PARTIAL NITRITATION/ANAMMOX PROCESS IN A MOVING BED BIOFILM REACTOR OPERATED AT LOW TEMPERATURES

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SUMMARY

The deammonification process (partial nitritation with anammox) has been used for several years to treat the ammonium rich supernatant in a cost effective way. However, still the introduction of anammox process into mainstream nitrogen removal is challenging due to less knowledge about how to deal with the slow growing anammox bacteria at the main stream conditions (low nitrogen concentrations (<80 mg N L^{-1}) and low temperatures (<20 °C). In this regard, the aim of the study was to investigate the deammonification process performance and efficiency under moderately low temperatures (from 19 °C to 10°C) and low nitrogen concentration in the influent (500 mg N to 45 mg NL^{-1}). This study also focused on evaluation of the influence of different environmental factors such as dissolved oxygen concentration, pH on the establishment and interaction between different microbial populations and how this will affect their activities. Therefore, the activity of different groups of microorganisms in the biofilm, was measured both in the long and short term during the entire operational study. Different online parameters such as dissolved oxygen, conductivity, pH, temperature and redox potential (ORP) were monitored. In addition, chemical analysis and microbial activity tests were performed. To investigate the microbiological behavior different microbial activity test such as Specific Anammox Activity test (SAA), Oxygen Uptake Rate test (OUR), and Nitrate Uptake Rate test (NUR) were carried out. A series of batch tests was performed to investigate the short term effect of temperature decrease on anammox biomass. Microbiological tests were also performed in cooperation with Chalmers University of Technology to investigate the stratification of the different groups of microorganism. High efficiency was observed from 19 to 16 °C and stable process performance was achieved down to 13 °C with low efficiency. However, the process lost stability at 10 °C. The activity of anammox bacteria dropped significantly with temperature decrease. To investigate low nitrogen concentration at low temperature (13 °C) the reactor was operated for ten months and the influent nitrogen was gradually decreased stepwise in six periods. Results showed that the activities of different groups of microorganisms decreased significantly and it became very crucial to find an optimum condition for the reactor. A relatively stable removal was possible though the stability of the performance was strongly dependent on the dissolved oxygen (DO) concentration and suppression of NOB (Nitrite Oxidizing Bacteria). In summary, dissolved oxygen became very crucial during the entire experimental period and optimizing the DO at different temperature and different concentration is very important to run the process in an efficient way.

SUMMARY IN SWEDISH

Deammonifikation (partiell nitritation följt av anammox) används sedan några år tillbaka för kväverening av varmt kväverikt rejektvatten från anaerob rötning på avloppsreningsverk (ARV). Däremot används inte ännu deammonifikation för kväverening från huvudströmmen av avloppsvatten vid ARV. Där är förhållandena, med lägre kvävehalter (<80 mg/L) och lägre temperaturer (<20°C), svårare för de långsamväxande anammoxbakterierna. Målsättningen med denna studie var att undersöka hur deammonifikationsprocessen fungerar vid låga temperaturer (19 °C – 10 °C) och låga kvävehalter (500 mg/L – 45 mg/L). I detalj var målsättningen att undersöka hur olika processparametrar, som halterna av löst syre och pH, påverkar samverkan mellan de olika mikrobiella grupperna och deras aktivitet. Försöken utfördes i pilotskala i en reaktor med biofilmer på bärare. Syrehalter, pH, temperatur och redox potential (ORP) mättes on-line. Kväveföreningar och en mängd vattenkemiska parametrar mättes med kemiska metoder. Aktiviteten för olika grupper av mikroorganismer mättes med olika test; Specifik anammoxaktivitet (SAA) för anammoxbakterier; Syreupptagningshastighet (OUR, oxygen uptake rate) för aeroba ammoniakoxiderand bakterier (AOB), aeroba nitritoxiderande bakterier (NOB) och aeroba heterotrofa bakterier; samt Nitratupptagnings (NUR, nitrate uptake rate) för denitrifikationsbakterier. För att i detalj undersöka hur temperatur påverkade anammoxaktiviteten genomfördes också SAA mätningar vid specifika temperaturer i enskilda mätomgångar. Mikrobiologiska undersökningar gjordes dessutom i samarbete med Chalmers Tekniska Högskola för att undersöka sammansättningen av anammoxbakterier, AOB och NOB och var i biofilmerna dessa grupper var lokaliserade. Resultaten visade att deammonifikationsprocessen var stabil med en hög avskiljningsförmåga vid 19 och 16 °C. Vid 13 °C, sjönk avskiljningsförmågan, men även vid denna temperatur var processen stabil. Vid 10 °C däremot kunde ingen stabil process erhållas. Anammoxbakteriernas aktivitet sjönk med sjunkande temperatur mellan 19 och 10 °C. För att undersöka deammonifikationsprocessen vid såväl låga temperaturer som låga kvävehalter genomfördes pilotförsök i tio månader vid 13°C med gradvis minskande kvävehalter. Resultaten visade på minskande aktiviteter vid de lägre halterna och det blev avgörande att finna optimala processförhållanden. Detta till trots kunde en relativt stabil process erhållas, där halterna av löst syre var en avgörande parameter. Sammanfattningsvis var syrehalterna viktiga under hela försöksperioden och optimering av dessa halter vid de olika temperaturerna avgjorde processens effektivitet.

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TABLE OF CONTENTS

LIST OF PAPERS

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

I. **Sultana, R**., Yang, J., Trela, J., Plaza, E., Wilén, B.M., Persson, F. (2014). Deammonification process performance and efficiency at moderate to low temperatures. Submitted to Chemical Engineering Journal.

II. Persson, F., **Sultana, R**., Wilén, B.M., Hermansson, M., Sörensson, F., Matsson, A., Plaza, E. (2013). One-stage nitritation-anaerobic ammonium oxidation at low temperatures in a moving bed biofilm reactor. In Proceedings of IWA Specialized Conference "Holistic Sludge Management, May 6-8, 2013 Västerås, Sweden.

III. **Sultana, R**., Plaza, E., Persson, F., Wilén, B.M. (2014). Partial nitritation/anammox with moderate to low nitrogen concentrations at 13°C. (Manuscript).

Other papers which are not appended in the thesis:

Persson, F., **Sultana, R.**, Suarez, M., Hermansson, M., Plaza, E. and Wilen, B. M. (2014). Structure and composition of biofilm communities in a moving bed biofilm reactor for nitritation-anammox at low temperatures. Bioresource Technology 154, 267-273.

Sultana, R., Yang, J., Trela, J., Plaza, E. (2013), Deammonification process performance and efficiency at different temperatures. In Proceedings of IWA Specialized Conference "Holistic Sludge Management, May 6-8, 2013 Västerås, Sweden.

Sultana, R., Bhattacharya, P. (2012), Evaluation of Groundwater chemistry in Shallow Aquifer System (dugwells). Proceeding of Polish-Ukrainian-Swedish seminar "Future Urban sanitation to meet new requirements for water quality in the Baltic sea region", Krakow, Poland. E. Plaza, E.Levlin (editors). TRITA LWR REPORT 3031.

ACRONYMS AND SYMBOLS

AOB	Ammonium Oxidizing Bacteria
AOR	Ammonium Oxidation Rate
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FA	Free Ammonia
FISH	Fluorescent in situ Hybridization
FNA	Free Nitrous Acid
HRT	Hydraulic Retention Time
MBBR	Moving Bed Biofilm Reactor
NOB	Nitrite Oxidizing Bacteria
NOR	Nitrite Oxidation Rate
NLR	Nitrogen Loading Rate
NRR	Nitrogen Removal Rate
NUR	Nitrate Uptake Rate
OM	Organic Matter
OUR	Oxygen Uptake Rate
qPCR	Quantitative Polymerase Chain Reaction
SAA	Specific Anammox Activity
TIC	Total Inorganic Carbon
TN	Total Nitrogen
UASB	Up flow Anaerobic Sludge Blanket
RBC	Rotating Biological Contactor
SBR	Sequencing Batch Reactor
WWTP	Wastewater Treatment Plant

ABSTRACT

The application of partial nitritation/anammox process to remove nitrogen from wastewater is a cost effective and sustainable approach since it can save energy and resources. It was applied successfully in treating ammonium rich waste streams (Wett, 2007; Abma et al., 2010). This is worth to use deammonification (partial nitritation/anammox) process in sewage treatment to create an energy positive environment and therefore, this has been studied extensively for last few years to investigate its applicability in mainstream condition where both temperature (10-20 °C) and nitrogen concentration (<100 mgNL^{-1}) are very low. In this regard, the aim was to investigate the partial nitritation/anammox process at moderate to low temperature and different nitrogen concentrations in a pilot scale MBBR. The study was divided into two steps. In first step, the long-term influence of low temperatures (from 19 to 10 °C) on one stage deammonification was investigated. Stable process performance with high efficiency (77%) was observed until 16 °C. Stable process with low efficiency (55%) was observed down to 13 ºC. However, at 10ºC nitrogen removal was very low and the process lost its stability. Batch tests showed that the potential activity of ammonia oxidizing bacteria (AOB) and anammox bacteria also decreased when the temperature was decreased from 19ºC to 10ºC. In second step the influence of low nitrogen concentrations at low temperature on the deammonification process was investigated. The concentration of influent nitrogen was gradually decreased from 500 mg L^{-1} to 45 mg L^{-1} NH_4-N at 13°C. Results showed that an MBBR operated at certain operating conditions can achieve maximum of 0.2 gNm^{-2}d^{-1} removal of nitrogen, at a loading rate of 0.3 gNm^{-2}d^{-1}. Although the total inorganic nitrogen removal rates showed potential, the suppression of nitrite oxidizing bacteria (NOB) was challenging. Quantitative PCR data showed that the biofilm biomass was dominated by anammox bacteria with considerably fewer AOB. The overall study shows the applicability of one-stage nitritation-anammox in MBBRs at low temperatures with moderate to low strength wastewater and highlights the importance of quantification and activity of AOB, NOB and anammox bacteria for understanding process performance.

Key words: Anammox, Deammonification, MBBR, Low temperature, Nitrogen

1. INTRODUCTION

1.1. Importance of nitrogen removal

Biological nutrients such as nitrogen (N), phosphorus (P) and potassium (K) are needed in great amount for the growth of living organisms. However, eutrophication can occur which damages the aquatic life rigorously if these nutrient concentrations (N, P) exceed the safe level. Wastewater from domestic households or industries is the main sources of nitrogen. For instance, municipal wastewater contains <80 mg N L^{-1} of NH_4-N (Metcalf & Eddy, 2003) whereas sludge liquor from digester contains 1000 mg L^{-1} of NH_4-N (Weisman, 1994). Some studies showed the landfill leachate may contain extremely high ammonium concentration varied from 1400 to a maximum of 2800 mg L^{-1} (Liang & Liu,

2008; Cema et al., 2009). Therefore, it is necessary to take actions to limit the discharge of nitrogen by different wastewater treatment plants (WWTP). In this regard, the legislation was set according to European Union Directive 91/271/EEC (EU commission, 1991) to regulate the discharge limit for nitrogen and phosphorus concentrations. According to this directive, the discharge limits are 10 mg L^{-1} for total nitrogen (TN) and 1 mg L^{-1} for total phosphorus (TP) for wastewater treatment plants connected to 100,000 person equivalents (P.E) if the water is discharged to sensitive areas. More stringent requirements were proposed by Baltic Sea Action Plan (Baltic Sea Action Plan, 2007) taken by different stakeholders including governments to restore the ecosystem of Baltic Sea. BSAP proposed that the wastewater treatment plants discharging to sensitive marine areas should remove 70-80% of the inflow nitrogen load and 90% of the inflow phosphorus load. Therefore, it is necessary for the countries around Baltic Sea to take actions to ensure the WWTP follow the new legislation. In this case the WWTP should treat the wastewater in an efficient way using both biological (secondary) and/or advanced treatment technologies. Biological nitrogen removal process should include innovative and novel ways which can be both energy positive and sustainable.

1.2. Biological nitrogen removal processes

The conventional way to remove nitrogen from WWTP is nitrification/denitrification process over nitrate.

