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OLEG ARTEMCHUK

Autotrophic nitrogen removal processes for nutrient removal from sidestream and mainstream wastewater

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Institute of Chemistry, Faculty of Science and Technology, University of Tartu, Tartu, Estonia.

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In memory of Professor Toomas Tenno

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LIST OF PUBLICATIONS INCLUDED IN THE THESIS

The current thesis is based on the following publications, which are listed below and referred by the Roman numerals in the text of thesis:

I. Zekker, Ivar; Markus Raudkivi; **Oleg Artemchuk**; Ergo Rikmann; Hans Priks; Madis Jaagura & Taavo Tenno (2020). **Mainstream-sidestream wastewater switching promotes anammox nitrogen removal rate in organic-rich, low-temperature streams.** Environmental Technology, 42(19), 3073–3082. DOI: https://doi.org/10.1080/09593330.2020.1721566

II. Zekker, Ivar; Kivirüüt, Aimar; Rikmann, Ergo; Mandel, Anni; Jaagura, Madis; Tenno, Toomas; **Artemchuk, Oleg**; Rubin, Sergio; Tenno, Taavo. (2019). **Enhanced efficiency of nitritating anammox SBR achieved at low decrease rates of oxidation-reduction potential.** Environmental Engineering Science, 36(3): 350–360. DOI: https://doi.org/10.1089/ees.2018.0225.

III. Zekker, Ivar; **Artemchuk, Oleg**; Rikmann, Ergo; Ohimai, Kelvin; Browmick, Gourav Dhar; Ghangrekar, Makarand; Burlakovs, Juris; Tenno, Taavo (2021). **Start-up of anammox SBR from non-specific *inoculum* and process acceleration methods by hydrazine**. Water. 13(3), 350. DOI: https://doi.org/10.3390/w13030350

PhD applicant performed reactor experiments, designed the studies, gave original ideas, processed data, interpreted results and write papers. In the two first articles, where the author carried out work on the design of the study, the stages of the experimental part were strictly prescribed to the recording of all processed data in specially created laboratory reactors. Additionally, PhD applicant wrote papers about work done in first two studies. For the third journal publication, the author was leading the work, including the technical aspects of the bioreactor, and the author also performed bioprocess control and wrote an article about achieved results. The author also carefully monitored all articles progressing to exclude errors that could occur within the articles, made special calculations using the appropriate formulas. Prepared graphs and drawings were used in the provided works.

Paper I Author performed ca 75% of experimental work and wrote the manuscript.

Paper II Author performed the experimental work (75%) and participated in the writing of the manuscript (50%).

Paper III Author operated the reactors, performed batch tests with hydrazine (90%), and wrote the manuscript (90%).

Author's contribution to the article denotes: "*" – minor contribution, "**" – moderate contribution, "***" – major contribution

Categories	Author's contribution		
Paper number	I	II	III
Writing the manuscript	***	**	***
Interpretation of results	***	**	***
Data processing, analysis	***	***	***
Design of study	**	***	***
Idea originality	***	***	***

LIST OF ABBREVIATIONS AND ACRONYMS

ANAMMOX	ANaerobic AMMonium OXidation process;
AOB	ammonium oxidizing bacteria;
BABE®	Bio-Augmentation Batch Enhanced technology;
C/N	carbon to nitrogen ratio;
CANON	Completely Autotrophic Nitrogen-removal Over Nitrite technology;
COD	chemical oxygen demand;
DEMON®	sequencing batch reactor based deammonification technology;
DIB	deammonification in biofilm;
DNA	deoxyribonucleic acid;
DO	dissolved oxygen;
FA	free ammonia (unionized ammonia in aqueous solution, also shown as $(NH_3)_W$);
FNA	free nitrous acid (undissociated HNO_2 in aqueous solution);
HRT	hydraulic retention time;
IC	inorganic carbon;
MBBR	moving bed biofilm reactor;
MLSS	mixed liquor suspended solid;
NOB	nitrite-oxidizing bacteria;
OLAND	technology of Oxygen-Limited Autotrophic Nitrification-Denitrification technology;
ORP	oxidation-reduction potential;
PANDA	technology of Partial Augmented Nitritation Denitritation with Alkalinity Recovery;
PCR	polymerase chain reaction;
qPCR	quantitative polymerase chain reaction;
RBC	rotating biological contractor;
rDNA	ribosomal deoxyribonucleic acid;
rRNA	ribosomal ribonucleic acid;
SAA	specific anammox activity;
SBR	Sequence Batch Reactor;
SHARON®	technology of Single reactor system for High activity Ammonium Removal Over Nitrite;
TN	total nitrogen;
TNLR	total nitrogen loading rate;
TNRE	total nitrogen removal efficiency;
TNRR	total nitrogen removal rate;
TOC	total organic carbon;
TSS	total suspended solids;
UASB	Upflow Anaerobic Sludge Blanket
WWTP	wastewater treatment plant;

16S rDNA	section of prokaryotic DNA that codes for a strand of ribosomal RNA, and which is used for measuring bacterial taxonomy, phylogeny, and the rate of divergence.

The capital letters in the explanatory text of designations of certain wastewater treatment technologies, genomic data analysis software, and certain biological processes indicate how an abbreviation is derived.

1. INTRODUCTION

Nowadays, increased amounts of wastewater generated pose threats to environment quality and people's health. Wastewater biological treatment is energy-efficient way to remove organic and inorganic pollutants from wastewater and diminish the probability of waste compounds entering to groundwater. Several biogases plant implementations will involve organic carbon conversion to biogas and nitrogen and phosphorus compounds presence in rejected water. Treatment of such waste streams is costly with traditional methods (nitrification-denitrification) needing addition of organic carbon back to the treatment process (often in the form of methanol). Among wastewater treatment technologies, anaerobic ammonium oxidation process (anammox) has been involved in autotrophic nitrogen removal in bench-scale, pilot- and full-scale technologies to eliminate nitrogen from wastewater with a low usage of organic carbon and usage of inorganic carbon (CO_2). Mainstream wastewater can be termed as municipal wastewater and sidestraem wastewater as rejected wastewater coming from the wastestream after surplus sludge digestion and after centrifugation of digested waste. Anammox process has been tried to involve both- for sidestream and with more difficulties involved to mainstream wastewater treatment using moving bed biofilm reactor (MBBR) and sequence batch reactor (SBR) technologies.

Compared to the treatment of sidestream wastewater, the main obstacles to overcome to achieve successful anammox application in the mainstream would be wastewater's high carbon to nitrogen ratio (C/N>1) and diminshed temperatures of wastewater (in Nordic country climates 12.5–19°C). Earlier studies on mainstream anammox wastewater treatment process have shown that low temperatures can pose problems for anammox biomass growth rates. To overcome the inhibiting effect of cold temperatures to anammox bacteria growth rates, mainstream systems can be inoculated with biomass cultivated in sidestream conditions. These practices would show great practical application for long-term adaption of suspended sludge, granular sludge or biofilms at sidestream conditions on starting up mainstream anammox treatment.

The biomass switching effects between 2 different streams (mainstream, sidestream) nitrogen components treatment were shown in current work (Paper I). The tests on one reactor MBBR system operation involved periodical changes in influent composition. The specific effect of colder temperatures and switching different wastewater flows (between low nutrient and high organic carbon concentrations) in a single reactor anammox process is not well known, specifically in biofilms.

Further information is needed for the treatment of nitrogen-rich wastewater streams with economical aspects on how to diminish dissolved oxygen (DO) needs in the process to save from electrical energy demand (Paper II). Nowadays, oxidation–reduction potential (ORP) has implemented as an important control parameter in SBR in the laboratory anammox applications, however,

many works have not achieved stable process control, concentrating only to observing the values of ORP, not controlling its changes after feeding and during process cycles. The ORP changes are happening due to the consumed and oxidized nitrogen species (nitrite and nitrate) changes and by the consuming of DO. The control of sequence of anammox SBR biomass ORP changes in different SBR phases (filling, aeration, anoxic phase, discharge phase, idling), enables us to screen the proper feeding pattern of the reactor and dose wastewater at optimum quantities to avoid inhibition by over or under oxidation. Batch tests can prove how DO is consumed in the aerobic stage by ammonium oxidizing bacteria (AOB); also, can show how nitrate is produced by nitrite oxidizing bacteria (NOB) and anammox bacteria and how it is consumed by denitrifying bacteria. Moreover, tests can show how DO is used by AOB, NOB, and aerobic heterotrophic bacteria. The changes in the ORP have effect on the composition of vital proteins for bacteria or are impacting the charge of mitochondrial membranes of the bacteria. ORP changes can be controlled by switching the aeration on/off or by changing the aerator-based intensity changes by using the signal and border values controlled by ORP probe.

Salinity and hydrazine effects on biomass are important parameters to estimate and know the anammox process behaviour (Paper III). Reaching optimum salinity levels will gain higher anammox activities and nitrogen removal rates. Anammox process intermediate- hydrazine, can affect anammox efficiency, but also diminish other process, such as denitrification effects to gain high autotrophic removals of nitrogen. Therefore, these effects were studied in the SBR and batch tests throughout the study.

Aims of study

To evaluate the effects of mainstream and sidestream switching on the anammox biofilm reactor operation and for batch tests, nitrogen removal rate estimation was done to find out the optimum feeding conditions for mainstream and sidestream treatment (Paper I). In our study, the anammox biofilm process was also studied in detail with the effect of a broad range of total organic carbon (TOC)/total nitrogen (TN) and chemical oxygen demand (COD)/TN ratios on the batch-scale nitrogen removal rate. Anammox and denitrifying bacteria operational taxonomic units in *inoculum* (i.e., reject water), mainstream and sidestream conditions were aimed to be determined by pyrosequencing analysis. There was aimed to increase the deammonification process, with TN removal, and SAA in the SBR biomass through ORP as a control parameter (Paper II). The goal was also to determine the optimum border ORP decrease rate values for efficient SBR operation and to confirm these for cultivated biomass in batch tests. The ORP, DO, ammonium, and nitrate patterns during aerobic/anoxic phase of an SBR process for achieving a high total nitrogen removal efficiency (TNRE) and total nitrogen removal rate (TNRR) were considered to be determined. The ORP decrease rate as a control indicator parameter ending the

treatment cycle when ammonium was depleted was investigated in connection with the determined bacteria.

This study also undertook the start-up of anammox bacteria with non-specific inocula seeding sludge (anaerobic and aerobic sludge) using hydrazine addition in the single sequence batch reactor (SBR) (Paper III). The main objective was to evaluate the effect of hydrazine addition on the specific anammox activity (SAA) and denitrification activity during different stages of the start-up process in order to accelerate the overall start-up of autotrophic nitrogen removal from scratch. An additional objective was to evaluate the effect of salinity on the start-up process and overcome the detrimental effect of excess salinity with hydrazine.

