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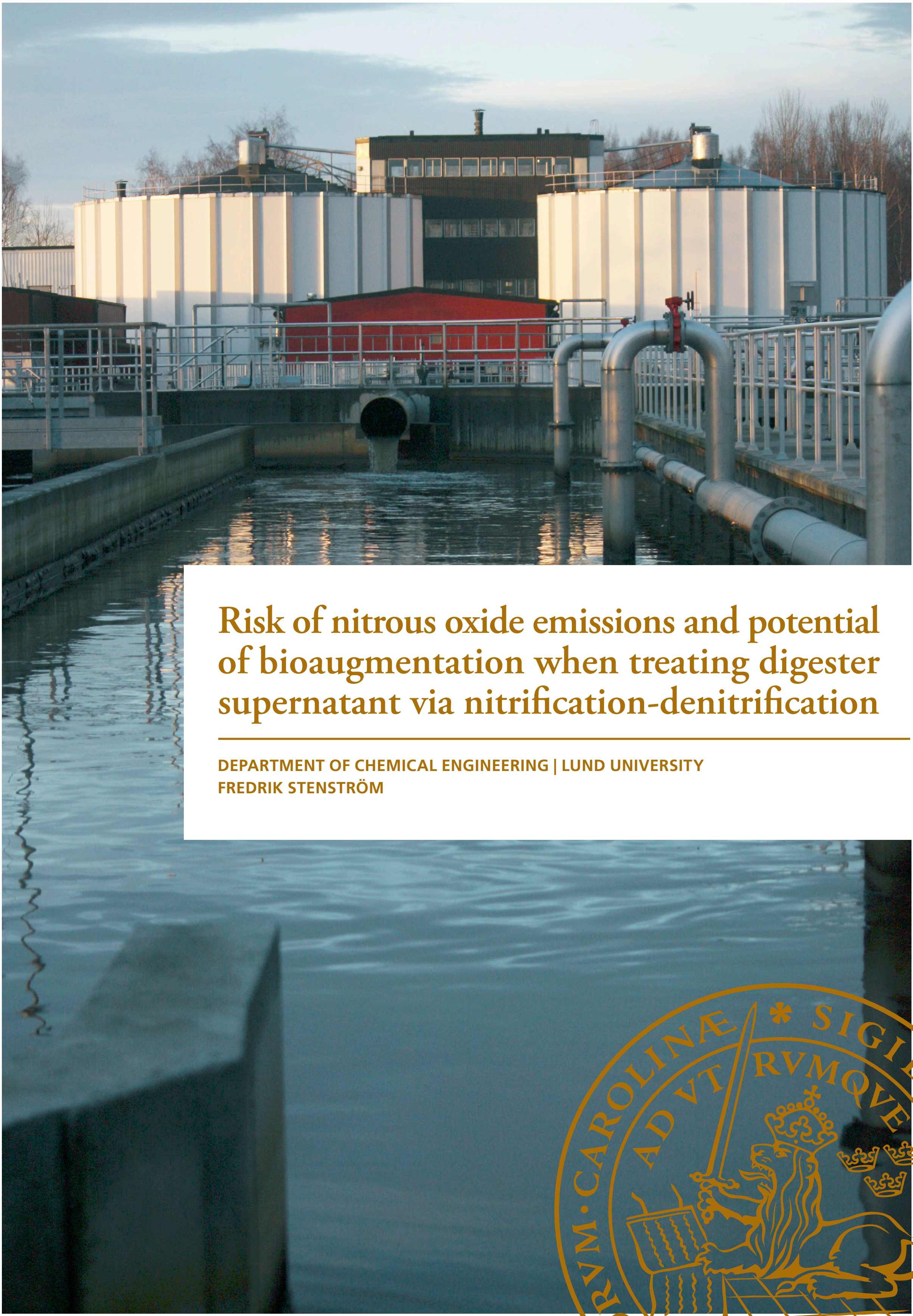

Risk of nitrous oxide emissions and potential of bioaugmentation when treating digester supernatant via nitrification-denitrification

DEPARTMENT OF CHEMICAL ENGINEERING | LUND UNIVERSITY
FREDRIK STENSTRÖM

Risk of nitrous oxide emissions and potential of bioaugmentation when treating digester supernatant via nitrification-denitrification

Fredrik Stenström

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Risk of nitrous oxide emissions and potential of bioaugmentation when treating digester supernatant via nitrification-denitrification

Abstract

This thesis examines two different impacts of sidestream treatment of digester supernatant via nitrification-denitrification in a sequenced batch reactor (SBR). One of the impacts is the detrimental formation of nitrous oxide, and the other is the positive boosting of nitrifiers to the mainstream process through bioaugmentation. The studies have been carried out in a full-scale wastewater treatment plant.

Different operating conditions were investigated in order to find thresholds for where the formation of nitrous oxide is obviously increased: low oxygen concentration during nitrification and low dosage of external carbon during denitrification. It was found that the nitrous oxide formation during nitrification was sharply increased when the oxygen concentration was lower than 1.0–1.5 mg O_2/L. It was also found that it is important to maintain a sufficient dosage of external carbon during denitrification to avoid formation of nitrous oxide during anoxic conditions. The emissions of nitrous oxide were considerably lowered with a carbon dosage corresponding to more than 4 kg COD/kg TN in the influent than with a carbon dosage of lower than 2.5 kg COD/kg TN. Nitrifier denitrification and incomplete denitrification are believed to be the main pathways under oxic and anoxic conditions, respectively. The nitrous oxide emissions were also modeled. It was shown that the model was capable of partly reproducing the emissions. However, additional work is required to predict the emissions with high certainty by simulation.

The boosting of nitrifiers from a sidestream reactor to the mainstream process has been studied: bioaugmentation. The effect of bioaugmentation was evaluated through nitrification rate measurements and analyses of nitrifiers by molecular methods. The measurements demonstrated that the nitrification rate increased by more than 40% during the coldest weeks and 25% during the whole studied period. The molecular methods showed an increased abundance of nitrifiers of 25% during the whole studied period, and thus consistent with the results from the nitrification rate measurements. Furthermore, the total number of nitrifying species increased during the bioaugmentation.

Key words

Bioaugmentation, digester supernatant, inoculation, nitrification-denitrification, nitrifiers, nitrous oxide emissions, reject water

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”Så som fadern har älskat mig, så har jag älskat er.
Bli kvar i min kärlek.”

Johannesevangeliet 15:9

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PREFACE

This thesis is the outcome of eight years of part-time study. During the studies, I have been associated with the department of Chemical Engineering at Lund University, but I have lived and been situated in the city of Örebro, 500 km north of Lund. This set-up was a prerequisite for the studies, but not uncomplicated. One of the things that I missed most with this set-up are the daily discussions with my colleagues at Lund University.

Before I started my PhD studies, I worked for several years as a project manager at Veolia Water Technologies – VA-Ingenjörerna. The company works in both consulting and as an entrepreneur, and so did I as a project manager. During those years, I achieved a broad knowledge of different wastewater processes and about the process of how to reconstruct an existing wastewater treatment plant (WWTP), or construct a new one. Nevertheless, I always found process design and the issues with regard to the biological processes to be the most thrilling part of the projects. When I finally got the chance to start as a PhD student and dig a little deeper into the biological processes, I gladly took the chance without a doubt.

During these years, I had the opportunity to learn a lot of new things. I found this process of absorbing new knowledge very stimulating, and I am grateful for that. Besides the studies described in this thesis, I also took on studies that are not presented. At the beginning of my PhD studies, I examined the α-value of wastewater mixed with digester supernatant at some WWTPs. I also made an attempt on modeling the bioaugmentation of nitrifiers in the WEST software (DHI). However, for different reasons, this was never completed.

Performing experiments in full scale is challenging. A study could be planned in meticulous details, but reality rules and anything can happen that might disturb or ruin experiments. The full-scale studies performed in the frame of this thesis are no exception: malfunction of the blower machine, unplanned stop of the decanter centrifuges, and troubles with the dosage of external carbon source were some of the problems that occurred and had to be handled.

Although many hours were spent in these studies, resulting in this thesis, I am well aware that this contribution is like a small piece of the puzzle, or like a small drop in the huge ocean of new advances in biological treatment of digester supernatant. But hopefully a drop that can make a difference.

ABSTRACT

This thesis examines two different impacts of sidestream treatment of digester supernatant via nitrification-denitrification in a sequenced batch reactor (SBR). One of the impacts is the detrimental formation of nitrous oxide, and the other is the positive boosting of nitrifiers to the mainstream process through bioaugmentation. The studies have been carried out in a full-scale wastewater treatment plant.

Different operating conditions were investigated in order to find thresholds for where the formation of nitrous oxide is obviously increased: low oxygen concentration during nitrification and low dosage of external carbon during denitrification. It was found that the nitrous oxide formation during nitrification was sharply increased when the oxygen concentration was lower than 1.0–1.5 mg O_2/L. It was also found that it is important to maintain a sufficient dosage of external carbon during denitrification to avoid formation of nitrous oxide during anoxic conditions. The emissions of nitrous oxide were considerably lowered with a carbon dosage corresponding to more than 4 kg COD/kg TN in the influent than with a carbon dosage of lower than 2.5 kg COD/kg TN. Nitrifier denitrification and incomplete denitrification are believed to be the main pathways under oxic and anoxic conditions, respectively. The nitrous oxide emissions were also modeled. It was shown that the model was capable of partly reproducing the emissions. However, additional work is required to predict the emissions with high certainty by simulation.

The boosting of nitrifiers from a sidestream reactor to the mainstream process has been studied: bioaugmentation. The effect of bioaugmentation was evaluated through nitrification rate measurements and analyses of nitrifiers by molecular methods. The measurements demonstrated that the nitrification rate increased by more than 40% during the coldest weeks and 25% during the whole studied period. The molecular methods showed an increased abundance of nitrifiers of 25% during the whole studied period, and thus consistent with the results from the nitrification rate measurements. Furthermore, the total number of nitrifying species increased during the bioaugmentation.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Att rena avloppsvatten är viktigt för miljön och för människan. Rening av avloppsvatten innebär främst att organiskt material och fosfor reduceras. Sedan 90-talet har det även införts krav på rening av kväve vid flera reningsverk i Sverige. Efter att vattnet har renats kvarstår en restprodukt vid avloppsreningsverket – slammet. Detta slam innehåller organiskt material, det rötas i rötkammare för att utvinna energirik gas. Efter rötningen tas en stor del av vattnet bort från slammet som då får en jordliknande konsistens och kan användas exempelvis som jordförbättringsmedel. Vattnet som tas bort från slammet kallas rejektvatten och innehåller hög halt av kväve. Rejektvattnet motsvarar endast ca 1% av vattenflödet till ett reningsverk men dess kväveinnehåll motsvarar ca 20% av kvävemängden till verket. Att införa separat rening av rejektvattnet kan därför ha stor betydelse för den totala kvävereningen vid avloppsreningsverk.

Det finns ett antal olika biologiska metoder för separat rening av rejektvatten. En av metoderna är via nitrifikation-denitrifikation i en satsvis biologisk reaktor, en så kallad SBR. Detta är fortfarande den vanligaste metoden även om det under de senaste åren utvecklats nya metoder som bland annat innebär lägre energiåtgång. Vid nitrifikation-denitrifikation omvandlas kvävet i avloppsvattnet med bakteriers hjälp till kvävgas och avgår till atmosfären, som redan till stor del består av just kvävgas.

Denna avhandling undersöker miljörisker samt möjligheter till förbättrad kväverening vid avloppsreningsverk som tillämpar rejektvattenrening via nitrifikation-denitrifikation i en SBR. En av de större miljöriskerna är att det bildas lustgas i stället för kvävgas. Lustgas är en kraftig växthusgas och har dessutom en nedbrytande effekt på ozonskiktet. Lustgasbildningen bör därför hållas så låg som möjligt. En förbättrad kväverening innebär att en större mängd kväve kan behandlas i reningsverkets befintliga bassänger. I takt med att städerna förtätas är detta en högintressant teknik eftersom den innebär en kompakt reningsprocess och att en utbyggnad av reningsverket kan undvikas.

Vi har undersökt lustgasproduktionen från en SBR för biologisk kväverening av rejektvatten vid Slottshagens avloppsreningsverk i Norrköping. Olika driftförhållanden har undersökts för att hitta tröskelvärden då lustgasproduktionen ökar i syfte att ge riktlinjer för vilka driftförhållanden som bör undvikas. Resultaten visar att bildningen av lustgas är betydligt lägre då reningsprocessen drivs med tillräckligt hög syrehalt och med tillräcklig dosering av kolkälla till bakterierna.

I denna avhandling har vi även undersökt om det är möjligt att förbättra kvävereningen i ett reningsverk genom att koppla samman verkets huvudlinje med dess rejektvattenbehandling. Syftet är att en av de viktigare bakterierna vid kvävereduktion – nitrifierarna – ökar i antal, därmed blir kvävereningen bättre i huvudlinjen. Våra resultat visar tydligt att antalet nitrifierare ökade och att den kraftigaste ökningen uppstod under de kallaste vinterveckorna; det är också då det behövs som bäst. Genom denna teknik kan därför en större mängd kväve renas vid ett reningsverk utan att fler bassänger behöver byggas.

LIST OF PAPERS

Paper I: Stenström, F., Tjus, K. & Jansen, J. la Cour. (2014). Oxygen-induced dynamics of nitrous oxide in water and off-gas during the treatment of digester supernatant. *Water Science & Technology*, *69*(1), 84–91.

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

Paper III: Stenström, F. & la Cour Jansen, J. (2016). Promotion of nitrifiers through side-stream bioaugmentation: a full-scale study. *Water Science & Technology*, *74*(7), 1736–1743.

Paper IV: Stenström, F. & la Cour Jansen, J. (2016). Impact on nitrifiers of full-scale bioaugmentation. *Accepted for publication in Water Science & Technology*, DOI: 10.2166/wst.2017.480.

Paper V: Stenström, F., Baresel, C., la Cour Jansen, J. (2013). Nitrous oxide production under varied C/N-ratio and DO in an SBR treating digester supernatant. Conference presentation, *NORDIWA 2013, The Nordic Wastewater Conference*, 8–10 October, Malmö, Sweden. The paper can be retrieved from http://lup.lub.lu.se/record/32d38a59-aafd-4a57-be63-d8413790bf3a.

MY CONTRIBUTION TO THE PAPERS

Paper I: I designed the full-scale experiment with my supervisor Jes la Cour Jansen. I performed the experiments at the WWTP with help with chemical analyses from Gertrud Persson at the Department of Chemical Engineering, Lund University. Kåre Tjus at IVL Swedish Environmental Research Institute performed the measurements of nitrous oxide in water and off-gas. I wrote the paper and received comments from the co-authors.

Paper II: I designed the full-scale experiment with my supervisor Jes la Cour Jansen. The data from the experiment were used in the modeling study performed by Erik Lindblom at Stockholm Vatten. Erik Lindblom wrote the manuscript for the paper, which I and the other co-authors commented on.

Paper III: I designed the full-scale experiment with my supervisor Jes la Cour Jansen. I performed the experiment at the WWTP, including the nitrification rate tests and some of the chemical analyses. I had help with many of the chemical analyses from the laboratory staff at the WWTP. I wrote the paper and received comments from Jes la Cour Jansen.

Paper IV: I designed the full-scale experiment with my supervisor Jes la Cour Jansen. I performed the experiment at the WWTP. Grab samples from the biological reactors were sent to DNA Sense in Aalborg for 16S rRNA amplicon sequencing. I analyzed the data from the amplicon sequencing. I wrote the paper and received comments from my supervisors.

Paper V: I designed the full-scale experiment with my supervisor Jes la Cour Jansen, except from the part with regard to the long period study of changed dosage of external carbon. This part was designed and analyzed by Christian Baresel at IVL Swedish Environmental Research Institute . I performed the experiments at the WWTP with help with chemical analyses from Gertrud Persson at the Department of Chemical Engineering, Lund University. Kåre Tjus at IVL performed the measurements of nitrous oxide in the off-gas. I wrote the paper and received comments from the co-authors.

RELATED PUBLICATIONS

Stenström, F., la Cour Jansen, J., Andersson Chan, A., Eliasson, M., Eriksson, Y., Marsteng, A-K., Sehlén, R. & Thelin, G. (2017). *Rejektvattenbehandling – en kunskapssammanställning* (Treatment methods for digester supernatant – a knowledge compilation), Report 2017-11, The Swedish Water and Wastewater Association, Stockholm, Sweden.

