WP2 – Nitrogen removal in mainstream

D 2.3: Process description for maintaining stable nitrogen removal using nitritation + anammox with MBBRs in mainstream water

The project "Full scale demonstration of energy positive sewage treatment plant concepts towards market penetration" (POWERSTEP) has received funding under the European Union HORIZON 2020 – Innovation Actions - Grant agreement° 641661

Deliverable 2.3	Process description for maintaining stable nitrogen removal using nitritation + anammox with MBBRs in mainstream water
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Abstract	Process description for maintaining stable nitrogen removal using nitritation + anammox with MBBRs in mainstream water. Assessment of performance in large-scale prototype trials including process stability at low temperature and measures to tackle loss of anammox biomass.

Dissemination level of this document

X	PU	Public
	PP	Restricted to other programme participants (including the Commission Services)
	RE	Restricted to a group specified by the consortium (including the European Commission Services)
	CO	Confidential, only for members of the consortium (including the European Commission Services)

Versioning and Contribution History

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Glossary

AOB	Ammonium Oxidizing Bacteria
AMX-stage	Anammox-stage
AnAOB	Anaerobic Ammonium Oxidizing bacteria (anammox)
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
MBBR	Moving Bed Biofilm Reactor
IFAS	Integrated Fixed Film Activated Sludge
NOB	Nitrite Oxidizing bacteria
N-stage	Nitritation-stage
OCT	Optical Coherence Tomography
RAS	Returned Activated Sludge
STAR	Superior Tuning and Reporting

Executive summary

POWERSTEP aims to demonstrate energy-positive wastewater treatment, which requires the utilization of the internal carbon in the wastewater to produce biogas. An increased carbon extraction for biogas production challenges conventional nitrogen removal, in which denitrifying bacteria depend on an easily accessible source of carbon. Hence, POWERSTEP focuses on novel concepts for nitrogen removal in the mainstream line, with a minimum requirement of carbon. One of these concepts is the mainstream deammonification process, in which autotrophic bacteria converts ammonia to nitrogen gas, resulting in low energy demand for aeration, lower sludge production and no need for carbon source.

This deliverable describes a technology for achieving stable nitrogen removal using the Moving Bed Biofilm Reactor (MBBR) technology, in which the biology was controlled by maintaining a thin biofilm thickness on the MBBR carriers. Two different approaches for mainstream deammonification were tested within the project. In the first phase, a two-stage nitritation + anammox concept treating mainstream and sidestream water in an alternating mode was conducted. In the second phase, the two-stage configuration was converted to a one-stage IFAS MBBR configuration, consisting of a regular one-stage MBBR with the addition of an external settler allowing sludge retention.

The main focus of this report is on the two-stage nitritation + anammox system, were the feasibility of the process was demonstrated in two 50 m^3 reactors located at Sjölunda WWTP (Malmö, Sweden). Both reactors were operated on reject water during start up, in order to boost ammonium oxidizing bacteria (AOB) and anammox growth, respectively. In order to prevent establishment of nitrite oxidizing bacteria (NOB), the N-stage was equipped with Anox K™Z-200 carriers, which limit the biofilm growth to a maximum of 200 µm, and the system was temporarily exposed to reject water. An on-line control system (STAR Utility Solutions™) was applied to secure stable operation.

The main focus of operation was on ensuring sufficient NOB suppression in the N-stage. Several strategies based on altering the reject exposure were implemented. The different alterations in reject exposure often resulted in a temporary suppression of NOB, although AOB were sometimes also negatively affected. However, two major drops in NOB activities were observed, most likely a result of a prolonged reject operation frequently exposing the biofilm in the nitritation reactor to pH ranging between 5.5 and 8.3. Although this study demonstrated that NOB can be suppressed in the mainstream, the availability of reject water may be limiting for the process.

In the second phase of the project, the two-stage nitritation + anammox configuration was reconstructed to a one-stage IFAS MBBR were the process consisted of one 50 m^3 MBBR (previous anammox reactor in the two-stage configuration) connected to a 50 m^3 settler for sludge retention. The reactor was operated with reject water feed in order to promote anammox growth for 217 days before the mainstream operation was initiated. No periodic reject water exposure was applied on the one-stage IFAS MBBR after starting the mainstream operation. Currently, a removal rate of 0.04 $kgNH_4\text{-}N/(m^3,d)$ has been achieved at 13°C and no NOB have been observed which is promising for further evaluation. However, the one-stage IFAS MBBR operation on mainstream water is still in an early phase and data is limited. Hence, any conclusions are still preliminary.

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1. Introduction

1.1. The energy positive wastewater treatment plant

Don't underestimate the power of wastewater. This sentence summarises the objective of the EU co-funded project POWERSTEP – a project led by research and industry players working to convert sewage treatment plants into power production facilities while still achieving high quality water treatment. In Europe, the municipal wastewater sector currently consumes the annual power generated by two large power plants. Concurrently, organic matter contained in municipal wastewater accounts for 12 times as much chemical energy potential. In conventional wastewater treatment, the majority of this organic matter, or carbon source, is generally being oxidized in aerobic biological treatment and/or used for nitrogen removal through denitrification. To achieve energy-positive wastewater treatment plants (WWTPs), however, this carbon source should instead be utilized to produce biogas.

An increased carbon extraction for biogas production typically challenges nitrogen removal in conventional wastewater treatment plants, given the dependence of denitrifying bacteria on an easily accessible source of carbon. Thus, POWERSTEP focuses on different concepts to overcome this barrier and guarantee extensive nitrogen removal with a minimum of carbon. One of the concepts tested is the deammonification process, in which autotrophic bacteria converts ammonia to nitrogen gas, resulting in low energy demand for aeration, lower sludge production and no need for carbon source.

1.2. Traditional nitrogen removal

Traditional nitrogen removal typically consists of two steps: aerobic oxidation of ammonia to nitrate (i.e. nitrification) and the anoxic conversion of nitrate to nitrogen gas by heterotrophic bacteria (i.e. denitrification). Nitrification is performed in two steps, by two groups of autotrophic bacteria. The first step is the oxidation of ammonia to nitrite performed by a group of ammonium oxidizing bacteria (AOB) (Equation 1). The best known AOB belong to the genera *Nitrosomonas* (Sliekers et al., 2002). However, *Nitrospira, Nitrosococcus,* and *Nitrosolobus* are also capable to convert ammonia to nitrite (Ahn, 2006).

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

The second step in nitrification is the conversion of nitrite to nitrate, performed by a group of nitrite oxidizing bacteria (NOB) (Equation 2). The main NOB in biological wastewater treatment belong to *Nitrobacter* and *Nitrospira* (Sliekers et al., 2002).

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \quad (2)$$

Denitrification can be performed by many different kinds of heterotrophic bacteria, using nitrate or nitrite as an electron acceptor in the absence of oxygen, and requires the availability of carbon (Equation 3). In wastewater treatment the carbon used for denitrification can either be sourced from the wastewater or added as an external carbon source, typically in the form of methanol, ethanol, acetate or glycerine.

$$2NO_3^- + H^+ + organic\ matter \rightarrow N_2 + HCO_3^- \quad (3)$$

1.3. Anammox, nitritation and the MBBR

The deammonification process relies on specialised autotrophic bacteria with anaerobic ammonium oxidation (AnAOB), also called anammox. Ammonium and nitrite are reduced under anoxic conditions without any requirements of organic carbon (Equation 4) and with up to 60% savings of oxygen compared to traditional nitrification-denitrification (Wett et al., 2013).

$$\mathrm{NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.26CH_2O_{0.5}N_{0.15} + 2.03H_2O} \quad (4)$$

The reaction for simultaneous performance of AOB and AnAOB is shown in Equation 5.

$$\mathrm{NH_4^+} + 0.804O_2 + 0.071HCO_3^- \rightarrow \mathrm{0.436N_2 + 0.11NO_3^-} + 0.009C_5\mathrm{H_7}O_2\mathrm{N + 0.028CH_2O_{0.5}N_{0.15}} + \mathrm{0.038H^+ + 1.46H_2O} \quad (5)$$

Since AnAOB use nitrite, it is essential that the deammonification process only contains AOB and AnAOB, and prevents the establishment of NOB. Another key challenge in the deammonification process is the slow growth of anammox bacteria. Hence, the first successful implementations of deamonification for wastewater treatment were done in high-strength sidestream wastewater from dewatering of sludge liquor (i.e. reject water), where high temperature, high ammonia concentrations and low carbon content secure favourable conditions for anammox growth. Today, a wide range of technologies for sidestream treatment with anammox can be found based on both suspended sludge, granules and fixed film, with more than 100 installations completed worldwide by 2014 (Lackner et al., 2014). However, only a minor part of the total nitrogen to be treated is found in the sidestream line, and the great benefit is not obtained until deammonification is applied in the mainstream line with maximised carbon extraction upstream.

Major challenges are still to be overcome for mainstream application of deammonification: (i) low temperature, (ii) low substrate concentration, (iii) high COD/N ratio, (iv) ability to retain anammox in the system, (v) efficient nitrite oxidizing bacteria (NOB) washout and (vi) final effluent quality (Xu et al., 2015). Out of all these challenges, an efficient NOB washout strategy and a robust and easy way to retain anammox in the system are seen as the most challenging ones (De Clippeleir et al., 2013; Gustavsson et al., 2012; Wett et al., 2013).

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The Moving Bed Biofilm Reactor (MBBR) process, where bacteria are growing as a biofilm on suspended carriers, offers the high flexibility required to meet these challenges. In the MBBR, slow growing bacteria will safely be retained in the reactor also at low temperatures and at high hydraulic flows, and the MBBR can be staged in series to promote enrichment of different groups of bacteria dependent on reactor conditions and substrate availability. But although the MBBR is ideal for retaining slow growing bacteria, NOB bacteria can still thrive at mainstream conditions, and out-compete the anammox for nitrite. Hence, careful operation strategies are required to ensure stable mainstream deammonification.

1.4. Mainstream deammonification

Deammonification for treatment of reject water in a one-stage MBBR (Christensson et al., 2013) (see Figure 1) is a state of the art technology and full scale processes have been installed worldwide (Lackner et al., 2014). Although one-stage MBBRs have been used to achieve deammonification under mainstream conditions (Gilbert et al., 2014; Gustavsson et al., 2014; Lemaire et al., 2014), the challenges of inhibiting NOB remains. In order to prevent NOB establishment and avoid oxygen inhibition of AnAOB, one-stage deamonification MBBRs must generally operate at low dissolved oxygen (DO) concentrations, which limits the activity of AOB. Since the AOB supplies nitrite to the AnAOB, a limited AOB activity results in limited nitrite availability for AnAOB, which slows down the overall removal rate of the process. In addition, if established in the biofilm, NOB can be difficult to inhibit without damaging the AnAOB population when grown in the same biofilm.

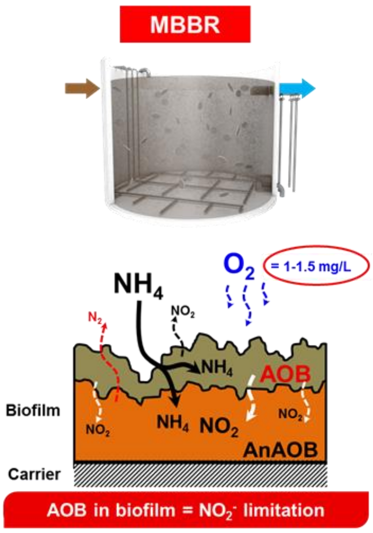

Figure 1: The concept of one-stage deammonification in the MBBR configuration

Interesting approaches to avoid NOB establishment in mainstream deammonification are the two-stage MBBR (Piculell et al., 2016a) (Figure 2) and the one-stage Integrated Fixed film Activated Sludge (IFAS) MBBR (Veuillet et al., 2014) (Figure 3) configurations, in which the AOB and AnAOB biomass are grown in separate biomass fractions.

In the first phase of the POWERSTEP project, the novel approach to mainstream deamonification with a two-stage MBBR configuration (Piculell et al., 2016a) was demonstrated in large-scale. In this two-stage configuration, the first reactor was aerated at high DO to achieve efficient nitritation (N-stage), followed by an anoxic, mechanically mixed anammox reactor (AMX-stage) (Figure 2). In order to ensure NOB suppression in the aerated stage, the biofilm thickness was maintained below 200 µm by using an engineered biofilm carrier, specifically developed for biofilm thickness control (Piculell et al., 2016). In addition, the feed to the N-stage was periodically switched from low-strength, low-temperature mainstream wastewater to reject water at high temperatures and concentrations. This sudden exposure to high substrate concentrations and temperatures was expected to inhibit NOB growth in the thin biofilm, and possibly also boost AOB activity (Piculell et al., 2016b). This concept had been shown feasible in achieving stable nitritation at mainstream conditions in both lab- and pilot-scale (Carlsson et al., 2016; Piculell et al., 2016a), but evaluation of full-scale implementation at real wastewater conditions and ambient temperatures remained to be performed.

