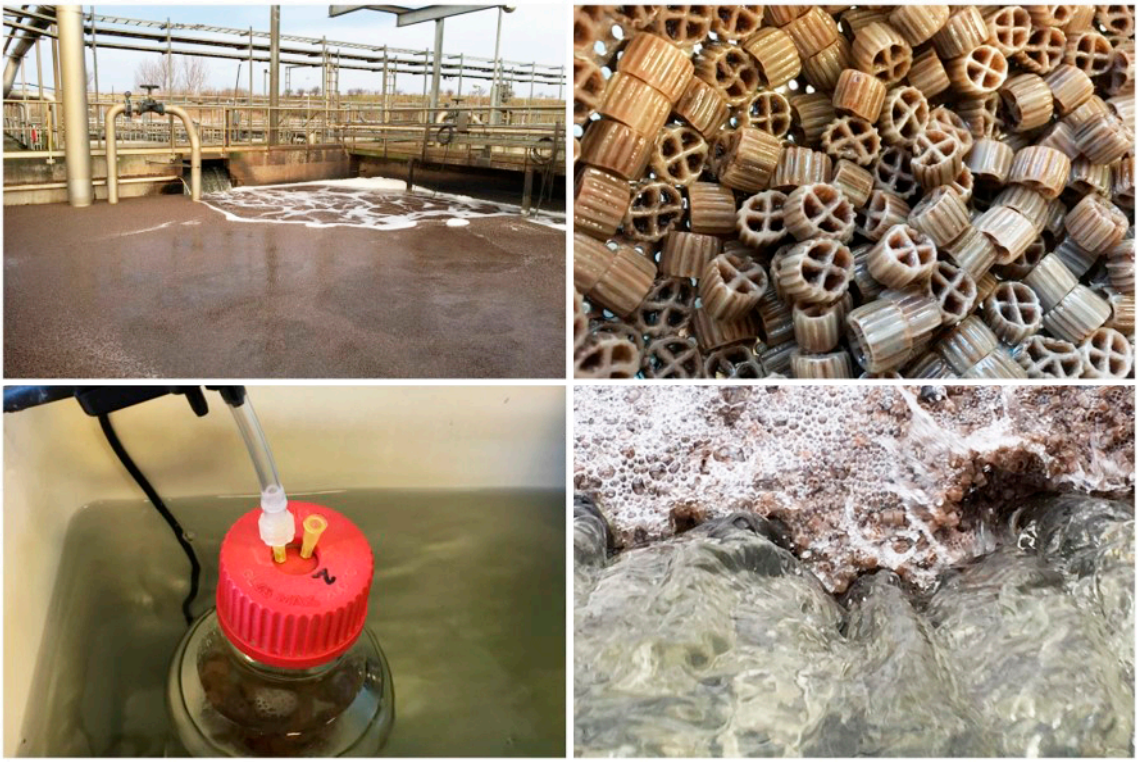
Monitoring of denitrifying activities in moving bed biofilm reactors with an ex-situ manometric batch test



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Water and Environmental Engineering
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by

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Picture on front page: The upper left and the lower right depict the inflow to the first MBBR. The upper right shows carrier material and the lower left shows the manometric reactor. Photo by Sofia Bårdskär.

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Preface

This thesis makes up the final part of my Master's degree in Biotechnical Engineering. The project was conducted at Water and Environmental Engineering, Department of Chemical Engineering at Lund University in cooperation with VA SYD. The experimental work has been performed at Sjölunda Wastewater Treatment plant in Malmö, Sweden.

I would like to express sincerest gratitude to my supervisors; David Gustavsson, Åsa Davidsson and Gabriel Persson for sharing your knowledge with me and for guiding me through this demanding project. I would also like to thank the personnel at Sjölunda WWTP for your valuable help and support and for providing all the necessary chemicals and instruments for my experiments. I would also like to thank Jes la Cour Jansen at Water and Environmental Engineering, Department of Chemical Engineering at Lund University for sharing your expertise regarding diffusion limitations.

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Abstract

Monitoring of denitrifying activities in moving bed biofilm reactors with an ex-situ manometric batch test

Emissions of reactive nitrogen to the environment is of major concern, e.g. since it is known to contribute to eutrophication and dead sea floors in water bodies. The capability to remove nitrogen from wastewater has therefore become a critical means of preserving and protecting the environment. Conventional nitrogen removal from municipal wastewaters usually includes nitrification together with denitrification. Due to more stringent legislation regarding effluent standards as well as higher loads, many wastewater treatment plants (WWTPS) in Sweden will have to be upgraded for increasing their degree of nitrogen removal. It is therefore important not only to be able to evaluate the efficacy of the existing facilities, but also to consider the implementation of new processes.

The aim of this Master's thesis was to adapt an ex-situ manometric batch test, developed for measuring anammox activity, to be used to measure denitrifying activity. The adapted test was used in order to study the activity on K1 carriers from the post-denitrification moving bed biofilm reactors at Sjölunda WWTP. Carriers from four different lines in the post-denitrification plant were analyzed.

Experiments were conducted with initial nitrate concentrations in the interval 25-150 mg N/L. The specific denitrifying activity turned out to be independent of initial nitrate concentrations in range 35-150 mg N/L and dependent on nitrate concentrations below 35 mg N/L. It was also revealed that COD/N ratios of 5.3-133.3 had no effect on the denitrifying activity, given that nitrate was not limiting. A small denitrifying activity was detected when no carbon source was added to the reactor, which indicated that the bacteria could use intracellular carbon as electron donor.

Experiments performed on the different lines showed varying specific denitrifying activities. However, operational results assessed over a period of four months, showed that all the lines in the post-denitrification plant had equal nitrate reduction efficacy. The operational data did not give any further information on how the capacity in the first zones differed from the capacity in the second zones. However, it was concluded by experimental assessment that the first zones had 3-4 times higher denitrifying activity than that of the second zones.

Sammanfattning

Övervakning av denitrifierande aktivitet på bärare i reaktorer med rörligt bärarmaterial med ett manometriskt labbtest

Utsläpp av kväve i naturen är ett stort problem eftersom det till exempel kan bidra till övergödning och så kallade döda bottnar i sjöar i akvatiska och marina miljöer. Förmågan att kunna avlägsna kväve från avloppsvatten har därför blivit ett viktigt medel för att bevara och skydda miljön. Kväveavskiljning från kommunala avloppsvatten sker vanligtvis med hjälp av nitrifikation och denitrifikation. Till följd av allt strängare lagstiftning gällande utsläppskrav samt högre belastningar, gör att många avloppsreningsverk i Sverige kommer att behöva utöka sin kväveavskiljande kapacitet. Det är därför viktigt att kunna utvärdera effektiviteten på befintliga anläggningar samt att överlägga införandet av nya processer.

Målet med detta examensarbete var att anpassa ett manometriskt labbtest, som tidigare utvecklats för att mäta anammoxaktivitet, för att kunna mäta denitrifikationsaktivitet. Den anpassade metoden användes sedan för att studera aktiviteten på K1-bärare från efterdenitrifikationsanläggningen på Sjölunda avloppsreningsverk. Aktiviteten på bärare från fyra olika linjer i denitrifikationsanläggningen analyserades.

Experiment utfördes med initiala nitratkoncentrationer i intervallet 25-150 mg N/L. Den specifika denitrifikationsaktiviteten visade sig vara oberoende av initiala nitratkoncentrationer i intervallet 35-150 mg N/L men beroende av startkoncentrationer under 35 mg N/L. Det visade sig också att COD/N kvoter mellan 5.3-133.3 inte hade någon effekt på denitrifikationsaktiviteten under förutsättning att nitrat inte var begränsande. En låg denitrifikationsaktivitet kunde detekteras utan tillsatt kolkälla, vilket antydde att bakterierna kunde använda intracellulärt lagrat kol som elektrondonator.

Experimenten utförda med bärare från de olika linjerna visade varierande denitrifikationsaktiviteter. Driftdatan som studerades under en fyramånadersperiod visade dock att alla linjer hade likvärdiga nitratreduktionseffektiviteter. Driftdatan gav ingen information om hur kapaciteten skiljde sig mellan de första och de andra zonerna. Det var dock påvisat genom experimentella försök att aktiviteten i de första zonerna var 3-4 gånger högre än aktiviteten i de andra zonerna.

Abbreviations and acronyms

Anammox	Anaerobic ammonium oxidation
AOB	Ammonia oxidizing bacteria
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
Manammox	Mainstream anammox
MBBR	Moving bed biofilm reactor
Nanammox	Nitritation-anammox
NOB	Nitrite oxidizing bacteria
NTF	Nitrifying trickling filter
NUR	Nitrate utilization rate
RPM	Revolutions per minute
SAA	Specific anammox activity
SDA	Specific denitrifying activity
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

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1 Background

Excess emissions of nitrogen and phosphorous compounds in the environment are known to be hazardous to lakes and water bodies. Even though nitrogen make up 80% of the elements in the atmosphere and is one of the most important compounds in living tissue, it becomes a pollutant when emissions are higher than the ecosystems can manage (Pitois *et al.*, 2001; Camargo & Alonso, 2006). The phenomenon is known as eutrophication, which has become an emerging concern in lakes, water streams and coastal areas over the last years. Eutrophication in water bodies contributes to a profileration of algal blooms which causes hypoxia at the sea floor, when degraded by microbes. The oxygen-deprived floors make it hard for other organisms, such as fish and plants, to survive (Hermansson *et al*., 2006; Conley *et al*., 2009). Nitrogen mainly enters the ecosystems through wastewater from households and industries and through discharge from agriculture.

Sweden is a member of the Helsinki Commission, which is an environmental policy maker for protection of the Baltic Sea area. In order to follow through with the commitments to the Baltic Sea Action Plan, Sweden will have to reduce its nitrogen load with 21,000 tons per year until 2021. This will take long-term investments in upgrades of the Swedish wastewater treatment plants (WWTPs) (Naturvårdsverket, 2012).

The WWTPs in Sweden have implemented nitrogen removal steps in order to decrease the discharge of nitrogen compounds. The nitrogen removal is based on biological processes, where biofilm systems becomes more and more common as alternatives to active sludge systems. Conventional enhanced nitrogen removal is often achieved through microbiological processes called nitrification and denitrification (Hermansson *et al*., 2006).

Sjölunda WWTP, located in Malmö, Sweden, has to expand its nitrogen removal capacity in order to meet the more stringent treatment standards and to be able to handle higher loads. Today, most of the denitrification at Sjölunda WWTP is carried out in moving bed biofilm reactors (MBBRs) as a post-treatment step (i.e after nitrification), and is therefore dependent on an external carbon source such as methanol. The available space and the nitrifying capacity are limited.

Several attempts have been made for investigating different processes as upgrading alternatives for the existing nitrogen removal facilities. Since 2012, two pilot projects called Manammox and Nanammox has been conducted in order to investigating the feasibility for implementing an anammox step in MBBRs (Gustavsson *et al*., 2015).

In order to evaluate the effectiveness of the processes, methods for analysis of the bacterial activity are crucial. Stefansdottir (2014) developed a method for measuring and evaluating the anammox activity on MBBR carriers from the Manammox pilot plant. The anammox activity was measured by a manometric batch test, where the pressure increment is proportional to the amount of produced nitrogen gas. The method showed to be reliable and reproducible and should enable further studies on the specific activity of the anammox bacteria.

In addition to investigating the benefits with implementing new processes, it is also important to evaluate the performance of the existing nitrogen removal facilities. It is interesting to see if

the manometric batch test is applicable on denitrification carriers and can be used as a complement when assessing the efficiency of the post-denitrification MBBR.

1.1 Aims

The main objective of this Master's thesis project was to adapt the manometric method, developed by Stefansdottir (2014), in order to study the denitrifying activity on the K1 carriers in the post-denitrifying MBBR at Sjölunda WWTP. The goal was to find suitable concentrations for full speed reactions. The Manometric batch test was then used to study the denitrifying capacity in the different lines and zones of the post-denitrification plant. This thesis project aimed to use the batch tests for monitoring variations of the specific denitrifying activities on the carriers in the post-denitrification plant and couple these variations with the operational results.

1.1.1 Hypotheses

- There is lower denitrifying activity on the carriers in the second zone compared to the first zone in the post-denitrifying MBBR, since the methanol dosage takes place in the first zone, which also has a higher nitrate concentration.
- There are different denitrifying capacities between the lines in the full-scale post-denitrifying MBBR.
- Higher substrate load in the full scale MBBR generates a higher specific denitrifying activities on the carriers
- There is some stored carbon source in the bacteria that can be utilized during carbon deficiency in the full-scale MBBR for denitrification

1.2 Limitations

There are several different bacterial groups in the biofilm, which have not been accounted for. The pressure meter used in the laboratory trials was not specific for nitrogen gas production from denitrifying bacteria. Other bacterial activities have been assumed to be negligible.

2 Theory

2.1 Conventional nitrogen removal strategies

In the recent years, the environmental regulation for nitrogen discharge has become more stringent. Emissions of ammonia and increased nitrogen loads are known to be hazardous to aquatic organisms and to cause eutrophication in lakes and watercourses. The removal of ammonium-nitrogen from wastewater has therefore become an important way to preserve and protect the environment (Wang *et al*., 2009).

The removal of nitrogen can be performed by several different technologies depending on the wastewater characteristics. Nitrogen removal technologies often involves oxidation of ammonium to nitrite (nitritation) or nitrate (nitrification) to be further reduced to dinitrogen gas via either denitrification (NO_3^- to N_2), denitritation (NO_2^- to N_2) or anammox (NO_2^- and NH_4^+ to N_2) (van Loosdrecht *et al*., 2016).

Since all organisms require nitrogen for DNA construction and protein synthesis, some bacteria can utilize nitrogen compounds either as an energy source (nitrification) or as a final electron acceptor in the respiratory electron transport chain (denitrification). These two biological processes are mediated by two different types of bacteria (nitrifiers and denitrifiers) and enable the transformation of ammonium nitrogen in wastewater to nitrogen gas (Hermansson *et al*., 2006).

2.1.1 Nitrification

Nitrification is a biological conversion of ammonium to nitrate in a two-step process. The first step, which can be seen in Equation 1, involves oxidation of ammonia to nitrite by ammonia oxidizing bacteria (AOB). The second step on the other hand, is carried out by nitrite oxidizing bacteria (NOB) and involves the oxidation of nitrite to nitrate according to Equation 2. Nitrification occurs under aerobic conditions (Wang *et al*., 2009).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \quad (1)$$

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

The two-step nitrification process involves mostly bacteria species from the *Nitrosomonas* genus, which mediate the ammonia oxidation (AOB), together with nitrite oxidizing species from the *Nitrobacter* and *Nitrospira* genus (NOB). Other bacteria that are also involved in the nitrification process are *Nitrococcus* and *Nitrosocystis* (Henze *et al*., 2002). Nitrifying bacteria are chemolithotrophic autotrophs that use the free energy generated from ammonia- and nitrite oxidation to form adenosine triphosphate (ATP), to be used for biomass production and CO_2 fixation (Wang *et al*, 2009). However, the energy yield from the oxidation of ammonium and nitrite is low (270 kJ/mol NH_4^+-N and 80 kJ/mol NO_2^--N, respectively), which contributes to a low growth rate. The low growth rate of the nitrifying bacteria is a major issue for the nitrification capacity in WWTPs (Henze *et al*., 2002).

2.1.2 Denitrification

Over the last century, the importance of denitrification has increased due to higher emissions of nitrogen coming from industries, chemical fertilizers and fossil fuels. It is estimated that approximately 50% of the biosphere nitrogen comes from fertilizers or fossil fuels (Chen *et al*., 2013).

Denitrification is the process where nitrogen is removed from the biosphere by conversion to dinitrogen (N_2), which is released into the atmosphere. In denitrification, the N-N bond formation of the intermediates nitric oxide (NO) and nitrous oxide (N_2O) is catalyzed to a lower oxidation state (Zumft, 1997). The steps of the denitrification process are seen in Equation 3.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (3)$$

Denitrification occurs under anoxic conditions, which means that nitrate and nitrite are used as electron acceptors for microbial respiration without the presence of oxygen. The bacteria that achieve denitrification are commonly known as denitrifiers, which are heterotrophic bacteria that are strongly dependent on organic carbon, such as methanol, as an electron donor of the denitrification process. Denitrifiers are facultative bacteria that can use either oxygen or nitrate as electron acceptors and can therefore survive under aerobic as well as anaerobic conditions (Wang *et al*., 2009). The stoichiometric relationship with methanol as carbon source can be seen in Equation 4, and the metabolism concerning biomass production is described in Equation 5.

$$6\,NO_3^- + 5\,CH_3OH \rightarrow 3\,N_2 + 5\,CO_2 + 7\,H_2O + 6\,OH^- \quad (4)$$

$$NO_3^- + 1.08\,CH_3OH + 0.24\,H_2CO_3 \rightarrow 0.056\,C_5H_7NO_2 + 0.47\,N_2 + HCO_3^- + 1.68\,H_2O \quad (5)$$

For a complete denitrification process, four enzymes are of the essence; nitrate reductase (NO_3^- → NO_2^-), nitrite reductase (NO_2^- → NO), nitric oxide reductase (NO → N_2O) and nitrous oxide reductase (N_2O → N_2). By definition, a denitrifier has to contain 2-3 of these enzymes and to be able to produce nitrous oxide or nitrogen gas (Chen *et al*., 2013). The genes encoding for denitrification reductases can be found, for instance, in *Pseudomonas* and *Paracoccus* strains where some important denitrifying species are *Pseudomonas stutzeri*, *Pseudomonas aeruginosa* and *Paracoccus denitrificans*. Denitrifying bacteria are gram-negative and can either be rod shaped (*P. stutzeri* and *P. aeruginosa*) or coccoid (*P. denitrificans*) (Zumft, 1997).

Alternative metabolic pathways

According to Ruiz *et al*. (2006), other pathways than denitrification are possible in an anaerobic reactor when nitrate and organic matter are present. Such pathway is for instant methanization, which is an anaerobic digestion of organic matter that produces methane gas. Methanization can be carried through if there is an excess of carbon in the reactor.

2.2 Anammox - anaerobic ammonium oxidation

The anaerobic ammonium oxidation process was described by Mulder *et al*. in 1995 based on the discovery that ammonium disappeared from a denitrifying fluidized bed reactor while treating wastewater from a methanogenic reactor. The conclusion was that ammonium together with nitrate as an electron acceptor can produce nitrogen gas during anaerobic circumstances. However, the bacteria that was able to perform anaerobic ammonium oxidation was still unknown. It was first in 1999 that Strous *et al.* managed to identify this missing link in the nitrogen cycle as autotrophic bacteria in the order *Planctomycetales*, by phylogenetic analysis of the RNA sequences.

The anammox bacteria are autotrophs and use hydrogen carbonate (HCO_3^-) as a carbon source, ammonium and nitrite as main electron donor and electron acceptor, respectively (van de Graaf *et al*., 1996). The general reaction formula can be seen in Equation 6.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (6)$$

Equation 7 shows the stoichiometry reaction, including the elemental composition of the biomass, calculated by Strous *et al*. (1998)

$$NH_4^- + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O \quad (7)$$

Trials performed by Strous *et al*. (1998) confirmed that the anammox bacteria have an extremely slow growth rate of 0.0027 h^{-1} and a generation time of 11 days at 32-33°C. One explanation for the slow growth rate is that the anammox bacteria has a slow consumption rate of ammonium. The specific activity of Anammox is seven times lower than that of aerobic ammonium oxidation (Strous *et al*., 1998)

2.3 Moving bed biofilm reactors, MBBR

The Moving Bed Biofilm Reactor (MBBR) is a method for water purification that was developed in Norway in the late 1980s and the early 1990s. Since then, the method has been successfully used for municipal wastewater treatment worldwide concerning BOD/COD-removal, nitrification and denitrification (Ødegaard *et al*., 2000; Rusten *et al*., 2006). In MBBRs, biomass grow on plastic carriers that move freely in the reactor, kept enclosed by sieving arrangements at the outflow. The MBBR technique can be implemented on aerobic, anaerobic and anoxic processes. The biofilm carriers are kept in motion by agitation set up from aeration in aerobic reactors, while the carrier movement is achieved by mechanical mixing in anaerobic and anoxic reactors. (Ødegaard, 1999).

One type of carrier material is the original Kaldnes carrier, also known as K1, which are cylindrical carriers made from polyethylene (Ødegaard *et al*., 2000). There are two types of K1 carriers, one heavy model and one light model. The K1 heavy carrier has a density of 0.98 g/cm^3, while density of K1 light is about 0.95 g/cm^3. The carrier that is used in the MBBRs at Sjölunda WWTP is K1 light. The carriers have a length of 7 mm and a diameter of 10 mm. The cylinders have grooves on the outside while the inside evenly divides the carrier into four zones (Ødegaard, 1999). The whole inner area of the carrier plus the area of the outer fins, constitute the effective surface area where the biofilm attaches and the reactions take place. On the K1 carriers the effective surface area is 490 mm^2 per piece or approximately 500 m^2/m^3 (Ødegaard *et al*., 2000). The biomass grows primarily on the inside of the carriers since the residues on the outside tend to wear of when the carriers clash into each other. In order to maintain free carrier suspension movement, it is recommended that the filling degree is kept below 70% (Rusten *et al*., 2006). The AnoxKaldnes™ K1 carriers are depicted in Figure 2-1.

Figure 2-1: The AnoxKaldnes™ K1 carriers showing the four zones on the inside, were the biofilm is attached, and the grooves on the outside. Photo by Bårdskär (2016)

The purpose of the development of the MBBR was to combine the advantages from the activated sludge process and biofilm processes and in the meantime avoid the disadvantages of the two. The MBBR process enables utilization of the whole tank for biomass growth, which makes it more space efficient than biofilter processes. Another advantage is that, contrary to the activated sludge process, the MBBR does not require a sludge recycling system, since the biomass is attached to the carriers that are kept in the reactor. The filling degree can also be adjusted in order to fit different degrees of nutrient removal needs (Ødegaard, 1999). One disadvantage with biofilm techniques is the diffusion limitation of substrates through the dense layer of bacteria (Henze *et al*., 2002).

2.4 Diffusion limitations in biofilms

In order for a reaction to take place in a biofilm, the substrates have to be transported in to the bacteria and the reaction products have to be transported out to the bulk. The mass transport in biofilms is enabled mainly by diffusion. The diffusion results in a bulk process, which, depending on the substrate concentration in the bulk, can attain either half or zero order reaction kinetics. A bulk reaction becomes zero order when the biofilm is fully penetrated, while the half order reaction implies that the biofilm is partly penetrated (la Cour Jansen & Harremoës, 1984). The penetration ratio can be described according to equation 8.

$$\beta = \sqrt{\frac{2D \cdot C^*}{k_{0f} \cdot L^2}} \tag{8}$$

Where, β is a dimensionless constant called the penetration ratio. D is the molecular diffusion coefficient in the biomass, (m^2/s). C^* is the substrate bulk concentration, (g/m^3). k_{0f} is the intrinsic zero order removal rate in the biofilm, (g/m^3s) and L is the thickness of the biofilm, (m).

Zero order bulk reactions are applicable for $\beta \geq 1$. This means that the reaction kinetics for the bulk process is dependent on the biofilm thickness rather than the substrate concentration, when the biofilm is fully penetrated. This can be seen in equation 9.

$$r_a = k_{0a} = k_{0f} \cdot L \tag{9}$$

r_a is the removal rate per unit area biofilm surface, (g/m^2s), and k_{0a} is the zero order removal rate per unit area, (g/m^2s).