1.2.1. Nitrification

Nitrification occurs by oxidizing ammonium to nitrate. Nitrite is formed as an intermediate in this reaction. Two different groups of microorganism include ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) are responsible for nitrification.

AOB convert ammonia to nitrite by the following stoichiometric equation:

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

The reaction for the oxidation of nitrite to nitrate by NOB is as follows.

$$NO_2^- + 1.5\ O_2 \rightarrow NO_3^- \quad (2)$$

Based on the growth yields for ammonium oxidizer and nitrite oxidizer, the reaction can be written as:

$$NH_4^+ + 1.83\ O_2 + 1.98\ HCO_3^- \rightarrow 0.021\ C_5H_7NO_2 + 1.041\ H_2O + 0.98\ NO_3^- + 1.88\ H_2CO_3 \quad (3)$$

The above equation shows that 4.18 g oxygen is required to oxidize per gram ammonium-nitrogen (Lin et al., 2009).

1.2.2. Denitrification

Biological conversion of nitrate to nitrite and nitrogen gas is occurred in denitrification process. In this process nitrate and nitrite are electron acceptors and carbon source acts as electron donor. Hence, denitrification occurs without the presence of oxygen. The process involves the transfer of electrons from

carbon substrate to nitrate or nitrite. Methanol is the most common external carbon source for this process.

The overall process equation shows:

$$NO_3^- + 1.08CH_3OH + 0.24\ H_2CO_3 \rightarrow 0.056\ C_5H_7NO_2 + 0.47\ N_2 + 1.68\ H_2O + HCO_3^- \quad (4)$$

The above equation indicates the requirement of organic carbon and nitrogen in this process.

1.2.3. Anammox Process

In nature inorganic nitrogen exists in different valence from +5 (nitrate) to -3 (ammonium, ammonia). After the discovery of anammox bacteria the nitrogen cycle was updated (Fig. 1). The anammox process bypasses the biological nitrification denitrifcation and converts ammonium (NH_4^+) to dinitrogen gas by using NO_2^- as electron acceptor.

The stoichiometric equation for anammox process (Strous et al. , 1998):

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (5)$$

The above equation shows that for every mole of NH_4-N, 1.32 moles of NO_2-N are consumed and 0.26 moles of NO_3-N are produced. The anammox reaction also provides alkalinity since 0.13 moles of H^+ are consumed according to the stoichiometry.

1.2.4. Partial nitritation/anammox (Deammonification)

The deammonification process is a novel way to treat ammonia rich wastewater by using partial nitritation coupled with anaerobic ammonium oxidation (anammox). The process requires two steps. In first step half of the NH_4^+ is oxidized to NO_2 by AOBs (partial nitritation) according to the equation 1. The remaining NH_4 and the produced NO_2 is used to produce dinitrogen (N_2) by anammox bacteria in second step according to equation 5.

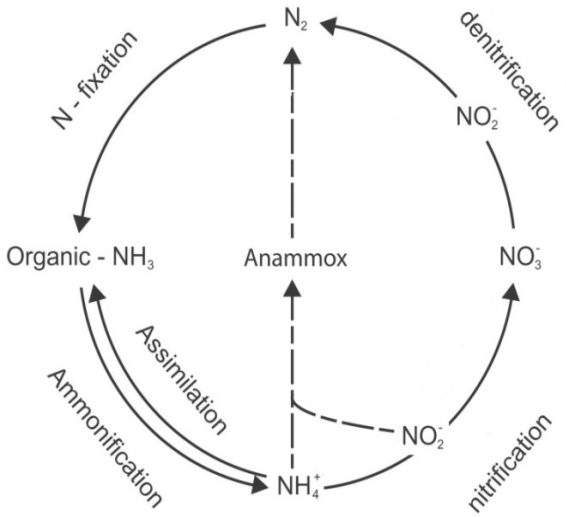

Fig. 1: Simplified Nitrogen cycle coupled with anammox (modification after Trimmer et al., 2003).

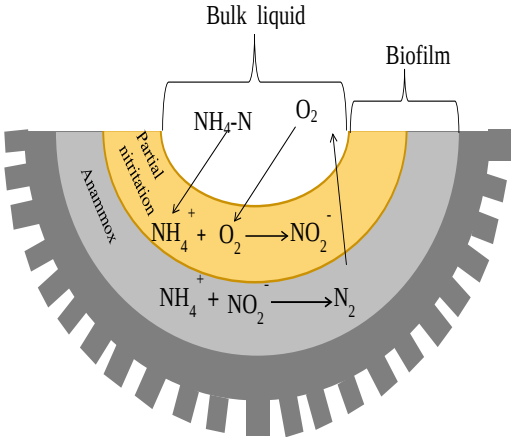

Fig. 2: Partial nitritation/anammox process in biofilm system.

The deammonification process can be applied in different system configuration. It can be either one stage deammonification or two stage deammonficiation. In two stage deammonification, nitritation and anammox process occur in separate reactors (Cema, 2009). However, in one stage deammonification, biomass is formed either by granules (Winkler et al., 2012) or biofilm grows on carriers, such as Kaldnes carriers (Szatkowska et al., 2007). In a one-stage deammonification process, partial nitritation occurs simultaneously with anammox and therefore, ammonium oxidizers co-exist with anammox bacteria and partial nitritation occur simultaneously with anammox as shown in Fig. 2. As it is shown, the outer thin layer is concentrated with aerobic ammonia oxidizers (AOB) and the inner layer of the biofilm contains anammox bacteria. The deammonification process has been successfully applied to treat ammonium rich wastewater after the discovery of anammox bacteria (Wett, 2007; Abma et al., 2010).

Advantages

The economic benefit of using deammonification is widely accepted over conventional nitrification denitrification process due to the cost reduction. Since anammox process require low carbon to nitrogen ratio therefore, deammonification saves about 60% of the aeration cost, 90% of the sludge handling and transport cost. Since no additional carbon is needed therefore, 100% cost is saved regarding external use of carbon compared to conventional nitrification/denitrification (Mulder, 2003). Study showed approximately 30–40% of the overall nitrogen removal costs can be saved (Fux & Siegrist, 2004). The concentration of oxygen in the system plays a key role in balancing the microbial activities, since 1.8 g O_2 per gram of nitrogen is required to achieve sufficient ammonium oxidation. In this case it is also important to maintain the optimal oxygen concentration to avoid excess nitrite production. Suppression of NOB to avoid excess nitrate production requires very low dissolved oxygen (DO) (e.g. 0.3 mg O_2 L^{-1}) in this process (Joss et al., 2009). Since energy cost is saved and no external carbon source is required therefore, application of

this process can create positive environment and maintain the strict requirements of nutrient discharge.

2. DEAMMONIFICATION PROCESS AT LOW TEMPERATURES WITH MODERATE TO LOW AMMONIUM CONCENTRATION

2.1. Different groups of microorganisms

As mentioned before in section 1.2, mainly three groups of chemolithoautotrophic bacteria namely aerobic ammonia oxidizers (AOB), aerobic nitritie oxidizers (NOB), and anaerobic ammonia oxiziders (Anammox) actively participate in deammonification process for the biological conversion of ammonia to nitrogen gas. All of these microorganisms can gain energy for their growth from the oxidation of nitrogen compounds.

Ammonia oxidizing bacteria (AOB)

Partial nitritation was carried out by AOB since these groups of microorganisms convert ammonia to nitrite under aerobic condition according to equation 1. The groups mainly comprise of genera *Nitrosomonas* and *Nitrospira*. Doubling time for AOB is 30 days and biomass yield is 0.013±0.019 g dry weight per gram of NH_3-N (Schmidt et al., 2003). Study showed at very low oxygen concentration (<0.8 mg L^{-1}) AOB use small amount of nitrite as terminal electron acceptor to produce nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen N_2 (Poth & Focht, 1985). Microbiological experiment with pure cultures showed that the optimal temperature for AOB is 35 °C (Grunditz & Dalhammar, 2001).

Nitrite oxidizing bacteria (NOB)

NOB has more versatility in comparison with AOB. This microorganism is much more sensitive to oxygen limitation than AOB. NOB oxidizes nitrite to nitrate by using oxygen according to equation 2. The well known genera for this group comprise of Nitrobacter and Nitrospira. The specific growth rate for NOB is 0.04 h^{-1}. The apparent activation energy of nitrite oxidation is 44 kJ mol^{-1}.These bacteria can be inhibited by hydroxylamine, ammonia and nitric oxide (Schmidt et al., 2003).The optimum temperature for this group of bacteria is 37 °C (Grunditz & Dalhammar, 2001).

Anammox bacteria

Engelberd Broda used thermodynamic calculation and predicted the existence of chemolithoautotrophic bacteria which can oxidize ammonium to nitrite as an electron acceptor (Broda, 1977). Mulder et al. (1995) confirmed this prediction after two decades later while doing experiment in a fluidized bed reactor. Strous et al.(1999a) reported the group belongs to the order planctomycetales based on the 16SrRNA gene sequence. The common genera for this group are Candidatus Brocadia anammoxidans. Anammox bacteria has a very low slow growth rate of 0.0027 h^{-1}. Doubling time is 11 days and biomass yield is 0.13 g dry weight per gram of NH_3-N (Schmidt et al., 2003).The apparent activation energy is 70 Kj mol^{-1} (Strous et al., 1999a). This

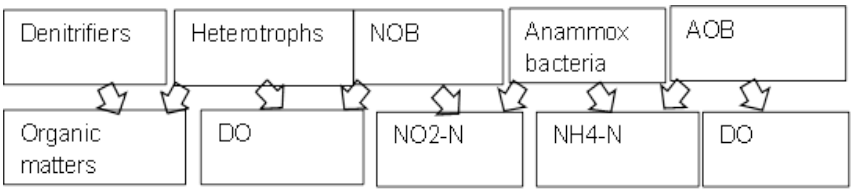

Fig. 3: Competition among different groups of micro-organisms.

group has very high affinity for substrate ammonia and nitrite. Studies showed the optimum temperature for anammox bacteria is 37°C and they are active within temperatures ranges from 6 to 43°C (Egli et al., 2001).

2.1.1. Competition among different group of microorganism

The competition for DO and organic carbon among these different groups of microorganism indicate that both NOB and AOB use DO and therefore, DO can be a limiting factor depending on the necessity and temperature in any certain condition (Fig. 3). At higher temperature AOB outcompeted NOB since AOB has higher affinity for oxygen compare to NOB. Therefore, subsequent nitrate production by NOB also can be prevented (Hellinga et al., 1998). But at low temperature DO concentration should be minimal since anammox bacteria can be inhibited by oxygen (Vazquez-Padin et al., 2009). Both the denitrifiers and heterotrophs compete for the organic matter (OM) and it is possible to avoid denitrifcation if the organic substance is low in the system.

2.2. Factors influencing partial nitritation/anammox process stability and efficiency

2.2.1. Temperature

Temperature is a key parameter in deammonification process since the effect of temperature influence the mass transfer and growth rate among different groups of microorganisms. In the nitrification process the rise of temperature creates two opposite effects including the increase of free ammonia inhibition and increased activity of the organisms. However, batch tests (Van Hulle et al., 2007) showed that temperatures between 35 and 45 °C are optimal for partial nitritation. Most of the studies on deammonification were carried out at temperature 30-35 °C since the optimal temperature for AOB and anammox bacteria are 35-40 °C (Isaka et al., 2008). Even partial nitritation/ anammox process was operated successfully in rotating biological contactor at 20 °C (Cema et al., 2007; Isaka et al., 2007). Strous (2000) reported the optimum temperature for the anammox biomass was 40 ± 3°C. So far few studies have been done to investigate the influence of low temperature on anammox process efficiency and bacteria's activity. Rysgaard et al., 2004 tested denitrification and anammox activity in marine sediment and reported that anammox bacteria showed activity at temperature between -2 to 30 °C with an optimum temperature of 12 ºC. Dalsgaard & Thamdrup (2002) showed

almost similar results while they were working with marine sediments from Baltic North Sea region. Dosta et al. (2008) investigated the effects of temperatures on the anammox activity both in short and long term. Study results showed that anammox bacteria reached the maximum activity at 35 – 45 °C in the short term batch tests and they operated successfully in laboratory scale SBR with granule sludge at 18 °C. Szatkowska & Plaza (2006) investigated the influence of temperature in anammox process by batch test temperature ranges from 24 to 31 °C. They reported a linear relationship between nitrogen removal and temperature. They observed that a sudden decrease in temperature affected anammox nitrogen removal rates. Hao et al. (2002) performed modeling based on the evaluation of temperature ranges from 15 to 40 ºC. The results showed a thicker biofilm or lower nitrogen load is required to have relatively high nitrogen removal efficiency at lower temperature. Recently Hu et al. (2013) operated successfully a nitritation /anammox SBR in lab scale at 12°C by using synthetic wastewater. They reported 90% of removal of nitrogen is possible even at 12 °C with influent nitrogen concentration of 70 mg N L^{-1}.