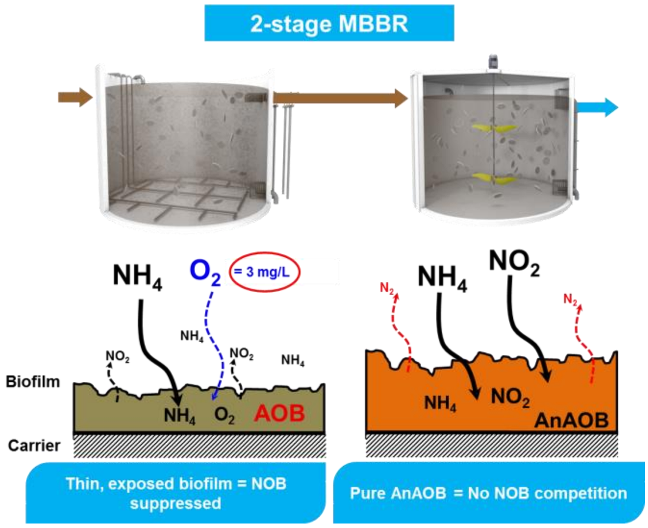

Figure 2: The concept of two-stage deammonification using MBBRs and biofilm control

In the second phase of the POWERSTEP project, a one-stage IFAS MBBR process for mainstream deammonification was evaluated. The IFAS process consists of a regular one-stage MBBR with the addition of an external settler allowing sludge retention (Figure 3). With this configuration, AnAOB preferentially grow in the biofilm while the aerobic AOB (and NOB) tend to grow in the suspended sludge. This robust physical separation between AnAOB-rich biofilm carriers and AOB-rich suspended sludge allows for control of the sludge age in the system and therefore selective wash-out of NOB while retaining anammox. The concept has been studied for mainstream treatment in pilot scale at real wastewater conditions with promising results (Lemaire et al., 2016), but remained to be validated in larger scale.

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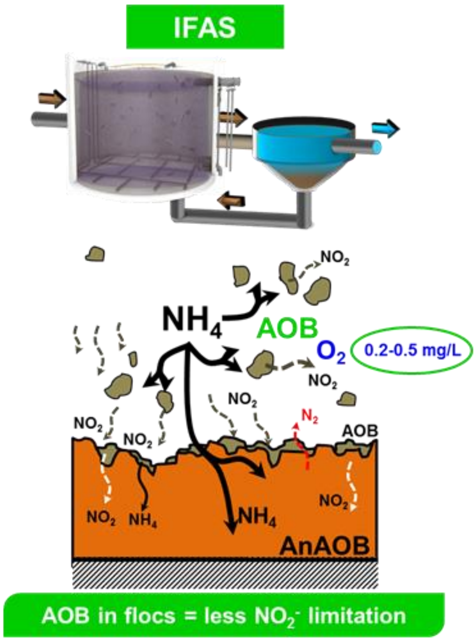

Figure 3: The concept of one-stage deammonification in IFAS MBBR configuration

2. Method

2.1. Overview

The overall strategy for the mainstream deammonification plant in the POWERSTEP project is to demonstrate the viability of the concept in full scale. Two different types of primary treatment were tested (Figure 4). Initially, the primary treatment consisted of a primary settler followed by a high rate activated sludge (HRAS) reactor, from which a low COD effluent was fed to the mainstream deammonification. Later in the project, the primary treatment was switched to a Hydrotech drumfilter, in which the COD could be removed by the combination of polymer, flocculation and fine mesh screening (connected to WP1 – for a more detailed description see deliverable *1.2 Design and performance of advanced primary treatment with microscreen)*.

When operating on the HRAS effluent, the two-stage MBBR configuration was applied, while for the second phase, the two-stage configuration was converted to a one-stage IFAS MBBR, due to the higher amount of soluble COD in the feed from the filtration unit, as compared to the HRAS effluent. During the second phase, different operation strategies of the filtration unit are currently tested, hence it is possible to test the effect of a varying influent COD/N ratio on the IFAS configuration.

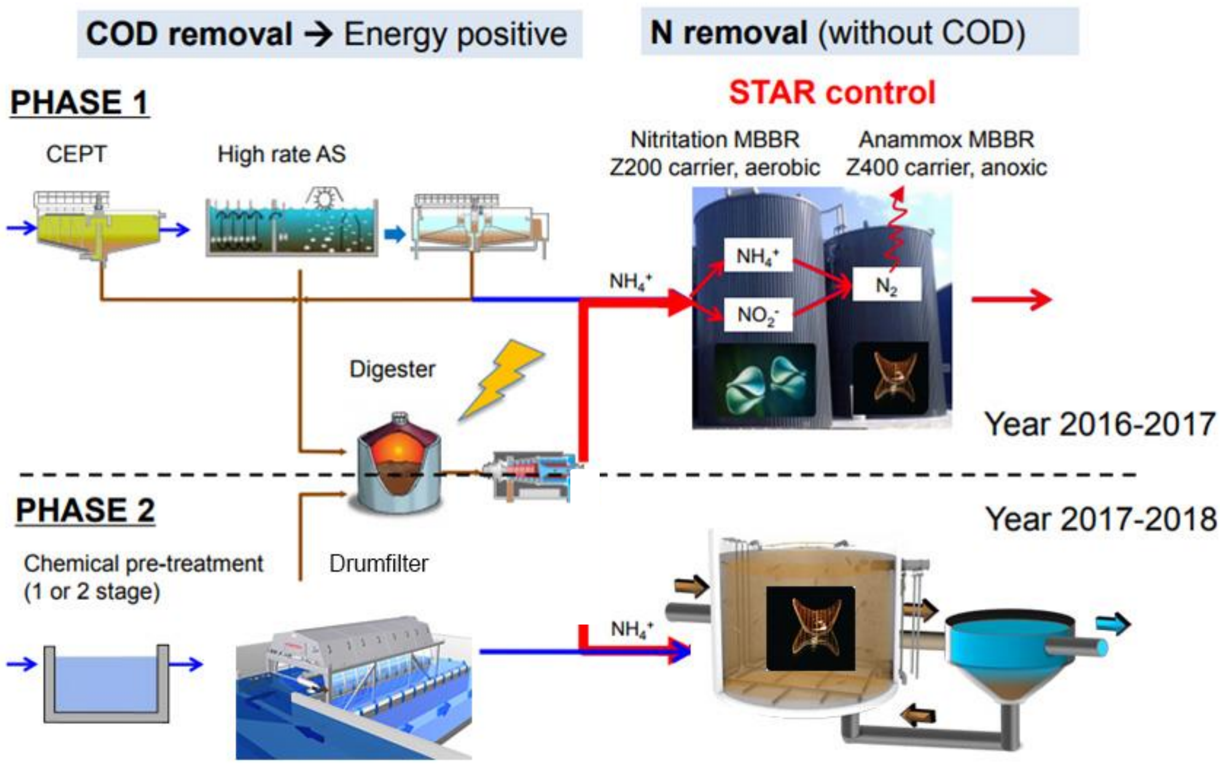

Figure 4: The two phases for the N removal using the HRAS effluent and two-stage nitritation + anammox configuration in phase 1 and Hydrotech Drumfilter effluent and one-stage IFAS configuration in phase 2.

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2.2. Case study and reactor configuration

The demonstration facility is located at Sjölunda wastewater treatment plant (300 000 p.e.) in Malmö, Sweden, which is treating the wastewater from Malmö municipality and its surroundings. Currently the plant consists of an inlet screen, grit chamber, primary settler, HRAS with sedimentation for COD removal, trickling filters for nitrification and MBBR for denitrification followed by dissolved air flotation, before the water is discharged into Öresund. The municipality plan to upgrade the plant within the next ten years, in order to increase the capacity while, ideally, improve the energy efficiency of the treatment process. Hence, it is an ideal location for the demonstration of mainstream deammonification. However, the plant also has a very dilute wastewater (average concentration of 27 mg NH_4-N /L in the HRAS effluent in 2015-2017). In combination with low temperatures in winter, the low concentrations in the HRAS effluent impose a major challenge for maintaining stable mainstream deammonification.

The two reactors used for the POWERSTEP project are part of a four-reactor configuration, operated by Veolia Water Technologies AnoxKaldnes. The four reactors are cylindrical with a 10 m^2 footprint and an active volume of 50 m^3 each.

2.2.1. Two-stage nitritation + anammox

In the N-stage, air was supplied through coarse bubble diffusers and a top mounted hyperbolic mechanical mixer (Invent) was installed to ensure carrier mixing at low aeration intensities. The reactor contained the new Anox K™ Z-200 (Figure 5), which controlled the biofilm thickness to maximum 200 µm (Piculell et al., 2016c), at a filling degree of approximately 40%.

The AMX-stage contained Anox K™ Z-400 (Figure 5), which controlled the biofilm thickness to maximum 400 µm, at a filling degree of approximately 40%. The AMX-stage was operated at anoxic conditions and mixing was accomplished with a top mounted hyperbolic mechanical mixer (Invent) at which additional mixing blades were added to achieve sufficient mixing of the carriers. Coarse bubble diffusers were installed in the reactor for air supplement if needed.

The N-stage was operated for 700 days, and the AMX-stage was started on day 135. Initially the reactors were operated individually on reject water to enable fast nitritation and anammox growth. Mainstream operation in the N-stage was initiated on day 250 while the AMX-stage was connected to the N-stage on day 360.

2.2.2. One-stage IFAS MBBR

The 50 m^3 IFAS reactor contained Anox K™ Z-400 at a filling degree of approximately 40% and the reactor was operated with periodical switches between anoxic and aerobic conditions. Air was supplied through coarse bubble diffusers and continuous mixing was accomplished with a mechanical mixer (Invent). The AMX-stage from the two-stage configuration was re-built to a one-stage MBBR on day 572 and the reactor was operated on reject water feed for 217 days in order to promote anammox growth. The reactor was connected to a settler with a volume of 50 m^3 to allow sludge retention on day 758 and mainstream operation was initiated on day 789.

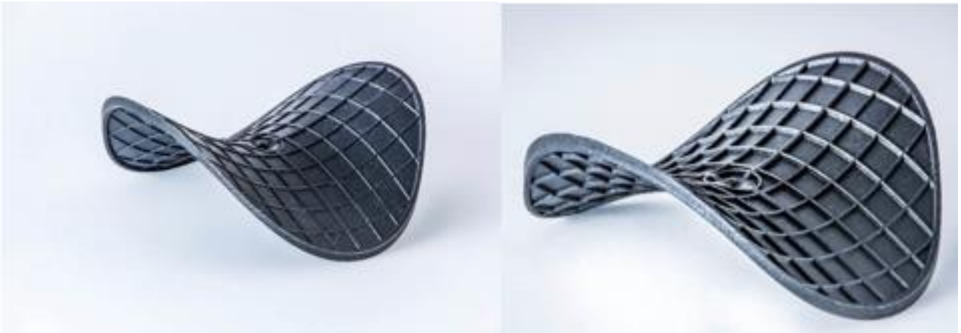

Figure 5: The Anox K^{TM} Z-200 carrier used in the nitritation stage of the plant (left). The Anox K^{TM} Z-400 carrier used in the anammox stage and the IFAS configuration (right).

2.3. Operation strategy

Over the course of the project, several different operational strategies were implemented and tested, with varying focus on performance, effluent quality and NOB suppression. In addition, the operation strategies differed between the two different MBBR configurations tested.

2.3.1. Two-stage nitritation + anammox

The advanced control strategy for the two-stage mainstream nitritation + anammox system was developed in collaboration between Veolia Water Technologies AnoxKaldnes in Sweden and Krüger in Denmark, using the Superior Tuning and Reporting (STAR) control program for online control and monitoring. The STAR system is a state-of-the-art control system allowing the long-term process optimisation of the conventional SCADA and PLC systems. The modular system can be tailored to any wastewater system, and the complexity of the control ranges from radar-based forecast control to simple unit-based PID regulations. In the POWERSTEP project, STAR*pro* platform was implemented, allowing comprehensive solutions and offering good overview of a large number of modules within the same system. Figure 6 shows the layout of the advanced control program.

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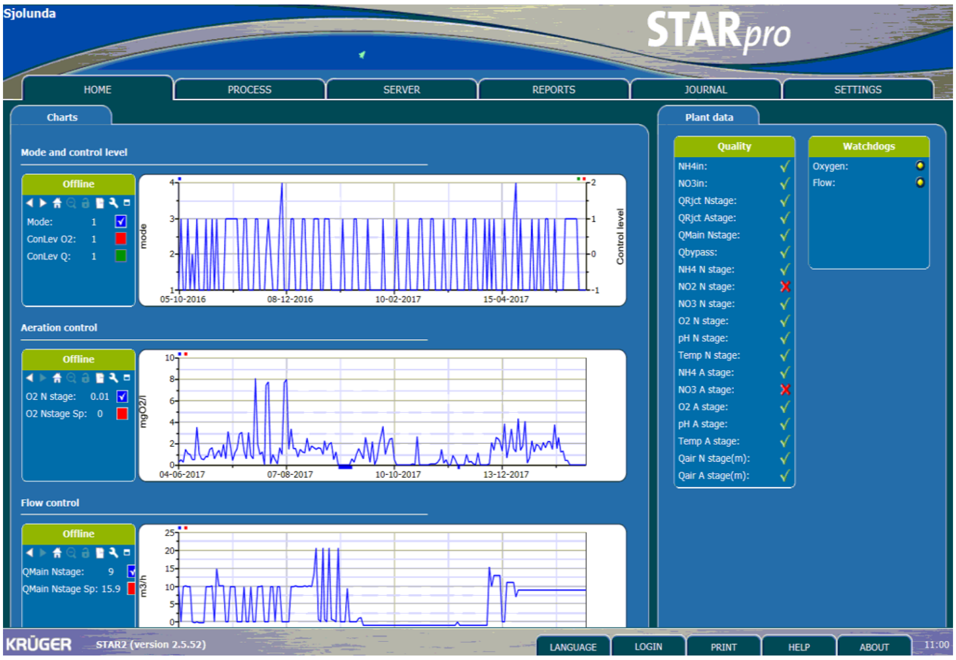

Figure 6. Layout of the STAR control program

The main control strategy for the two-stage configuration was applied on the N-stage, with the focus on supressing the NOB activity and boost AOB growth. The N-stage was intermittently operated with mainstream water and reject water as feed. The different feeding schemes are hereafter referred to as the operation modes, and the operation changed between the modes in a cyclic manner. These modes were 1) operation on mainstream water, 2) switch from mainstream to reject water, 3) operation on reject water, and 4) switching from reject to mainstream water. During operation on reject water, the flow rate and aeration intensity were controlled to reach alternating pH (set points altered through the course of the project). Furthermore, the operation time at the pre-defined pH values were controlled with the STAR program. The AMX-stage was by-passed during reject water periods to avoid inhibition of the anammox bacteria by the strong water coming out from the N-stage.

