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THE DEAMMONIFICATION PROCESS IN MOVING BED BIOFILM REACTORS

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SUMMARY

In order to decrease the risk of eutrophication in natural water bodies, the requirements for wastewater treatment plants' (WWTPs) discharge has become stricter than previously. Around 15-20% of the nitrogen load in the mainstream comes from reject water which is produced in sludge treatment line. The partial nitritation/anammox process, which is also called deammonification, appears to be a good alternative to treat reject water. However, for this process to be effective, control strategies which are reliable and require less maintenance are needed. In this thesis, different control strategies were studied at pilot scale in order to optimise the deammonification process operated in a Moving Bed Biofilm Reactor (MBBR) for reject water treatment. Meanwhile, the processes were monitored by microbial activity tests, Specific Anammox Activity (SAA), Oxygen Uptake Rate (OUR), and Nitrate Uptake Rate (NUR) tests, in order to measure the anammox, ammonium and nitrite oxidizers and denitrifiers activity, respectively. Intermittent aeration and redox (ORP) as control parameters were studied. The results showed that intermittent aeration, with 15min non-aerated period in one hour cycle, could reduce the aeration time without any loss of process efficiency. A Redox value of pE=0 gave the best operational condition. When the redox value was fixed at 0, the aeration could adjust itself even if there was different nitrogen loads applied in the system.

Pilot scale plants with deammonification MBBR were operated at decreasing temperatures and diluted influent nitrogen concentrations towards to mainstream conditions. The reactor was run at different temperatures (25-19°C) to test the process stability and it showed that the process started to become unstable when the temperature was at 19°C. Free ammonia (FA) concentration in the reactor increased even though the nitrogen load and DO concentration were adjusted. Moreover, a new treatment line with combined Upflow Anaerobic Sludge Blanket (UASB) reactor and MBBR with deammonification process was established, with the aim of being applied in the mainstream treatment. The study results indicated that when the influent of deammonification process shifted from reject water to UASB effluent, which corresponded to an inlet ammonium concentration of 100 mg/l, the deammonification started to show unstable performance. During the whole operation, anammox and ammonium oxidizers were the dominating groups of bacteria.

Due to the increased attention on global warming and greenhouse gas emissions, WWTPs have begun to identify the major sources of these emissions within their own plants, especially nitrous oxide (N_2O), which is produced mainly as a byproduct of the nitrogen removal process. In this study, nitrous oxide was measured and compared in the pilot and a in a full scale deammonification process treating reject water. Between 0.4 - 2 % of the nitrogen load in both full scale and pilot scale was converted to nitrous oxide and the results indicated that there was no significant emission difference when the deammonification process performed with continuous or intermittent aeration. It was determined that the production and consumption of nitrous oxide was dependent on the nitrogen loads and DO concentration applied in the system.

SAMMANFATTNING

För att minska risken för övergödning i naturliga vattendrag har kraven på reningsverkens utgående vatten blivit strängare än tidigare. Ca 15 – 20 % av kvävebelastningen i huvudströmmen kommer från rejektvatten som producears i slambehandlingen. Partiell nitritation/Anammox-processen, som också kallas deammonifikation, verkar vara ett bra alternativ för behandling av rejektvatten. Dock för att processen skall vara effkektiv behövs goda kontrollstrategier som är tillförlitliga och kräver mindre underhåll. I denna avhandling har olika kontrollstrategier studerats i pilotskala i syfte att optimera deammonifikationsprocessen i en reaktor med MBBR-teknik (med biofilm på biobärare) för rejektvattenbehandling. Processen har övervakats genom mikrobiella aktivitetstester; Specifik AnammoxAktivitet (SAA), syreupptagningshastighet (OUR) och nitratupptagningshastighet (NUR), för att mäta aktiviteten hos anammoxbakterier, ammoniumoxiderare, nitritoxiderare och denitrifierare. Intermittent luftning och redox (ORP) som styrparametrar har testats. Resultaten visar att intermittent luftning i entimmarscykler med 15 min icke-luftad period kan minska luftningstiden utan förlust av processeffektivitet. Ett redoxstyrvärden på pE = 0 gav bästa driftsförhållandena. När redoxstyrvärdet sattes till 0 mV justerade luftningen sig själv även då olika kvävebelastningar användes i systemet.

Pilotförsök med deammonifikations-MBBR genomfördes vid lägre temperaturer och i inflödet utspädda kvävekoncentrationer närmare huvudströmsförhållanden. Reaktorn kördes vid olika temperaturer (19 - 25 °C) för att testa processens stabilitet, och processen började bli instabil vid temperaturen 19 °C. Koncentrationen av fritt ammonium (FA) i reaktorn ökade då trots att kvävebelastningen och syrehalten justerades. Dessutom etablerades en ny behandlingslinje där en UASB-reaktor (Upflow Anaerobic Sludge Blanket) och en deammonifikationsprocess kombinerades, i syfte att kunna tillämpas i huvudströmsbehandling. Resultaten från studien indikerade att när inflödet till deammonifikationsprocessen ändrades från rejektvatten till utflödet från UASB:n, som motsvarar en ammoniumkoncentrationen i inflödet på 100 mg/l, började deammonifikationsprocessen att visa instabil prestanda. Under hela driften var de dominerande bakteriegrupperna Anammox- och ammoniumoxiderande bakterier (AOB).

På grund av den stora uppmärksamheten på global uppvärmning och utsläpp av växthusgaser, har de viktigaste källorna till utsläpp av växthusgaser från reningsverk identifierats, särskilt lustgas (N_2O), som i reningsverk mestadels produceras i kvävereningsprocessen. I denna studie mättes och jämfördes lustgasutsläpp i piloten och i en fullskalig deammonifikationsprocess för behandling rejektvatten. Mellan 0,4 och 2 % av kvävebelastningen i både fullskale och pilotskala omvandlades till lustgas. Resultaten visade att det inte fanns någon stor skillnad i utsläpp mellan deammonifikationsprocess som utförs med kontinuerlig eller intermittent luftning. Det framkom att produktionen och konsumtionen av lustgas var beroende av kvävebelastningen och syrehalten i systemet.

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LIST OF PAPERS

This thesis is based on results presented in the following papers, which are appended at the end of the thesis:

I. Zubrowska-Sudol, M., **Yang, J.,** Trela, J., Plaza, E. 2010. Evaluation of deammonification process performance at different aeration strategies. *Water Science & Technology*. **63** (6), 1168-1176.

II. **Yang, J.,** Trela, J., Zubrowska-Sudol, M., Plaza, E. 2015. Intermittent aeration in one stage partial nitritation/anammox process. *Ecological Engineering.* **75,** 413-420.

III. **Yang, J.,** Trela, J., Plaza, E., Wahlberg, O., Levlin, E. 2015. Oxidation-reduction potential (ORP) as a control parameter in a single-stage partial nitritation/anammox process treating reject water. Journal of Chemical Technology & Biotechnology. DOI: 10.1002/jctb.4849

IV. **Yang, J.,** Trela, J. Plaza, E., Tjus, K. 2013. N_2O emissions from a one stage partial nitrification/anammox process in moving bed biofilm reactors. *Water Science & Technology*. **68** (1), 144-152.

V. **Yang, J.,** Trela, J., Plaza, E. 2015. Nitrous oxide emissions from one-step partial nitritation/anammox processes. In: *Proceedings of the IWA conference "World Water Congress & Exhibition 2014"*, 21-26 September 2014, Lisbon, Portugal. Submitted to *Water Science & Technology*, under review.

Other publications which are not appended in the thesis:

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Yang, J. 2012. Controlling and monitoring of deammonification process in moving bed biofilm reactor. TRITA LWR LIC 2065.

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Trela, J., Malovanyy, A., **Yang, J.**, Plaza, E., Trojanowicz, K., Sultana, R., Wilen, B-M., Persson, F., Baresel, C. 2014. Deammonification Synthesis report 2014. IVL rapport Nr B 2210.

Malovanyy, A., **Yang, J.,** Trela, J., Plaza, E. 2015. Combination of upflow anaerobic sludge blanket (UASB) reactor and partial nitritation/anammox moving bed biofilm reactor (MBBR) for municipal wastewater treatment. *Bioresource Technology*. **180**,144-153.

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Lindblom, E., Arnell, M., Flores-Alsina, X., Stenström, F., Gustavsson, D. J. I., **Yang, J.,** Jeppsson, U. 2016. Dynamic modelling of nitrous oxide emissions from three Swedish sludge liquor treatment systems. *Water Science & Technology*. **73** (4), 798-806.

ACRONYMS AND SYMBOLS

AOB - Ammonium-Oxidising Bacteria

AMO - Ammonia Monooxygenase

Anammox - Anaerobic Ammonium Oxidation

BOD - Biochemical Oxygen Demand

COD - Chemical Oxygen Demand

DO - Dissolved Oxygen

FA - Free Ammonia

FISH - Fluorescent In Situ Hybridisation

FNA - Free Nitrous Acid

HAO - Hydroxylamine Oxidoreductase

HPLC - High Performance Liquid Chromatography

HRAS - High Rate Activated Sludge

HRT - Hydraulic Retention Time

HZO - Hydrazine Oxidase

IFAS - Integrated Fixed Film Activated Sludge

MBBR - Moving Bed Biofilm Reactor

MW - Municipal Wastewater

NL - Nitrogen Load

NLR - Nitrogen Load Rate

NiR - Nitrite Reductase

NOB - Nitrite Oxidizing Bacteria

N_2OR - Nitrous Oxide Reductase

NRBC - Non-woven Rotating Biological Contactor

NRR - Nitrogen Removal Rate

NUR - Nitrate Utilization Rate

ORP - Oxidation Reduction Potential

OUR - Oxygen Uptake Rate

PID - Proportional–Integral–Derivative

RBC - Rotating Biological Contactor

SAA - Specific Anammox Activity

SBR - Sequencing Batch Reactor

SNAD - Simultaneous partial Nitrification, Anammox and Denitrification

SRT - Solids Retention Time

TP - Total Phosphorus

TN - Total Nitrogen

TSS - Total Suspended Solids

UASB - Upflow Anaerobic Sludge Blanket

VFA - Volatile Fatty Acid

VSS - Volatile Suspended Solids

WWTP - Wastewater Treatment Plant

ABSTRACT

Deammonification process appears to be a good alternative to treat reject water which is produced in the sludge treatment line in WWTPs. However, for this process to be effective, control strategies which are reliable and require less maintenance are needed. In this thesis, control strategies were studied at pilot scale in order to optimise the deammonification process operated in a Moving Bed Biofilm Reactor (MBBR) for reject water treatment. The processes were monitored by microbial activity tests, Specific Anammox Activity (SAA), Oxygen Uptake Rate (OUR), and Nitrate Uptake Rate (NUR) tests, in order to measure the anammox, ammonium/nitrite oxidizers and denitrifiers activity. Aeration and redox as control parameters were tested. The results showed that intermittent aeration, with 15min non-aerated period in a one hour cycle, could reduce the aeration time without loss of process efficiency. A redox value of pE=0 gave the best operational condition even if there were different nitrogen loads applied in the system.

Pilot scale deammonification MBBR was tested at decreasing temperatures and diluted influent nitrogen concentrations towards to mainstream conditions. The reactor was run at different temperatures (25-19°C) to test the process stability and it was seen that the process started to become unstable when the temperature was at 19°C. Moreover, the combined treatment line, Upflow Anaerobic Sludge Blanket (UASB) reactor and MBBR with deammonification process, was established with the aim of being applied in mainstream treatment. The study results indicated that when the influent of the deammonification process shifted from reject water to UASB effluent (NH_4^+-N=100 mg/l), the process began to show unstable performance.