ABBREVIATIONS

ANAMMOX	ANaerobic AMMonium OXidation
AOB	Ammonia-oxidizing bacteria
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
DO	Dissolved oxygen
HRT	Hydraulic retention time
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
N	Nitrogen
NH_4^+	Ammonium
NOB	Nitrite-oxidizing bacteria
NO_2^-	Nitrite
NO_3^-	Nitrate
PO_4^{3-}	Phosphate
RAS	Return activated sludge
SRT	Solids retention time
TN	Total nitrogen
TSS	Total suspended solids
VSS	Volatile suspended solids
WAS	Waste activated sludge
WWTP	Wastewater treatment plant

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1 INTRODUCTION

1.1 BACKGROUND

Biological wastewater treatment is the largest biotechnological industry in the world (Mielczarek et al., 2013), and it is crucial for protecting the environment and human health. The purpose of a wastewater treatment plant (WWTP) is to remove the constituents in the wastewater that cause oxygen depletion and eutrophication — essentially carbon, nitrogen and phosphorus.

Digester supernatant is formed during the dewatering of digested sludge. The nitrogen load to a WWTP corresponds to 12–14 g per person and day. Thirty to forty percent of this are found in the total suspended solids (TSS) from the mainstream process and are conveyed to the sludge handling (Siegrist, 1996). When the sludge is degraded during digestion, the concentration of some constituents increases, for example nitrogen. About 50% of the nitrogen conveyed to the digesters are found in the digester supernatant. Consequently, the amount of nitrogen from the sludge handling corresponds to 15–20% of the nitrogen in the influent to a WWTP (Siegrist, 1996). However, the highly concentrated digester supernatant constitutes only 0.5–1.5% of the influent flow rate to a WWTP.

Some of the most characteristic features of digester supernatant are the high concentration of nitrogen and the high temperature. These qualities make it favorable for separate treatment. Furthermore, the low concentration of chemical oxygen demand (COD) is an advantage for some of the treatment methods. The mole ratio of alkalinity/NH_4-N is often 1.1 or higher (alkalinity as HCO_3). Typical compositions, according to literature, of ordinary wastewater and digester supernatant are compared in Table 1.1. The same comparison is presented in Table 1.2 but with data collected from WWTPs in Sweden and northern Europe according to Stenstrom et al. (2017).

A high degradation of sludge is desired in order to gain as much energy as possible (in the form of methane gas) to achieve a well-stabilized product and to minimize shipping costs. The development of improved techniques for a higher degradation of sludge is constant. Moreover, more stringent standards for hygienization of sludge are expected in Sweden and in many other European countries, implying that a higher degradation of sludge is likely at many WWTPs. A higher degradation of organic matter will lead to a higher concentration of degradants in the digester supernatant, with a higher concentration of nitrogen among others (Carrère et al., 2010). Besides, in order to decrease the energy consumption when heating the sludge, many WWTPs aim to increase the TSS concentration to the digesters. This will imply higher retention times in the digesters and result in an increased concentration of nitrogen in the digester supernatant.

Table 1.1. Typical composition of municipal wastewater and digester supernatant.

		Municipal wastewater	Digester supernatant
BOD_7	(mg/L)	125–400 [a, b]	300–4000 [d]
COD	(mg/L)	250–800 [a]	700–9000 [d]
TN	(mg/L)	20–70 [a]	120–800 [d]
NH_4-N	(mg/L)	12–45 [a]	100–500 [d]
TP	(mg/L)	4–12 [a]	15–300 [d, e]
TSS	(mg/L)	120–400 [a]	500–10000 [d]
Alkalinity	(mg HCO_3/L)	180–430 [c, d]	180–2500 [d, f]
Temperature	(°C)	8–20	25–35

[a] Metcalf & Eddy, 2003.
[b] Calculated according to $BOD_7 = BOD_5 * 1.15$.
[c] Converted from ekv/m^3. The alkalinity of the potable water in the area has an impact on the value.
[d] Henze et al., 2002.
[e] The highest values are formed at WWTPs with enhanced biological phosphorus removal.
[f] Converted from ekv/m^3.

Table 1.2. Typical composition of municipal wastewater and digester supernatant, according to Stenström et al. (2017).

		Municipal wastewater		Digester supernatant	
		Interval	Median	Interval	Median
BOD_7 [b]	(mg/L)	150–340	230 (n=8) [a]	140–700	200 (n=4)
COD	(mg/L)	370–620	500 (n=7)	200–2500	850 (n=4)
TN	(mg/L)	27–52	37 (n=8)	700–1800	1100 (n=6)
NH_4-N	(mg/L)			620–1500	900 (n=9)
TP	(mg/L)	3.3–9.3	5.2 (n=8)	15–240	60 (n=6)
TSS	(mg/L)			200–1700	470 (n=7)
Alkalinity	(mg HCO_3/L)			4000–5300	4900 (n=4)
Temperature	(°C)			23–35	28 (n=9)

[a] ”n” is the number of values (WWTPs) for interval and median.
[b] BOD concentration given as BOD_5 has been recalculated according to $BOD_7 = BOD_5 * 1.15$.

The main task for a treatment plant intended for digester supernatant is to remove the content of nitrogen, and if performed in a sustainable, economic and low energy-consumption way, the better it is. After this has been fulfilled, one of the worst things that can occur is if the treatment plant produces emissions of nitrous oxide (N_2O), because this is one of the most potent and hazardous greenhouse gases. In contrast, one of the best things that can occur is if it can be used for the extra boost of nitrifiers to the mainstream process, like in bioaugmentation.

N_2O is one of the most potent greenhouse gases. It is 298 times more potent as a climate gas than carbon dioxide, based on a time horizon of 100 years (Forster et al., 2007). Furthermore, it is the third largest contributor to climate change, after carbon dioxide and methane (Forster et al., 2007), and has been identified as the single most important ozone-depleting gas emitted in the 21st century (Ravishankara et al., 2009). Over the last few decades, emissions from nitrous oxide in biological wastewater treatment have been elucidated. Because the concentration of nitrogen is considerably higher in digester supernatant than in normal municipal wastewater, the risk for higher emissions is obvious. Many studies have been performed during the last 10–15 years to understand what process conditions trigger or mitigate nitrous oxide emissions from wastewater treatment.

Among the different processes available for treating digester supernatant, bioaugmentation from a separate reactor is the only one that obviously improves the mainstream process. Consequently, when a separate treatment of the digester supernatant is combined with a deliberate inoculation to the mainstream process, there is a double effect: reducing the total nitrogen (TN) load in the digester supernatant and boosting the mainstream process.

Bioaugmentation means inoculation of bacteria, and in this case it is inoculation of the slowest growing bacteria in conventional wastewater treatment — the nitrifiers. Because nitrifiers grow slowly and are sensitive to cold temperature, the volume needed for nitrification dominates the biological reactors.

1.2 AIM

This thesis aims to evaluate different risks and possibilities of treating digester supernatant via nitrification-denitrification in a sidestream sequenced batch reactor (SBR). The risks concern the formation and emission of nitrous oxide, and the possibilities refer to the positive boosting of nitrifiers from the sidestream treatment to the mainstream process. To meet this aim, the following research questions need to be answered:

- In order to reduce the emission of nitrous oxide from sidestream plants, is it possible to discern different thresholds in the operating conditions where the production of nitrous oxide is obviously increased?
- As a complement to on-site measurements of nitrous oxide emissions, is it possible to model and predict the emissions in an accurate way?

- If bioaugmentation is applied, would the nitrifying capacity in the mainstream process be obviously increased?
- Does bioaugmentation have any impact on the microbial composition and diversity of nitrifiers in the mainstream process?

1.3 OUTLINE OF THE THESIS

Chapters 2 and 3 present basic knowledge of biological treatment of nitrogen and of digester supernatant, respectively. Also, in chapter 3 some different benefits and drawbacks with separate treatment of digester supernatant are discussed. The more experienced reader can proceed directly to chapters 4–6, where the performed experiments and the results are presented.

In chapter 4, the different analytical methods, experimental set-ups and experimental plans are described.

The outcome from the studies of nitrous oxide emissions are presented and discussed in chapter 5 and in **Papers I, II** and **V**.

The results from the studies of bioaugmentation are presented and discussed in chapter 6 and in **Papers III** and **IV**.

Finally, a conclusion of this thesis is presented in chapter 7, and some suggestions of futures studies are outlined in chapter 8.

2 BIOLOGICAL TREATMENT OF NITROGEN

Nitrogen is a prerequisite for all living organisms. It is included in nucleic acids and amino acids, which constitute the basis of DNA and proteins. Nevertheless, nitrogen in wastewater will cause eutrophication in the recipients if it is not removed. In the WWTP process, the main methods of reducing nitrogen in the wastewater is through assimilation, nitrification-denitrification or, introduced in recent decades, through deammonification (anammox).

The nitrogen cycle is shown in Figure 2.1. The main pathways for nitrogen removal in wastewater treatment are discussed in the following chapters.

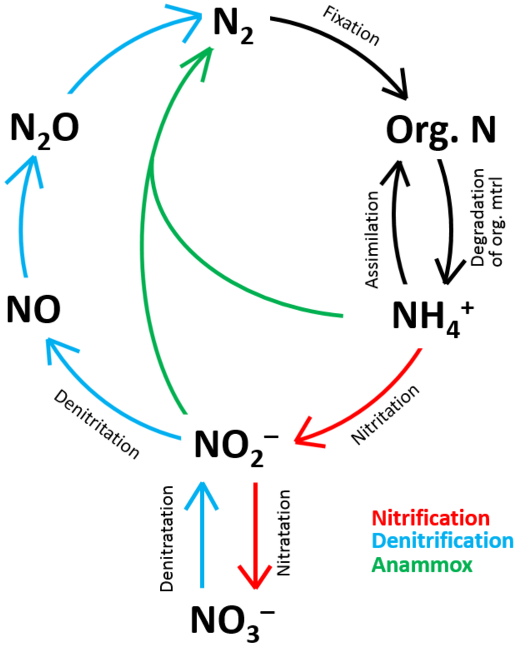
N2
Fixation
Org. N
N2O
Assimilation
Degradation of org. mtrl
NO
NH4+
Denitritation
Nitritation
NO2−
Denitratation
Nitratation
Nitrification
Denitrification
Anammox
NO3−

Figure 2.1. The nitrogen cycle including the main forms of nitrogen and major pathways.

At WWTPs, nitrogen in wastewater is absorbed and used by microorganisms for their metabolism, so-called assimilation. Bacteria assimilate ammonium, and if ammonium is unavailable several different denitrifying bacteria can transform nitrate to ammonium and use it in its metabolism (Halling-Sørensen & Jørgensen, 1993). Assimilation accounts for about 20% of the nitrogen reduction at a municipal WWTP (Ekama & Wentzel, 2008b). Nevertheless, the magnitude of assimilation depends on the solids retention time (SRT). The higher the SRT the lower the sludge production and, consequently, the lower the assimilation of nitrogen. Because nitrifiers only constitute 2–4% of the biomass at a WWTP (Ekama & Wentzel, 2008b), the largest part of nitrogen assimilation is performed by heterotrophs.

Apart from assimilated into the bacteria cells in the biological reactors, nitrogen is also included in particulate material from the primary settlers. Altogether, 30–40% of the nitrogen that enters a WWTP is conveyed to the sludge handling (Siegrist, 1996). The sludge treatment at the plant most often includes digestion. During the sludge digestion, about 50% of the nitrogen in the sludge is released as ammonium and found in the digester supernatant (Siegrist, 1996). Consequently, about 20% of the nitrogen that enters a WWTP could be reduced in separate sidestream treatment. A conceptual process scheme of a typical WWTP with conventional nitrification-denitrification is shown in Figure 2.2 together with a mass balance for nitrogen.

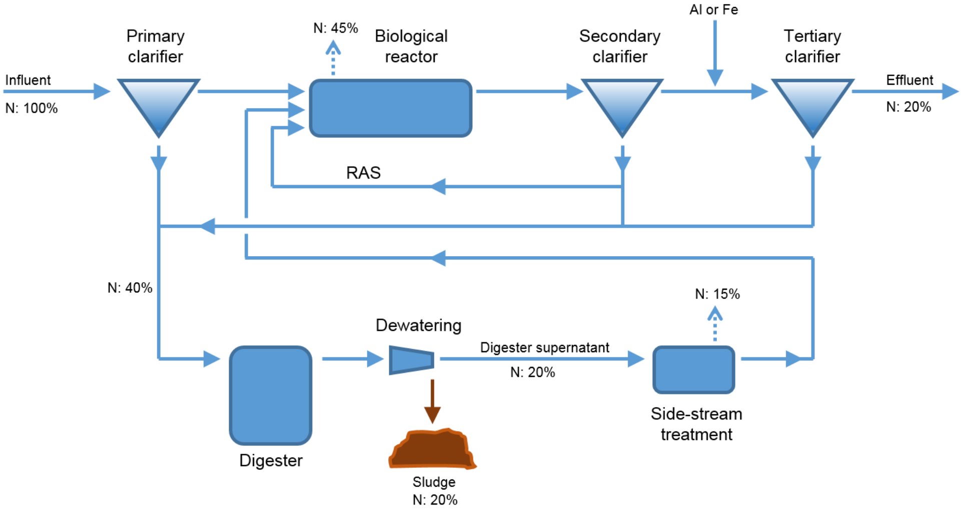
Al or Fe
Primary clarifier
N: 45%
Biological reactor
Secondary clarifier
Tertiary clarifier
Influent
N: 100%
Effluent
N: 20%
RAS
N: 40%
N: 15%
Dewatering
Digester supernatant
N: 20%
Side-stream treatment
Digester
Sludge
N: 20%

Figure 2.2. Conceptual process scheme of a WWTP applying nitrogen removal and separate treatment of digester supernatant. A rough mass balance for nitrogen (N) is included.

2.1 NITRIFICATION

Nitrification is the oxidation of ammonium (NH_4^+) performed by, for example, autotrophic bacteria, via hydroxylamine (NH_2OH) to nitrite (NO_2^-) by ammonia-oxidizing bacteria (AOB), which is further oxidized to nitrate (NO_3^-) by nitrite-oxidizing bacteria (NOB). AOB and NOB use NH_4^+ and NO_2^-, respectively, as the electron donor (i.e., energy source), oxygen as the electron acceptor, and carbon dioxide as the carbon source.

Simplified partial reactions for nitrification are:
$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+$ (nitritation, performed by AOB)
$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$ (nitratation, performed by NOB)

The simplified total reactions is:
$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + 2\ H^+$

The theoretical consumption of oxygen for nitrification is 4.57 g O_2/g N. From the above reactions it can be seen that 75% of the oxygen is consumed in the nitritation: 3.43 g O_2/g N. The other 1.14 g O_2/g N is consumed in the nitratation. Furthermore, the reactions show that hydrogen ions are produced during nitritation and the alkalinity is decreased. The consumption of alkalinity corresponds to 8.71 g HCO_3^-/g NH_4^+-N. The actual electron donor for AOB is un-ionized ammonia (NH_3) and not ammonium. Similarly, the actual electron donor for NOB is un-ionized nitrous acid (HNO_2) and not nitrite (Anthonisen et al., 1976).

A more complete total reaction of nitrification also includes cell growth of bacteria (Crites & Tchobanoglous, 1998):
$NH_4^+ + 1.863\ O_2 + 0.098\ CO_2 \rightarrow$
$\rightarrow 0.0196\ C_5H_7O_2N + 0.98\ NO_3^- + 0.0941\ H_2O + 1.98\ H^+$

In this reaction, the chemical term $C_5H_7O_2N$ represents new biomass of bacteria. From the reaction it is shown that the oxygen consumption and production of hydrogen ions become somewhat lower when the cell growth is included. This is because some part of the ammonium is incorporated in new cells instead of being oxidized.

In conventional biological nitrogen removal, nitrification is a slower process than denitrification. Moreover, it is more affected by a low temperature than denitrification, implying that a bigger volume is needed for nitrification during the cold season. Consequently, nitrification is the process that has the strongest influence on the design of the biological reactors' volume.

Nitritation, the oxidation of ammonium to nitrite, is included in all biological treatment methods of digester supernatant. Thereafter, the continued treatment varies depending on which process route will be used: continued oxidation (conventional nitrification), denitritation to form nitrogen gas, or biochemical reaction with ammonium to form nitrogen gas (anammox). Because nitrification or more precisely nitritation is included in all biological treatment methods of digester supernatant, nitrification will be studied somewhat closer. Nitrification is affected by several different parameters. The most important parameters are well described in literature (Parker & Wanner, 2007; Metcalf & Eddy, 2003; Henze et al., 2002), which are:

- Temperature
- Dissolved oxygen (DO) concentration
- Concentration of substrate (ammonium concentration)
- pH and alkalinity
- Toxic substances

2.1.1 Temperature

Nitrifiers are sensitive to temperature, and more sensitive than heterotrophs (Henze et al., 2002; Metcalf & Eddy, 2003). One of the reasons for this is that different species of heterotrophs can dominate the bacteria community at different temperatures. Psychrophilic heterotrophs can dominate at a lower temperature and mesophilic heterotrophs can dominate at a higher temperature (Wijffels et al., 1995).