2.2.2. *Dissolved Oxygen (DO)*

Oxygen concentration influences significantly the nitrification process if it is considered as rate limiting factor in MBBR (Hem et al., 1994). Study showed air saturation less than 0.5% can provide a stable interaction between *Nitrosomonas* as aerobic microorganisms and *Planctomycetales* as anaerobic bacteria (Sliekers et al., 2002). Canziani with his coworkers showed dissolved oxygen is the main controlling factor though free ammonia (>2.5 mg L^{-1}) also can influence the process (Canziani et al., 2007). Low DO concentration (<0.5 mg L^{-1}) can achieve stable inhibition of the NOBs. Guo et al. (2009) studied long-term effect of DO on partial nitrification performance and interestingly they did not observe any influence of low DO on nitrite accumulation. Cema et al. (2011) studied the influence of DO in a MBBR and observed that increase of biofilm thickness does not have any influence on nitrogen removal even if the oxygen concentration was high or low. According to their study even higher DO can achieve higher nitrogen removal but also leads to higher nitrate production. Model simulations carried out by Hao et al. (2002) indicated that the maximum nitrogen removal rate could be possible if the dissolved oxygen concentration can be kept pace with the ammonium surface load. Zubrowska-Sudol et al. (2011) studied the influence of different aeration strategy on deammonification process while operating pilot scale MBBR and concluded that an optimal aeration strategy is needed considering both DO concentration and the ratio between time of non-aerated and aerated phases.

2.2.3. *pH*

Different studies showed different pH values for nitrification. However, the optimum pH for both AOB and NOB ranges

between 7 and 8 (Van Hulle et al., 2010). Since AOB use NH_3 as substrate therefore they prefer slightly alkaline environment. However, at certain pH values NH_3 and HNO_2 can exhibit inhibitory effects. Hellinga et al. (1998) observed the growth rate of NOB decreased at pH 7. However, variation for AOB at this certain pH condition is negligible. Nitrification rate will decrease due to CO_2 stripping if the pH is below 7 (Wett & Rauch, 2003). The optimal pH interval for anammox is 6.7–8.3 with an optimum of 8 as mentioned by Strous et al. (1999b). Low pH (6.5±0.01) and free ammonia (FA) concentrations (0.5 mg L^{-1}) were the critical parameters for stable anammox activity in MBBR (Jaroszynski et al., 2011).

2.2.4. Different inhibitors

Several inhibitors effect and influence the activity and efficiency of anammox process. Many studies have already been conducted to observe and show the impact and effect of different inhibitors regarding the anammox process.

Nitrite

Controlling the nitrite concentration is very important since excess nitrite production can inhibit the anammox bacteria activity. However, exact threshold value of nitrite inhibition is not known so far. Dapena-Mora et al. (2007) reported that the concentration of nitrite up to 350 mg L^{-1} can create 50% inhibition of the anammox process. Different studies showed different nitrite concentration can inhibit the anammox process completely (eg. Strous et al., 1999; Fux et al., 2004; Egli et al., 2001). Dosta et al. (2002) observed that the ratio of nitrite to ammonium consumption decreased from 1.38:1 to 1.05:1 while decreasing the temperature from 30 to 18 °C. Egli et al.(2001) showed the anammox genera *Kuenenia Stutgarttiensis* has a very low tolerance to nitrite (>5 mM).

Free ammonia (FA)

FA inhibits nitrification at high pH (>8), whereas free nitrous acid HNO_2 inhibits at low pH (<7.5). In literature different threshold values were proposed for nitrification inhibition (Anthonisen et al., 1976; Van Hulle et al., 2007). Anthonisen et al. (1976) reported that FA inhibition for AOB occurred at NH_3 concentrations of 8–120 mg N L^{-1} and HNO_2 concentrations of 0.2–2.8 mg N L^{-1}. Li et al. (2012) studied the influence of free ammonia and reported that high FA (<20 mg L^{-1}) resulted in the deterioration of the system. According to another study FA concentration exceeding the value 2 mg N L^{-1} can inhibit the process and reduce nitrogen removal (Jaroszynski et al., 2012).

Organic matter(OM)

Nitrogen rich wastewater with low OM is favourable for anammox since anammox process is autotrophic. Anammox bacteria cannot compete with denitrifiers due to slower growth rate if certain level of organic matter is present in the wastewater (Strous et al., 1999). Anammox bacteria and denitrifiers can coexist and play an

important role in treating streams with less biodegradable organic carbon (Ruscalleda et al., 2008). Many studies showed maintaining low carbon to nitrogen (C/N) ratio (<0.5) in influent stream can create suitable condition for anammox (Winkler et al., 2011). Study showed severe inhibition to anammox bacteria can occur if methanol and ethanol is used even at very low concentration (<1 mM) (Kartal et al., 2007).

2.3. Earlier studies

Deammonification (Partial Nitritation/anammox) requires good operations strategy to get high performance efficiency at low temperatures. One of the strategies is to produce necessary biomass in a separate reactor with favorable temperature for anammox bacteria and decreased the temperature gradually. After that the adapted biomass can be used as seed culture in another reactor which is operated at low temperature.

Until now extensive research have been done to investigate the feasibility of the partial nitritation/anammox process (De Clippeleir et al., 2011; Ma et al., 2011; Winkler et al., 2012; Lotti, 2013). The viability of anammox process under low and moderate temperature conditions was tested by different studies as well (Hendrickx et al., 2012; Ma et al., 2011; Ma et al., 2013). An overview of these studies is shown in Table 1. Recently, Wett et al. (2013) investigated the feasibility of anammox based deammonification in mainstream condition. They tested bioaugmentation, enrichment and performance of anammox organisms in mainstream treatment at three different experimental scales. Two different technologies were investigated include selective cyclone to retain anammox granules and intermittent

Table 1: Overview of nitritation/anammox process operated at moderate to low temperatures.

Reactor type	Influent	Temp (°C)	Influent nitrogen (mgL^{-1})	HRT (h)	N load (gNm^{-3} d^{-1})	NRR (gNm^{-3} d^{-1})	References
SBR (granule)	Synthetic	18	300	24	300	290	Dosta et al., 2008
Fixed bed	Synthetic	18	340	24	320	280	Isaka et al., 2008
SBR (granule)	Supernatant	15	200-350	24	700	200	Vazquez padin et al., 2011
Anoxic aerobic granular sludge	Supernatant	18±3	150	3	1200	900	Winkler et al., 2012
Gaslift (granular sludge)	Synthetic	20	69	5.3	310	260	Hendrickx et al., 2012
UASB	Synthetic	30-16	20	2.28	2400	2280	Ma et al., 2013
RBC	Synthetic	15	55	1	1100	500	Declippeleir et al., 2013
*SBR (lab scale)	Synthetic	30-12	580-70				Hu et al , 2013
**SBR		15					Wett et al., 2013

*90% removal was observed

**This study was performed at three different experimental scales

aeration to suppress NOB. According to their study the success of mainstream deammonification depends on NOB repression and prevention of nitrate formation. However, their study is quite promising towards future research.

The studies so far conducted can be divided into some different reactor configurations as given below:

Rotating Biological Contactor (RBC)

The deammonification process in mainstream condition has been investigated by De Clippeleir et al. (2013) at different temperature from 29 °C to 15 °C and a COD/N increase from 0 to 2 in RBC with low nitrogen concentration (55-60 mg NH_4-N L^{-1}). This study showed that nitrogen removal rates of 0.5 g N $L^{-1}d^{-1}$ can be maintained at low temperature (15°C) and low nitrogen concentration (69 mg N L^{-1}) with moderate COD levels. This study also reported that nitric oxide (NO) can be a potential regulator for competition between anammox and NOB for nitrite.

Moving Bed Biofilm Reactor (MBBR)

Very few studies have been conducted regarding the operation of the MBBR for deammonification process at low temperature. Earlier Szatkowska et al. (2007) studied deammonfication process to treat supernatant in MBBR at 25°C. However, George et al. (2013) performed a comparative study to investigate the process performance and stability between biofilm carriers (MBBR) and suspended growth biomass. Both system were operated at 30°C. They reported that MBBR systems displayed significantly higher NH_4^+ removal rates (0.65±0.18 kg N $m^{-3}\cdot d^{-1}$) relative to suspended growth counterparts (0.36±0.08 kg N $m^{-3}\cdot d^{-1}$) over a six month period. However, MBBR showed significant variability in performance.

Sequencing Bed Reactor (SBR)

Few studies were performed regarding the operation of SBR at lab scale with low temperature and low nitrogen concentration. For instance, Guo *et al.* (2010) studied effects of temperature decrease using domestic wastewater in a SBR operated from 25 to 15°C. They reported stable process with decreased specific ammonia oxidation rate. They observed high nitrite accumulation ratio (above 95%) at low temperatures. Hu et al. (2013) investigated the feasibility of the deammonification process at temperature as low as 12 °C using low influent nitrogen concentration (70 mg N L^{-1}). They observed high anammox and AOB activity which can successfully remove more than 90% of the supplied nitrogen.

Up flow Anaerobic Sludge Blanket reactor (UASB)

Few studies showed potential removal can be obtained while operating UASB with low influent nitrogen concentration at low temperature. Ma et al. (2013) investigated anammox activity in an UASB reactor to treat low strength wastewater under moderate and low temperatures condition. They achieved very high nitrogen removal rate (NRR) of up to 5.72 kg N $m^{-3}d^{-1}$ with a hydraulic retention time (HRT) of 0.12 h at 30 °C. They have observed

decreased efficiency from 92.31% to 78.45% during the operation at 16 °C.

2.4. Challenges

Few challenges have been discussed in literature considering the deammonification process at low temperature and low influent ammonium concentration.

Low temperature can be considered as the first challenge since the deammonification process is needed to operate at low temperature (10–15 °C). Several studies already reported the negative impact of low temperature condition on the activity of the separate microbial groups (Dosta et al., 2008; Hendrickx et al., 2012). Only a few studies showed the long-term effect of temperature (below 20 °C) with lower nitrogen concentration (<100 mg N L^{-1}) on the microbial balances of anammox bacteria, and AOB and NOB (Vazquez-Padin et al., 2011; Winkler et al., 2011). At low temperature maintaining the balance between NOB and AOB are more challenging since the growth rate of NOB will become higher than the growth rate of AOB (Hellinga et al., 1998). Therefore, it will not be possible to wash out NOB based on overall or even selective sludge retention strategies.

Low nitrogen concentration is the second challenge to apply deammonification process in mainstream condition since domestic wastewater contain around 30–100 mg N L^{-1} and 113–300 mg COD L^{-1} (Metcalf & Eddy, 2003). However, earlier studies showed that high nitrogen conversion rates can be obtained at nitrogen concentrations of 30–60 mg N L^{-1} and at short HRT of 1–2 h (De Clippeleir et al., 2011).

Suppression of NOB can be considered as the most challenging approach at temperature ranges from 10–20 °C and at nitrogen concentration ranges between 30–60 mg N L^{-1} (low free ammonia and low nitrous acid).

Another challenge is the input of organics at moderate levels (90–240 mg biodegradable COD L^{-1}) in the wastewater. Competition may occur among heterotrophic denitrifiers with AOB in the presence of organics for oxygen. The heterotrophoic denitrifiers also can compete with anammox for nitrite or organics in the presence of organic matter since certain anammox bacteria can consume organic acid and take part into denitrification (Kartal et al., 2007).