WWTPs also started to identify the major sources for greenhouse gas emission in the plants due to global warming, especially nitrous oxide. N_2O was measured and compared in the deammonification process treating reject water in this study. Between 0.4% and 2 % of the nitrogen load was converted to N_2O in pilot and full scale studies. The results indicated that there was no significant emission difference when the process was performed with continuous or intermittent aeration; the production and consumption of N_2O was dependent on the nitrogen loads and DO concentration applied in the system.

Key words: anammox; deammonification; intermittent aeration; moving bed biofilm reactor (MBBR); N_2O emission; redox (ORP) control; partial nitritation; reject water; upflow anaerobic sludge blanket (UASB).

1. INTRODUCTION

1.1. Background

Large amounts of nitrogen and phosphorus released into natural waterbodies can cause eutrophication, which leads to an increase in the

ecosystem's primary productivity and also results in a shortage of oxygen and serious reductions in water quality (OECD, 1982). Sources of nitrogen entering natural ecological systems include domestic wastewater, industrial production and agriculture fertilisation (Gao et al., 2002, Jin et al., 2006, Le et al., 2010).

In order to prevent eutrophication in the Baltic Sea, the Baltic Sea Action Plan has been developed by various stakeholders, including governments and other organisations representing both private and public sectors, to restore its good ecosystem status. To achieve this, countries around the Baltic Sea ensure that discharges from WWTPs are treated in an efficient way. In Stockholm, there are four WWTPs: Henriksdal and Bromma WWTPs are located in the centre of Stockholm, Käppala WWTP is to the east and Himmerfjärden WWTP is to the south-west of Stockholm. Effluent requirements differ depending on the location of the treatment plants. The average values of common pollutants in the discharge and requirements are shown in **Table 1.**

Table 1. The average values of BOD, nitrogen and phosphorus in discharge in 2014 (Himmerfjärdsverket, 2014, StockholmVatten, 2014, Käppalaförbundet, 2014).

WWTP	Effluent (mg/l)			Requirement (mg/l)		
	BOD	TN	TP	BOD	TN	TP
Henriksdal and Bromma	2.9	8.6	0.16	8	10	0.3
Käppala	<2	8.2	0.20	8	10	0.3
Himmerfjärden	6.2	8.2	0.32	8	8	0.4

Different methods have been developed to remove nitrogen from wastewater in WWTPs, for instance biological nitrogen removal process, air stripping (Siegrist, 1996, Li et al., 1999), chemical precipitation to form struvite, and ion exchange technology (Wang et al., 2006, He et al., 2007, Thornton et al., 2007). Most WWTPs use biological treatment to remove nitrogen.

1.2. Nitrogen cycle

The conversions and reactions of different forms of nitrogen compounds are shown in **Fig. 1.** Inorganic nitrogen exists in nature from a valence of +5 (nitrate) to -3 (ammonium, ammonia). The atmosphere is the largest pool for nitrogen, with approximately 78 % of the modern atmosphere formed of nitrogen gas. Classical transformations in the nitrogen cycle include nitrogen fixation, nitrification and denitrification. The possibility for anaerobic ammonium oxidation using ammonium as the energy source and nitrite as the electron acceptor was predicted by Broda in 1977 based on thermodynamics (Broda, 1977). To minimise the nitrogen concentration in effluent from WWTPs, the traditional way of removing nitrogen from WWTPs is the process of nitrification/denitrification. After

the discovery of anammox bacteria, technologies have been developed based on the anammox reaction.

The nitrification process is a two-step autotrophic process. First, ammonium is oxidised to nitrite by ammonium-oxidising bacteria (AOB) (**Eq 1**) and then nitrite is oxidised to nitrate by nitrite-oxidising bacteria (NOB) (**Eq 2**).

The stoichiometry reactions are:

$NH_4^+ + 1.382O_2 + 1.982HCO_3^- \rightarrow 0.018C_5H_7O_2N + 0.982NO_2^- + 2.927H_2O + 1.891CO_2$ (**Eq 1**)

$NO_2^- + 0.003NH_4^+ + 0.488O_2 + 0.010H_2CO_3 + 0.003HCO_3^- \rightarrow 0.003C_5H_7O_2N + NO_3^- + 0.008H_2O$ (**Eq 2**)

When considering the nitrogen conversion alone, simple ways of writing the reactions are:

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$ (**Eq 3**)

$NO_2^- + 0.5O_2 \rightarrow NO_3^-$ (**Eq 4**)

Denitrification is performed by heterotrophic bacteria under anoxic conditions converting nitrite/nitrate to nitrogen gas while using organic matter (illustrated by methanol in **Eq 5**) as a source of carbon and energy:

$6NO_3^- + 5CH_3OH \rightarrow 2N_2 + 5CO_2 + 7H_2O + 6OH^-$ (**Eq 5**)

It can be seen from **Eq. 5** that biodegradable organic matter is needed for the denitrification process. If there is no available organic matter present in the wastewater, the addition of an external carbon source is required. Methanol is commonly added in the denitrification process in WWTPs due to its low cost in comparison with ethanol. For the reduction of 1 g NO_3^--N during denitrification, 2.85 g COD are required (Bernet et al., 1996).

Due to an increased focus on nitrous oxide emission, the reactions related to nitrous oxide are also included in **Fig. 1**. The key metabolic pathways of N_2O production are 1) Nitrifier denitrification under anoxic condition; 2) autotrophic ammonia oxidation changing from anoxic to aerobic condition (Kim et al., 2010, Chandran et al., 2011, Law et al., 2012a); 3) heterotrophic denitrification. Meanwhile, denitrifiers are able to convert nitrous oxide into nitrogen gas (Schneider et al., 2013).

1.3. Anammox reaction

The anammox reaction, which means ANaerobic AMMonium OXidation, using ammonium as an energy source and nitrite as an electron acceptor, was first described by Broda in 1977 (Broda, 1977). He pointed out that one group of lithotrophic bacteria had not yet been detected which could oxidise ammonia to nitrogen with O_2, nitrite or nitrate. The anammox reaction was first observed in the Gist Brocades Fermentation Company in the Netherlands in 1986 (Mulder et al., 1995). The presence of anammox bacteria was later reported in the Black Sea

(Kuypers et al., 2003), in the Golfo Dulce in Costa Rica (Dalsgaard et al., 2005), and in the Baltic Sea (Gebhardt et al., 2004, Engström et al., 2005). Today, it is acknowledged that the anammox reaction plays a significant role in the marine system and that 30-50 % of the N_2 produced from the oceans is from the anammox reaction (Dalsgaard et al., 2005).

The stoichiometry of the process has been described by Strous *et al.* (1998):

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2H_2O \quad \textbf{(Eq. 6)}$$

According to **Eq. 6**, the removal of 1 mole of NH_4^+-N by anammox bacteria requires 1.32 mole of NO_2^--N and has 0.26 mole of NO_3^--N production. Since most of the nitrogen present in the wastewater is in the form of ammonium, about half of the ammonium needs to be oxidised to nitrite before the anammox reaction occurs. The theoretical maximum nitrogen removal efficiency is 88.8 %. Hydrogen carbonate is used in the reaction as the carbon source, which decreases the alkalinity in the system. Biomass yield is 0.066 mole C/mole NH_4^+-N. The Gibbs free energy calculated by Strous is 357 kJ/mole NH_4^+-N (Strous, 2000).

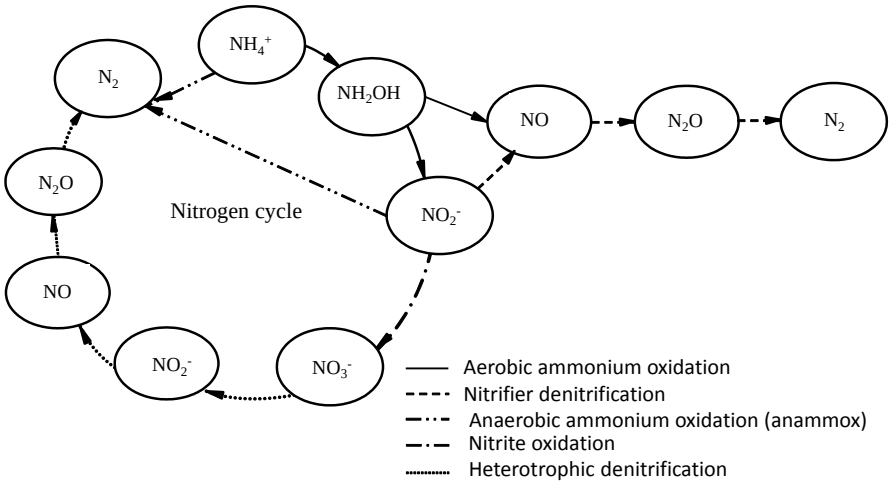

Fig 1. Nitrogen cycle.

Van de Graaf *et al.* (1997) found that the intermediates for the anammox reaction were hydrazine and hydroxylamine. Schalk *et al.* (1998) confirmed that hydrazine was an intermediate of the anammox process. Important enzymes were hydroxylamine oxidoreductase (HAO), which was purified by Schalk *et al.* (2000), and hydrazine oxidase (HZO), which was purified by Shimamura *et al.* (2007). It has been proposed that the mechanism of the anammox reactions converted ammonium and hydroxylamine, which was converted from nitrite, into hydrazine and then oxidised to form nitrogen gas (Schalk et al., 1998).

Moreover, it has been found that anammox bacteria (species of *Candidatus "Brocadia fulgida" and "Candidatus Anammoxoglobus propionicus"*) have another reaction route to oxidise volatile fatty acids with nitrate as an electron acceptor, while forming ammonium with

nitrite as an intermediate (Guven et al., 2005, Kartal et al., 2008). Anammox bacteria do not incorporate fatty acids into biomass, but fully oxidise them into CO_2 thereby maintaining a low biomass yield (Kartal et al., 2007).

Anammox bacteria *"Brocadia"," Kuenenia", "Candidatus Scalindua brodae", "Candidatus Scalindua wagneri", "Candidatus Anammoxoglobus propionicu"* have been found in wastewater, while "*Candidatus Scalindua sorokinii", "Candidatus Scalindua Arobica"* have been discovered in seawater (Cema, 2010).

2. DEAMMONIFICATION PROCESS

Partial nitritation/anammox, which is also called the deammonification process, was introduced by University of Hanover and applied to remove nitrogen from leachate (Hippen et al., 2001). This term has subsequently been more commonly used in biofilm processes (Seyfried et al., 2002, Rosenwinkel and Cornelius, 2005, Plaza et al., 2011).

Deammonification is a two-step process that includes partial nitritation and anammox reaction. It can be performed in either one or two reactors. In the first step, part of the ammonium is oxidised to nitrite (**Eq. 3**) and then anammox bacteria use ammonium and nitrite as substrates and produce nitrogen gas, which proceed at anaerobic conditions (**Eq. 6**).

In one stage biofilm deammonification process, biomass forms a biofilm to allow simultaneous performance of nitritation and the anammox process. The biofilm can either be in granule form (Winkler et al., 2012) or grow on carriers, such as Kaldnes carriers (Szatkowska et al., 2007). In a one-stage deammonification process, ammonium oxidisers exist in the outer layer of the biofilm and anammox bacteria are present in the inner layer. Therefore, anammox bacteria can avoid oxygen, which have an inhibition effect on anammox bacteria.

The one-stage deammonification process with different reactor configurations has been studied by several research groups, such as granular sludge applied in SBR (Mulder et al., 1995), biofilm on Kaldnes carriers used in MBBR (Rosenwinkel and Cornelius, 2005) and on a rotating biological contactor (RBC) (Cema et al., 2007).