Nitrifiers have a temperature optima at 30–35 °C. A higher temperature than 35–40 °C will result in a dramatically reduced activity, shown in Figure 2.3.

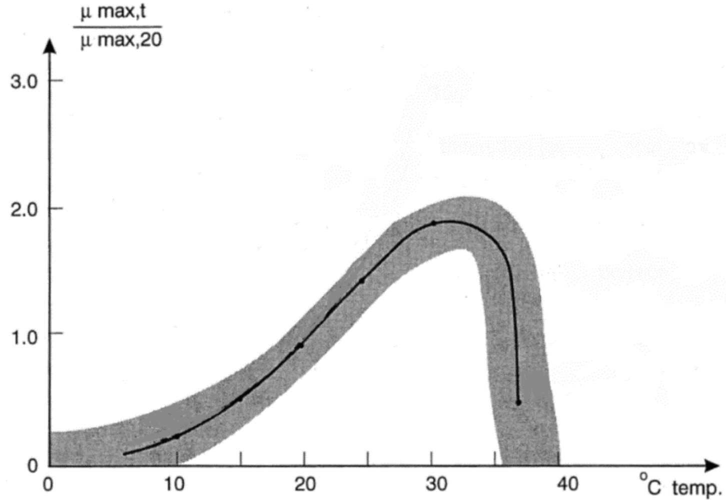

Figure 2.3. Maximal nitrification rate as a function of temperature (modified from Henze et al., 2002). Printed with permission from the authors.

The temperature correction factor for the specific growth rate of nitrifying bacteria ranges from 1.072 to 1.127 (Head & Oleszkiewicz, 2004). In colder climate regions, the temperature difference between the mainstream process and a sidestream reactor for digester supernatant could be 15–25 °C. Hence, the required SRT needs to be considerably longer in the mainstream process than in the sidestream reactor. This will result in a substantially bigger volume needed for nitrification of the same amount of ammonium in the mainstream process compared to separate treatment. Moreover, the nitrification rate is greatly reduced by a sudden temperature drop than by a gradual temperature decrease (Hwang & Oleszkiewicz, 2007). The big difference in the nitrification rate at different temperatures implies a big difference in required reactor volume. In a historical perspective, this has been one of the major arguments for separate treatment of digester supernatant. AOB and NOB have different optimal growth rates at different temperatures. At a temperature lower than 20–25 °C, NOB grow faster than AOB and vice versa at a higher temperature (Hellinga et al., 1998).

2.1.2 DO concentration

The nitrification rate is affected by the DO concentration and the transfer of oxygen. In turn, the efficiency of the oxygen transfer to the microorganisms is affected by the size and density of the bioflocs or the thickness of a biofilm. The affinity for oxygen is lower for nitrifiers than for heterotrophs (Henze et al., 2002). This implies that the highest growth rate for nitrifiers is achieved at a higher DO concentration than for heterotrophs. In an activated sludge system, the nitrification rate is commonly specified to increase up to a DO concentration of 3–4 mg O_2/L, and is then unaffected even if the DO concentration is further increased (Metcalf & Eddy, 2003). The correlation between nitrification rate and a DO concentration up to 3 mg O_2/L is shown in Figure 2.4.

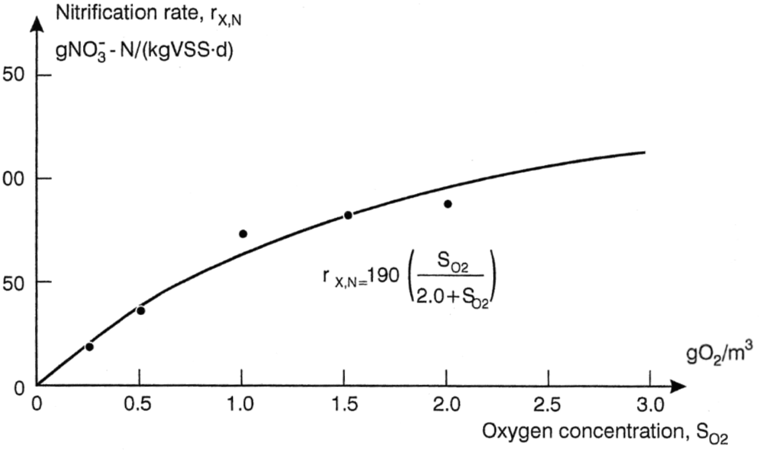

Figure 2.4. Nitrification rate as a function of oxygen concentration in an activated sludge system (Henze et al., 2002). Printed with permission from the authors.

2.1.3 Substrate concentration

The true substrate for nitrifiers is ammonia and nitrous acid, which are in equilibrium with ammonium and nitrite, respectively. The nitrification rate is often described as a relationship to the concentrations of ammonium and nitrite, which, is not quite correct. Many studies of nitrification rates show that the rate depends on the ammonium concentration up to a certain concentration (e.g., Downing et al., 1964). When the concentration is further raised the nitrification rate will not increase. Thus, above this ammonium concentration the relationship seems to be of a zero reaction order. Different studies show different results of how high this certain ammonium concentration is. In a simulation study on nitrification of ammonium to nitrite in an SBR, Gao et al. (2010) showed that the nitrification rate increased up to an ammonium concentration of 5–15 mg NH_4^+-N/L, which is shown in Figure 2.5 A, where it also outlines how different DO concentrations affect the nitrification rate. Dinçer & Kargi (2000) performed a study that revealed that the nitrification rate increased up to a ammonium concentration of 30–50 mg NH_4^+-N/L (see Figure 2.5 B). It is noteworthy that the nitrification rate was slightly increased even above this concentration, which is a benefit with regard to nitrification of digester supernatant.

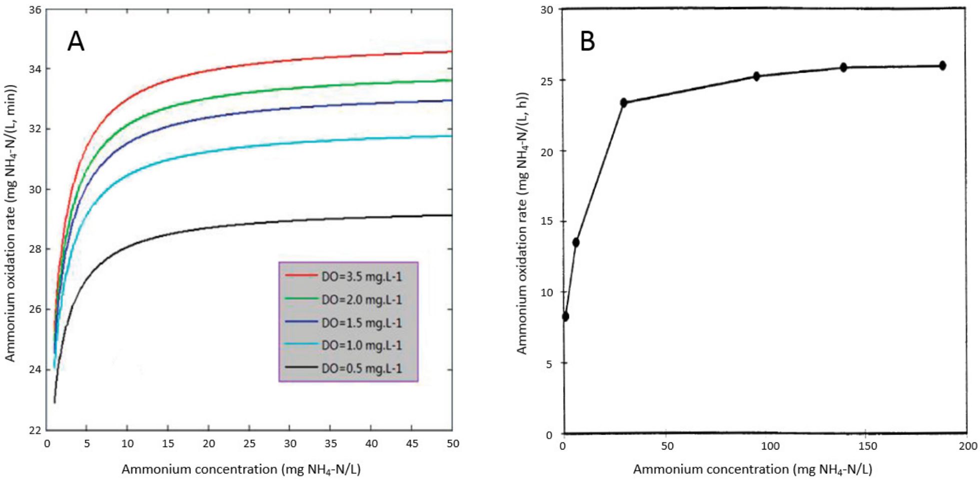

Figure 2.5. Nitrification rate as a function of ammonium concentration. A: Results from a simulation study at different DO concentrations (Gao et al., 2010). Note that the y-axis does not start at zero. B: Results from a lab-scale study of nitrification rate in activated sludge (Dinçer & Kargi, 2000). Printed with permission from American Chemical Society and Elsevier, respectively.

2.1.4 pH and alkalinity

Nitrifiers are more sensitive to changes in pH than heterotrophs. Extracted from different studies, Sharma & Ahlert (1977) and Shammas (1986) compiled how pH affects nitrification. The compilations refer to nitrifiers as a group (AOB + NOB) and show a large range for the optimal pH. Nevertheless, the optimal pH could be stated to be in the range of 8 ± 0.5. However, it should be emphasized that the optimal pH differs between different nitrifiers and different WWTPs. Park et al. (2007) performed a study with different AOB and NOB and showed that the optimal pH was slightly higher for AOB than for NOB: 8.2 ± 0.3 and 7.9 ± 0.4, respectively. Furthermore, the pH range within which more than 50% of the nitrification rate was maintained was wider for AOB than NOB; 3.1 ± 0.4 and 2.2 ± 0.4, respectively.

The nitrification rate declines rapidly outside the optimal pH range. The affect of this is accented in biological methods that include a varying pH, as in processes based on different batches such as for an SBR. An example of the narrow range for optimal pH is illustrated in Figure 2.6 from a lab-scale study of Massone et al. (1998) at activated sludge. Optimal pH in the study was in the range of 7.6–8.5. Outside this range, the nitrification rate was halved at pH 7.4 and 8.9, respectively.

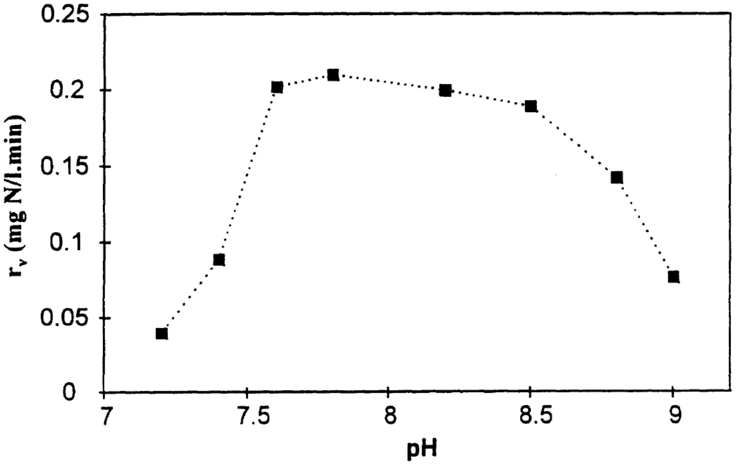

Figure 2.6. Nitrification rate as a function of pH during nitrification in an activated sludge process, from a lab-scale study performed by Massone et al. (1998). Printed with permission from the Water Environment Federation.

The alkalinity is decreased during nitrification. Theoretically, 8.71 g HCO_3^- is consumed per 1 g oxidized NH_4^+-N. The decrease of pH during nitrification will be limited as long as the alkalinity is high. Nevertheless, if the alkalinity drops below 50 mg HCO_3^-/L, the pH becomes unstable (van Loosdrecht, 2008). This will imply a more accentuated decrease in pH at a continued alkalinity drop. At pH < 5.8 the nitrification stops (Henze et al., 2002). The impact on nitrification from pH and alkalinity is further discussed in chapter 2.1.5.

2.1.5 Inhibiting conditions and substances

Free ammonia (NH_3) and free nitrous acid (HNO_2) have an inhibiting effect on nitrifiers if the concentrations are too high. Simultaneously, these components are also the substrate (electron donors) for AOB and NOB, respectively. The concentrations of free ammonia and free nitrous acid vary with pH, temperature, ammonium concentration, and nitrite concentration. From Anthonisen et al. (1976), the following can be stated with regard to nitrifiers, free ammonia and free nitrous acid:

- AOB are inhibited by:
  - NH_3 at concentrations $\geq$ 10–150 mg/L
  - HNO_2 at concentrations $\geq$ 0.2–2.8 mg/L

- NOB are inhibited by:
  - NH_3 at concentrations $\geq$ 0.1–1.0 mg/L
  - HNO_2 at concentrations $\geq$ 0.2–2.8 mg/L (as for AOB)

- The range for when inhibiting occurs, according to the intervals above, can depend on:
  - Acclimatization of the bacteria at high concentrations
  - Temperature
  - The amount of nitrifiers

It should be noted that NOB are inhibited by lower concentrations of free ammonia than AOB.

More recent research results suggest that the inhibition effect from high concentration of free ammonia and free nitrous acid is somewhat exaggerated, and that low concentration of bicarbonate (alkalinity) has a stronger impact on inhibition of nitrifiers (Wett & Rauch, 2003). CO_2 makes up the carbon source for nitrifiers. Furthermore, CO_2 is in equilibrium with HCO_3^-, and when the concentration of HCO_3^- is low (i.e., low alkalinity) carbon source is lacking, which implies inhibiting of nitrification. Because the alkalinity drops when the pH declines, deficiency of carbon will also emerge when pH declines.

Nitrifiers are more sensitive to toxic substances than heterotrophs (Blum & Speece, 1991, 1992; Ren, 2004; Principi et al., 2006). Nitrifiers are inhibited by many different organic and inorganic substances (compiled in Henze et al. (2002), among others).

2.2 DENITRIFICATION

Denitrification is the stepwise reduction of nitrate (NO_3^-) via nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O) to nitrogen gas (N_2) by heterotrophic denitrifiers. Heterotrophic denitrifiers use organic carbon as both the electron donor and carbon source, and NO_3^- and NO_2^- as the electron acceptor.

Denitrifiers are facultative organisms; they can use oxygen as well as nitrate or nitrite as electron acceptor. They gain more energy when oxygen is used as electron acceptor, which entails that no denitrification is performed during aerobic conditions. Contrary to nitrifiers, there are numerous denitrifying species. Furthermore, they are not as sensitive to toxic compounds as nitrifiers (Metcalf & Eddy, 2003).

The biochemical reaction of denitrification can be written in several ways, with different types of carbon sources and nitrogen compounds. Furthermore, the denitrification reaction can be expressed in several steps but, unlike nitrifiers, the same group of bacteria can perform all the different steps. A common way to express the reaction is with methanol as a carbon source:

$6\ NO_3^- + 2\ CH_3OH \rightarrow 6\ NO_2^- + 2\ CO_2 + 4\ H_2O$ (denitratation) and

$6\ NO_2^- + 3\ CH_3OH \rightarrow 3\ N_2 + 3\ CO_2 + 3\ H_2O + 6\ OH^-$ (denitritation)

The resulting total reaction is:

$6\ NO_3^- + 5\ CH_3OH \rightarrow 3\ N_2 + 5\ CO_2 + 7\ H_2O + 6\ OH^-$

From the reaction it can be stated that one mole of nitrate (or nitrite) will result in one mole of hydroxide. This corresponds to an alkalinity increase of 4.35g HCO_3^-/g NO_3^--N. Half of alkalinity consumed by nitrification is thereby regained during denitrification. The theoretical consumption of COD is 2.86 g COD/g NO_3^--N for the total reaction, if the formation of new biomass is not included. As can be seen from the reactions, 40% of the carbon source can be saved if the denitrification starts with nitrite instead of nitrate.

Another way to express the denitrification reaction is by using the organic matter in the wastewater as a carbon source and to include the formation of new biomass, which also includes the assimilation of ammonium nitrogen (Henze et al., 2002):

$0.52\ C_{18}H_{19}O_9N + 3.28\ NO_3^- + 0.48\ NH_4^+ + 2.80\ H^+ \rightarrow$

$\rightarrow C_5H_7O_2N + 1.64\ N_2 + 4.36\ CO_2 + 3.8\ H_2O.$

In this reaction, $C_{18}H_{19}O_9N$ denotes the organic matter in the wastewater and $C_5H_7O_2N$ denotes newly formed biomass. When the formation of new biomass is included, the COD consumption becomes larger than the theoretical. The following equation can be used to calculate the COD consumption during denitrification and assimilation (Metcalf & Eddy, 2003):

$COD/NO_3^-\text{-}N = 2.86/(1–1.42 * Y_H)$, where:
1.42 = relationship between COD/VSS, and
Y_H = sludge production when new heterotrophs are formed
(g $VSS_{\text{new biomass}}$/g COD_{reduced}).

Practically, 4–15 g COD/g NO_3^--N is consumed during denitrification (Kampas, 2007). A lowest consumption of 3.5–4 g COD/g NO_3^--N is mentioned in Kujawa & Klapwijk (1999). COD is also consumed for reduction of organic matter if oxygen is present. The DO concentration should therefore be kept as low as possible in anoxic zones.