3. OBJECTIVES OF THE THESIS

The deammnoification is the most promising biological nitrogen removal process which is extensively used for ammonium rich wastewater. Though the process has several challenges but it is worth to use deammonification in mainstream condition to create an energy positive environment. Therefore, the aim of the thesis was set to investigate the following objectives:

- To study the deammonification process at low temperatures and moderate to low influent nitrogen concentrations.
- To investigate the processes performance and strategies to reach stable operational conditions.
- To evaluate the influence of different environmental factors on the establishment and interaction between different microbial populations and how this will affect their activity.

4. METHODOLOGY

4.1. Pilot plant at Hammarby Sjöstadsverket

4.1.1. Description of the pilot plant

The two years study was performed with one stage deammonification process in a pilot scale MBBR. The reactor working volume was 200 litres and 40% of the reactor volume was filled with Kaldnes carriers K1 type (Fig. 4a). The specific surface area of the carriers was 500 m^2 m^{-3}. The MBBR was situated in Hammarby Sjöstadsverket research Station. During two years of the study different physical parameters such as redox, pH and DO inside the reactor were continuously measured by online sensors (Fig. 4b). DO inside the reactor was controlled by PID controller (Cerlic AB, Sweden). Temperature was continuously monitored and regulated by compact controller (JUMO GmbH & Co. KG, Germany). Both heater and cooler were used to control and maintain temperature inside the reactor (Julabo AB, Sweden). Well mixing was provided by a stirrer (50 rpm) with two blade propeller and air was supplied from the bottom of the reactor using two bar air pressure

Fig. 4: One stage deammonification system in pilot scale MBBR at Hammarby sjöstadsverket: A) Moving bed biofilm reactor B) Control and monitoring panel.

Table 2: Operations Strategy to run the MBBR at different temperatures.

Days	Temp (°C)	Follow up of pilot plant	Follow up batch tests	Intensive short term batch tests	Microbiological tests *		
					PCR	qPCR	FISH, CLSM, ISR-FISH
1-146	19	yes	yes	(6 SAA tests in series)	yes	yes	yes
147-298	16	yes	yes	(15 SAA tests in series)	yes	yes	yes
299-396	13	yes	yes	(25 SAA tests in series)	yes	yes	yes
397-431	10	yes	yes	(15 SAA tests in series)	yes	yes	yes

4.1.2. Operations strategy for decreasing the temperature

During the temperature study the reactor was continuously fed with supernatant from sludge dewatering after anaerobic digestion from a municipal wastewater treatment plant situated in Bromma, Stockholm. The influent NH_4^+-N concentration was 850 to 1000 mg L^{-1} and COD ranged from 470 to 600 mg L^{-1} O_2. The pH, conductivity, and alkalinity of the influent was 8-8.5, 8-9 mS cm^{-1} and 68-70 mmol L^{-1}, respectively over the study period. The operations strategy was to change the temperature at certain nitrogen load. Therefore, the temperature was set to decrease gradually started from 19 to 10 °C (3 °C in each step). The reactor was operated at 19, 16, 13 and 10 °C at different time period from July 2011 till August 2012. Duration of each period varied depending on the process stability, performance and efficiency. Nitrogen load was comparably high during the pilot plant operation at 19°C. During the whole study the hydraulic retention time changed depending on the nitrogen loading and ammonium concentration in the supernatant. Though initially (19 °C) nitrogen loading was relatively high but nitrogen loading was kept constant with a value of 1 g $Nm^{-2}d^{-1}$ when the pilot plant was operated at 16, 13 and 10 °C. An overview of the operation study is detailed in Table 2.

4.1.3. Operations strategy for decreasing the influent nitrogen concentration

During concentration change, the reactor was continuously fed with diluted supernatant. The supernatant was collected from the sludge dewatering from Himmerfjärden WWTP, Stockholm and tap water was used as diluent. The MBBR was operated for ten months and the duration was divided into six periods. The concentration during period I, period II and period III were 500 mg L^{-1}, 250 mg L^{-1} and 125 mg L^{-1}. However, later it was observed that less gradual decrease was necessary to reach process stability.

Table 3: Operations Strategy to run the MBBR at different influent nitrogen concentrations at 13°C.

Period	Days	Inflow Nitrogen (mgL^{-1})	Follow up of pilot plant	Follow up batch tests	Microbiological tests *		
					PCR	qPCR	FISH, CLSM, ISR-FISH
Period I	1-58	500	Yes	yes	yes	yes	yes
Period II	59-100	250	Yes	yes	yes	yes	yes
Period III	101-133	175	Yes	yes	yes	yes	yes
Period IV	134-190	125	Yes	yes	yes	yes	yes
Period V	191-259	80	Yes	yes	yes	yes	yes
Period VI	260-303	45	Yes	yes	yes	yes	yes

*Microbiological tests were performed in cooperation with Chalmers University

Therefore, the concentration during the period IV was set 125 mg L^{-1} and the period V was 85 mg L^{-1}. However, during period VI the target was to achieve mainstream condition and so the concentration was set as 45 mg L^{-1}. During the whole operational study the MBBR was operated at 13 °C. An illustration of the operational plan has been given in Table 3.

4.2. Analysis

To monitor the process performance, grab samples were taken from both influent and effluent once or twice in a week depending on the hydraulic retention time. Before carrying out the analysis, all water samples were filtered by using 0.45 μm filter paper. The different forms of nitrogen components (NO_2^--N, NH_4^+-N and NO_3^--N), alkalinity and COD were analyzed by using Dr. Lange test cuvettes and XION 500 spectrophotometer. The measurements and frequency of sampling was carried out extensively. Both online parameters and experimental data were analyzed and the results have been discussed in different papers. The discussion in different papers have been synthesized in the thesis.

4.2.1. Measurement of microbial activity

To follow up the process performance in the MBBR, some batch tests were performed as shown in Table 4.

Specific anammox activity (SAA)

To monitor the operation of the pilot plant in long term, Specific Anammox Activity tests (SAA) were performed following the methodology described by Dapena-Mora et al. (2007). The tests were based on the nitrogen gas pressure measurement. The operating temperature for the SAA tests was 25 °C. The tests were performed both in long term and short term. Short term SAA tests were performed as a series of batch tests to investigate the short term effect of temperature on the anammox biomass activity and long term SAA tests were performed to follow up the process.

Table 4: Summary of the microbial activity tests used in paper I and paper III

Tools for measurement of microbial activity	Operating temperature in the water bath	Measurement parameters	Groups of micro-organism investigated	References
SAA	25 °C	Nitrogen gas pressure	Anammox	Dapena-Mora et al. (2007) Modified by Yang (2012)
NUR	25°C	Nitrate consumption rate	Denitrifiers	Yang 2012
OUR	25°C	Oxygen consumption rate	AOB, NOB and Heterotrophic denitrfiers	Surmacz-Górska et al.(1996) Gut et al. (2005)

Oxygen uptake rate tests (OUR)

To measure the activity of different groups of bacteria, OUR tests were performed following the methodology given by Surmacz-Górska et al. (1996) and Gut et al. (2005). In this test, a three neck bottle with a total volume of 1.56 L was filled with diluted supernatant (tap water was used as diluent) to make NH_4-N concentration in the diluted supernatant 100 mg L^{-1}. Two selective inhibitors, 17mM sodium chlorate ($NaClO_3$) to inhibit the nitrite oxidation by NOB and 43 μM Allylthiourea (ATU) for ammonium oxidation by AOB, were used. Sodium chlorate and ATU were dosed after five and ten minutes interval since the test started. Data logger TESTO® 251 connected to a dissolved oxygen electrode was used to record the DO concentrations changes. The test was performed triplicates at 25 °C to get the average value of oxygen consumed by different groups of bacteria and the consumption was measured as g O_2 $m^{-2}d^{-1}$.

Nitrate uptake rate tests (NUR)

NUR test was performed by measuring the maximum nitrate utilization rate as described by Yang (2012) to evaluate the activity of heterotrophic denitrifiers. A container (1.5 L) was filled with diluted supernatant (diluted with distilled water) and 10 ml $NaNO_3$ solution was dosed to make the initial NO_3-N concentration of 100 mg L^{-1}. Nitrogen gas was flushed in the container continuously to achieve DO concentration of 0.5 mg O_2 L^{-1}. After that the container was tight closed and kept in water bath to maintain a temperature of 25 °C. Calculation was based on the nitrate consumption rate as a function of time.

4.3. Calculations

Nitrogen removal rate (NRR), ammonium oxidation Rate (AOR), Nitritrite oxidation rate (NOR), denitrification were calculated based on the data obtained from chemical analysis. The formula was derived using the stoichiometric equation for anammox (Strous et al., 1998).

Chemical oxygen demand (COD) g O_2 m^{-3}

Volumetric flow rate m^3d^{-1} (Q)

Total surface area of carriers in the reactor m^2m^{-3} (SSR)

Total inorganic nitrogen removal ΔN (g N m^{-3}) = $[NH_4^+\text{-}N_{influent}] - ([NH_4^+\text{+-}N_{effluent}] + [NO_2^-\text{-}N_{effluent}] + [NO_3^-\text{-}N_{effluent}])$

Nitrogen removal rate, NRR (g $Nm^{-2}d^{-1}$) = $\frac{\Delta N}{SSR} \times Q$

Aerobic ammonium oxidation rate, AOR (g N $m^{-2}d^{-1}$) = $\frac{[NH_4^+\text{-}N_{influent}] - [NH_4^+\text{-}N_{effluent}] - \frac{\Delta N}{2.04}}{SSR} \times Q$

Nitrite oxidation rate (assuming no denitrification and no NO_3 in the influent),

NOR ($gNm^{-2}d^{-1}$) = $\frac{[NO_3\text{-}N_{efluent}] - \frac{0.26 \times \Delta N}{2.04}}{SSR} \times Q$

COD removal, $\Delta COD = \frac{[COD_{influent}] - [COD_{effluent}]}{SSR} \times Q$

Denitrification (based on 4.2 g COD consumed per gram of nitrogen) (g N $m^{-2}d^{-1}$) = $\frac{\Delta COD}{4.2}$

Free ammonia (FA) and free nitrous acid (FNA) concentration was calculated as described in Anthonisen et al. (1976).

5. RESULTS AND DISCUSSIONS

5.1. Study on decreasing the temperature (paper I and paper II)

5.1.1. Nitrogen removal with decreasing temperatures

High fluctuation of the nitrogen removal was observed during the pilot plant operation at 19 °C, since controlling dissolved oxygen concentration was very crucial at this period. At the beginning of the pilot plant operation at 16 °C, the load was kept 1 g N m^{-2} d^{-1} and maintained constant during the rest of the study. The highest average nitrogen removal rate (0.9 g N $m^{-2}d^{-1}$) was observed during this time with this constant nitrogen load. During the operation at 13 °C, the removal rate was 0.5 g N $m^{-2}d^{-1}$ and it dropped very sharply to 0.16 g N $m^{-2}d^{-1}$ at 10 °C. Lowering the temperature decreased the specific activities of different groups of microorganism (AOB and anammox bacteria) and the process performance (Vlaeminck et al., 2012; Dosta et al., 2008; Isaka et al., 2008). Therefore, decreased nitrogen removal was observed

Table 5: Operational results during the study on temperature decrease (Adapted from paper I)

Temp °C	NLR $gNm^{-2}d^{-1}$	NRR $gNm^{-2}d^{-1}$	DO mgL^{-1}	NH_4-N effluent mg L^{-1}	NO_2-N effluent mg L^{-1}	NO_3-N effluent mg L^{-1}	FA mg L^{-1}
19	2.2±0.5	1.6±0.4	1.4±0.3	122±81.8	11.1±5.2	110.2±45	2.9±3.0
16	1.0±0.3	0.9±0.1	1.2±0.4	45±52.9	17.7±13	130.9±55	0.3±0.5
13	1.0±0.0	0.5±0.1	1.2±0.2	302±142	8.7±3.0	86.9±47	3.9±3.3
10	1.0±0.0	0.2±0.1	1.7±0.2	540±43.7	19.4±20	54.9±34	19.7±12

with decreasing temperature. DO concentration played key role during this time. Different values of dissolved oxygen concentrations were set to enhance the aerobic oxidation and therefore oxygen input was increased for AOB simultaneously avoiding inhibition of the anammox bacteria. The strategy to find optimal operating condition by setting different values of DO was successful since nitrite accumulation in the effluent did not occur except at 10°C. DO concentration was set as 1.5 mg O_2 L^{-1} at 19°C (Table 5). However, it was observed that despite of high DO the pH inside the reactor increased and high ammonium concentrations led to FA inhibition at this time. High DO during the pilot plant operation at 16 °C resulted in high nitrate and lower ammonium concentration in the effluent (Fig. 5). The highest nitrogen removal was observed during this period. DO concentrations was decreased to 1.15 from 1.5 mg L^{-1} and the removal efficiency was increased at this certain DO condition. During the period at 13°C, with high DO, stable process condition with lower efficiency was observed. However, nitrogen removal rates were gradually decreasing due to increased activity of NOB during the pilot plant operation at 10 °C. During the operation at 13°C, the ammonium oxidation rate was rather low and the ammonium concentration in the effluent was increased to 301±142 mg L^{-1}. When the temperature was 10 °C, high concentrations of ammonium (average 540 mg L^{-1}) and nitrite (average 19 mg L^{-1}) in the effluent indicated that both AOB and anammox activity decreased significantly due to low temperature (Fig. 5). Increased nitrate concentration was observed at the end of pilot plant operation at 13 °C indicating increased nitratation due to DO fluctuation. However, nitrate production decreased again during the pilot plant operation at 10 °C.