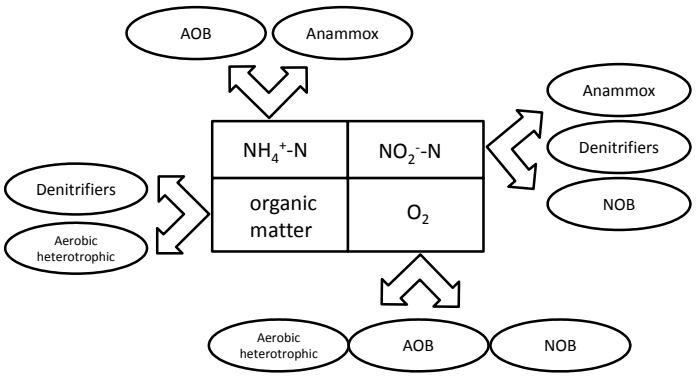

Fig. 2. Competition between different microorganisms in the one-stage deammonification process.

In the one-stage deammonification process, anammox bacteria and ammonium oxidizers are the dominant group in the system. Heterotrophic bacteria and NOB are very hard to avoid if there are available substrates. Simultaneous partial nitrification, anammox and denitrification (SNAD) processes treating wastewater with an approximate C/N ratio of 0.5 have recently been developed in different reactor configurations, including in a UASB (Upflow Anaerobic Sludge Blanket) reactor (Lan et al., 2011), a non-woven rotating biological contactor (NRBC) (Chen et al., 2009) and a sequencing batch reactor (SBR) (Xu et al., 2010). **Fig. 2** shows the competition between the different groups of microorganisms on the same substrate. The characteristics of the influent and the operating conditions determine the competition results.

2.1. Deammonification process for reject water treatment

2.1.1. Factors influencing the process

Dissolved oxygen

In the one-stage deammonification process, DO is necessary for NO_2^--N production by ammonium oxidizers. A low DO concentration will lead to a deficiency of NO_2^--N for the anammox reaction, while high NOB activity can be caused by a high DO concentration in the system. Anammox bacteria need anaerobic conditions and DO is a reversible inhibitor. The activity of the anammox bacteria was reversibly inhibited even by oxygen concentration below 0.5 % air saturation with intermittent aeration (Strous et al., 1997).

The activity of ammonium oxidizers and NOB is significantly influenced by DO concentration. In the activated sludge system with nitrification, a good process performance could be achieved when DO exceeds 2 mg/l (Trela, 2000) and nitrite oxidation was strongly restricted at DO of 0.5 mg/l (Hanaki et al., 1990).

Hyungseok *et al.* (1999) showed that nitrate formation could be prevented efficiently in one-stage partial nitrification and denitrification processes by frequent switching between aerobic and anoxic phases. The aeration should switch off before approximately half of the ammonium oxidised to nitrite and further converted to nitrate. Wantawin *et al.* (2008) studied different oxygen supply modes including continuous aeration, intermittent aeration and different HRT in sequencing batch biofilm reactors for nitrogen removal from synthetic wastewater. Their results showed that different aeration modes influenced most the NH_4^+-N and NO_3^--N concentrations in the effluent, which were the parameters indicating system efficiency.

Temperature

Microorganism growth rate, mass transfer (oxygen transfer characteristics of the system) and chemical equilibrium were influenced by temperature (Collins and Incropera, 1978, Van Hulle et al., 2010).

Pure cultures of *nitrosomonas sp.* and *nitrobacter sp.* have an optimum temperature of 35 °C and 38 °C respectively (Grunditz and Dalhammar, 2000). It was possible to effectively outcompete NOB at temperatures above 25 °C with a low solids retention time (SRT) due to a more rapid increase in the specific growth rate of AOB than of NOB (Hellinga et al., 1998, Jetten et al., 1998). Partial nitritation was successfully performed at a temperature of between 15 °C and 30 °C. However, the efficiency of the nitritation process decreased dramatically when the temperature was below 15 °C (Yamamoto et al., 2006).

Many studies showed that the optimum temperature for operation of the anammox process was between 35 °C and 40 °C (Szatkowska and Plaza, 2006, Yang et al., 2006). The highest anammox activity has been observed at 37 °C (Egli et al., 2001). No anammox activity was observed at 45 °C and anammox activity at 11 °C was approximately 24 % of that at 37 °C. The optimum temperature for the anammox biomass has been observed to be 40 ± 3 °C (Strous, 2000). The activity changes according to the Arrhenius law at between 20 °C and 37 °C. Dalsgaard *et al.* (2002) and Rysgaard (2004) reported that anammox bacteria showed activity at a temperature of 15 ºC and 12 ºC respectively.

Studies on partial nitritation/anammox process have mostly been carried out at temperatures of between 20 and 30 °C (Szatkowska and Plaza, 2006, de Graaff et al., 2010, Liang et al., 2011, Yang et al., 2011). It has been proven that partial nitritation/anammox process could be successfully operated in a rotating biological contactor at 20 °C (Cema et al., 2007). Similar results have been reported by Isaka *et al.* (2007) and the process could be operated at a temperature of 20-22 °C. The short and long-term effects of temperature on the anammox process have been tested. Study results showed that anammox bacteria reach maximum activity at 35-45 °C in the short-term batch tests and a laboratory-scale SBR with granule sludge had been successfully operated at 18 °C (Dosta et al., 2008), while SBR losing its stability when the temperature decreased to 15 °C. Hao *et al.* (2002) performed a model-based evaluation of temperature on a partial nitrification and anammox biofilm process. The simulation was performed at a temperature range between 15 and 40 ºC. The results showed that if the temperature decreased, the activities of the organisms decreased, which means that thicker biofilms were needed or the nitrogen load should be decreased to maintain a high nitrogen removal at lower temperatures.

pH and alkalinity

The level of pH value influenced the free ammonia and nitrous acid concentration (Anthonisen et al., 1976). An increase of free ammonia will be observed with a rise in pH value when there are constant temperatures and concentrations of ammonium. A decrease in pH value leads to a rise in nitrous acid concentration. Anthonisen *et al.* (1976) showed that ammonium oxidisers were inhibited at a free ammonia concentration of 120 mg/l and nitrous acid concentration of 2.8 mg/l.

The pH value was usually kept in the range of 7.5 to 8.5 in the partial nitrification process (Van Hulle et al., 2010). The optimum pH value was 8.1 for *nitrosomonas sp.* and 7.9 for *nitrobacter sp.* (Grunditz and Dalhammar, 2000). Strous *et al.* (1999) observed anammox activity between pH values of 6.7 and 8.3 with an optimum pH value of 8. There was activity of anammox bacteria at pH values below 9 (Egli et al., 2001).

For nitrification and the anammox process, HCO_3^- is the carbon source for the reaction and the construction of new bacteria cells. Alkalinity reduces during the nitrification process. Each mole of NH_4^+ oxidised will consume two moles of HCO_3^- (Trela, 2000). Synthesis of new bacteria cells and decomposition of organic nitrogen will influence the alkalinity value. Bagchi *et al.* (2010) showed that alkalinity could be used as a controlling parameter in a single-stage partial nitritation and anammox reactor. Tokutomi *et al.* (2010) indicated that nitrite and nitrate accumulation could be controlled by the addition of different alkalinity sources ($NaHCO_3$ or NaOH respectively).

Organic matter

The anammox reaction is autotrophic. Wastewater containing a low concentration of organic matter and a high nitrogen content is favourable for the anammox process. It has been reported that with the presence of a certain level of organic matter, anammox bacteria cannot compete with denitrifiers due to a slower growth rate (Strous et al., 1999). Many studies have recently shown that with a C/N ratio below 0.5 in the influent, anammox bacteria could maintain and outcompete heterotrophic bacteria in the system (Winkler et al., 2012). Ahn *et al.* (2004) had successfully treated piggery waste in a UASB anammox reactor, reporting competition between anammox and denitrification. Tang *et al.* (2010) concluded that biodegradable COD: N cannot be above 2.9 if anammox is required to be the dominant way of nitrogen removal.

It has been reported that some small organic chemical compounds, such as methanol and ethanol, have an irreversible inhibition effect on anammox bacteria (Guven et al., 2005, Isaka et al., 2007). Recently it has been established that anammox bacteria have the capacity to use small molecular organic matter with nitrate as the electron acceptor, while forming ammonium with nitrite as the intermediate (Guven et al., 2005, Kartal et al., 2008). Anammox bacteria do not incorporate the organic matter into biomass, but completely oxidise it into CO_2, thereby maintaining a low biomass yield (Winkler et al., 2012).

2.1.2. Controlling and monitoring the process

The one-stage deammonification process is a promising nitrogen removal method to apply in WWTPs. With more applications ongoing, proper control strategies are needed for successful operation. Various control strategies have been developed based on different reactor configurations and forms of biomass. Wett (2007) proposed a control strategy based on pH value combined with continuous feeding. Joss *et al.* (2009) have

developed a control strategy based on the on-line measurement of ammonium, nitrate and oxygen and cycle feeding. Szatkowska (2005) suggested that conductivity can be used as a monitoring parameter to evaluate the system. Redox potential using as monitoring parameter has also been under consideration (Lackner and Horn, 2012).

For the one-stage deammonification process, oxygen supply and nitrogen loads are the control parameters and other available on-line measurements, for instance ammonium, nitrate, nitrite, DO, pH value, conductivity and redox potential value, are the monitoring parameters. In order to supply the correct volume of oxygen to a system that could have different nitrogen loads, monitoring parameters are used to help adjust the oxygen supply. The control strategy can be simplified to identify a reliable parameter that can provide integrated information about all the possible chemical and physical processes in the treatment system. This parameter is also expected to be durable, low cost and easy to use. Therefore, the physical parameter control is more popular due to its price and convenience (Lackner et al., 2014).

Based on the experiences from different aeration control strategies, redox potential could be a promising monitoring tool for the process. Redox potential has been widely studied for monitoring nitrification/denitrification and anaerobic digestion (Martinez, 2007, Tanwar et al., 2008), and can provide information that other parameters are unable to reveal, such as the shift between aerobic, anoxic and anaerobic conditions (Lackner and Horn, 2012). An in-depth investigation using redox potential value as a control parameter for the one-stage deammonification process is needed.

2.2. Nitrous oxide emission

Greenhouse gas emissions have attracted much attention due to the great concerns about global warming. Among the greenhouse gases, nitrous oxide (N_2O) has a global warming potential of 265-298 CO_2-equivalents for a 100-year timescale and therefore can play a significant role in global warming. N_2O emissions can occur as direct emissions in gaseous form from wastewater treatment plants (WWTP) or as indirect emissions in liquid phase in WWTP discharge. IPCC guidelines require policy makers to estimate the N_2O emissions from WWTPs. Therefore, more and more research studies carried out to investigate the N_2O formation and emission from the wastewater treatment process. From the process point of view, nitrous oxide can be formed in both aerobic and anoxic nitrogen transformation processes in WWTPs. In most cases, nitrous oxide is the by-product of nitritation and denitrification processes (Firestone et al., 1979, Lipschultz et al., 1981, Freitag and Bock, 1990, Kim et al., 2010). Nitrous oxide can be produced by the enzymes ammonia monooxygenase (AMO) or hydroxylamine oxidoreductase (HAO) in ammonium-oxidising bacteria (AOB) and by the enzyme nitrite reductase (NiR) in heterotrophic bacteria. Meanwhile, enzymes of nitrous oxide reductase (N_2OR) in heterotrophic bacteria can degrade nitrous oxide into nitrogen gas (Rosenwinkel et al., 2013). Under limited

oxygen or completely anoxic conditions, nitrous oxide can be generated by a nitritation step (Schmid et al., 2001). The causes of denitrifiers producing nitrous oxide can be low pH (Focht, 1974), limited organic carbon (Hanaki et al., 1992) and the presence of inhibitors *etc.* (Schulthess et al., 1995). Nitrous oxide can be consumed by the denitrification process when optimal conditions for the reaction are attained. Schneider *et al.* (2013) showed that N_2O was completely reduced to N_2 once nitrite is depleted. N_2O had not been expected to be produced by anammox bacteria because it had not been detected as an intermediate product in the anammox reaction.