At lower substrate concentrations in the bulk, the diffusion limitation leads to partial penetration of the biofilm and the bulk process follows half order. Half order bulk reactions are dependent on substrate concentrations and are valid for $\beta < 1$, see Equation 10.

$$r_a = k_{\frac{1}{2}a} \cdot C^{*\frac{1}{2}} = \sqrt{2 \cdot D \cdot k_{0f}} \cdot C^{*\frac{1}{2}} \quad (10)$$

Where $k_{1/2a}$ is the half order rate constant per unit area, ($g^{1/2}/m^{1/2}s$). Figure 2-2 depicts a process transitioning from half order reaction kinetics to zero order reaction kinetics when the biofilm is fully penetrated.

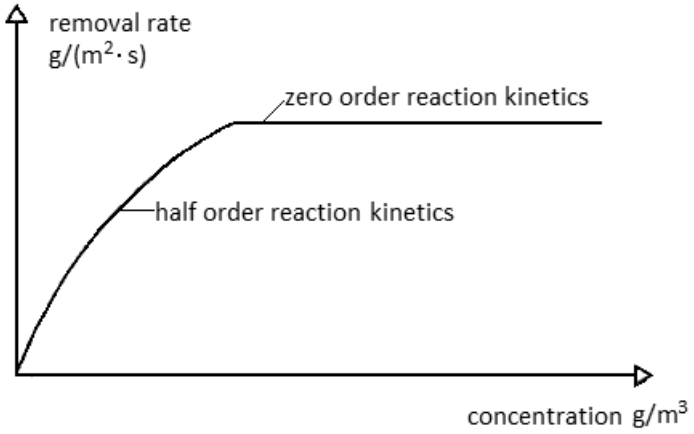

Figure 2-2: Reaction kinetics for half order and zero order reactions. When the removal rate reaches zero order reaction rate, the biofilm is fully penetrated with substrate. Adapted with permission from la Cour Jansen (1983).

The substrate that penetrates the biofilm the least becomes rate limiting. Either the electron acceptor or the electron donator can be the limiting component in redox processes. When the bulk process follows half order reaction kinetics, Equation 11 below can be used in order to investigate which substrate that is rate limiting and thus determines the reaction rate (la Cour Jansen & Harremoës, 1984).

$$\frac{C_d^*}{C_a^*} = \frac{D_a \cdot k_{0fd}}{D_d \cdot k_{0fa}} = \frac{D_a}{D_d} \cdot M \quad (11)$$

Where, C^*_d and C^*_a are the bulk concentrations of the electron donor and the acceptor, (g/m^3). D_d and D_a are the coefficients for molecular diffusion for electron donor and the acceptor, (m^2/s). K_{0fd} and k_{0fa} are the intrinsic zero order removal rate in the biofilm for the electron donator and the acceptor, (g/m^3s) and M is the stoichiometric consumption rate, (g/g).

2.5 Factors influencing the denitrification process

2.5.1 Dissolved oxygen

It has been widely accepted that biological denitrification is a strictly anaerobic process and that dissolved oxygen has a negative effect on denitrification activity (Gomez *et al*., 2002).

When oxygen is available, denitrifiers may prefer aerobic respiration over denitrification as metabolic route, since the presence of oxygen represses the synthesis of nitrate reductase in the denitrification process (Wang *et al*., 2009). However, Davies *et* al. (1989) provided evidence for aerobic denitrification in *P. denitrificans* and *P. aeruginosa*. It was revealed that the utilization of nitrate depended on the growth and maintenance conditions of the bacteria. Aerobically grown cells showed rapid conversion of nitrate to nitrogen gas or nitrous oxide in the presence of oxygen. The highest nitrate utilization rate was found in cells that were grown and maintained anaerobically, while the lowest rate was found in cells grown anaerobically and maintained aerobically.

Based on the observations by Gomez *et al.* (2002), it was found that the presence of 4.5 mg O_2/L resulted in a decreased nitrate removal and an increase in nitrite accumulation. However, the negative effects varied depending on the carbon source used. Denitrification with alcohols such as methanol and ethanol was less effected by dissolved oxygen, compared to denitrification with sucrose.

2.5.2 The effect of COD/N ratio

The proportion between the COD concentration and the nitrogen concentration has shown to have a crucial effect on the denitrification efficiency. Ruiz *et al.* (2006) concluded that the COD/N ratio had an effect on the utilization route of the organic matter. Denitrification was found to be the main utilization route for low ratios (COD/N<10) while methanization was found to be prevailing for a COD/N ratio of 100. The nitrate removal was poor at a COD/N ratio of 1, while a ratio of 5 achieved a nitrate elimination of almost 100%.

Akunna *et al*. (1992) found the methanization route to be dominant for COD/N ratios above 53. Denitrification was dominant for ratios under 8.86, while both denitrification and methanization occurred for ratios between 8.86 and 53. However, it is widely accepted that methanization is only possible after the denitrification is finished, since nitrate has an inhibitory effect on methane production (Akunna *et al*., 1992; Ruiz *et al.*, 2006).

2.5.3 Temperature

Carrera *et al.* (2004) concluded from their studies that the denitrification process is more affected by temperature in the interval 6-10°C than in the interval 10-25°C. The highest denitrification rate found in that same study was at 25°C. Studies on cold sediments conducted by Rysgaard *et al.* (2004), showed that the highest obtained denitrification rate was found at a temperature of 24°C. Findings in the same study implied that low temperatures results in lower denitrification rates. At a temperature of -1.3°C, the denitrification rate was 17% of that of the optimum at 24°C. Pfenning & McMahon (1996) also found that decreasing temperature resulted in lower denitrification rates. Their trials on riverbed sediments resulted in a 77% decrease in N_2O-production when changing the temperature from 22°C to 4°C.

2.5.4 pH

Lee & Rittmann (2003) observed the effect of pH on denitrification and concluded that the optimum pH for autotrophic denitrification can be found in the range 7.7-8.6. In their study, a significant decrease in nitrate removal rate and an increased nitrite accumulation was observed for pH levels above 8.6. Lee & Rittmann (2003) also concluded that some field applications may need pH control since the pH rose by 1.2 units when there was no phosphate buffer added. For experiments conducted with buffer, the pH rose with 0.1-0.3 units.

Trials conducted by Saleh-Lakha *et al*. (2009) concluded that the expression of denitrification genes in *Pseudomonas mandelii* was significantly lower in cells grown at pH 5 compared to cells grown at pH 6-8. Bergaust *et al.* (2010) saw the same pattern with low gene expression at pH 6 in *Paracoccus denitrificans*. Knowles (1982) suggests that the most optimal pH for denitrification can be found in the range 7-8.

2.6 Strategies for measuring bacterial denitrifying activity

In order to evaluate the performance of biological nitrogen removal processes, batch activity tests can be performed. Batch activity tests are applicable under aerobic, anaerobic as well as anoxic conditions and can be used to assess the kinetic rates of processes such as nitrification, denitrification and anammox. Different tracking techniques are available for process kinetics assessment such as chemical, titrimetric and manometric tracking (van Loosdrecht *et al*., 2016). Chemical tracking means that the substrate concentrations are assessed over time, while titrimetric tracking involves pH-static titration to processes that affect the pH of the solution. Manometric tracking is applicable to processes that produce soluble gases such as N_2 and includes measurement of the pressure increment caused by such gases.

Depending on the type of process (nitrification, denitrification or anammox) and the tracking technique, several different batch activity tests can be performed for nitrogen removal evaluation (van Loosdrecht *et al*., 2016).

2.6.1 Manometric batch tests

Manometric batch tests are based on the measurement of nitrogen gas production and can be used for kinetic description of biological denitrification and anammox activity (Dapena-Mora *et al*., 2007; Ficara *et al*., 2009). Provided that the reaction takes place in a gas-tight reactor, the pressure increment is proportional to the conversion of nitrate to nitrogen gas. The pressure difference can be converted to the amount of produced gas by using the ideal gas law, see Equation 12 (Ficara *et al*., 2009; van Loosdrecht *et al*., 2016).

$$p(t) = n_{N_2}(t) \cdot \frac{R \cdot T}{V_{HS}} \tag{12}$$

Where p(t) is the overpressure, V_{HS} is the volume of the headspace (L) and n_{N2} is the number of moles N_2 released into the headspace of the reactor. R is the ideal gas constant and T is the temperature in Kelvin.

According to Sanchez *et al*. (2000), the maximum specific denitrification activity can be calculated from the maximum gas production in accordance with Equation 13.

$$SDA_m = \frac{dN_2/dt}{X \cdot V_r} \; (g\ N_2 - N/(g\ VSS \cdot d)) \tag{13}$$

Where SDA_m is the maximum specific denitrifying activity, dN_2/dt is the maximum N_2 production rate, X is the microbial concentration (g VSS/L) and V_r is the volume (L) of aqueous phase in the reactor. Equation 13 is also applicable for determination of the specific anammox activity, SAA (Dapena-Mora *et al*., 2007).

Manometric batch tests has been applied to studies on denitrification activity (Buys *et al*., 2000; Sanchez *et al*., 2000; Ficara *et al*., 2009) and on anammox activity (Dapena-Mora *et al*., 2007;

Scaglione *et al*., 2009; Lotti *et al*., 2012). All the tests were conducted on sludge in the studies mentioned, except from Lotti *et al*. (2012) where granules were used.

At Sjölunda WWTP, studies have been performed where the manometric batch test has been applied in order to evaluate the anammox activity on AnoxKaldnes™ K1 carriers (Gustavsson, 2013; Stefansdottir, 2014; Okhravi, 2015).

Equipment and execution

There are certain criteria that the reactor, used for the batch tests, has to fulfill regardless of the nature of the experiment. For instance, the reactor has to be air-tight and to be able to avoid oxygen intrusion under anoxic conditions. It also has to be able to maintain a desirable temperature, to provide pH control and to allow sufficient mixing conditions (van Loosdrecht *et al*., 2016). In order to eliminate oxygen from the reactor and to obtain anoxic conditions, the liquid phase and the head space can be flushed with nitrogen gas (Buys *et al*., 2000; Dapena-Mora *et al*., 2007; Lotti *et al*., 2012). The reactor can preferably be sealed with a rubber septum, which allows for the additions of substrates by injections. The septum also enables pressure stabilization if secondary needles are inserted, acting as a passage for surplus gas (Ficara *et al*., 2009; Lotti *et al*., 2012). After the substrate additions, the overpressure is recorded by a manometric device, which should include a pressure transducer inside a measuring head that is attached to the reactor, together with a data logging system (Scaglione *et al*., 2009; Lotti *et al*., 2012).

2.6.2 Nitrate utilization rate (NUR) batch tests

As an alternative for the manometric test method, the denitrifying activity can be assessed with the nitrate utilization rate (NUR) test. The NUR test is a chemical tracking method for characterization of denitrifiers in biomass and for characterization of wastewater (Kristensen *et al*., 1992; van Loosdrecht *et al*., 2016). Provided that the initial concentration of nitrate is known, the decrease of nitrate can be assessed over time. NUR can then be calculated from the slope of the curve where the nitrate concentration is plotted versus time (Henze, 1986; Kristensen *et al*., 1992).

3 Sjölunda wastewater treatment plant

3.1 General information about the overall process

Sjölunda WWTP, situated in Malmö, is one of the largest wastewater treatment plants in Sweden, built for 550,000 population equivalents regarding COD removal. The plant receive wastewater, corresponding to an average wastewater flow of 1,650 L/s, from the greater part of Malmö and Burlöv together with parts of the municipalities of Lomma, Staffanstorp and Svedala.

Sjölunda WWTP was built and taken into operation in 1963. Several upgrades have been made thereafter regarding nutrient removal from the wastewater, due to more stringent effluent standards. The upgrade in 1998 was made in order to implement nitrification in trickling filters and post-denitrification in MBBRs. The aim was to achieve effluent standards of 12 mg BOD_7/L, 10 mg total-N/L and 0.3 mg total-P/L (Hanner *et al.*, 2003; Mases *et al.*, 2010). The process configuration of Sjölunda WWTP can be seen in Figure 3-1.

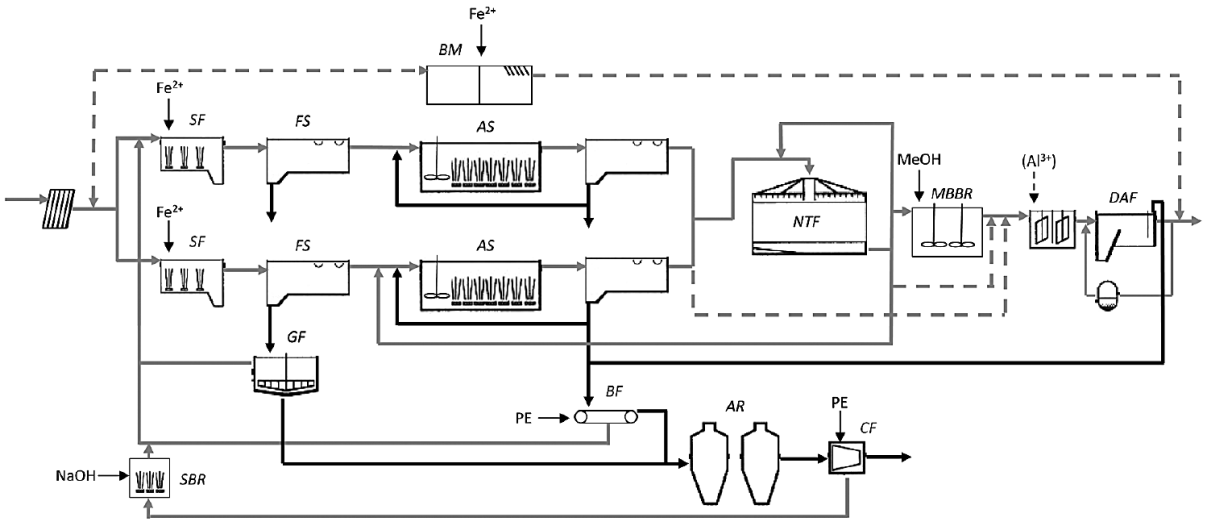

Figure 3-1: Process configuration of Sjölunda WWTP where SF is the grit removal, FS is the primary clarification, AS is the activated sludge plant, NTF are the nitrifying trickling filters, MBBR are the moving bed biofilm reactors, DAF is the dissolved air flotation, GF and BF are the sludge thickening plants, AR are anaerobic digesters, CF are dewatering centrifuges, SBR is the sequencing bath reactor for sludge liquor treatment and BM is the wet weather overflow plant. Picture taken from Gustavsson et al. (2012) with permission from Magnus Persson, editor of Journal of Water Management and Research

The water treatment process includes mechanical, biological and chemical treatment, together with further processing of the separated sludge. The purpose of the mechanical treatment is to separate objects and grit from the wastewater. This is done by filtration and sedimentation. The chemical treatment allows the removal of phosphorus through precipitation by adding a ferrous-based chemical to the wastewater. The biological steps include an activated sludge plant for COD removal, nitrifying trickling filters (NTFs) and post-denitrification MBBRs for nitrogen removal. Before the effluent wastewater is released into the strait of Öresund, the wastewater passes through a flotation plant where the floc-forming microorganisms are removed.

After the grit removal step, the grit is washed and dewatered in order to eliminate organic material so it can be used in soil construction. The sludge from the process is treated and digested and can be converted into biogas and utilized as fertilizer.

3.2 The full-scale post-denitrification plant

At Sjölunda WWTP, the denitrification is carried out in MBBRs as a post-treatment step after nitrification in trickling filters. The denitrification plant consists of six lines with two MBBR zones in each line with a total reactor volume of 6,234 m^3 and a maximum hydraulic load of 2,200 L/s. The total influent is divided between the six lines. The flow rate of each line is regulated by a chute at the inflow, which can be moved up or down and thereby limit the overflow. The chute on line six is positioned higher, which contributes to a more limited overflow and thus a lower flow rate. Since water streams take the easiest way out due to hydraulic forces, a restricted flow to the last line is necessary in order to force the water out to the other lines.

The reactors are filled with K1 carriers from AnoxKaldnes™, Sweden, to a filling degree of 50% (Aspegren *et al*., 1998). Each MBBR is kept agitated by three mechanical stirrers, situated at one of the sides of each reactor. The carriers are not mixed between the lines or the zones and are kept enclosed in the reactors by barriers and sieving mechanisms. The sieves are situated between the zones and at the outflow of each line. In order to remove jammed up carriers in the sieves and to facilitate MBBR movement, the sieves are flushed with air 2-6 times a day, depending on the season. More flushing sessions are required during the winter in order to prevent the water from freezing and due to higher wastewater load. The sieves are flushed for five minutes at each occasion. A brief outline of the post-denitrification plant is shown in Figure 3-2.

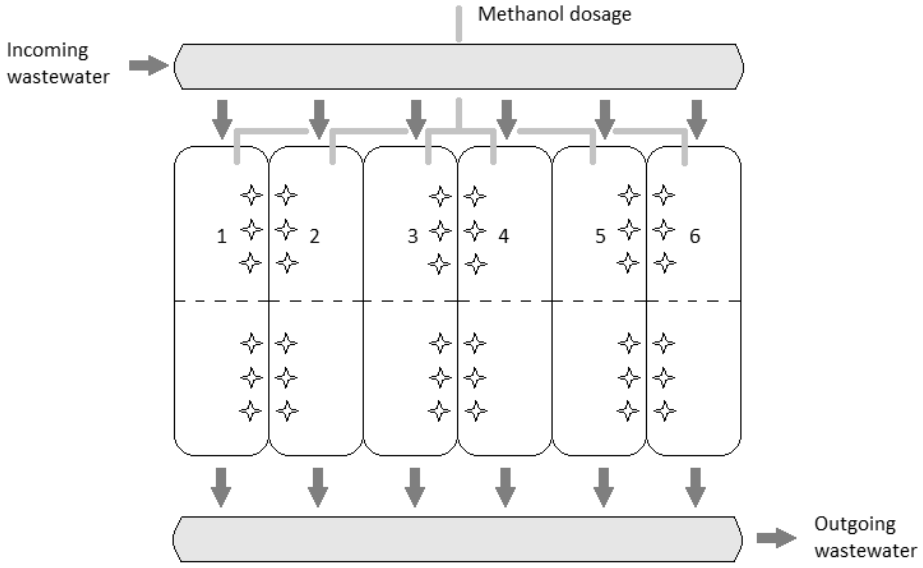

Figure 3-2: A brief outline of the post-denitrification plant. The mechanical stirrers are situated at one side of each reactor and are marked as stars. The dashed line shows the boundaries between the zones. Methanol is added close to the inflow of each line and is marked as grey lines. The MBBRs receive effluent from the nitrifying trickling filters, which after the denitrification step, continues to the flotation plant. Methanol is dosed at the inflow of each line. Picture by Bårdskär (2016)

Methanol is added as a carbon source in the first zone of each line and is stored in four containers with a volume of 62.5 m^3 each. The methanol dosage varies depending on the nitrate load, COD content in the carbon source and a wanted inlet ratio of COD/N for each line (Mases *et al.*, 2010). The COD and nitrate concentrations are determined by online measurements of each line and methanol is then added accordingly to get a COD/N ratio of approximately 4.

The total denitrification capacity at the design temperature of 10°C is 2,000 kg NO_x^--N/d, which corresponds to a denitrification rate of 1.2 g NO_3^--N/(m^2·d) (Hanner *et al.*, 2003; Mases *et al.*, 2010). Two basins of the full-scale post denitrification plant are depicted in Figure 3-3.

Figure 3-3: The post-denitrification facilities at Sjölunda WWTP showing the inflow to Line 1, Zone 1 to the left and Line 1, Zone 2 to the right. The chute at the inflow regulates the flow rate. The basins are filled with AnoxKaldnes™ K1 carriers depicted in Figure 2-1. Photo by Bårdskär (2016)

4 The experimental set-up

4.1 Sampling

In order to get samples from the full-scale post-denitrification plant a plastic container attached to a long metal stick was submerged directly into the basin of choice. The carriers were taken from a spot in the basin where there was good agitation without foaming. The container was dipped into the reactor long enough to collect at least 240 carriers to be used in the manometric batch activity test. The carriers were then transported to the laboratory where the experiments were conducted.

After the completed experiments, the collected carriers were poured back to the reactor of origin.

4.2 The manometric batch activity test

The manometric batch activity test used in this project was developed by Stefansdottir (2014) to be used on carriers from an anammox reactor. The purpose of this project was to conduct the test on carriers from the full-scale post denitrification plant. To be able to do that, the test would have to be adapted to be used on the carriers in the post-denitrification MBBRs. The principle of the test was to measure the maximum specific activity on the carriers by logging the pressure increment of produced nitrogen gas as a function of time. The pressure increment is proportional to the ability of the bacteria to convert the substrates into nitrogen gas.

4.2.1 Method and devices

The following description is adapted from the method that Dora Stefansdottir developed during her Master's thesis in Water and Environmental Engineering in 2014

From the taken sample, 240 carriers were manually counted and carefully washed with water in order to remove as much solids as possible. The carriers were put into a 1 L glass flask together with a magnetic bar. The flask was placed on a scale and 750 g distilled water (i.e. 750 mL) and 22 mL of phosphate buffer (5 mg P/mL) were added in order to maintain a steady pH of around 7.75 throughout the experiment. The flask was then closed with a lid and a septum. The water bath, that the flask was kept in during the test, was made from a plastic cooler together with a temperature regulator and a magnetic stirrer. The temperature was set to 20°C and the magnetic stirrer was set to 400 rpm. The temperature was allowed to stabilize for 15 minutes before flushing the gas and the liquid phase of the reactor with nitrogen gas for 10 minutes. A pressure meter was connected with a needle through the septum and atmospheric pressure equilibrium was achieved by putting a separate needle through the septum. The pressure was allowed to stabilize for 30 minutes before nitrate (5 mg N/mL) and methanol were added with a syringe through the septum. The initial concentrations of the substrates varied depending on which experiment that was supposed to be conducted.

The logging started after the addition of nitrate and methanol. The experiments were carried out for 80 or 120 minutes. The pressure meter was programmed to log one value each minute. At the end of each experiment the volume of the reactor head space was measured. This was done by weighing the reactor and then filling the head space with water. The weight difference was then converted to the corresponding volume by division with the density of water.

The pressure meter used in the laboratory trials was GMH 5150 from Greisinger electronic GmbH (Regenstauf, Germany). The pressure meter was connected to a sensor, GMSD 350MR from Greisinger electronic GmbH, which measured the relative pressure in the interval -199-350 mbar. The pressure meter was connected to the computer via USB 5100 from Gerisinger electronic GmbH. A computer program, GSOFT 3050 from Greisinger electronic GmbH, was installed on the computer to process the logged values. The recorded data was then exported to Excel 2013 where all the calculations were performed.

The equipment used for conducting manometric batch tests can be seen in Figure 4-1.