2. LITERATURE OVERVIEW

Nutrient-rich liquid waste streams with a high C to N quantitative ratio are generated in many waste management processes (domestic and industrial) (Jardin & Hennerkes, 2012). Rejected water (sidestream) generated in an aerobic digestion tank is an example of N-rich wastewater, containing high N and low organic carbon concentrations (with a chemical oxygen demand (COD) to total nitrogen (TN) quantitative ratio below 1). Numerous anaerobic ammonium oxidation (anammox) method applications are originated for sidestream wastewater treatment with an appropriately low COD/TN (C/N<1) quantitative ratio everywhere in the world (Lotti et al., 2015; Rikmann et al., 2014). Anammox treatment has major savings compared to standard treatment processes (nitrification-denitrification), therefore, its usage for municipal waste stream (mainstream) handling could be very important (Zekker et al., 2016). For 4000 L pilot scale anammox granular biomass system, municipal wastewater treatment achieved average total nitrogen removal rate (TNRR) of 182 (± 46) g N $m^{-3}d^{-1}$ at 19 (± 1)°C (Lotti et al., 2015). For conventional 200 L pilot mainstream-like low temperature MBBR treatments, TNRRs have been 1.67 g N m^{-2} d^{-1} at 19°C whereas at 13°C and 16°C, removal rate was 0.55 and 0.81 g N m^{-2} d^{-1}, respectively (Persson et al., 2014). Note, that for sludge-based systems volumetric TNRRs are given whereas for biofilm systems, often carriers area-specific TNRRs are given. For mainstream treatment, anammox application has several obstacles, like: diminished temperature, elevated organic carbon concentration (Gilbert et al., 2014; Udert et al., 2008) and therefore a high quantitative ratio of total organic carbon/total nitrogen (TOC/TN). In addition, the anammox method efficiency decreases sharply at temperatures below 15°C or at temperature values more than 40°C (Ni et al., 2010). Elevated TNRR of two kg of N per m^{-3} d^{-1} has been associated with anammox reactors treating low-concentrated wastewater acquainted to municipal waste stream at a low temperature of 16°C (Ma et al., 2013). However, TNRR dropping from 0.465 kg N m^{-3} d^{-1} to zero kg N m^{-3} d^{-1} has occurred at 29°C for treatment of similar stream. A TNRR of 0.46 kg N m^{-3} d^{-1} at 12.5°C during a reactor treating pretreated municipal wastewater has conjointly determined (Laureni et al., 2015). A doable choice to overcome the problems associated with long-term mainstream treatment is the periodic switching of biomass between the hotter sidestream and the colder mainstream to be efficient in biofilm reactors in autotrophic TNRR sustaining. In this way, anammox microorganism will have time to adapt in low TOC/TN ratio and hot temperature sidestream treatment conditions. The foremost common downside of high organic carbon presence in mainstream conditions would be the competition for substrates between autotrophic (anammox) and heterotrophic microorganisms (denitrification). Mature anammox biomass might short-termly tolerate TOC concentrations around one thousand mg C L^{-1} (Jin et al., 2012; Fernández et al., 2012) and TOC/TN quantitative ratio around 0.4/1 (Shu et al., 2016). Application of anammox method at elevated organic carbon to TN

concentration ratios (TOC/TN or COD/TN) is, therefore, a matter of interest. Industrial wastewaters from numerous chemical and energy manufacturing industries, (pretreated) manure (Bernet & Béline, 2009) may be treated by anammox method when the association of autotrophic bacterial efficiencies being tailored to high organic carbon concentrations. Moving bed biofilm reactor (MBBR) may be promising reactor type for anammox mainstream and sidestream treatment systems, as biomass for this type of bioreactor is connected to the inner biofilm layer, enabling sufficient retention of biomass and tolerance against other bacteria caused inhibition (Mehrdad et al., 2014; Rikmann et al., 2014; Zekker et al., 2012). As typically nitrite is an inhibiting and limiting substrate for anammox microorganisms in sewage treatment systems (Bettazzi et al., 2010; Raudkivi et al., 2017), partial endogenous nitrate-reducing organisms occurring in mixed biomass consortia, may be participating as nitrite utilizing group against high concentrations at certain treatment intervals (Shu et al., 2016).

Novel solutions, like periodic ORP values management, are necessary for optimizing performance of treatment processes of nitrogen-rich wastewaters (supernatant from anaerobic digestion, landfill leachate). Coupling partial nitrification (ammonium reaction to nitrite) with anammox method during a single tank treatment via a method known as deammonification has gained increasing attention (Rikmann et al., 2018; Zekker et al., 2014), however, nitrogen removal improvement will take a protracted time while not a specific management mechanism to anammox bacteria is carried out (Lackner et al., 2015; Lackner & Horn, 2012b). Autotrophic N removal may be administrated with a high efficiency within the case of a self-controlled system or ORP-controlled (Dapena-Mora et al., 2006; Lackner et al., 2012). ORP values of +120 to -40 mV (vs. standard hydrogen electrode (SHE)) are found to be optimum within one cycle of anoxia of anammox SBR, with ORP showing much higher sensitivity dynamics within 120 mV units in the case of interval aeration applied, whereas DO is fluctuated solely within 0.3 increments (Lackner & Horn, 2012b). On-line variables used for management of deammonification processes embody coupling of the subsequent parameter's control separately such as: pH (Rikmann et al., 2018), conductivity and ORP (Lackner et al., 2012), DO (Jin et al., 2008), NH_4^+-N and NO_3^--N control measures by ion selective sensors application (Lackner & Horn, 2012b). The ORP parameter will summarize and mirror the concentration of DO, the conduction of NH_4^+-N and NO_3^--N, organic compounds, activity of microorganisms, and a few cyanogenetic compounds within the reactor with only 1 detection (Holman & Wareham, 2003). Mistreatment of wastewater is associated by a poor aeration management in wastewater treatment plant (WWTP) systems, which can be supported by periodic ORP measuring, which will discover the rates of the ORP decrease from the slopes of ORP–time curve at ammonium depletion points. As DO measurements among the required values vary for nitritation–anammox (0.02–0.2 mg L^{-1}) systems or lacks in signal stability, the ORP provides consistent information conjointly at low DO levels, as it has also ealier been reported (Holman & Wareham, 2003).

Events like the depletion of organic carbon or ammonium within the biofilm at low-oxygen conditions, and aerobic/anoxic/anaerobic conditions changes, may without a delay be detectable by on-line ORP profiles. The precise logical program supporting ORP management must be created for the SBR operational cycles for efficient N removal management as anammox biomass has low inhibition thresholds towards nitrite, free ammonia (FA), and DO concentrations. ORP might be a lot more appropriate and a lot more consistent parameter for anammox system management and helps to shorten the lengths of the operational cycles (both aerobic and anoxic) compared with pH or DO control, because of ORP's wider values range (several hundred mV) (Lackner & Horn, 2012b). The broad values of ORP signal permits to outline the shorter lengths of aerobic and anoxia phases to realize higher specific substrate removal rates at shorter treatment times, thereby lowering water treatment prices. Optimized ORP borderline value set-up allows actual detection of depletion of ammonium and DO, or accumulation of excess nitrite and nitrate, avoiding operational disturbances like overloading, underloading, overaeration, and under-aeration of waste treatment reactors (Tanwar et al., 2008). For the treatment of high-strength wastewaters, fast signal responses are of utmost importance to avoid inhibition. Hence, to discover the result of the ORP decline rate on substrate depletion and on the activity of fragile floccular deammonification biomass, associate degree in-depth study of ORP management is needed. Optimized ORP drop rates supporting substrate consumption rate in every treatment step of associate degree SBR cycle might considerably shorten the total treatment method time. For the deammonification method, while not inhibition, further aeration management as double management is of essential importance once applying ORP change-based method maintenance. The latter has not nonetheless been shown to be a stable management parameter. Sufficient amount of intervals between hypoxia and aerobic phases and controlled DO levels are needed to shorten water treatment cycles and to avoid the direct restrictive effects caused by substrates-free ammonia and free acid (FNA) on the anammox bacteria and to limit the expansion of accumulation of nitrate (van der Star et al., 2007). Management of DO concentrations and ORP values could be a key issue for operational inhibition-sensitive floc-based anammox systems like SBR (Vázquez-Padín et al., 2009). DO reversible inhibition on associated degree anammox bacterium activity in an SBR was reportable at on top of bound values: >0.3 mg O_2 L^{-1} (Udert et al., 2008), >0.5 mg O_2 L^{-1} (Lackner & Horn, 2012a), and 0.7 mg O_2 L^{-1} (De Clippeleir et al., 2009). The appliance of the SBR system for the deammonification method operation permits enables easier management of wastewater retention time, and guarantees even distribution of substrate and fine biomass retention. Microbial consortia ought to be developed involving AOB, anammox bacteria, and a minor quantities of heterotrophic denitrifiers (van der Star et al., 2007). In different articles (Schaubroeck et al., 2012), high specific anammox activities (SAAs) (range 141–700 mg N g^{-1} VSS d^{-1}) were achieved for the granular anammox SBR within the treatment of artificial wastewater, whereas per (Udert et al., 2008), the organic fraction was

conjointly removed from autoclaved supernatant and from diluted source-separated urine. Floccular sludge systems have not been studied in terms of considering the combinatory results of ORP decrease rates and DO management in case of anammox method. Therefore, this thesis studied the floccular anammox process control by ORP decrease rate.

The slow rate of growth of anammox bacteria has driven the requirement for optimum parameters for the expansion and cultivation of anammox microorganisms (Rikmann et al., 2014; Xu et al., 2018). Because the acquisition of the foremost appropriate anammox biomass is costly, anaerobic sludge can be expeditiously used for the start-up of anammox bacterium cultivation, ending with a home-grown biomass additional tailored to the needed effluent concentrations (Rikmann et al., 2018).

The development of associated anammox biomass has shown interest to be used in the suspended sludge-based sequence batch reactor (SBR) system, that embodies flexibility of operation, compactness (Mace & Mata-Alvarez, 2002), stability and resiliency, with a short biomass recovery time (Laureni et al., 2019). AOB are found at the outer surface of the biomass, where they consume DO, and, inside the associated hypoxic zones, anaerobic bacteria thrive, facilitating the co-existence of AOBs and anammox bacteria (Zekker et al., 2020). The salinity at optimal concentration of 3–10 g NaCl L^{-1} has enhanced the formation of anammox granular sludge, whereas higher salinities (30 g NaCl L^{-1}) (Windey et al., 2005) have benefitted on the anammox process (Jin et al., 2012). Increased salinity leads to a high-pressure level of the bacterium, which might cause dormancy or plasmolysis of bacterium. Compared to denitrifiers, anammox bacteria have shown adaptation to high saline concentrations (Cho et al., 2020; Jin et al., 2012). Although, the exerted repressive impact on anammox bacteria by NO_2^- is reversible within a short time (Lotti et al., 2012), it can be recovered effectively by hydrazine as a reducing agent addition (Zekker et al., 2012). The anammox method itself is affected by reducing compounds, such as hydrazine (N_2H_4) and nitric oxide (NO) gas presence, as these anammox intermediates at optimal concentration promote TNRR (Schalk et al., 1998). Furthermore, reducing agent hydrazine is employed within the production of additional energy for anammox bacteria, and this consequently helps within the acceleration of growth of the anammox biomass assuring the start-up method efficiency. The external addition of N_2H_4 limits the buildup of NO_3^- and the production of a problematic greenhouse gas- N_2O (Ganesan & Vadivelu, 2019). The extra N_2H_4 might directly alter the anammox ratio so additional proton is employed per mole of regenerated ammonium ion, since additional protons and electrons alter anammox reaction, whereas nitrate production is diminished in the process. This transformation within the anammox reaction is free of other metabolic activities of different bacteria present in the biomass. Anammox bacterium is able to unambiguously metabolise associated hydrazine as an energy source (Schalk et al., 1998). The utilization of a non-specific aerobic, anaerobic, and fermentation biomass has found very little application within the cultivation of anammox bacteria so far, with the utilization of the anammox intermediate hydrazine

to accelerate the anammox process and destimulate different heterotrophic bacteria activity. Hydrazine addition facultatively shortens the non-anammox-specific biomass cultivation time, which has not been given wide attention so far. Some works have highlighted the positive impact of the reducing agent enhancing anammox biomass TNRR, with associated optimum concentration of 4 mg of N_2H_4 L^{-1} (Yao et al., 2013). Experiments performed inside the associate anammox system showed that the addition of 2–5 mg of N_2H_4 L^{-1} considerably improved TNRE ($p < 0.01$) and raised the method stability. This N_2H_4 concentration has been beneficial to anammox bacteria while not harming AOBs, however inhibiting nitrite oxidizing bacteria (NOBs) (Xiang & Gao, 2019). However, systematic studies of the impact of N_2H_4 on all major groups of bacteria concerned in N removal (anammox, AOBs, NOBs, denitrifiers) are lacking. N_2H_4 addition contributes to a speedy recovery of the anammox biomass from nitrite inhibition, however its impact within the case of harmful effects from excessive salinity is additionally attainable. A significantly higher hydrazine concentration (>15 mg of N_2H_4 L^{-1}) was discovered to weigh down the positive effect of hydrazine to nitrogen removal (Xiang & Gao, 2019). If hydrazine dosing enables a significant reduction from the start-up time and from the recovery time of the inhibition-damaged anammox biomass, it is simply applied during a WWTP operation, employing a supply of hydrazine salt (also called hydrazinium sulfate salt, $N_2H_6SO_4$), delivered by a dosing pump in the form of liquid chemical solution. Hydrazine salt is easy to supply to WWTP, its not volatile, poses no pollution risks, is simple to handle, and has low risks for its storage. The objective is to optimize the impact of N_2H_4 addition on the specific anammox activity (SAA) and denitrification activity throughout different stages of the anammox start-up to accelerate the autotrophic nitrogen removal. A further aim is to show the impact of salinity on the anammox start-up and overcome the harmful impact of excess salinity with hydrazine addition.