A wide range of N_2O emissions have been measured by different studies. N_2O emission fractions vary from 0.01-1.8 % of nitrogen load in 12 full-scale activated sludge processes in the United States (Chandran, 2011). Desloover *et al.* (2012) reported that values of nitrous gas emissions evaluated at full-scale WWTPs range from 0.01 to 3.3 % of the nitrogen load in different studies. De Graaff *et al.* (2010) showed that the emission of nitrous oxide (N_2O) corresponded to 0.6-2.6 % (average 1.9 %) of the total nitrogen load in the partial nitritation process. During full-scale reject water treatment studies by Kampschreur *et al.* (2008), the N_2O production was 1.7 % and 0.6 % of the nitrogen load from the partial nitritation reactor and the anammox reactor respectively.

2.3. Deammonification process towards mainstream application

The trend in WWTPs is moving from an energy consumption unit towards a nutrient recovery and energy production unit. From the process point of view, the organic matter in wastewater should be collected as much as possible and further used to produce biogas. Nutrients such as nitrogen and phosphorus should be removed by a low-cost solution or recovered. In the WWTPs, organic matter removal and collection can be achieved by anaerobic treatment (*e.g.* a UASB reactor), aerobic treatment (*e.g.* high-rate activated sludge (HRAS)) or other physical methods (precipitation, coagulation and flocculation *etc.*) (Versprille et al., 1985, Mahmoud et al., 2004, Guida et al., 2007). Phosphorus can be recovered by precipitation or removed by biological process. Since the deammonification process is a lower cost technology than the traditional nitrification/denitrification process, its successful application in the mainstream would have a considerable economic benefit. However, applying the deammonification process in the mainstream still presents a challenge.

The deammonification process has been successfully applied in reject water treatment. Compared with reject water, municipal wastewater has ammonium concentrations of 40-60 mg/l, temperatures of 10-25 °C and an unstable COD/N ratio. The main challenge for mainstream deammonification application is maintaining anammox bacteria and suppressing NOB under low ammonium concentrations and variable temperatures and COD/N conditions in long term.

Many research groups have undertaken studies on deammonification performance at low nitrogen concentrations and temperatures using

synthetic wastewater, pre-treated wastewater or a mixture of the two (Vazquez-Padin et al., 2011, Hendrickx et al., 2012, Jin et al., 2013, Du et al., 2014, Hendrickx et al., 2014, Lotti et al., 2014,). Wett *et al.* (2013) reported a deammonification SBR system treating full-scale HRAS effluent. Lotti *et al.* (2013) presented treatment of municipal wastewater after HRAS at 19 °C in a granular deammonification reactor, establishing that the highest nitrogen removal efficiency is 49 %. De Clippeleir *et al.* (2013) operated a RBC for a year and decreased the temperature from 30 to 14 °C, decreasing nitrogen removal efficiency from 54 % to 34 %. To suppress NOB, Kornaros *et al.* (2010) suggested using intermittent aeration to out-select NOB, while some studies used FNA to inhibit NOB. Long-term operations are needed to evaluate the microorganisms' evolution.

3. OBJECTIVES OF THE THESIS

This study is divided into three parts: control strategies for deammonification MBBR treating reject water, deammonification MBBR towards to mainstream application and nitrous oxide emissions from the deammonification process (**Fig. 3**).

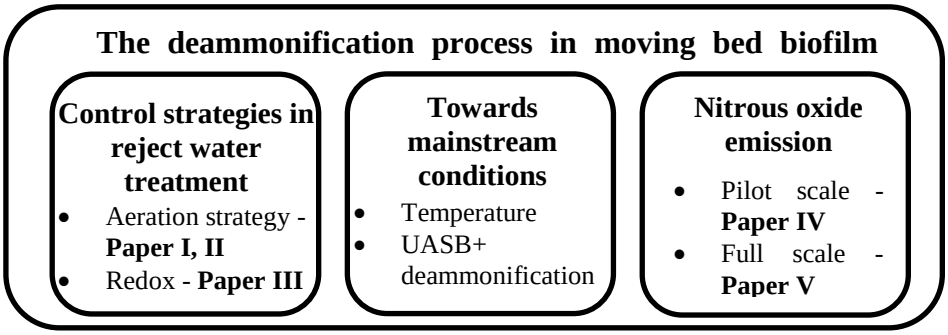

Fig. 3. Overview over the research with respect to the main objectives.

Suitable control strategies could improve process performance and efficiency and lead to operation cost reductions. The first part of the study was to find a reliable control strategy in order to optimise and control the deammonification process treating reject water. The specific goals were:

- to evaluate different aeration strategies to control the deammonification process;
- to monitor the evolution of microorganisms with changing operating conditions;
- to investigate the possibility of using redox potential to control the process.

The objectives of the research on deammonification towards mainstream application were

- to evaluate the usage of combined UASB and deammonification process to treat municipal wastewater;

- to assess the influence of different temperatures on the deammonification process.

In order to investigate the greenhouse emission from the deammonification MBBR process, nitrous oxide was measured in both the pilot and full-scale reactors. The goals of the research were:

- to monitor the nitrous oxide emission from the deammonification MBBR process;
- to identify the forms and periods of nitrous oxide emission under intermittent aeration;
- to calculate nitrous oxide production and consumption under different operational conditions;
- to discuss the factors influencing nitrous oxide emission and production.

4. MATERIAL AND METHODS

Most of the experimental study was carried out at the Hammarby Sjöstadsverk research facility, which is located to the south-west of Stockholm, Sweden. The facility was built to study and develop innovative technologies in wastewater treatment. It is now also a place for testing and demonstrating new solutions and equipment in the field of wastewater treatment. Part of the study was undertaken in Himmerfjärden WWTP, which is a municipal WWTP located in the southwest of Stockholm. It serves a population of 275,000 and has an inflow of around 109,700 m^3/d wastewater. Himmerfjärden WWTP has a separate reject water treatment using deammonification process, making it the first full-scale deammonification plant in Sweden. The treated reject water recirculates back to the mainstream in WWTP.

4.1. Laboratory-scale study

A laboratory scale study was carried out to investigate the influence of different aeration strategies on the deammonification process.

Three series of deammonification batch tests were carried out using different DO concentrations (2, 3 and 4 mg/l). Four batch tests were performed for each series of tests with different R (0, 1/3, 1 and 3). R stands for the ratio between the duration of the non-aerated and aerated phase in a one-hour operation cycle. Each batch test was operated for four hours and the sample was taken at the beginning and end of each period. For continuous aeration, samples were taken every half an hour. The inoculated sludge was a biofilm carrier with both anammox and ammonium oxidizers in the biofilm. The substrate was diluted reject water with an initial NH_4^+-N concentration of 150 mg/l. Concentrations of different nitrogen compounds were analysed by AQUATEC-TECATOR 5400 ANALYZER. Detailed information is in **Paper I**.

4.2. Pilot-scale study

4.2.1. Description of MBBRs

Two moving bed biofilm reactors (MBBRs), each with a working volume of 200 l, are in operation at the Hammarby Sjöstadsverk research station. Forty percent of each reactor volume, which is 80 l, was filled with Kaldnes carriers (K1) as the biofilm carrier. Air was supplied from the bottom of the reactor and regulated by a PID controller in each reactor. Thorough mixing conditions were achieved by both a mechanical stirrer and aeration. A heater was installed in reactor 1 (R1) to maintain the temperature at 25 °C. In reactor 2 (R2), a heater and a cooler were connected to regulate the temperature. Reject water as influent was continuously fed into the reactors and on-line instruments were installed to measure pH, redox, conductivity, temperature and DO concentration. Detailed information about influent characteristics is given in **Papers II, III**. Influent and effluent samples were taken from the reactors according to the different HRT during the operation. The method for the chemical analysis is presented in section **4.2.3**. During the operation, the activities of different groups of microorganisms were monitored (section **4.2.4**).

4.2.2. Control strategy

During the operation period of reactor 1, the proportional-integral-derivative (PID) controller was firstly connected to the DO online sensor and the reactor operation was based on the DO control (see **Paper II**). Nitrous oxide emissions were measured under the intermittent aeration (**Paper IV**). The aeration was then controlled by redox potential value in the reactor (**Paper III**). After testing different control strategies for reject water treatment, R1 was put into operation for a mainstream application of the deammonification process. The reactor was connected to a UASB reactor treating municipal wastewater. The influent of the deammonification reactor (R1) was the mixture of UASB effluent and reject water (**Table 2**).

The second reactor was operated under different temperatures, starting at 25 °C and falling to 19 °C (3 °C stepwise change). The reactor was controlled by the DO concentration (Trela, 2014) (**Table 2**).

Table 2. Operational conditions in two pilot scale reactors.

Study	**R1**	**R2**
Control strategies in reject water treatment		
Aeration	×	
Redox	×	
Towards mainstream conditions		
temperatures		×
UASB+deammonification	×	
N_2O emissions		
Pilot scale	×	×

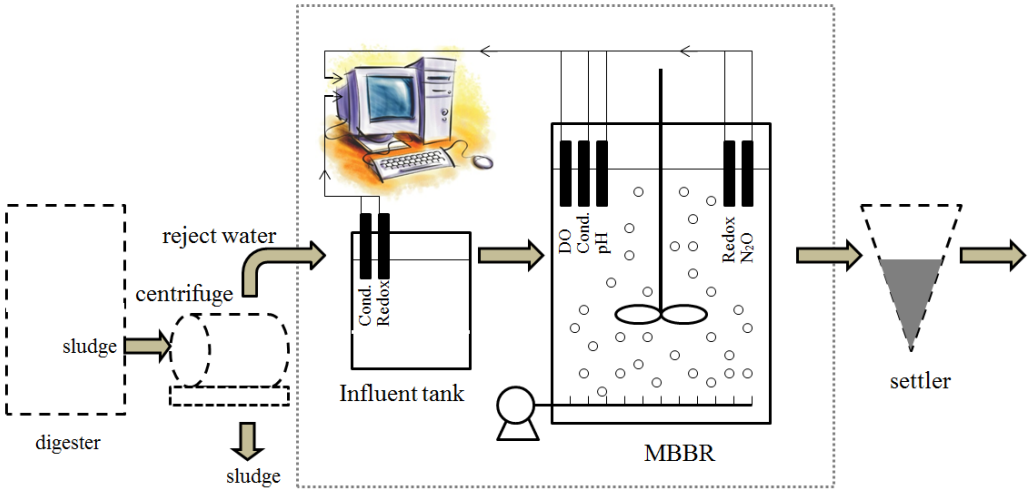

Fig. 4. The experimental setup testing deammonification in reject water treatment application.

4.2.3. Analysis methods

Samples of both influent and effluent from the pilot-scale reactor were collected and analysed for NH_4^+-N, NO_2^--N, NO_3^--N, COD and alkalinity using Dr. Lange kits after being filtrated through 0.45 µm filter. To measure the concentrations of total suspended solids (TSS) and volatile suspended solids (VSS) in the biocarriers, biofilm was removed from the biocarriers and washed with distilled water. The removed biomass was filtered using glass fibre filters, and TSS and VSS were measured according to standard methods.

4.2.4. Activity tests for different microorganisms

Specific anammox activity test (SAA)

To measure the activity of anammox bacteria, SAA tests were performed. The methodology was modified from Dapena-Mora *et al.* (2007) and was based on the pressure measurement of nitrogen gas produced by anammox bacteria.

