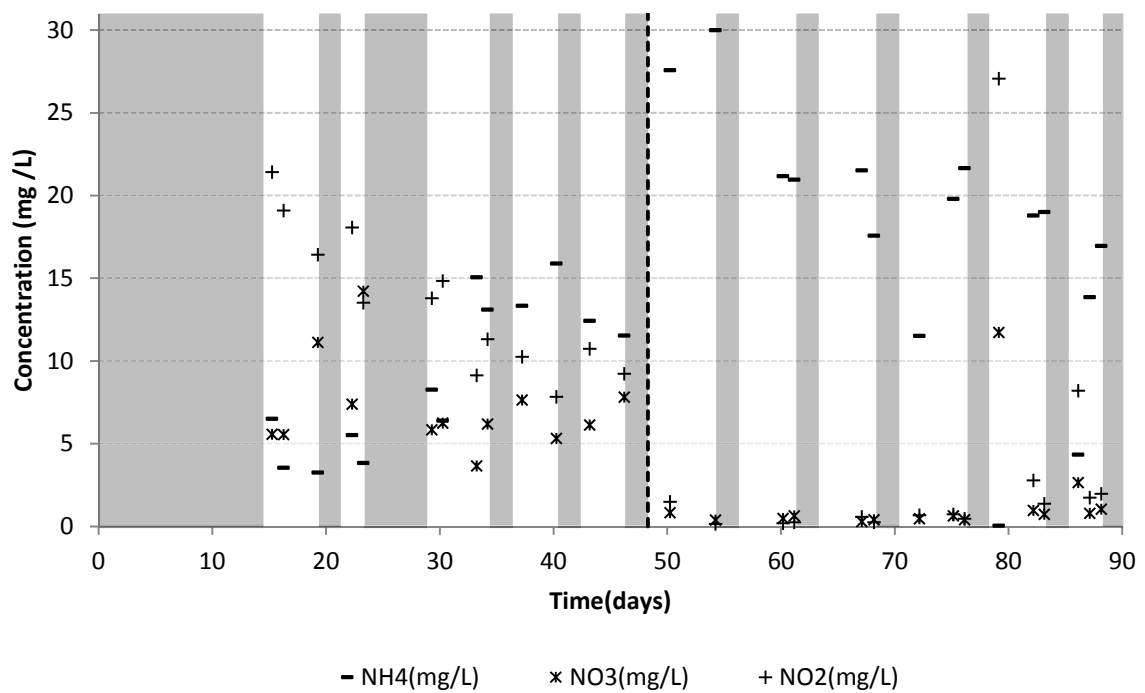


Figure IV.2 Concentrations of NH₄-N, NO₃-N and NO₂-N in R1. The marked areas in the figure illustrate RP operations. The dashed line illustrates the time when switching to MPB occurred.