Anammox process

Disappearance of some of accumulated NH_4^+ in the anoxic marine environments was observed as early as 1965 (Mulder et al., 1995). Based on this fact, the hypothesis was proposed that a group of unknown microorganisms produced N_2 when oxidating NH_4^+ by using NO_3^- as an electron acceptor. In 1977, the possibility of oxidation of NH_4^+ by using both- NO_2^- and NO_3^- as electron acceptors was confirmed *via* thermodynamic calculations and the existence of anaerobic ammonium oxidising (anammox) bacteria in nature was already predicted and developed further (Mulder et al., 1995). The anammox process was first discovered in a denitrifying fluidized bed reactor treating reject water (supernatant from a methanogenic reactor) and was confirmed by nitrogen and redox balance in continuous-flow experiments (Egli et al., 2001). The autotrophic anammox bacteria belonging to the phylum *Planctomycetales* can convert NH_4^+ to N_2 with nitrite as an electron acceptor under anoxic conditions. Eq. (1) represents the anammox catabolism. Combining the anammox catabolism

and anabolism with the experimentally obtained yield of bicarbonate / ammonium (0.066 mol HCO_3^-/1 mol NH_4^+) results in the Eq. (2) of the overall anammox reaction (Jetten et al., 1998):

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O \quad (1)$$

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2\uparrow + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (2)$$

Equations (1) and (2) have been applied as intrinsic stoichiometries of anammox process since 1999 in almost all studies worldwide. However, in a recent study with the operation of high purity free suspended cell anammox bacteria, a different stoichiometric equation has been proposed (Fernández et al., 2012):

$$NH_4^+ + 1.146\ NO_2^- + 0.071\ HCO_3^- + 0.057\ H^+ \rightarrow 0.986\ N_2\uparrow + 0.161\ NO_3^- + + 0.071\ CH_{1.74}O_{0.31}N_{0.20} + 2.002\ H_2O \quad (3)$$

Deammonification represents a short-cut in the bacterial conversion of NH_4^+ to N_2 and comprises two steps: about a half the amount of NH_4^+ is oxidised to NO_2^- and then, residual NH_4^+ and NO_2^- are transformed to molecular nitrogen in an anoxic pathway. The first step involves aerobic oxidation of NH_4^+-N by AOB, in a process called nitritation.

In the second step, the anammox reaction occurs, leading to the formation of N_2 and a production of a small amount of NO_3^-. Nitrate can also be formed in the oxidation of nitrite by NOB. The latter is considered undesirable in deammonification, since it reduces the efficiency of anammox process. To some extent, denitrification occurs in anammox and deammonification systems (removing NO_3^-), depending on the availability of substrate. Due to low C/N ratio in rejected water and high content of ammoniacal species (500÷1000 mg NH_X-N·L^{-1} in Tallinn WWTP and up to 1300 mg NH_X-N·L^{-1} in Tartu WWTP (Rikmann et al., 2018)), an anammox-based technology would be suitable to treat the reject water from anaerobic sludge treatment separately from mainstream in order to reduce the total nitrogen load in the WWTPs.

Various reactor configurations have been used to start-up the anammox process in laboratory, pilot and industrial scale, including fluidized bed reactors (Lotti et al., 2014), sequencing batch sludge-based (Sliekers et al., 2002) and biofilm reactors (Zekker et al., 2012), up-flow anaerobic sludge blankets (Rikmann et al., 2014) and membrane bioreactors (Xu et al., 2014), among other reactor types (Wett, 2006). Examples of industrial autotrophic nitrogen removal technologies requiring inoculation at start-up phase include SHARON® (Kampschreur et al., 2008), CANON (Cema et al., 2006), OLAND (Jeanningros et al., 2010), DEAMOX (Kalyuzhnyi et al., 2006), BABE® and PANDA (Salem et al., 2004, Sharp et al., 2020), DIB and DEMON (Wett, 2006), Anita™Mox (Veuillet et al., 2014) and CANDO (Scherson & Criddle, 2014) technologies.

3. MATERIALS AND METHODS

Anammox process TNRRs and efficiencies were estimated after anammox bacteria growth in MBBR and in SBR systems. MBBR and SBR systems as well as 800 mL batch test vessels were involved in the experiments to perform efficient anammox process, nitrogen removal and test process efficiencies and rates depending on COD/TN, TOC/TN, oxidation-reduction potential (ORP), salinity values and hydrazine concentrations. Microbiological analyses were done to assess changes in microbial composition during reactor operation under certain parameters (i.e., mainstream versus sidestream treatment).

Redox potential control can be done earlier in anammox studies and as wastewater is typically not heterogenous, redox potential can be controlled at optimum values for efficient process with less air and electricity consumption. That broadens the redox potential control applications in real WWTP operation.

3.1. Anammox enrichment in moving bed biofilm reactor (MBBR) (Paper I)

A plexiglass moving bed biofilm reactor (MBBR) (with 20 L liquid volume) was applied for the enrichment of anammox microorganisms at desired temperatures (21.6 (± 2)°C prior to sidestream and 16.5 (± 3.5)°C for mainstream). The reactor was equipped with a water jacket and the desired temperatures were maintained by an Assistant 3180 (Assistant, Germany) waterbath thermostat. Anammox biofilm was earlier developed onto the biofilm of the carriers's surface, whereas carriers were of polyethylene material (Bioflow 9, Aquamyc, RVT Process Equipment GmbH, Germany). Continuously fed conditions of anaerobic digester supernatant (NH_4^+ source) gained from one of the Estonian municipal wastewater treatment plant (WWTP) were created. Mainstream-like wastewater was made from NH_4Cl and OECD synthetic wastewater. The carriers used with a specific surface area ≈800 m^2 m^{-3}, were filled about 50% of the liquid volume of the reactor (an overall surface area being 1.78 m^2). The anammox process was previously operated for prolonged time of 2260 days before presented operational system was operated with changed wastewater flows. Reactor run performed by PhD applicant consisted of 2 periods: mainstream wastewater supplementing phase with diminished nutrient quantities (<100 mg NH_4^+-N L^{-1}) and a low temperature of 16.5 (± 3.5)°C; and reject water (sidestream) supplementing phase with high nutrients concentration (~1000 mg NH_4^+-N L^{-1}) and temperature of 21.6 (± 2)°C. Standard deviations of temperatures in mainstream and sidestream operation periods were (± 3.5)°C and (± 2)°C, respectively.

These phases (mainstream and sidestream) were changed after every 8 weeks. The value of pH was measured with a pH meter (Evikon, Estonia) being averagely at 7.16 (±0.65) and 7.37 (±1.25) for mainstream and sidestream

operation, respectively. DO concentration was maintained by DO controller (Elke sensor, Estonia) at the range up to 1.5 mg L^{-1}.

The MBBR system's total nitrogen loading and removal rate (TNLR and TNRR, correspondingly) were calculated based on feed flow rate, influent and effluent ammonium, nitrate and nitrite parameters, and carriers' total specific surface area/ reactor volume and daily feed flow rate present. Flow rate of the feed varied during reactor operation from 4.67–14.5 L d^{-1}. Biofilm total suspended solids (TSS) content was determined as previously shown (Zekker et al., 2017).

3.2. SBR inoculation and operation (Paper II)

The sequence batch reactor was inoculated with *inoculum* received from the pilot-scale SBR plant (3 m^3) performing nitritation–anammox process in purifying reject water from nitrogen compounds (Rikmann et al., 2018). Ammonium-containing (600–1300 mg N L^{-1}) diluted digester effluent water from the Tartu wastewater treatment facility (Estonia) was applied for the reactor influent.

The anammox reaction was studied in a 9.6 L system equipped with a water jacketed and thermostated environment at 25.0 (±0.5) ℃ (using Assistant 3180 thermostate, Germany). The reactor was maintained with ion-selective online sensor (Hach-Lange, Germany) involving NO_3^--N and NH_4^+-N measuring and probes for ORP (Ponsel, France), pH (Elke Sensor, Estonia) and DO (Ponsel, France) detection were used. The essential controlling of DO was based on the ORP. Optimum value of ORP decrease rate was set for the airflow to be stopped when the certain, such as ORP decrease values of 0.4, 0.9, and 1.65 mV min^{-1} were reached. The alternative tertiary DO control within aerobic conditioned time was DO value-based: airflow was disconnected at DO contents <0.5 mg L^{-1} (after 400 days of operation); till 1.5 mg L^{-1} (earlier than 220 days of operation). Anoxia within the reactor was defined 5 min after finishing of the aerobic cycle, whereas DO content decreased towards 0 mg L^{-1}. An extensively prolonged – 37.5–48 h lasting hydraulic retention time (HRT) was involved to protect against bacterial inhibition, otherwise inhibition could be happening by supplementation of the non-diluted feed. The pH value was within 6.5–8.2 as suitable for nitritation anammox performance.

Consecutive stages were involved in SBR operation: 15 min of reactor feeding, aerated conditions for 3– 30 min, anaerobic conditions for 30–60 min, settling time of 1 h and a 15 min effluent discharge phase.

The lengths of current stages overall were 37.5–48 h. The rates of ORP decrease were optimized at determined values within 0, 0.4, and 1.65 mV min^{-1}. At these ORP decrease rates, the biomass settling stage started, and followingly purified effluent was coming out of the reactor and water samples were taken.

TNREs and TNRRs were computed at concentrations of ammonium present on the feed and effluent, flow rate, influent and effluent nitrite, nitrate con-

centrations, and volatile suspended solid (VSS) content of SBR using below shown formulas:

$$TNRE=(TN_{inf} - TN\_{eff})/ TN\_{inf} \times 100\%, \quad (3)$$

$$TNRR=Q \times (TN_{inf} - TN_{eff})/TN_{inf} \quad (4)$$

where $[N]_{inf}$ and $[N]_{eff}$ are the sums of nitrogen contents (NO_3^--N, NO_2^--N, NH_4^+-N) in the influent and effluent, respectively (g N/ day) and Q shows daily flow rate, L/day.

The specific activity of anammox biomass sufficient to nutrient removal depends on the wastewater needed to be treated and hydraulic retention time applied. For municipal wastewater, biomass removal rate of 100 g N m^3 d^{-1} is sufficient whereas for rejected water biomass nitrogen removal rate needs to be around 1000 g N m^3 d^{-1} when 1-day hydraulic retention time is applied.

3.3. SBR operation at different salinity levels (Paper III)

In the thesis, an SBR involving a volume of 20 L was applied, with a height of reactor of 52 cm and an inner diameter of 25 cm. Initial stage of the SBR work was a reactor feeding process with NH_4Cl (24 g of N L^{-1}), $NaNO_2$ (30 g of N L^{-1}), or reject water, applying a peristaltic pump (Seko, Italy). The reactor was mechanically stirred at rates of 200 (±5) revolutions per minute (rpm). The SBR content was settled for 30 min within each cycle. Effluent discharge was done followingly. Involved temperature within SBR was 30 (±0.5) ℃, and the hydraulic retention time (HRT) was set at 2–3 days. The reactor was maintained with a thermostate and a water jacket. HRT was ensured by a replacement of water of 10 L just at the end of the settlement to diminish the changes caused by elevated nitrate content and salts influence (Tenno et al., 2016, 2018) within the SBR.

3.4. *Inoculum* and Operation Conditions in the Bioreactor (Paper III)

5 L of anaerobic sludge and aerobic sludge were suplemented to the reactor toward the start of of inoculation. The amount of *inoculum* in the SBR was determined by a mixed liquor-suspended solids (MLSS) content. The MLSS content at the beginning of operation and after inoculation was 10.8 (±0.25) g L^{-1}, and the MLSS was 9.0 (±0.05) g L^{-1} following seven days of operation, bringing the process activity down due to the nitrifying and denitrifying bacteria' elimination to the effluent. Following fourteen days of operation, the SBR was operated under a plastic hood to overcome NO_2^- oxidizing to NO_3^-. The salinity level and nitrogen species (NH_4^+ and NO_2^-) contents were controlled by supplemented $NaNO_2$ (30 g of N L^{-1}), NH_4Cl (24 g of N L^{-1}) and

rejected wastewater. The latter consisted suitable mineral components and NH_4^+–N (up to 1400 mg of N L^{-1}) (Zekker et al., 2020). Within the biological activity period, the total NH_4^+ and NO_2^- content in the inflow was approximately 100–150 mg of N L^{-1} to reproduce the TN content available in the mainstream wastewater. The TN concentration in the effluent and influent water was measured in mg of N L^{-1} of summary NO_2^-, NO_3^- and NH_4^+ concentrations after the samples were centrifuged at 4500 g.