The Kaldnes carriers collected from MBBR were washed in a phosphate buffer (0.14 g KH_2PO_4/l and 0.7 g K_2HPO_4/l) before the tests to avoid flocculent sludge attachment and biofilm detachment. The pH value of the phosphate buffer was kept at 7.8 (Hach Lange pH meter). Fifteen Kaldnes carriers together with the phosphate buffer were introduced in a vial (with a total volume of 38 ml) to reach a volume of 24 ml. The vial was then closed with a gas-tight septum. Nitrogen gas was supplied continuously inside the vial with syringe needles for 10 min to remove air from both the liquid and gas phases. The vials were then kept in a water bath to maintain the temperature inside vials at 25 ºC. After half an hour of thermal stabilisation, substrates of NH_4Cl and $NaNO_2$ were added (5 ml for each substrate) into each vial to reach a concentration of 70 mg NH_4^+-N/l and 70 mg NO_2^--N/l. The gas pressure was checked every

thirty minutes by the pressure transducer (Centrepoint Electronics). Specific anammox activity was calculated based on the maximum slope of the curve with accumulative pressure over time. The results of the tests are presented using the unit of g $N/m^2 \cdot d$.

Oxygen uptake rate tests (OUR)

The tests aimed to measure the activity of heterotrophic bacteria, ammonium oxidizers (mainly *nitrosomonas sp.*) and nitrite-oxidising bacteria (NOB) (mainly *nitrobacter sp.*) based on measurements of the oxidation rate of NH_4^+-N, organic matter content and NO_2^--N (produced in the test) by the subsequent addition of selective inhibitors of *nitrobacter sp.* and *nitrosomonas sp.*, which were $NaClO_3$ and ATU (*allylthiourea*) in the testing sample (Surmacz-Gorska et al., 1996).

A volume of 100 ml Kaldnes biofilm carriers was gently washed in distilled water to wash away the attached particles and flocculent sludge before the test. The reject water from sludge dewatering after the digester (the same as the influent for the MBBR) was diluted 10 times with distilled water to reach a NH_4^+-N concentration of 100 mg/l and COD concentration of 70 mgO_2/l. Diluted reject water was left for thirty minutes until the particles in it had settled. Only the upper part of the diluted reject water was used in the test to avoid influence from flocculent sludge and particles. The pH value in the diluted reject water was 7.5 (WTW pH meter). A 1.56 litre three-neck round bottle was filled with diluted reject water to 80 % of the volume. The bottle was placed on a magnetic stirrer and kept in a water bath to maintain a temperature of 25 ºC. The temperature inside the bottle stabilised after thirty minutes and the air was supplied by a sparger to allow dissolved oxygen concentration inside the bottle above 6 mg/l. A volume of 100 ml of Kaldnes biofilm carriers was inoculated, and more diluted reject water was filled inside the bottle until no space was left. Two necks on the side of the bottle were closed with a rubber septum and a DO electrode was installed in the middle neck of the bottle (Hach Lange DO meter). 17 mM of $NaClO_3$ and 12 mg/l of ATU were subsequently added at 5-7 min and 10-12 min. The whole test took around 15-20 min. The activity of heterotrophic bacteria, ammonium oxidizers and NOB were calculated according to the maximum slope of oxygen consumption rate over time. The results of the tests are described using the unit g $O_2/ m^2 \cdot d$.

Nitrate utilisation rate test (NUR)

The test was undertaken to evaluate the activity of heterotrophic denitrifiers by measuring the maximum nitrate utilisation rate over a four-hour period. Reject water (the same as influent) was diluted twice with distilled water to derive a COD concentration of 350 mgO_2/l. The pH value in the diluted reject water was around 8. The reject water contained an alkalinity concentration of 4 g $CaCO_3$/l and the pH value barely changed during the test. One litre of diluted reject water was poured into a 1.5 L plastic beaker. The beaker was placed on a magnetic stirrer in a water bath to keep the temperature at 25 °C. Nitrogen gas was supplied continuously into the diluted reject water to remove oxygen

from the liquid phase. A volume of 400 ml Kaldnes biofilm carriers, which had been gently washed as described in the OUR tests, were introduced into the container after DO concentration was less than 0.5 mg/l and the temperature had stabilised. The addition of $NaNO_3$ solution achieved an NO_3^--N concentration of 100 mg/l. After that, parafilm was used to cover the beaker and avoid oxygen diffusion into the liquid phase, and then nitrogen gas was supplied on the water surface during the test. Liquid samples were taken every hour and the concentration of NO_3^--N was analysed by Dr Lange tests (LCK340). The activity of the denitrifiers was calculated based on the nitrate consumption rate as a function of time. The results of the tests are using the unit of g $N/m^2 \cdot d$.

4.3. Nitrous oxide measurement

4.3.1. Experimental setup in the pilot-scale reactors

Nitrous oxide emission was measured in the pilot-scale MBBRs, which is described in section **4.2**. When nitrous oxide was being measured, the reactor was covered with a plastic board to achieve a closed system during measurement. Aeration was supplied from the bottom of the reactor (Q2) and an external pump, which had a constant airflow Q1 (flow meter 1), was used to collect the air above the water surface. As the reactor was being operated with intermittent aeration and there were periods without aeration, an extra inlet of air (Q3 for dilution and constant air flow) and flow meter 3 were added. Thus $Q_1=Q_2+Q_3$. Unisense nitrous oxide microelectrode and Teledyne analytical instruments (Model GFC-7002E) were used to measure the nitrous oxide concentration in the liquid and gas phase respectively. More detailed information is given in **Paper IV**.

4.3.2. Nitrous oxide measurement in the full-scale treatment process

Full scale treatment plant consisted of two parallel lines (L1 and L2), each with a volume of 700 m^3 divided into three zones (Z1, Z2 and Z3). Each zone was filled with about 32 % biofilm carriers (K1), which allowed the anammox and ammonium oxidizers to attach. Four months before nitrous oxide emissions were measured, the first line shifted from a one to two-step deammonification process with the Z1 partial nitritation process followed by the Z2 and Z3 anammox process. Biofilm carriers were removed from the first basin and nitrifiers were suspended to grow in Z1 with continuous aeration. The biomass in Z2 and Z3 was not changed and both basins were kept under anoxic conditions. DO, pH, conductivity and temperature were monitored on-line in both treatment lines.

Nitrous oxide in the liquid phase was measured using an on-line Unisense microelectrode. To measure nitrous oxide in the gas phase, a flux chamber, 0.9 m × 0.9 m in size, floating on the water surface was used and acted as gas sampler. Gas samples and dilution air were collected by a sample pump and further transferred to the on-line measurement equipment after the cooling system and water trap unit. Concentrations of different nitrogen compounds (nitrate and ammonium)

were measured continuously with on-line sensors connected to the floating chamber. Measurements of nitrous oxide in both liquid and gas phases were carried out in anammox reactors (L1Z2 and L1Z3) in L1 and all the zones in L2 (L2Z1, L2Z2, L2Z3). **Paper V** contains more detailed information on this.

4.3.3. Calculations

To calculate the amount of nitrous oxide emissions, calculations were undertaken for nitrous oxide in both the gas and liquid phases.

$$N_2O-N/N_{load}(\%)=\frac{\frac{C_{N_2O_{(l)}}\times 44}{10^6}\times Q_{effluent}\times 24\times\frac{28}{44}+\frac{C_{N_2O}\times 1.94\times Q_{airflow}\times 24\times A_{reactor}}{A_{sampler}\times 10^6}\times\frac{28}{44}}{N_{load}}$$

To calculate the amount of nitrous oxide existing in the reactor:

$$N_2O_{(l)(gN_2O/reactor)}=\frac{C_{N_2O_{(l)}}\times 44}{1000}\times V_{reactor}$$

To calculate the nitrous oxide production/consumption:

$$N_2O_{(g)(gN_2O/\min/reactor)}=\frac{C_{N_2O_{(g)}}\times 1.94\times Q_{airflow}}{1000\times 60}\times\frac{A_{reactor}}{A_{sampler}}$$

$$\Delta N_2O_{(gN_2O/\min/reactor)}=(N_2O_{(l)(gN_2O/reactor)_{(t+1)}}-N_2O_{(l)(gN_2O/reactor)_{(t)}})\times$$

$$\Delta t-\frac{(\frac{C_{N_2O_{(l)}(t+1)}+C_{N_2O_{(l)}(t)}}{2})\times 44\times Q_{effluent}}{1000\times V_{reactor}}+N_2O_{(g)(gN_2O/\min/reactor)_{(t)}}$$

Where:

N_2O-N – nitrogen emission in nitrous oxide form ($gN/m^3/d$);

N_{load} – nitrogen load in the reactor ($gN/m^3/d$);

44 – molar weight of N_2O;

$Q_{effluent}$ – effluent from the reactor (m^3/h);

$Q_{airflow}$ – airflow for the aeration (m^3/h);

28 – molar weight of N_2 in N_2O;

$C_{N2O(g)}$ – N_2O concentration in the off gas (ppm);

1.94 – 1ppm N_2O = 1.94 N_2Omg/l;

$A_{sampler}$ – the water surface area covered by sampler (m^2);

$A_{reactor}$ – the surface area of the reactor (m^2);

$N_2O_{(l)}$ – the total amount of N_2O in the reactor (gN_2O/reactor);

$C_{N2O\,(l)}$ – the N_2O concentration in the liquid phase (mmol/l);

$N_2O_{(g)}$ – the total amount of N_2O emitted from the reactor (gN_2O/min/reactor);

ΔN_2O – the production / consumption of the N_2O (gN_2O/min/reactor).

5. RESULTS AND DISCUSSION

5.1. Reject water treatment with a partial nitritation/anammox process

5.1.1. Aeration strategies

In order to investigate and evaluate the influence of different aeration strategies on the deammonification process, both laboratory and pilot-scale research were carried out. The laboratory study was a primary short-term study to establish a starting point for the pilot-scale study.

Laboratory-scale batch tests

In all the batches tested, a gradual decrease in ammonium and total nitrogen and an increase in nitrate concentration were observed. The highest nitrogen removal efficiency was 69.5 % in all the tested aeration strategies, when the DO concentration was 4 mg/l and R equalled 1/3. The lowest nitrogen removal was obtained when the DO concentration was 2 mg/l and R equalled 3. Not enough oxygen was supplied into the system and this led to a high ammonium concentration in the effluent in this batch test. A previous study has shown that the bottle neck for the one-stage deammonification process is the production of nitrite (Cema et al., 2011). In the present test results, nitrite concentration was influenced by the different aeration strategies. It continuously increased and reached a plateau under continuous aeration. When the system was operated under intermittent aeration, the concentration of nitrite increased during the aerated period and decreased during the non-aerated period. The actual production of nitrite was higher than the observed value because most of the produced nitrite was immediately consumed by anammox, denitrifiers or NOB. The highest nitrite production rate was achieved in the aerated period when the DO concentration was 4 mg/l and R was 1/3. The results also showed that with the same DO concentration in the aerated period, the highest nitrogen removal efficiency was when R equalled 1/3. When using intermittent aeration with R = 1/3, a higher DO concentration led to better nitrogen removal. More detailed information is given in **Paper I**.

Due to the promising results obtained in the laboratory tests, a further investigation of the intermittent/continuous aeration was undertaken in the pilot-scale study.

Pilot-scale study

Process performance

The whole pilot-scale experiment was divided into six periods. The nitrogen loads were over 3.5 $gN/m^2/d$ from period I to period IV. It decreased to 2.5 $gN/m^2/d$ from period V to period VI due to the unstable process in period IV. The nitrogen removal efficiencies varied between 82 % and 88 % during the test period, except for period IV. The highest nitrogen removal efficiency of 88 % was obtained in period II with a DO concentration of 3.5 mg/l and R of 1/3. The results showed that the introduction of intermittent aeration with R = 1/3 allowed a decrease in the total amount of aeration without any loss in process efficiency. On-

line cycle measurement showed that the ammonium concentration decreased in the aerated period but increased in the non-aerated period. The main reason for this was that there was continuous influent in the pilot-scale reactor. During the aerated period, ammonium consumption was higher than the ammonium introduced from the influent, therefore ammonium concentration decreased. During the non-aerated period, ammonium consumption was lower than the introduced ammonium, then an increase in ammonium concentration was observed. The dynamic changes to the nitrate followed the supply of aeration. Nitrate was produced when there was aeration, and was not produced when there was no aeration. The concentration of nitrite was very low in the effluent. The nitrite produced was consumed immediately and was unable to accumulate in the system. Intermittent aeration with R 1/3 gave similar nitrogen removal efficiency with continuous aeration under the different nitrogen loads. A higher DO concentration of 3.5 mg/l in the aerated period in intermittent aeration resulted in the highest nitrogen removal, which was consistent with the laboratory study.