Additionally, a strategy was implemented in STAR to control effluent quality during mode 1, in the following manner:

1. The set point for the flow of mainstream water to the N-stage was determined by the effluent NH_4-N concentration from the AMX-stage through a piece-wise linear function. The user defined the range in flow and concentrations by setting the function.
2. The set point for the DO in the N-stage was determined by the NO_2-N/NH_4-N ratio in the N-stage through a piece-wise linear function. The user defined the range in DO and ratio by setting the function.
3. The bypass flow over the AMX-stage could be set manually to a fixed value, and was hence not adjusted automatically.
4. The aeration could be set to intermittent, by defining time on/off in the graphical user interface. During 'on time' the calculated DO set point from 2) was used, while the set point was zero during 'off time'.

For complete information about the control strategy, see deliverable 2.2 Process description for maintaining stable nitritation of mainstream water using MBBR at low temperature.

2.3.2. One-stage IFAS MBBR

A new operational strategy was developed for the one-stage IFAS MBBR process, mainly focusing on effluent quality. Differentially from the two-stage configuration, there were no switching between reject water feed and mainstream feed. In the one-stage IFAS MBBR configuration, the system could not be exposed to as intense NOB suppression methods as in the two-stage configuration since that would be detrimental to the anammox. However, to simulate a common ammonium concentration in the mainstream wastewater at European conditions, the mainstream feed was spiked with a small amount of reject water in a mixing chamber, before the feed was pumped to the IFAS MBBR. The flow rate of mainstream and reject water, respectively, was controlled to reach desired ammonium concentration in the influent to the IFAS MBBR. Additionally, the aeration intensity and the load to the IFAS MBBR were controlled. The one-stage IFAS MBBR was operated with intermittent aeration during the whole mainstream period. Howerever, the duration of the aerated and non-aerated phases varied as well as DO SP due to different influent carachteristics and operation of the reactor. The focus was on aerating for a certain amount of time at a DO set point between 1.0-1.5 mgO_2/L under which ammonia was converted to nitrite. Thereafter, during the non-aerated period, a rapid reduction of oxygen conentration was expected and consumption of ammonia and nitrite at anoxic conditions was facilitated. The target suspended solids concentration in the IFAS reactor was 1.5 - 2.5 gTSS/L and around 4 gTSS/L in the returned activated sludge to be above the critical SRT for nitritation. A function for wasting the sludge was available in case these values were exceeded. A new version of the STAR control program, based on the strategy described above, was developed for on-line control and monitoring of the process.

2.4. Analytical methods

2.4.1. Sampling and on-line measurements

Grab samples were taken from the N-stage and AMX-stage (phase 1), the IFAS MBBR (phase 2) and the influent every week day, and 24-h composite samples were taken frequently when operating on mainstream wastewater. The samples were analysed for NH_4-N, NO_2-N and NO_3-N using Hach Lange cuvettes (LCK 304, 342 and 399, respectively), or using a Gallery Plus Automated Photometric Analyzer (Thermo Scientific). Additionally, when operating in IFAS mode, both grab samples and composite samples from the influent, the IFAS reactor, the returned activated sludge (RAS) and the effluent from the settler were taken and analysed for COD and TSS using a Gallery Plus Automated Photometric Analyzer (Thermo Scientific).

In addition to sampling, the daily variation of pH, temperature, NH_4-N and NO_3-N were logged on-line using a ISEMax sensor (CAS40D, Endress + Hauser), while the reactors also contained sensors for logging DO (Hach Lange) and the N-stage even contained

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a sensor for logging NO_2-N (s::can) and N_2O-N (Unisense). Air flow as well as the influent of mainstream and/or reject water was measured using Endress + Hauser flow meters.

In order to monitor N_2O emissions, a gas analyser (Horiba VA3000) was installed to measure the off-gas composition from the IFAS reactor. The reactor was covered and sealed to avoid air leakage, and gas was sampled from a chimney at the top of the reactor. The gas analyser continuously logged the volumetric fraction of O_2, CO_2 and N_2O in the sample gas, which was used together with the measured air flow to calculate the N_2O emissions according to Frey (1989) and Parravicini et al. (2015).

2.4.2. **Batch trials**

In order to determine the maximum AOB, NOB and AnAMX capacity in the nitritation, anammox and later the IFAS reactor, carriers were removed on a regular basis and tested for maximal specific activity in lab-scale batch reactors.

AOB and NOB batch trials were performed in parallel, testing carriers (400 pieces per trial) collected from the N-stage on the same day and rinsed carefully in tap water. The trials were performed in 3 L reactors, containing 1.5 L of synthetic medium. For the AOB trials the medium consisted of 50 mgNH_4-N/L, while the NOB trial medium contained 30 mgNO_2-N/L. For both trials the medium also contained a pH buffer ($NaHCO_3$), trace minerals and some PO_4-P to avoid substrate limitation. All trials were performed at 20°C, DO was adjusted to 2 mg/L, by combining air and N_2-gas at a fixed total air flow of 2-3 L/min, and pH was adjusted to 7 by the gradual addition of 0.5 M sulphuric acid. Both the AOB and NOB trials lasted for one hour, with samples being extracted every 10 minutes. Each sample was analysed (see above for methods) to detect changes in NO_2-N and NO_3-N (NOB trials) as well as NH_4-N (AOB trials only) over time, for which the rate of ammonia and nitrite oxidation could be calculated. During IFAS operation, both carriers and sludge were sampled for AOB and NOB batch trials. In those trials, the temperature was set to 15°C and the DO was kept high (> 5 mg/L) by only adding air at a flow of 3 L/min. The sludge was sampled from the IFAS reactor and concentrated by settling and removing the clarified phase. Then 1 L of the sampled sludge was mixed with 1.5 L of the synthetic medium containing either 50 mgNH_4-N/L for AOB trials or 30 mgNO_2-N/L for NOB trial. Otherwise, the batch trials were performed according to the procedure described above.

Anammox batch trials were performed on 400 pieces of carriers collected from the anammox reactor or the IFAS reactor and rinsed carefully in tap water. The trials were performed in 3 L reactors, containing 3 L of synthetic medium consisting of 50 mgNH_4-N/L, 50 mgNO_2-N/L, pH buffer ($NaHCO_3$), trace minerals and some PO_4-P to avoid substrate limitation. During the trials, DO was kept bellow 0.05 mgO_2/L, by continuous inflow of N_2-gas at a fixed flow of 3 L/min, and pH was adjusted to 7.5 by the gradual addition of 0.5 M sulphuric acid. Anammox trials on carriers from the AMX stage from the two-stage configuration were performed at 30°C, while the anammox trials on carriers from the IFAS configuration were performed at 30°C and 15°C at each occasion to evaluate both the maximum capacity and the effect of temperature change and potential temperature adaption. Each anammox trial lasted for 42 or 60 minutes, with samples being extracted every 6 or 10 minutes depending on activity. Each sample was analysed (see above for methods) to detect changes in NH_4-N and

NO_2-N over time, for which the rate of ammonia and nitrite consumption could be calculated.

2.4.3. **Biofilm characteristics**

The biofilm on the Anox K™ Z-200 and Anox K™ Z-400 carriers from the N-stage, AMX-stage and IFAS-stage respectively were characterized by several methods in order to determine the biofilm thickness, mass, structure and microbial composition. During each batch trial, the carriers were imaged using a stereo microscope (OLYMPUS SZ-ET, Olympus Corporation, Japan). In addition, the total mass of the biofilm was determined on a sample of 20 carriers after each trial (see Piculell et al. (2016b) for method). At three occasions, Z200 carriers from the N-stage were sent to Karlsruhe Institute of Technology (Germany) for imaging with optical coherence tomography (OCT), which is used to quantify the biofilm thickness (Piculell et al., 2016b). For OCT, a sample of five carriers was randomly picked and imaging was performed in three pre-selected grid-compartments on each carrier.

Biomass was also extracted for microbial community analysis. For each batch trial, biomass was collected from 3 carriers and stored in the freezer until being sent for DNA extraction, library preparation, DNA sequencing (Illumina MiSeq, 2x301bp) and bioinformatic processing by DNASense in Aalborg, Denmark (dnasense.com). In total, twelve samples from the N-stage, and three samples from the AMX-stage were analysed during the project period, and additional two samples have been collected from the IFAS reactor for future analyses.

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3. Results

In the following chapter, the results obtained during mainstream operation are presented. First, all results from the reactor performance, batch trials and biofilm characteristics from the two-stage nitritation + anammox configuration are presented, thereafter followed by results from the one-stage IFAS MBBR operation.

3.1. Two-stage nitritation + anammox

3.1.1. Reactor performance

Nitritation Performance

Figure 7 shows the inlet water characteristics and load to the two-stage nitritation + anammox process during mainstream conditions. Figure 8 and 9 show removal rates and concentrations in the N-stage, mainly focusing on mainstream conditions.

After initiating mainstream operation in the N-stage on day 250, several different operational issues resulted in frequent switching to reject for the first 50 days (Figure 7): On day 270 the utility had issues with receiving the effluent from the POWERSTEP reactors, why the system had to be fed reject water during 10 days while the utility changed some equipment. Later, on day 290, some re-construction on site caused an additional operation stop, why reject water was fed to the N-stage to ensure that the system would not starve.

After day 300 the operational issues seized, and a stable period of 40 days with nitritation on mainstream feed followed by regular switches to reject water on a weekly basis according to plan (Figure 7). During mainstream conditions, the N-stage was fed 15 m^3/h of wastewater (fixed flow), and hence the nitrogen load varied with the inlet concentration to the reactor (Figure 7). During the initial mainstream phase (until day 340) the nitrite accumulation ratio ($NO_2N/(NO_2N+NO_3N)$) in the N-stage was around 0.9, with a nitrite to ammonia ratio varying between 1.2 and 1.8 depending on the load. The ammonia concentration in the reactor was below 5 mg/L due to high nitritation capacity. Unfortunately, the load could not be increased to avoid substrate limitation due to limited pump capacity.

Around day 340 and onwards there was a clear build-up of nitrate in the reactor, indicating the establishment of NOB in the system (Figure 8 and 9). In order to supress the NOB, the reject feeding phase around day 360 was prolonged, resulting in a noticeable drop in nitrate production. Unfortunately nitrate started to build up again shortly after this, probably due to the temperature in the mainstream feed dropped below 20°C (Figure 7).

During day 380-395 the reject water supply was limited due to reconstruction of the digesters at the site, why reject feeding was turned on and off in a discontinuous manner. Hence, sampling was limited during this time period. As seen in Figure 7, the temperature in the mainstream feed dropped gradually due to seasonal changes, until it reached the lowest value of 11°C on day 513. Additionally, the inlet ammonia concentration also dropped considerably during the same period. Combined, these factors enhanced NOB competitiveness requiring additional strategies for NOB suppression.

During day 405-480 the reject feeding phase with alternating pH and concentrations of free ammonia and free nitrous acid (see Chapter 2.3.1) was looped three times in a row with 3-6 days of mainstream operation between the reject phases. Hence, the duration and amount of reject feeding before switching back to mainstream influent was increased compared with previous operation. The ammonium removal rate during this period was around 0.075 kgNH$_4$-N/(m^3,d), and the nitrate production was low after switching back to mainstream water. Unfortunately, the NOB seemed to recover after a few days of mainstream operation and nitrate production increased gradually until the next reject phase (Figure 8 and 9). This pattern was observed repeatedly; therefore, a new NOB suppression strategy was initiated.

Between day 483-550 the aeration was changed from continuous to intermittent aeration with an oxygen set point of 1-2 mgO$_2$/L during aerated period. Additionally, the looping of the reject feeding phase was stopped and instead, the pH limits during the reject phase were adjusted to achieve elevated concentrations of free ammonia and free nitrous acid. Combined, these changes in operation resulted in a lower nitrate production (Figure 8) but unfortunately, also the ammonium removal rate dropped (to approximately 0.05 kg/m^3,d). Similar operation was continued during days 550-667, but with the addition of extra reject feeding periods with a combination of high concentration of free ammonia followed by high concentration of free nitrous acid for several days in a row. The flow rate during mainstream feed was decreased from 15 m^3/h to a fixed 10 m^3/h on day 594, in order to reach higher nitrite concentration in the reactor due to longer HRT.

The last attempt to supress the NOB was performed on day 678 to 680, were the strategy was to temporarily expose the reactor to extremely low pH (<5) by the manual addition of sulphuric acid. This action proved to be very effective, since the N-stage could be successfully operated with stable nitrite production on only mainstream feed (i.e. no reject exposure) for almost 20 days before nitrate started to build up in the reactor again (Figure 8 and 9).