5.1.2. Inhibitory effect of FA

Low temperature sharply decreased the activity of AOB and led to

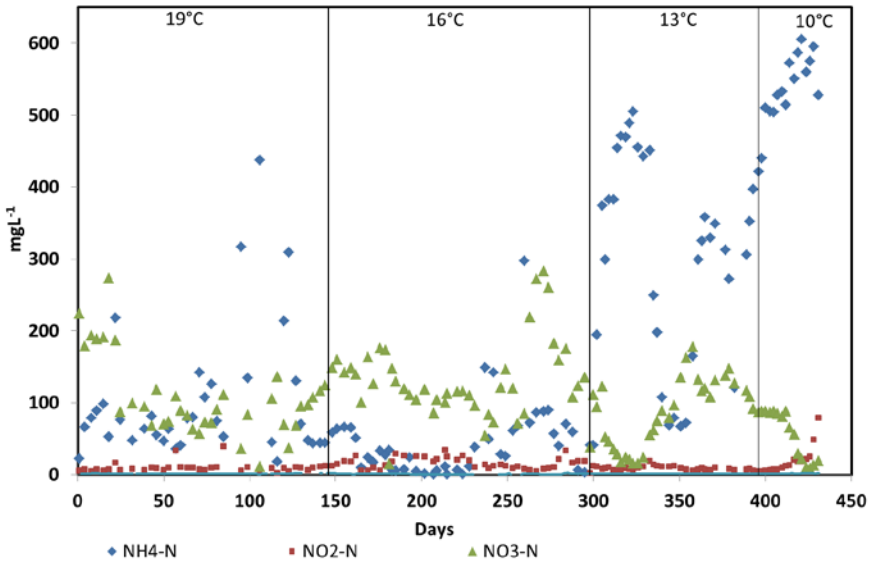

Fig. 5: NH_4-N, NO_3-N and NO_2-N concentration in the effluent at different operating temperatures in the MBBR.

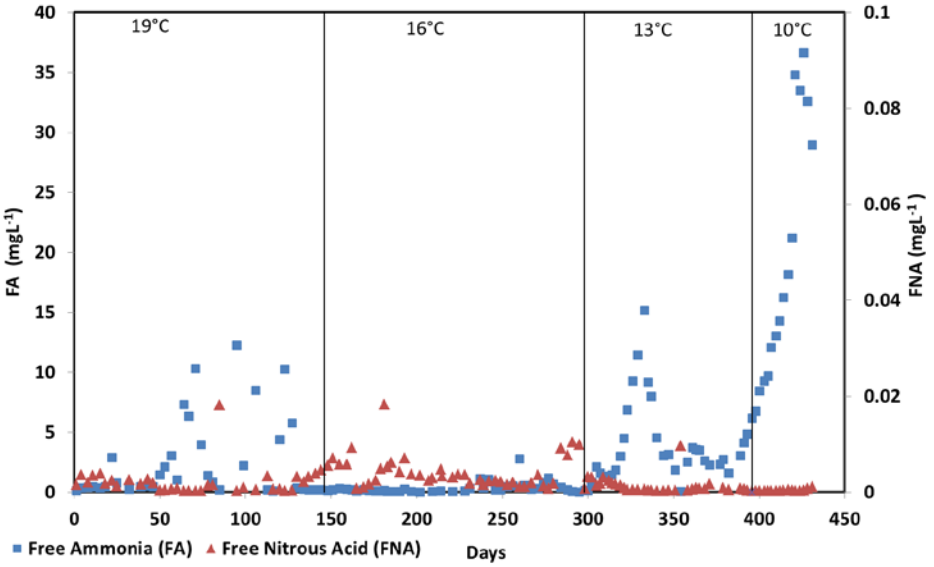

Fig. 6: FA and FNA concentrations inside the MBBR operated at different temperature condition.

high concentration of ammonium and FA. During the pilot operation at 19 °C the inhibitory effect of FA was observed in few cases whereas during operation at 16°C there was no FA inhibition occurred in the MBBR (Fig. 6). Literature studies showed that high FA concentration (>2 mg L^{-1}) decreased the nitrogen removal significantly and decreased the anammox activity (Jaroszynski et al., 2012). High concentration of ammonium in the effluent and high pH led to FA inhibition according Anthonisen et al. (1976) which was observed during the pilot plant operation at 13 °C and continued towards 10 °C. When the temperature was 10 °C, high concentrations of ammonium and nitrite in the effluent indicated that both anammox and NOB lost part of their activities due to low temperatures and high concentrations of FA (Fig. 6). However, Hu et al. (2013) did not observe accumulation of nitrite even at 12 °C. Interestingly, increased nitrate at 13°C period indicating high NOB activity despite the rather high FA concentrations. Since there is a threshold limit for NOB to tolerate FA which is inhibitory to AOB ((Li et al., 2012) and probably FA concentration during 13 °C and 10 °C exceeded this threshold value. However, it is clear that ammonium oxidation decreased due to FA inhibition during this period. Less FNA (<0.02 mg L^{-1}) concentration was observed during the temperature study.

5.1.3. Interaction of the different groups of microorganisms

Activities of different groups of microorganisms at different temperature indicate that AOB showed higher activity value than heterotrophs and NOB during the most of the operational period (Fig. 7). Decreasing the temperature did not influence the predominating role of AOB among the oxygen consumers. Both heterotrophic bacteria and AOB decreased activity with lowing temperature. NOB did not show decrease of activity with changing

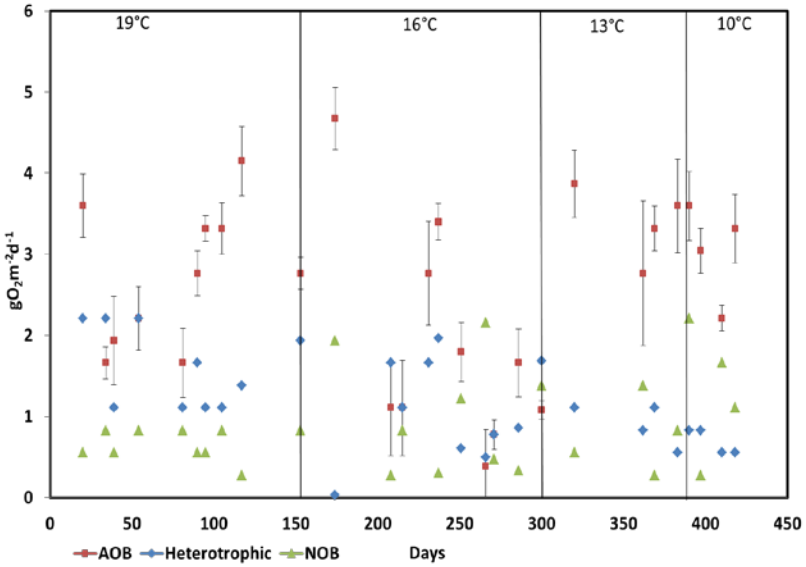

Fig. 7: Activities of different groups of microorganism measured as OUR on the biocarriers during the pilot plant operation at different temperatures.

temperature in this study. AOB activity decreased significantly from 19 to 16 °C, which was 36% of activity. Therefore, the crucial point for AOB adapting the low temperature condition existed between 19 to 16 °C, which is consistent with the results obtained by Yamamoto et al. (2008). He reported that the efficiency of nitritation process decreased significantly when temperature was 15°C. Heterotrophic bacteria decreased 25% of activity when the temperature dropped from 19 to 16 °C. Anammox bacteria played dominant role for nitrogen removal during the whole operational periods. The activity of Anammox bacteria was much higher than denitrifying bacteria during pilot plant operation at 19 °C and 16 °C (Table 6). Both Anammox and denitrifying bacteria decreased their activities with the dropping of temperature. Activity of anammox bacteria dropped significantly (40% of reduction in activity) with temperature decrease from 19°C to 16°C (Table 6). Denitrifying bacteria also showed 50% less activity at 16°C compare to 19°C. Results indicated that decreasing temperature from 19 °C to 16 °C was critical for bacteria adapting to the low temperature. Similar results were observed by Lotti (2013). They reported that for all the biomasses cultivated at temperature lower than 20 °C, the sharper effect of temperature decrease occurred in the lowest range of temperature (10-15 °C). However, nitrogen removal and ammonium oxidation in the reactor was much less as compared to the potential activity of anammox bacteria and AOB during 13 and 10 °C (Table 6). The reason could be the inhibition of FA during this temperature period inhibits the ammonium oxidation and low temperature inhibits the anammox activity and resulted in very low nitrogen removal. Denitrifiers contributed very little to the total nitrogen removal during the whole operational study.

Table 6: Nitrogen conversion measured in the activity test and in the MBBR at different temperature.

		g N $m^{-2}d^{-1}$					
		Nitrogen conversion in the activity test			Nitrogen conversion in the reactor		
Temp in MBBR (°C)	AOB	NOB	Anammox activity	Denitrifier activity	Aerobic ammonium oxidation	Nitrite oxidation	Denitrifi-cation
19	0.79	0.66	2.30	0.14	1.16	0.16	0.07
16	0.67	0.58	1.84	0.08	0.64	0.09	0.03
13	0.77	0.45	1.83	0.06	0.39	0.07	0.03
10	0.81	1.03	1.53	0.03	0.18	0.06	0.01

The results obtained from OUR batch tests indicated nitrogen conversion by AOB as measured by activity tests was almost consistent and did not change significantly with temperature decrease (Table 6). As discussed in paper II, the average specific nitrogen conversion rates were higher at 16 °C than at 13 °C for AOB and anammox bacteria. Earlier study revealed that bacteria present in different layers of biofilms are not equally active, since the variation of availability of substrate carbon and electron acceptors (Gieseke et al., 2005). In this study, there observed an extensive depth distribution of anammox bacteria, as shown by fluorescence in situ hybridization (FISH) in together with confocal microscopy (data not shown) in the biofilm. Since the AOB were located at a thin layer at the water interface of the biofilm therefore, they could be less affected by mass transport. The anammox bacterial community was maintained at a high density in the biofilm even at low temperature (13-16 °C) while the AOB community was considerably smaller. Therefore, the cell specific conversion rates were much higher for AOB than for the anammox bacteria.