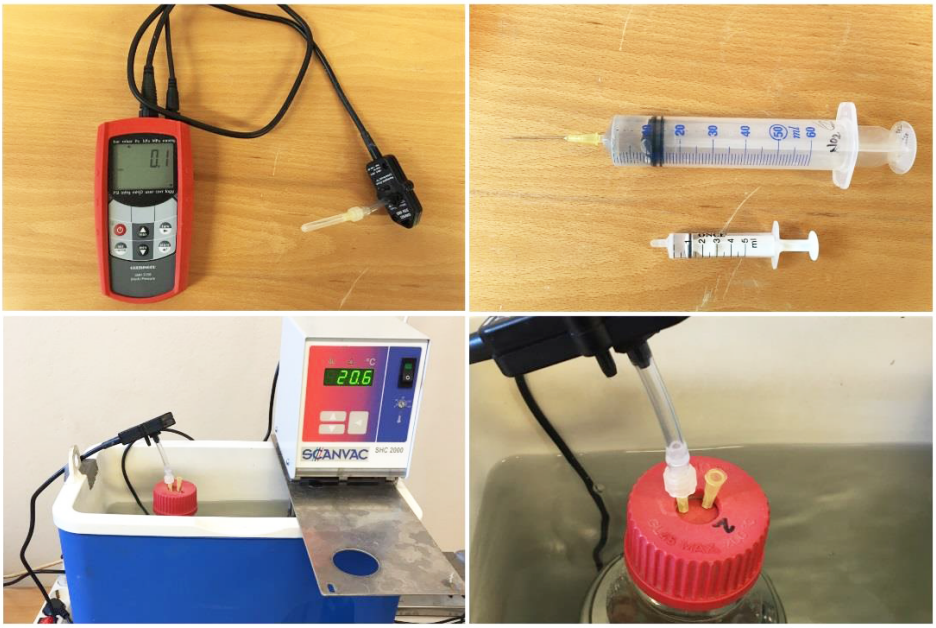

Figure 4-1: A schematic picture of the equipment used during the experiments. The pressure meter with sensor can be seen at the top left. The top right picture shows the syringes used to add the substrates to the reactor. The water bath with submerged reactor can be seen in the bottom left picture. The bottom right picture shows the pressure sensor and the stabilization needle through the septum.

The adapted method had only a few alterations from the original procedure. In the original procedure, the water bath was set to 28°C and the logging time was 120 minutes. Since the original method was used to measure anammox activity, 20 mL nitrite and 20 mL ammonium were added to the reactor in order to achieve a nitrite concentration of 125 mg N/L. Other than these differences, all the other steps were performed according to the original procedure.

Chemicals

The experiments performed to study the denitrifying activity on the post-denitrification plant, were conducted with additions of nitrate and methanol. The nitrate solution had a concentration of 5 mg N/mL and was prepared by adding 15.18 g $NaNO_3$ to a 500 mL vial that was filled with distilled water. The vial was placed on a magnetic stirrer to the point where the salt was completely dissolved. The solution was then stored in a fridge.

Pure methanol was taken from a tap in connection to the methanol distributors of the post-denitrification plant. The COD value of the methanol is measured once a month by the staff at

the laboratory. The COD value for methanol usually lies between 1.1-1.2 kg O_2/L. For COD calculations in this project, a COD value of 1.2 kg/L has been used.

In excess of the added substrates, a phosphate buffer of 5 mg P/mL was used in both cases in order to achieve a suitable pH of 7.75 for the reactions. The phosphate buffer was prepared by mixing 32.35 g $Na_2HPO_4 \cdot 2H_2O$ with 2.84 g NaH_2PO_4 in a 200 mL vial. The vial was then filled with 200 mL distilled water. The solution was placed on a magnetic stirrer and heated to 50°C for two hours. After the preparation, the buffer was stored warm in order to avoid crystallization.

The nitrate solution and the phosphate buffer was prepared by the staff at Sjölunda WWTP.

pH measurements

Due to the lack of proper measuring instruments, the pH was only measured at the last three conducted experiments. The pH was measured in the reactor before flushing with nitrogen gas and after the experiment was finished. The instrument used was pH/conductor meter SevenGo duo from Mettler Toledo AG (Schwerzenbach, Switzerland).

4.3 Specific denitrification activity (SDA)

The specific denitrifying activity was measured using the method developed by Stefansdottir (2014), which is based on the methodology orchestrated by Dapena-Mora *et al.* (2007). The Principle is to measure the pressure increment, which is proportional to the conversion of nitrate to nitrogen gas. The amount of moles of produced gas can be calculated using the ideal gas law, see Equation 14.

$$p \cdot V_{HS} = n \cdot R \cdot T \tag{14}$$

Where p is the pressure (mbar), V_{HS} is the gas volume of the headspace of the reactor (m^3), n is the amount of moles (mol), R is the gas constant (0.08314 mbar·m^3/(K·mol)) and T is the temperature (K).

The nitrogen conversion rate can be obtained if the nitrogen gas production is assessed over time. The maximum slope of the curve in Equation 15 gives the maximum nitrogen gas production rate.

$$\frac{dN_2}{dt} = \alpha_{max} \ \ (mol\, N_2/min) \tag{15}$$

Where α_{max} is the maximum nitrogen gas production rate.

The specific denitrifying activity can then be calculated using Equation 16 below. The methodologies described by Dapena-Mora *et al.* (2007) and Sanchez *et al.* (2000) are based on sludge with known biomass concentration in g VSS/L. When a manometric batch test is applied on carriers from an MBBR, the calculations are performed with accounts for the active biofilm surface area of the carriers, rather than biomass concentration.

$$SDA = \frac{\alpha_{max} \cdot M_{wN_2} \cdot 24 \cdot 60}{x \cdot a_e} \ \ (g\, N_2 - N/(m^2 \cdot d)) \tag{16}$$

Where the molar weight of nitrogen gas is designated as M_{WN2} (g/mol). X is the number of carriers in the reactor and a_e is the effective area of each carrier (m^2). In order to get the conversion from minutes to day, the expression is multiplied with 60 min/h and 24 h/d.

4.4 Laboratory sessions on the post-denitrification MBBR

4.4.1 Initial concentration measurements

The manometric batch activity test was conducted with different initial concentrations of nitrate in order to determine at which concentration the highest production rate can be achieved. The purpose of this test was to find a suitable substrate concentration to be used when studying the different capacities between the lines and the zones of post-denitrification plant. The carriers used in these experiments were collected from Line 1, Zone 1.

According to Stefansdottir (2014) the specific anammox activity was independent of initial concentrations of nitrite in the interval 75-125 mg N/L. Since the method has not yet been used to study the denitrifying activity on the carriers in the full-scale post-denitrification plant, the first experiment was conducted with an initial nitrate concentration of 75 mg N/L. The three subsequent experiments were conducted with higher nitrate concentrations than that of the first set. These where 100, 125 and 150 mg N/L. Another two sets of experiments were performed with lower concentration than that of set 1.1 (50 and 25 mg N/L). The calculations for the substrate and COD concentrations were done with an assumed final volume of 750 mL. In the two first sets of experiments, 6.25 mL methanol was added, which corresponds to a COD concentration of 10,000 mg/L. This volume turned out to be miscalculated, which is why the tests for 75 and 100 mg N/L were repeated with lower COD concentrations. 1 mL methanol was added for experiment 1.4-1.8, which corresponds to a COD concentration of approximately 1,600 mg/L, and 0.5 ml methanol was added to experiment 1.9, which corresponds to a COD concentration of 800 mg/L. Except for the substrates and the different substrate concentrations, experimental sets 1.1-1.9 were performed according to the original procedure. The conditions for the initial concentration measurements can be seen in Table 4-1.

Table 4-1: Initial concentration measurements with a logging time of 120 minutes.

Experiment	*[NO_3^--N] (mg N/L)*	*[COD] (mg/L)*	*COD/NO_3^--N*
1.1	75	10,000	133.3
1.2	100	10,000	100
1.3	125	1,600	12.8
1.4	150	1,600	10.7
1.5	50	1,600	32
1.6	50	1,600	32
1.7	25	1,600	64
1.8	100	1,600	16
1.9	75	800	10.7

In order to further investigate the process at lower nitrate concentrations, another seven sets of experiments with concentrations ranging between 35-75 mg N/L, were conducted. The logging time was shortened to 80 minutes since it was suspected that the nitrate would not be sufficient enough to maintain the reaction for 120 minutes. Different COD concentrations were added. Regarding experiment set 2.7, 0.25 mL methanol was added resulting in a COD concentration of 400 mg/L. The purpose was to see how the process behaved with a lower COD/NO_3^--N ratio. See Table 4-2 for the experiment conditions.

Table 4-2: Initial concentration measurements with a logging time of 80 minutes.

Experiment	*[NO_3^--N] (mg N/L)*	*[COD] (mg/L)*	*COD/NO_3^--N*
2.1	50	1,600	32
2.2	75	1,600	21.3
2.3	35	1,600	45.7
2.4	50	800	16
2.5	50	1,600	32
2.6	75	1,600	21.3
2.7	75	400	5.3

4.4.2 Reference tests

The reference tests were conducted in order to investigate how the process behaved in the absence of substrates. The first test was performed without any addition of methanol or nitrate, whatsoever. In the second trial, 125 mg N/L nitrate was added without any addition of carbon source. In the third trial, 1 mL of methanol was added without any addition of nitrate. All the other steps in the experiment was performed according to the original procedure with a 120-minute logging time. The carriers in these three experiments were also collected from Line 1, Zone 1. The experiment plan can be seen in Table 4-3.

Table 4-3: The conditions for the reference tests, logging time 120 minutes.

Experiment	*[NO_3^--N] (mg N/L)*	*[COD] (mg/L)*
3.1	-	-
3.2	125	-
3.3	-	1,600

4.4.3 Specific denitrifying activity tests on the different lines and zones

Experimental trials were conducted in order to study the denitrifying capacity of the different lines and zones of the post-denitrifying MBBR. The experiments were performed with an initial nitrate concentration of 75 mg N/L and an initial COD concentration of 400 mg/L. In order to achieve the desired COD concentration, 1 mL methanol was diluted with 3 mL distilled water. 1 mL diluted methanol was added to the reactor which resulted in a COD/NO_3^--N ratio of 5.3. The processes were logged for 80 minutes.

Another five trials were performed on Line 1, Zone 1 in order to see if the SDA was affected by rising outside temperature. As opposed to the initial concentration measurements that were performed in February/March, the new series of experiments were performed in the second half of April. The experiment conditions can be seen in Table 4-4.

Table 4-4: Experiments conducted on the different lines and zones of the full-scale post-denitrification facilities. The experiments were conducted with nitrate and COD concentrations of 75 mg N/L and 400 mg COD/L, respectively, and a logging time of 80 minutes. The "sets" column describes how many times each experiment was repeated.

Line:Zone	*Sets*	*[NO_3^--N] (mg N/L)*	*[COD] (mg/L)*	*COD/NO_3^-*
1:2	3	75	400	5.3
3:1	2	75	400	5.3
3:2	2	75	400	5.3
5:1	2	75	400	5.3
5:2	2	75	400	5.3
6:1	2	75	400	5.3
6:2	2	75	400	5.3
1:1	5	75	400	5.3

A time table for each conducted experiment can be found in Appendix I and a more explicit description of the manometric method can be seen in Appendix II.

4.4.4 Evaluation of the operational results

The operational results from Sjölunda WWTP are managed by a computer program called eWASTE. The program collects data points from sensor signals at the plant and from grab samples analyzed in the laboratory. The data was traced and assessed for the time period January 1 to April 27 2016. The Nitrate load and reduction together with the COD/N ratio were retrieved from eWASTE and with calculated Excel 2013, according to chapter 4.5.3.

4.5 Calculations

4.5.1 Diffusion limiting substrate

The diffusion limiting substrate was calculated according to Equation 11 together with the stoichiometric relationship in Equation 5. The molecular diffusion coefficient for nitrate and methanol in pure water is $1.6 \cdot 10^{-4}$ m^2/d and $0.8 \cdot 10^{-4}$ m^2/d, respectively (Henze *et al.*, 2002). According to the experiments conducted by la Cour Jansen & Harremoës (1984) the change of limiting substrate takes place at a bulk concentration ratio of 2 g methanol per g nitrate. The calculation can be seen in Equation 17, below.

$$\frac{C^*_{MeOH}}{C^*_{NO_3}} = \frac{D_{NO_3} \cdot k_{0fMeOH}}{D_{MeOH} \cdot k_{0fNO_3}} = \frac{1.6 \cdot 10^{-4}}{0.8 \cdot 10^{-4}} \cdot \frac{1.08}{1} = 2.16\, g\ MeOH/g\ NO_3^- - N \quad (17)$$

By using the relationships in Equations 18 and 19, the ratio can be converted to g COD/g N, which is the unit that is used at Sjölunda WWTP.

Volume for 2.16 g methanol:

$$\rho_{MeOH} = 792\ g/L\ \rightarrow V_{MeOH} = \frac{2.16\ g}{792\ g/L} = 0.00273\ L \quad (18)$$

The COD value for the methanol used was 1.2 kg/L. This was measured by the staff at the Sjölunda WWTP laboratory. The amount of COD for the calculated volume is then:

$$0.00273\ L \cdot 1200\ \ g/L = 3.3\ g \rightarrow 3.3\ g\ \ COD/g\ NO_3 - N \quad (19)$$

This implies that nitrate limits the reaction rate when the COD/N ratio is higher than 3.3 and COD limits the reaction rate when the ratio is lower than 3.3. It is worth noting that this is a rough estimation of the critical COD/N ratio. The diffusion coefficients correspond to pure water values and no diffusion through the biofilm nor the biofilm thickness have been taken into account. At Sjölunda WWTP the COD/N ratio is being held at a base value of 4.

4.5.2 The specific denitrifying activity

The specific denitrifying activity was calculated using the terminology in section 4.3 by a previously prepared calculation sheet in Excel 2013. The calculations for the experiments were conducted with accounts for the nitrogen gas in both the gas phase and the liquid phase of the reactor. In order to do so, the soluble nitrogen gas in the liquid phase was calculated using Henry's law in accordance with Equation 20.

$$C_{T,N_2} = K_{H,cp} \cdot p = 6.1 \cdot 10^{-4} \cdot \frac{1}{1013} \cdot e^{1300 \cdot \left(\frac{1}{T} - \frac{1}{298.5}\right)} \cdot p\ (mol/L) \quad (20)$$

Where $K_{H,cp}$ is Henrys constant (mol/(L·atm)), which is converted to mbar by division with 1013. T is the actual temperature in Kelvin and p is the actual pressure (mbar) in the reactor.

From Equation 20, the pressure of soluble nitrogen gas in the liquid phase could be calculated using Equation 21.

$$p_{sol} = C_{TN_2} \cdot V_L \cdot R \cdot \frac{T}{V_{HS}}\ (mbar) \quad (21)$$

Where V_L is the liquid phase volume (L), R is the ideal gas constant (0.08314 mbar·m^3/(k·mol), T is the temperature (K) and V_{HS} is the volume of the gas phase in the reactor (m^3).

The total pressure of produced nitrogen gas in the reactor was obtained by adding p_{sol} to every data point recorded by the pressure meter. The total production of nitrogen gas can then be calculated with Equation 22, assuming that ideal conditions occur.

$$N_2prod = \frac{p_{tot} \cdot V_{HS}}{R \cdot T} \cdot 1000\ (mmol) \quad (22)$$

Where p_{tot} is the total pressure of the nitrogen gas in the liquid phase and the gas phase in the reactor (mbar). A factor of 1000 is used in order to convert the expression from mol to mmol.

As mentioned earlier, the pressure meter recorded data once each minute. This means that the Nitrogen gas production could be assessed over time as an increasing curve with one data point each minute. The nitrogen gas production rate was then calculated though linear regression of a set of ten consecutive data points. By using the function SLOPE in Excel 2013, the nitrogen production rate was calculated for every 10-minute interval of the nitrogen gas production

curve. The new set of data points were plotted as a function of "end of interval" time. The maximum production rate was found when the nitrogen gas production rate curve reached a plateau. By using the function MAX in Excel 2013, the maximum production rate could easily be determined. The maximum production rate can be described according to Equation 23.

$$\frac{dN_2}{dt} = \alpha_{max}\ (mmol/min) \tag{23}$$

Where α_{max} is the maximum slope of the curve from linear regression from 10 consecutive data points.

The maximum specific denitrifying activity was then calculated using Equation 24.

$$SDA_{max} = \frac{\alpha_{max} \cdot 0.001 \cdot M_{N_2} \cdot 24 \cdot 60}{240 \cdot 0.00049}\ (g\ N_2/(m^2 \cdot d)) \tag{24}$$

Where M_{N2} is the molar weight of nitrogen gas (g/mol), 0.001 is the factor for converting mmol to mol and 24·60 is to convert minutes to days, 240 is the number of carriers in the reactor and 0.00049 is the effective area of each carrier (m^2).

4.5.3 Calculations of the operational results

The operational data was retrieved from eWASTE and transferred to Excel 2013 where the calculations were performed and the graphs were plotted.

The total effective biofilm area of the lines in the post-denitrifying MBBR

The total volume of the MBBRs is 6,234 m^3. With a 50% filling degree of carriers and an effective biofilm area of 500 m^2/m^3, the total effective area per line can be calculated according to Equations 25 and 26.

$$Total\ effective\ area = 6234 \cdot 0.5 \cdot 500 = 1{,}558{,}500\ m^2 \tag{25}$$

The effective area per line is then:

$$Effective\ area/line = \frac{1{,}558{,}500\ m^2}{6} = 259{,}750\ m^2 \tag{26}$$

Nitrate load and reduction

The nitrate load for the whole post-denitrifying MBBR and for each line was calculated according to Equations 27 and 28, while the reductions were calculated according to Equations 29 and 30.

$$Nitrate\ load = \frac{NO_{3,in}^{-} \cdot F \cdot 3{,}600 \cdot 24 \cdot 0.001}{1{,}558{,}500}\ (g\ N/(m^2 \cdot d)) \tag{27}$$

Where $NO^{-}_{3,in}$ is the incoming concentration of nitrate in the wastewater (mg/L), F is the flow rate to the post-denitrifying MBBR (L/s), 0.001 is the factor for converting mg to g and 3,600·24 is the factor for converting seconds to days.

$$Nitrate\ load/line = \frac{NO_{3,in}^{-} \cdot F_L \cdot 3{,}600 \cdot 24 \cdot 0.001}{259{,}750}\ (g\ N/(m^2 \cdot d)) \tag{28}$$

Where F_L is the specific flow rate to the certain line in question (L/s)

$$Nitrate\ reduction = \frac{(NO_{3,in}^{-} - NO_{3,out}^{-}) \cdot F \cdot 3{,}600 \cdot 24 \cdot 0.001}{1{,}558{,}500}\ (g\ N/(m^2 \cdot d)) \quad (29)$$

Where $NO_3^-{}_{,out}$ is the outgoing nitrate concentration in the wastewater from the post-denitrifying MBBR.

$$Nitrate\ reduction/line = \frac{(NO_{3,in}^{-} - NO_{3,out,L}^{-}) \cdot F_L \cdot 3{,}600 \cdot 24 \cdot 0.001}{259{,}750}\ (g\ N/(m^2 \cdot d)) \quad (30)$$

Where $NO_3^-{}_{,out,L}$ is the outgoing nitrate concentration in the wastewater from each line.

COD load and COD/N ratio

The COD load and the COD/N ratio were calculated in accordance with Equations 31 and 32.

$$COD\ load/line = \frac{F_{ML} \cdot 1{,}200 \cdot 24}{259{,}750}\ (g\ COD/(m^2 \cdot d)) \quad (31)$$

Where F_{ML} is the methanol flow for the line in question (L/h), 1,200 is the COD concentration in methanol (g/L) and 24 is the factor for converting hour to day.

$$COD/N\ ratio = \frac{COD\ load/line}{Nitrate\ load/line}\ (g\ COD/g\ N) \quad (32)$$

5 Results and discussion

5.1 Initial concentration measurements

The initial concentration measurements were conducted with the purpose to find a suitable concentration, generating the highest denitrifying activity possible, to use in further experiments on the post-denitrification plant. Seven different concentrations within the range of 25-150 mg N/L were investigated. The aim was to find the transition state where the process alters from obeying half order reaction kinetics to zero order reaction kinetics. A high concentration of COD was used in order to be certain that the carbon source would not be a limiting factor in the experiments. If nothing else is stated, 1 mL methanol, corresponding to a COD concentration of 1,600 mg/L, was added to the reactor.

5.1.1 120-minute tests

The first sets of experiments were performed with a logging time of 120 minutes in accordance with the original procedure. As seen in Figure 5-1, the highest amount of produced nitrogen gas was found at an initial nitrate concentration of 100 mg N/L and a COD concentration of 1,600 mg/L. At this substrate concentration, a nitrogen gas production of 1.32 mmol was achieved. When the COD concentration was higher (10,000 mg/L) at experiment set 1.2, a nitrogen gas production of 1.22 mmol was achieved. Initial nitrate concentrations of 125 and 150 mg N/L resulted in 1.15 and 1.19 mmol produced nitrogen gas, respectively.

At a low initial nitrate concentration of 25 mg N/L, the production of nitrogen gas reached a plateau half way through the experiment. This was probably due to the fact that the reactor ran out of nitrate and thus slowed the reaction rate down. The experiment done with 25 mg N/L ended up with 0.50 mmol produced nitrogen gas. For experiment sets 1.1 and 1.6 (75 and 50 mg N/L), 1.10 and 1.13 mmol nitrogen gas was produced, respectively. The results can be seen in Table 5-1.

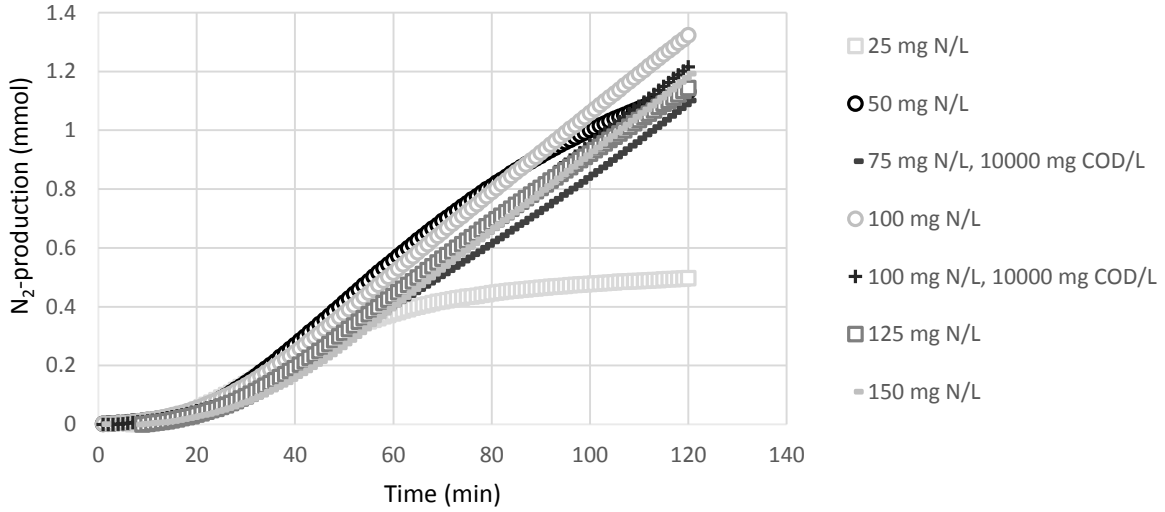

Figure 5-1: The nitrogen gas production for the initial concentration experiments. The highest production was found at a nitrate concentration of 100 mg N/L, while the lowest production was found at 25 mg N/L. If nothing else is stated, the COD concentration was 1,600 mg/L.