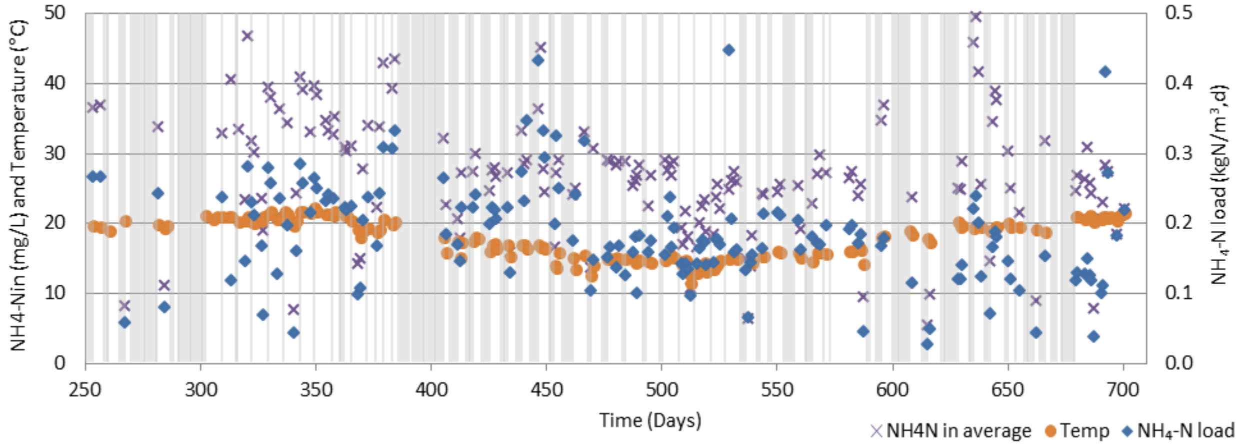

Figure 7: Characteristics of the inlet water to the N-stage over time during mainstream operation (vertical grey lines represent days with reject feed).

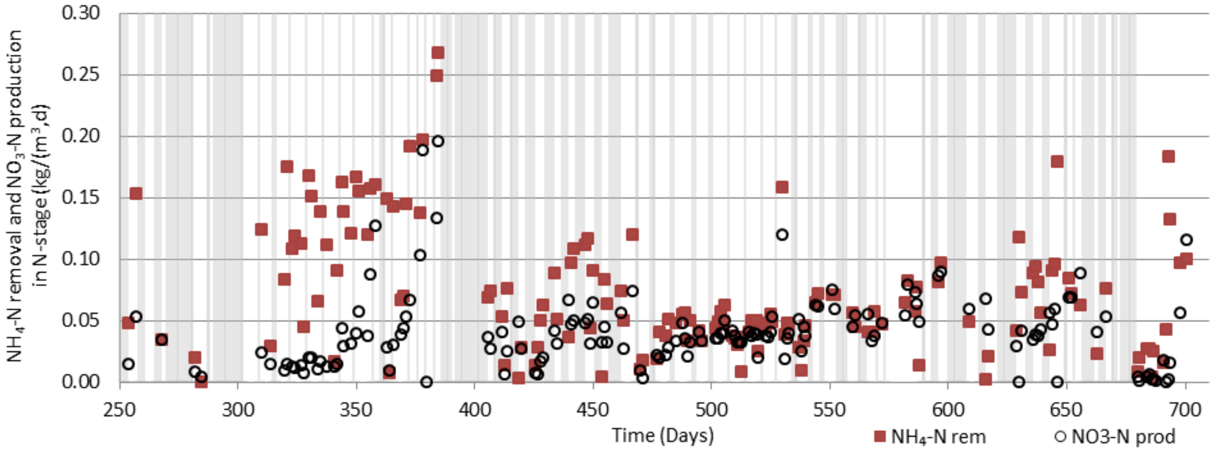

Figure 8: Removal rates and production rates in the N-stage during continuous operation on mainstream water (vertical grey lines represent days with reject feed).

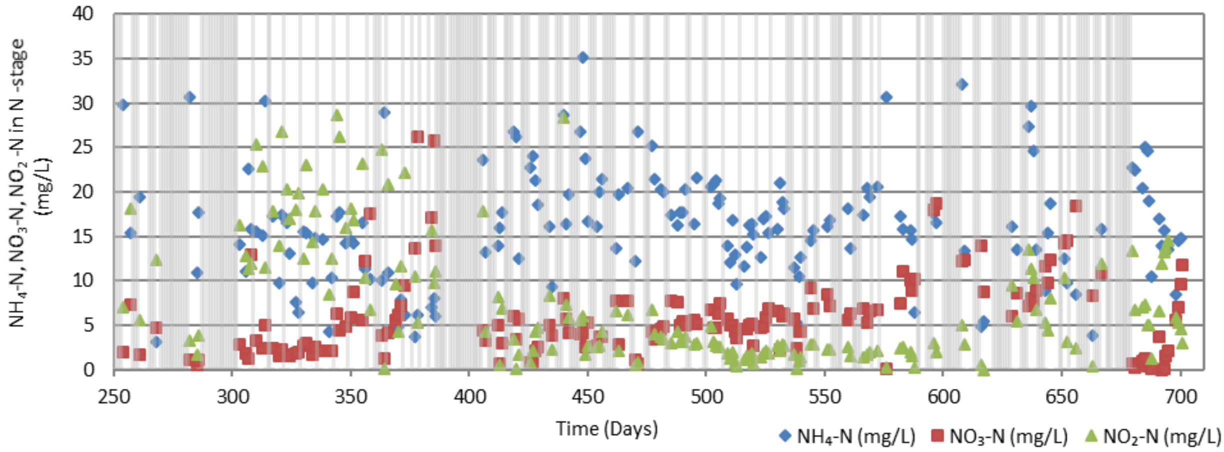

Figure 9: Reactor concentrations in the N-stage during continuous operation on mainstream water (vertical grey lines represent days with reject feed). The y-axis is limited to 40 mg/L hence, the concentrations during reject water feeding are not shown in the figure.

Anammox Performance

The AMX-stage was started up on operational day 135 (with respect to the N-stage). Establishment of anammox bacteria on the carriers was accomplished by operating on reject water and aerating the reactor. The AMX-stage was connected to the N-stage on day 360. After connecting the reactors, the aeration was switched off and the AMX-stage was operated at anoxic conditions, receiving effluent from the N-stage. The AMX-stage was by-passed during reject operation of the N-stage to avoid inhibition of the anammox bacteria by the strong water coming out from the N-stage. The AMX-stage was disconnected from the N-stage on day 572 and operation on reject water was again initiated to regain the anammox activity.

Figure 10 shows the ammonium load, ammonium removal and ammonium removal efficiency of the AMX-stage during the period when the reactor was connected to the N-stage. The vertical grey lines represent the days at which the AMX-stage was by-passed due to reject water operation of the N-stage. Figure 11 shows the ammonium, nitrate and nitrite concentrations in the reactor during the same period.

Between day 367 to 390 the load to the anammox reactor was only around 0.03 kgNH4-N/(m³,d), due to high efficiency of the N-stage, low effluent ammonia concentration from the N-stage and a fixed by-pass flow. The removal efficiency during this period varied between 40 and 98% (Figure 10) and the effluent ammonia concentration was below 8 mg/L. The nitrite concentration in the AMX-stage varied between 6 and 26 mgNO2-N/L, highly depending on the effluent quality from the N-stage. Unfortunately, from day 390 to 410, the reactor was operated on reject water due to operational issues with the N-stage. To maintain the biology during that period, air was supplied to the reactor during reject water feeding.

From day 410 the reactor was fed with effluent from the N-stage again. Between day 410 and day 470, the load was between 0.05 and 0.24 kgNH4-N/(m³,d) with an average of 0.12 kgNH4-N/(m³,d). The ammonia removal efficiency was between 10% and 40% with effluent ammonia concentration fluctuating between 3 mg/L and 30 mg/L (Figure 11). The nitrite concentration varied between 0 and 3.7 mg/L with an exception on day 447 where the concentration was measured to 5.6 mg/L (Figure 11). The inlet nitrite concentration (effluent from the N-stage) was almost zero during the period from day 450 to 480, limiting the activity in the anammox reactor and resulting in high effluent ammonia concentration (Figure 11).

From day 470 to day 572, the load was relatively stable around 0.06 to 0.1 kgNH4-N/(m³,d). Unfortunately, the removal efficiency gradually decreased to below 20% on day 551. It can be seen in Figure 11, that the nitrite concentration in the anammox reactor was below 1 mg/L from day 520 to day 572 with only three exceptions were the concentration increased slightly, indicating that the low nitrite concentration caused the low removal rate due to substrate limitation.

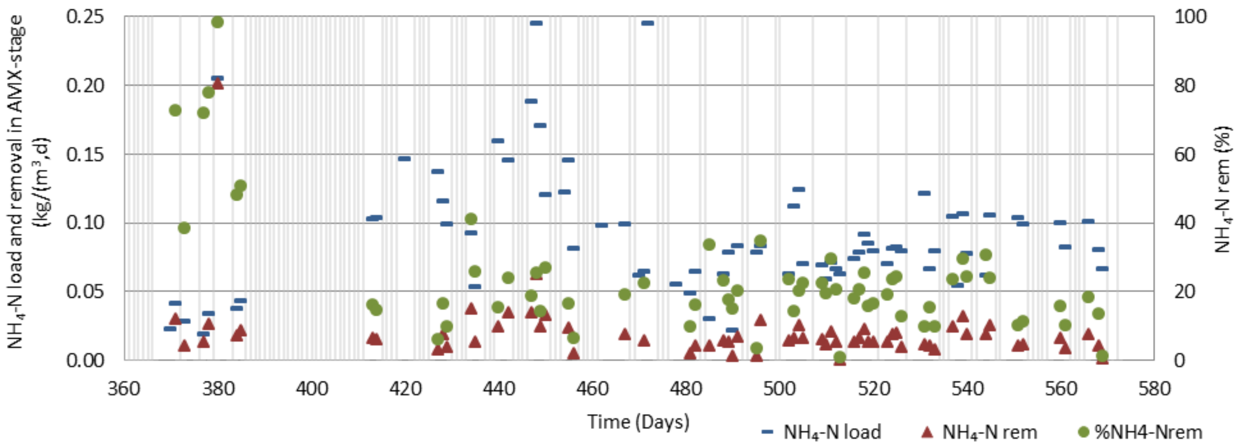

Figure 10: Load, removal rate and efficiency in the AMX-stage during continuous operation on mainstream water (vertical grey lines represent days with reject operation when the AMX-stage was by-passed except during day 390-410 were the AMX-stage was fed reject water).

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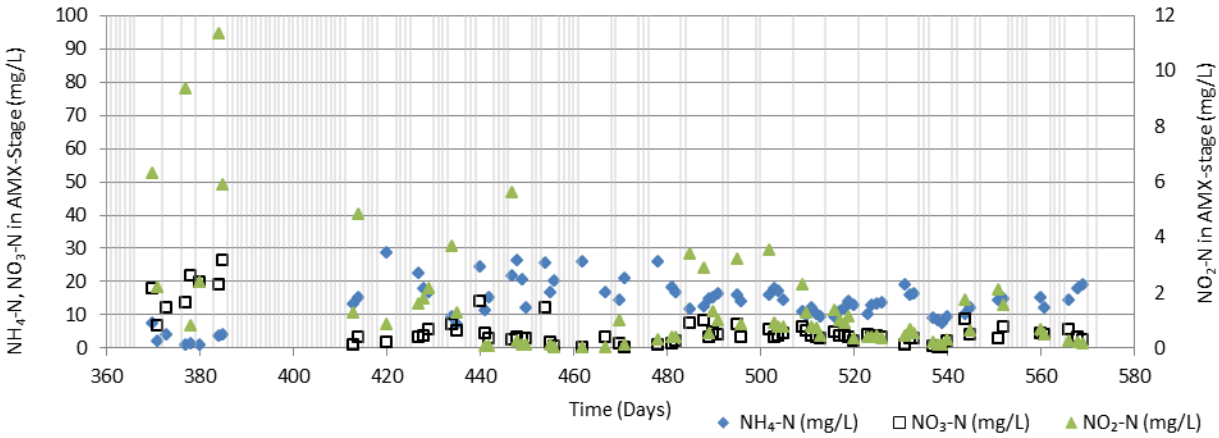

Figure 11: Reactor concentrations in the AMX-stage during continuous operation on mainstream water (vertical grey lines represent days with reject operation when the AMX-stage was by-passed except during day 390-410 were the AMX-stage was fed reject water)

Figure 12 presents the total nitrogen removal for the two-stage nitritation + anammox system during steady state mainstream operation. As seen in Figure 12, the measured total nitrogen removal for the two-stage system varied from 5% to 44% with an average of approximately 23%. The reactors were not connected during the period where the N-stage was working optimal (day 300-340) and influent concentrations to the AMX-stage would have been ideal. Hence, the results presented are from periods were the total nitrogen removal is strictly limited by the reduced performance of the N-stage.

STAR was initiated on day 61 and used over the course of the project for monitoring and control. Although there were some limitations in the accuracy of the online sensors, the implementation of STAR enabled a smooth transition between the different operation modes (see Chapter 2.3.1), especially for the nitritation reactor. Control of the effluent quality was however limited by the sensor accuracy and hence only implemented for short periods of time.