3.5. Analytical methods

Effluent and influent contents of the NH_4^+-N, NO_2-N and NO_3^--N (TN- the sum of those) were determined spectrophotometrically by the method shown by (Greenberg et al., 1992). VSS, HCO_3^- and TSS contents were measured according to (Greenberg et al., 1992). Water samples were centrifuged at 4,000 g for 10 min and solids were removed from the samples before analysis. The pH of the samples was measured using a pH meter coupled to a Jenway pH electrode (Germany), and the DO was measured using a Marvet Junior (MJ2000, Estonia) DO analyser. TOC concentrations were measured with a multi-N/C UV-Vis analyser (Analytic Jena).

3.6. Chemical Analysis and Calculations

A Metrohm chromatograph (model 930 Compact IC Flex 1) supplied with column for anions determination (Metrosep A Supp 5–100/4.0) was used for measurements of nitrate/nitrite and salinity using 3.2 mM of Na_2CO_3 and 1.0 mM $NaHCO_3$ solutions as eluents. Cuvette tests of Hach-Lange LCK 514 and LCK 314 for chemical oxygen demand (COD) measurements were applied by PhD applicant according to the instructions set by manufacturer, while the tests were run on a spectrophotometer of Hach-Lange DR 2800. Nessler's method based on colorimetry (Hach Lange 8038 method) was involved for measuring ammonium. Hydrazine was measured applying HydraVer reagent (Hach-Lange, Germany) using the spectrophotometer for detecting light absorbance of prepared samples. MLSS measuring was done as per (Greenberg et al., 1992). For pH determination, pH meter E6115 supplied by Evikon was applied.

Hydrazine concentration was measured by PhD applicants from batch tests media. Hydrazine supplementation to wastewater treatment reactor was done using time-controlled peristaltic pump (Paper III). Hydrazine is not toxic if small quantities of hydrazine sulfate are added. In the form of hydrazine sulfate salt, it is not a volatile and hazardous substance. It can be volatile if hazardous hydrazine wastes (fuel waste or others) are stored at open athmosphere where they are not rapidly oxidized.

TNRRs and TNREs were computed from the measured influent flow rate, effluent and influent ammonium, nitrate, nitrite measures, and the rate of wastewater flow to the bioreactor as described above.

For calculating the SAA, regressions of concentrations of nitrogen consumption were used, while the result division by determined MLSS content (g/L) as well as by time (h) was done. Statistical analyses were done by Pearson correlation (two-tailed) for determination of the correlations for parameters. Furthermore, t-tests by Student's method were made applying the M.S. Excel toolpack for statistics. The level of significance for measurements was taken at $p < 0.05$.

Furthermore, F-test and the two-way t-tests, respectively were applied for homogeneity of variances in groups and the distinction between group means being analysed.

3.6.1. Pyrosequencing

DNA extraction and amplification

Microbial analyses of *inoculum*, rejected water, mainstream operation biomass, and sidestream operation adapted biomass from the MBBR and from the operation of SBR were analyzed by pyrosequencing. DNA extraction was done with PowerBiofilm® DNA Sample Kit for isolation of DNA (Mo Bio Laboratories Inc., U.S.A.) using instructions set by the manufacturer. Extraction was done with 0.13 g of inoculation sludge and 0.09 g of sludge adapted to 5 MBBR carriers (TSS 0.017 g carrier^{-1}) or 25–50 mg of SBR bacterial mass. Thereafter, the V3-V4 hypervariable regions of the 16S rRNA gene were PCR amplified and sequenced by Illumina MiSeq 2×250 v2 platform (Estonian Genome Center). Used universal primers were: ARC344F (5′-ACGGGGYGCAGCAG GCGCGA-3′ (Raskin et al., 1994) and Arc806R (5′-GGACTACVSGGGTAT CTAAT-3′ (Takai & Horikoshi, 2000).

3.6.2. Taxonomic profiling

Sequence data was analysed using BION-meta open-source programme according to author's instructions. Firstly, forward sequences were cleaned at both ends using a 99.5% minimum quality threshold for at least 18 of 20 base pairs (bp) for 5′-end and 28 of 30 bases for the 3′-end. Secondly, reads less than 230 bp were eliminated. After that, chimeras were cleaned, and reads were grouped by oligonucleotide similarity at a level of 95% (k-mer length of 8 bp, step size 2 bp). Consensus readings were aligned to the SILVA reference 16S rDNA database (v123) using a word length cut-off of 8 and a similarity cut-off of 90% (Quast et al., 2013). Further details about the microbial measurement techniques involved are discussed in (Mandel et al., 2019).

4. RESULTS AND DISCUSSION

This thesis established results on wastewater treatment by MBBR operation through mainstream and sidestream switching (Paper I), SBR control mechanisms by ORP (Paper II), and by proper salinity and hydrazine levels (Paper III). Followingly, reactors results shown in thesis are presented along with their comparison with other researches for potential usage in water sector.

4.1. The anammox system mainstream treatment on the moving bed biofilm reactor operation.

The effect of switching the reactor influent between mainstream-like and sidestream wastewater was studied on the moving bed biofilm reactor operation (Paper I).

To begin, the MBBR system was operated with both – mainstream and sidestream wastewater because there were no previous scientific reports on the long-term effect of switching the reactor influent between mainstream-like and sidestream wastewater on anammox biomass stability. Anammox bacteria activity typically decreases over time at low temperatures (Zekker et al., 2015) and with a high organic carbon to nitrogen ratio present in mainstream feeding (Lotti et al., 2015). Therefore, after a certain period, the MBBR's feeding media were switched between mainstream and sidestream wastewater (around 8 weeks). Anammox bacteria were fed with mainstream wastewater for a longer period at a time. Denitrification often began to dominate over the anammox process during long-term mainstream feeding (8 weeks), but the process efficiencies were reversed when reject water feeding was resumed. The longer anammox bacteria are fed to mainstream wastewater with an extremely high TOC/TN ratio, the less active they become due to additional low temperature inactivation effects. The anammox process was sustained again during the subsequent warm temperature sidestream treatment. Performance of the biofilm reactor with mainstream-like wastewater at 16.5 (±3.5) °C resulted in the maximum TNRR of 61 g N m^{-3} d^{-1} (Figure 1). Standard deviation for temperature within mainstream operation was (± 3.5)°C. Average TNRR achieved for sidestream wastewater treatment was 180 (±140) g N m^{-3} d^{-1} and for mainstream it was 20 (±15) g N m^{-3} d^{-1}. Therefore, standard deviations for TNRRs for sidestream and sidestream wastewater treatment were (±140) and (±15), respectively. High anammox TNRRs were achieved already within first months, so long-term operation for achievement of anammox stability is not often needed.

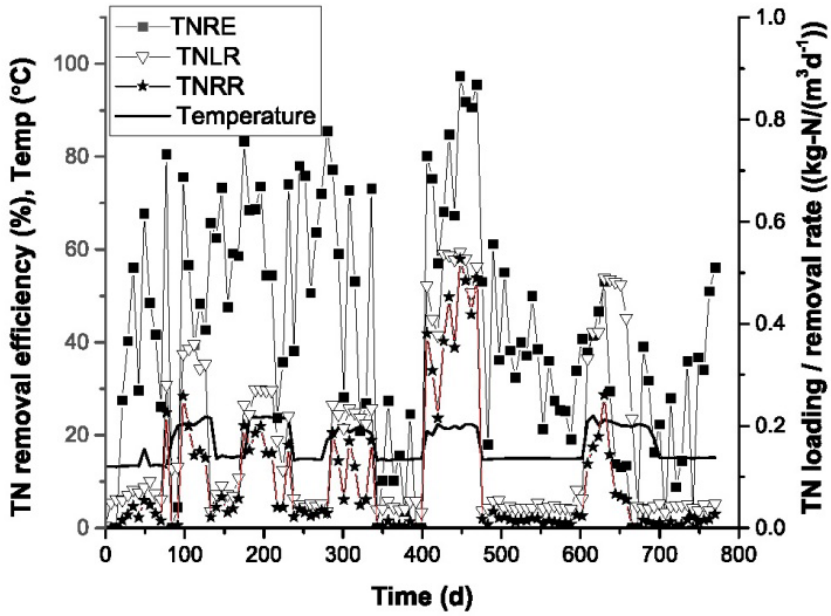

Figure 1. TNREs, TNLRs and TNRRs during mainstream and sidestream MBBR operation days depending on the temperature change.

The maximum TNRR gained for sidestream operation was 527 g N m^{-3} d^{-1}, after the fourth cycling with rejected and municipal wastewater flows, demonstrating the long-term sustainability of biomass to perform the anammox process. The highest TNRR of 61 g N m^{-3} d^{-1} was achieved after the second cycling with mainstream for reactor feeding with municipal wastewater-like synthetic feed, with similar TNRRs achieved in the fifth, fourth, third mainstream treatment steps. Following the achievement of high TNRRs in the fourth sidestream wastewater cycling, the fifth cycling with mainstream wastewater was extended (19 weeks) to determine biomass capability to treat organic-rich wastewater in the long-term with a high COD concentration in the mainstream-like influent. Effluent COD was also lower during the last cycling (107 (±48) mg L^{-1}), after receiving a high COD/TN ratio of 1/1 in the influent (Figure 2) to cope with the long mainstream feeding period. Standard deviation for effluent COD value was (±48) mg L^{-1}. The average TNLR was 60 (±10) g N m^{-3} d^{-1}, with a TNRR of 20 (±15) g N m^{-3} d^{-1}. Standard deviation for TNLR was (±10) g N m^{-3} d^{-1} and (±15) g N m^{-3} d^{-1} for TNRR. The average TNRR for mainstream feeding was not significantly lower in the fifth period (16 g N m^{-3} d^{-1}) than in the previous four feedings by mainstream (*p-value* >0.05). It was discovered that bacterial adaptation to mainstream feed occurs after several cycles with both influents.

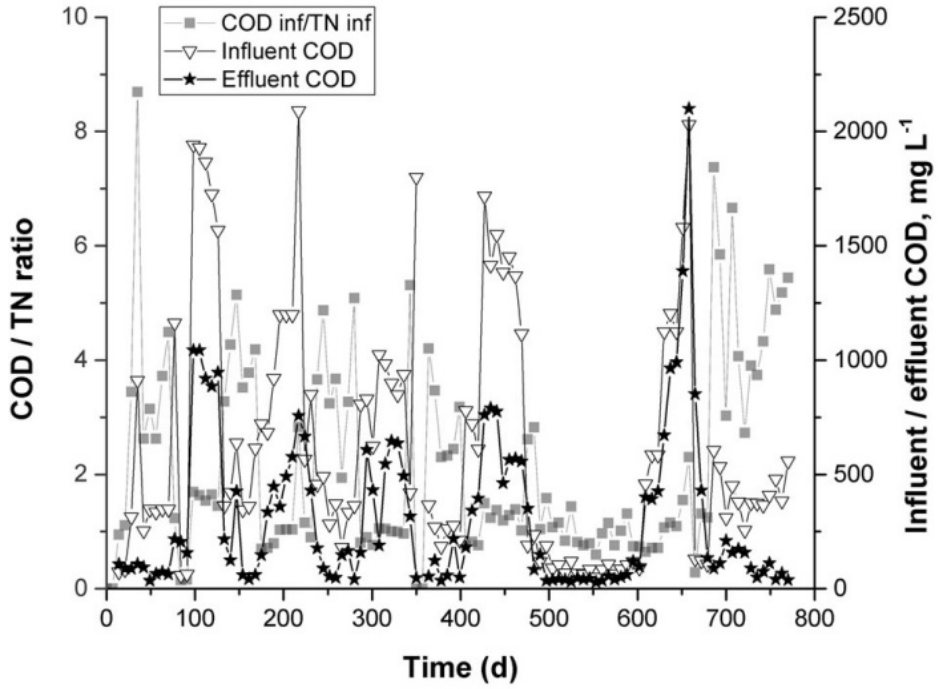

Figure 2. Influent COD/influent TN ratios, influent and effluent COD concentrations during mainstream and sidestream MBBR operation days.