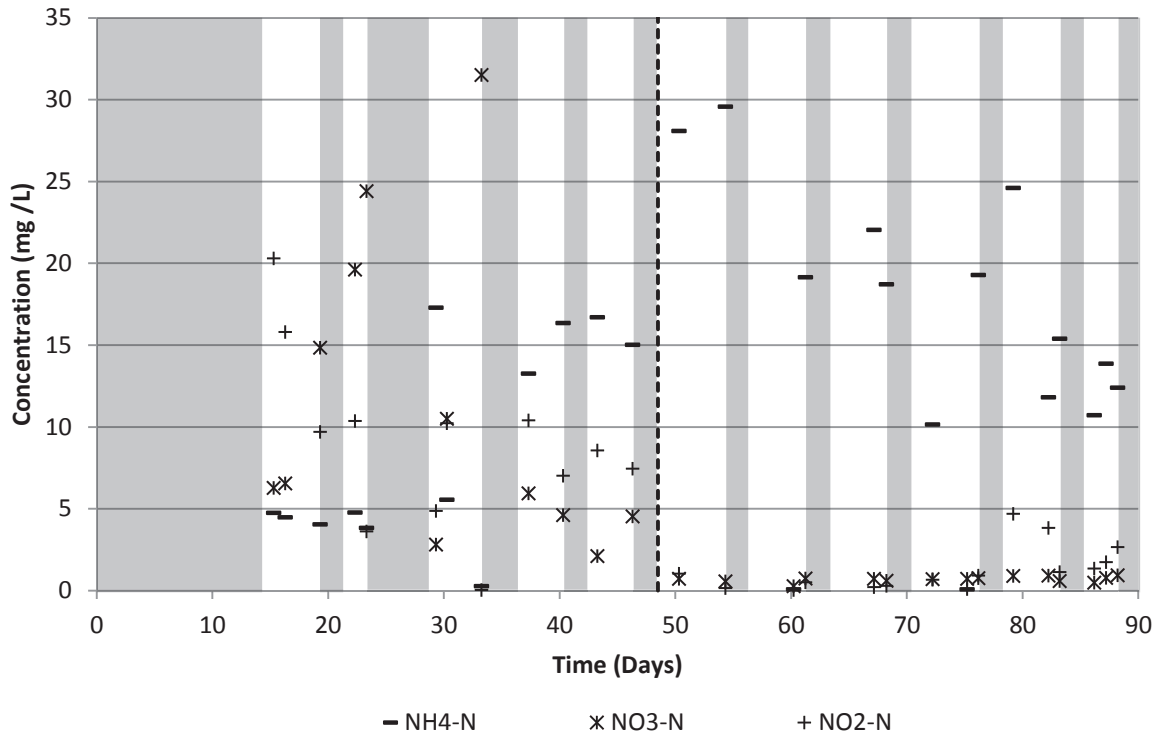


Figure IV.3 Concentrations of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ in R2. The marked areas in the figure illustrate RP operations. The dashed line illustrates the time when switching to MPB occurred.

Appendix V

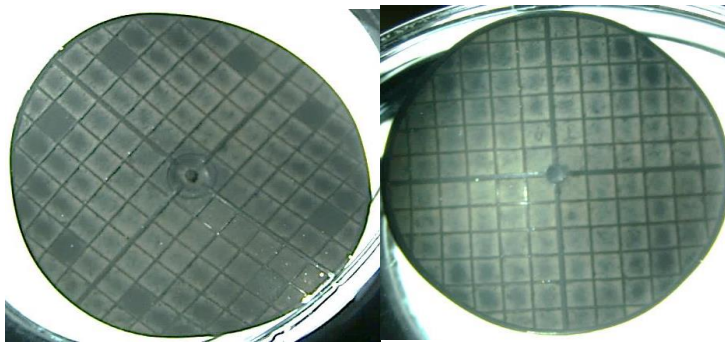


Figure V.1 Microscopic images of the carriers in R2 during the MPA. The date of the picture is 22/12/2016 (operation day 49).

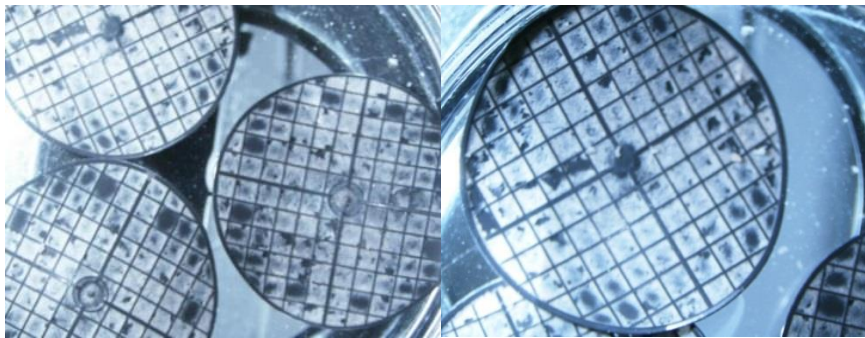


Figure V.2 Microscopic images of the carriers in R2 during the MPB. The date of the picture is 17/01/2017 (operation day 76).