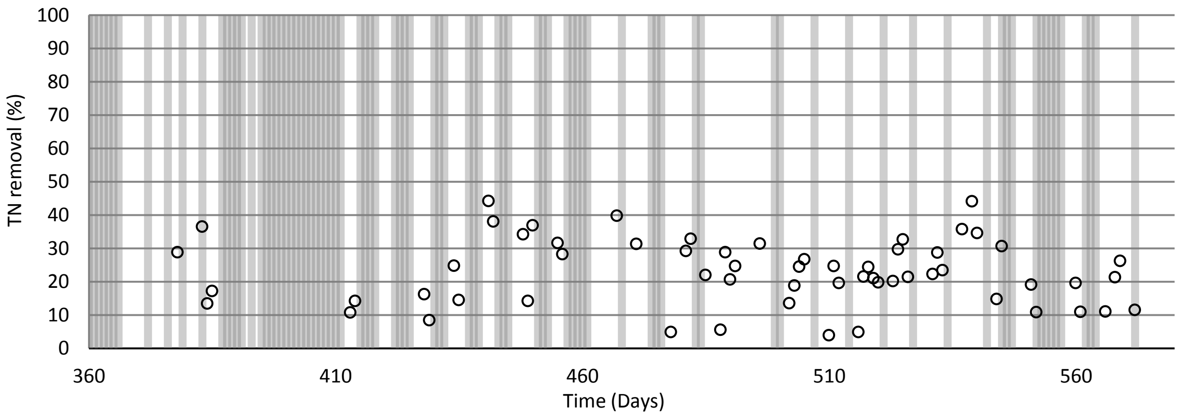

Figure 12: Total nitrogen removal (%) in the two-stage process during mainstream operation (vertical grey lines represent days with reject operation when anammox stage was bypassed except during day 390-410 were the AMX-stage was fed reject water)

3.1.2. Batch trials and biofilm characteristics

Observed activities in batch trials of AOB and NOB, as well as the relative abundance from DNA sequencing on the carriers from the N-stage can be seen in Figure 13. AOB activity reached an activity of approximately 0.8 gN/(m^2,d) when operating on reject water during start up. After switching to mainstream operation (day 250), the rate dropped to approximately 0.5 gN/(m^2,d). No NOB activity was detected in the batch trials up until day 330, after which NOB activity was observed and confirmed in microbial analysis (Figure 13). As the NOB gradually increased in the biofilm, AOB activity remained relatively stable around 0.5 gN/(m^2,d), although the abundance of AOB dropped (Figure 13).

Several attempts were made to suppress the NOB development by altering the reject exposure (varying the duration, load and/or pH in the reactor during reject feed as described in Chapter 3.1.1) but the achieved effect on NOB activity was only temporary (Figure 13) and NOB recovered fast. After the intense reject exposure around day 360 (see Figure 7-9), the NOB activity was noticeably decreased. However, the AOB activity was also affected by the aggressive exposure, and dropped to below 0.4 gN/(m^2,d) (Figure 13).

In the following days, AOB and NOB activity recovered, both peaking on day 400, after which the long term exposure to reject again resulted in a drop in activity for both bacteria groups. Trials performed days 404-517 show that the AOB activity decreased from 0.6 to 0.4 gN/(m^2,d) while the NOB activity increased to 0.4 gN/(m^2,d) without any observed effect of the aggressive reject water treatment. From day 517 to 601, the AOB activity recovered slightly to 0.6 gN/(m^2,d) at the same time as the relative abundance of the AOB in the biofilm decreased to below 5% after day 404 and remained low until day 601. Unfortunately, NOB activity increased dramatically and reached the maximum rate of 1 gN/(m^2,d) in the trial performed on day 601. The relative abundance of NOB also increased gradually, peaking at 10% on day 601.

A considerable drop in NOB activity was observed after 7 days of reject exposure between day 601 and 608 (Figure 13), while the AOB activity was not equally affected during the same time span. Rather, the abundance of AOB in the biofilm increased to almost 20% while the NOB abundance dropped to 5%. Both NOB and AOB activity had decreased dramatically in trials performed on day 681, where NOB activity dropped down to almost zero whereas the AOB activity decreased to 0.4 gN/(m^2,d). Following day 681 the N-stage was operated on only mainstream feed (i.e. no reject exposure) for almost 20 days before nitrate started to build up in the reactor again. The recovering NOB activity was confirmed in the final batch trial, where the NOB activity had increased to 0.4 gN/(m^2,d). AOB activity had also increased during the prolonged mainstream operation, to almost 0.6 gN/(m^2,d).

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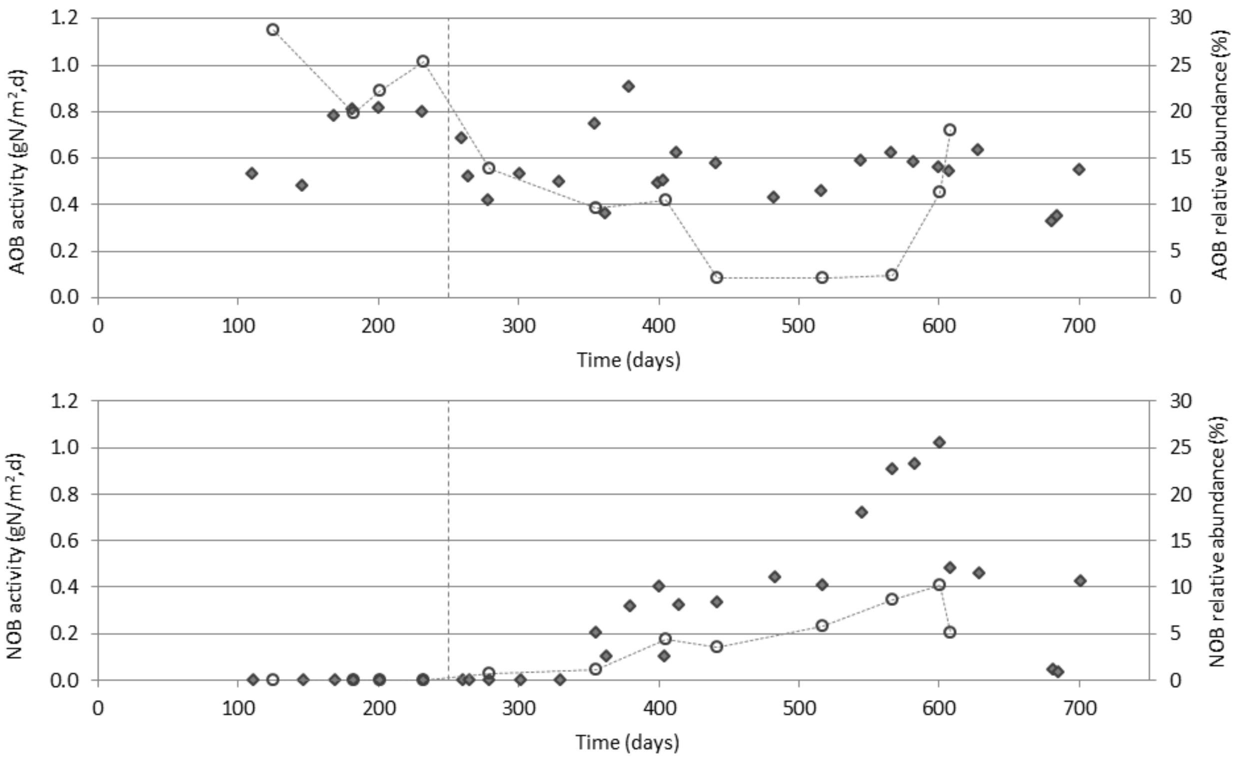

Figure 13: Observed activity in batch trials (diamonds) and relative abundance from DNA sequencing (circles) of AOB (top) and NOB (bottom) in carriers from the N-stage over time. Dashed vertical line represents switch to mainstream operation.

Multivariate statistics (PCA) of the microbial community indicated a gradual community shift in the N-stage after changing from reject to mainstream operation. The most abundant bacteria in the nitritation biofilm were the AOB *Nitrosomonas*, while *Nitrobacter* was the only observed NOB. Biofilm thickness remained thin on the nitritation carriers throughout the study and carrier biomass was relatively stable around 5.2 to 7.0 mgTSS/carrier during mainstream operation.

Figure 14 shows the results from the OCT measurements on the carriers from the N-stage. During start-up of the N-stage, a thin biofilm developed on the Anox K^{TM} Z-200 carriers, and when the first round of OCT images were taken on day 200, the biofilm was compact (13% porosity) with an average thickness of 77 µm. After switching the influent to mainstream, the biofilm thickness decreased to approximately 50 µm on day 355 and the porosity increased to around 27%. OCT measurements indicate that the thickness and porosity of the biofilm remained relatively stable throughout the mainstream operation and was measured to 50 µm and 29% respectively on day 605.

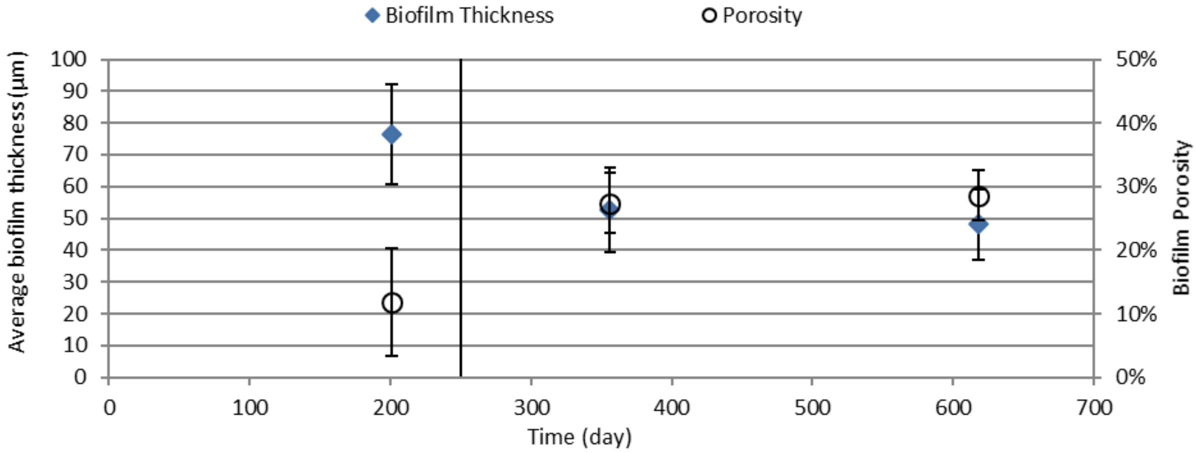

Figure 14: The average biofilm thickness and porosity of Anox K^{TM} Z-200 carriers from the N-reactor sampled at three different operational days, as analysed using OCT imaging. The black line represents the day when mainstream operation was initiated.

Observed activities in batch trials with anammox bacteria, as well as the biomass measurements and relative abundance from DNA sequencing on the carriers from the AMX-stage can be seen in Figure 15.

After start-up on day 135, anammox activity increased steadily with the exception of a sudden drop in activity on day 309, as a consequence of a week-long operational stop due to some un-related activities at the site. However, the activity recovered fast and reached 8 gN/m^2,d on day 357 (Figure 15), after which the reactor was connected to the nitritation effluent on day 360. When operating on mainstream water, the anammox activity dropped gradually to 1.7 gN/(m^2,d) on day 573. The activity drop correlated with biomass loss (Figure 15), which dropped from 8 g/m^2 down to 3 g/m^2. To avoid complete biofilm loss, the mainstream operation was paused and the feed was switched to reject water. From the microbial analysis, it was found that the most abundant bacteria in the biofilm was *Candidatus Brocadia*, with a stable abundance between day 468 and 546 (Figure 15).

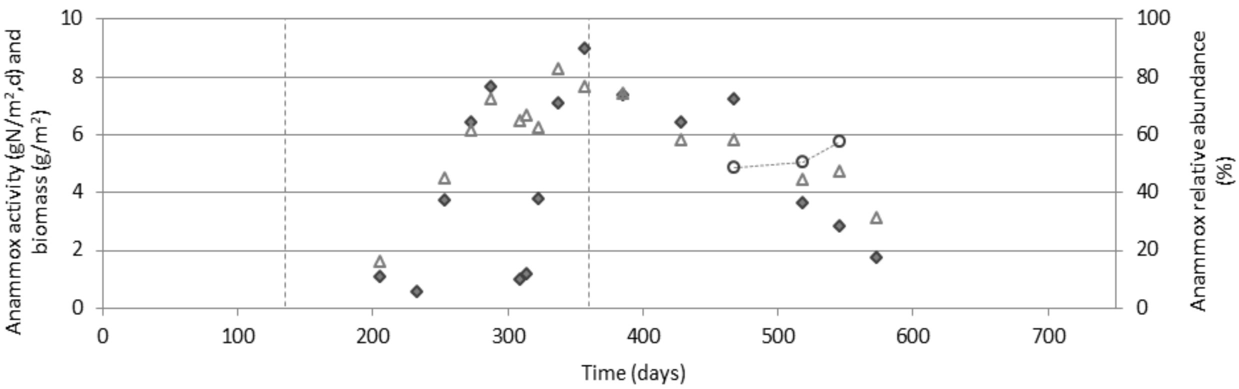

Figure 15: Observed anammox activity in batch trials (diamonds), measured biomass (triangles) and relative abundance of anammox bacteria from DNA sequencing (circles) in carriers from the AMX-stage over time. Dashed vertical lines represents start-up of reactor (day 135) and connection with N-stage (day 360)

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The carriers from both the N-stage (Figure 16) and the AMX-stage (Figure 17) were imaged on a regular basis using a stereomicroscope to visually analyse the biofilm. After switching to continuous operation on mainstream wastewater, the biofilm on the carriers from the N-stage remained thin, with seemingly thicker biofilm along the edges of the grid compartments and only a very thin biofilm layer in the centre of the compartments as seen in Figure 16. No visible changes in the biofilm in the N-stage appearance were observed during the mainstream operation. The observed loss of biomass in the AMX-stage (Figure 15), could also be seen visually (Figure 17).