Microorganism activity

FISH analysis was performed to identify the species of bacteria (**Paper II**). It showed that the dominant bacteria in the biofilm were anammox bacteria and ammonium oxidizers. Activity test results showed that the average value of anammox bacteria activity was 4.2 times higher than that of the denitrifiers. The dominant bacteria responsible for nitrogen removal were anammox bacteria. However, both anammox bacteria and denitrifier activity was limited within the pilot-scale study due to a deficiency in the substrate. The study results showed that anammox bacteria could outcompete heterotrophic denitrifying bacteria when the ratio of C/N in influent was below 0.5 at 25 °C, which was similar to other studies (Chen et al., 2009, Xu et al., 2010, Lan et al., 2011). In this study, denitrifying bacteria were restricted by the limited organic carbon source.

The activity of ammonium oxidizers was on average 60 % of the total maximum oxygen uptake rate (including ammonium oxidizers, NOB and aerobic heterotrophic bacteria). Ammonium oxidizers activity was 2.5 times that of heterotrophic bacteria. NOB had the lowest activity, which was 15 % of the total maximum oxygen uptake rate. Ammonium oxidizers successfully outcompeted the heterotrophic bacteria and NOB because of the low amount of oxygen supply. Ammonium oxidizers in the biofilm were not the only source for NO_2^--N production in the reactor. The activity of different groups of microorganisms in the suspended sludge was measured during the reactor operation. No activity of anammox and heterotrophic bacteria was detected in the suspended sludge. A large fraction of the bacteria in the suspended sludge was ammonium oxidizers, which made a major contribution to NO_2^--N production in the system. More detailed information can be found in **Paper II.**

5.1.2. Redox potential (ORP) control

During the entire operational period under redox control, the average value of the nitrogen removal rate was 2.18 $gN/m^2/d$ and the maximum value was 2.98 $gN/m^2/d$. The mean value of nitrogen removal efficiency was 83.9 % and the maximum value was 98.3 %. The first part of the experimental study was carried out under similar nitrogen loads. When the set point of pE was +1, DO concentration was 1.41 mg/l, which was the highest value among all the tested periods. The effluent concentration of ammonium increased and the concentration of nitrate decreased when the redox value set point was reduced from 1 to -1. Nitrite in the effluent was very low during the entire operational period at less than 10 mg/l. The highest nitrogen removal efficiency was obtained when ORP was fixed at pE=0. When pE=-1, the increased concentration of ammonium and decreased concentration of nitrate in the effluent suggested that ORP of -1 weakened the rate of nitritation and less ammonium was oxidised due to low DO concentrations in the system. When pE was +1, the ratio between the measured and calculated amount of nitrate produced was 1.36, which indicated that around 26 % of the nitrate in the effluent was produced by NOB. NOB activity was stimulated when pE =1.

The second part of the study was performed when the ORP set point was fixed at a constant value of 0 mV. The increased nitrogen loads led to an increased airflow in order to maintain a stable pE value in the system. DO concentrations varied between 0.78 and 1.11 mg/l. The experimental results showed that with a fixed constant pE value of 0, DO concentration and pH value were adjusted automatically and resulted in stable and appropriate operational parameters values. Increased nitrogen loads led to increased concentrations of ammonium and nitrate in the effluent. The nitrogen removal efficiency decreased from 89 % to 83 %, which was still relatively high. When pE=0, the system maintained relatively high N removal efficiencies even though nitrogen loads varied. It was also found that when the redox value was fixed at 0mV, the airflow rate of aeration and nitrogen loads had a linear relationship. This meant that the process with redox control could respond to sudden changes in nitrogen loads caused by nitrogen concentration in the inflow or inflow rate (**Paper III**).

5.1.3. Temperature influence

Process performance

The reactor initially started with a nitrogen load of 3.3 g $N/m^2/d$ at 25 °C and nitrogen removal efficiency was 82 %. When the temperature decreased gradually (in 3 °C steps) from 25 °C to 22 °C, the nitrogen removal efficiency dropped from 82 % to 72 %, even though the nitrogen loads were almost the same (Sultana et al., 2013). The ammonium concentration in the effluent was higher than the value obtained at 25 °C. In order to maintain the high nitrogen oxidation rate, a higher DO concentration, which was 2.3 mg/l, was used in period II compared to that in period I. Nevertheless, the increase in DO concentration from 2.0 to 2.3 mg/l did not have a significant effect on ammonium oxidation. In order to avoid increased FA concentration in the reactor, a lower nitrogen

load of 2.7 gN/m^2/d was applied in the system in period III when the temperature was 19.6 °C. DO concentration decreased together with the nitrogen load, returning to 2 mg/l (**Table 3** & **Fig. 5, 6A**). However, the nitrogen removal efficiency did not improve and the nitrogen removal rate dropped sharply. Increased pH value and a high ammonium concentration in the reactor led to FA accumulation (**Fig. 6B**).

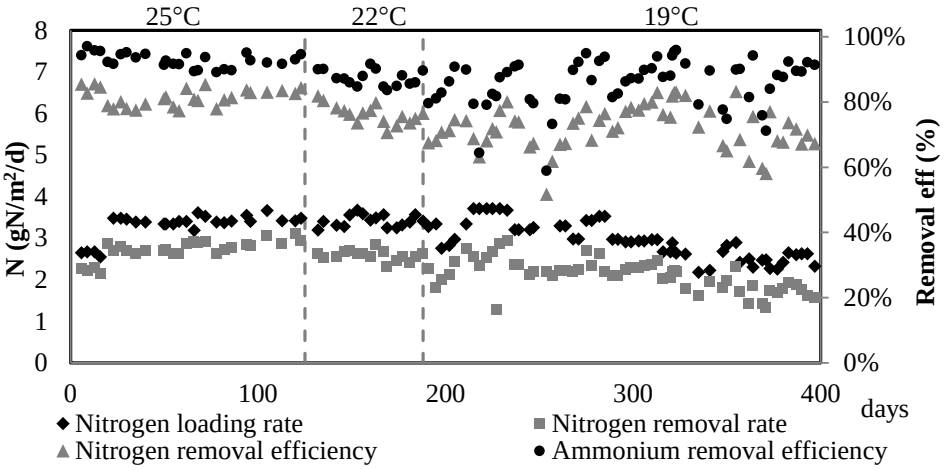

Fig. 5. Reactor operation under different temperatures.

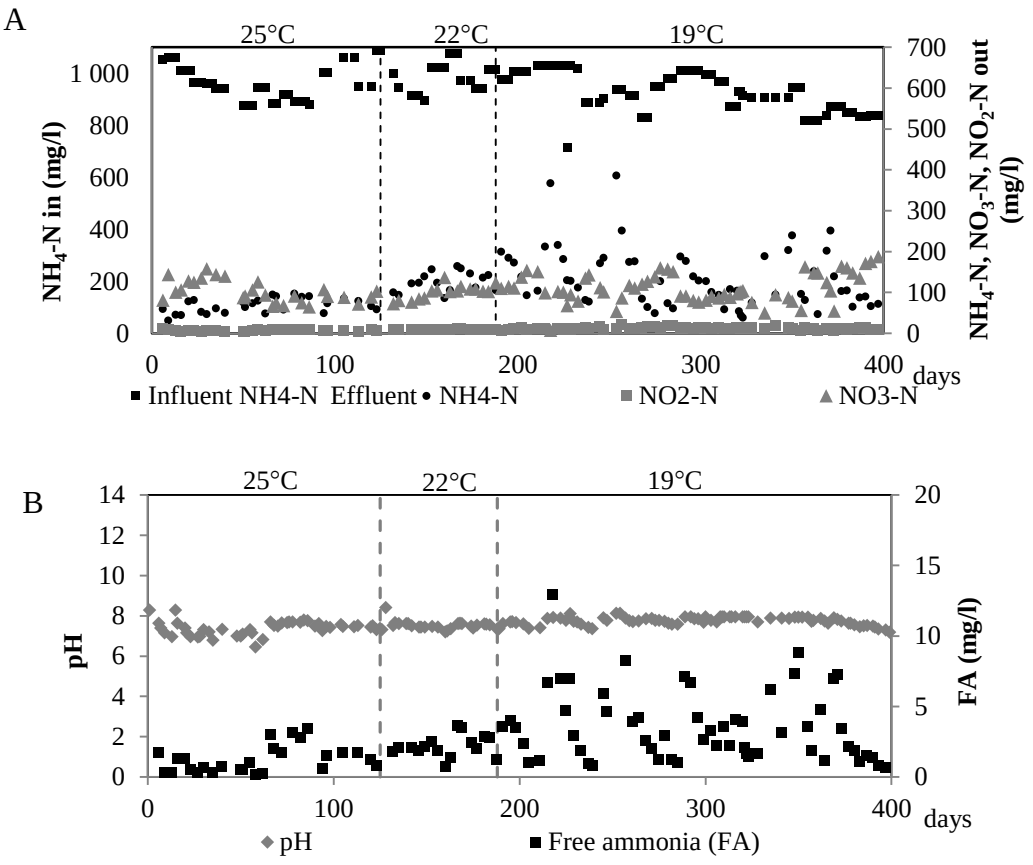

Fig. 6. A) concentrations of different nitrogen compounds in the reactor; B) pH and FA concentration in the MBBR.

During the entire operational period, the reactor performance and efficiency were influenced by temperature, nitrogen loads and DO concentrations. The increased concentrations of ammonium in the effluent from period I to period III indicated that ammonium oxidation rates decreased rapidly when the temperature dropped. The rise in DO concentrations or lowering of nitrogen loads did not improve ammonium oxidation. FA concentration in period III at reached 8 mg/l. Jaroszynski *et al.* (2012) reported that FA concentrations above 2 mg/l had a negative impact on the anammox rate. However, successful operation of nitritation-anammox reactors at higher FA concentrations, approximately 3 mg/l, has been reported by Vlaeminck *et al.* (2009).