The nitrogen gas production rates can be seen in Figure 5-2 and the conversion to the maximum specific denitrifying activity can be seen in Table 5-1. For initial nitrate concentrations below 50 mg N/L, the substrate seemed to be fully consumed before the experiments were finished. This can be seen as declining production rate curves. For the measurement done with 25 mg N/L, the specific denitrifying activity reached 3.38 g N/(m^2·d) before the curve declined after 40 minutes. At a nitrate concentration of 50 mg N/L, the specific denitrifying activity reached 5.03 g N/(m^2·d), which also turned out to be the highest SDA of all initial concentration experiments. However, the production rate curve declined after 60 minutes for said experiment.

For initial nitrate concentrations of 100, 125 and 150 mg N/L, a plateau was reached after 50-60 minutes, indicating that the reaction worked at full speed with complete penetration of the biofilm. For experiment sets 1.2 and 1.8 (100 mg N/L with different COD concentrations) SDAs of 4.78 and 4.65 g N/(m^2·d) were obtained, respectively. Initial nitrate concentration measurements performed with 125 and 150 mg N/L resulted in SDAs of 4.35 and 4.67 g N/(m^2·d), respectively. Experiment set 1.1 (75 mg N/L) had a slow increase in nitrogen production rate and ended up with a specific denitrifying capacity of 4.41 g N/(m^2·d).

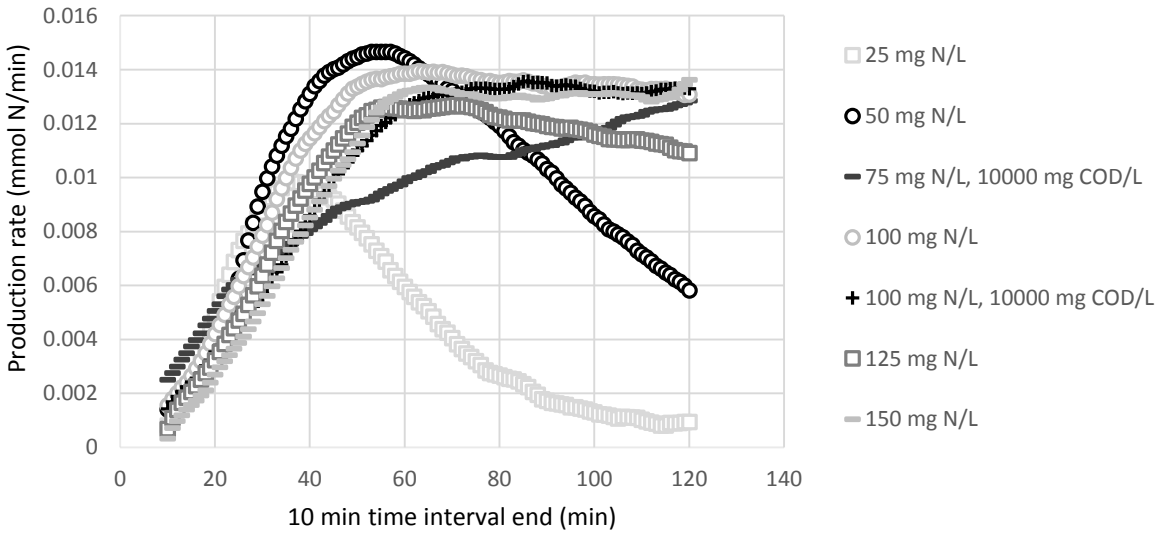

Figure 5-2: The nitrogen gas production rate vs. time for the initial concentration experiments. The highest production rate was found at initial nitrate concentration of 50 N/L, while the lowest production rate was found at 25 mg N/L. For initial nitrate concentrations exceeding 100 mg N/L, a plateau was reached after 50-60 minutes. If nothing else is stated, the COD concentration was 1,600 mg/L

The COD concentration did not seem to affect the specific denitrifying activities. However, it seems that a lower COD concentration allows the process to reach the maximum reaction speed faster. One possible explanation is that a higher COD concentration obstructs the diffusion of nitrate through the biofilm.

As shown in the graphs, there was no guarantee that the highest nitrate concentration would yield the highest SDA or nitrogen gas production. All concentrations except for 25 mg N/L (set 1.7) seemed to reach full reaction speed. The mean SDA for all experiments except for 1.7 was 4.67 g N/(m^2·d) with a variation of only 5%*. The variation was most likely due to environmental factors such as temperature, biofilm structure etc., rather than substrate concentration.

Table 5-1: A conversion chart showing the maximum specific denitrifying activity and the maximum production rate for the 120 minutes experiments

Experiment	*[NO_3^--N] (mg/L)*	*[COD] (mg/L)*	*N_2-production (mmol)*	*Max. production rate (mmol N/min)*	*SDA (g N/(m^2·d))*
1.1	75	10,000	1.10	0.0128	4.41
1.2	100	10,000	1.22	0.0136	4.65
1.3	125	1,600	1.15	0.0127	4.35
1.4	150	1,600	1.19	0.0136	4.67
1.6	50	1,600	1.13	0.0147	5.03
1.7	25	1,600	0.50	0.0098	3.38
1.8	100	1,600	1.32	0.0139	4.78

See chapter 5.1.3 for results from experiment sets 1.5 and 1.9

* The term "variation" simply refers to the ratio standard deviation to mean value.

5.1.2 80-minute tests

The 80-minute experiments sets 2.1-2.7 were conducted in order to further investigate how the process behaved at lower initial nitrate concentrations. Earlier experiences from experiment set 1.1-1.9 revealed that the initial nitrate concentrations had to exceed 50 mg N/L in order to supply the bacteria with enough nitrate to be able to maintain the reaction for 120 minutes. Since it was expected that the nitrate would not last at lower initial concentrations, the experiment time was shortened to 80 minutes.

As seen in figure 5-3, the highest production of nitrogen gas (0.85 mmol) was achieved with an initial nitrate concentration of 75 mg N/L and a COD concentration of 400 mg/L. The experiment conducted with 75 mg N/L and a higher COD concentration of 1,600 mg/L, resulted in 0.81 mmol produced nitrogen gas. Experiment sets 2.4 and 2.5 were both conducted with a nitrate concentration of 50 mg N/L and COD concentrations of 800 and 1600 mg/L, respectively. Experiment set 2.4 resulted in a nitrogen gas production of 0.80 mmol, while experiment set 2.5 yielded a production of 0.73 mmol nitrogen gas. Regarding set 2.3, with an initial nitrate concentration of 35 mg N/L, a production of 0.70 mmol nitrogen gas was achieved. The results can be seen in Table 5-2.

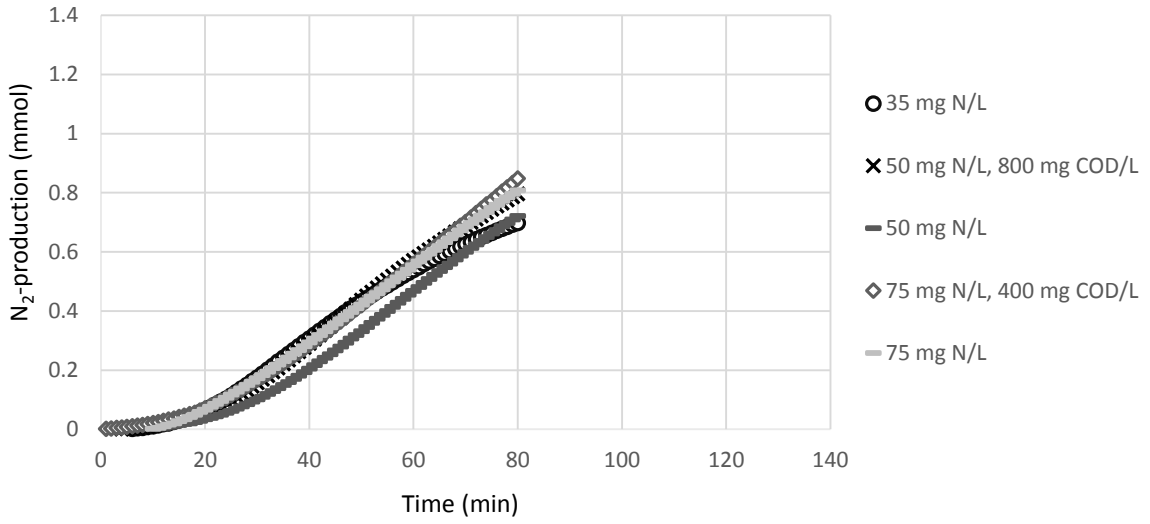

Figure 5-3: The nitrogen gas production for the experiments conducted with a logging time of 80 minutes. The highest production was found at an initial nitrate concentration of 75 mg N/L and a COD concentration of 400 mg/L. If nothing else is stated, the COD concentration was 1,600 mg/L.

The production rates for the 80-minute tests are shown in Figure 5-4 and the conversions to maximum specific denitrifying capacity are shown in Table 5-2. The highest specific denitrifying activity of 5.01 g N/(m^2·d) was found at set 2.7 when the initial nitrate concentration was 75 mg N/L and the COD concentration was 400 mg/L. The other experiment done with an initial nitrate concentration of 75 mg N/L and 800 mg COD/L resulted in a SDA of 4.58 g N/(m^2·d). For the two 50 mg N/L experiments (sets 2.4 and 2.5) the SDA was higher at lower COD concentration. The results for sets 2.4 and 2.5 were 5.01 and 4.67 g N/(m^2·d), respectively. The lowest specific denitrifying activity of 4.57 g N/(m^2·d) was obtained when the initial nitrate concentration was 35 mg N/L.

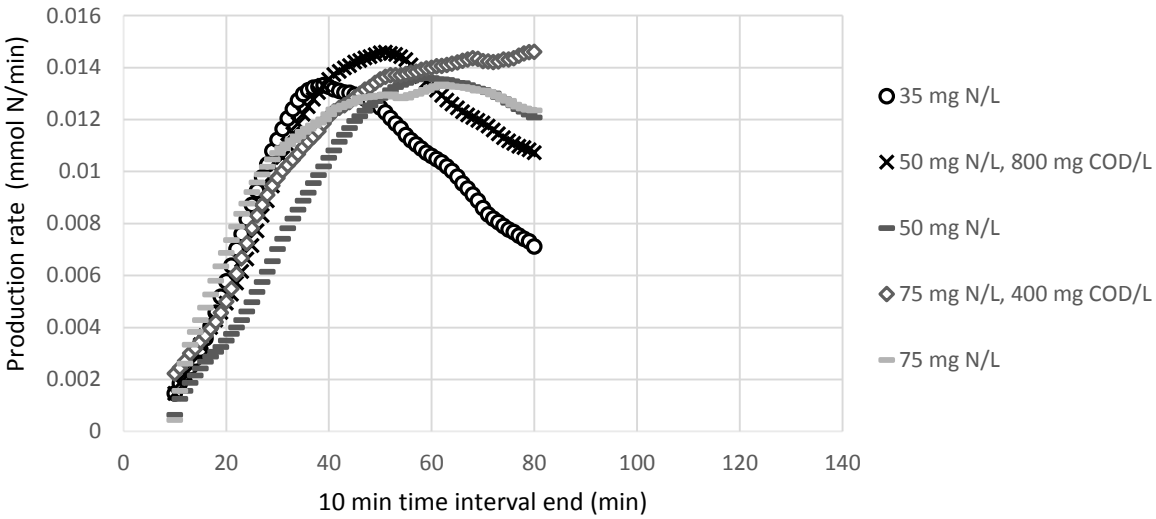

Figure 5-4: The nitrogen gas production rate vs. time for the initial concentration experiments of 80 minutes. The highest production rate was found at an initial nitrate concentration of 75

mg N/L, while the lowest production rate was found at an initial nitrate concentration of 35 mg N/L. If nothing else is stated, the COD concentration was 1,600 mg/L.

It can be seen in the production rate graph that the maximum reaction rate was obtained faster with lower COD concentration for the experiments performed with an initial nitrate concentration of 50 mg N/L. However, this trend was not valid for the experiments conducted with an initial nitrate concentration of 75 mg N/L. The production rate seemed to accelerate faster with a COD concentration of 1600 mg/L, but started to decline shortly thereafter. The SDA variation for the experiments was still low. The mean SDA value for all 80-minute tests were 4.77 g N/(m^2·d) with a variation of only 4%.

Table 5-2: A conversion chart showing the maximum specific denitrifying activity and the maximum production rate for the 80-minute experiments

Experiment	*[NO_3^--N] (mg/L)*	*[COD] (mg/L)*	*N_2-production (mmol)*	*Max. production rate (mmol N/min)*	*SDA (g N/(m^2·d))*
2.3	35	1,600	0.70	0.0133	4.57
2.4	50	800	0.80	0.0146	5.01
2.5	50	1,600	0.73	0.0136	4.67
2.6	75	800	0.81	0.0133	4.58
2.7	75	400	0.85	0.0146	5.01

See chapter 5.1.3 for results from experiment set 2.1 and 2.2.

5.1.3 Measurement noise

On several occasions, the experiments resulted in discontinuous nitrogen gas production graphs due to measurement noise. These experiments were sets 1.5, 1.9, 2.1 and 2.2. The discontinuous graphs contributed to a higher apparent derivative in the intervals were the leaps occurred. This in turn, resulted in high and deceptive values for the specific denitrifying activity on the carriers. In order to rectify such faulty graphs, the average modulation of the nitrogen gas production graphs was adjusted to cover a larger interval for derivative calculations in order to cancel out the measurement noise. Figure 5-5 depicts the discontinuous graphs for nitrogen gas production for initial nitrate concentrations of 50 mg N/L and 75 mg N/L. In all four cases, the leaps occurred within 60 minutes after logging start. Problems with measurement noise occurred during both 80-minute experiments (2.1 and 2.2) and 120-minute experiments (1.5 and 1.9).

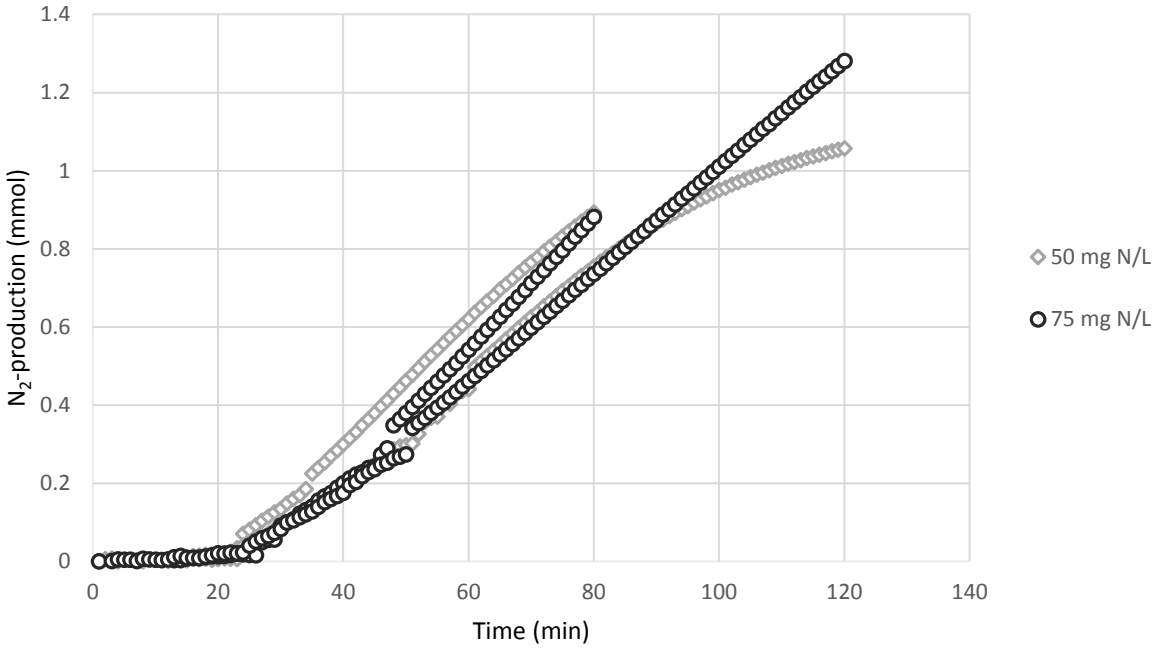

Figure 5-5: Nitrogen gas production graphs from experiments affected by measuring noise. The 50 mg N/L experiments can be seen as light grey lines where the 120-minute experiment corresponds to set 1.5 and the 80-minute experiment corresponds to set 2.1. The 75 mg N/L experiments can be seen as dark grey lines where the 120-minute experiment corresponds to set 1.9 and the 80-minute experiments corresponds to set 2.2.

The default settings of the Excel 2013 calculation sheet enabled derivative estimations of the nitrogen gas production graphs in time intervals of 10 minutes. As seen in Figures 5-6 and 5-7 below, the derivative becomes deceptively high in the discontinuous intervals resulting in significant peaks in the production rate charts. The average modulation was adjusted to cover a 30-minute time interval in all cases resulting in new graphs shown as lines. By calculating the derivatives in larger time intervals, the peaks were evened out, which resulted in more accurate values for the specific denitrifying activities.

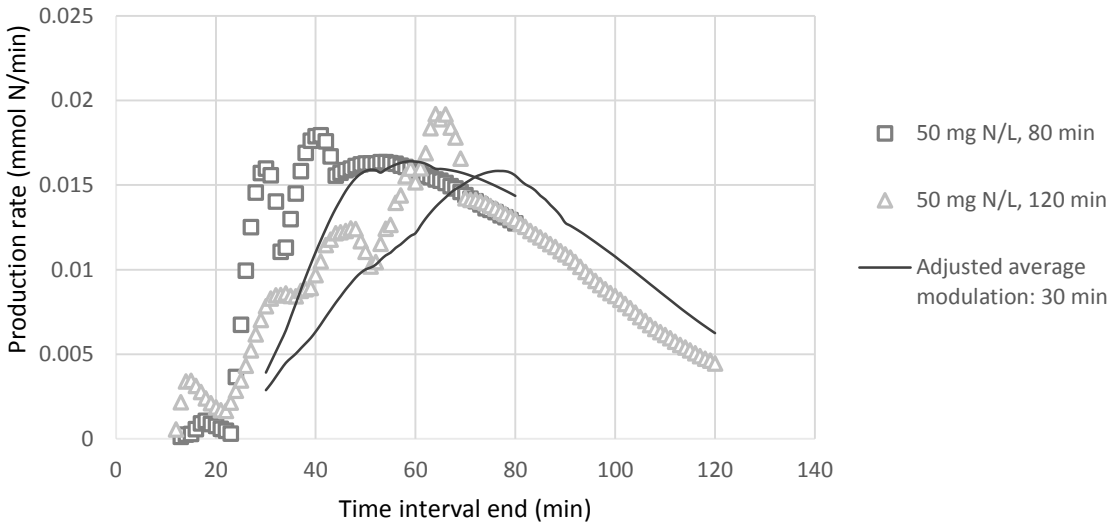

Figure 5-6: The nitrogen production rate curves for experiment set 1.5 and 2.1, both conducted with an initial nitrate concentration of 50 mg N/L. Experiment set 1.5 had a 120-minute logging time while set 2.1 had 80-minute logging time. The result from the adjusted average modulation can be seen as dark grey lines.

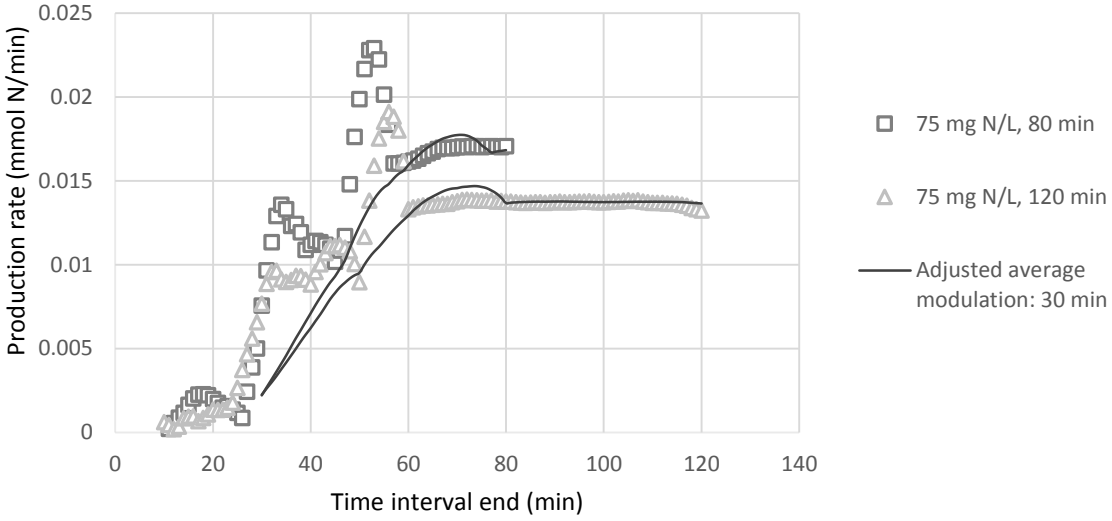

Figure 5-7: The nitrogen production rate curves for experiment set 1.9 and 2.2, both conducted with an initial nitrate concentration of 75 mg N/L. Experiment set 1.9 had a 120-minute logging time while set 2.2 had 80-minute logging time. The result from the adjusted average modulation can be seen as dark grey lines.

Table 5-3 shows how the value for the specific denitrifying activity changes with altered intervals for derivative estimations. The original values can be seen in column 3, while the values after adjusted average modulation can be seen in column 4.

Table 5-3: The specific denitrifying activity before and after adjusted average modulation for experiments 1.5, 1.9, 2.1 and 2.2. Linear regression was performed in a time interval of 30 minutes.

Experiment	*[NO_3^--N] (mg/L)*	*[COD] (mg/L)*	*SDA (g N/(m^2·d))*	*New SDA* (g N/(m^2·d))*
1.5	50	1,600	6.58	5.44
1.9	75	800	6.55	5.04
2.1	50	1,600	6.15	5.63
2.2	75	1,600	7.86	6.09

* After adjusted average modulation

As seen in Table 5-3 and Figures 5-6 and 5-7, the specific denitrifying activity became significantly lower when the average modulation was adjusted. Regarding experiment set 1.5 the SDA dropped 1.14 units, while experiment set 1.9 showed a SDA decrease of 1.51 units after adjusted average modulation. The SDAs of experiment set 2.1 and 2.2 decreased with 0.52 and 1.77 units respectively.

It was suspected that the reactor was leaking gas since the rubber septum was old and worn out, which might have contributed to the measurement noise. The rubber septum was exchanged to a new one on March 8 in order to achieve a more gas-tight reactor.

5.1.4 The effect of COD to nitrate ratio

Figure 5-8 depicts the SDAs of the initial concentration measurements test versus the COD/N ratio. As seen in the figure, it seems that the COD/N ratio did not have much effect on the denitrifying activity. The two experiments conducted with an initial nitrate concentration of 100 mg N/L resulted in a SDAs that only differed 0.13 units, while the COD/N ratio differed by a factor of 6 between the two. The same pattern could be observed for the experiments conducted with an initial nitrate concentration of 75 mg N/L. The COD/N ratio differed by a factor of 25 between the two experiments. However, the tests resulted in SDAs that only varied with 0.6 units. It is worth noting that the COD/ratios for some of the initial concentration measurements were very high, due to miscalculations. The highest ratio tested was 133.3 g COD/g N, while the lowest ratio was 5.3.

The majority of the experiments resulted in specific denitrifying activities between 4-5 g N/(m^2·d) regardless of the COD/N ratio. The exception is the experiment performed with an initial nitrate concentration of 25 mg N/L, which only reached a SDA of 3.38 g N/(m^2·d). The reason for this might be due to the fact that the nitrate concentration was too low for the reaction to reach zero order kinetics.