5.1.4. Effect of temperature decrease on anammox activity

Short term SAA tests were performed to investigate the influence of temperature on anammox activity (Fig. 8). Microbial analysis (qPCR) confirmed the number of copies of anammox bacteria at different temperature and data were presented in Persson et al. (2014). Under the same temperature condition in the MBBR, the anammox activity in the short term SAA test decreased gradually from 25 °C to 5 °C (Fig. 8). The activity of anammox bacteria was comparable under the temperature condition of 13, 16 and 19 °C in the MBBR, especially when the short term SAA tests performed at 20, 15 and 10 °C. Therefore, a tendency of adaptability was observed in the MBBR during 16 and 13 °C. Similar study was carried out by Dosta et al. (2008) and they observed the adaptation of biomass to low temperatures during the operation of the SBR with short term activity tests. When the MBBR was operated at 10°C, significant drop of anammox activity was observed in the short term tests. The calculated activation energy (50 kj mol L^{-1} for 16 °C) and (61kj mol L^{-1} for 13 °C) based on the short term batch

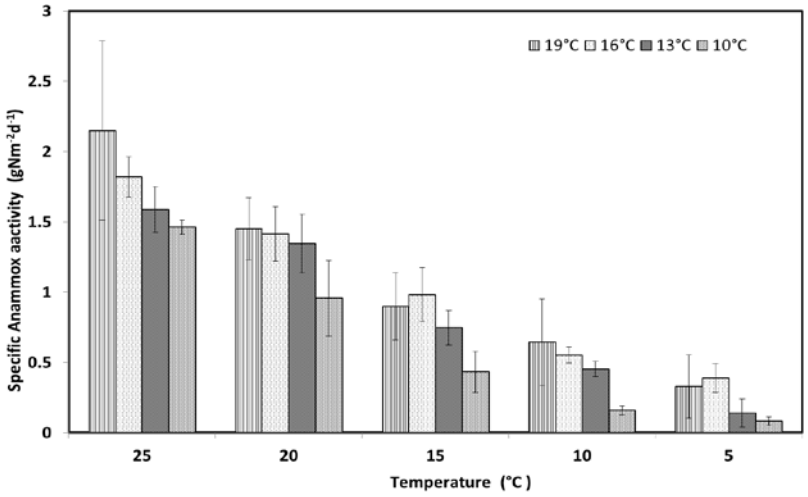

Fig. 8: Effect of temperature on anammox activity (adapted from paper I).

tests were comparable with the value obtained by Rysgaard et al. (2004).

5.2. Study on decreasing the influent nitrogen concentration (paper III)

5.2.1. Nitrogen conversion by different groups of microorganisms

Changing the influent nitrogen concentration at constant temperature (13 °C) showed that potential nitrogen removal could be possible while operating the MBBR with influent nitrogen concentration as low as 85 mg L^{-1}. However, below this concentration the removal decreased and process lost stability. During the period I and II, NH_4-N concentration in the effluent was high (Table 7). In the period I, the nitrogen removal efficiency was low due to low ammonium oxidation and unstable process performance. AOB oxidation was 0.33 g N $m^{-2}d^{-1}$ during the period I (Fig. 9). However, AOB oxidation decreased to 0.07 during the period III and it increased again 0.18 g N $m^{-2}d^{-1}$ during the period V when the nitrogen load was 0.27 g N $m^{-2}d^{-1}$. Nitrogen conversion by AOB was low compared to nitrogen conversion by NOB which indicated that AOB could not compete with NOB in some occasion. Increased activity of NOB during the period I, and period IV and period VI was observed. The biofilm biomass in the

Table 7: Operational results with different influent nitrogen concentration in the MBBR (Adapted from paper III).

	Influent	Effluent			Nitrogen removal efficiency (%)
	NH_4-N (mg L^{-1})	NO_3-N (mg L^{-1})	NO_2-N (mg L^{-1})	NH_4-N (mg L^{-1})	
Period I	496±32	98±44	13±7.2	306±68	16.6±8
Period II	248±14	44±13	7±1.5	149±33	28.6±9
Period III	176±6	10±8	3±2.0	121±11	25.7±6
Period IV	126±13	16±13	3±1.6	61±23	32.0±13
Period V	85±7	22±14	2±1.6	32±23	36.4±11
Period VI	44±4	15±6	2±0.3	21±5	16.8±7

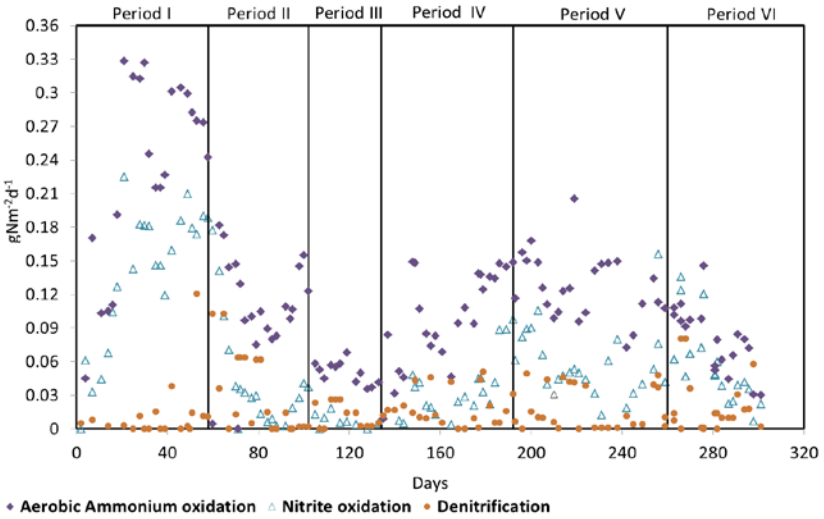

Fig. 9: Measurement of aerobic ammonium oxidation, nitrite oxidation and denitrification in different periods.

reactor was approximately 7±0.6 g L^{-1} during the entire operation study. This value was much higher than the value reported by Gut et al. (2006), where they observed 2.3 g L^{-1}. However, the average potential activity of anammox bacteria (SAA) was very low (0.3 g N $m^{-2}d^{-1}$) even at this high biofilm biomass. The nitrite accumulation during the whole operational study was not observed. Growth of anammox bacteria is always associated with nitrate production since anammox bacteria oxidize part of the nitrite which is used as electron acceptor for cell synthesis (Kartal et al., 2013). We know that the stoichiometric ratio of nitrate production to ammonium consumption should be 0.26 if we only consider the anammox process (Strous et al., 1998). However, for one stage deammonification process the stoichiometric ratio of nitrate production to removed ammonium should be 0.1. In this MBBR the ratio was much higher (0.26) in the period V and

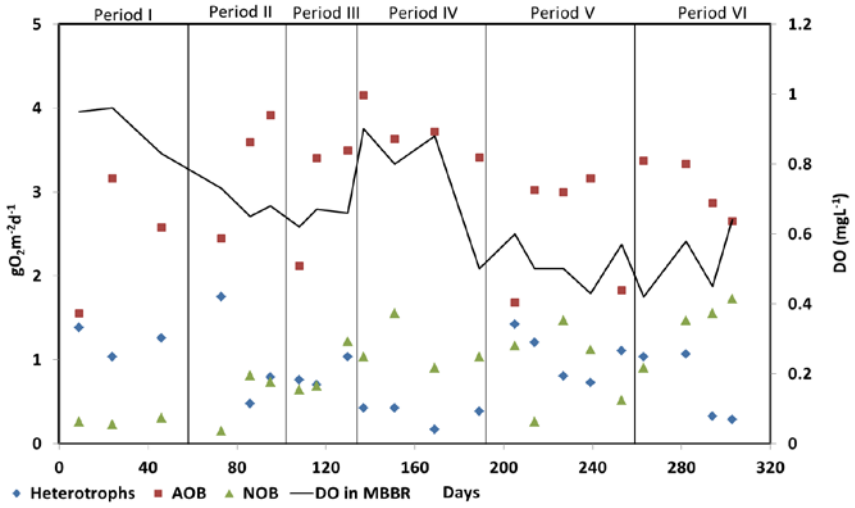

Fig. 10: Activity of different groups of microorganism as measured by OUR on the biocarriers and DO concentration in the reactor in different periods.

period VI clearly indicating that activity of NOB increased significantly. Aerobic ammonium oxidation decreased from period I to period III indicating decreased AOB activity during the beginning of the study. The ammonium oxidation increased from period IV to period V and highest removal was observed during the period V with highest average removal efficiency (36%).The lowest removal was observed during the period VI with very low ammonium oxidation indicating that AOB and anammox bacteria could not adapt at this substrate condition and therefore they showed very low activity. OUR results showed that AOB were the most predominating oxygen consumers among different groups of bacteria (Fig. 10). However, the average activity of AOB was similar and no profound changes occur during the whole study. In the beginning of each period the AOB activity decreased with decreased substrate concentration but recovered after some time. Though dissolved oxygen concentration was higher in period V but still oxidation by AOB was not potentially high.

5.2.2. Competition between AOB and NOB

While operating the MBBR at low temperature, one of the strategies could be to create a strict selection of AOB above NOB by limiting the DO. Depending on the maximum growth rate, NOB outcompete AOB below 15 °C and the optimal growth of AOB can be achieved at 28 °C (Hellinga et al., 1998). Therefore, one of the ways to suppress the NOB could be to limit the DO concentration. However, in this MBBR even with very low DO concentration (0.4 mg L^{-1}) in period VI could not sufficiently suppress the NOB activity. Estimation of nitrogen flux measured for AOB, NOB and anammox bacteria indicated increased NOB activity with concentrtion decrease (Fig. 11). The potential for anammox bacteria was relatively low at different period and the average anammox activity decreased to 0.25 g N $m^{-2}d^{-2}$ when the influent nitrogen concentration was 45 mg N L^{-1} from 0.4g$Nm^{-2}d^{-1}$ when the influent nitrogen concentration was 175 mg N L^{-1}.

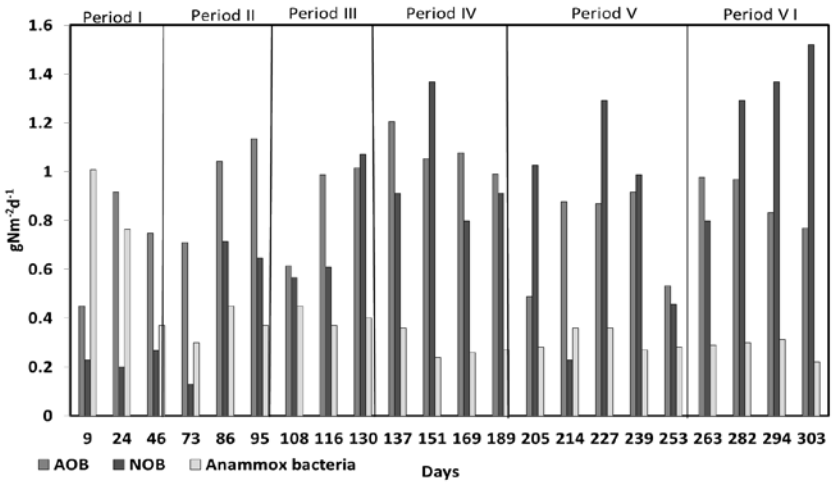

Fig. 11: Nitrogen flux estimation for AOB, NOB and anmmox bacteria based on the potential measured by activity tests.

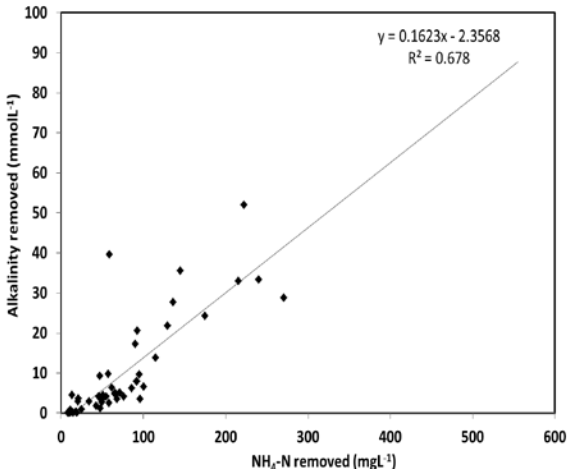

Fig. 12: Correlation between alkalinity and ammonium removed.