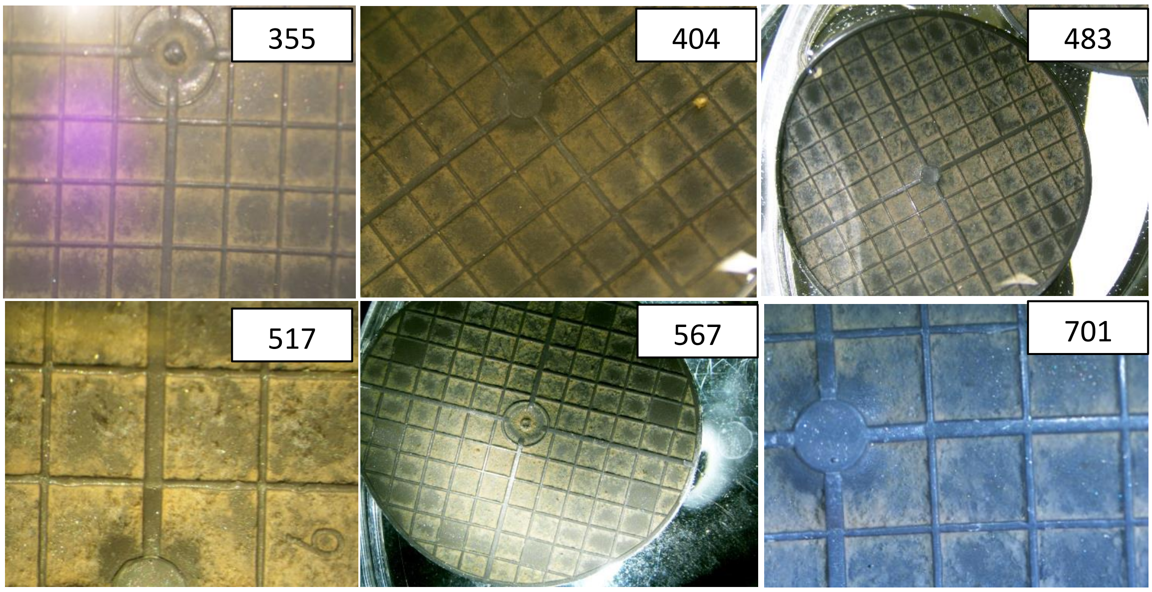

Figure 16: The biofilm on Anox K™ Z-200 carriers from the N-stage seen from above using a stereomicroscope on different operational days during mainstream operation.

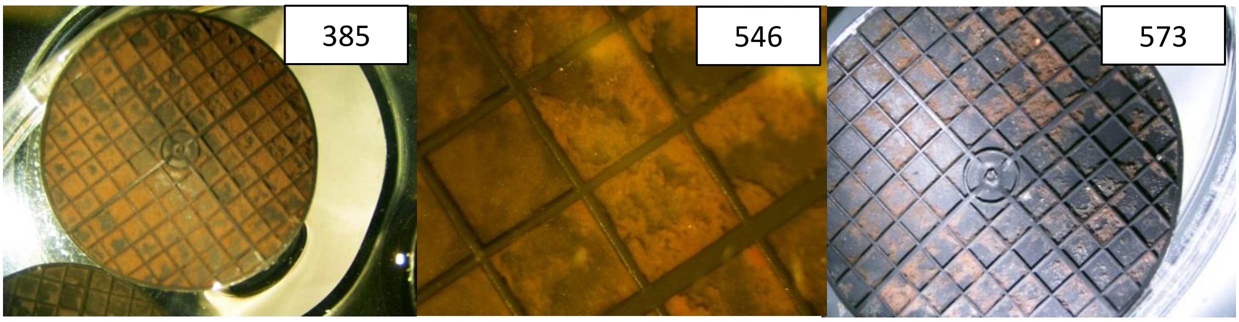

Figure 17: The biofilm on Anox K™ Z-400 carriers from the anammox reactor seen from above using a stereomicroscope on different operational days during mainstream operation.

3.2. One-stage IFAS MBBR

3.2.1. Reactor performance

The anammox reactor from the two-stage nitritation + anammox configuration was disconnected from the N-stage on day 572, and operated on reject water feed with continuous aeration for a faster re-establishment of anammox biofilm on the carriers. Due to reconstruction of one MBBR to a settler, the anammox reactor was not connected to a settler until on day 758 and IFAS operation was started. For a faster built-up of sludge in the process, the system was seeded with sludge originated from an SBR reactor for reject water treatment at the Sjölunda WWTP. Initially, the one-stage IFAS MBBR was operated on reject water feed to reach stable conditions, but on day 789 the influent was switched to the HRAS effluent mainstream feed. Day 839 the feed was switched from HRAS effluent to the primary Hydrotech drumfilter effluent. The one-stage IFAS MBBR was operated with intermittent aeration during the whole mainstream period. During operation on HRAS effluent mainstream feed, the duration of the aerated and non-aerated period was 5 minutes and 20 minutes, respectively. During operation on the primary Hydrotech drumfilter effluent, the aerated period was increased to 20 minutes and the non-aerated period was decreased to 10 minutes. The RAS flow rate was fixed at 9 m^3/h and no excess sludge production was observed during the reported period. The SRT could not be determined due to a varying suspended solids concentration in the IFAS reactor because of varying influent TSS concentration.

Figure 18 shows the ammonia load, ammonia removal and produced nitrate over reduced ammonia during reject water feed, both during MBBR start up and after initiating IFAS operation.

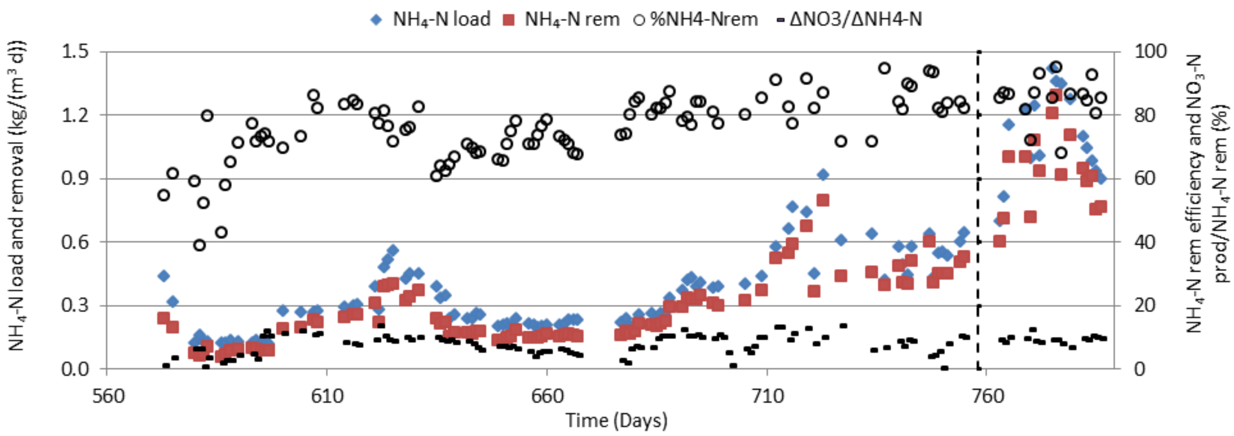

Figure 18: Ammonia load, ammonia removal and produced nitrate over reduced ammonia during reject water feed. The dashed line on day 758 represents the day when the settler was connected to the MBBR.

During reject water operation, the ammonia load and removal gradually increased, reaching a stable ammonia removal efficiency above 80%. When IFAS operation was initiated, the load and removal increased further, to 1.1 $kgNH_4\text{-}N/(m^3,d)$ and 0.94 $kgNH_4\text{-}N/(m^3,d)$ respectively. The ratio between produced nitrate and consumed ammonia was below the stoichiometric 11% (equation 5) during the whole period of reject water feed, indicating the absence of NOB activity.

On day 789, mainstream operation was initiated on effluent from the high rate activated sludge (HRAS) reactor. The shift from reject water to mainstream water only was performed stepwise by gradually decreasing the reject flow and increasing the mainstream flow. On day 794, the process was operating only on mainstream water. Figure 19 shows the ammonia load, ammonia removal and produced nitrate over reduced ammonia during mainstream water feed, and the dashed line represents the day when the primary treatment was switched to a Hydrotech drumfilter. Figure 20 shows influent ammonia concentrations as well as the reactor concentrations during mainstream operation.

Due to problems with reject water treatment at the utility from day 798, no reject water was available for spiking the influent to simulate a common ammonia concentration in the mainstream wastewater at European conditions, as initially planned. Therefore, the load to the process was dependent on the ammonia concentration in the mainstream feed. Additionally, due to unreliable sensor values, the STAR control strategy could unfortunately not be applied on the process. The average concentration of the HRAS effluent during the reported period was: NH_4-N=31 mgN/L and sCOD=55 mg/L. The one-stage IFAS MBBR was operated with intermittent aeration during the whole mainstream period. Howerever, the duration of the aerated and non-aerated phases varied due to different operational strategies.

From day 796 to 827, the load varied from 0.07 to 0.2 kgNH_4-N/(m^3,d) with an average of 0.13 kgNH_4-N/(m^3,d), as a result of feed concentrations (fixed flow 10-11 m^3/h) and the removal rate varied between 0.02 and 0.12 kgNH_4-N/(m^3,d) with an average of 0.06 kgNH_4-N/(m^3,d). The ammonia removal efficiency varied from 27% to 76% during the same period. The ratio for produced nitrate over consumed ammonia was below 11%, with low nitrite and nitrate concentrations in the reactor. The ammonia concentration varied between 4-20 mg/L, with the exception of days 819-824 were the ammonia concentration increased due to problems with the blower resulting in no aeration. The temperature in the reactor was around 15°C after switching to mainstream water only due to winter conditions.

On days 830-838, the load to the IFAS reactor was decreased to reach ammonia concentration around 3-5 mg/L and a total nitrogen concentration below 10 mgN/L in the effluent. During the same period, grab samples from the reactor were taken two times a day, in the morning and in the afternoon, due to unreliable sensor values at low concentrations. Due to winter conditions, the temperature in the reactor decreased down to 13°C during this period, and the removal rate decreased to 0.03 - 0.04 kgNH_4-N/(m^3,d) (Figure 19) but despite the low temperatures, total nitrogen concentration in the effluent was measured below 10 mgN/L at several occasions. On average, the total nitrogen concentration was 15 mgN/L from the grab samples taken in the morning and 12 mgN/L from the samples taken during the afternoon. The main contribution to the total nitrogen concentration were the ammonia and nitrite, and the concentration of ammonia was notably lower in both the influent and the effluent during the afternoon (Figure 20).

On day 839, the feed was changed to the effluent from the Hydrotech drumfilter (average concentration during the reported period was: NH_4-N=32 mgN/L, sCOD=100.8 mg/L and TSS=83.5). As seen in Figure 20, the ammonia concentration in the effluent increased to around 13 mg/L, while the nitrite and nitrate concetrations dropped to almost zero. Thereby, the TN concentartion in the effluent was equal to the ammonia concentration (10.5 mgN/L on average). The TN removal efficiency varied from 56% to 65% (Figure 21).

During operation on the effluent from the Hydrotech filter, the total aeration was increased considerably by prolonging the aerated time and shortening the non-aerated time.

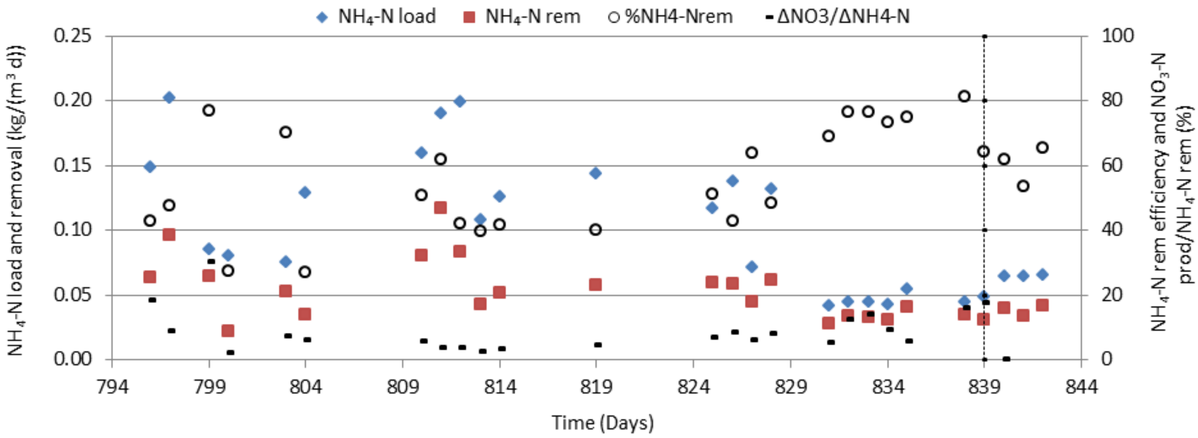

Figure 19: Ammonia load, ammonia removal and produced nitrate over reduced ammonia during mainstream feed. The dashed line on day 839 represents the day when the IFAS was connected to the Hydrotech drumfilter.