The data points within the grey square corresponds to values that required adjusted average modulation.

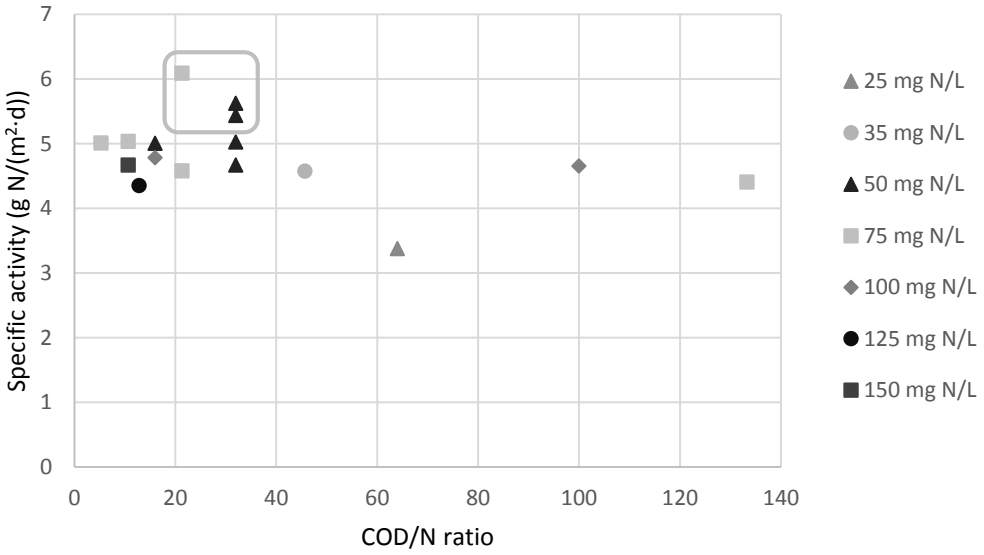

Figure 5-8: The specific denitrifying activity vs. the COD/N ratio. The COD/N ratio did not seem to have much effect on the specific denitrifying activity. The marks within the square corresponds to values that were still somewhat high after being adjusted with a different average modulation.

According to Akunna *et al*. (1992), methanization is possible for COD/N ratios above 8.86. Nevertheless, it has been known that methanization is only possible after the denitrification is finished (Akunna *et al*. 1992; Ruiz *et al.* 2006). With this in mind, it has been ruled out that methanization would have been an issue during the experiments, with an exception for set 1.7 (25 mg N/L). As seen in Figure 5-2, the production rate was almost down to zero at the end of the experiment, which implies that the nitrate was scarce and the onset of methanization could have been possible. Regarding the other experiments, it seems that total depletion of nitrate was not an issue within the duration of 80 or 120 minutes.

5.1.5 The specific denitrifying activity vs. initial concentration

In order to choose a suitable initial nitrate concentration for further trials on the full-scale post-denitrification plant, the specific denitrifying activity was plotted against the initial concentration for each conducted experiment. Figure 5-9 shows how the specific denitrifying activity varies with initial nitrate concentration. The idea was to use a concentration that lies within the range where the specific denitrifying activity becomes independent of the initial nitrate concentration and full penetration of the biofilm is enabled. For the carriers used in these experiments (Line 1, Zone 1), a fully penetrated biofilm was obtained with initial nitrate concentrations above 35 mg N/L. This implies that the reaction followed zero order reaction kinetics for initial nitrate concentrations above 35 mg N/L, while the reaction followed half order reaction kinetics for concentrations below 35 mg N/L. According to la Cour Jansen & Harremoës (1984), the transition state where the reaction switches from half order to zero order, occurs at initial nitrate concentrations of around 30 mg N/L. The measuring points, whose average modulation were adjusted, are described with black triangles. Those measuring points were not taken into account when choosing a suitable initial nitrate concentration.

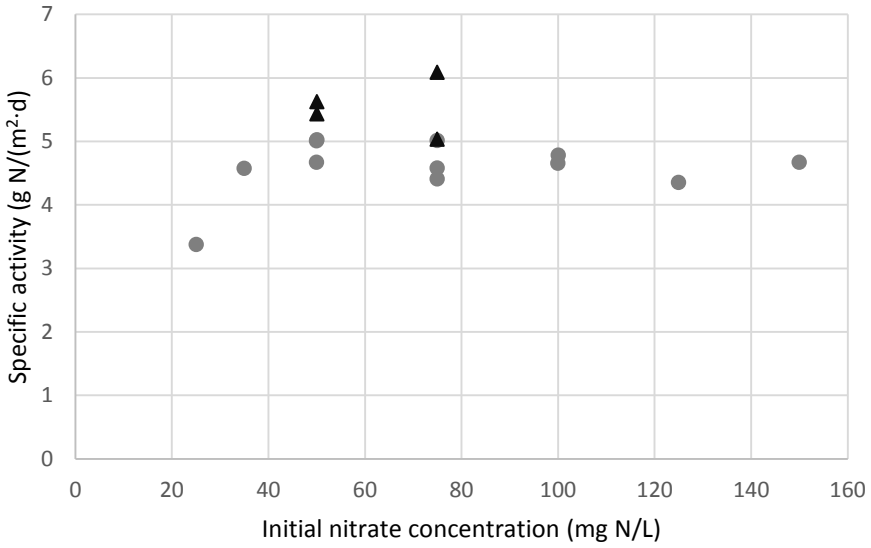

Figure 5-9: The specific denitrifying activity vs. initial nitrate concentration. The plateau was reached at initial nitrate concentrations above 35 mg N/L, which implied the transition from half order reaction kinetics to zero order reaction kinetics. The modified values for the specific denitrifying activity for experiments 1.5, 1.9, 2.1, and 2.2 can be seen as black triangles.

When it came to choosing an initial nitrate concentration to use in further experiments, it was important that full penetration of the biofilm was enabled for optimal denitrifying activity. In this case, it was possible to choose from initial nitrate concentrations within the range of 35-150 mg N/L. The initial nitrate concentrations of 35 and 50 mg N/L were considered to be unsuitable to be used in further trials, since the experiments resulted in declining production rate curves within 60 minutes after logging start. From the results of the initial nitrate concentration measurements it was concluded that an initial nitrate concentration of 75 mg N/L was suitable to use in further experiments. By choosing a concentration that was at the lower end of the range, it was possible to avoid unnecessary wasting of the nitrate solution.

As mentioned earlier the COD/N ratio should exceed the critical value of approximately 3.3 g COD/g NO_3^--N. By adding 1 mL of a 1:3 methanol-water solution to the reactor, resulting in a COD concentration of 400 mg/L, a COD/N ratio of 5.3 was achieved.

When Stefansdottir (2014) developed the method for measuring anammox activity, the activity was independent of initial nitrite concentrations between 75-125 mg N/L. The chosen initial nitrite concentration that was used in further experiments was 125 mg N/L. It seems like the denitrifying activity becomes concentration independent at lower initial nitrogen concentrations compared to that of the anammox activity. This can be explained by the fact that nitrite has a diffusion coefficient of $0.9 \cdot 10^{-4}$ m^2/d in pure water, which is much lower than that of nitrate, which is $1.6 \cdot 10^{-4}$ m^2/d (Henze *et al*., 2002). Nitrate can in fact diffuse more efficiently through the biofilm than nitrite and thus obtain zero reaction kinetics at lower concentrations.

Further information and values from the initial concentration experiments can be found in Appendix III.

5.2 Reference tests

The reference tests were performed in order to further investigate the behavior of the process during substrate limitations and carbon deficiency. A total of three tests were conducted; one with nitrate addition, one with methanol addition and one with no additions whatsoever. Note that the reference tests were performed before the initial concentration measurements were finished, which is why the nitrate addition experiment was conducted with a nitrate concentration of 125 mg N/L instead of 75 mg N/L. As seen in Figure 5-10, the highest nitrogen gas production was found when only nitrate was added to the reactor. The addition of nitrate only, contributed to a total nitrogen gas production of 0.25 mmol. This is however an almost five-fold decrease from experiment set 1.3, which was conducted with a nitrate concentration of 125 mg N/L together with a COD concentration of 1,600 mg/L. The two other experiments, one performed with the addition of methanol corresponding to 1,600 mg COD/L and the other performed without any additions at all, both showed negligible production of nitrogen gas. In the case were methanol was added, a nitrogen gas production of 0.04 mmol was achieved. Furthermore, 0.02 mmol nitrogen gas was obtained during the zero addition test.

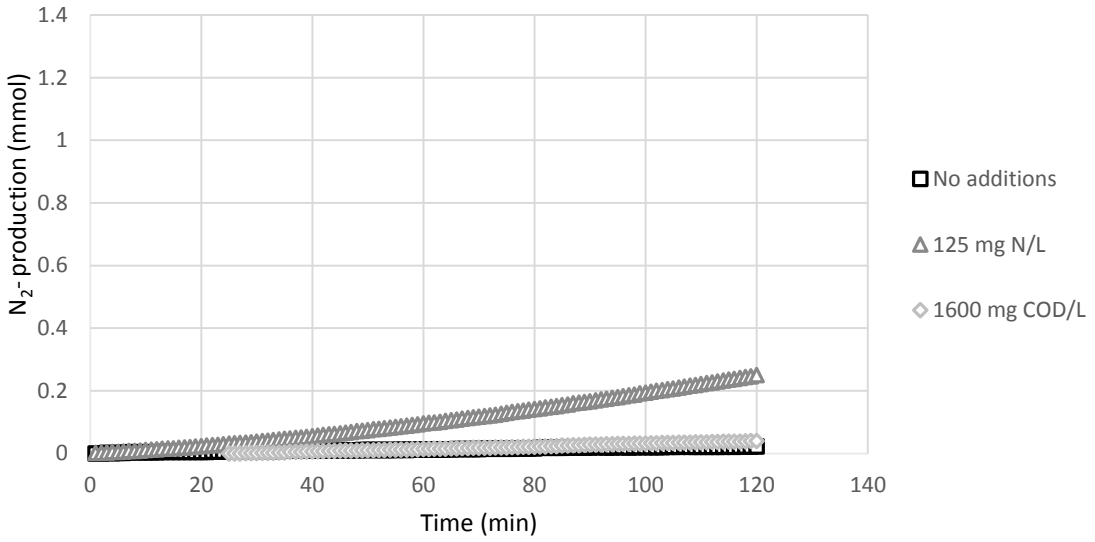

Figure 5-10: The nitrogen gas production from the reference tests. The highest nitrogen gas production was found at the experiment conducted with 125 mg N/L. The two experiments performed with methanol and no additions at all, showed a negligible nitrogen gas production.

The production rate curves from the reference test followed, as expected, the same trend as the gas production curves. This can be seen in Figure 5-11. The highest production rate of 0.0029 mmol/min, corresponding to a SDA of 0.98 g N/(m^2·d), was achieved by the test performed with nitrate addition. The zero addition test and the methanol addition test obtained SDAs of 0.13 and 0.21 g N/(m^2·d), respectively.

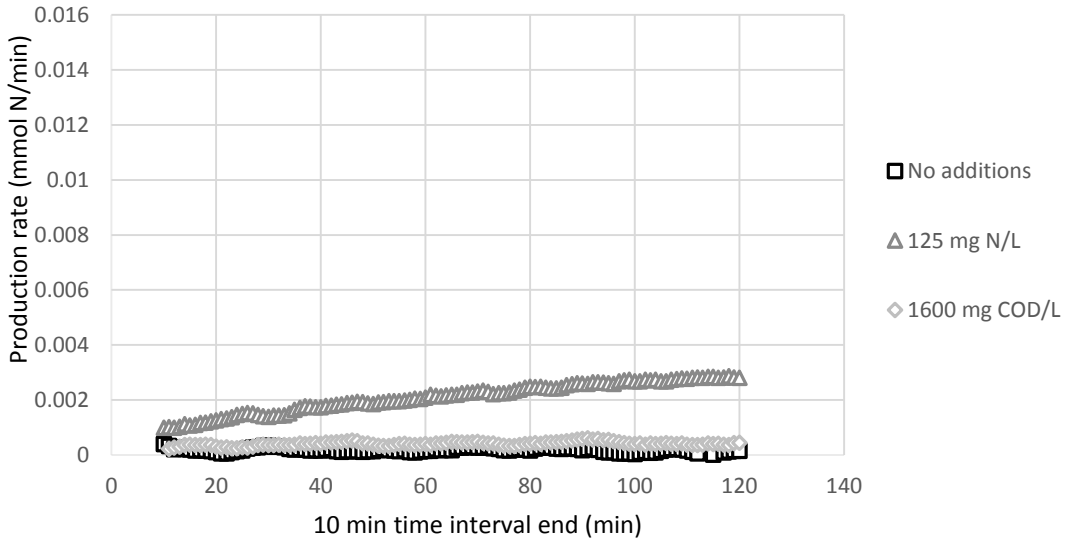

Figure 5-11: The nitrogen gas production rate vs. time for the reference tests. The highest production rate was achieved when nitrate was added.

From the reference tests, it could be concluded that it is possible to detect a small denitrification activity when the denitrifying bacteria have access to nitrate. This can be explained by the fact that the bacteria have some stored carbon that can be used through endogenous respiration during carbon deficiency in the wastewater. It is known that denitrification is possible without external carbon sources when integrated into an enhanced biological phosphorous removal process. Coats *et al*. (2011) concluded that the denitrification process was mainly driven by glycogen, which was stored intracellularly from organic acids in the wastewater. Even though enhanced phosphate removal is unrelated to the post-denitrification MBBR at Sjölunda WWTP, glycogen storage could be possible given that the cells have access to organic acids.

Regarding the other two experiments, the minimal detection of denitrification activity was most likely due to the fact that there had been some nutrient residues left on the carriers when the experiments started. The reason as to why the carriers should be rinsed with tap water before starting the experiments is to eliminate as much residue as possible without risking any loss of biofilm.

It should be mentioned that it is uncertain if the small detection of activity for the COD experiment and the zero addition test was due to denitrification or other bacterial activities. The pressure meter used was not selective for nitrogen gas, which means that the pressure increment from the COD experiment could also be due to a production of methane gas.

Additional results from the reference tests can be seen in Appendix IV.

5.3 Specific denitrifying activity of the different lines and zones

The specific denitrifying activity was measured on the different lines and zones of the post-denitrification plant in order to see how the activity and the denitrifying capacity on the carriers varied between the different lines. Experiments were performed on four of the lines in the post-denitrification plant, including tests on both zones. The first tests were performed on Line 1,

Zone 2. After that followed experiments on Lines 3:1, 3:2, 5:1, 5:2, 6:1 and 6:2. After the experiments had been performed on Line 6:2, tests were conducted again on Line 1:1 in order to detect any differences from the initial concentration measurements.

If nothing else is stated, the experiments were conducted with an initial nitrate concentration of 75 mg N/L and a COD concentration of 400 mg/L. The logging time for the experiments was 80 minutes.

5.3.1 Experiments conducted on first-zone carriers from the different lines

The results from Line 1 are presented after Lines 3-6

Lines 3-6

The Nitrogen gas production curves of line 3, 5 and 6 can be seen in Figure 5-12. Line 5:1 turned out to have the highest nitrogen gas production of 1.34 and 1.25 mmol for test 1 and test 2, respectively. Test 1 of Line 3:1 resulted in a production of 1.20 mmol nitrogen gas, while the result from test 2 was 1.10 mmol of produced gas. The second test of Line 3:1 resulted in a discontinuous graph, which had to be adjusted for further assessments (see section 5.1.3). Line 6:1 showed tendencies to have a lower nitrogen gas production compared to the other lines tested. The production of gas was 0.98 mmol for both tests conducted.

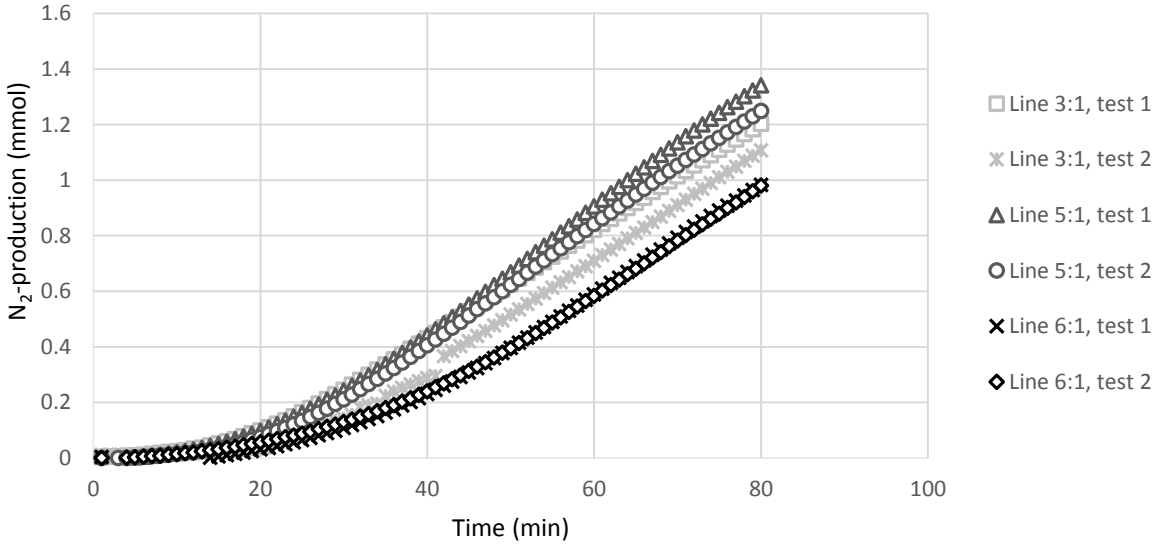

Figure 5-12: The nitrogen gas production from the tests on the first zones of different lines. Line 5:1 obtained the highest nitrogen gas production, while Line 6:1 obtained the lowest production. Two tests were conducted on each line.

The results of the production rates can be seen in Figure 5-13 and the conversions to maximum specific denitrifying capacity is shown in Table 5-4. Line 5:1 seemed to have the highest SDA of the different lines tested with 8.13 and 7.58 g N/(m^2·d) for tests 1 and 2, respectively. Line 3:1 had SDAs of 6.76 and 6.91 g N/(m^2·d), while Line 6:1 achieved SDAs of 6.86 and 7.01 g N/(m^2·d). The second test of Line 3:1 resulted in a discontinuous nitrogen gas production graph, which was why the production rate curve was calculated from time intervals of 30 minutes.

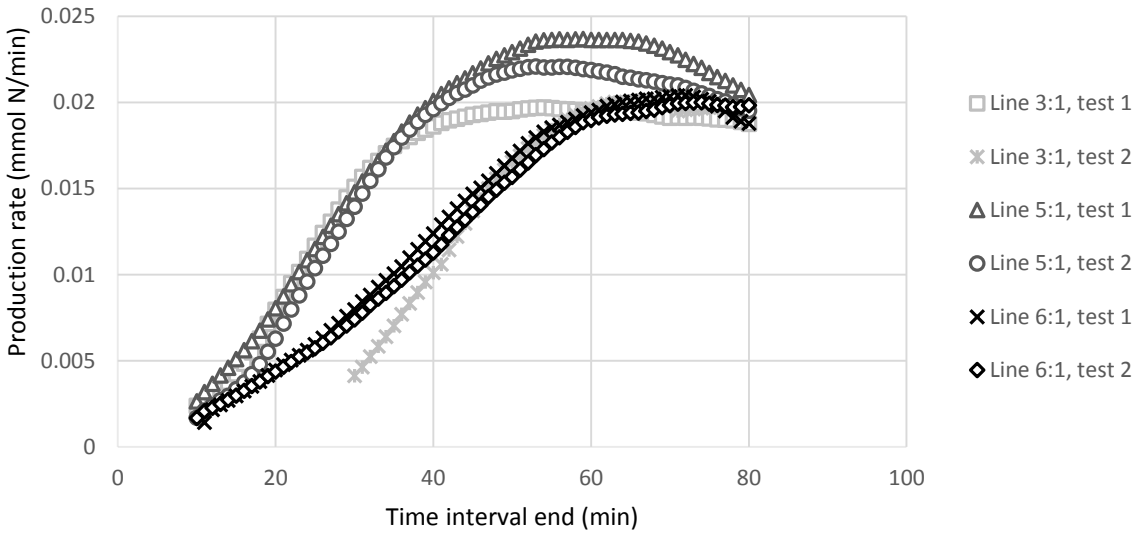

Figure 5-13: Nitrogen gas production rate curves of the first zone of the different lines. The highest production rate was found in Line 5, while the lowest production rate was found in Line 6. Test 2 of Line 3:1 was adjusted with a 30-minute interval for derivative calculations.

Line 6:1 had higher SDAs than that of Line 3:1 even though it turned out to have lower nitrogen gas productions. This reveals that the conversion rate from nitrate to nitrogen gas is crucial for the denitrifying activity, rather than nitrogen gas production. However, it is worth to mention that test 1 of Line 3:1 reached maximum production rate faster than that of Line 6:1. Both Line 3:1 and Line 5:1 showed faster accelerating nitrogen production rates than that of Line 6:1, which can be seen from the curves. This faster acceleration might be due to a more efficient diffusion of nitrate and methanol through the biofilm. The reason for this might be that the carriers in Line 6:1 are used to lower loads than that of the carriers from the other lines, since the flow rate is lower.

The mean values of the maximum specific denitrifying activities for the different lines can be seen in Table 5-4 below. As expected, the highest mean SDA was found at Line 5:1. Line 3:1 and 6:1 had mean SDAs with a difference of only 0.10 units between the two. What also can be mentioned is that the standard deviation of Line 5:1 is higher than that of the other lines. However, the variation was still only 3%. Both Line 3:1 and 6:1 had data points close to the mean with only 1% variation. The variation between the lines can depend on different things. For instance, denitrification is performed by living organisms that are affected by factors in the surroundings, so daily variations are not unusual. These factors might be, temperature, nutrient availability, and so on.

Table 5-4: A conversion chart showing the maximum production rate of nitrogen gas and the maximum specific denitrifying activity of the first zones of the different lines in the post denitrification plant.

Line:Zone	*Max. production rate (mmol N/min)*	*N_2-production (mmol)*	*SDA (g N/(m^2·d))*	*Mean SDA ± standard deviation*
3:1	0.0197	1.20	6.76	6.84±0.08
	0.0201*	1.10	6.91*	
5:1	0.0237	1.34	8.13	7.86±0.27
	0.0221	1.25	7.58	
6:1	0.0200	0.98	6.86	6.94±0.08
	0.0204	0.98	7.01	

*Value after adjusted average modulation

Line 1

A series of tests were performed on Line 1:1 after the experiments on line 3, 5, and 6 had been conducted. The purpose was to follow the specific denitrifying activity over time and to see if there would be any alterations in SDA during rising outside temperature. In this section, the temperatures mentioned corresponds to the diurnal wastewater temperature on the days when the tests were conducted. The first experiment was conducted on April 13, while the last test was performed on April 27. A total set of five tests were conducted within said period. The results from the nitrogen gas production is depicted in Figure 5-14. The first test had the highest nitrogen gas production of 1.29 mmol, while test 5 had the lowest production of 0.77 mmol. The wastewater temperatures when tests 1 and 5 were conducted were 15.6 and 11.8°C, respectively. Tests 2, 3 and 4 achieved nitrogen gas productions of 0.95, 1.00 and 1.05 mmol, respectively. The wastewater temperature when those tests were conducted were 15.2°C for test 2 and 15.8°C for tests 3 and 4. It can be seen in the figure that the slope of the curves significantly increases after 30-40 minutes, which indicate higher production rates.