Different types of carbon sources will give different heterotrophic sludge yields and different denitrification rates. Untreated wastewater gives a sludge yield (Y_H) of 0.45 g VSS/g $COD_{red.}$ (Ekama & Wentzel, 2008b). External carbon sources like methanol and ethanol give a lower sludge yield than untreated wastewater. There is a big variation between reported sludge yields for methanol and ethanol. The sludge yield for methanol is reported as 0.12 g VSS/g $COD_{red.}$[1] (Siegrist, 1996); 0.18 g VSS/g $COD_{red.}$ (Metcalf & Eddy, 2003); and 0.32 g VSS/g $COD_{red.}$[2] (Mokhayeri et al., 2009). The sludge yield for ethanol is reported to be in the same range as for methanol or up to 10–15% higher (Christensson et al., 1994; Nyberg et al., 1996). Mokhayeri et al. (2009) report a sludge yield for ethanol of 0.37 g VSS/g $COD_{red.}$[3].

Some part of the nitrogen will be assimilated to the new heterotrophic biomass during denitrification. If ammonium is not available, heterotrophs can transform nitrate to ammonium and subsequently assimilate it into the new biomass. The sludge yield will then become somewhat lower (Henze et al., 2002). The denitrification rate is nearly independent of the nitrate concentration. From a practical view the reaction can be regarded as a zero reaction order. A decreased denitrification rate can be noted only if the nitrate concentration falls below 2–3 mg NO_3^--N/L (Henze Christensen & Harremoës, 1977).

Parameters that clearly affect the denitrification rate are:

- Temperature
- Type of carbon source
- pH
- DO concentration
- Toxic substances

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[1] Converted from 0.17 g TSS/g COD_{red}.
[2] Converted from 0.45 g COD (new biomass)/g COD_{red}.
[3] Converted from 0.53 g COD (new biomass)/g COD_{red}.

2.2.1 Temperature

The denitrification rate increases with an increasing temperature up to a temperature of 32 °C. A maximal denitrification rate is found between 32–40 °C and is nearly constant. At a temperature excessing 45 °C, the denitrification is rapidly declining. The impact of temperature on the denitrification rate differs for different carbon sources. The denitrification rate increases 5–8% per °C up to a temperature of 32 °C (see chapter 2.2.2).

2.2.2 External carbon sources

Readily biodegradable carbon sources give a higher denitrification rate than slowly biodegradable carbon. External carbon sources like methanol and ethanol give a higher denitrification rate than the organic matter in untreated wastewater. Carbon from endogenous respiration will give among the lowest denitrification rates (see Figure 2.7).

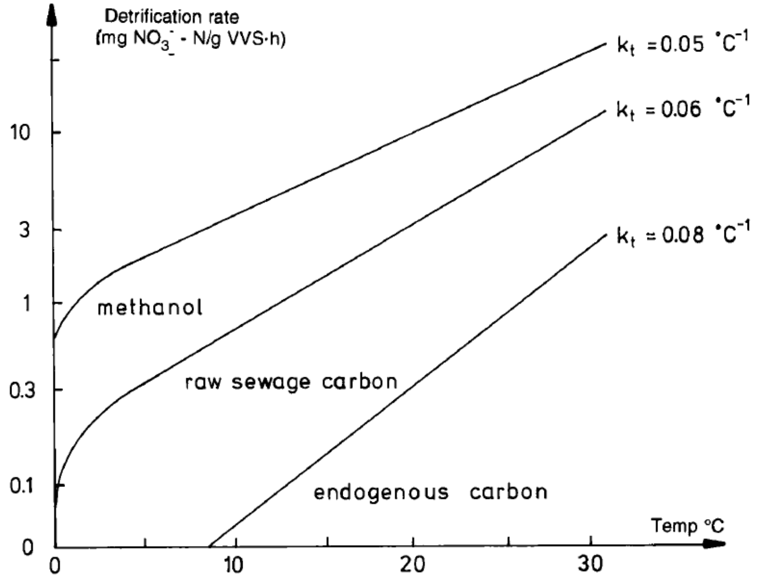

Figure 2.7. Denitrification rate as a function of temperature for different carbon sources (Halling-Sørensen & Jørgensen, 1993 (based on Henze Christensen & Harremoës, 1977)). Printed with permission from Elsevier.

2.2.3 pH

Denitrifiers are not as sensitive as nitrifiers for pH (Metcalf & Eddy, 2003). The interval for optimal pH is wider than for nitrifiers — between 7–9. However, outside this range, the denitrification rate declines rapidly (see Figure 2.8). Most of the denitrifiers are more sensitive to changes in temperature than changes in pH (Lu et al., 2014). At low pH there is a risk that the denitrification reaction stops at N_2O, implying an increased risk for nitrous oxide emissions (Kampschreur et al., 2009a) (see also chapter 3.7.5).

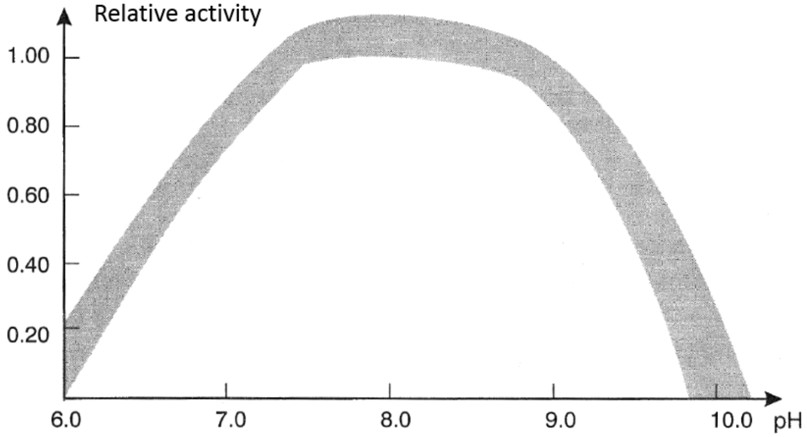

Figure 2.8. Denitrification rate as a function of pH (modified from Henze et al., 2002). Printed with permission from the authors.

2.2.4 DO concentration

Because heterotrophs are facultative organisms and prefer oxygen instead of nitrate or nitrite as an electron donor, oxygen is inhibiting for denitrification. Even very low DO concentrations of 0.1 mg/L are inhibiting to denitrifiers (Oh & Silverstein, 1999). When oxygen is present, organic matter is consumed without any denitrification. When an external carbon source is added to the process, it will be consumed under aerobic conditions, resulting in a higher consumption of external carbon and extra costs. Furthermore, the anoxic volume (or the anoxic phase) will not be used, which will reduce the denitrification and the total nitrogen reduction.

The transition from aerobic to anoxic conditions should be designed to prevent oxygen from being brought into the anoxic zone (or anoxic phase). Moreover, the DO concentration in return activated sludge (RAS) streams and nitrate recirculation should preferably be kept low.

2.2.5 Water depth

CO_2 is produced during denitrification. It will be transformed in the water from liquid phase to gas phase and leave the system, resulting in an increase of pH. The partial pressure of CO_2 is increased at a deeper water depth, which will suppress the transition to a gas phase and lead to a lower pH increment in the system. In turn, this will imply a lower denitrification rate and can result in increased costs for an external carbon source. Hellinga et al. (1998) stated that the denitrification is not inhibited at a water depth lower than 4–5 m.

2.2.6 Toxic substances

Denitrification is inhibited by several different substances of which free nitrous acid is one of them. The concentration of free nitrous acid is correlated to the concentration of nitrite, pH and temperature (described in chapter 2.1.5). Glass et al. (1997) stated that free nitrous acid has an inhibiting effect on denitrification starting at a concentration of 0.02 mg HNO_2-N/L. According to Ma et al. (2010), the inhibition is complete at a concentration of 0.2 mg HNO_2-N/L. Denitrifiers are also inhibited by substances comprising sulfide and organic substances containing, for example, acetylene, cyanide and different pesticides (Knowles, 1982).

2.3 ANAMMOX

Anammox is an acronym for ANaerobic AMMonium OXidation. A shortcut in the nitrogen cycle is used that results in a lower oxygen consumption and no need for COD (see Figure 2.1). The knowledge of the anammox process is relatively new. Nevertheless, a paper was published in 1932 presenting that nitrogen gas was produced by a yet unknown fermentation process in the sediments of Lake Mendota, Wisconsin, USA (Allgeier et al., 1932). Forty-five years later, it was described that it should exist a chemolithotrophic bacteria capable of oxidizing ammonium to nitrogen gas with nitrate as an electron acceptor (Broda, 1977). During the latter part of 1980s, signs of anammox activity were noted when the ammonium concentration was reduced in a denitrifying reactor (van de Graaf, 1990; Mulder et al., 1995).

The fact that the anammox bacteria were found in a WWTP (Devol, 2003) is somewhat unusual; the wastewater industry is characterized by applying discoveries from other disciplines, not the other way around. Globally, 30–50% of the nitrogen gas production in the oceans is considered to be performed by anammox bacteria (Devol, 2003).

The first full-scale anammox reactor began operation in 2002 at Dokhavens WWTP in Rotterdam, the Netherlands (van der Star et al., 2007). The establishment of new anammox plants has been quite fast: in 2014, there were more than 100 full-scale plants in operation worldwide (Lackner et al., 2014). Of these plants, 29 are in Germany, holding most of the plants in the world, while the Netherlands has 19 plants (Ali & Okabe, 2015).

The anammox bacteria need nitrite to oxidize ammonium to nitrogen gas. The process can be divided into two steps: a first anaerobic step where about half of the ammonium is transformed to nitrite by AOB, and a second step for the anammox reaction. Together, the two steps are called deammonification. The process can be operated in different ways. One way is to operate the process in two different reactors, applying partial nitritation in the first reactor and anammox in the second. It can also be operated in the same reactor with alternating aerobic/anaerobic conditions to achieve alternating nitritation/anammox, or with a simultaneous nitritation/anammox process. When a simultaneous nitritation/anammox process is applied, a low DO concentration is generally practiced to obtain a distinction between aerobic and anaerobic microenvironments in granules or biofilms, for instance.

Simplified partial reactions for deammonification are:
$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2\ H^+$ (nitritation, performed by AOB)
$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$ (anammox)

The simplified total reactions is:
$NH_4^+ + 0.75\ O_2 \rightarrow 0.5\ N_2 + 1.5\ H_2O + H^+$

A more complete total reaction of deammonification also includes cell growth of bacteria (Strous et al., 1998):

$$\begin{aligned}&NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow \\ &\qquad 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O\end{aligned}$$

In this reaction, the chemical term $CH_2O_{0.5}N_{0.15}$ represents new biomass of autotrophic bacteria. The following can be noted from the formula:

- More than half of the initial nitrogen (57%) is nitrite nitrogen.
- 0.26 mole NO_3^--N is formed per 2.32 mole N. This is equal to 11% of the initial nitrogen. Consequently, at most 89% of the initial nitrogen can be transformed to nitrogen gas in a nitritation-anammox process (without any present denitrifiers).
- The growth of new biomass is low: only 0.066 mole $CH_2O_{0.5}N_{0.15}$ per 2.32 mole of N.

When cell growth is included to the nitritation reaction it can be written (WERF Nutrient Challenge, 2014):

$$\begin{aligned}&NH_4^+ + 1.404\ O_2 + 0.0743\ HCO_3^- \rightarrow \\ &\qquad \rightarrow 0.0149\ C_5H_7O_2N + 0.985\ NO_2^- + 1.03\ H_2O + 1.911\ H^+\end{aligned}$$

When cell growth is included, the sum of the reactions can be written (WERF Nutrient Challenge, 2014):

$$NH_4^+ + 0.804\ O_2 + 0.071\ HCO_3^- \rightarrow 0.436\ N_2 + 0.111\ NO_3^- + 0.009\ C_5H_7O_2N + 0.028\ CH_2O_{0.5}N_{0.15} + 1.038\ H^+ + 1.46\ H_2O$$

The deammonification process is characterized by a low oxygen consumption. The need for oxygen is 57% lower than at conventional nitrification-denitrification (the value is about the same, no matter if cell growth is included or not). Because partial nitritation and the anammox reaction is performed by autotrophic bacteria, there is no need for COD (see Figure 2.9).

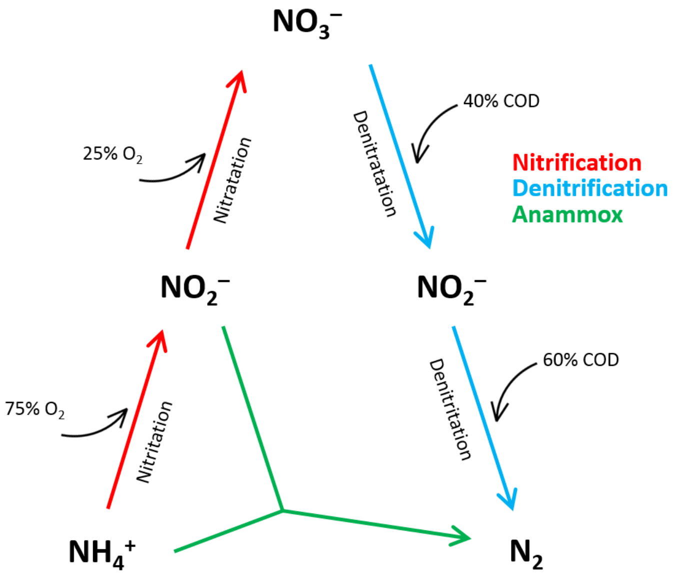

Figure 2.9. Pathway of the anammox reaction compared to conventional nitrification-denitrification.

The anammox bacteria grow considerably slower than do denitrifiers and nitrifiers. The generation time is about 11 days at a temperature of 32–33 °C (Strous et al., 1998). The slow growth rate implies that a high SRT is imperative; the anammox bacteria need to be retained in the system for at least one generation. Eleven percent of the total nitrogen in an anammox process is formed to nitrate. The reason for this is probably that the energy (electrons) needed for the reduction of CO_2 during the carbon fixation is retrieved from the oxidation of nitrite to nitrate (Strous et al., 1998).

The growth rate of anammox bacteria is highly affected by the temperature. The temperature optima is 30–40 °C (Strous et al., 1999; Egli et al., 2001). The process can be performed in a range of 6–43 °C, but the reaction rate is greatly reduced under 15 °C and over 40 °C (Zhu et al., 2008). The specific activity of anammox bacteria can drop about ten times when the temperature is decreased from 30 °C to 10 °C (Lotti et al., 2015). Consequently, it is challenging to implement the anammox process in the mainstream process (Cao et al., 2017), with low temperature as one of the difficulties. However, it is less difficult to implement and operate it as a sidestream process.

The anammox bacteria have a characteristic red color that is caused by the heme c group of the protein cytochrome c that plays an important role in anammox metabolism (Jetten et al., 2009). It is found on the outside of the inner membrane of the mitochondria. In 2015, about 19 species and broadly 6 genera were identified in the taxonomic group of anammox bacteria (Ali & Okabe, 2015).

Anammox bacteria occur as free cells, flocs in active sludge, and granules, and in biofilms. They tend to clump together into aggregates, which is the case in processes that include granules or biofilms. When the circumstances are right, granules are formed spontaneously. Some important conditions for granule formation are: high shear forces created by turbulence (Arrojo et al., 2006); continuously altering conditions of feast/famine (Liu & Tay, 2004); and a short settling time that favors the dense granules over slow settling bioflocs that are washed out from the system (Morgenroth et al., 1997).

3 BIOLOGICAL TREATMENT OF DIGESTER SUPERNATANT

The history of biological treatment of digester supernatant is not old. Internationally it has been practiced for about 40 years and in Sweden for about 25 years. Nevertheless, it has been developed from being regarded as a problem to a means of boosting the mainstream process (bioaugmentation), and showing the way to more energy- and resource-efficient treatment methods for the mainstream process (anammox). In this chapter, some different processes are described deeper. The benefits and drawbacks with separate treatment of digester supernatant are discussed at the end of the chapter.

3.1 HISTORICAL BACKGROUND

During the 1910s, Germany decided that sludge from WWTP should be returned to agriculture as fertilizer. Thus, to enable this, the sludge had to be dewatered, which was performed in presses and centrifuges. Germany seems to be one of the first countries that applied dewatering of sludge (Hatton, 1921). Nevertheless, when dewatering was applied, reject water was produced as a side product. The fact that reject water contains a high concentration of nitrogen was noted by Fowler (1920), who suggested that it should therefore be returned to agriculture.

Internationally, separate treatment of digester supernatant has been applied since the 1930s (Rudolfs & Gehm, 1939). In the USA in the 1950s, digester supernatant was considered a problem at WWTPs. It was stated that when digester supernatant was conveyed back to the influent of the plants it caused among others: extra load to the biological reactors; extra load to recipients; odor problems when dissolved gases was stripped from aerated reactors or trickling filters; and increased consumption of chemicals. Moreover, it was recommended that an equalization tank be used to avoid peak flows to the mainstream process (Kappe, 1958). Until the 1970s, digester supernatant could be treated by chemical flocculation, sedimentation, aeration (to reduce organic matter), filtration, stripping, and flotation (Malina & DiFilippo, 1971). Nevertheless, during the 1970s, biological treatment of digester supernatant was introduced to reduce the nitrogen concentration (Prakasam & Loehr, 1972).