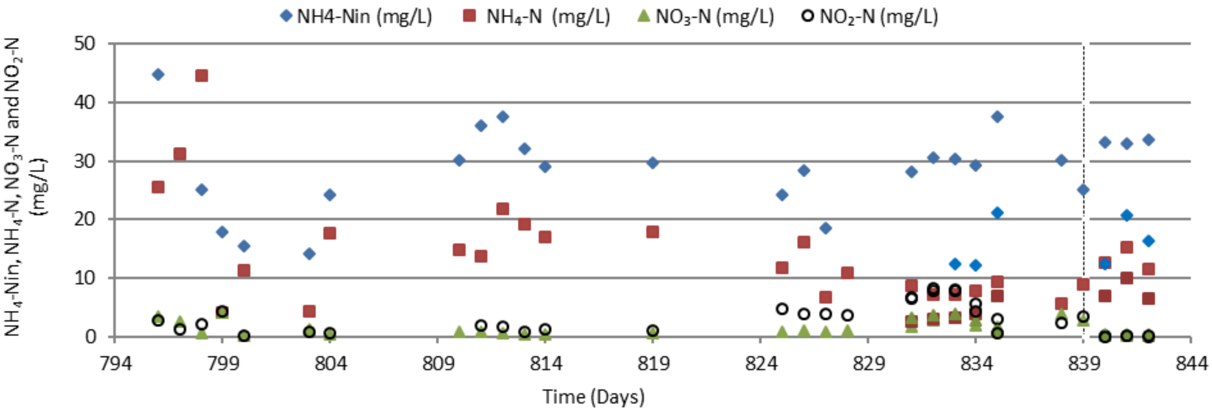

Figure 20: Influent and effluent concentrations in the one-stage IFAS MBBR during mainstream operation. The dashed line represents the switch from HRAS effluent to effluent from the Hydrotech drumfilter.

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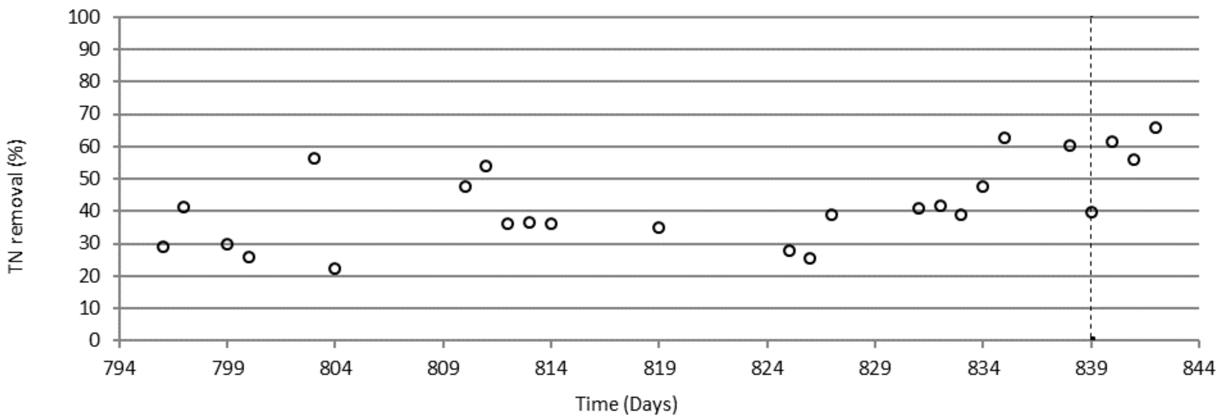

Figure 21: Total nitrogen removal (%) in the one-stage IFAS MBBR during mainstream operation: The dashed line represents the switch from HRAS effluent to effluent from the Hydrotech drumfilter.

The concentration of suspended solids in the influent, the IFAS reactor and in the effluent, as well as the Sludge Volume Index (SVI) in the reactor, are presented in Figure 22. Due to unreliable values from the measurements of the TSS in the RAS flow, results from RAS measurements are not presented. Between day 796 and 800, sampling was limited due to some reconstruction of the mixer. As of day 801, the reactor was continuously mixed at low speed to enhance reactor mixing during non-aerated phases. After day 809, the TSS concentration in the IFAS reactor was relatively stable between 1.3 and 2.0 g/L during operation on effluent from high rate activated sludge. However, the TSS concentration increased considerably after changing the feed to effluent from the Hydrotech drumfilter on day 839. The concentration of suspended solids in the effluent was below 0.06 g/L during the reported period. The SVI varied typically between 200-350 mL/g (with an outlier on day 832) and with no noticeable effect from switching the feed.

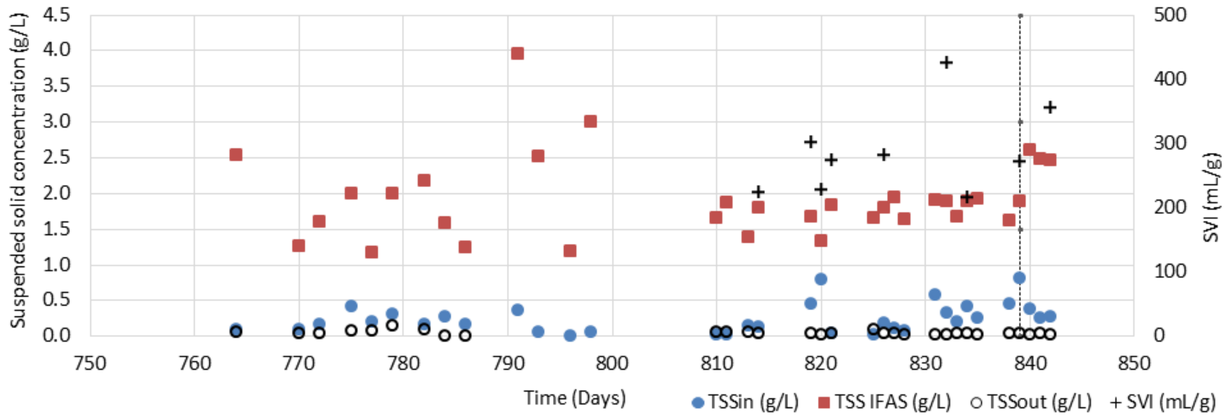

Figure 22: Concentration of suspended solids in the influent, IFAS reactor and effluent (g/L) and SVI (mL/g) sampled during mainstream operation. The dashed line represents the day when the feed was changed to effluent from the Hydrotech drumfilter.

Currently, sampling of reliable data for long-term observation of N_2O-N emissions have not been achieved due to problems with the equipment and re-construction of the process configuration. Additionally, only observations of N_2O-N in gas phase were accomplished due to complications with the liquid N_2O-N sensor. Preliminary results (Figure 23) indicate a low production of N_2O-N emission in the one-stage IFAS MBBR configuration, were the percentage of produced N_2O-N over consumed NH_4-N has been below 0.2%.

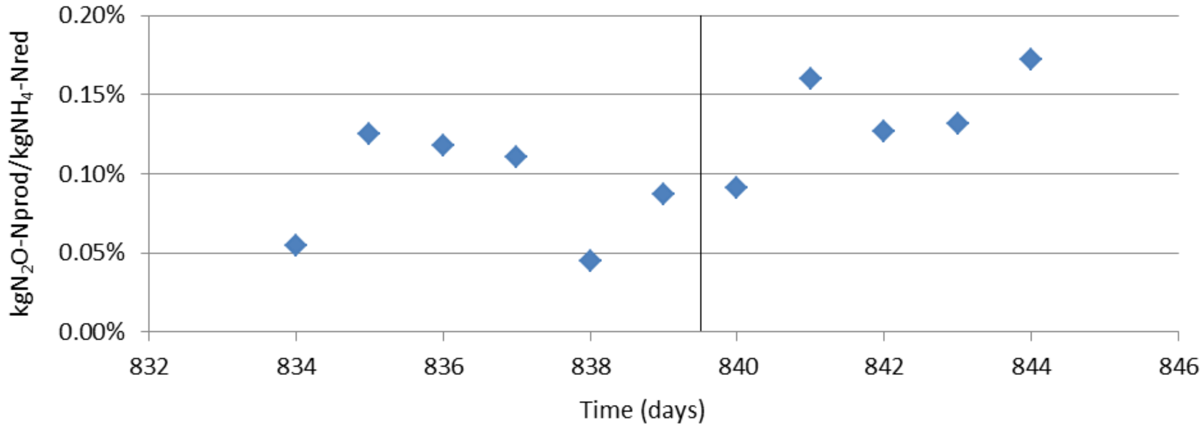

Figure 23: Produced N_2O-N over reduced NH_4-N over time. The black line represents the time when the feed was changed to effluent from the Hydrotech drumfilter.

3.2.2. Batch trials and biofilm characteristics

As previously mentioned, the anammox reactor from the two-stage configuration was disconnected from the N-stage on day 572 and operated on reject water for re-establishment of the anammox bacteria on the carriers. Initially, the anammox activity was 1 gN/(m^2,d) with a biomass of 2.6 g/m^2 (Figure 24). The activity and biomass content remained low until day 720, when it increased to 6.0 gN/(m^2,d) and 5.1 g/m^2, respectively. The activity increased further up to 7.5 gN/(m^2,d) before the settler was connected to the MBBR and IFAS operation was initiated on day 758. After switching the feed on day 789, the activity trials were conducted at two both 30°C and 15°C to evaluate temperature dependence and adaption of biomass (Figure 24). In the trials performed at 30°C, the activity was 8.3 gN/(m^2,d) on day 802 and 7.2 gN/(m^2,d) on day 833, indicating that the anammox capacity remained after the change from reject water to mainstream water influent. In trials performed at 15°C, the activity was 1.0 gN/(m^2,d) at both occasions, which clearly indicates the effect of low temperature on the removal rate. The biomass measured on day 802 and 833 was 8.2 g/m^2 and 7.2 g/m^2, respectively.

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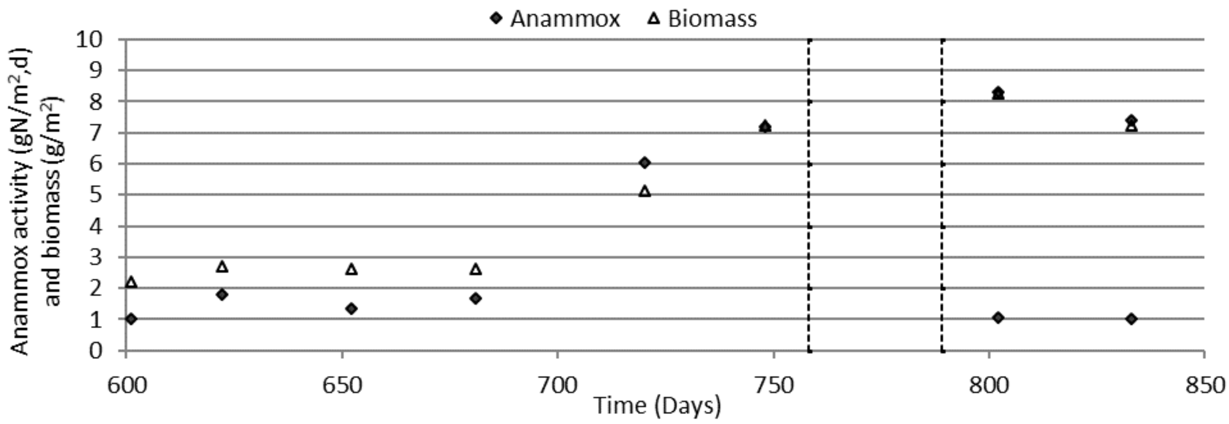

Figure 24: Observed anammox activity in batch trials (diamonds) and measured biomass (triangles) in carriers from the one-stage IFAS reactor over time. Dashed vertical lines represents connection to the settler (day 758) and start of mainstream operation (day 789)

For analysing the maximum nitritation rate and the potential NOB appearance in the IFAS process, batch trials on AOB activity and NOB activity were performed on the carriers and the sludge respectively on day 833 (Table 1). From the AOB activity trials, activity of 0.15 gN/(m^2,d) was observed for the biofilm growing on the carriers. The AOB activity in the sludge phase was 6.1 mgN/(gTSS,h). Interestingly, no NOB activity was observed in the batch trials, neither on the carriers nor in the sludge (Table 1).

Table 1: AOB and NOB activity at 15°C and DO>5mg/L on the carriers and in the sludge sampled on day 833.

AOB activity		NOB activity	
Carriers	Sludge	Carriers	Sludge
0.15 gN/(m^2,d)	6.1 mgN/(gTSS,h)	-	-

4. Discussion and Conclusions

The objective of this project was to demonstrate a technology for achieving stable nitrogen removal in the mainstream. During the course of the project, two technologies were applied and evaluated; the two-stage nitritation + anammox MBBR and the one-stage IFAS MBBR.

4.1. Two-stage nitritation + anammox

The feasibility of the two-stage nitritation + anammox process was demonstrated in two 50 m^3 reactors. Both reactors were operated on reject water during start up, in order to boost AOB and anammox growth, respectively. The N-stage was operated for 700 days, initiating mainstream operation on day 250, while the AMX-stage was operated for 437 days (between day 135 to 572), being connected to the N-stage between day 360 and 572 (except for a minor break on days 390-410) (Figure 9 and 11). In order to prevent NOB establishment, the N-stage was equipped with Anox K™ Z-200 carriers which limit the biofilm growth to a maximum of 200 µm, and the system was temporarily exposed to reject water.

Initially the demonstration plant aimed for good effluent quality from the whole system, but due to NOB establishment on the carriers in the N-stage, as observed after day 330 (Figure 9 and 13), the focus of operation shifted to ensuring sufficient NOB suppression in the N stage. Several strategies were implemented, mainly focusing on altering the reject exposure scheme, as summarized below.