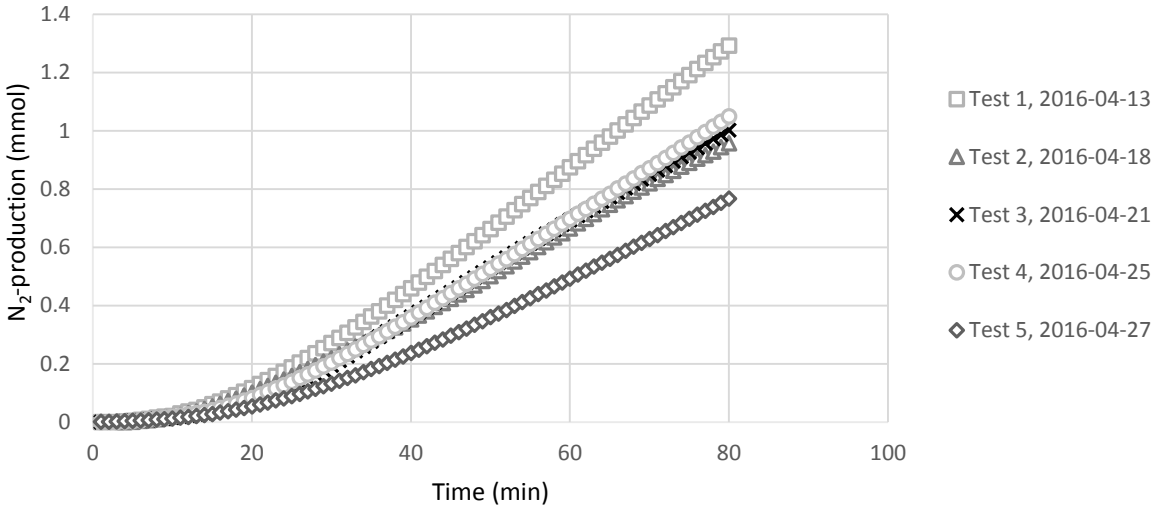

Figure 5-14: The nitrogen gas production curves from the series of tests conducted on Line 1, Zone 1. The highest production was achieved by test 1, while the lowest production was found at test 5.

The nitrogen gas production rate curves are shown in Figure 5-15 and the conversion to specific denitrifying activity is demonstrated in Table 5-5. In accordance with the nitrogen gas production curves, the highest and the lowest maximum production rates were found at test 1 and test 5, respectively. Test 1 had a SDA of 7.29 g N/(m^2·d), while test 5 had a SDA of 4.75 g N/(m^2·d). Tests 3 and 4 reached the plateau after approximately 40 minutes and obtained SDAs of 5.81 and 6.13 g N/(m^2·d), respectively. As seen in the figure, test 3 had a slightly declining production rate curve after the plateau was reached. Test 2 reached the maximum after 60 minutes with an SDA of 5.67 g N/(m^2·d). After the maximum was reached, the curve started to decline. The mean SDA for all tests were 5.93 with a variation of 14%.

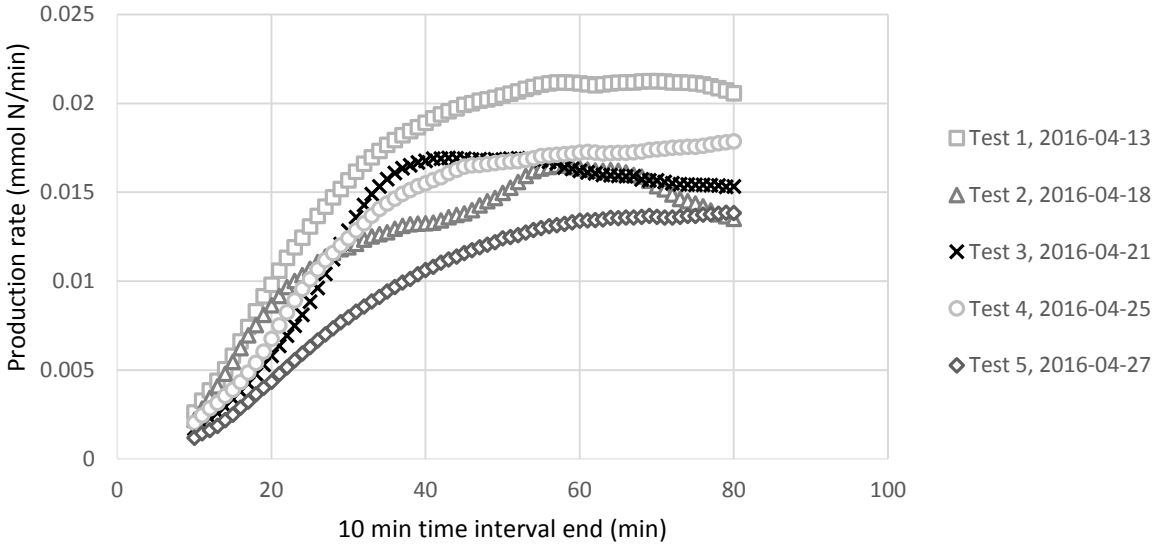

Figure 5-15: The production rate curves from the different tests conducted on Line 1, Zone 1. The highest production rate was found at test 1, while the lowest production rate was found at test 5.

It is expected that the denitrifying activity increases with rising temperature. This is because the biomass growth rate increases with rising temperature up to a certain limit (Pietikäinen *et al.*, 2005). However, since the temperatures for test 1-4 are quite similar, it is hard to conclude why the SDA for test 1 was much higher than that of the other tests. According to Carrera *et al.* (2004), alterations of temperatures in the interval 10-25°C do not have a very high effect on the denitrifying activity. The reason as to why test 1 had a higher SDA than the other tests might have other explanations than temperature. For instance, there could have been leftover nutrient residues on the carriers after washing. There could also have been contamination if the syringes were not sufficiently cleaned.

When test 5 was performed the outside temperature had been relatively low for several days, which contributed to a decreased water temperature in the basin. Thus, it seems more likely that the low SDA of test 5 was coupled to low outside temperature. Note that the temperatures stated is only diurnal averages. The actual temperature in the water could be higher or lower than the diurnal average when the carriers where sampled.

In order to be able to make a fair assessment of the temperature effect, it would be preferable to compare experiments conducted in the winter with experiments conducted in the summer. When the last initial concentration measurement was performed on March 15, the average water temperature was 14.8°C. The SDA for this experiment was 5.01 g N/(m^2·d). This indicates that the water temperature did not vary much throughout the experimental course of this project.

Table 5-5: A comparison of the denitrifying capacity at different temperatures and the conversion of the maximum production rate of nitrogen gas to the maximum specific denitrifying activity of the different tests of Line 1, Zone 1.

Experiment date	*Water temperature (°C)*	*N_2-production (mmol)*	*Max. production rate (mmol N/min)*	*SDA (g N/(m^2·d))*	*Mean SDA ± standard deviation*
13/4	15.6	1.29	0.0213	7.29	
18/4	15.2	0.95	0.0165	5.67	
21/4	15.8	1.00	0.0169	5.81	5.93±0.82
25/4	15.8	1.05	0.0179	6.13	
27/4	11.8	0.77	0.0138	4.75	

5.3.2 Experiments conducted on second-zone carriers from the different lines

The results, regarding nitrogen gas production from the experiments conducted on the second zones of the lines, can be seen in Figure 5-16. The nitrogen gas production was significantly lower when second-zone carriers were tested compared to that of the first-zone carriers (see section 5.3.1). This indicates that the first zone carriers have a much higher denitrifying capacity than that of the second zone.

Carriers from Line 1:2 achieved the highest produced nitrogen gas of 0.27 mmol. Line 3:2 and 5:2 both produced 0.21 mmol nitrogen gas, while Line 6:2 had a production of 0.19 mmol

nitrogen gas. Note that it is only the results from the tests that did not require adjusted average modulation that are shown here. The rest can be seen in Appendix V.

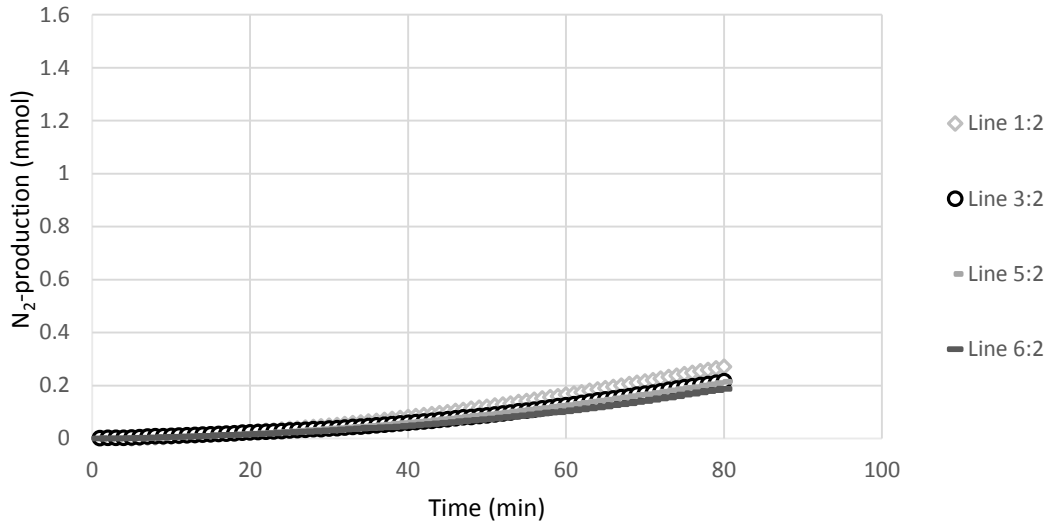

Figure 5-16: The nitrogen gas production curves from the first tests conducted on the second zones of the different lines.

The results regarding the nitrogen gas production rates can be seen in Figure 5-17 and the conversion to specific denitrifying activity can be seen in Table 5-6. The results in Figure 5-17 corresponds to the tests that did not require adjusted average modulation. The highest SDA shown in the graph was 1.90 g N/(m^2·d), which was obtained by carriers from Line 1:2. Line 3:2 had a SDA of 1.62 g N/(m^2·d), while Line 5:2 and 6:2 had SDAs of 1.58 and 1.59 g N/(m^2·d), respectively. The Results can be seen in Table 5-6.

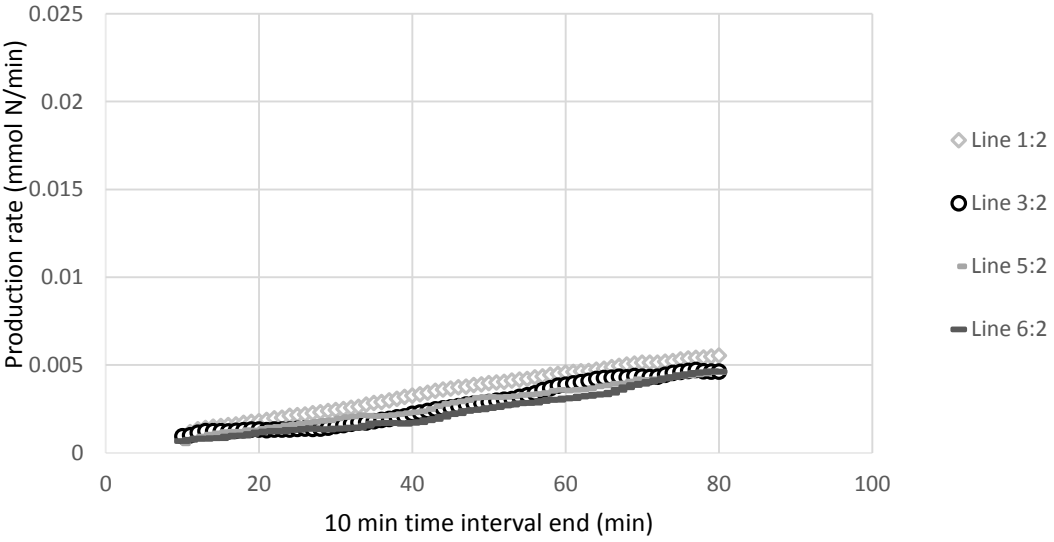

Figure 5-17: The nitrogen gas production rate curves from the second zones of the different lines.

Table 5-6 include the results from the tests that had to be adjusted with larger time intervals for derivative calculations. Even though adjustments have been made, some of the values are relatively high. Regarding Line 3:2, there was a 0.46-unit difference between the test that needed adjustments with the test that did not. This resulted in a variation of 12%. For Line 5:2, the difference between the tests were 0.47 units and the variation was 13%. Three tests were performed on Line 1:2, whereof two tests needed adjustments. The variations for Line 1:2 and Line 6:2 were 10% and 2%, respectively.

Table 5-6: A conversion chart showing the maximum production rate of nitrogen gas and the maximum specific denitrifying activity of the second zones of the different lines in the full scale post denitrification plant. Note that this table includes results from the graphs in Appendix V.

Line:Zone	*N_2-production (mmol)*	*Max. production rate (mmol N/min)*	*SDA (g N/(m^2·d))*	*Mean SDA ± standard deviation*
1:2	0.27	0.0055	1.90	1.76±0.17
	0.22	0.0054*	1.86*	
	0.19	0.0044*	1.52*	
3:2	0.15	0.0060*	2.08*	1.85±0.23
	0.21	0.0047	1.62	
5:2	0.21	0.0046	1.58	1.82±0.23
	0.21	0.0060*	2.05*	
6:2	0.19	0.0046	1.59	1.62±0.03
	0.24	0.0048*	1.64*	

*Value after adjusted average modulation

From experience, it seemed to be harder to conduct the manometric batch test on the second-zone carriers compared to that of the first-zone carriers. The tests performed on second-zone carriers seemed more prone to be affected by measuring noise. This might be a result of a thinner and a more sensitive biofilm since more bacteria are exposed to the surface. In a thicker biofilm the surface bacteria will act as a protective coat for the inner bacteria. It is also worth noting that a nitrate concentration of 75 mg N/L is immensely higher than the second-zone bacteria are used to (more of that in chapter 5.5).

The production rate results from the other tests performed on the second zones of the different lines can be seen in Appendix V.

5.3.3 Comparisons between the two MBBR zones

The reason as to why the specific denitrifying capacity was higher in the first zone compared to that of the second zone, can be explained by the direction of the wastewater flow. As mentioned earlier, the wastewater enters the MBBR in the first zone before passing through to the second zone. Another reason is that methanol is added in the first zone. This indicates that the

denitrifying bacteria in the first zone receives water high in nutrients that can be utilized for cell growth and survival. The consumption of the nutrients in the first zone causes a waning nitrate and COD concentration gradient through the MBBR. When the wastewater enters the second zone, the depletion of nutrients is substantial, causing the second zone bacteria to starve.

From studies by Peyton (1996), it was observed that the biofilm thickness was dependent on substrate concentrations. Based on the observations it was concluded that a higher substrate concentration contributed to a thicker biofilm. This phenomenon can be seen in Figure 5-18. The pictures on the upper left and the lower left depicts carriers that were sampled from Line 3:1 and Line 1:1, respectively. The pictures on the upper right and the lower right shows carriers sampled from Line 3:2 and Line 6:2, respectively. It is clearly visible that the first zone carriers have more biofilm compared to that of the second zone carriers, since they are much darker in colour. The carriers from the first zones also appeared to be much heavier. It was discovered that 240 first-zone carriers weighed approximately 20 grams more than the same amount of second-zone carriers. Almost no biofilm could be detected by visual inspection on the second-zone carriers.

Figure 5-18: A comparison of the carriers from different lines and zones of the full-scale post-denitrification plant. The upper row depicts carriers from Line 3 where zone 1 is shown to the left and zone 2 are shown to the right. The bottom row shows carriers from Line 1 zone 1 to the left and carriers from line 6 Zone 2 to the right. Photo by Bårdskär (2016)

Overall, the tests on the different lines and zones concluded that the SDA of the second zone carriers were about 3-4 times lower than the SDA of the first zone carriers.

5.4 pH measurements

The pH was supposed to be measured at each experiment. But due to the lack of proper instrumentation, pH was only measured at the last three experiments conducted. These were test 3, 4, and 5, in the series on Line 1, Zone 1. The results can be seen in Table 5-7.

Table 5-7: The pH before and after the experiment start. The pH was measured before the nitrogen gas flushing and after the logging was finished.

Test	*pH before*	*pH after*
3	7.80	7.87
4	7.76	7.88
5	7.73	7.78

The phosphate buffer seemed to be reliable since the pH was kept in a range that was desirable for denitrification, which according to Knowles (1982) was pH 7-8. The buffer was designed to maintain a pH around 7.75. Experiment 4 had the highest rise in pH of 0.12 units. This test had also the highest denitrifying activity and nitrogen gas production of the three experiments. The pH for experiment 3 and 5 rose with 0.07 and 0.05 units, respectively.

5.5 Operational results

The operational results were obtained from the data program eWASTE, which is a platform for all data results from online measurements as well as grab samples measured in the laboratory. The purpose was to further investigate the full-scale denitrifying process regarding nitrate load and reduction and also to study the operational differences between the lines. The operational data do not provide any information about the different zones in the lines, but rather account for each line as a whole. The operational results from the period 2016-01-01 to 2016-04-27 were studied. It should be mentioned that it is only nitrogen in terms of nitrate that has been studied in order to facilitate comparisons to the experimental results. The amount of ammonium and nitrite that enters the denitrification MBBRs is small since the main part is consumed in the nitrifying trickling filters.

5.5.1 Wastewater and methanol flows

Wastewater flow

The wastewater flow rate to the different lines of the post-denitrification plant can be seen in Figure 5-19. Lines 1-5 have basically the same flow rate since the chutes are positioned at the same height. This is why Lines 1-5 are depicted with the same line in the graph. Line 6 have a higher positioned chute than that of the other lines and thus have a lower flow rate, which can be seen as the light grey line in the graph. The average wastewater flow to Line 1-5 was 217 L/s with a 9% variation per line for the period 1 Jan-27 April, which can be seen in Table 5-8. The average flow to Line 6 was 172 L/s with a 12% variation. The total average wastewater flow to the post denitrification plant for the same period was 1,257 L/s. the variation for the total wastewater flow for the given period was 10%.

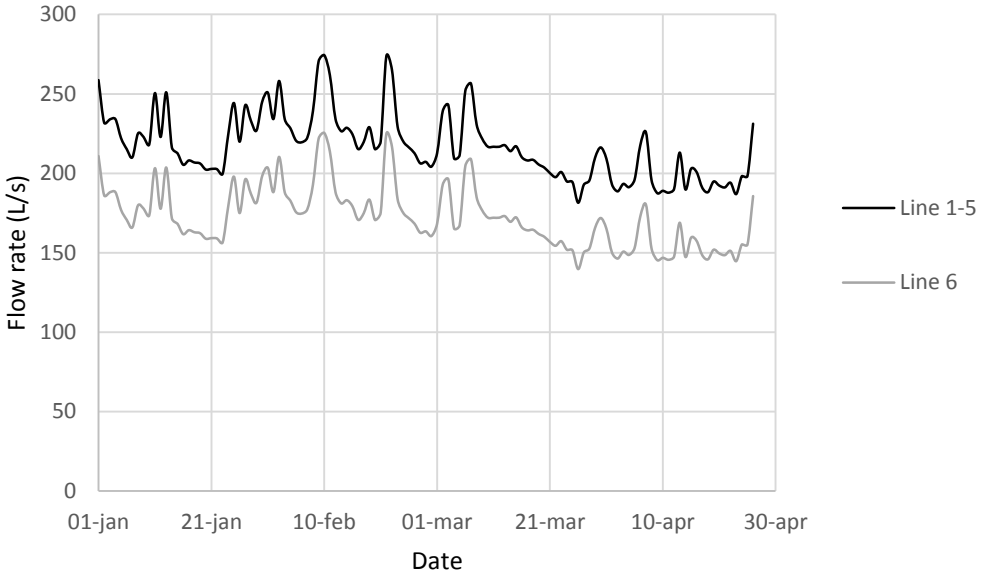

Figure 5-19: The wastewater flow of the different lines of the denitrification plant for the period 1 Jan-27 April. Line 1-5 have the same flow rate while Line 6 have a lower flow rate.

There were significant peaks on February 10 and on February 22. The trend seemed to be decreasing after March 6. On Sjölunda WWTP, the average wastewater flow rate is higher during the winter than that of the summer. During the summer, many inhabitants of Malmö go on vacation, which contributes to a lower flow. The dip at the end of February overlapped with the "February break", while the dip on March 26 coincided with the Easter holidays.

Table 5-8: The mean values and standard deviations for the flow to the different lines and the average total wastewater flow to the denitrification plant.

	Wastewater flow mean ± standard deviation (L/s)	*Total flow mean ± standard deviation (L/s)*
Line 1-5	217±20	1,257±120
Line 6	172±20	

Methanol flow

The methanol flow to the post-denitrification plant is regulated depending on the nitrogen content in the wastewater, the COD content in the methanol together with a desired COD/N ratio in each line. The methanol flow to the different lines during the pertaining period can be seen in Figure 5-20 and the mean values together with standard deviations for each line is shown in Table 5-9. Lines 1-5 had similar average flow rates within the range of 45-49 L/h and variations between 6-7%. Line 2 had the highest mean flow during the period inspected, while Line 5 had the lowest average methanol flow of Lines 1-5. The flow to Line 6 was lower than that of the other lines, which depends upon the lower wastewater flow to said line. The average flow for Line 6 was 34 L/s and a 6% variation during the studied period.

All the lines experienced a significant dip in methanol flow at February 10, even though the wastewater flow increased on this day. The flow to Line 1-5 went down to approximately 35 L/h, while the flow to Line 6 decreased to around 25 L/h. The trend was decreasing after March 21, which might be coupled to decreasing wastewater flow rates.

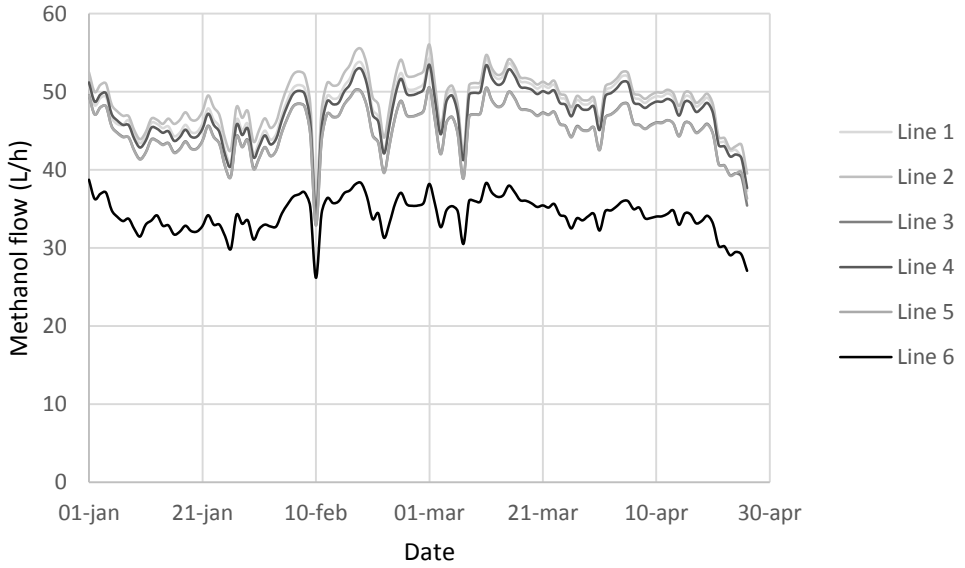

Figure 5-20: The methanol flow to each line in the post-denitrification plant. Line 6 has a lower methanol flow due to the lower wastewater flow.