5.2.3. Inorganic carbon limitation

While decreasing the influent nitrogen concentration stepwise, it was observed that AOB could not significantly oxidize the ammonia though the potential activity of AOB was dominant. One of the reasons could be the limited inorganic carbon since the diluted supernatant had very low alkalinity. Based on experimental study and modelling, Guisasola et al. (2007) showed that AOB could be limited by total inorganic carbon (TIC) concentration less than 3 mmol C L^{-1}. However, in this study the influent alkalinity was never below 3 mmol C L^{-1}. Therefore it can be assumed that AOB was not inhibited by limited inorganic carbon. This was supported by additional batch tests of AOB activity (data not shown). Correlation between the alkalinity reduction and the ammonium removal showed linear (regression value 0.68) (Fig. 12). The relationship indicates that equal amount of alkalinity was removed during the equal amount of NH_4^+-N removal.

5.3. Directions for operation of full scale deammonification process in mainstream conditions

Stable and potential nitrogen removal can be achieved even at low temperature and low influent concentration. However, the challenges exist due to oxygen control since both AOB and NOB compete for oxygen and the affinity for oxygen determine the AOB and NOB activity at low temperature. Therefore, it is very crucial to provide an optimal oxygen concentration at low temperature.

Significant nitrogen removal was possible when the pilot plant MBBR was operated at 16 °C so it is important to keep the temperature as low as 16 °C but if needed to go further down then it would be difficult to operate.

Suppression of NOB is still challenging which is already accepted by researchers (Wett et al., 2013) and different options were proposed to suppress NOB. Regarding suppression of NOB while DO is limiting factor, it is very important keep a proper DO since at low temperature (<15 °C) NOB is more prone to oxygen than AOB.

The study was performed by following continuous aeration strategy. However, to suppress NOB, intermittent aeration strategy can be investigated together with FA formation by increasing the pH inside the reactor. In this case external use of base can be a good option.

6. CONCLUSIONS

Operation of the MBBR with the partial nitritation/anammox process at low temperature and low nitrogen concentration is challenging. However, the following general conclusions have been derived after two years study.

- Stable and potential nitrogen removal can be obtained at low temperatures (19-13 °C) with high ammonium concentrations (850 to 1000 mg L^{-1}).
- Dissolved oxygen concentration became very crucial while operating at low temperature and low nitrogen concentration.
- Stability of the process at low influent nitrogen concentration at 13 °C strongly dependent on the suppression of NOB.
- AOB are the predominating oxygen consumers among the oxygen consumers.
- Stable nitritation-anammox biomass is possible in the biofilm irrespective of low temperature and low nitrogen concentration.

In particular, the following conclusions can be derived while operating the MBBR at different temperature and nitrogen concentrations.

- The average nitrogen removal efficiency decreased significantly with the temperature drop from 71.9 ± 8.5% at 19 °C to 15.9 ± 8.7% at 10 °C. Free ammonia inhibition occured treating the supernatant at low temperature (13-10°C) and decreased nitrogen removal was observed with temperature decrease.
- Anammox bacteria played the dominating role in the nitrogen removal during the entire operational study. The average anammox activity decreased from 2.3 N $m^{-2}d^{-1}$ at 19 °C to 1.5 g N $m^{-2}d^{-1}$ at 10 °C. Therefore, the average potential anammox activity was comparatively high during the entire temperature study.
- Anammox bacteria activity did not decrease significantly with decreasing temperature until the pilot plant was operated at 13 °C and activity of anammox bacteria was observed even at 5 °C. Denitrifiers showed a decrease in activity with temperature decrease.
- The biggest drop of activity of AOB occurred when temperature decreased from 19 to 16 °C. Decreasing the temperature did not influence the dominating role of AOB among oxygen consumers. Increased NOB activity was

observed in some occasions but the average NOB activity did not change with decreasing the temperature.

- Decreasing the inflow nitrogen concentration did not influence the dominating role of AOB among oxygen consumers. The potential activity of anammox bacteria decreased while decreasing the inflow nitrogen concentration gradually from 500 mg to 45 mg L^{-1} at 13 °C. Denitrification was also observed at different influent nitrogen concentration.

7. FUTURE RESEARCH

It is very important to investigate the partial nitritation/anammox process performance and efficiency at low temperature and low nitrogen concentration since the knowledge can be applied to create an energy positive sewage treatment facility. The study will help to develop better knowledge about the behavior of different groups of microorganism towards mainstream condition (low temperature and low influent nitrogen).

However, few challenges occurred during the study include the inhibitory effect of FA and maintaining low hydraulic retention time. In this case it is important to perform more research to keep a suitable pH so that inhibition by FA can be minimized but also NOB suppression can be possible.

More research should be carried out with different aeration strategies since in this study we only consider continuous aeration. Therefore, NOB suppression could be possible by shifting the continuous aeration towards intermittent aeration.

Another possible way for the suppression of NOB could be increasing the FA concentration in the reactor which could eliminate the NOB. In this regard increasing the pH or adding alkali could be one way to suppress the NOB activity. However, determining the optimal dissolved oxygen concentration should be taken into consideration to suppress the NOB at low temperature.

It is very important to decrease the AOB wash out from the MBBR. This can be done by integrating internal recirculation of sludge produced during the partial nitritation /anammox process. In this case, installation of integrated fixed film activated sludge process (IFAS) could be suitable solution to the existing MBBR operated at low temperature and low nitrogen concentration.

REFERENCES

Abma W. R., Driessen W., Haarhuis R. and van Loosdrecht M. C. M. (2010). Upgrading of sewage treatment plant by sustainable and cost-effective separate treatment of industrial wastewater. *Water Science and Technology* **61** (7), 1715-1722.

Anthonisen A. C., Loehr R. C., Prakasam T. B. and Srinath E. G. (1976). Inhibition of nitrification by ammonia and nitrous acid. *Journal of Water Pollution Control Federation* **48** (5), 835-852.

Broda E. 1977. Two kinds of lithotrophs missing in nature.Z *Allg Mikrobiol* **17** (6), 491-493.

Canziani R., Emondi V., Garavaglia M., Malpei F., Pasinetti E. and Buttiglieri G. (2006). Effect of oxygen concentration on biological nitrification and microbial kinetics in a cross-flow membrane bioreactor (MBR) and moving-bed biofilm reactor (MBBR) treating old landfill leachate. *Journal of Membrane Science* **286** (1-2), 202-212.

Cema G. (2009). Comparative study of different anammox systems. PhD Thesis, TRITA LWR PHD 1053, 72 p.

Cema G., Plaza E., Trela J. and Surmacz-Górska J. 2011. Dissolved oxygen as a factor influencing nitrogen removal rates in a one-stage system with partial nitritation and Anammox process. *Water Science and Technology* **64** (5), 1009-1015.

Cema G., Szatkowska B., Plaza E., Trela J. and Surmacz-Gorska J. (2006). Nitrogen removal rates at a technical-scale pilot plant with the one-stage partial nitritation/Anammox process. *Water Science and Technology* **54** (8), 209-217.

Cema G., Wiszniowski J., Żabczyński S., Zabłocka-Godlewska E., Raszka, A. and Surmacz-Górska, J. (2007). Biological nitrogen removal from landfill leachate by deammonification assisted by heterotrophic denitrification in a rotating biological contactor (RBC). *Water Science and Technology* **55** (8-9), 35-42.

Cema G., Raszka A., Stachurski A., Kunda K., Surmacz-Górska J., Plaza E. (2009). A one-stage system with partial nitritation and Anammox processes in Rotating Biological Contactor (RBC) for treating landfill leachate. In: Proceedings of the IWA Conference Processes in Biofilms: Fundamentals to Applications, Davis, USA, 13-16 September 2009.

EU Commission, (1991). Directive 91/271/EEE concenring urban wastewater treatment.

Dalsgaard T. and Thamdrup B. (2002). Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. *Applied Environmental Microbiology* **68**(8), 3802-3808.

Dapena-Mora A., Fernandez I., Campos J. L., Mosquera-Corral A., Mendez R. and Jetten M. S. M. (2007). Evaluation of activity and inhibition effects on Anammox process by batch tests based on the nitrogen gas production. *Enzyme and Microbial Technology* **40** (4), 859-865.

De Clippeleir H., Vlaeminck S. E., De Wilde F., Daeninck K., Mosquera M., Boeckx P., Verstraete W. and Boon N. (2013). One-stage partial nitritation/anammox at 15 degrees C on pretreated sewage: feasibility demonstration at lab-scale. *Applied Microbiology and Biotechnology* **97**(23), 10199-10210.

De Clippeleir H., Yan X., Verstraete W. and Vlaeminck S. E. (2011). OLAND is feasible to treat sewage-like nitrogen concentrations at low hydraulic residence times. *Applied Microbiology and Biotechnology* **90**(4), 1537-1545.

Dosta J., Fernandez I., Vazquez-Padin J. R., Mosquera-Corral A., Campos J. L., Mata-Alvarez J. and Mendez R. (2008). Short- and long-term effects of temperature on the Anammox process. *Journal of Hazardous Material* **154**(1-3), 688-693.

Metcalf,Eddy (2003). Wastewater Engineering, Treatment disposal and reuse. McGraweHill, Newyork.

Egli K., Fanger U., Alvarez P. J. J., Siegrist H., van der Meer J. R. and Zehnder A. J. B. (2001). Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. *Archives of Microbiology* **175**(3), 198-207.

Fux C., Marchesi V., Brunner I. and Siegrist H. (2004). Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors. *Water Science and Technology* **49**(11-12), 77-82.

Fux C. and Siegrist H. (2004). Nitrogen removal from sludge digester liquids by nitrification/denitrification or partial nitritation/anammox: environmental and economical considerations. *Water Science and Technology* **50**(10), 19-26.

Gieseke A., Nielsen J. L., Amann R., Nielsen P. H. and de Beer D. (2005). In situ substrate conversion and assimilation by nitrifying bacteria in a model biofilm. *Environ Microbiol* **7**(9), 1392-1404.

Grunditz C. and Dalhammar G. (2001). Development of nitrification inhibition assays using pure cultures of Nitrosomonas and Nitrobacter. *Water Research* **35**(2), 433-440.

Guo J., Peng Y., Huang H., Wang S., Ge S., Zhang J. and Wang Z. (2010). Short- and long-term effects of temperature on partial nitrification in a sequencing batch reactor treating domestic wastewater. *Journal of Hazardous Material* **179**(1-3), 471-479.

Guo J., Peng Y., Wang S., Zheng Y., Huang H. and Wang Z. (2009). Long-term effect of dissolved oxygen on partial nitrification performance and microbial community structure. *Bioresource Technology* **100**(11), 2796-2802.

Gut L., Plaza E. and Hultman B. (2005). Oxygen Uptake Rate (OUR) tests for assessment of nitrifying activities in the deammonification system, In: Integration and optimization of urban sanitation system, Joint Polish-Swedish Reports, No 13. Royal Institute of Technology, Stockholm, 2005, TRITA-LWR-Report 3018

Gut L., Plaza E., Trela J., Hultman B. and Bosander J. (2006). Combined partial nitritation/Anammox system for treatment of digester supernatant. *Water Science and Technology* **53**(12), 149-159.

Hao X., Heijnen J. J. and Van Loosdrecht M. C. (2002). Model-based evaluation of temperature and inflow variations on a partial nitrification-ANAMMOX biofilm process. *Water Research* **36**(19), 4839-4849.

Hellinga C., Schellen A. A. J. C., Mulder J. W., van Loosdrecht M. C. M. and Heijnen J. J. (1998). The SHARON process: An innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology* **37**(9), 135-42.

Hem L. J., Rusten B. and Odegaard H. (1994). Nitrification in a Moving-Bed Biofilm Reactor. *Water Research* **28**(6), 1425-1433.

Hendrickx T. L., Wang Y., Kampman C., Zeeman G., Temmink H. and Buisman C. J. (2012). Autotrophic nitrogen removal from low strength waste water at low temperature. *Water Research* **46**(7), 2187-2193.

Hu Z., Lotti T., van Loosdrecht M. and Kartal B. (2013). Nitrogen removal with the anaerobic ammonium oxidation process. *Biotechnology Letter* **35**(8), 1145-1154.