In Sweden, one of the first signs of biological treatment of digester supernatant is from 1982: nitrification of digester supernatant was studied in lab-scale (Mossakowska, 1994). Furthermore, studies were performed on the biological treatment of digester supernatant in an activated sludge application during the 1980s at Bromma WWTP, Stockholm; both in lab-scale and pilot-scale. The ammonium concentration was reduced about 60% during the studies (Tendaj-Xavier, 1985). The first full-scale plant for treating digester supernatant in Sweden was an SBR for nitrification-denitrification, commissioned in 1991 at Nykvarn WWTP, in the city of Linköping.

In 2006, the separate treatment of digester supernatant was applied at 16 municipal WWTPs in Sweden, wherein the most common configuration was nitrification-denitrification in an SBR (Nikolic & Sundin, 2006). In the same year, an SBR configuration was also the most common method for the separate treatment of digester supernatant in Germany (Jardin et al., 2006). In 2015, nitrification-denitrification in an SBR was still the most common way of treating digester supernatant internationally (Bowden et al., 2015), in spite of the fact that more resource- and energy-efficient methods had been applied for nearly two decades.

3.2 TREATMENT IN THE MAINSTREAM PROCESS

In 2014, 139 WWTPs were identified in Sweden that applied sludge digestion (Statens energimyndighet, 2015). Today, about 15 of the WWTPs apply separate treatment of digester supernatant. Among those, there are a few deammonification processes, one SHARON configuration, and one plant using nitritation only with alkali dosage. However, the most dominant configuration is nitrification-denitrification in an SBR. If the practice of treating digester supernatant in the RAS stream should be included, there are about 25 WWTPs. Thus, the most common method of treating digester supernatant in Sweden today is in the mainstream process.

3.3 NITRIFICATION-DENITRIFICATION IN AN SBR

Nitrification-denitrification in an SBR is a variant of the activated sludge process. The phrase “activated sludge” is strongly associated with a plant consisting of bioreactors and settling tanks. However, this association is somewhat misguiding because it actually refers to the bio flocs that are formed naturally when the residence time of the sludge is longer than the hydraulic residence time (i.e., SRT > HRT). The bio flocs are also formed in an SBR configuration. Thus, the SBR is a variant of the activated sludge process but with the distinction that the bioreactor and settling tank are included in the same volume.

Today, nitrification-denitrification in an SBR is still the most common separate treatment method of digester supernatant, in Sweden and internationally. The reason why SBRs have gained such popularity could be explained by the lower cost of building one tank instead of two. Moreover, the even distribution of the digester supernatant flow rate over a day implies a minimal risk of hydraulic overload. Another explanation could be the flexibility of the SBR; it is easy to change between pre-denitrification and post-denitrification. It is also easy to change the time intervals for different phases, for instance. Normally, SBRs are preceded by a buffer tank to enable a batchwise pumping to the reactor. Some SBRs are also followed by an equalization tank to avoid hydraulic overload of the following reactors.

The treatment of digester supernatant in an SBR could be operated with pre-denitrification or post-denitrification. Usually, the inherently low COD/N ratio implies that an external carbon source is difficult to avoid during the denitrification phase. An SBR cycle usually lasts for 6–8 hours, and a typical cycle could be constituted by the following phases:

1. Filling and simultaneous aeration (nitrification)
2. Aeration (nitrification)
3. Mixing (denitrification)
4. Settling
5. Decantation
6. Withdrawal of excess sludge

Higher reaction rates are reached when treating digester supernatant than ordinary wastewater. The higher temperature in the digester supernatant is one of the main reasons for this, but the higher concentration of nitrogen can also give a higher reaction rate. The use of an external carbon source, which are much more common in sidestream treatment than in the mainstream process, also gives a higher reaction rate. A volumetric reduction rate of nitrogen of 0.25–0.35 kg TN/m^3.d is common when treating digester supernatant in an SBR.

On-line sensors for monitoring pH, mixed liquor suspended solids (MLSS), DO, NH_4^+-N, NO_3^--N, and water level and temperature are frequently used in SBRs. Different control strategies are also used such as a straight time control of the different phases, which is a simple control strategy. To achieve higher reaction rates, the process could be controlled in the range of optimal pH. Nevertheless, this strategy can result in a higher addition of an external carbon source to achieve a higher pH.

3.4 NITRITATION-DENITRITATION

Nitritation-denitritation can be performed in different configurations, for example in an SBR or utilizing the SHARON process.

The difference between nitritation-denitritation and nitrification-denitrification is that the oxidation/reduction to/from nitrate is omitted. Ammonium is oxidized to nitrite only, followed by denitritation. When applying the nitrite route, theoretically 25% of oxygen is saved and 40% less COD is consumed. As a consequence, the sludge production is decreased by about 40% (van Loosdrecht, 2008). In order to avoid nitratation, AOB activity should be promoted and NOB activity impeded. This could be arranged in different ways. One way is to operate the process at a pH that give concentrations of free ammonia or free nitrous acid that inhibit NOB but not AOB. As an example, NOB are more inhibited by high concentrations of free ammonia than are AOB as a consequence of an elevated pH (Hellinga et al., 1998) and high ammonium concentrations. Another way is to keep a low DO concentration during aerobic phases; AOB have a higher affinity for DO and, furthermore, NOB have a longer lag phase than AOB when changing from anoxic to aerobic conditions (Katsogiannis et al., 2003). Consequently, AOB have an advantage over NOB during frequently changed anoxic/aerobic conditions. Yet, another way is to apply a low SRT because NOB grow slower than AOB in a temperature above 20–25 °C (Hellinga et al., 1998). By keeping a shorter aerobic SRT in the reactor than the generation time of NOB, the NOB will be washed out of the system before they reproduce. The oxidation of ammonium will thereby be stopped at nitrite. This is used in the SHARON process (see Figure 3.1).

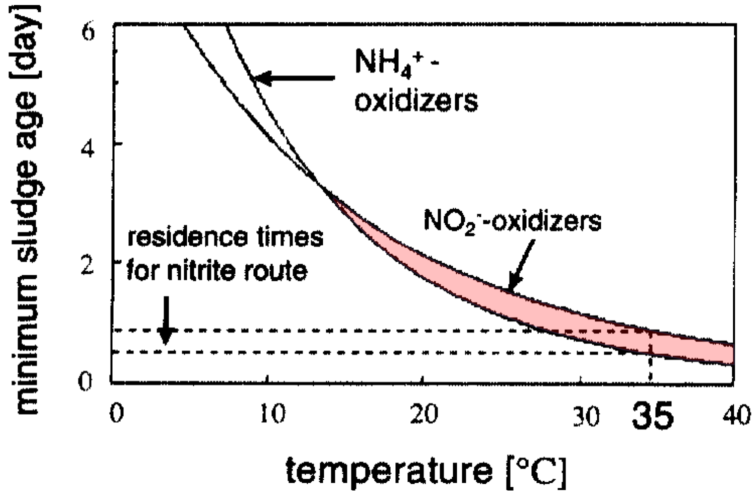

Figure 3.1. Minimum SRT for AOB and NOB at different temperatures (Hellinga et al., 1998). In the red area, AOB are reproduced but not NOB. Printed and modified with permission from IWA publishing.

To promote AOB and meanwhile impede the growth of NOB, Figure 3.1 shows that this could be performed only in a narrow range for the aerobic SRT: 0.5–0.9 d at a temperature of 35 °C. Nevertheless, there are some other factors that effect the growth rate of nitrifiers, the pH value among these. At an increasing pH, the minimum aerobic SRT for AOB is decreased, meanwhile the aerobic SRT for NOB is increased. This implies that the range that the aerobic SRT should be kept within is widened, shown in Figure 3.2.

The different ways to give AOB an advantage over NOB can also be combined.

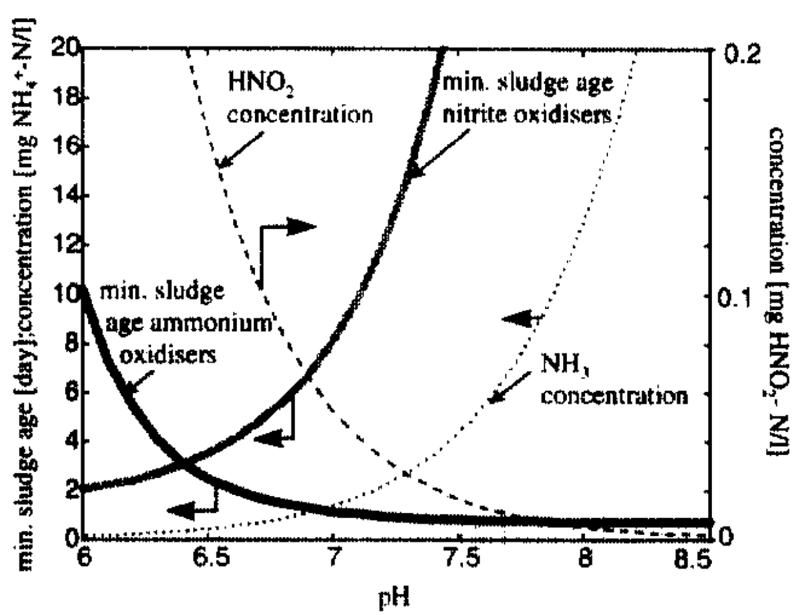

Figure 3.2. Minimum SRT for AOB and NOB at different pH (at a temperature of 35 °C, ammonium concentration of 130 mg/L and nitrite concentration of 300 mg/L (Hellinga et al., 1998)). Printed with permission from IWA publishing.

During a lab-scale study performed by Queiroz et al. (2011), a stable nitritation-denitritation was achieved in an SBR by keeping a high pH (8.3) and a low DO concentration (1.0 mg/L). Nevertheless, denitritation was inhibited during concentrations of nitrite higher than 70 mg/L. In a full-scale study of nitritation-denitritation, the process was operated by maintaining a medium low DO of 1.3 mg/L and letting the pH drop to 6.7–7.1 at the end of the aerobic phase. However, the denitrification was inhibited during the study, which coincided with a low pH and, consequently, high concentrations of nitrite and free nitrous acid (Gustavsson et al., 2011b).

3.5 BIOAUGMENTATION

The principle of bioaugmentation is to continuously inoculate nitrifiers from a sidestream reactor to the mainstream process. Hence, the number of nitrifiers in the mainstream process increases, which, consequently, gives a higher nitrification rate in the mainstream process (van Loosdrecht, 2008). Because bioaugmentation aims to both reduce the nitrogen concentration in the digester supernatant and boost the mainstream process, bioaugmentation differs from other treatment methods of digester supernatant.

At many WWTPs applying separate sidestream treatment, the treated water head to the mainstream process. Even if the conventional nitrification-denitrification is used in the sidestream treatment as well as in the mainstream process, the environments differ widely with regard to temperature, ammonium concentration, alkalinity, and pH. These distinctions can give totally different communities of nitrifiers. The result can be that nitrifiers grown in the sidestream process may not be well acclimated to the conditions in the mainstream process, and consequently do not reproduce there. To enable the nitrifiers from the sidestream reactor to add an extra boost to the mainstream process, the nitrifiers should originate from the mainstream process in order for them to be adapted to the two different conditions. This can be accomplished by continuously conveying RAS from the mainstream process to the sidestream plant (Salem et al., 2003). The principle of bioaugmentation is shown in Figure 3.3.

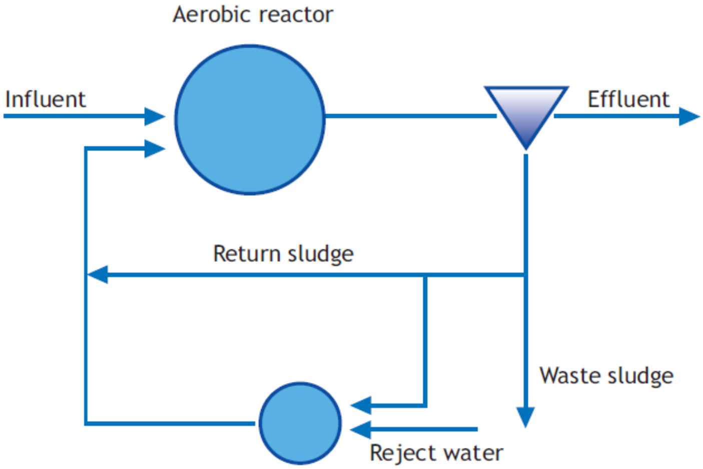

Figure 3.3. The principle of bioaugmentation. A fraction of RAS from the mainstream process is conveyed to the sidestream plant and back to the mainstream process (van Loosdrecht, 2008). Printed and modified with permission from IWA publishing.

In conventional nitrification-denitrification, the nitrifiers typically constitute less than 4% of the bioreactors biomass in the mainstream process (Ekama & Wentzel, 2008b). Because the nitrifiers grow slowly, it is crucial with a SRT high enough for the nitrifiers to reproduce. Accordingly, the aerobic SRT is one of the main parameters to achieve an effective nitrogen removal. Enhancing the number of nitrifiers in the mainstream process through bioaugmentation would imply that a higher amount of nitrogen could be reduced in the same reactor volume, or that the MLSS concentration could be decreased while using the same reactor volume. The latter means a lower endogen respiration, entailing a lower oxygen and energy consumption. Furthermore, if the sludge surface load is too high to the secondary settling tanks, bioaugmentation allows for keeping the same quantity of nitrifiers in the system at a lower sludge concentration. Thus, bioaugmentation should not be regarded only as a method of nitrogen removal in the sidestream process, but also a means to enhance the nitrogen removal in the mainstream process.

Because the nitrifiers are very sensitive to temperature, the largest effect from bioaugmentation is gained at a low temperature in the mainstream process. In a full-scale study of bioaugmentation, it was found that the nitrification rate was increased by 41% at a temperature in the mainstream process of 8 °C (**Paper III**). In another full-scale study at Garmerwolde WWTP, the Netherlands, the nitrification rate increased about 60% in the mainstream process (Salem et al., 2004). The flow rate of the RAS stream to the sidestream reactor is of importance for the bioaugmentation effect. In **Paper III**, the effect from different RAS flow rates on the bioaugmentation effect was examined: 10%, 30% and 100% RAS flow rates of digester supernatant flow rate were surveyed. The effect of bioaugmentation was obvious at 10% but was largest at 100%. To gain the largest possible effect from bioaugmentation, the SRT should be kept low in the sidestream reactor to minimize the decay of nitrifiers (Salem, 2004).

For WWTPs that practice treatment of digester supernatant via nitrification-denitrification in an SBR, it is easy to transform the process to bioaugmentation. Consequently, bioaugmentation could be regarded as an interesting configuration for these plants, in particular plants that balance at the limit to the permissible effluent standards.

3.6 OTHER PROCESSES

Several different processes have been developed during the recent decades. Many of them are marketed or patented with registered names. This includes different configurations of the deammonification process.

SHARON is an acronym for Single reactor system for High activity Ammonia Removal Over Nitrite[4] (Notenboom et al., 2002). As the acronym suggests, ammonium is not oxidized all the way to nitrate but only to nitrite. Because 25% less air volume and 40% less carbon source are required, theoretically the process is cheaper and more environmentally friendly than conventional nitrification-denitrification. In addition, the sludge production is about 40% lower (van Loosdrecht, 2008). Nitritation and denitritation are performed in a completely mixed reactor with intermittent aeration. The feeding to the reactor is continuous, unlike an SBR. The first SHARON plant was commissioned in 1997 at the Utrecht WWTP, the Netherlands (Grontmij, n.d.). The only SHARON reactor in Sweden is located at Nykvarn WWTP in Linköping and was commissioned in 2009 (Stenström et al., 2017). There are slightly more than 10 SHARON reactors installed worldwide. The reactor in Linköping seems to be one of the last installations.

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[4] Because the process later on has been practiced in two separate reactors, the acronym has been changed to Stable High Ammonia Removal Over Nitrite.