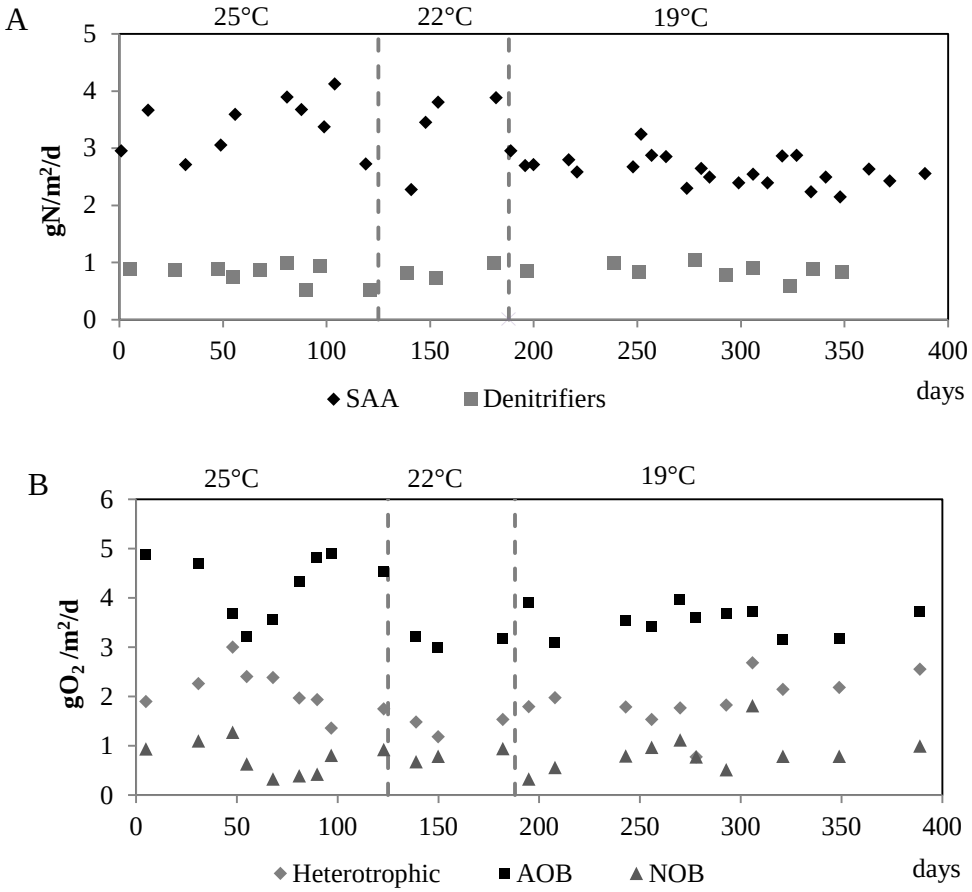

Fig. 7. Activities of different microorganisms in the MBBR at different temperatures: A) anammox and denitrifier activities, B) activities of oxygen consumers.

Table 3. Operating conditions and results.

	Period I (1-125 days)	Period II (126- 188 days)	Period III (189-546 days)
Operational condition			
Temperature (°C)	25.0±0.2	22.0±0.8	19.6±1.2
HRT (days)	1.5±0.23	1.5±0.06	1.7±0.35
DO (mgl^{-1})	2.0±0.3	2.3±0.1	2.0±0.3
pH	7.4±0.6	7.5±0.2	7.6±0.5
Performance			
Nitrogen loading ($gN/m^2/d$)	3.3±0.3	3.4±0.6	2.7±0.4
Nitrogen removal ($gN/m^2/d$)	2.7±0.2	2.6±0.1	2.0±0.4
NH_4-N (in) (mgN/l)	943±60	999±47	869±99
N removal efficiency (%)	81.5±0.0	76.3±3.1	71.9±8.5
NH_4-N (eff) (mgN/l)	67.4±18.1	125.9±24.0	121.6±81.8
NO_2-N (eff) (mgN/l)	7.3±1.8	9.5±0.8	11.1±5.2
NO_3-N (eff) (mgN/l)	102.1±26.8	100.9±17.2	110.2±44.7
Free ammonia (mgN/l)	1.2±0.9	2.2±0.8	3.5±3.7

Activities of different microorganisms

The activities of different groups of microorganisms indicated that ammonium oxidizers showed higher activity value than heterotrophic bacteria and NOB for most of the operational time (**Fig. 7**). The average values of ammonium oxidizers, heterotrophic bacteria and NOB were 3.1, 1.6 and 0.75 g $O_2/m^2/d$ respectively. The activity of ammonium oxidizers was two times greater than that of heterotrophic bacteria and four times that of NOB. During the entire study ammonium oxidizers were the biggest oxygen consumers. Decreasing the temperature did not influence the predominant role of ammonium oxidizers among the oxygen consumers. Both heterotrophic bacteria and ammonium oxidizers decreased activity at lower temperatures. Heterotrophic bacteria lost 9 % of their activity when the temperature reduced from 25 °C to 19 °C while ammonium oxidizers lost 17 %. Yamamoto *et al.* (2006) reported that the efficiency of the nitritation process decreased dramatically when the temperature was 15 °C.

The average activity value of anammox bacteria and denitrifiers was 2.9 and 0.83 $gN/m^2/d$ respectively. The activity of denitrifiers was stable throughout the entire operational period. The reduced temperature did not have any significant influence on the denitrifiers' activity. Anammox bacteria activity decreased by 22 % when the temperature was reduced. However, anammox bacteria were still the dominant group of microorganisms in nitrogen removal.

The results of the microbial activity tests on the biofilm carriers and calculated nitrogen removal rate in the pilot scale MBBR are compared in **Table 4**. It shows that the nitritation rate was higher in the reactor than the values obtained in the activity test. The reason for this was that there was suspended sludge in the MBBR, which was mainly consisting of ammonium oxidizers and partly NOB. The nitrogen removal rate in the

reactor was lower than the SAA value, which was due to limited substrate (NO_2^--N) in the reactor and the presence of inhibitors such as free ammonium (FA).

Table 4. Comparison of the results of MBBR operation and activity tests at different temperatures.

$gN/m^2/d$	Activity test			Calculated nitrogen conversion in the reactor			Ratio		
Temperature in MBBR (°C)	OUR AOB	OUR NOB	SAA	AOB	NOB	NRR	AOB (reactor /OUR)	NOB (reactor /OUR)	NRR /SAA
25	1.24	0.67	3.38	1.77	0.11	2.70	1.43	0.16	0.80
22	1.01	0.71	3.38	1.70	0.06	2.60	1.69	0.08	0.77
19	0.92	0.71	2.60	1.34	0.15	2.00	1.46	0.20	0.77

5.1.4. Research towards mainstream application

Process performance

In order to test the possibility and stability of combining UASB reactor and the MBBR with deammonification process in the treatment of municipal wastewater, the influent of the pilot-scale reactor was changed from just reject water to a mixture of UASB effluent and reject water. The UASB influent was municipal wastewater from Henriksdal WWTP. The effluent of UASB contained the following average concentrations: TN 45 mg/l, COD 54 mgO_2/l and NH_4-N 30 mg/l (Malovanyy et al., 2015). For the deammonification process, the entire operation period was divided into eight periods. **Table 5** shows the ratio of UASB effluent and reject water.

The deammonification process started by treating reject water with an ammonium concentration of 884 mg/l in the influent. The nitrogen removal efficiency was 86 % (period I). From period II, the influent of the deammonification was diluted reject water with an influent ammonium concentration of 463 mg/l. In order to maintain a similar nitrogen load to that in period I, HRT decreased to 1.33 days. However, the nitrogen removal efficiency dropped significantly to 63 %. Most of the nitrogen in the effluent was ammonium. Although DO concentration was increased from 0.8 to 1.14 mg/l, the ammonium oxidation was still not sufficient. The main reason for this was that the suspended sludge concentration in the reactor decreased considerably from 178 to 82 mg/l, in which there were mostly ammonium oxidizers. Due to the low ammonium oxidation rate, the nitrogen removal rate decreased in period III when the proportion of the UASB effluent increased in the influent MBBR. The concentration of ammonium was decreased but the nitrogen removal efficiency did not improve. From period IV, the fraction of nitrate concentration increased in the effluent. The increase in the nitrate concentration in the effluent suggested that the activity of NOB increased in the system, resulting in a low nitrogen removal efficiency (**Fig. 8**).

Table 5. Performance of deammonification MBBR during transition to mainstream conditions.

Period		I	II	III	IV	V	VI	VII	VIII
Days of operation		1-40	41-54	55-70	71-84	85-98	99-126	127-157	158-220
inflow	R[1] UASB : RW	0:100	50:50	70:30	80:20	85:15	90:10	95:5	100:0
	NH_4-N (mg/L)	884	463	252	194	146	108	67	31
	COD (mg/L)	404	194	95	84	72	56	56	37
	Alkalinity (mmol/L)	71	38	21	17	14	10	8	5
outflow	TN (mg/L)	141	259	154	87	60	68	46	40
	NH_4-N (mg/L)	51	143	80	30	39	23	16	7
	NO_2-N (mg/L)	4.5	3.7	2.6	1.8	1.3	0.4	0.3	0.1
	NO_3-N (mg/L)	66	25	21	34	18	38	22	14
	COD (mg/L)	275	148	94	70	61	48	37	24
	Alkalinity (mmol/L)	9.9	12.2	12.6	4.6	5.0	3.2	2.1	2.1
VSS (mg/L)		178.2	81.9	31.3	28.2	23.5	18.9	23.4	13.4
Biofilm VS (mg/m^2)		13.4	14.0	14.0	14.4	14.9	13.2	12.9	10.0
DO (mg/L)		0.80	1.14	0.84	0.91	1.36	0.9	0.62	0.5
pH		7.34	7.62	7.23	6.84	6.80	6.54	6.66	6.66
ORP (mV)		33	53	96	154	124	124	104	134
NLR (g $N/m^2/d$)		1.79	1.74	1.22	1.21	0.84	0.69	0.44	0.20
Efficiency (%)		86	63	59	66	63	44	42	35
NRR (g $N/m^2/d$)		1.60	1.07	0.70	0.84	0.54	0.34	0.19	0.06
HRT (days)		2.45	1.33	1.06	0.81	0.95	0.78	0.76	0.77

[1] Volumetric ratio between UASB reactor effluent and reject water.

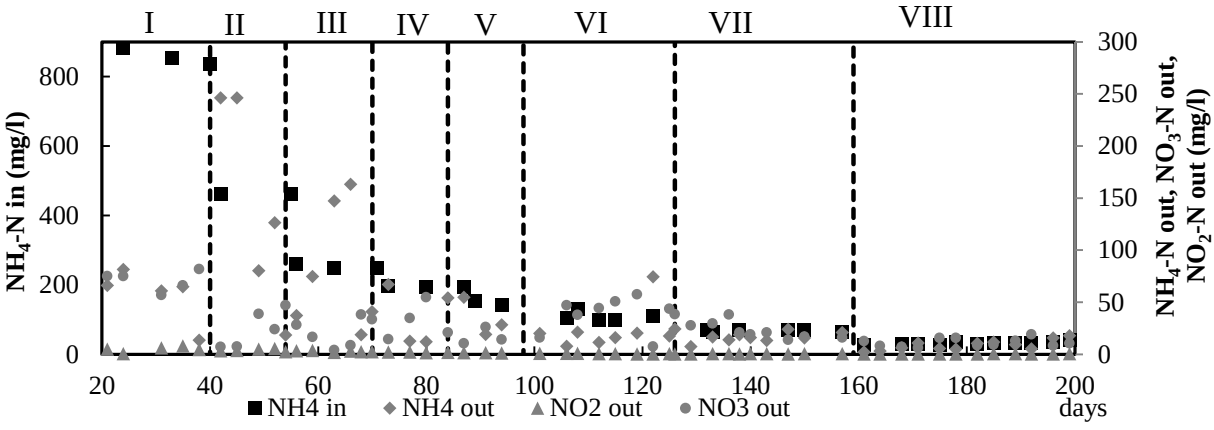

Fig. 8. Operational results with the mixture of UASB effluent and reject water as deammonification process influent.

Microorganism activity

One of the challenges in the application of the deammonification process in mainstream treatment is maintaining anammox and ammonium oxidizers as the dominant groups of microorganisms in the system to suppress NOB activity.

The activity test results showed that the activity of anammox bacteria was high and stable from period I to period V. The significant drop in activity (decrease of 27 %) occurred in period VI when the ammonium concentration was close to 100 mg/l and HRT was 18 h. During the 200 days of operation, the activity of the denitrifiers was consistently low. Anammox bacteria were the dominant bacteria responsible for nitrogen removal.