Increasing the TNLR above 250 g N m^{-3} d^{-1} during the sidestream influent-involved period, it also caused increasing TNRR around 200 g N m^{-3} d^{-1}. The system involving increased TNLRs enhanced the maximum TNRE achieved to 96% during the treatment of sidestream wastewater with a moderate average TNRE of 51 (±27) %. Standard deviation for TNRE during treatment of sidestream wastewater was (±27) %. 8 weeks after, when TNLR was decreased to 50 g N m^{-3} d^{-1} by using mainstream wastewater-like feed, effluent ammonium concentration decreased to less than 70 mg N L^{-1} and the average TNRR diminished approximately to 20 g N m^{-3} d^{-1}. The values of two other ion contents, such as nitrite (average 11 mg N L^{-1}) and nitrate (average 33 mg N L^{-1}) were low in the effluent (Figure 3). Mainstream feeding period showed low average TNRE achieved – 35 (±23) %. Standard deviation for the TNRE in treatment of mainstream wastewater was (±23) %.

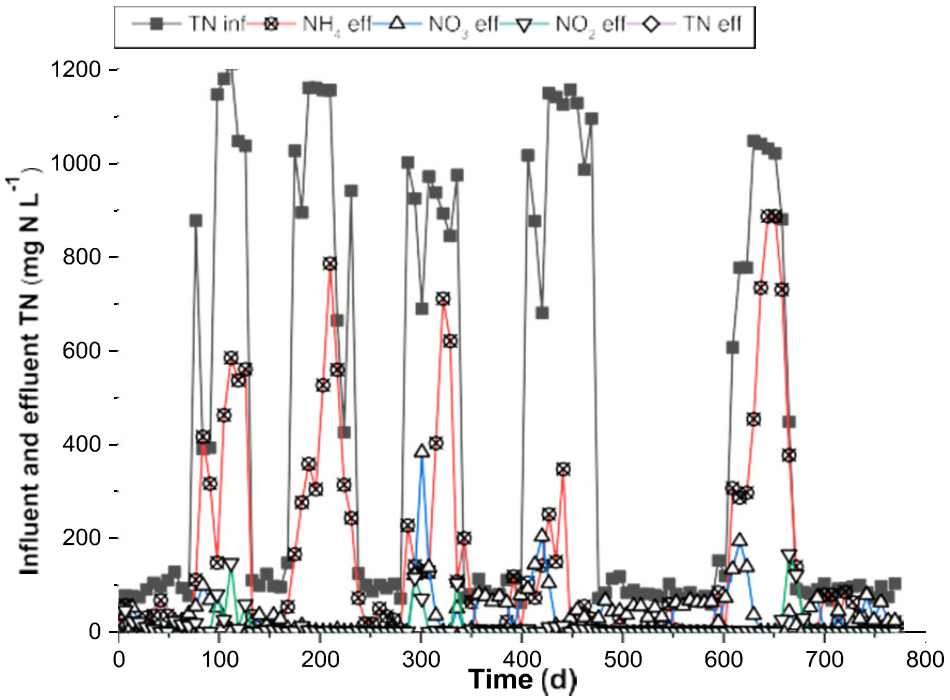

Figure 3. Influent and effluent TN forms concentrations during mainstream and sidestream MBBR operation days.

For anammox treatment process, it has become essential to show NO_2^- $_{consumed}$ to NH_4^+ $_{consumed}$ ratio to calculate pure anammox process efficiency and differ it from other processes, such as heterotrophic denitrification. Nitrite and ammonium simultaneous consumption are present only with anammox reaction. Perfect anammox NO_2^- $_{consumed}$ to NH_4^+ $_{consumed}$ ratio has been proven experimentally to result at 1.32/1 (Strous et al., 1999). In theory, broader values of NO_2^- consumed/NH_4^+ consumed ratios of 1.0–1.5 are suitable for anammox treatments, too. Anammox efficiency has been differed previously by different concentrations of organic carbon, which have limited the ratio NO_2^- $_{consumed}$ to NH_4^+ $_{consumed}$ with enhanced organic carbon (Hassan et al., 2018).

The average ratios of consumption of NO_2^- /NH_4^+ $_{consumed}$ for sidestream influent and mainstream influent-related operation, were 1.10/1 and 1.67/1, respectively. For sidestream operation cycle, the ratio was less and for mainstream cycle it was more than depicted as optimum consumption ratio of 1.32/1 presented earlier (Strous et al., 1999**;** Zekker et al., 2013), showing elevated nitrite consumption efficiency during mainstream step. The average ratios of NO_3^- $_{produced}$ /NH_4^+ $_{consumed}$ for the sidestream influent and mainstream influent feeding time were 0.88/1 and 0.34/1, respectively. The ratio was increased for the sidestream operation, but remained same for mainstream operation compared to stoichiometrical ratio (Strous et al., 1999). It was due to generation of nitrate was performed by anammox bacteria (7.6% of total bacteria), not by

NOBs (*Nitrospira*, *Nitrobacter* 0% of total bacteria) during sidestream treatment. Stoichiometry was also altered by the changes in the denitrifiers abundance detected during mainstream (0.4% of *Pseudomonas* presence) and sidestream-feeding (0.1% of *Pseudomonas* presence) cycles. Anammox and denitrifying bacteria abundance was increased during mainstream operation as compared with results from sidestream treatment stage.

4.1.1. The effect of the COD/TN ratio

For mainstream treatment phase, an elevated COD/ TN ratio of 3.2/1 was represented. The average COD/TN ratio for mainstream was more than twice higher than for sidestream operation: 3.2/1 (±1.9/1) and 1.1/1 (±0.72/1), correspondingly (Figure 2). In the mainstream, COD/TN ratio has been normally between 3.7/1–2.9/1 (shown by (Ji et al., 2018) and for sidestream around 1/1 or below that. Lower COD concentrations (300 mg L^{-1}) detectance within mainstream period was presented. For sidestream period of operation, corresponding values were around 1090 mg L^{-1}.

The MBBR recovered from low TNRRs achieved within mainstream operation after changing feed to reject water along with a limited COD/TN of 1.1/1. TNRRs recorded were 150–200 g N m^{-3} d^{-1}. Within the municipal wastewater treatment, normally, COD concentration is 250–800 mg L^{-1}, occasionally it is higher for WWTPs receiving industrial wastewater (Jalilzadeh et al., 2013). Elevated COD content and even more, an elevated COD/TN ratio kept at prolonged period, could increase heterotrophic denitrifiers quantity and make a shift of the relative abundance of bacterial consortia towards decreased anammox bacteria quantities. But, according to microbial analyses, anammox bacteria quantities were determined to be higher in case of mainstream treatment as compared to sidestream treatment (see microbial analyses part). Anammox process being limited at high COD concentrations due to increase in heterotrophic bacteria growth is reversible.

High effluent ammonium concentration and pH of 8.2 (±0.37) present in the reactor during sidestream operation period (600 mg N L^{-1}) can inhibit NOBs growth by free ammonia (FA) exceeding 10 mg N L^{-1}, thus, mainly nitrite as a substrate for anammox process is produced (Figure 3). The inhibitory FA concentrations are 6 mg N L^{-1} to NOBs, 16 mg N L^{-1} for AOBs, to anammox bacteria 10–17 and 50 mg N L^{-1} to heterotrophic denitrifiers (Zekker et al., 2014).

4.1.2. MBBR microorganisms determined by pyrosequencing

The microbial community present in *the inoculum* and in the anammox biofilm during mainstream and sidestream feeding was described in Figure 4.

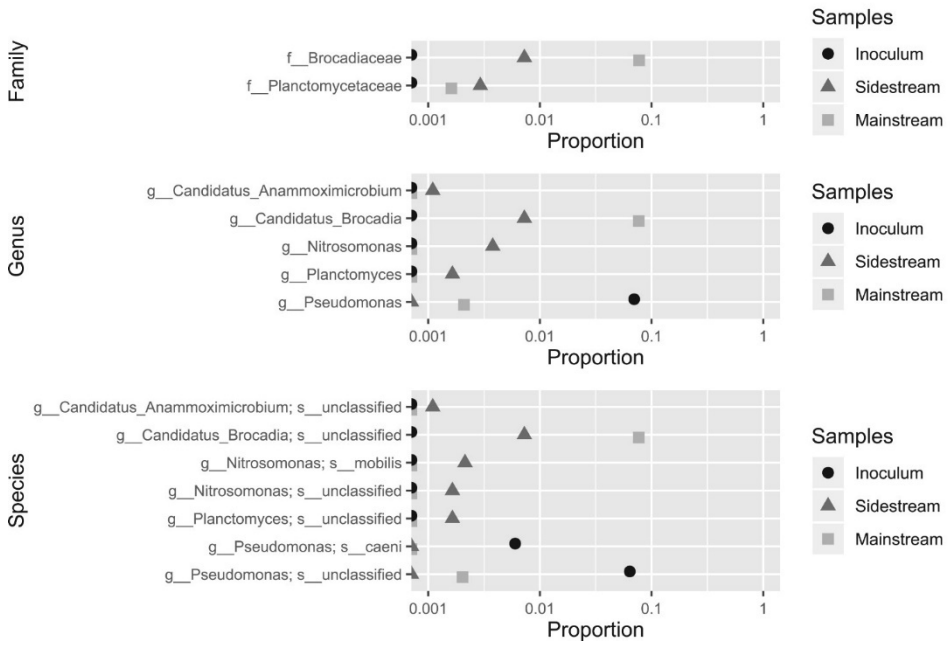

Figure 4. Shifts in microbial communities between inoculum, sidestream and mainstream period.

Regarding to anammox bacteria, DNA sequences belonging to uncultured *Planctomycetales* bacterium *Candidatus Brocadia* were detected at the highest quantities from biomass taken during mainstream operation (7.6%), followed by the anammox species *Planctomyces* and *Candidatus anammoximicrobium*, the quantities of which were highest for the sidestream operation period, followed by mainstream operation and *inoculum* bacterial abundances. Earlier, using ammonium, nitrite and bicarbonate has enabled to achieve the enrichment of *Candidatus Brocadia fulgida* (Kartal et al., 2007) and *Planctomycetes clone P4* (Zekker et al., 2017) in the reactor biomass. Aside from anammox bacteria, aerobic ammonium oxidizing organisms were found in the biomass during the sidestream operation, whereas *Nitrosomonas* unclassified species were present in low proportions in the mainstream and *inoculum*. *Nitrosomonas mobilis*, an aerobic ammonium oxidizing bacterium previously discovered in low-oxygen conditions (Zekker et al., 2017) was one of those discovered. Additionally, the aforementioned aerobic bacteria could have performed incomplete denitrification (NO_2 and NO reduction).

The biofilm did not contain nitrite oxidizing bacteria such as *Nitrospira*, which have been present in the anammox MBBR under very low oxygen conditions, as previously discovered (Park & Noguera, 2008).

4.2. ORP-controlled nitritation–anammox process in SBR (Paper II)

For 600 days, single nitritation–anammox SBR was run to study the impact of different ORP decrease rates on the achievement of efficient aerobic/anoxic nitrogen removal, as well as high process's efficiencies. The deammonification nitrogen removal is limited by nitrate and ammonium high values, which could be sustained low by using ORP and aeration control without importantly lowering feed TN loads in the SBR.

The reactor was operated in four phases: one without ORP control and three with aeration and cycle time management based on the ORP values decrease rate.