- Increasing frequency of exposure
- Prolonging exposure scheme
- Looping exposure scheme
- Increasing/decreasing pH set-points
- Expose system to extreme pH (<5)
- Intermittent aeration during mainstream operation

The temporal switch to reject feed often resulted in a temporary suppression of the NOB, although AOB were sometimes also negatively affected. During the initial mainstream operation the ammonia removal in the N stage was 0.10-0.15 kgNH_4-N/(m^3,d), which increased to 0.20-0.25 kgNH_4-N/(m^3,d) when NOB was established (Figure 8). When the reject exposure became more pronounced around day 400, ammonia removal dropped to 0.08 kgNH_4-N/(m^3,d), and then further down to 0.05 kgNH_4-N/(m^3,d) when suppression became even more aggressive (Figure 8). However, the declining rates in full scale may also be a result of declining temperature in the mainstream (Figure 7). This theory is strengthened by the lack of decline in AOB rates as observed in batch trials, where the temperature was controlled to 20°C and AOB activity varied around 0.5 gN/(m^2,d) throughout the mainstream operation (Figure 13). AOB abundance did, however, drop in the biofilm during the colder months, which, together with stable biomass content, suggests a community shift due to temperature change (Figure 13), which was further confirmed by PCA analysis.

Although a temporary suppression of NOB was observed from the reject exposure, the overall NOB activity gradually increased, peaking at 1 gN/(m^2,d) in batch trials on day

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600 (Figure 13). It is unclear whether NOB establishment was a result of changes in operational conditions (for example by staying for too long in mainstream mode before switching to reject mode or not being suppressed enough during reject exposure) or, independent on operation strategy, the result of NOB establishing over time at favourable mainstream conditions.

The trend of increasing NOB presence did however shift, with significant drops in NOB activity sometime between day 601 and 608, as well as between day 629 and 681 (Figure 13). These two major drops in NOB activities were most likely the result of a prolonged reject operation where the scheme was looped repeatedly, hence frequently exposing the reactor to extreme FA and FNA. In addition, the temperature in the mainstream increased from 15°C to 20°C between day 580 and 620 (Figure 7), which may also have helped in recovering the nitrite production.

In conventional treatment processes, the most common AOB and NOB are *Nitrosomonas* and *Nitrospira*, respectively (Daims et al., 2001). However, while *Nitrosomonas* were the only AOB found in the biofilms, *Nitrobacter* was the only present NOB in the N-stage. It has been shown that *Nitrospira* thrives at low nitrite concentrations, which are the most common conditions in conventional treatment, while *Nitrobacter* thrives at high nitrite conditions (Blackburne et al., 2007). As the N-stage was operating at high nitrite, *Nitrobacter* rather than *Nitrospira* would develop. However, *Nitrobacter* also has a higher threshold for inhibition of both FA and FNA (Blackburne et al., 2007), meaning that, unless the inhibiting FA and/or FNA thresholds are exceeded during reject exposure, the NOB would have been boosted rather than inhibited by the high concentrations of nitrite. This is an additional explanation for the nitrate accumulation in the system and the requirements of extreme pH during reject exposure to achieve successful NOB inhibition.

Biofilm thickness in the N-stage never reached the maximum allowed thickness of 200 µm, but remained around 50 µm (Figure 14). It is noticeable that this extremely thin biofilm still achieved nitrifying rates similar to conventional municipal systems with normal biofilms (Rusten et al., 1995). Potentially the thin biofilms were a result of high shear forces due to high aeration intensities. Also, the extreme conditions during reject exposure may have inhibited some organisms which usually build up thicker biofilms in MBBRs, such as microanimals and heterotrophic bacteria.

The performance of the AMX-stage was always limited by the performance of the nitritation. The total TN removal of the process was low, around 20-30%, partially due to limited nitrite production in the N-stage. However the anammox capacity also dropped gradually after switching to mainstream operation, as observed in batch trials (Figure 15). Some decrease in anammox activity was expected due to the colder temperatures, but the considerable activity drop also strongly correlated with biomass loss (Figure 15), suggesting that the loss in activity could have been a result of decay due to starvation, and detachment of biomass due to the mechanical mixing in operation also under non-feeding conditions. It is therefore anticipated that the total nitrogen removal in the two-stage process would have been higher if the AMX-stage was connected to the N-stage during ideal operation of the N-stage and without substrate limitation in the AMX-stage. The abundance of anammox bacteria (*Candidatus Brocadia*) was stable while the activity and biomass content dropped

(Figure 15), indicating that the change in anammox activity was not a result of a community shift. However the samples for DNA analysis are limited and no strict conclusions can be drawn.

Although this study demonstrated that NOB can be suppressed in the mainstream, it may be that more reject is needed than what is practically feasible in relation to the mainstream water availability. However it should be noted that the inlet water at Sjölunda is exceptionally challenging for mainstream deammonification, due to the low temperatures in winter and diluted inlet concentrations. It can hence be anticipated that NOB suppression would require less reject water at other plants where inlet concentrations are higher. However, if successful nitritation and anammox can be achieved at Sjölunda, its implementation at other municipal treatment plants would be very likely.

4.2. One-stage IFAS MBBR

In the second phase of the project, the two-stage nitritation + anammox configuration was reconstructed to a one-stage IFAS MBBR were the process consisted of one 50 m^3 MBBR (previous anammox reactor in the two-stage configuration) connected to a 50 m^3 settler for sludge retention. Initially, the reactor was operated as a MBBR without sludge retention and on reject water feed in order to promote anammox growth. The reactor was fed reject water between day 572 and 789, after which the MBBR was connected to a settler on day 758 and IFAS operation initiated. The reactor was seeded with sludge from an SBR at the utility, which resulted in a rapid accumulation of sludge. Mainstream operation was started on day 789, initially on effluent from high rate activated sludge between days 789-839, after which the influent was changed to effluent from a Hydrotech drumfilter (Figure 18 and 19). No periodic reject water exposure was applied on the one-stage IFAS MBBR after starting the mainstream operation. The one-stage IFAS MBBR operation on mainstream water is still in an early phase, and data from mainstream operation is limited.

The reactor was operated on reject water for longer time than expected due to slow re-establishment of anammox biofilm on the carriers (Figure 23) and more time consuming labour with reconstruction of the process than estimated. During the initial mainstream operation, the ammonia load was 0.07-0.2 kgNH_4-N/(m^3,d) and the removal rate was 0.02-0.12 kgNH_4-N/(m^3,d), with an effluent ammonia concentration varying between 4-20 mg/L (Figure 19 and 20). The removal rate was clearly affected by the temperature in the process, which was 15°C when the mainstream operation was initiated, decreasing down to 13°C over the following month. The TSS concentration in the IFAS process varied between 1.3-2 gTSS/L during mainstream operation on effluent from high rate activated sludge, with average effluent TSS of 0.03 gTSS/L.

Because of the highly varying effluent quality, the load was significantly decreased to observe the removal rates achieved at low effluent concentrations, preferable <10 mgN/L. Since the sensors were considered unreliable at these low effluent concentrations, process control was solely relying on grab samples that were taken two times a day during that period. The load was decreased to 0.04-0.05 kgNH_4-N/(m^3,d), but the effluent requirements were not met, with a total nitrogen in the effluent varying

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between 8.5-17.8 mgN/L (average of 14 mgN/L). However, since the ratio between produced nitrate over consumed ammonia was lower than the stoichiometric 11%, and no NOB activity was observed (neither in the full-scale process nor in the NOB batch trials, see Figure 21 and Table 1), the process could potentially have been operated more aggressively with increased aeration resulting in a more efficient removal. An increased aeration strategy increases the risk of NOB establishment. However, NOB establishment would mainly occur in the sludge phase due to the low operational DO. Hence, NOB could be washed out of the reactor, which gives more flexibility compared to if NOB would establish in the biofilm.

From the batch trials on carriers and sludge on day 833, it was observed that AOB appearance and nitritation capacity was mainly found in the sludge phase (6.1 mgN/(gTSS,h)), but AOB activity was also found on the carriers (0.15 gN/(m^2,d)) (Table 1). These results suggest that in the full scale IFAS MBBR, the nitritation is mainly found in the sludge phase (≥85%) and only a small fraction (≤15%) potentially occurs on the carriers. The AOB activity on the carriers is most likely originating from when the reactor was operated as pure MBBR (before connecting the reactor to a settler). However, since the IFAS reactor is operated at oxygen limited conditions, and not high DO like in the batch trials, the nitritation is believed to almost solely take place in the sludge phase in the full scale.

According to the anammox batch trials, the maximum capacity remained high after the shift from reject water feed to mainstream water feed, where the activity was 8.3 gN/(m^2,d) on day 802 and 7.2 gN/(m^2,d) on day 833. However, in anammox trials performed at 15°C, the activity was 1 gN/(m^2,d) at both occasions, clearly showing the high dependency of temperature on the activity. These results correlate with the low removal rates observed in the full-scale process, when the temperature decreased down to 13°C during the reported period. Generally, the nitritation reaction is considered to be the rate limiting step in mainstream deammonification processes (Wett et al., 2013). However, according to observed results, it could have been the anammox reaction that was the rate limiting step at these cold conditions and relatively high nitritation capacity in the sludge phase. However, some adaption to the low temperature can potentially take place. Also, an increased biomass would potentially further improve the removal rates of the process.

After changing the feed to effluent from the Hydrotech filter, the load was stable at 0.06 kgNH_4-N/(m^3,d) and the removal rate was 0.04 kgNH_4-N/(m^3,d) on average. The TN out was lower compared to previous operation (TN varying between 6-15 mgN/L), and the effluent mainly contained ammonia while both nitrite and nitrate concentrations were below 0.5 mg/L. These results, combined with the increased concentration of suspended solids in the IFAS reactor (from 1.8 to 2.5 gTSS/L), suggest that heterotrophic bacteria are increasing in the sludge phase. With heterotrophic bacteria involved in the nitrogen removal, control of the process to avoid NOB establishment becomes more complex, since heterotrophs are capable of using both nitrite and nitrate as electron acceptors at anoxic conditions. Moreover, the heterotrophs consume oxygen during COD reduction and compete with AOB and NOB for oxygen. Hence, the importance of regular batch trials and sampling for DNA analysis becomes increasingly important, in order to observe the evolution of the microbial community during the final months of the project.

Concerns arose regarding the suspended sludge quality during the initial mainstream operation of the one-stage IFAS MBBR, with high SVI factor and poor settling characteristics (Figure 21). Poor sludge quality may limit the SRT and thereby overall nitritation capacity,

Preliminary results from the N_2O-N measurements indicated a low production of N_2O-N (<0.2%). This could partially be explained by the low nitrite production in the one-stage process, as nitrite production and N_2O-emissions often correlate, but the values are considerably lower than found in conventional nitrogen removal (Massara et al., 2017). Most likely, the measurement range of the analyser is too large to pick up the low emissions from the IFAS. Hence, the equipment needs further improvements before any strict conclusions can be made. Improved on-line measurements of N_2O-N will therefore be continued and further evaluated.

4.3. Final remarks

The implementation of deammonification technology has the potential to change conventional wastewater treatment plants from being energy consuming to becoming energy self-sufficient or even energy producing systems. Successful mainstream deammonification depends on stable anammox activity and efficient suppression of NOB. The most common approaches for NOB repression are designed for reject water processes, characterised by high ammonia concentration and high temperature. These suppression strategies are not applicable for mainstream processes due to much lower ammonia concentration in the mainstream water and the lower and more varying temperature.

In the scope of this project, the feasibility of two-stage nitritation + anammox MBBR and the one-stage IFAS MBBR for mainstream deammonification were evaluated. The technologies have different advantages and challenges. In two-stage nitritation + anammox MBBR system, with AOB growing in biofilms, a compact solution with a high accumulation of nitrifying biomass may be enabled. Additionally, NOB can be supressed in the aerated zone without affecting the anammox. Hence, ideal conditions for each of the desired bacteria group can be achieved and potentially, even the start-up process and establishment of anammox biofilm on the carriers may be facilitated. In this study, high nitritation and anammox capacity was initially achieved. But after long-term operation, increment of NOB was observed in the nitritation reactor affecting the total performance of the process. However, even if more reject water is potentially required than what is practically feasible in relation to the mainstream water availability, it was demonstrated that the NOB can be suppressed when alternating the feed between mainstream water and reject water.

In the one-stage IFAS MBBR configuration, suppression of NOB might be enhanced when operated in one stage, due to competition for nitrite between the anammox and NOB. Furthermore, since the anammox tend to grow in the biofilm of the carriers and the nitrifiers in the suspended sludge, a control of the sludge age in the system and therefore selective wash-out of NOB while retaining anammox is possible. Additionally,

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the process solution has showed potential to handle variation in COD load well (Lemaire et al., 2016). Within the project, one-stage IFAS MBBR has been evaluated for a very short period and more time is needed for further evaluation, but promising pilot results over long time has been demonstrated (Lemaire et al., 2016). For a successful implementation of mainstream deammonification, an advanced process control strategy, like STAR control, is required. This, in turn, requires reliable and accurate sensors, which was observed as one of the major challenges in this project.

With this project, steps towards full-scale implementation of deammonification process for nitrogen removal in the mainstream have been taken. Both the one-stage IFAS MBBR and two-stage MBBR process have shown promising results, but several subjects need to be addressed in order to implement the technologies on a broader scale. Further development of reliable and efficient NOB repression strategies is required and will increase the robustness of the technologies. Concurrently, the start-up strategy, particularly for the anammox biofilm, also needs to be evaluated and optimized for a competitive implementation. Finally, with a suitable control strategy and reliable equipment, further process optimisation with regard to energy consumption and N_2O-N emissions can be achieved.

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