Table 5-9: The mean values and the standard deviations for the methanol flow to the different lines.

	Methanol flow *mean ± standard deviation (L/h)*
Line 1	48±3
Line 2	49±3
Line 3	45±3
Line 4	47±3
Line 5	45±3
Line 6	34±2

5.5.2 Nitrate load and reduction

Overall nitrate load and reduction for the whole post-denitrification plant

The nitrate concentration of the ingoing and outgoing wastewater in the post-denitrification plant can be seen in Figure 5-21. The average nitrate concentration in the incoming wastewater

was on average 16.6 mg N/L with a 12% variation during the period 1 Jan-27 April. The average outgoing nitrate concentration in the wastewater was 1.25 mg N/L during the same period. However, the variation for outgoing nitrate concentration was 32% for the given period. The mean values and standard deviations for the incoming and outgoing nitrate concentrations can be seen in Table 5-10.

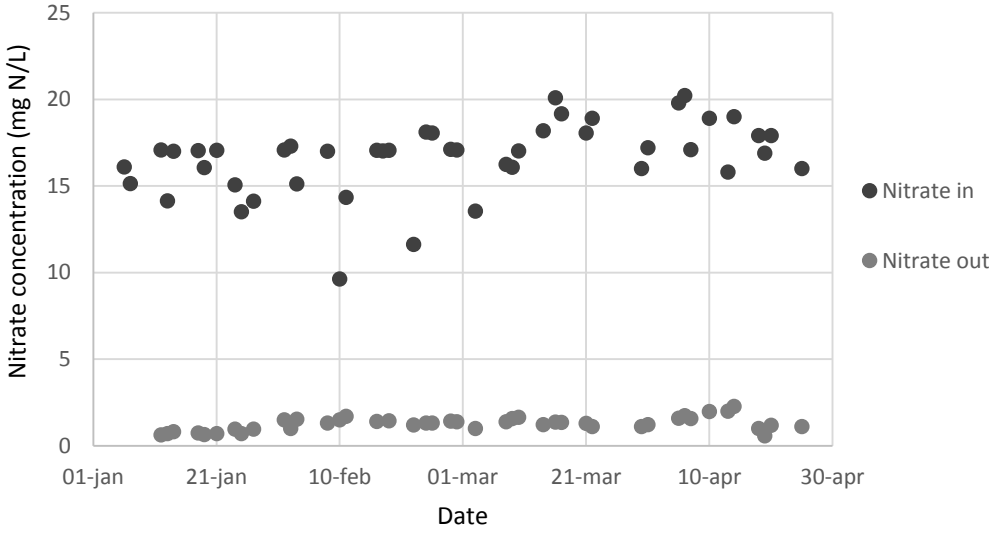

Figure 5-21: The incoming and outgoing nitrate concentrations of the full-scale post denitrification plant.

The incoming nitrate concentration reaches its lowest point of 9.6 mg N/L on February 10, which might provide the explanation as to why the methanol flow was low on this day. The incoming nitrate concentration of 11.6 mg N/L on February 22 also causes a dip in the methanol flow chart. The nitrate concentration in the incoming wastewater reaches its highest point of 20.2 mg N/L on April 6. The overall incoming concentration seemed to increase after March 14.

The outgoing nitrate concentration had the highest point of 2.26 mg N/L on April 14, whereas the lowest outgoing concentration was 0.56 mg N/L on April 19.

Table 5-10: The mean values and standard deviations for incoming and outgoing nitrate concentrations.

	Mean ± standard deviation (mg/L)
$[NO_3^-\text{-}N]_{in}$	16.6±2.0
$[NO_3^-\text{-}N]_{out}$	1.25±0.4

Figure 5-22 shows the nitrate load and the nitrate reduction converted to g N/(m^2·d). The nitrate load was calculated by multiplying the incoming nitrate concentration with the flow rate and then divide with the total effective area of the carriers in the post-denitrification plant. The

nitrate load did not always follow the same trend as the nitrate concentration shown in Figure 5-21, due to fluctuating incoming wastewater flow. For instance, the peak in nitrate load on February 2 cannot be seen as an increase in nitrate concentration in Figure 5-21. The high wastewater flow on this day, which can be seen in figure 5-19, contributed to an increase in nitrate load. On April 6 however, both the wastewater flow and the nitrate concentration were high, which resulted in a peak in the nitrate load chart.

The mean value for the nitrate load during the studied period was 1.16 g N/(m^2·d) with a variation of 9%, which can be seen in Table 5-11. In the meantime, the average reduction of nitrate was 1.08 g N/(m^2·d) with a 10% variation. This implies that the reduced nitrate constitutes 93% of the total incoming nitrate to the post-denitrification plant.

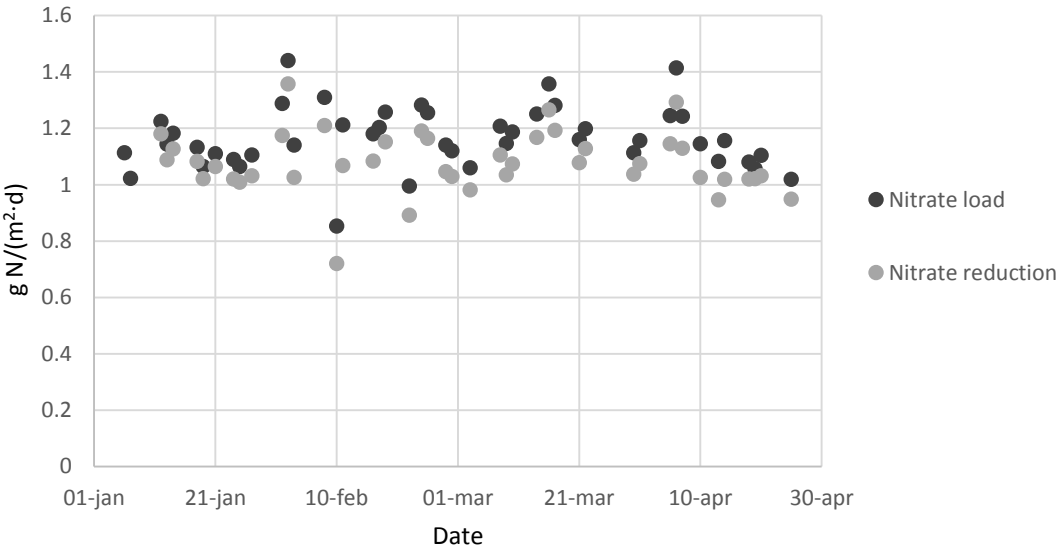

Figure 5-22: The nitrate load and reduction for the whole post-denitrification plant. The nitrate load and reduction reaches their lowest points om February 10. Significant peaks can be seen on February 2 and April 6.

Table 5-11: The mean values and standard deviations for the nitrate load and reduction, together with the nitrate removal efficacy of the whole post-denitrification plant.

	Mean ± standard deviation (g N/(m^2·d))	*Nitrate removal efficacy (%)*
NO_3^--N load	1.16±0.11	93
NO_3^--N reduction	1.08±0.11	

It can be concluded from the observations in eWASTE, that the overall reduction for the post-denitrification plant in the period studied, was close to the denitrification rate of 1.2 g N/(m^2·d) at the design temperature of 10°C. The result looks promising, however, it should be mentioned that the denitrification rate is usually studied as an annual average as opposed to the four months that were studied in this project. This implies that the average denitrification rate could change

if the whole year is accounted for. In 2004-2009, the annual average denitrification rate at Sjölunda WWTP was 0.90 g NO_x-N/(m^2·d) and the average load was 1.08 g NO_x-N/(m^2·d), while the average reduction was 84% (Mases *et al.*, 2010), see Appendix VI. Since then, it is plausible that the load has increased since the municipality is expanding and thus releases more nitrogen, which might be the reason as to why the nitrate load and reduction was higher for the period that was studied in this project.

Nitrate load and reduction for the different lines

The nitrate load for the different lines is depicted in Figure 5-23. Since Line 1-5 have the same chute settings and thus theoretically equal flow rates, the nitrate loads for said lines are basically identical. The light grey dots in the picture depicts the values for Line 1-5, while the dark grey dots are values from Line 6. Due to the lower flow rate, the nitrate load in Line 6 was lower than that of Lines 1-5. The mean nitrate load values for the different lines are shown in Table 5-12. For Lines 1-5, the average nitrate load for the period was 1.20 g N/(m^2·d), while the mean value for Line 6 was 0.96 g N/(m^2·d). All the lines had a variation of 9%.

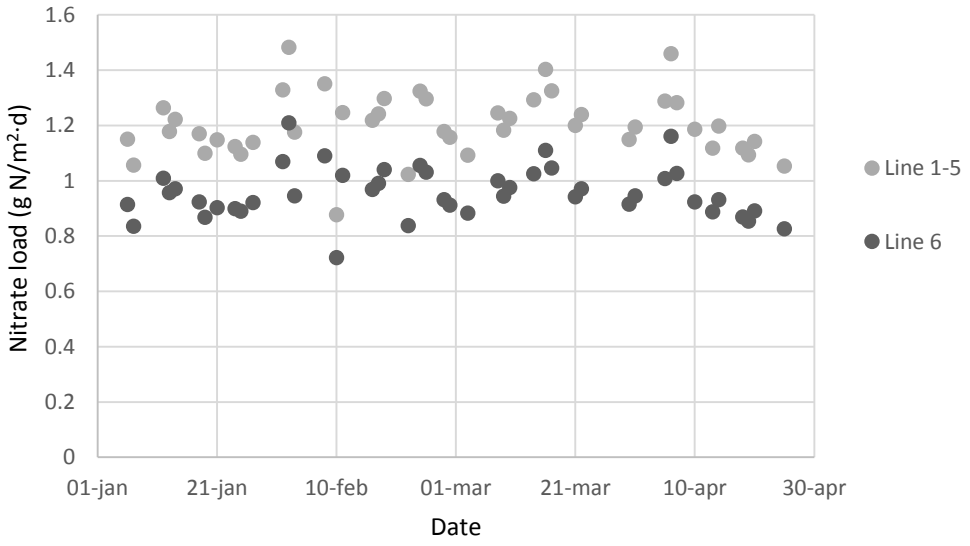

Figure 5-23: The nitrite load for the different lines. Note that the values for Line 1-5 share the same markers since they have equal load. Line 6 have a lower flow rate and thus a lower nitrate load.

The nitrate loads for the different lines follows the same trend as the nitrate load for the whole plant, which is reasonable. Peaks occurred on February 2 and on April 6, while a dip was seen on February 10.

Table 5-12: The mean values and standard deviations for the nitrate load of the different lines. The values for Line 1-5 are basically the same since they have the same flow rate. Line 6 has a lower flow rate and thus a lower nitrate load.

Line	*Nitrate load mean ± standard deviation (g N/(m^2·d))*
1-5	1.20±0.11
6	0.96±0.09

The nitrate reduction for each line can be seen in Figure 5-24. Compared to the previous figures of the operational results, Figure 5-24 contains significantly fewer data points. As mentioned earlier, eWASTE compiles results from online measurements as well as laboratory measurements. The data is stored as column vectors and if the results are retrieved on different days, the vectors are incompatible, which obstructs the calculations. The result is that some measurements are left blank.

The nitrate reduction seemed to be equal for Line 1-5 throughout the time period in question, with an exception for February 22 when Line 3 had a lower reduction. As expected, Line 6 had the lowest nitrate reduction of the lines due to the lower nitrate load. The mean values for the nitrate reduction together with the nitrate removal efficacy in the different lines can be seen in Table 5-13. Line 1, 4 and 5, all had an average nitrate reduction of 1.18 g N/(m^2·d). With a nitrate load of 1.20 g N/(m^2·d), this accounted for a nitrogen removal efficacy of 98% for said lines. Line 2 and 3 had an average nitrogen reduction of 1.16 g N/(m^2·d) and a corresponding nitrogen removal efficacy of 97%. Line 6 had a nitrogen reduction of 0.93 g N/(m^2·d). Since the nitrogen load was 0.96 g N/(m^2·d), Line 6 had a nitrogen removal efficacy of 97%. The nitrate reduction variations lied between 9-11% for the lines.

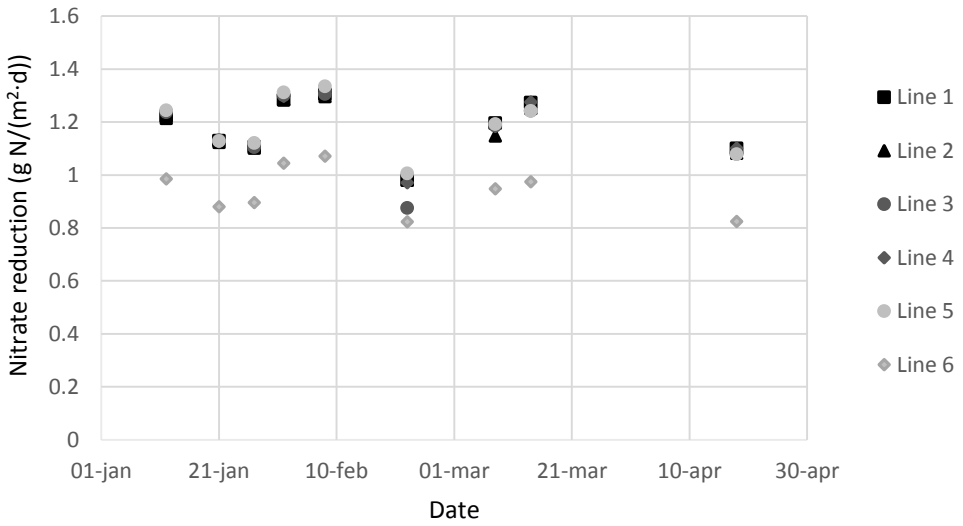

Figure 5-24: The nitrate reduction in the different lines in the post-denitrification plant. Line 6 had a lower nitrate reduction than that of the other lines. Line 1-5 seemed to have similar nitrate reductions.

Table 5-13: The mean values and standard deviations for the nitrate reduction and the nitrate removal efficacy of the different lines.

Line	*Nitrate reduction mean ± standard deviation (g N/(m²·d))*	*Nitrate removal efficacy (%)*
1	1.18±0.10	98
2	1.16±0.10	97
3	1.16±0.13	97
4	1.18±0.11	98
5	1.18±0.10	98
6	0.93±0.08	97

It can be seen in Table 5-13 that the nitrogen removal efficacies for the different lines were much higher than the value for the whole post-denitrification plant, which was 93%. The reason for this might be due to the fewer data points for the different lines, which might result in inaccurate mean values for the nitrate reductions. In this case, it seems that the mean values for the nitrate reductions in the different lines became deceptively high. The average value for the nitrate reductions for all lines shown in Table 5-13 was 1.13 g N/(m^2·d), while the average reduction for the whole post-denitrification plant was 1.08 g N/(m^2·d). These two numbers should be equal in theory.

With this in mind, it seems hard to make an accurate assessment of the nitrate reduction in the different lines, due to the few data points. What can be said is that it seems that the different lines have equal nitrate removal efficacies regardless of the few data points. It also seems that the reduction potential of Line 6 equals that of the other lines, in spite of having a lower nitrate load.

5.5.3 Carbon load

The COD/N ratio of the different lines can be seen in Figure 5-25 and the average value for each line is shown in Table 5-14. As mentioned earlier the desired COD/N ratio of the whole post-denitrification plant is approximately 4. The methanol is added to the basins in the post-denitrification plant with regards to COD content in the carbon source and incoming wastewater nitrate concentration. The average COD/N ratio for the whole plant during the studied period was 4.3 g COD/g NO_3^--N, which lies close to the desired value of 4. The variation for the whole plant during the same period was 7%. The ratios vary between the lines 1-5 even though the nitrate load is theoretically the same for these lines. Line 2 had the highest reported COD/N ratio average of 4.5, which can be coupled to the high methanol flow rate of 49 L/h (see Table 5-9 in section 5.5.1). Line 1 and 4 had equal COD/N ratio average of 4.4 and methanol flows of 48 and 47 L/h, respectively. Line 3 and 5 both had a COD/N ratio of 4.2 and methanol flows of 45 L/h. Line 6 had, as expected, the lowest reported COD/N ratio of 4.0, which can be coupled to the low methanol flow rate of 34 L/h.

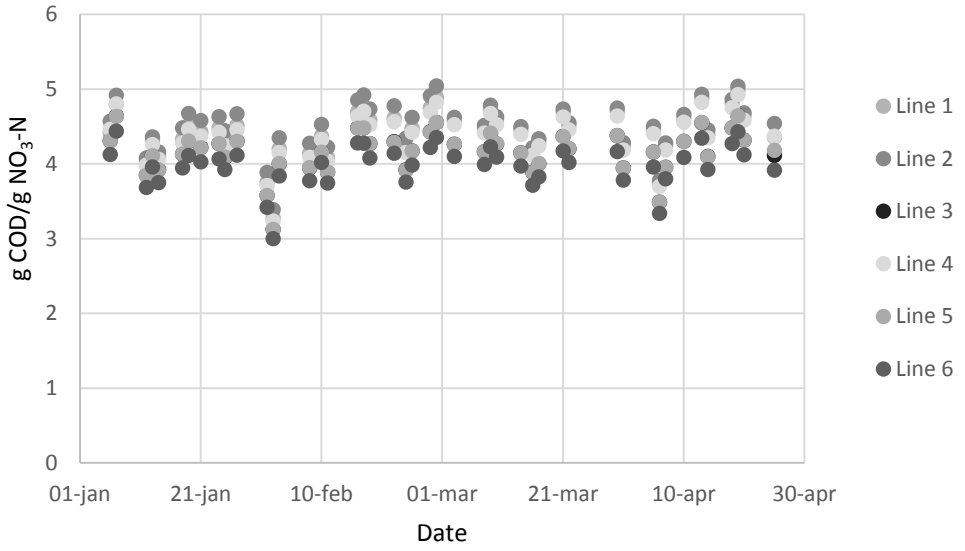

Figure 5-25: The COD/N ratio in the different lines of the post-denitrification plant. The COD/N ratios varied between 3-5 for the period studied, with an average ratio of 4.3.

Table 5-14: The mean values and standard deviations for the COD/N ratios in the different lines.

Line	*COD/N ratio mean ± standard deviation (g COD/g NO_3^--N)*	*Total COD/N ratio mean ± standard deviation (g COD/g NO_3^--N)*
1	4.4±0.3	4.3±0.3
2	4.5±0.3	
3	4.2±0.3	
4	4.4±0.3	
5	4.2±0.3	
6	4.0±0.3	

The strategy for carbon dosage at Sjölunda WWTP is to have as much COD consumed in the denitrifying MBBRs as possible. The carbon load should be high enough in order to sustain a desirable denitrification potential, but low enough so that the denitrification plant does not leak COD. This is of the essence since there are no steps after the denitrification MBBRs that can remove COD from the wastewater. The policy is that if external chemicals are needed, they should be consumed in the very basin where they were added.

For the two following chapters (5.6 and 5.7), it has been assumed that the dinitrogen production rate corresponds to the nitrate reduction rate. This is a reasonable conjecture since, according to equation 4, it takes two moles of nitrate to produce one mole of nitrogen gas while the nitrogen part in nitrogen gas is twice the part of nitrogen in nitrate.

5.6 Comparison between the experimental and the operational results

As mentioned before, the operational data do not give any further information about the differences between the two MBBR zones in each line. The nitrate reduction potential obtained from the operational data is an average for the whole line, where deviations between the zones may occur. In this regard, it is beneficial to be able to use the manometric batch test for further investigation of the different lines and zones.

The experimental results showed more significant differences regarding nitrogen gas production rates between the lines than what could be concluded from the operational results. Based on the observations from the manometric tests, it seemed like Line 5:1 had the highest capacity and that Line 1:1 had the lowest detected activity. Regarding the second zones, Line 3:2 and Line 5:2 had the best denitrifying capacity while the lowest capacity was found in Line 6:2. However, based on the observations from the operational results it was concluded that Lines 1-5 had basically the same nitrate reduction capacity. Line 6 had the lowest capacity due to lower nitrate load and flow rate. Nevertheless, it was found that all the lines had equal percental reduction efficacy regardless of nitrate load.

There are several explanations as to why the experimental results differed from the operational results. First of all, the operational results were assessed over a time period of four months, while the narrow time frame of this project only allowed for two or three tests per zone. Several experiments on the different lines would have to be performed in order to make a more accurate assessment.

The flow rates to the different lines were calculated based on the total flow rate to the denitrification plant and the positions of the chutes. It might be problematic to adjust the chutes in a way that the flow through the reactors becomes exactly the same. This means that the actual flow rate might differ from the theoretical value. It is also important to consider the effective area of each line. There is no guarantee that the lines contain exactly the same amount of carriers and have an equal effective area of 259,750 m^2. Therefore, it is possible that there actually were some variations between Lines 1-5 concerning nitrate load and reduction that the operational results did not show.

5.7 Hypothetical reduction potentials with equal activity in both zones

From the SDA measurements, it was concluded that the activity in Zone 1 was approximately 3-4 times higher than that of Zone 2, at any line (see section 5.3). With this knowledge, it is possible to hypothetically estimate the extent of the capacity if both of the zones would have equal activity and how much nitrate reduction that then would be manageable.

Assuming that the denitrification rate is 1.2 g N/(m^2·d), which is the capacity at the design temperature of 10°C, and that the activity of Zone 1 is four times higher than that of Zone 2, the activity of the whole post-denitrification plant would then correspond to 1.92 g N/(m^2·d), provided that both zones have equal denitrifying activity. Given that all the lines have the same capacity and flow rate, in theory, it would be feasible to manage a nitrate reduction of 34.6 mg N/L. Assuming a 93% reduction efficacy, the nitrate load would correspond to 37.2 mg N/L. This would however, lead to an effluent nitrate concentration of 2.6 mg N/L, which is much higher than the current nitrate emission of 1.25 mg N/L (see section 5.4.2).

If the calculations are made accounting for the separate lines, then a nitrate reduction of 26.6 would be manageable for Line 1-5, assuming a denitrification rate of 1.92 g N/(m^2·d) and a flow

rate of 217 L/s per line. The reduction for Line 6 was estimated to 26 mg N/L, provided that the denitrification rate is 1.49 g N/(m^2·d) and that the wastewater flow is 172 L/s. Assuming a 93% reduction efficacy, this would result in a load of 28.6 mg N/L to Line 1-5 and 28 mg N/L to Line 6. The effluent nitrate concentration would then be 2 mg N/L for all lines. The calculations can be seen in Appendix VII.

It seems that, if both zones in each line were to have equal denitrifying activity, the load would have to be much higher in order to achieve an increased activity. Table 5-15 shows how much reduction that would be possible if the denitrifying activity worked at full speed, as determined by the experimental trials. Both zones were assumed to have equal activity and a nitrate reduction of 93%. The high SDAs are tied to higher loads. Even if the specific denitrifying activities are high, the resulting effluent concentrations are unacceptable and much higher than 1.25 mg N/L that was observed from the operational data for the given period. The stringent effluent standards hinder Sjölunda WWTP from releasing more nitrate than it already does.