Isaka K., Date Y., Kimura Y., Sumino T. and Tsuneda S. (2008). Nitrogen removal performance using anaerobic ammonium oxidation at low temperatures. *FEMS Microbiology Letters* **282**(1), 32-38.

Isaka K., Sumino T. and Tsuneda S. (2007). High nitrogen removal performance at moderately low temperature utilizing anaerobic ammonium oxidation reactions. *Journal of Bioscience and Bioengineering* **103**(5), 486-490.

Jaroszynski L. W., Cicek N., Sparling R. and Oleszkiewicz J. A. (2011). Importance of the operating pH in maintaining the stability of anoxic ammonium oxidation (anammox) activity in moving bed biofilm reactors. *Bioresource Technology* **102**(14), 7051-7056.

Jaroszynski L. W., Cicek N., Sparling R. and Oleszkiewicz J. A. (2012). Impact of free ammonia on anammox rates (anoxic ammonium oxidation) in a moving bed biofilm reactor. *Chemosphere* **88**(2), 188-195.

Jetten M. S., Strous M., van de Pas-Schoonen K. T., Schalk J., van Dongen U. G., van de Graaf A. A., Logemann S., Muyzer G., van Loosdrecht M. C. and Kuenen J. G. (1998). The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews* **22**(5), 421-437.

Joss A., Salzgeber D., Eugster J., Konig R., Rottermann K., Burger S., Fabijan P., Leumann S., Mohn J. and Siegrist H. (2009). Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR. *Environmental Science and Technology* **43**(14), 5301-5306.

Kartal B., de Almeida N. M., Maalcke W. J., Op den Camp H. J., Jetten M. S. and Keltjens J. T. (2013). How to make a living from anaerobic ammonium oxidation. *FEMS Microbiology Reviews* **37**(3), 428-461.

Kartal B., Kuypers M. M., Lavik G., Schalk J., Op den Camp H. J., Jetten M. S. and Strous M. (2007). Anammox bacteria disguised as

denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. *Environmental Microbiology* **9**(3), 635-642.

Kartal B., van Niftrik L., Rattray J., van de Vossenberg J. L., Schmid M. C., Sinninghe Damste J., Jetten M. S. and Strous M. (2008). Candidatus 'Brocadia fulgida': an autofluorescent anaerobic ammonium oxidizing bacterium. *FEMS Microbiology Ecology* **63**(1), 46-55.

Li S., Chen Y. P., Li C., Guo J. S., Fang F. and Gao X. (2012). Influence of free ammonia on completely autotrophic nitrogen removal over nitrite (CANON) process. *Applied Biochemistry and Biotechnology* **167**(4), 694-704.

Liang Z. and Liu J. (2008). Landfill leachate treatment with a novel process: anaerobic ammonium oxidation (Anammox) combined with soil infiltration system. *Journal of Hazardous Materials* **151**(1), 202-212.

Lin Y.-M., Tay J.-H., Liu Y. and Hung Y.-T. (2009). Biological Nitrification and Denitrification Processes. In: *Handbook of Environmental Engineering*, Humana Press, Totowa NJ, pp. 539-588.

Lotti T., Kleerebezem, R., van Lossdrecht, M. C.M. (2013). Effect of temperature on anammox bacteria cultivated under different conditions. In: *Nutrient Removal and Recovery 2013: Trends in Resource Recovery and use*, WEF/IWA, Vancouver,Canada.

Ma B., Peng Y., Zhang S., Wang J., Gan Y., Chang J., Wang S., Wang S. and Zhu G. (2013). Performance of anammox UASB reactor treating low strength wastewater under moderate and low temperatures. *Bioresource Technology* **129**, 606-611.

Ma B., Zhang S., Zhang L., Yi P., Wang J., Wang S. and Peng Y. (2011). The feasibility of using a two-stage autotrophic nitrogen removal process to treat sewage. *Bioresource Technology* **102**(17), 8331-8334.

Mulder A. (2003). The quest for sustainable nitrogen removal technologies. *Water Science and Technology* **48**(1), 67-75.

Mulder A., Graaf A. A. v. d., Robertson L. A. and Kuenen J. G. (1995). Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microbiology Ecology* **16**(1995), 177-184.

Ni S. Q., Ni J. Y., Hu D. L. and Sung S. (2012). Effect of organic matter on the performance of granular anammox process. *Bioresource Technology* **110**, 701-705.

Persson F., Sultana R., Suarez M., Hermansson M., Plaza E. and Wilen B. M. (2014). Structure and composition of biofilm communities in a moving bed biofilm reactor for nitritation-anammox at low temperatures. *Bioresource Technology* **154**, 267-273.

Plaza E., Stridh S., Örnmark J., Kanders L. and Trela J. (2011). Swedish Experience of the Deammonification Process in a Biofilm System. In: *Nutrient Recovery and Management 2011* Water Enviornment Federation, Miami, USA, pp. 1067-1079.

Poth, M.and Focht, D.D. (1985). 15N kinetic analysis of N2O production by Nitrosomonas europaea: an examination of nitrifier denitrication.*Applied.Environmental.Microbiology*. 49, 1134-1141.

Ruscalleda M., Lopez H., Ganigue R., Puig S., Balaguer M. D. and Colprim J. (2008). Heterotrophic denitrification on granular anammox SBR treating urban landfill leachate. *Water Science and Technology* **58**(9), 1749-1755.

Rysgaard S., Glud R. N., Risgaard-Petersen N. and Dalsgaard T. (2004). Denitrification and anammox activity in Arctic marine sediments. *Limnology and Oceanography* **49**(5), 1493-1502.

Schmidt I., Sliekers O., Schmid M., Bock E., Fuerst J., Kuenen J. G., Jetten M. S. M. and Strous M. (2003). New concepts of microbial treatment processes for the nitrogen removal in wastewater. *FEMS Microbiology Reviews* **27**(4), 481-492.

Sliekers A. O., Derwort N., Gomez J. L., Strous M., Kuenen J. G. and Jetten M. S. (2002). Completely autotrophic nitrogen removal over nitrite in one single reactor. *Water Research* **36**(10), 2475-2482.

Strous M. (2000). Microbiology of anaerobic ammonium oxidation. PhD thesis, Technical University of Delft, The Netherlands.

Strous M., Fuerst J. A., Kramer E. H., Logemann S., Muyzer G., van de Pas-Schoonen K. T., Webb R., Kuenen J. G. and Jetten M. S. (1999a). Missing lithotroph identified as new planctomycete. *Nature* **400** (6743), 446-449.

Strous M., Heijnen J. J., Kuenen J. G. and Jetten M. S. M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology* **50**(5), 589-596.

Strous M., Kuenen J. G. and Jetten M. S. (1999b). Key physiology of anaerobic ammonium oxidation. *Applied Environmetnal Microbiology* **65**(7), 3248-3250.

Surmacz-Górska J., Gernaey K., Demuynak C., Vanrolleghem P. and Verstraete W. (1996). Nitrification monitoring in activated sludge by oxygen uptake rate (OUR) measurements. *Water Research* **30**(5), 1228-1236.

Szatkowska B., Cema G., Plaza E., Trela J. and Hultman B. (2007). A one-stage system with partial nitritation and Anammox processes in the moving-bed biofilm reactor. *Water Science and Technology* **55** (8-9), 19-26.

Szatkowska B. and Plaza E. (2006). Temperature as a factor influencing the Anammox process performance. Water and Environmental Management Series No 12, Young Researchers 2006, 51-58, IWA publishing, London, UK.

Trimmer M., Nicholls J. C. and Deflandre B. (2003). Anaerobic ammonium oxidation measured in sediments along the Thames estuary, United Kingdom. *Applied Environmetnal Microbiology* **69**(11), 6447-6454.

U. Wiesmann (1994). Biological nitrogen removal from wastewater, Advances in Biochemical Engineering/Biotechnology (51) 113–154.

van de Graaf A. A., De Bruijn P., Robertson L. A., Jetten M. S. M. and Kuenen J. G. (1997). Metabolic pathway of anaerobic ammonium oxidation on the basis of I5N studies in a fluidized bed reactor. *Microbiology* **143**, 2415-2421.

van de Graaf A. A., Mulder A., de Bruijn P., Jetten M. S., Robertson L. A. and Kuenen J. G. (1995). Anaerobic oxidation of ammonium is a biologically mediated process. *Applied Environmental Microbiology* **61**(4), 1246-1251.

van der Star W. R., van de Graaf M. J., Kartal B., Picioreanu C., Jetten M. S. and van Loosdrecht M. C. (2008). Response of anaerobic ammonium-oxidizing bacteria to hydroxylamine. *Applied Environmental Microbiology* **74**(14), 4417-4426.

Van Hulle S. W. H., Vandeweyer H. J. P., Meesschaert B. D., Vanrolleghem P. A., Dejans P. and Dumoulin A. (2010). Engineering aspects and practical application of autotrophic nitrogen removal from nitrogen rich streams. *Chemical Engineering Journal* **162**(1), 1-20.

Van Hulle S. W. H., Volcke E. I. P., Teruel J. L., Donckels B., van Loosdrecht M. C. M. and Vanrolleghem P. A. (2007). Influence of temperature and pH on the kinetics of the Sharon nitritation process. *Journal of Chemical Technology and Biotechnology* **82**(5), 471-480.

Vazquez-Padin J. R., Fernandez I., Morales N., Campos J. L., Mosquera-Corral A. and Mendez R. (2011). Autotrophic nitrogen removal at low temperature. *Water Science and Technology* **63**(6), 1282-1288.

Vazquez-Padin J. R., Pozo M. J., Jarpa M., Figueroa M., Franco A., Mosquera-Corral A., Campos J. L. and Mendez R. (2009). Treatment of anaerobic sludge digester effluents by the CANON process in an air pulsing SBR. *Journal of Hazardous Materials* **166**(1), 336-341.

Well, G., F., Shi, Y.-J., Joss, A., Bürgmann, H., Johnson, D.,R., Morgenroth, E., (2013). Comparing Process Performance and Stability Between Replicate MBBR and Suspended Growth Combined Nitritation-Anammox Reactors. In: 9th International Conference on biofilm reactors, IWA, Paris, France.

Wett B., Omari A., Podmirseg S. M., Han M., Akintayo O., Brandon M. G., Murthy S., Bott C., Hell M., Takacs I., Nyhuis G. and O'Shaughnessy M. (2013). Going for mainstream deammonification from bench to full scale for maximized resource efficiency. *Water Science and Technology* **68**(2), 283-289.

Wett, B. (2007): Development and implementation of a robust deammonification process. *Water Science and Technology*, **56**(7), 81-88

Wett B. and Rauch W. (2003). The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. *Water Research* **37**(5), 1100-10.

Winkler M. K., Kleerebezem R. and van Loosdrecht M. C. (2012). Integration of anammox into the aerobic granular sludge process for main stream wastewater treatment at ambient temperatures. *Water Research* **46**(1), 136-144.

Winkler M. K. H., Kleerebezem R., Kuenen J. G., Yang J. J. and van Loosdrecht M. C. M. (2011). Segregation of Biomass in Cyclic Anaerobic/Aerobic Granular Sludge Allows the Enrichment of Anaerobic Ammonium Oxidizing Bacteria at Low Temperatures. Environmental Science and Technology **45**(17), 7330-7337.

Vlaeminck S. E., De Clippeleir H. and Verstraete W. (2012). Microbial resource management of one-stage partial nitritation/anammox. Microbiology and Biotechnology **5**(3), 433-448.

Yamamoto T., Takaki K., Koyama T. and Furukawa K. (2008). Long-term stability of partial nitritation of swine wastewater digester liquor and its subsequent treatment by Anammox. Bioresource Technology **99**(14), 6419-6425.

Yang J. (2012). Controlling and monitoring of deammonification process in moving bed biofilm reactor. Licentiate thesis. TRITA LWR LIC 2065, 29p.

Zubrowska-Sudol M., Yang J., Trela J. and Plaza E. (2011). Evaluation of deammonification process performance at different aeration strategies. Water Science and Technology 63(6), 1168-1176.