The ammonium oxidizers in the biofilm were the largest oxygen consumer. It was relatively high from period I to period V. At the end of period VI varying between 5.1 to 4.6 $gO_2/m^2/d$. At the end of period VI, the activity of the ammonium oxidizers decreased to 2.5 $gO_2/m^2/d$. NOB and heterotrophic bacteria in the biofilm retained low activity during the entire operational period. Activity of ammonium oxidizers in sludge showed a considerable decrease from period I to period VIII. Meanwhile, the activity of heterotrophic bacteria increased (**Fig. 9).**

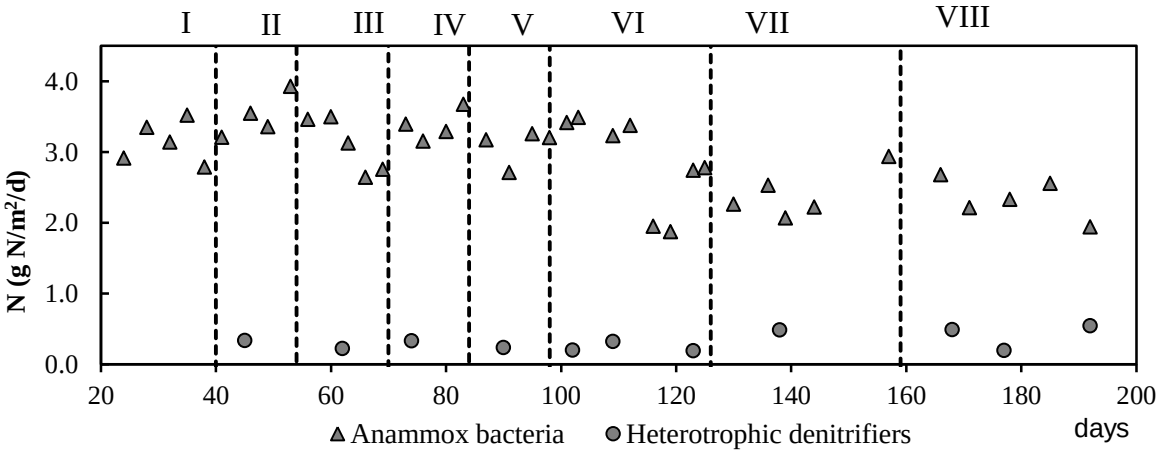

Fig. 9. Activities of anammox bacteria and heterotrophic denitrifiers during the period of being fed the UASB effluent and reject water mixture.

5.2. Nitrous oxide emission from deammonification process

In order to evaluate the greenhouse effect of the deammonification process, nitrous oxide emission was measured in both the pilot-scale and full-scale deammonification processes.

5.2.1. Pilot-scale reactors

During the measurement of nitrous oxide in the pilot-scale reactors, R1 was operated under different aeration strategies and R2 under different temperatures.

The measurements of nitrous oxide in R1 were carried out for 85 days. The average nitrogen load was 2.3 $gN/m^2/d$ and the nitrogen removal efficiency was 86 %. Nitrous oxide emission to the gas phase was 0.6 gN/d when the nitrogen load was 92 gN /d, which meant that around

0.65 % of the nitrogen load was converted into nitrous oxide in the gas phase. Nitrous oxide production increased to 1.88 gN/d when the nitrogen load applied to the reactor was 112 gN/d, indicating that 1.7 % of the nitrogen load was emitted into the gas phase as nitrous oxide. There was only 0.48 g N_2O-N/d presenting in the liquid phase, which was around 0.043 % of the nitrogen load. During the measurement, the first reactor was running with intermittent aeration. However, no difference in N_2O emission under continuous and intermittent aeration was detected (**Paper IV**).

The nitrogen load in R2 was 84 gN/d during the 45 days of nitrous oxide measurements. Nitrous oxide emitted into the air was 0.4-1.2 gN/d, which indicated that 0.4-1.6 % of the nitrogen load was converted to nitrous oxide and emitted to the gas phase. Nitrous oxide emitted with effluent accounted for just 0.02 % of the nitrogen load.

The results from both R1 and R2 showed that 0.4-2 % of the nitrogen load was emitted as nitrous oxide to the air and less than 0.05 % was released as nitrous oxide dissolved in the effluent. These results are comparable with those obtained by Kampschreur *et al.* (2008) and Okabe *et al.* (2011). The range of nitrous oxide emissions from R1 and R2 was 0.09-2.34 g N_2O/d and 0.4-1.4 g N_2O/d respectively, which were lower than the results obtained when measuring emissions from the full-scale plant (Kampschreur et al., 2009).

5.2.2. Full-scale reactors

During the measurements of nitrous oxide, influent supernatant in both lines contained an average ammonium concentration of 1476 mg/l, DOC of 380 mg/l, alkalinity of 4705 mg $CaCO_3$/l and pH of 7.8. The mean value of temperature was 28 °C in both lines. L1Z1 contained suspended sludge 450-550 mg/l and DO concentration was close to 3 mg/l. L1Z2 and L1Z3 were under anaerobic conditions. Air was intermittently supplied to all zones in L2 with 50 min aerated phase and 10 min non-aerated phase. DO level ranged between 1.8 to 3 mg/l in L2 during the aerated phase.

During the measurements, the nitrogen loads were 88 and 188 kg N/d in L1 and L2, respectively (Jönsson et al., 2015). The average nitrogen removal efficiencies were 75 % and 81 % respectively. The average values of nitrous oxide emissions were 0.12 kgN/d and 0.95 kgN/d, which was 0.12 % and 0.51 % of the nitrogen loads and 0.19 % and 0.62 % of the removed nitrogen respectively from L1 (considering only anammox reactors) and L2 (**Paper V**). These values are comparable with the value obtained in the other studies (**Table 6**).

In the L1 treatment with two-step partial nitritation/anammox processes, measurements took place in Z2 in day 1-7 and Z3 in day 8-11. Effluent from the partial nitritation step, which contained high concentrations of nitrite and the remaining free oxygen, caused higher nitrous oxide emissions in L1Z2. In the last treatment zone (Z3), the amount of nitrous oxide emissions was very low at close to zero.

In the one-step partial nitritation/anammox process (L2), the nitrogen load in the treatment line was almost constant at around 188 kgN/d. N_2O emission was higher at the beginning of the measurement and during days 23-25. That was because DO concentration was less than 2.5 mg/l during the measurement duration. On days 14 and 26, the aeration was stopped for a certain period of the time. As a result, the nitrous oxide emission was much lower than the values on the other days because most of the produced nitrous oxide was in the liquid phase and had meanwhile been consumed. Very little nitrous oxide was stripped out in the gas phase. Further results are presented in **Paper V.**

Table 6. Nitrous oxide emissions from different studies.

Processes	N_2O emission/TN in inflow	Reference
Granular sludge reactor	2%	(Castro-Barros et al., 2015)
CANON process	0.14-7.25%	(Xiao et al., 2014)
Partial nitrification	5%	(Kong et al., 2013)
Partial nitrification-anammox process	0.1-4%	(Okabe et al., 2011)
Partial nitritation	0.6-2.6%	(de Graaff et al., 2010)
Partial nitrification-anammox process	0.36-1.7%	(Kampschreur et al., 2008)
Deammonification full scale one step process	0.51%	This study
Deammonification full scale two step process (partial nitritation step)	0.12%	This study
Deammonification pilot scale one step process	0.4-2 %	This study

6. CONCLUSIONS

The aim of this study was to optimise deammonification MBBR for reject water treatment. Nitrous oxide emissions were then monitored and evaluated. Finally deammonification MBBR was connected with UASB with the aim of treating municipal wastewater.

The conclusions for controlling the deammonification process on reject water treatment are:

- The use of intermittent aeration in a one-stage partial nitritation/anammox process treating reject water can decrease aeration time without losing nitrogen removal efficiency. A 15-min non-aerated phase in a one-hour operation is a recommended operation strategy based on this study.
- Intermittent aeration offered good conditions for anammox bacteria and successfully maintained anammox bacteria as the dominant group in the reactor. Meanwhile, intermittent

aeration suppressed NOB and kept the nitrate concentration low in the effluent.

- The redox value can be used as a control parameter for the one-stage nitritation/anammox process. The highest nitrogen removal rate and efficiency were obtained when pE=0 among the tested redox values (pE= -1, 0, +1).
- When pE= 0, the nitrogen removal efficiencies were above 70 % under the different nitrogen loads applied. The amount of the air supply to the system showed a linear correlation with the nitrogen loads.

The conclusions from testing the deammonification process towards to mainstream conditions are:

- The system became unstable when the temperature was reduced from 25 to 19 °C. The significant activity reductions in ammonium oxidizers and anammox bacteria occurred when the temperature decreased to 19 °C. FA started to accumulate in the system and caused the inhibition effect.
- During the entire operational period (25 to 19°C), anammox and ammonium oxidizers were the dominant bacteria in the MBBR reactor.
- Ammonium oxidizers and anammox bacteria were dominated in deammonification process after UASB treatment during entire tested period.
- Testing decreasing influent nitrogen concentration shows that it was difficult to balance DO, nitrogen load and HRT to achieve high nitrogen removal efficiency when the nitrogen concentration in the influent was less than 100 mg/l.

The conclusions for nitrous oxide measurements are:

- In the pilot-scale reactor, nitrous oxide emission was 0.4-2% of the nitrogen load. In the full scale deammonification process, 0.51% of the total nitrogen load was converted to nitrous oxide.
- Between 80-90 % of nitrous oxide emissions were in the gas phase and the rest were emitted in the liquid phase in the pilot plant discharge.
- In the pilot scale reactor, nitrous oxide was mainly produced in the aerated period and consumed in the non-aerated period when the nitrogen load was 2.5 and 3.3 $gN/m^2/d$ with DO 1.5 mg/l.
- Nitrous oxide production occurred during the non-aerated period in the full-scale treatment plant due to over-aeration during the aerated period.

- Both dissolved oxygen and nitrogen loads influenced nitrous oxide production. However, with regard to nitrous oxygen emissions, air stripping was the main factor.

General conclusions:

- Both intermittent aeration and redox control could be used as control strategies to optimise the deammonification process treating reject water. Intermittent aeration could decrease the cost of operation without losing process efficiency. Redox control could adjust the aeration automatically with the changes of nitrogen loads.
- Although deammonification process has being applied in the full-scale reject water treatment, however, to use deammonification process in mainstream is still a challenge. Both ammonium oxidizers and anammox bacteria could be maintained in the deammonification reactor when the temperature was 19°C. The combined UASB reactor and MBBR with deammonification was an option to treat mainstream wastewater.
- Nitrous oxide production was related to the nitrogen loads and oxygen supplied in the deammonification process. Nitrous oxide emission was significantly influenced by the nitrogen loads, supplied oxygen and air flow in the reactor.

7. FURTHER RESEARCH WORK

Based on the studies undertaken, the following research is recommended:

- Mainstream deammonification application would be a major economic benefit for WWTP. Reliable control systems are needed to ensure process performance. The most commonly used control strategies are based on ammonium/nitrate concentration in the effluent or DO control. Other low-cost alternatives based on pH and redox should be developed. A successful control system should be able to enhance the activity of anammox and ammonium oxidizers but suppress that of NOB.
- The deammonification process has been successfully applied using different reactor configurations, SBR, granular sludge and MBBR systems have mainly been used. This study shows that suspended sludge in the biofilm reactor mainly consist of ammonium oxidizers. Therefore, the development of an integrated fixed film activated sludge (IFAS) system would be an efficient way of enhancing the ammonium oxidation rate and could be successfully applied in both mainstream and side stream in future.
- This study investigated the combination of UASB reactor and MBBR with demmonification process to treat municipal wastewater. In this specific case the UASB is used as an

effective method of removing dissolved organic matter, but other methods could be used in future combinations, such as enhanced precipitation and High Rate Activated Sludge (HRAS).

- The measurement of greenhouse gas emissions from wastewater treatment systems is a new research area and the development of suitable measurement equipment is still in progress. As measurements are normally carried out in the field, robust and reliable equipment is needed.
- Future studies should be also focused on understanding how N_2O is produced and which factors influence N_2O emissions.

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