ANAMMOX® is a deammonification process, marketed by the Dutch company Paques. The process is based on granules and can be designed as one- and two-stage reactor. The first ANAMMOX® reactor was commissioned in 2002 in Rotterdam at Dokhaven WWTP as a two-stage reactor, preceded by a SHARON reactor for partial nitritation (van der Star et al., 2007). Since 2006, Paques has designed the ANAMMOX® reactors as one-stage reactors because of a lower investment cost, which is the most common configuration of ANAMMOX® reactors today (Lackner et al., 2014). The process is developed in cooperation with Delft University of Technology and the University of Nijmegen. Many of the ANAMMOX® reactors are installed at municipal WWTPs. However, most of the plants are aimed at treating industrial wastewater. Lackner et al. (2014) made a compilation of installed deammonification plants. From the compilation it was found that out of more than 100 deammonification plants, 21 constituted ANAMMOX® reactors, and out of these 18 constituted one-stage reactors. Out of the 21 reactors, 6 were treating digester supernatant or a mix of industrial wastewater and digester supernatant. The anammox bacteria are aggregated in granules.

The ANITA™ Mox process is a moving bed biofilm reactor (MBBR) and a variant of the deammonification process. It is developed and marketed by AnoxKaldnes, a part of Veolia Water Technologies. The anammox bacteria attach and grow at plastic carriers. It is a one-stage process: the partial nitritation and the anammox takes place in the same reactor. It is operated with continuous feeding and aeration. The first ANITA™ Mox reactor started operation in 2010 at Sjölunda WWTP, Malmö, Sweden. It treats a smaller part of the digester supernatant produced at the plant. In total, about 10 ANITA™ Mox plants have been installed (Veolia Water Technologies, n.d.). Only some years after the first ANITA™ Mox reactor was commissioned, the process was further developed to include a settling tank and sludge retention: Integrated Fixed-Film Activated Sludge (IFAS) ANITA™ Mox. In lab-scale studies, a four times higher volumetric nitrogen removal rate was noted compared to without sludge retention (Veuillet et al., 2014). Furthermore, IFAS ANITA™ Mox can better withstand a higher COD/N ratio than without sludge retention (Lemaire et al., 2015). The volume for the settling tank typically constitutes 15% of the reactor volume.

DEMON® is another configuration of the deammonification process. The name is an acronym for DE-amMONification. The DEMON® process is developed and patented by the University of Innsbruck, Austria. The first DEMON® reactor began operation in 2004 at Strass WWTP, Austria (Wett, 2006), close to Innsbruck. The

DEMON® process is currently the most common configuration of deammonification plants. In a survey by Lackner et al. (2014), where more than 100 full-scale anammox reactors were included, it was stated that 40% of the plants were a DEMON® configuration. More than 70% of these treated digester supernatant. Partial nitritation and anammox is accomplished in the same reactor, as the DEMON® process is designed as a one-stage reactor. Unlike many other types of SBRs, feeding to the reactor is performed in both aerated and unaerated phases, and only during settling and decantation phases is there no feeding to the reactor (Wett, 2006). Accordingly, the preceding buffer tank can have a limited volume and the fluctuations of alkalinity in the reactor are smaller. Nevertheless, there are some DEMON® reactors that apply continuous feeding. There exist both activated sludge flocs and granules in these reactors. Compared to the ANAMMOX® process, the granules in a DEMON® reactor have a smaller size. Because of the big difference in growth rates between AOB and anammox bacteria, different SRTs are applied in the system. The practiced SRT for AOB are a few days, while the SRT for anammox bacteria are several weeks. To achieve the difference in SRT, AOB and anammox bacteria must be separated. This is performed by a hydrocyclone, where the difference in density for activated sludge flocs and granules is used. The excess sludge is pumped to the hydrocyclone and the activated sludge flocs (mainly AOB) are conveyed out of the system, whereas the granules (mainly anammox bacteria) are headed back to the reactor.

3.7 BENEFITS AND DRAWBACKS WITH SEPARATE TREATMENT OF DIGESTER SUPERNATANT

The most distinct advantage of the separate treatment of digester supernatant is the decreased concentration of nitrogen in the effluent from a WWTP. Related to this, and as an alternative to expanding the mainstream process, it is a benefit that introducing a sidestream treatment does not intrude on the operation of the mainstream process during the construction period. Another advantage is that the nitrogen in the digester supernatant can be treated in a more compact reactor and/or in a less energy consuming way than what is possible in the mainstream process. This has been accentuated in recent years, as methods are available that are less energy consuming than what is possible in the mainstream process.

A general disadvantage is that a separate sidestream treatment will mean another process to be involved in and to control.

At some WWTPs without separate sidestream treatment, there are problems with temporary nitrogen loads to the mainstream process if the digester supernatant is headed directly from the dewatering process. A way of solving this problem is to install a buffer tank. The digester supernatant could then be headed to the mainstream process evenly around the clock or during the night when the load to the plants is lower.

In addition to the above arguments, there are some other advantages and disadvantages for the separate treatment of digester supernatant. Some of these are presented below.

3.7.1 High concentration of nitrogen

The TN concentration in digester supernatant is often in the range of 700–1800 mg/L (Stenström et al., 2017). That is about 15–50 times higher than in municipal wastewater. An obvious increment in nitrification rates is achieved up to ammonium concentrations of 5–15 mg/L (Gao et al., 2010). At ammonium concentrations above that, the nitrification rate becomes almost constant; however, not totally constant. There are studies that demonstrate an increasing nitrification rate at higher ammonium concentrations (e.g., Dinçer & Kargi, 2000), even though the increment is limited. Nevertheless, it can be noted that a high ammonium concentration does not have any limiting effect on the nitrification rate, provided that there is no inhibitory concentrations of free ammonia or free nitrous acid.

3.7.2 Low concentration of organic matter

A low concentration of organic matter means a low COD/N ratio. This is beneficial to many methods of sidestream treatment. In particular for the deammonification processes because a high COD concentration can give an undesired growth of heterotrophs (Güven et al., 2005).

In general, the higher the portion of nitrifiers in the biomass, the higher the nitrification rate. At a low COD/N ratio, the heterotrophs will be disfavored. In turn, autotrophs that use inorganic CO_2 as a carbon source will be promoted such as nitrifiers and anammox bacteria. On the contrary, if the heterotrophs are favored in a high COD/N ratio, the portion of nitrifiers will be diminished, leading to a lower nitrification rate per kg VSS and higher oxygen consumption due to an increased endogenous respiration.

In treatment methods that include denitrification, COD is required as a carbon source for heterotrophs. Because the low concentration of COD in digester supernatant is not enough for denitrification, an external carbon source is usually added. Several external carbon sources are more easily degradable than the organic matter in raw municipal wastewater, for example methanol and ethanol, and give less sludge production (Nyberg et al., 1992). Because of the reduced sludge production, it could not be excluded that a larger share of heat is generated during the degradation of external carbon sources, resulting in a larger temperature increase. Additionally, since the amount of heterotrophs (sludge) is diminished, the portion of nitrifiers in the biomass consequently becomes larger per kg VSS.

With regard to methods of sidestream treatment that include denitrification and the practice of adding an external carbon source: if the amount of COD in the raw wastewater would have been enough to denitrify the digester supernatant, the cost of the external carbon source is an extra cost. Furthermore, the added carbon source generates extra sludge.

In systems where only nitrification is practiced, the portion of nitrifiers of the biomass should be large and, consequently, high nitrification rates should be achieved. However, Dytczak et al. (2008) demonstrated the opposite when they compared two different systems: one with continuous nitrification (with alkali dosage to avoid pH decrease) and the other with alternating nitrification-denitrification. The highest nitrification rate was found in the system of nitrification-denitrification because fast-growing nitrifiers like nitrosomonas and nitrobacter were selected. In the system of continuous nitrification, slow-growing nitrifiers such as nitrosospira and nitrospira dominated.

3.7.3 The impact of temperature

When the temperature is increased in a biological reactor, the increased rate of biochemical reactions implies that the SRT of the system could be decreased with maintained reduction of substrates (Ekama & Wentzel, 2008b). Furthermore, when the SRT is decreased, the consumption of oxygen from endogenous respiration is also decreased (Ekama & Wentzel, 2008a). Altogether, the increased reaction rate results in decreased oxygen consumption.

The solubility of DO is decreasing with increasing temperature (Metcalf & Eddy, 2003). As an example, the solubility of DO in water at a temperature of 10 °C (pure water at an atmospheric pressure of 760 mm Hg) is 11.02 mg O_2/L. The solubility of DO in water at a temperature of 30 °C is 7.54 mg O_2/L. Consequently, more oxygen and energy is consumed to aerate the high temperature water.

Another effect of a high water temperature is that the aging of rubber is accelerated. In an SBR for treatment of digester supernatant at Slottshagen WWTP in Norrköping, Sweden, it was found that the oxygen transfer via the fine bubble rubber membranes has been greatly reduced after only a few years of operation (Stenström et al., 2017). This is considerably less time than expected at a lower temperature.

3.7.4 Impacts on the mainstream process

Introducing a separate treatment for the digester supernatant can be regarded as a relief of the mainstream process. However, it may also have a negative impact on the mainstream process. Nitrifiers need ammonium (as electron donor) to grow. When a separate sidestream treatment is introduced, the amount of ammonium to the mainstream process is decreased. This will result in a decrease of nitrifiers in the mainstream process. In order to compensate for this, the SRT in the mainstream process must be increased, which, in turn, will lead to a higher endogenous respiration and a higher oxygen consumption (Ekama & Wentzel, 2008a). The effect of this on the mainstream process depends on a number of factors and needs to be considered in each case.

Treated water and excess sludge from a sidestream plant are usually conveyed to the biological reactors in the mainstream process; thus, bacteria are transferred to the mainstream process. Depending on whether there are any similarities between the sidestream treatment and the mainstream process, this transfer of bacteria may imply an inoculation to the mainstream, which can potentially increase the rate for the biochemical reaction. For example, if activated sludge with conventional nitrification-denitrification is applied in both the sidestream reactor and the mainstream process, nitrifiers from the sidestream reactor can then continuously be transferred to the mainstream process. Furthermore, there are some techniques that are based on a deliberate inoculation to the mainstream process such as the BABE® process (Salem et al., 2004) and the EssDe® process (Wett et al., 2012).

The deliberate inoculation of bacteria from a sidestream plant to the mainstream process constitutes a way of enhancing the biological processes in the mainstream. It has the potential of speeding up the biological conversion rates and, consequently, allowing reactors to be designed in a more cost-effective manner.

3.7.5 Emissions of nitrous oxide

An undesired effect of the biological treatment of digester supernatant is emissions of nitrous oxide (N_2O). Because of the higher nitrogen concentrations in the digester supernatant, higher N_2O emissions are generally found from a sidestream plant than from a mainstream process.

The anammox process has shown generally lower N_2O emissions than other nitrogen removal pathways. It has been assumed that N_2O is not included in the anammox metabolism (Kampschreur et al., 2008); other bacteria than anammox are believed to be the source of N_2O emissions in deammonification reactors. Nevertheless, several studies pointed out that the anammox process could be a potential source of N_2O emissions. Kartal et al. (2007) suggest that NO detoxification by anammox could be a potential source of N_2O production. In a study of N_2O emissions pathways from a single-stage nitritation-anammox granular reactor, it was found that 30% of the produced N_2O was formed in the anammox bacteria-dominated anoxic zone of the granules, thus possibly mediated by the anammox pathway (Ali et al., 2016).

The magnitude of N_2O emissions can vary greatly between different process configurations. Moreover, the emission can differ considerably between two plants with the same process configuration. Because of the large divergences of N_2O emissions from different plants, it is recommended to perform measurements onsite if the emissions from a specific reactor are wanted. However, sidestream reactors based on conventional nitrification-denitrification or nitritation-denitritation, with a reasonably prosperous biological process, seem to have N_2O emission corresponding to about 4% of the reduced nitrogen (**Paper V**; Gustavsson et al., 2011a). If the biological process is not in balance, the emissions are probably larger. Processes based on deammonification generally cause lower N_2O emissions. In an ANITA™ Mox reactor in Växjö, Sweden, emissions of N_2O-N corresponded to 0.75% of the reduced nitrogen (Christensson et al., 2013). In an ANAMMOX® plant in Olburgen, the Netherlands, the corresponding value was 1.7% (Kampschreur et al., 2009b). In a DEMON® reactor in Strass WWTP, Austria, a sewage treatment plant, the corresponding value was 0.9–1.3% (Weissenbacher et al., 2012). In contrast, the emissions of N_2O-N from a DEMON® reactor in Odense, Denmark corresponded to 2–9% of the reduced nitrogen (Stenström et al., 2017).

The big differences in nitrous oxide emissions from plant to plant elucidate the need for a deeper understanding of the mechanisms behind the forming of nitrous oxide.

4 EXPERIMENTAL PLANS AND ANALYTICAL METHODS

The studies included in this work have been performed at the SBR for treatment of digester supernatant at Slottshagen WWTP, located in Norrköping, Sweden. A description of the methodologies used during the experiments is included in this chapter, together with a description of the plant.

4.1 EXPERIMENTAL PLAN FOR NITROUS OXIDE EMISSIONS

The purpose of the study in **Papers I** and **V** was to examine three different process conditions believed to enhance emissions of nitrous oxide: low oxygen concentration during nitrification, low C/N-ratio during denitrification, and low concentrations of DO present during denitrification. Furthermore, any correlation between high nitrous oxide emissions and high concentration of nitrite was to be studied in all studies by measuring the nitrite concentration by frequently taken grab samples. The plan was to stepwise aggravate the process conditions during the three studies in order to find critical thresholds when the emissions were obviously increased. Later, it was noticed that the lowest possible frequency for the frequency controlled blower machine was not low enough to maintain denitrification, and the experiment of keeping a low DO present during denitrification was therefore relinquished.

Measurements were made from February through May 2012 in three campaigns and in a longer-term study. Each campaign lasted approximately 36 h. Before Campaigns 1 and 3, the process in the SBR had been accidentally disturbed, which resulted in low pH and high NO_2^--N concentrations. Between and after the campaigns, the N_2O emissions in off-gas were measured without performing any chemical analyses in order to study the longer-term effect of ethanol dosage on N_2O emissions (**Paper V**).

Campaign 1 – Reduced DO concentration

Campaign 1 was performed from February 29 through March 1, 2012. This campaign comprised four cycles in which DO was reduced stepwise during the nitrification phases. The DO set points in these successive cycles were 2.0, 1.2, 0.9, and 0.6 mg/L. The ethanol dosage to the SBR was accidentally interrupted for several days just before the campaign started. This resulted in low pH and high

NO_2^--N concentrations, which in turn resulted in high N_2O emissions from the start of the campaign, before the DO was reduced. Therefore, the results from Campaign 1 are only sparingly presented.

Campaign 2 – Reduced DO concentration (again)

Campaign 2 was performed from March 20–21, 2012. This campaign comprised four SBR cycles, each with a different DO set point: cycle 1, 2.0 mg/L (normal DO set point at the plant); cycle 2, 0.9 mg/L; cycle 3, 2.0 mg/L (recovery); and cycle 4, 0.5 mg O_2/L. The measurements included the online recording of N_2O levels in water and off-gas for all cycles. Grab samples were taken from the SBR throughout cycles 1, 2 and 4. Some grab samples were also taken at the beginning of cycles 3 and 5 to follow how the process was affected by the reduced DO level in the preceding cycles. Several grab samples were taken and mixed together to form a composite sample of the influent and effluent during cycles 1, 2 and 4 in order to calculate mass flows.

Campaign 3 – Reduced ethanol dosage

Campaign 3 was performed from March 28–29, 2012. During the campaign, the ethanol dosage was varied in four successive cycles: 2.0 (normal ethanol dosage at the plant), 1.0, 2.0 (recovery), and 0 L/m^3. In addition, the process in the SBR was disturbed by a low influent volume to the SBR some days before the campaign started, which meant a lower ethanol dosage, in turn resulting in low pH and high NO_2^--N concentrations in the SBR.

4.2 EXPERIMENTAL PLAN FOR BIOAUGMENTATION

The study described in **Papers III** and **IV** was performed from December 2013 to May 2014. The SBR was started for the season at the end of October 2013, using activated sludge from the mainstream as inoculum. A stable process was established after two weeks. A pipe was temporarily installed for pumping RAS from the Augmented Train to the SBR, shown in Figure 4.4.

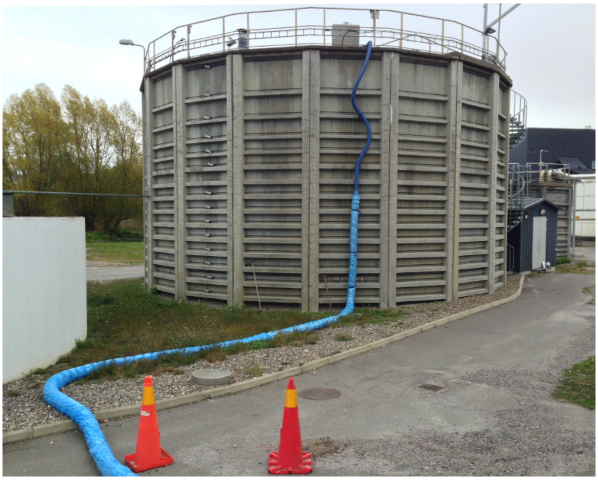

Figure 4.4. The SBR at Slottshagen WWTP and the temporarily installed pipe for pumping RAS to the SBR.