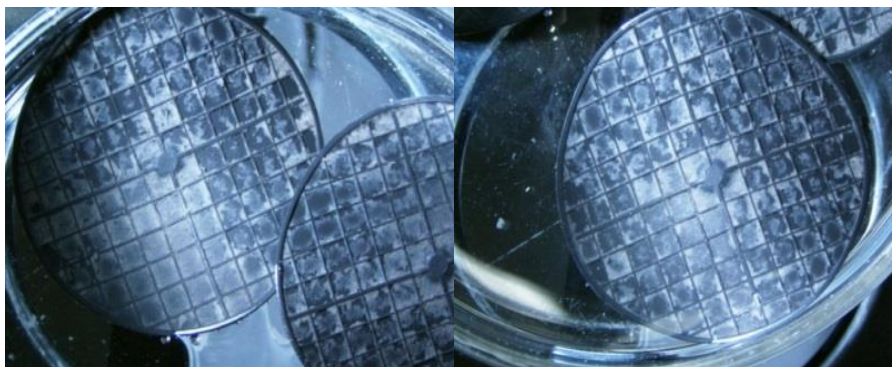


Figure V.3 Microscopic images of the carriers in R2 after the RP during MPB. The date of the picture is 19/01/2017 (operation day 78).

Appendix VI

Bakterier, våra små hjälpredor!

Den här texten är en populärvetenskaplig sammanfattning av ett examensarbete som utfördes på VA-teknik vid Institutionen för kemiteknik och Veolia Water Technologies AB.

Doaa EL Halabi
Mars 2017

Förorenat vatten såsom avloppsvatten kan orsaka stora skador på miljön. Därför finns det ett behov av att behandla avloppsvatten för att bli av med ämnen som organiskt material, kväve och fosfor). Problem med avloppsvatten har observerats under lång tid, och reningsverk har med tiden blivit mer avancerade i takt med att olika processkombinationer utvecklats och testats.

Vattenrening kan ske med olika metoder. En av dessa metoder är den biologisk rening, där bakterier används för att rena vattnet. Vattenrening kan även ske genom kemiska (kemikalier tillsatser) och mekaniska metoder (till exempel filtrering). Dessa metoder kan även kombineras för utveckling av en mer lämplig och energieffektiv vattenreningsprocess.

Avloppsvatten innehåller organiskt material, kväve och fosfor som bakterierna kan använda sig av för att kunna leva och föröka sig. Det organiska materialet kan med fördel användas för biogasproduktion, och därför har studier fokuserat på att utveckla processer för vattenrening där mindre organiskt material behövs. I det här examensarbetet studerades en processkombination för vattenrening där inget organiskt material behövs. Processkombinationen bygger på ett filtreringssteg som kombineras med kemikalietillsats för att maximera reduktionen av det organiska materialet. Det avskilda organiska materialet kan användas för produktion av biogas. Därefter kompletteras reningen med en biologisk process där inget organiskt material behövs.

Målet var att utreda effekten av filtreringssteget som skulle föregå den biologiska processen. Utvärdering av filtreringssteget i labbskala visade en låg reduktion av det lösliga organiska materialet, därför var det viktigt att testa effekten av det lösliga organiska materialets tillgänglighet på den biologiska processen som följer filtreringsteget.

Resultaten visar att tillgängligheten av organiskt materialet är avgörande för processkombinationen. En ökad tillgänglighet på det organiska materialet resulterade i utveckling av snabbväxande mikroorganismer. Detta visar att olika tillgänglighetsgrader av organiskt material har en stor effekt på variationen av mikroorganismers tillväxt, vilket leder till svårigheter i processtyrningen. Dessutom kunde ett spännande fenomen observeras, nämligen att bakterier har förmågan att äta varandra för att överleva. Bakterierna renar naturligtvis inte avloppsvatten för att de är snälla och vill hjälpa till, utan reningsprocessen styrs av deras fundamentala behov.