At the beginning (0–210 days), the SBR operation based on anammox N-removal was performed with no ORP value limitations. The average value of gained TNRR was 90 (±31) g N m^{-3} d^{-1} (with biomass specific activity of 76 mg N g^{-1} VSS d^{-1}). The TNRE achieved averagely resulted in 80% (Fig. 5). During the operation with no controlling of ORP, the highest TNRR of 160 g N m^3 d^{-1} (SAA 89 mg N g^{-1} VSS L^{-1}) with a highest TNRE of 95% was achieved during a 132-day operation period without ORP control. Despite the high influent TN values (500–800 mg N L^{-1}), low average effluent TN values achieved were: 3 mg NH_4^+-N, 0.5 mg NO_2^--N, and 64 mg NO_3^-–N L^{-1}. A smaller average TNRR of 90 g N m^{-3} d^{-1} (SAA 76 mg N g^{-1} VSS L^{-1}) with a sufficiently high average TNRE of 80% was gained within a 210-day run (Fig. 5). As a comparison, a maximum TNRE was achieved in Paper I at 96% during the treatment of sidestream wastewater in MBBR with a moderate average TNRE of 51 (±27) %. The maximum TNREs were similar between two systems, showing repetitiveness of experiments. Lower average TNRE for MBBR (Paper I) compared to SBR (Paper II) was achieved due to the effects of periodical introduction of mainstream wastewater in case of MBBR.

Moreover, a significantly enhanced average TNRRs at 120 g N m^{-3} d^{-1} were gained in the following period between 211–600 days by the SBR's operation cycle being completed with a certain ORP decrease rate values (such as: 0.4, 0.9 and 1.65 mV min^{-1}), guided by the OPR measuring device. Furthermore, within various operation periods, batch assays were done at beforementioned ORP decrease rate values fixed during testing and regulated for reactor cycle completion. Maximum SAA achieved was 4.4 (±1.8) mg N g^{-1} VSS h^{-1} when the ORP decrease rate value of 0.4 mV min^{-1} was applied in batch assays (Table 1). Standard deviation for SAA measuring was (±1.8) mg N g^{-1} VSS h^{-1}.

Following 258 days of SBR run at ORP monitoring control fixed at 1.65 mV min^{-1}, mean NO_3^- produced was according to stoichiometry of anammox pro-

cess predicted (95 mg NO_3^--N L^{-1}). At the final days of the period, NOB got adapted to conditions maintained and further lowering of DO values through ORP control was needed. Thereafter, the decrease rate of the ORP was declined more to 0.9 mV min^{-1} to limit nitrate formation enabled by excessive aeration and NOB adaption (Fig. 6).

461 days and afterwards, SBR process and operation with the lowest ORP decrease value was fixed at the 0.4 mV min^{-1}. TNRRs peaked at the largest values of 220 (±32) g N m^{-3} d^{-1} with TNLR applied being 230 g NH_4^+-N m^{-3} d^{-1}. Standard deviation for TNRRs at the period was (±32) g N m^{-3} d^{-1}.

ORPs changing at low range of –150 to 0 mV during operation, emphasized more that negative ORP values are optimal for SBR operation. Average TNREs gained after 461 days were 60%. During the 461–600 days, a high average TNRR of 100 g N m^{-3} d^{-1} with a moderate average TNRE (68 (±50%)) was achieved due to involving of ORP parameter set-up at low ORP decline rate values of 0.4 mV min^{-1}, which limited DO content below <0.5 mg L^{-1}. Standard deviation for TNRE at the period was (±50) %.

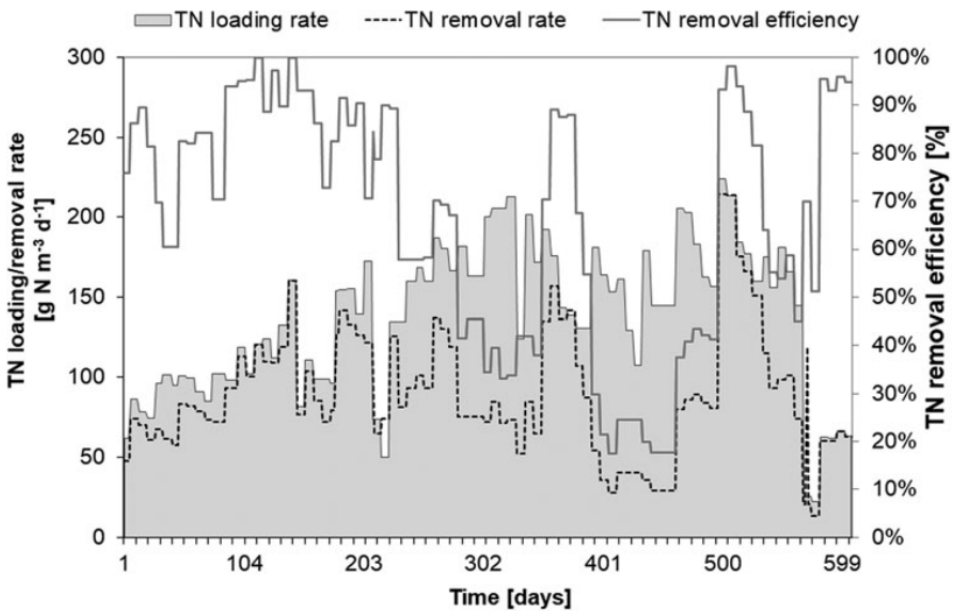

Figure 5. TNREs and TNRRs are shown in relation to the ORP decline rates within SBR operation. TNRR- total nitrogen removal rate; ORP- oxidation – reduction potential; TNRE- total nitrogen removal efficiency.

Table 1. Specific Anammox Activities and TNRRs depending on the ORP and decrease rates of ORP within reactor operation and batch analyses SAA, specific anammox activity; ORP, oxidation–reduction potential; VSS, volatile suspended solid; TNRR, total nitrogen removal rate

Reactor operation time (days)	Reactor TNRR (g N m^{-3} d^{-1})	Aerobic/ anoxic period lengths (min min^{-1})	ORP range (mV)	ORP decrease rate (mV min^{-1})	VSS (g L^{-1})	Batch SAA (mg N g^{-1} VSS h^{-1})
0–210	90	30/30	-150 to +50	4.2	1.37 (±0.64)	1.35 (±0.45)
211–421	83	15/40	-50 to +150	1.65	2.55 (±0.95)	1.9 (±0.5)
422–460	40	15/40	-250 to +30	0.9	2.49 (±0.14)	3.4 (±1.5)
461–600	100	5/60	-150 to -60	0.4	2.28 (±0.52)	4.4 (±1.8)

Nitrite concentration averagely in the effluent during this step was sufficiently low: 7 (±1.5) mg N L^{-1}. Standard deviation for effluent nitrite concentration at the period was (±1.5) mg N L^{-1}.

Higher applied pH of the influent at 8.21 (±0.15), and elevated influent NH_4^+ concentrations (500–800 mg N L^{-1}), presented on day 0–50, enabled efficient NOB suppression being ensured through the elevated FA concentration presence during each start of feeding (>10 mg NH_3-N L^{-1}). Then, the effluent NO_3^--N concentration decreased from 70 to 18 mg N L^{-1}. According to De Clippeleir et al. (2009), enhancement of anoxic stage duration has sustained limited nitrate presence and eliminated inhibition by nitrite, the $NO_3^-N_{formed}/NH_4^+N_{removed}$ ratio staying at optimum anammox-reaction ratio range of 1.32/1 or near to it.

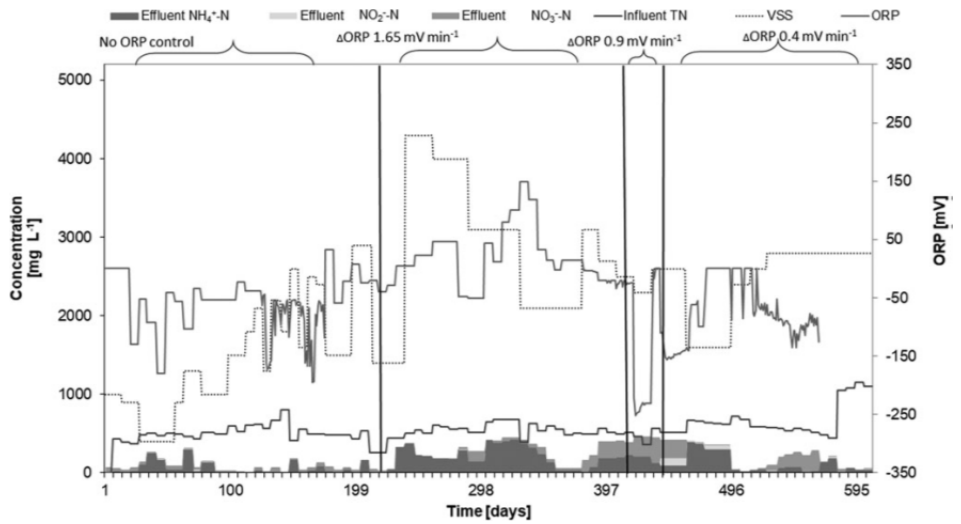

Figure 6. TN concentrations, VSS, ORP changes in the influent and effluent during reactor operation at different ORP values.

4.2.1. Pyrosequencing and qPCR observations

Microorganisms' parametrical abundances within the biomasses of SBR at different stages during the reactor run (days 76–600) and influent *inoculum* (rejected water) composition were measured. According to pyrosequencing tests performed on the bacterial mass sampled at 482th day, 3 related anammox micro-organisms' strains were seen (*Candidatus Brocadia anammoxidans, Candidatus Anammoximicrobium, uncultured Planctomycetales bacterium clone P4*) in the *inoculum* and SBR biomass. *Candidatus Kuenenia* strains, which are also typical anammox organisms' strains, were not detected. Various heterotrophic bacteria were determided from the reactor, too.

Uncultured *Planctomycetales bacterium clone P4* (Gen- Bank: DQ304521) were determined from the biomasses sampled on day 482, increasing from 2.8×10^4 to 1.6×10^6 copies g^{-1} TSS (Figure 7). *Planctomycetales* bacteria re-presented in bacterial biomass belong to the order *Brocadiales*, being closely related to strains, such as *Candidatus Brocadia fulgida*.

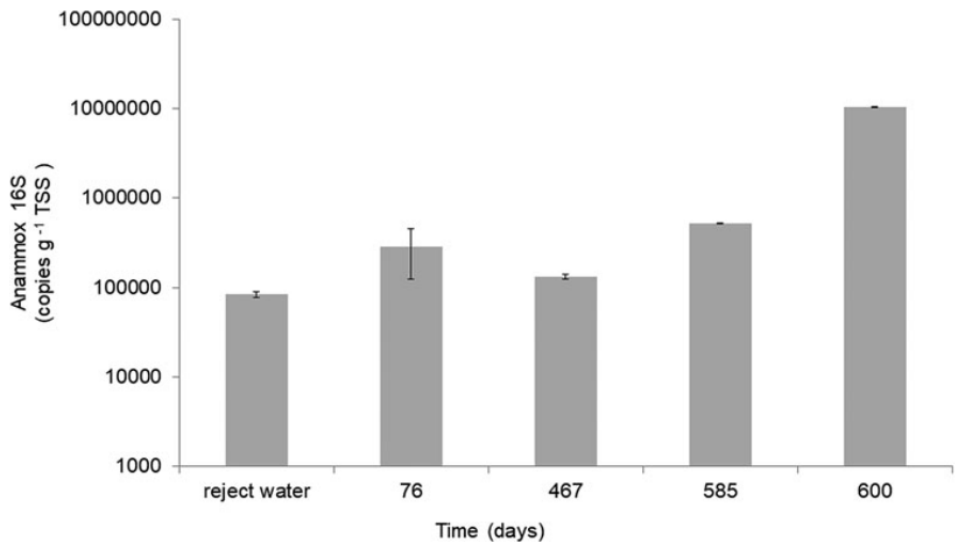

Figure 7. Gene copy numbers of anammox 16S rRNA at different reactor operation days.

Among aerobic N-oxidizers, *Nitrosomonas sp*. and *Nitrosomonas mobilis* at equal shares (0.2%) were present in the biomass taken on the 482nd day from SBR with *Nitrobacter spp*. and *Nitrospira* species (unwanted NOBs) not being present. The absence of the latter is attributed to low nitrate production in SBR. *Anammoxoglobus/Brocadia*-like species have been typically shown to be present in the autotrophic wastewater treatment reactors (van der Star et al., 2008).

Bacterial sampling and analyses along with reactor operation data proved there was success in treating anaerobic tank effluent by a process operation based on the control of ORP values and ORP decrease rate in the anammox system.