Table 5-15: The hypothetical loads and reductions for the different lines, given that the SDA measured in the experimental trials were accomplished. The SDAs presented here are average values for both zones in the corresponding line.

Line	*mean SDA (g N/(m^2·d))*	*Load (mg N/L)*	*Reduction (mg N/L)*	*Outgoing concentration (mg N/L)*
1	3.85	57.4	53.3	4.1
3	4.35	64.8	60.3	4.5
5	4.84	72.0	67.0	5.0
6	4.28	80.4	74.8	5.6

As observed from the experimental results, the denitrifying activity is strongly dependent on the nitrate concentration up to a certain limit. The nitrate load to the post-denitrifying MBBR is much lower than 35 mg N/L, where the SDA becomes independent of initial nitrate concentration. The denitrifying activity will then follow half order reaction kinetics and it is therefore reasonable to say that higher nitrate concentration in the MBBR will contribute to higher reaction rates.

It seems unreasonable to achieve equal denitrifying activity in both of the zones in the post-denitrifying MBBRs. The nitrate and the COD will be depleted on its way out which leaves starving biofilms on the second-zone carriers. A more even nitrate distribution in the reactors could be achieved with higher stirring speed. However, an increased stirrer speed might pose a higher strain on the carriers, which could lead to the loss of biofilm.

6 Conclusions

The experimental research has been conducted on K1 carriers from the post-denitrification MBBRs at Sjölunda WWTP. The following conclusions have been featured based on the observations from the conducted experiments and from the operational data analysis:

- The specific denitrifying activity turned out to be independent of initial nitrate concentrations in the interval of 35-150 mg N/L, while the activity was dependent on initial nitrate concentrations below 35 mg N/L.
- Higher load in the post-denitrifying MBBR contributes to a higher denitrifying capacity. This is because the load is much lower than 35 mg N/L, which means that half order kinetics is adopted and the reaction rate is concentration dependent.
- The specific denitrifying activity showed to be independent of the COD/N ratio in the interval of 5.3-133.3 g COD/g N, given that nitrate was not limiting.
- There was a low denitrifying activity detected on the carriers during carbon deficiency, which implies that denitrifiers can utilize intracellular carbon as electron donor.
- The first zones in the post-denitrification plant had approximately a 3-4 times higher denitrifying activity than that of the second zones.
- All the lines in the post-denitrification plant had equal nitrate reduction efficacy. However, the reduction rate for one line was lower than that of the other lines due to lower flow rate.

7 Further work

Additional experiments can be conducted with the manometric batch test for future investigations of the denitrifying activity on the carriers in the post denitrification plant. For instance, it would be interesting to further study the how the temperature affects the denitrifying activity. The experiments in this project were performed during a period where the temperature had only small variations. In this regard, it would be more informative to compare test performed during the summer and the winter.

Regarding experiments on the different lines and zones, tests on Line 2 and 4 could preferably be conducted since those lines were not covered in the experimental trials.

Neither the operational nor the experimental data give any information on how the concentration varies in the lines. In order to estimate how much nitrate that is consumed in the first zone, water samples from different part of the line could be analyzed. It is suggested that water from the inflow to Zone 1, the inflow to Zone 2 and the outflow is investigated.

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9 Appendices

Appendix I: A timetable for all the conducted experiments

Appendix II: Manual for the manometric activity batch test

Appendix III: Additional results from the initial concentration measurements

Appendix IV: Additional results from the reference tests

Appendix V: Additional results and graphs from the measurements from the different lines

Appendix VI: Operational results from Sjölunda WWTP 2002-2009, annual averages

Appendix VII: Additional calculations

Appendix VIII: Populärvetenskaplig sammanfattning (in Swedish)

Appendix I

A timetable for all the conducted experiments.

Table A-1: All the experiments conducted during this project are listed in this table.

Date	*Type*	*$[NO_3^-]$ (mg/l)*	*V_{NO3} (ml)*	*V_{MeOH} (ml)*	*Comments*
2016-02-11	Initial concentration measurements	75	11.25	6.25	120 min
2016-02-11		100	15	6.25	120 min
2016-02-15		125	18.75	1	120 min
2016-02-16		150	22.5	1	120 min
2016-02-22		50	7.5	1	120 min Ugly graph
2016-02-22		25	3.75	1	120 min
2016-02-24	Reference tests	-	-	-	120 min
2016-02-24		125	18.75	-	120 min
2016-02-29		-	-	1	120 min
2016-02-29	Initial concentration measurements	50	7.5	1	120 min
2016-03-02		50	7.5	1	80 min Ugly graph
2016-03-03		75	11.25	1	80 min Ugly graph
2016-03-33		35	5.25	1	80 min
2016-03-04		50	7.5	0.5	80 min
2016-03-07		100	15	1	120 min
2016-03-08		75	11.25	0.5	120 min Ugly graph
2016-03-09		50	7.5	1	80 min
2016-03-11		75	11.25	1	80 min
2016-03-15		75	11.25	0.25	80 min
2016-03-16	Line 1, Zone 2	75	11.25	0.25	80 min
2016-03-16		75	11.25	0.25	80 min Ugly graph

2016-03-21		75	11.25	0.25	80 min Ugly graph
2016-03-21	Line 3, Zone 1	75	11.25	0.25	80 min
2016-03-22		75	11.25	0.25	80 min Ugly graph
2016-03-22	Line 3, Zone 2	75	11.25	0.25	80 min Ugly graph
2016-03-30		75	11.25	0.25	80 min
2016-03-30	Line 5, Zone 1	75	11.25	0.25	80 min
2016-03-31		75	11.25	0.25	80 min
2016-03-31	Line 5, Zone 2	75	11.25	0.25	80 min
2016-04-04		75	11.25	0.25	80 min Ugly graph
2016-04-04	Line 6, Zone 1	75	11.25	0.25	80 min
2016-04-12		75	11.25	0.25	80 min
2016-04-12	Line 6, Zone 2	75	11.25	0.25	80 min
2016-04-13		75	11.25	0.25	80 min Ugly graph
2016-04-13	Line 1, Zone 1	75	11.25	0.25	80 min
2016-04-18		75	11.25	0.25	80 min
2016-04-21		75	11.25	0.25	80 min
2016-04-25		75	11.25	0.25	80 min
2016-04-27		75	11.25	0.25	80 min

Appendix II

Manual for the manometric activity batch test

1) Start the water bath and select a temperature for the experiment. The water bath will start to beep if the water level is too low. There should be enough water to cover the reactor but the lid should be kept over the surface.
2) Place a magnetic stirrer at the bottom of the water bath and set it to 400 RPM.
3) Sample carriers from the chosen reactor in the post-denitrification MBBR by sinking a plastic bucket attached to a long stick. Pour the carriers into a colander and take them to the laboratory room.
4) Rinse the carriers carefully with tap water.
5) Count 240 carriers and put them into a 1 L reactor.
6) Add 750 mL distilled water and 22 mL phosphate buffer to the reactor.
7) Put a magnet bar into the reactor and put on a septum and a lid.
8) Place the reactor on the magnetic stirrer in the water bath and leave it for 15 minutes for temperature stabilization.
9) Measure the pH in the reactor.
10) Flush the liquid phase of the reactor with nitrogen gas for 10 minutes and the headspace for 1.5 minutes. Put the lock and the septum back on the reactor immediately after removing the gas distributor.
11) Start the pressure meter and tare it. Connect the sensor to the reactor through a needle trough the septum. Add a separate needle through the septum and leave it be for 30 minutes for pressure stabilization. The pressure should stabilize at 0 mbar.
12) Add 11.25 ml nitrate (5 mg NO_3^--N/mL) and 0.25 mL methanol with a syringe and needle through the septum. Since methanol is volatile, it can preferably be diluted. Then dilute 1 mL methanol with 3 mL distilled water and add 1 mL of the solution to the reactor.
13) Let the pressure once again stabilize at 0 mbar. Remove the separate needle and start logging by pressing the "start logging recorder" in the computer software.
14) Run the reaction for 80 minutes
15) Stop logging by pressing the "stop button" in the computer software.
16) Measure the pH once again and weigh the reactor.
17) Fill the reactor headspace completely with water and note the weight difference.
18) Transfer the logged data to Microsoft Excel. Make sure to store the file on a USB.
19) Pour carriers back to the reactor of origin. Make sure to pick up the magnet bar first.

Appendix III

Additional results from the initial concentration measurements

Table A-2: Additional information from the initial concentration measurements. The experiments starting with "1" are the 120-minute experiments, while the 80-minute experiments start with "2".

Experiment	*[NO_3^--N]/[COD] (mg/L)*	*Pressure increment (mbar)*	*Volume headspace (10^{-6} m^3)*	*N_2-production (mmol)*	*Max. production rate (mmol N/min)*	*SDA (g $N/m^2 \cdot d$)*
1.1	75/10,000	112.2	240.0	1.10	0.0128	4.41
1.2	100/10,000	123.9	240.0	1.22	0.0136	4.65
1.3	125/1,600	119.6	234.6	1.15	0.0127	4.35
1.4	150/1,600	124.8	233.7	1.19	0.0136	4.67
1.5	50/1,600	104.0	248.6	1.06	0.0192/0.0158*	6.58/5.44*
1.6	50/1,600	113.4	243.8	1.13	0.0147	5.03
1.7	25/1,600	48.8	249.8	0.50	0.0098	3.38
1.8	100/1,600	140.9	229.5	1.32	0.0139	4.78
1.9	75/800	130.9	239.4	1.28	0.0191/0.0147*	6.55/5.04*
2.1	50/1,600	89.0	245.0	0.89	0.0179/0.0164*	6.15/5.63*
2.2	75/1,600	95.3	226.8	0.88	0.0229/0.0178*	7.86/6.09*
2.3	35/1,600	73.4	233.1	0.70	0.0133	4.57
2.4	50/800	83.7	233.0	0.80	0.0146	5.01
2.5	50/1,600	71.3	248.8	0.73	0.0136	4.67
2.6	75/1,600	84.4	234.6	0.81	0.0133	4.58
2.7	75/400	84.3	245.6	0.85	0.0146	5.01

*Before and after adjusted average modulation.

Appendix IV

Additional results from the reference tests

Table A-3: Additional results from the reference tests.

Experiment	*Pressure increment (mbar)*	*Volume head-space (10^{-6} m^3)*	*N_2-production (mmol)*	*Max. production rate (mmol N/min)*	*SDA (g N/m^2·d)*
No additions	2.09	256.6	0.022	0.00039	0.13
125 mg N/L	25.0	242.0	0.247	0.00286	0.98
1600 mg COD/L	4.09	250.9	0.042	0.00060	0.21

Appendix V

Additional results and graphs from the measurements from the different lines

Table A-4: Additional information from the experiments regarding the different lines and zones.

Line:Zone	*[$NO3^-$-N]/[COD] (mg/L)*	*Pressure increment (mbar)*	*Volume headspace (10^{-6} m^3)*	*N_2-production (mmol)*	*Max. production rate (mmol N/min)*	*SDA (g $N/m^2 \cdot d$)*
1:1	75/400	129.2	244.8	1.29	0.0213	7.29
		96.5	242.8	0.95	0.0165	5.67
		102.5	239.3	1.00	0.0169	5.81
		102.7	250	1.05	0.0179	6.13
		75.6	247.8	0.77	0.0138	4.75
1:2	75/400	27.9	238.6	0.27	0.0055	1.90
		22.9	238.6	0.22	0.0067/0.0054*	2.29/1.86*
		17.4	264.5	0.19	0.0057/0.0044*	1.96/1.52*
3:1	75/400	120.1	244.1	1.20	0.0197	6.76
		109.4	245.0	1.10	0.0236/0.0201*	8.09/6.91*
3:2	75/400	14.6	253.9	0.15	0.0065/0.0060*	2.23/2.08*
		19.6	268.6	0.21	0.0047	1.62
5:1	75/400	134.5	244.4	1.34	0.0237	8.13
		127.1	240.3	1.25	0.0221	7.58
5:2	75/400	19.3	270	0.21	0.0046	1.58
		19.2	268.8	0.21	0.0067/0.0060*	2.30/2.05*
6:1	75/400	100.2	239.3	0.98	0.0200	6.86
		95.3	251.4	0.98	0.0204	7.01
6:2	75/400	16.9	271.1	0.19	0.0046	1.59
		21.4	270.8	0.24	0.0058/0.0048*	1.99/1.64*

* Before and after adjusted average modulation.

The following figures (Fig. A-1 and Fig. A-2) depicts the results from the experiments conducted on the second zones that were not presented in the chapter 5.3.2.

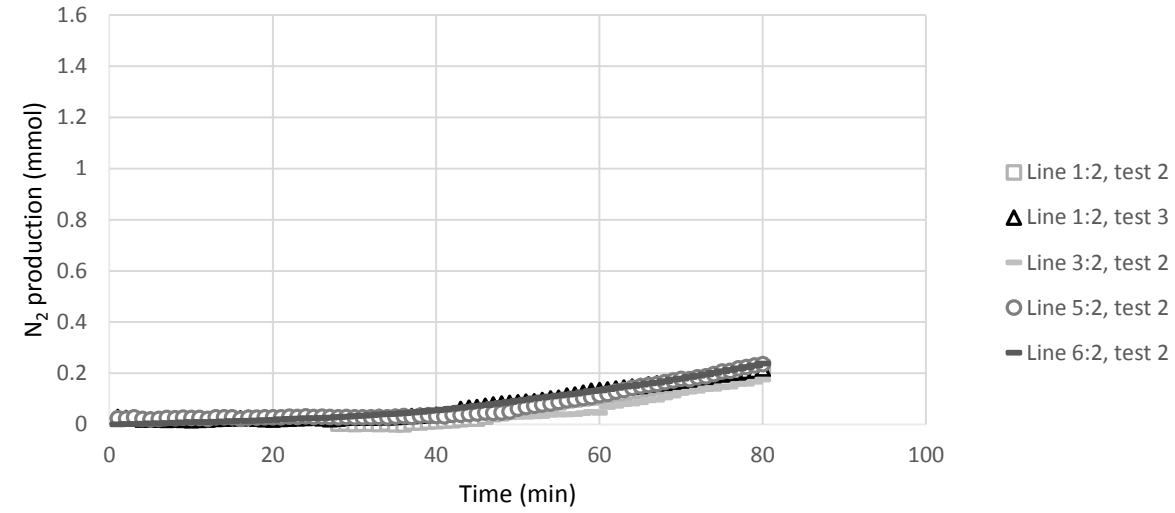

Figure A-1: The nitrogen gas production curves from the second zones of the different lines

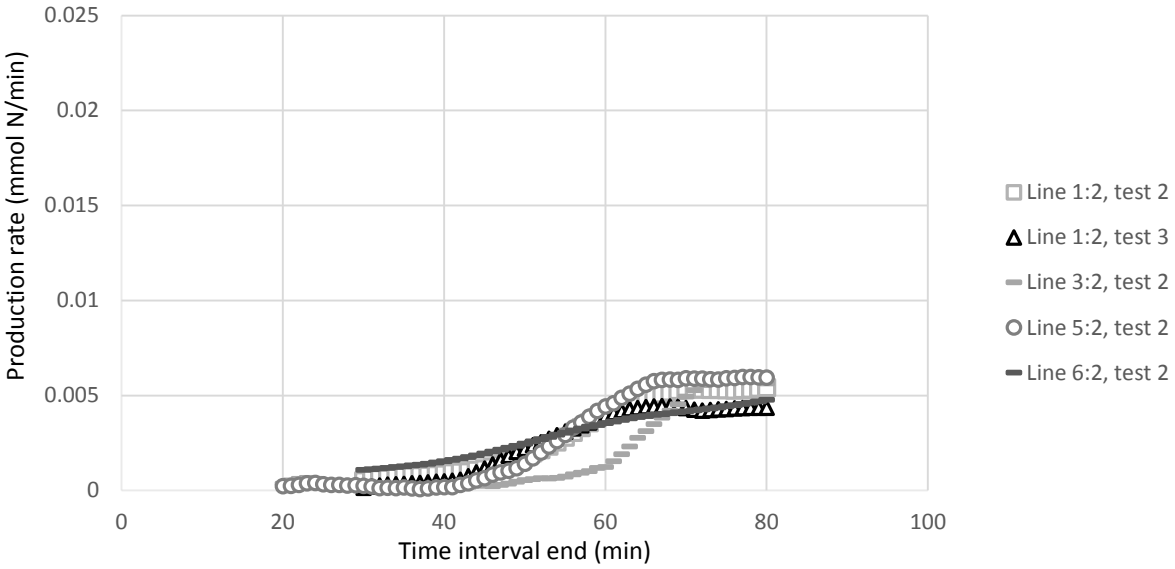

Figure A-2: The production rate curves from the second zones of the different lines. Tests 1:2:2, 1:2:3 and 6:2:2 had an adjusted average modulation of 30 minutes.

Appendix VI

Operational results at Sjölunda WWTP 2004-2009, annual averages

The following values have been obtained from Mases *et al.* (2010).

Table A-5: the operational results for the years 2004-2009. The results are presented as annual averages.

Year	*Load (g NO_x-N/(m^2·d))*	*Denitrification rate (g NO_x-N/(m^2·d))*	*Reduction (%)*
2004	1.16	1.01	88
2005	1.11	0.91	83
2006	1.07	0.90	85
2007	1.10	0.89	82
2008	1.06	0.89	84
2009	0.99	0.82	83
Average 2004-2009	1.08	0.90	84

Appendix VII

Additional calculations

Reduction potential for the whole plant

The first zone was assumed to have a four times higher capacity than that of the second zone.

$$\frac{4x + x}{2} = 1.2\ g\ N/(m^2 \cdot d)$$

Where, 1.2 is the design reduction rate for the whole denitrification plant (g N/(m^2·d)). Solving for x, gives a reduction rate of 1.92 g N/(m^2·d), if both zones have the same capacity.

The total amount of reduced nitrate per liter wastewater is then:

$$\frac{1.92 \cdot 1{,}558{,}500 \cdot 1{,}000}{24 \cdot 3{,}600 \cdot 1257} = 34.6\ mg\ N/L$$

Where, 1,558,500 is the total effective area (m^2), 1257 is the average flow rate (L/s) 1,000 is the conversion from g to mg, 24 and 3,600 are used to convert days into seconds.

$$\frac{34.6}{0.93} = 37.2\ mg\ N/L$$

Assuming a removal efficacy of 93%, the total load would then be 37.2 mg/L.

Reduction potential for Line 1-5

The average reduction capacity for Line 1-5 was assumed to be 1.2 g N/(m^2·d) and that the first zones had a four-fold higher capacity.

$$\frac{1.92 \cdot 259{,}750 \cdot 1000}{24 \cdot 3{,}600 \cdot 217} = 26.6\ mg\ N/L$$

Where, 259,750 is the total effective area (m^2) and 217 is the wastewater flow (L/s)

$$\frac{26.6}{0.93} = 28.6\ mg\ N/L$$

An assumed nitrogen reduction of 93% results in a Nitrate load of 28.6 mg N/L.

Reduction potential for Line 6

The average reduction capacity of Line 6 was assumed to be 0.93 g N/(m^2·d) and that the first zone had a four-time higher capacity than that of the second zone. The capacity of the first zone would then be 1.49 g N/(m^2·d).

$$\frac{1.49 \cdot 259{,}750 \cdot 1{,}000}{24 \cdot 3{,}600 \cdot 172} = 26.0\ mg\ N/L$$

Where 172 is the flow rate (L/s)

$$\frac{26.0}{0.93} = 28.0\ mg\ N/L$$

An assumed nitrogen reduction of 93% results in a Nitrate load of 28.0 mg N/L.

The nitrate loads and reductions for the different lines with SDAs obtained from the experiments were calculated in the same fashion as above. The wastewater flow to Line 1, 3, and 5 were assumed to be 217 L/s, while the flow to Line 6 was 172 L/s. The effective area of the reactor was 259,750 m^2 and the nitrate reduction was assumed 93%.

Appendix VIII

Populärvetenskaplig sammanfattning

Övergödning av sjöar och hav sker till följd av för höga halter av näringsämnen som kväve och fosfor i naturen. Ökat utsläpp av dessa näringsämnen bidrar till en ökad tillväxt av alger som orsakar syrebrist på sjöbotten och därmed förhindrar överlevnad av andra vattenlevande organismer. Förmågan att kunna avskilja kväve och fosfor från avloppsvatten är därför ett viktigt medel för att kunna bevara och skydda miljön, vilket är avgörande för en hållbar samhällsutveckling.

För att kunna uppfylla sina åtaganden till Helsingforskommissionen måste flera svenska avloppsreningsverk uppgradera och utvärdera sina kvävebortföringsanläggningar de kommande åren. I vanliga fall renas avloppsvattnet med hjälp av biologiska processer, som nitrifikation och denitrifikation, där kvävet omvandlas till kvävgas som sedan kan släppas ut i atmosfären. Nackdelen med dessa processer är att det krävs externa kolkällor som till exempel metanol, för att uppnå denitrifikationsaktivitet samt att den nitrifierande kapaciteten är oftast begränsande till följd av långsam bakterietillväxt. På Sjölunda avloppsreningsverk i Malmö utvärderas möjligheten att införa nya kvävereningsprocesser som inte kräver externa kolkällor, som alternativ till den befintliga anläggningen. Samtidigt som nya alternativ evalueras är det även viktigt att studera kapaciteten för den befintliga anläggningen. Genom att utvärdera aktiviteten hos de kväveomvandlande bakterierna kan processernas effektivitet undersökas närmare.

På Sjölunda avloppsreningsverk består denitrifikationsanläggningen av sex linjer av bioreaktorer med rörligt bärarmaterial med två zoner i varje linje. Avloppsvattnet inträder i den första zonen i varje linje och det är även där metanolen tillsätts. Denna studie ämnade att undersöka aktiviteten i denitrifikationsanläggningen med hjälp av ett manometriskt labbtest som mäter tryckökningen, vilken är proportionell mot producerad mängd kvävgas.

Initiala koncentrationsmätningar med nitrat påvisade att aktiviteten var koncentrationsberoende för koncentrationer under 35 mg N/L. För initiala nitratkoncentrationer över 35 mg N/L var aktiviteten konstant. När en konstant aktivitet uppnås har biofilmen blivit fullständigt penetrerad med substrat. Experiment utan tillsatt kolkälla visade en låg denitrifikationsaktivitet vilket påvisade att denitrifierande bakterier kan lagra kol intracellulärt som kan användas vid kolbrist. Experiment utförda utan tillsatt nitrat visade försumbar denitrifikationsaktivitet.

Denitrifikationsaktiviteten mättes på bärare i fyra av anläggningens sex linjer. Aktiviteten visade sig variera mellan de olika linjerna. Vid jämförelse av de olika zonerna upptäcktes det att de första zonerna hade cirka 3-4 gånger högre aktivitet än de andra zonerna. Anledningen till detta antogs vara att de första zonerna har mer tillgång på näringsämnen då avloppsvattnet kommer in den vägen och även att det där som metanol tillsätts.

När driftdatan från verket analyserades under en fyramånadersperiod visade det sig, till skillnad från de experimentella resultaten, att alla linjer hade likvärdiga nitratreduktionseffektiviteter. Slutsatsen var då att variationerna jämnade ut sig då ett längre tidsperspektiv betraktades. Det intressanta med de experimentella resultaten var att skillnader mellan de olika zonerna kunde undersökas närmare då driftdatan endast betraktade varje linje i sin helhet.