The flow rate of RAS to the SBR was stepwise increased relative to the flow rate of the digester supernatant to the SBR: starting with 0%, it was stepped up to 10%, 35% and finally 100%, corresponding to 0.08%, 0.28% and 0.80% of the RAS flow rate in the Augmented Train, respectively. The RAS was pumped to the SBR during the denitrification phase. Nitrification tests were performed roughly every second week on water from the Augmented Train and the Reference Train. The samples were collected from the last aeration basin in each train. On most occasions when nitrification tests were performed, multiple water samples were collected and analyzed to enable mass balances and other calculations. The temperature in the mainstream process varied from 8 °C to 15 °C and in the SBR from 24 °C to 35 °C. The temperature in the SBR was not noticeably affected by the stepwise increased share of cold RAS. The heat generated by biological reactions (Jewell & Kabrick, 1980), enhanced by ethanol dosage, kept the temperature at approximately 30 °C. The lowest temperature in the SBR occurred in mid March and coincided with a week-long blower failure. During these days, the cyclic pH fluctuations were greatly diminished, confirming the reduced biological activity.

In order to inoculate as large an amount of nitrifiers as possible, the SRT in the SBR was decreased during the last two months of the study. This was accomplished by omitting the sedimentation phase and letting the mixers operate during the decantation phase so that the SRT was equal to the HRT. Although the aim was to decrease the SRT in the mainstream process during the last months of the study, this was not possible due to practical restrictions in the waste activated sludge (WAS) treatment system.

4.3 SLOTTSHAGEN WWTP AND THE SBR

The full-scale studies performed (**Papers I–V**) took place at Slottshagen WWTP, located in Norrköping, Sweden (see Figure 4.1). The WWTP mainly treats municipal wastewater from Norrköping. It is designed for 200,000 population equivalents (PE), defined as 70 g BOD_7/(PE*day), a total nitrogen load of 2240 kg/d, and a flow rate of 2000 m^3/h. The actual load corresponds to 135,000 PE, a TN load of 1650 kg/d, and a flow rate of 1900 m^3/h.

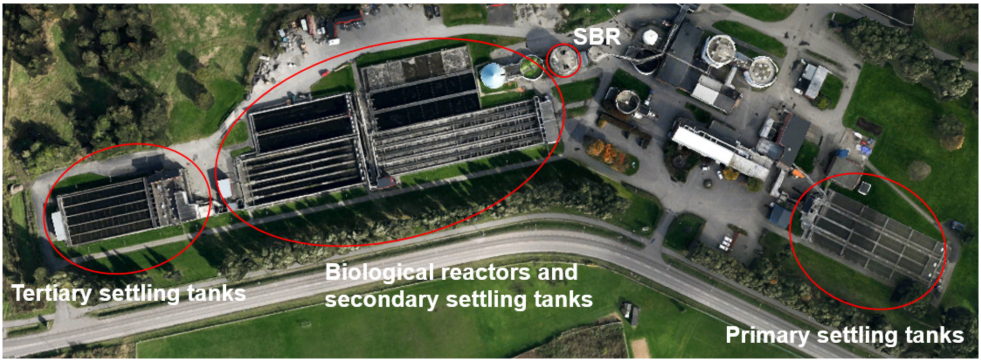

Figure 4.1. Slottshagen WWTP in Norrköping, Sweden (photo from Google Earth).

The WWTP is an activated sludge plant comprising pre-precipitation, biological reactors with pre-denitrification and contact stabilization, and post-precipitation. The biological treatment in the mainstream process consists of two separated trains and is composed of a modified Ludzack-Ettinger (MLE) process (Metcalf & Eddy, 2003), in addition to aerated stabilization reactors on the RAS flow. The typical composition of the influent/effluent to/from the biological treatment is: TN, 30/6 mg/L; NH_4^+-N, 23/2 mg/L; NO_3^--N, 1/2 mg/L; COD, 220/35 mg/L; PO_4^{3-}-P, 1/0.2 mg/L; and BOD_7, 115/2 mg/L. Ethanol is added as an external carbon source when needed, and is dosed only when the nitrate in the effluent from the biological treatment is higher than a pre-set value. The concentration of mixed liquor suspended solids (MLSS) is controlled based on the water temperature, indirectly controlling the SRT in the system. A separate pump is used for the withdrawal of WAS, pumping from the RAS stream in order to maintain the required MLSS concentration in the reactors. The flow rate from the frequency-controlled WAS pump is set manually. Ferric chloride is used for chemical precipitation of phosphorus. The fact that the mainstream process is composed of two separate trains makes the plant perfect for comparative studies. This is used in **Papers III** and **IV**, where bioaugmentation is applied in one of the trains and the other train is a reference.

The sludge is pumped to two mesophilic digesters. The sludge is dewatered in centrifuges, and the digester supernatant is piped to a buffer tank with a water volume of 200 m^3, from which it is pumped into an SBR. The SBR is in operation from November to May to ensure that the legislatively permitted TN level in the effluent from the mainstream process is fulfilled during the cold period of the year. During the start-up, the SBR is partly filled with excess sludge from the mainstream process (both trains). Parts of the digester supernatant are added cautiously and the SBR cycle is started. After 1–2 weeks, the SBR receives all produced digester supernatant. The covered SBR has a volume of 1000 m^3 and constitutes 3% of the total volume of the biological treatment in the mainstream process. The SBR has been operated with varying pre-denitrification or post-denitrification. In recent years and during the studies of bioaugmentation (**Papers III** and **IV**), it has been operated with post-denitrification. However, during the study of nitrous oxide emissions (**Papers I, II** and **V**), it was operated with pre-denitrification. It is run with a cycle length of 8 h. Ethanol is added to the SBR as a carbon source during denitrification. The influent flow in an ordinary cycle for the SBR is normally 70 m^3 The flow rate of the digester supernatant typically constitutes 0.5% of the flow rate of influent to the WWTP, whereas the TN load to the SBR averages about 15% of the TN load to the plant. The treated water from the SBR is directed to the biological reactors of the mainstream process.

The typical composition of the digester supernatant in the influent to the SBR is: TN, 1,200 mg/L; NH_4^+-N, 1,000 mg/L; NO_3^--N, 5 mg/L; COD, 2,000 mg/L; PO_4^{3-}-P, 40 mg/L; HCO_3^-, 90 mM; pH, 8.0; and temperature, 28 °C. In an ordinary cycle for the SBR there is an influent flow of 70 m^3. Under normal operating conditions, the SBR has a temperature of 30 °C, a concentration of MLSS of 3000 mg/L, a pH fluctuation of 1.3 units (typically 6.2–7.5), and a DO set point during nitrification of 2.0 mg/L. The HRT is 4.7 ± 0.3 d. The SRT is 15 ± 3 d. Removal of TN and NH_4^+-N is typically 80% and 95%, respectively. In the SBR, these parameters normally fluctuate in the following ranges: NH_4^+-N, 30–90 mg/L; NO_2^--N, 10–25 mg/L; and NO_3^--N, 90–150 mg/L. Two stirrers ensure good mixing during denitrification, using a total power input of 13.2 W/m^3. A variable frequency blower with a maximum capacity of 2400 Nm^3/h provides air during nitrification; the air is distributed through fine bubble membrane diffusers.

4.4 CHEMICAL ANALYSES AND INSTRUMENTATION

Several online sensors were installed permanently at the SBR: DO (LDO; Hach Lange), pH (1000 TR; Elmacron, Norrköping, Sweden), MLSS concentration (Solitax; Hach Lange), ethanol dosage (Mass 2100; Siemens, Munich, Germany), blower airflow (FCI, ST50-EF32BN0A), SBR water level (7060; MJK, Säffle, Sweden), and temperature (MCR-SL-PT100-UI-200; Phoenix Contact, Blomberg, Germany).

During the studies of nitrous oxide emissions (**Papers I, II** and **V**), some temporarily online sensors were installed at the SBR. A microsensor (N_2O-R; Unisense, Aarhus, Denmark) was used for the online measurement of N_2O concentration in water. An ultra trace nitrous oxide analyzer (model GFC-7002E; Teledyne Analytical Instruments, City of Industry, CA, USA) and a single-beam infrared spectrophotometer (MIRAN 1B; Foxboro Co., Foxboro, MA, USA) were used for the online measurement of N_2O concentration in off-gas.

In **Papers I, II** and **V**, grab samples for analysis were taken frequently at various time intervals from the SBR. The samples for analysis of soluble parameters were immediately filtered through a 0.45-µm filter. All samples were kept cold and analyzed within 18 h. In **Papers III** and **IV**, grab samples were taken from the biological reactors in the mainstream process and at the end of the aerated phase in the SBR. In order to enable mass balance calculations in **Papers III** and **IV**, grab samples were also taken at the influent and effluent to/from the SBR and to/from the two trains in the mainstream process. All chemical analyses (**Papers I–V**) were performed using commercial cuvette test kits (Hach Lange, Düsseldorf, Germany) and a Xion 500 spectrophotometer (Hach Lange). Test kits LCK 303 and LCK 304 were used for NH_4^+-N, LCK 341 and LCK 342 for NO_2^--N, LCK 339 for NO_3^--N, LCK 138 for TN, LCK 114 for COD, LCK 348 for total phosphorus, and LCK 348 for soluble PO_4^{3-}-P. MLSS, mixed liquor volatile suspended solids (MLVSS), and alkalinity were determined according to Swedish standard methods SS-EN 872:2005, SS 028112-3, and SS 028139-1, respectively.

4.5 MEASUREMENT OF N_2O IN WATER AND OFF-GAS

In **Papers I, II** and **V**, the concentration of nitrous oxide was measured in the water, as well as the emissions stripped to the air. Accordingly, the correlation between these two parameters could be studied. When measuring N_2O in the off-gas, a 0.81 m^2 floating gas hood was used to collect the gas from the water surface, shown in Figure 4.2. An extra air inlet was introduced to the hood to enable gas flow to the off-gas analyzer during periods without aeration. The total air flow was measured using a mass flow meter (model 6441; Testo, Lenzkirch, Germany) and a variable area meter, in other words a rotameter (Brooks Instrument, Hatfield, PA, USA). A dilution system with a maximum dilution of 40 times was used to measure high N_2O concentrations in the off-gas.

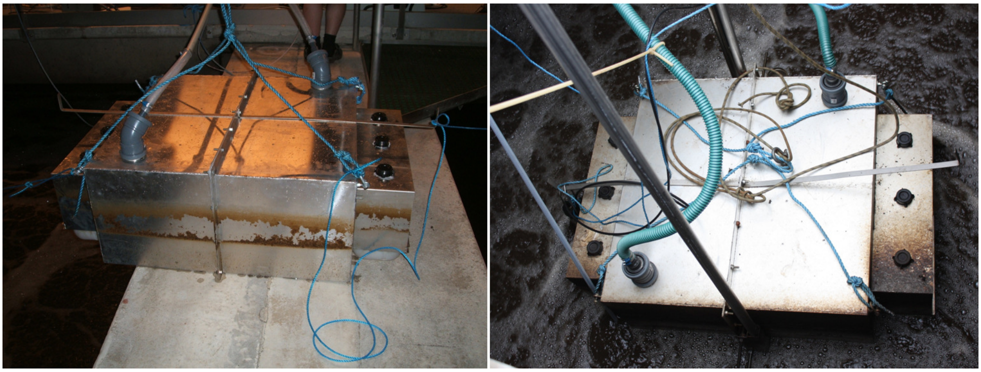

Figure 4.2. The floating gas hood for collecting of the gas from the water surface. Left: Situated above a basin. Right: In use in the SBR at Slottshagen WWTP.

4.6 NITRIFICATION RATE TESTS

Nitrification rate tests in **Paper III** were performed in the laboratory. The nitrification tests were performed in accordance with Kristensen et al. (1992). A 400 mL portion of each sample was continuously aerated at 20 °C to a DO concentration of 5.0–6.5 mg/L during the test. Ammonium was added to an initial concentration of about 40 mg/L, along with nutrients and alkalinity. Samples of 7 mL were withdrawn every 20 minutes and were immediately filtered. The experimental set-up for the nitrification rate tests is shown in Figure 4.3. Samples were analyzed for NH_4^+-N, NO_3^--N, and NO_2^--N. The ammonium utilization rate (AUR) was determined from the production rate of nitrate plus nitrite, and as a control, also determined from the utilization rate of ammonium. Concentrations of MLSS and MLVSS were determined to enable calculations of utilization rates related to suspended solids.

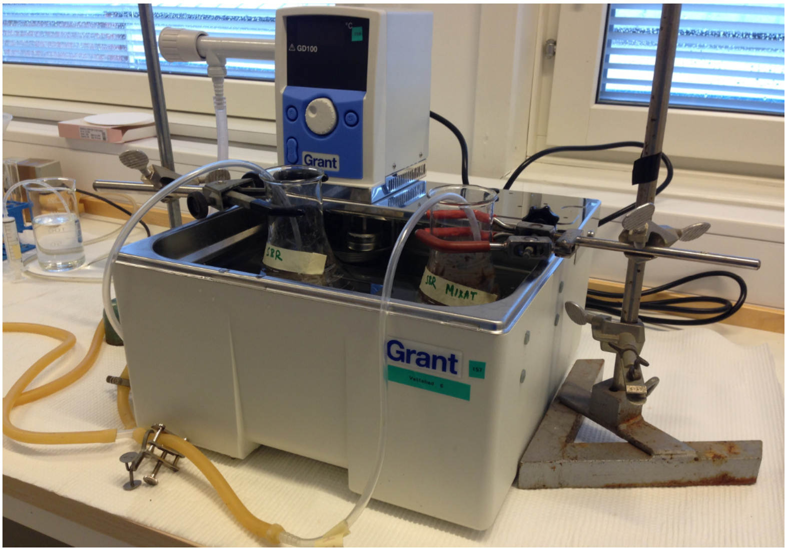

Figure 4.3. The experimental set-up for nitrification rate tests. Water bath for the keeping of a stable temperature (20 °C) and hoses for the aeration of the samples.

4.7 16S rRNA AMPLICON SEQUENCING

Grab samples were taken every time a nitrification test were performed (**Papers III** and **IV**), approximately every second week, from the SBR and the two separate trains in the mainstream process. 16S rRNA amplicon library preparation (V1–3), DNA sequencing and 16S rRNA amplicon bioinformatics processing were performed in the same way as described in Matturro et al. (2016). The samples were homogenized and immediately frozen. They were later analyzed by 16S rRNA amplicon sequencing, targeting the bacterial variable region 1–3 (V1–3), performed by DNASense, Aalborg (Denmark). The method comprises DNA extraction from all bacteria through a molecular process followed by DNA sequencing. The bacterial DNA have specific "fingerprints" which are used to identify the species by matching with a database. DNA was extracted through the FastDNA SPIN Kit for soil (MP Biomedicals, USA), using 4x the normal bead beating to enable recovery of bacteria that are difficult to lyse (Albertsen et al., 2015).

5 NITROUS OXIDE EMISSIONS

The formation of nitrous oxide in water and emissions to the off-gas were studied under varying oxygen concentrations in **Paper I**. In **Paper II**, an approach for modeling the results from **Paper I** was performed. In **Paper V**, the study on formation of nitrous oxide related to the carbon dosage was presented.

Studies performed in full-scale WWTPs demonstrate that N_2O emissions vary significantly from plant to plant and depending on the process configuration. In a study of 25 activated sludge plants, Wicht and Beier (1995) found that N_2O emissions ranged from 0% to 14.6% of nitrogen load, while in a study of seven WWTPs with various configurations, Foley et al. (2010) found that N_2O emissions ranged from 0.6% to 25.3% of denitrified nitrogen. These ranges are immense and indicate that the way of operating a plant could have a great impact on the emissions. Furthermore, because such high N_2O emissions have been found in mainstream processes, considerably higher emissions could be expected in sidestream processes treating digester supernatant with much higher concentrations of nitrogen. This is also demonstrated in **Papers I** and **V**.