4.3. Sequence batch reactor performance at varied salinity and hydrazine levels (Paper III)

As salinity is strongly affecting anammox activity, its effect was also studied in the current thesis (Paper III). Division of SBR into three phases during the start-up of SBR was done based on salinity levels represented.

4.3.1. SBR Inoculation, Operation and Operational Phases

At the initiation of the reactor N-removal, *inoculum* gained from anaerobic and nitrification–denitrification water treatment processes was grown to anammox N- removal carrying out microbial consortium. The TNRR and TNRE determination of the anammox SBR in its performance was split into 3 stages. The first stage was the bacterial activation and acclimatization stage, with the use of only nitrite supplementing in the form of $NaNO_2$ (0–64 days). The second stage was the elevated salinity stage (content of 18.2 g Cl^- L^{-1}) (65–110 days), carried out by the supplementation of $NaNO_2$ and NH_4Cl as nitrogen and salinity contents. Salinity was enhanced by the supplementation of NH_4Cl and $NaNO_2$, enabling Cl^- and Na^+ enhanced measures. The 3rd stage consisted of supplementation of anaerobic tank effluent and nitrite as N feeding contents (111–194 days), achieving proper salinity conditions (0.5–2 g Cl^- L^{-1}). The salinity decline was measured by the end of the NH_4Cl addition in the feed.

The starting NH_4^+-N content in the reactor being 220 mg of N L^{-1}, continued to drop after treatment despite supplemental feeding of $NaNO_2$, NH_4Cl and anerobic tank effluent as N feeding contents. SBR resulted around 80% of TNRE on average during the end of 110-day operation (Figure 8) maintained by low organic carbon environment and optimized salinity values. The TNLR increased at day 0 from 0.012 kg N m^{-3} d^{-1}, up to 0.1 kg N m^{-3} d^{-1} after the end of operation days. Corresponding TNRRs registered were maximally around 0.08 kg N m^{-3} d^{-1} (Figure 8).

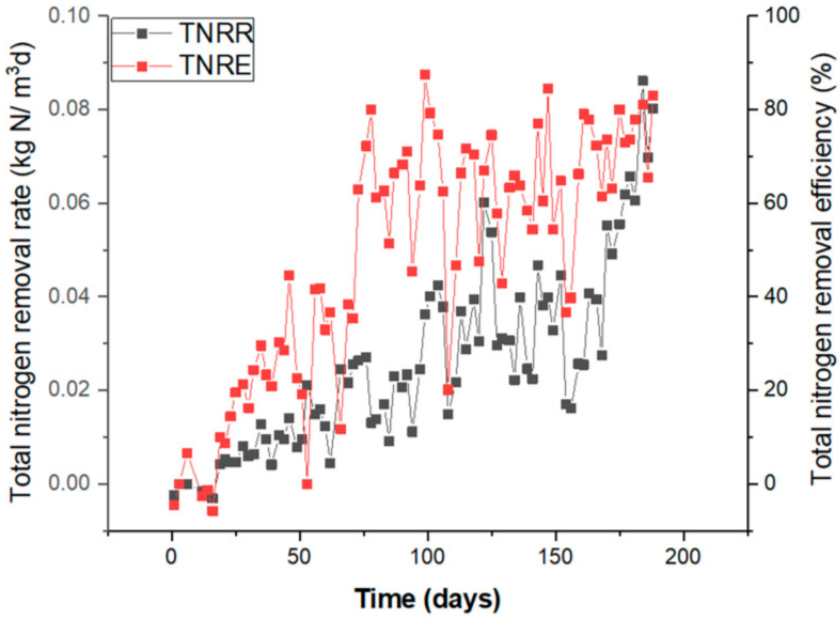

Figure 8. Operation of the SBR with related TNREs and TNRRs registered during 194 days of reactor run.

4.3.2. Adapting/Activity increase Stage

The adapting/activity increase stage was presented for 65 days of reactor operation and was named as a diminished salinity stage (<0.2 g Cl L^{-1}). NH_4^+, NO_2, and organic pollutants came from the *inoculum* as the main substrates represented in the SBR. The changes between the effluent and influent NH_4^+-N contents showed an increase in the effluent NH_4^+-N content in the SBR, whereas the NO_2^--N in the effluent dropped a lot in comparison to influent (Figure 8) by the organic carbon source aid coming from *inoculum*. Anammox microorganisms could grow together with bacteria mediating nitrate reduction dissimilatorily to ammonium (DNRA), shown by the decline of nitrite and nitrate at the initial step (Castro-Barros et al., 2017; Shu et al., 2016). Some of the NO_2^--N losses observed in this phase could be occurring because of denitrifying bacteria activity. Organic contents shown to be affecting bacterial processes during adaptation stage acted as carbon sources for denitrifying organisms, with a subsequent co-utilization of NO_2^--N (Figure 9).

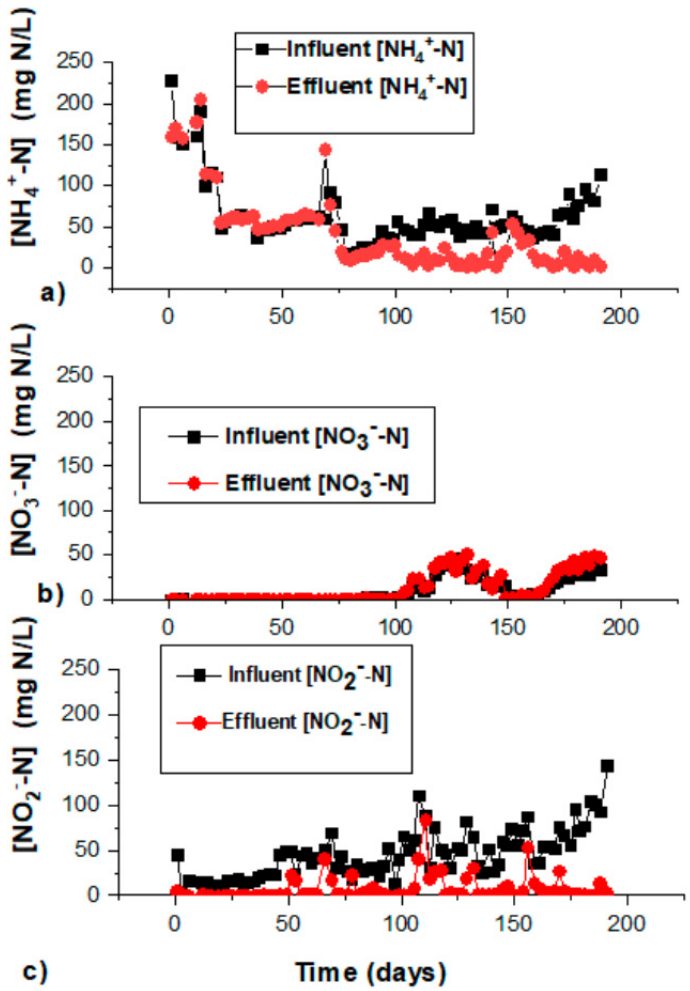

Figure 9. Changes in the effluent and influent (a) NH_4^+ content; (b) NO_3^- content; and (c) NO_2^- content during reactor operation.

TNRE within the low saline stage was between 20% to 40% (Figure 8). The calculation of the TN contents removed in the stage was mostly gained from NO_3^--N and NO_2^--N utilisation (Figures 9b, 9c). NH_4^+ content was changing little at the start showing limited anammox activity. Denitrification reaction was mostly occurring at the beginning.

4.3.3. High Salinity Phase

Within the stage, the anammox process was happening with a decline in nitrite and ammonium contents, without important effect of elevated salinity present in the reactor. Enhancement of the salinity of SBR was observed up to values 10–18 g of Cl^{-} L^{-1}. This stage was occurring during days 65–110 (Figure 9).

TNRRs seen were maximally at 0.05 kg N m^{-3} d^{-1}; TNREs shown were at 67–87%. Contents of NaCl at 3–15 g of NaCl L^{-1} have shown to be enhancing a retention of the microbial mass and helping to formulate anammox granules (Dapena-Mora et al., 2007), and theoretically wash-out denitrifying microorganisms. Choosing such salinity values was due to earlier researches stating that 6–10 g of salinity can stimulate AOBs activity and anammox performance, whereas enhanced values (30 g of NaCl L^{-1}), limit the anammox process (Dapena-Mora et al., 2007; Okabe et al., 2011; Oshiki et al., 2011). Above 30 g L^{-1} of NaCl, AOB and anammox performance have shown a decline from 95% and 43% for low-salt tolerant microbes, correspondingly, whereas for the AOB and anammox biomass adapted to elevated salinity, the decline has been lower- 59% and 24%, respectively (Windey et al., 2005). In our experiments, the TNRR declined for 30% in case of increased salinity stage application as compared with suitable salinity stage TNRR values achieved.

The stoichiometric ratio of NO_2^{-}-N/NH_4^{+}-N in the outflow of SBR was 2.52 (±0.51)/1 at the start of phase and below 1.31/1 in further treatment, receiving a value of 1.03 (±0.16)/1 as average (Table 2). NOB activity was diminished at the high saline stage being smaller than at the optimized saline stage (Figure 9), as shown previously (Windey et al., 2005). As per earlier researches, there were depicted as follows: *Candidatus Kuenenia stuttgartiensis* could adapt to elevated saline conditions with contents of 10% KCl, 90% NaCl (Kartal et al., 2006). High salinity negative effects for anammox organism can also be reversed by lowering the salinity (which was done in our last stage). Overall, majority of the anammox species are thriving in freshwater conditions, but *Brocadia anammoxidans* can handle limited salinity as shown earlier (Jin et al., 2012; Windey et al., 2005).

Table 2. The anammox ratio based on the stoichiometric mass balance of NO_2^{-}-N/NH_4^{+}-N within the SBR operation phases. ± represents standard deviation of 3 replicate measurements.

Operation stage (Days)	**NO_2^{-}-N/NH_4^{+}-N**
66–99	2.52 (±0.51)/1
101–125	1.03 (±0.16)/1
126–145	1.12 (±0.12)/1
146–194	1.33 (±0.06)/1

4.3.4. Optimum Salinity Phase

There has been demonstrated at 110–194 days of SBR running, that the optimized anammox process behavior can be achieved at optimum salinity values. The conditions with optimized salinity were depicted at values less than 0.5 g of Cl^{-} L^{-1} within the stage, bringing much better TNRRs than achieved at high concentrations of 18.2 g of Cl^{-} L^{-1} represented within the earlier step. The operation of SBR in the current stage at optimized salinity values was maintained by the stop of NH_4Cl supplementation and the appliance of anaerobic tank effluent water as ammonium source. The achieved TNRE maximum value was seen at 87% (Figure 8) with TNRR values seen at 0.08 kg N m^{-3} d^{-1} during the period with optimized salinity. The stoichiometric ratio of NO_2^{-}-N/NH_4^{+}-N was at 1.12 (±0.12)/1 during days 126–145. The stoichiometric NO_2^{-}-N/NH_4^{+}-N ratio was changed during following period to averagely to 1.33 (±0.06)/1 on 146–193 days, showing that the SBR biomass gained sufficiently efficient anammox performance.

Previous studies have highlighted the successful anammox start-up by using different inocula. The start-up has been achieved at a low temperature using Upflow Anaerobic Sludge Blanket (UASB) and a membrane bioreactor setups (Wu et al., 2018; Zekker et al., 2014). By applying traditional activated sludge biomass in the reactor, the launching of the anammox system has been done during 110 days (Wang et al., 2012).

Table 3 presents the represented TNRRs gained within the initiation of the anammox process for various systems presented in the research field. Various setups were run at distinctive conditions, such as the optimized temperature and reactor configuration, and with anammox type of biomass. The adjacent conditions have been considered to gain the initiation of the anammox system; the efficiency, and the TNRR has been varied with respect to various parameters. The successful launching of anammox system has been carried out at a limited temperature (10–20 °C) within SBR system coupled with membranes to achieve a TNRR of 0.027 kg N m^{-3} d^{-1}, as a comparison TNRR in gas-lift reactor achieved has been at 0.29 kg N m^{-3} d^{-1} (Hendrickx et al., 2014; Hendrickx et al., 2012). Hu et al. (2013) results with TNRR of 0.028 kg N m^{-3} d^{-1} have been lower than that gained in the current study (0.08 kg N m^{-3} d^{-1}).

Table 3. Comparison of anammox TNRRs for various reactors and types of sludge.

Reactor	Temperature (°C)	Start-Up Time (Days)	Volume of Reactor (L)	TNRR (kg N m^{-3} d^{-1})	Include Nitritation	Sludge Type	Reference
RBC	29	300	2.5	0.60	+	Start-up	(De Clippeleir et al., 2013)
SBR/MBR	10	727	4.2	0.027	-	Mixed activated sludge	(Hendrickx et al., 2014)
MBR	35	110	4.8	0.159	+	Conventional activated sludge	(Wang et al., 2012)
Gas-lift reactor	20	253	4.5	0.29	_	Anammox sludge	(Hendrickx, et al., 2012)
SBR	30	345	5	0.028	+	Start-up	(Hu et al., 2013)
SBR	30	193	20	0.127	-	Start-up with activated and anaerobic sludge	Current study

5. CONCLUSIONS

Possible mainstream treatment application of anammox biomass via feed switching between warmer sidestream and colder mainstream wastewater with a high organic carbon concentration was investigated in the current thesis (Paper I). Long-term tests in the biofilm reactors showed average TNRRs of 180 (±140) g N m^{-3} d^{-1} for sidestream wastewater treatment and 20 (±15) g N m^{-3} d^{-1} for mainstream. Standard deviations of TNRRs for sidestream and mainstream wastewater treatments were (±140) and (±15) g N m^{-3} d^{-1}, respectively.

Based on the performed experiments, it can be concluded that nitrogen removal occurs in the treatment of the mainstream, through both- denitrification and the anammox process with a decline in TNRRs for sequential mainstream introduction to MBBR. Nitrogen removal for the mainstream feed at high TOC/TN ratios of 3.2/1 was still carried out.

The degree of fluctuation of the batch tested TNRR was affected by the organic content value. However, for anammox bacteria, a more suitable sidestream environment with higher temperatures and lower organic content was most appropriate. The highest TNRR in the batch test was achieved at a low COD concentration of 480 mg L^{-1}, reflecting a TNRR of ≈5 mg N g^{-1} TSS h^{-1}. For a high COD concentration of 2600 mg L^{-1} (TOC/TN = 8/1), TNRR decreased similarly in both feeding media to 1.6 mg N g^{-1} TSS h^{-1}. The anammox microorganism's *Brocadia* enrichment in deammonification biofilm showed 7.6% of bacterial abundance out of all bacteria during mainstream feeding. Therefore, long-termly cultivated autotrophs dominating the biofilm can remove nitrogen also from mainstream through the anammox process when wastewater flows between the sidestream and mainstream are periodically switched.

The nitritation–anammox process was sustained in the floccular SBR system in the long term (600 days) (Paper II). The low ORP decrease rate of 0.4 mV min^{-1} enabled the TN loading rate to be increased from 128 to 230 g N m^{-3} d^{-1}, achieving a maximum TNRR of 220 g N m^{-3} d^{-1} at ORPs of -70 to 0 mV. Deammonification-related microorganisms contained in the SBR system resulted in an average TNRE of 63% and a TNRR of 100 g N m^{-3} d^{-1} (SAA of 85 mg N g^{-1} VSS d^{-1}). The most efficient nitrogen removal, without accumulation of ammonium and nitrate in the reactor through the control of ORP, was achieved from days 500 to 511 on, with a high achieved TNRR of 220 g N m^{-3} d^{-1}. During the whole ORP-controlled period, high average TNRRs were achieved-83 (±32) g N m^{-3} d^{-1}. It was discovered that the suitable times for the aeration and anoxic phases could be 3–5 min and 60 min, respectively.

Findings suggested a real-time ORP control was suitable for monitoring of nitritation–anammox SBR operation during operation period. Various N-converting and anammox organisms were detected from the samples taken from the SBR, such as *Candidatus Brocadia fulgida* and uncultured *Planctomycetales bacteria clone P4* strains. Uncultured *Planctomycetales bacterium clone P4*

(GenBank: DQ304521) quantity on days 76–600 was increased from 2.8×10^4 up to 1.6×10^6 copies g^{-1} TSS.

Based on the tests carried out, it can be concluded that ORP as a control parameter is able to reduce the energy requirements for aeration, at the same time maintaining a high autotrophic nitrogen removal rate.

Another SBR system operation was carried out at 30 (±0.5) °C, which worked for a period of 193 days to assess salinity impact on anammox process. The start-up of the anammox cycle was accomplished within 65 days of development of the biomass. The operation of SBR was begun with a non-anammox-based *inoculum* sludge, applying a hydraulic retention time (HRT) maintenance at 2–3 days during the start-up time frame. Different salinity conditions were attempted for anammox start-up. Reject water gave the ideal salinity of 0.5–2 g of Cl^- L^{-1}, with a fundamentally higher nitrogen removal rate than seen in the tests during the high salinity operation time (18 g of Cl^- L^{-1}). A typical summary nitrogen removal efficiency of 80% was achieved at the ideal salinity period.

The addition of trace amounts of hydrazine short-termly was able to enhance anammox activity after tests with a high C/N ratio were maintained. The start-up of the anammox process for a suspended biomass was successful, with hydrazine significantly accelerating anammox activity (Paper III).

Main Challenges for the Treatment of Real Wastewater by Anammox Process

Overall, the essential difficulties for the treatment of raw wastewater through the anammox process are connected with the process optimisation to fulfill full-scale operation strategies for the development and sustaining of an anammox biomass. Making automatization of the SBR setup is vital for the full-scale anammox technique execution. The propagation of anammox organism entities can be accomplished through the guidance and supplementation of hydrazine to high-saline and medium-saline conditions and at inhibiting circumstances. Assessment of the effect of moderate temperature on anammox biomass is required to improve microorganisms' activity for the purification of mainstream wastewater at low temperature (standard wastewater conditions). Adjusting of microorganisms' activity with a high C/N proportion within wastewater should synchronously endeavor anammox and denitrifiers activities, which thus should not restrain anammox process application in mainstream conditions. Ideal amounts of hydrazine conveyed to the anammox system could contend with denitrifiers in such circumstances. Microbial assessment at larger scope at the beginning of operation is required for a process improvement, essentially to achieve the greatest anammox microorganisms' quantities in various operating conditions and for various waste flows.

6. SUMMARY IN ENGLISH

The increase in the amount of generated waste today threatens the quality of the environment and human health. Biological treatment of wastewater is an energy-efficient way to remove organic and inorganic pollutants from wastewater and to reduce the likelihood of residues entering the groundwater. The implementation of several biogas plants involves the conversion of organic carbon into biogas and the presence of elevated nitrogen and phosphorus compounds in the effluent. Treatment of such waste streams is costly with traditional methods (nitrification-denitrification), which require adding organic carbon back to the treatment process (often in the form of methanol). Among wastewater treatment technologies, the anaerobic ammonium oxidation process (anammox) has been incorporated into autotrophic nitrogen removal in pilot and full-scale technologies for nitrogen removal from waste with low organic carbon content.

The mainstream can be called domestic sewage, and the side stream centrifuged anaerobic sludge reject water. The latter is formed after the fermentation and centrifugation of excess sludge. Attempts have been made to incorporate the anammox process into both sidestream and mainstream treatment using moving media biofilm reactor (MBBR) and batch scrubber (SBR) technologies.

The main obstacles to be overcome in order to achieve a successful use of the anammox process in the mainstream are the high carbon to nitrogen ratio of the waste (C/N > 1) and the low temperature of the wastewater (12.5–19 °C present in the Nordic climate). Previous studies using the mainstream anammox process have shown that low temperatures can cause problems with anammox biomass growth. To overcome the inhibitory effect of cold temperatures on the growth rate of anammox bacteria, the mainstream system can be inoculated with biomass grown under side stream conditions.

Changes in redox potential (ORP) affect the composition of proteins vital to bacteria or the charge of bacterial mitochondrial membranes. ORP changes can be controlled by turning aeration on/off or changing aerator-specific intensity changes using the ORP sensor signal and appropriate value ranges.

The salinity of the wastewater and the effect of hydrazine are important parameters to evaluate the course of the anammox process. Achieving an optimal salinity level increases the activity of the anammox process and the rate of nitrogen removal. An intermediate in the anammox process, hydrazine, can affect the efficiency of the anammox process but also reduces other processes, such as denitrification, to achieve high nitrogen removal autotrophically.

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8. SUMMARY IN ESTONIAN

Autotroofsed lämmastikuärastuse protsessid lämmastikuärastuseks kõrval- ja peavoolureoveest

Tekkivate jäätmekoguste suurenemine ohustab tänapäeval keskkonna kvaliteeti ja inimeste tervist. Reovee bioloogiline puhastus on energiasäästlik viis orgaaniliste ja anorgaaniliste saasteainete eemaldamiseks reoveest ning jääkainete põhjavette sattumise tõenäosuse vähendamiseks. Mitmete biogaasijaamade rakendamine hõlmab orgaanilise süsiniku muundamist biogaasiks ning kõrgenenud sisaldusega lämmastiku- ja fosforiühendite esinemist heitvees. Selliste jäätmevoogude töötlemine on kulukas traditsiooniliste meetoditega (nitrifikatsioon-denitrifikatsioon), mis nõuab orgaanilise süsiniku lisamist tagasi töötlemisprotsessi (sageli metanooli kujul). Reoveepuhastuse tehnoloogiate hulgas on anaeroobset ammooniumi oksüdatsiooni protsessi (anammox) kaasatud autotroofse lämmastiku eemaldamisse piloot- ja täismahus tehnoloogiates lämmastiku eemaldamiseks reoveest vähese orgaanilise süsiniku kasutamisega.

Peavoogu võib nimetada olmereoveeks ja kõrvalvoogu väduks, viimane tekib pärast liigmuda kääritamist ja tsentrifuugimist. Anammox protsessi on püütud kaasata nii kõrvalvoo kui ka peavoo puhastusse, kasutades liikuvate kandjatega biokilereaktorit (MBBR) ja annuspuhastuse reaktori (SBR) tehnoloogiaid.

Võrreldes kõrvalvoo puhastamisega, on peamised takistused, mis tuleb ületada, et saavutada edukas anammox protsessi kasutamine peavoos: reovee kõrge süsiniku ja lämmastiku suhe (C/N>1) ja reovee madal temperatuur (põhjamaade kliimas 12,5–19 °C). Varasemad uuringud peavoo anammox protsessi kasutamise kohta on näidanud, et madalad temperatuurid võivad tekitada probleeme anammox biomassi kasvule. Et ületada madalate temperatuuride pärssivat mõju anammox bakterite kasvukiirusele, saab peavoo süsteemi nakatada kõrvalvoolu tingimustes kasvatatava biomassiga.

Muutused redokspotentsiaalis (ORP-s) mõjutavad bakterite jaoks elutähtsate valkude koostist või mõjutavad bakterite mitokondriaalsete membraanide laengut. ORP muutusi saab juhtida aeratsiooni sisse/välja lülitamisega või aeraatoripõhiste intensiivsuse muutuste muutmisega, kasutades ORP-anduri signaali ja sobivate väärtuste vahemikke.

Reovee soolsus ja hüdrasiini mõju on olulised parameetrid, mille abil hinnata anammox protsessi kulgu. Optimaalse soolsuse taseme saavutamine suurendab anammox protsessi aktiivsust ja lämmastiku eemaldamise kiirust. Anammox protsessi vaheühend – hüdrasiin, võib mõjutada anammox protsessi efektiivsust, kuid kahandada ka muid protsesse, näiteks denitrifikatsiooni, et saavutada kõrge lämmastiku eemaldamine autotroofselt.

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PUBLICATIONS

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List of publications:

1. Zekker, Ivar; Markus Raudkivi; **Oleg Artemchuk;** Ergo Rikmann; Hans Priks; Madis Jaagura & Taavo Tenno (2020) **Mainstream-sidestream wastewater switching promotes anammox nitrogen removal rate in organic-rich, low-temperature streams.** Environmental Technology, 42(19), 3073–3082. DOI:https://doi.org/10.1080/09593330.2020.1721566
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