As explained in **Paper I**, the process conditions for the biological nitrogen removal were deliberately deteriorated in order to investigate different thresholds of increased formation of nitrous oxide. Consequently, the emissions of nitrous oxide achieved from different campaigns varied greatly, which was presented in **Paper V** (see Table 5.1). Beside the fact that the process conditions were deliberately worsened, the high emissions were also caused by the fact that the process conditions in the full-scale SBR differed considerably before each campaign started. In some cycles, the amount of N_2O-N in the off-gas exceeded 100% of the TN in the influent, because the TN in the SBR's bulk liquid was also transformed into N_2O. This occurred in both Campaign 2 when the DO was reduced and in Campaign 3 when the ethanol dosage was reduced.

Even if the biological process in the SBR was aggravated intentionally, the tremendous fluctuations of nitrous oxide emissions shown in Table 5.1 demonstrate that the way a biological treatment plant is operated cannot be overstated.

Table 5.1. Maximum, minimum and average values for various parameters in the SBR in all three campaigns. Values are achieved from on-line sensors (pH, N_2O in off-gas (ppm) & N_2O in water) chemical analyses (TN_{filtr}, NH_4-N, NO_3-N, NO_2-N & COD_{filtr}), and calculations (N_2O-N in off-gas (% of TN in influent per cycle)). From **Paper V**.

		pH	N_2O in off-gas (ppm)	N_2O-N in off-gas (% of TN in influent per cycle)	N_2O in water (µmol/L)	TN (filtr.) (mg/L)	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	COD (filtr.) (mg/L)
Campaign 1	Max.	8.1	4900	8.9%	846	543	160	188	86	723
	Min.	5.6	1	3.5%	1	280	64	96	1	651
	Average	7.1	420	6.5%	200	431	109	143	45	690
Campaign 2	Max.	8.7	56,400	108%	1640	218	69	110	66	482
	Min.	6.2	0	5.1%	2	156	0	41	8	382
	Average	7.2	1600	32%	343	184	37	75	34	437
Campaign 3	Max.	8.3	76,200	218%	1180	365	123	120	124	701
	Min.	5.5	5	34%	9	235	22	37	66	607
	Average	6.8	4270	83%	285	281	73	76	90	642

5.1 N_2O FORMATION AND CARBON DOSAGE

The ethanol dosage was stepwise increased over time as part of a longer-term study to discern whether the N_2O in the off-gas would be affected. The original ethanol dose of 2.0 L/m^3 digester supernatant in the SBR was increased in steps of 0.5 L/m^3, ending at 3.5 L/m^3. This corresponds to an approximate increment of COD from dosed ethanol of 2.3 to 4.1 kg COD/kg TN in the influent. The increased ethanol dosage resulted in lower N_2O emissions in the off-gas and a more stable process without separate peak emissions (Figure 5.1). The decreased N_2O emissions corresponds to, on average, from > 12% to < 3% of TN influent.

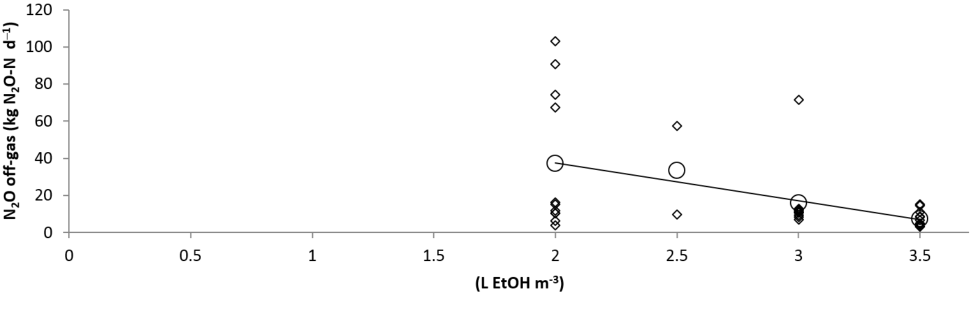

Figure 5.1. N_2O-N in off-gas from the SBR from March 15 to May 2, 2012, over different dosages of ethanol (diamonds) and with average values for each dosage (circles). Data affected by the modified operation during the campaigns are excluded. Emissions of 10 kg N_2O-N/d in the off-gas correspond to approximately 3.5% of the TN in SBR influent. From **Paper V**.

The results of decreasing N_2O emissions with increasing COD/N-ratio aligns with findings by Hanaki et al. (1992), who studied N_2O emissions at different COD/N-ratios (1.5, 2.5, 3.5, and 4.5) and noticed that more than 10% of the nitrogen load was emitted at a COD/N-ratio of 1.5, compared to virtually 0% at a COD/N-ratio of 3.5 at the same SRT. Furthermore, the importance of a sufficient COD/N-ratio was also demonstrated in **Paper I**: dissolved nitrous oxide accumulated in the water during denitrification but was immediately reduced when the ethanol dosage started. The suggested mechanism for N_2O formation here is incomplete denitrification by heterotrophs.

5.2 N_2O FORMATION AND DO

The immediate effect of decreased DO concentrations was revealed in Campaign 2, presented in **Paper I**. When the DO concentration was reduced from 2.0 to 0.9 mg/L in cycles 1 and 2, the formation of N_2O increased in both water and off-gas. The increment of N_2O in water was 65.6%, comparing N_2O formed during the nitrification phases with TN in the influent. The vast majority of N_2O formed in these cycles was produced during the nitrification phases. In cycles 3–5, much more N_2O was formed in water than in cycles 1 and 2. Nevertheless, the N_2O emissions in off-gas were still low during denitrification because the formed N_2O accumulated in the water during denitrification and was stripped off when the aeration started.

A stepwise reduction of the DO concentration revealed an obvious correlation between decreased DO and increased N_2O concentrations in the water (**Paper I**), shown in Figure 5.2. When the DO concentration was below 1.0–1.5 mg/L, the N_2O concentration in the water increased.

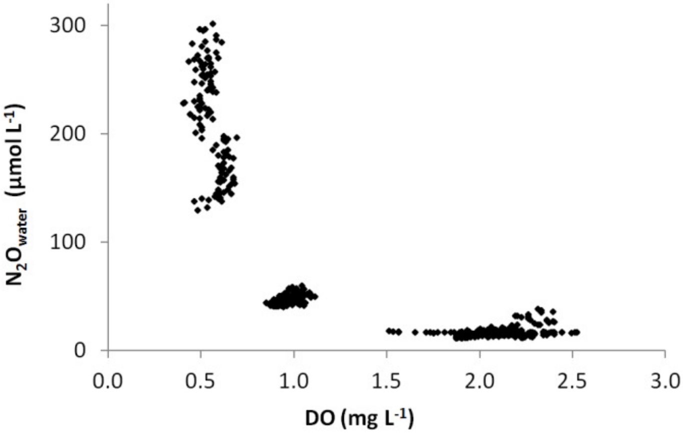

Figure 5.2. Concentration of N_2O in water over the last 2 h of the nitrification phase versus DO. The N_2O concentration in water increased when the DO concentration was reduced. The graph is based on data from cycles 1, 2 and 4, Campaign 2. From **Paper I.** Printed with permission from IWA Publishing.

The pathways suggested here for forming of N_2O are aerobic denitrification of NO_2^- performed by AOB, so-called nitrifier denitrification. Another pathway of N_2O generation during aerobic conditions is when N_2O is formed during hydroxylamine oxidation. Nevertheless, because the NO_2^- concentration was high during the study, nitrifier denitrification is a stronger N_2O formation pathway than is hydroxylamine oxidation (Wunderlin et al., 2012). The results coincide with Zheng et al. (1994), who observed a significant decrease of N_2O production when DO was increased from 0.5 mg/L to 1.7 mg/L.

5.3 N₂O FORMATION AND NITRITE CONCENTRATION

When the DO concentration was reduced in Campaign 2, the NO_2^- concentration increased and, in turn, the N_2O concentration increased in both water and off-gas. This was demonstrated in **Paper I** and is shown in Figure 5.3. The correlation between simultaneously high concentrations of NO_2^- and N_2O has been observed before in several studies, as reviewed by Kampschreur et al. (2009a). Foley et al. (2010) also observed a strong correlation between high production of N_2O and a high NO_2^- concentration. However, it was stressed that it is difficult to clearly point out the predominant mechanism of N_2O production because NO_2^- is simultaneously a product, a substrate and an inhibitor, and can be formed and utilized under both aerobic and anoxic conditions.

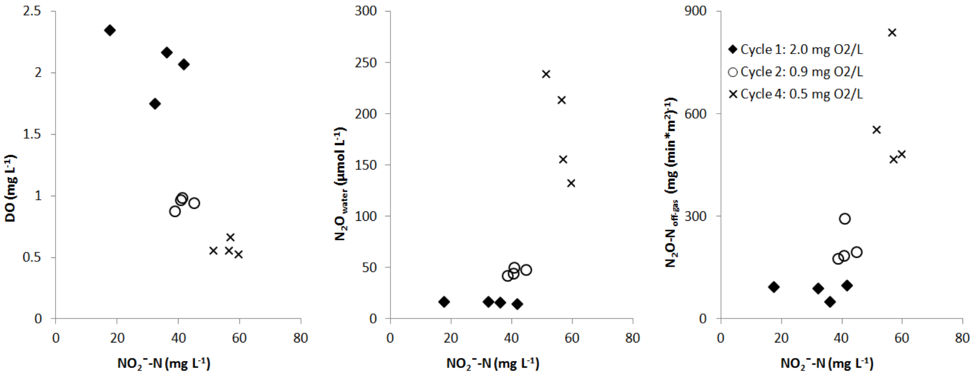

Figure 5.3. Concentrations of DO and N_2O in water and in off-gas versus NO_2^- over the last 2 h of the nitrification phase of cycles 1, 2 and 4, Campaign 2. N_2O in off-gas is expressed in mg/(min*m^2) of water surface. From **Paper I**. Printed with permission from IWA Publishing.

5.4 MODELING OF N₂O EMISSIONS

Results from the modeling of N_2O emissions from the SBR (**Paper II**) supports one of the main results from **Paper I**: nitrifier denitrification was an important reason for N_2O emissions during aerated phases. The applied AOB denitrification model could successfully describe the biological processes and the behavior of N_2O formation in the SBR. Nevertheless, there were some adjustments needed. The original ASMN inhibition term for S_{NO} was replaced by S_{NO2} inhibition (Zhou et al., 2008) since no information on the NO concentrations was available. Furthermore, the drastic shift between complete and no inhibition because of a low availability of readily biodegradable substrate (S_S) could not be captured by the original ASMN model, and motivated the extension with an additional model component representing ethanol, $S_{S,EtOH,5}$ [g COD/m^3].

However, there is potential for some improvements in the model, as the SBR cycles chosen for simulation did not include the more extreme fluctuations of N_2O formation. Moreover, during the latter part of the denitrification phases, when the ethanol dosage had stopped, there were simulated peaks of N_2O formation in the SBR that were not measured. As a consequence, the model generated false peaks of N_2O in the off-gas when the aeration started, also described in **Paper II**.

In spite of many different attempts to model the formation of N_2O, reviewed in Mannina et al. (2016), the existing models still contain limitations and there is a need for models that better describe the N_2O formation (Mannina et al., 2016).

6 BIOAUGMENTATION

The purpose of bioaugmentation is to enhance the nitrifiers in the mainstream process and hence enable a capacity increase in the mainstream process. Consequently, bioaugmentation constitutes both treatment of digester supernatant and boosting of the mainstream process.

Two full-scale studies of bioaugmentation have been performed at Slottshagen WWTP, Norrköping. The mainstream process at the WWTP has an appropriate configuration for the study, composed by two separate trains where one of them can serve as a reference: the Augmented Train and the Reference Train.

6.1 NITRIFICATION RATE TESTS

The nitrification rate test was used to examine the difference between the Augmented Train and the Reference Train during the study. Accompanying the nitrification rate tests, mass balances were established.

Different RAS flow rates were conveyed to the SBR from the mainstream process: 0%, 10%, 35% and 100% of the digester supernatant flow rate to the SBR. Altogether, nitrification rate tests were performed on 11 different occasions. Results from the nitrification rate tests were grouped into different categories based on the RAS flow rate to the SBR and on the varied SRT in the SBR. The resulting nitrification rates from these categorized groups are shown in Figure 6.1a. A normalization of the results from the two trains was performed to enable an adequate comparison, described in detail in **Paper III**. The maximal increase in nitrification rate was observed during the coldest period. The nitrification rate was by then increased by 41% in the Augmented Train (Figure 6.1b).

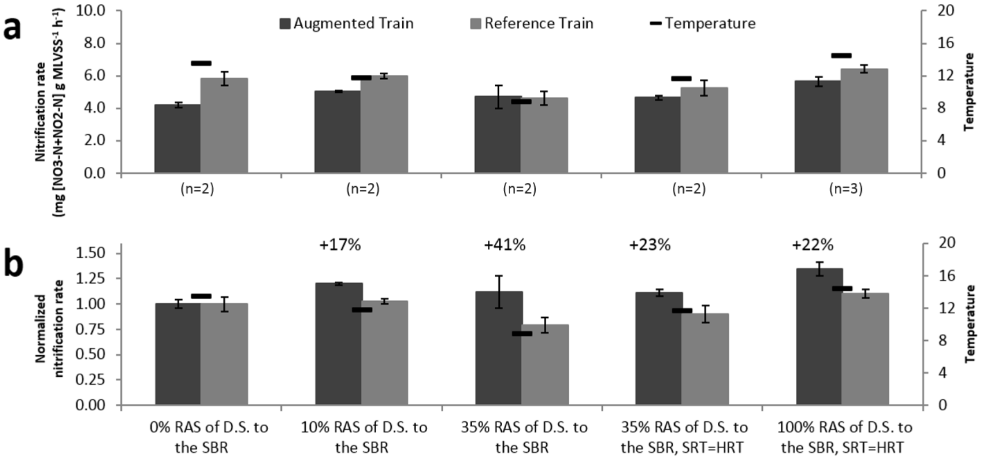

Figure 6.1. Results from the Augmented Train and the Reference Train during the experiment, grouped into categories based on the RAS flow rate of digester supernatant (D.S.) flow rate to the SBR. (a) Nitrification rates, and (b) normalized nitrification rates. From **Paper III**. Printed with permission from IWA Publishing.

To reveal how the increased nitrification rates from the bioaugmentation correlated with the temperature in the mainstream process, weekly uncategorized and normalized data were used. The normalized nitrification rates in the Augmented Train were compared with those in the Reference Train. The results showed that the highest increment of nitrification rate was achieved when 100% RAS of digester supernatant flow rate was applied, stepwise followed by 35% and 10% RAS of digester supernatant flow rate. This suggests that a higher RAS recirculation to the SBR implies a larger increment of the nitrification rate. In this comparison, it was found that the highest increment in the nitrification rate for the Augmented Train coincided with the lowest temperature and was 58% higher than in the non-bioaugmented Reference Train. This is in the same range as was measured in a study by Berends et al. (2005).

The increased nitrification rate over the whole bioaugmented period averaged 25%. This is in agreement with Hommel et al. (2006), who observed a 23% increment in nitrification activity in a resembling full-scale study. In order to obtain a low decay of nitrifiers, Salem et al. (2004) stated that the optimum SRT in the sidestream plant should not be higher than 0.5–2 d. The applied minimum SRT during the study was 2.5–10 d. Consequently, there was a potential to reach even higher nitrification rates than was measured during the study.

6.2 16S rRNA AMPLICON SEQUENCING

A total of 33 samples were analyzed by 16S rRNA amplicon sequencing from all three reactors to identify and count the bacteria species and their abundance (**Paper IV**).

The relative abundance of AOB and NOB were examined. In the Augmented Train, Reference Train and in the SBR the relative abundance of nitrifiers varied between 1.5–3.5%, 1.2–3.2 and 1.4–7.8%, respectively. Eleven different AOB species were read: ten of those were of the genus *Nitrosomonas*, one was of the genus *Nitrosospira* (in a few samples only and at very low abundances). Five different NOB species were detected: three of the genus *Nitrospira* and two of the genus *Candidatus Nitrotoga*. The study also included examination of different strategists. K-strategists have a low growth rate and high substrate affinity, and consequently benefit from low substrate concentrations. In contrast, r-strategists have a high growth rate but a low substrate affinity, and therefore thrive in high substrate concentrations. Any change from r-strategists to K-strategists was not observed for AOB or NOB in any of the three reactors, in contrast to the results of a pilot-plant study that were reported by Pei et al. (2015).

Before the bioaugmentation commenced, there were in total barely 1,000 different species in the SBR. After the bioaugmentation started, that is when RAS from the mainstream process began pumping to the SBR, and the number increased to more than 1,700 species in a few weeks; an obvious effect on the SBR from bioaugmentation. The difference between the two mainstream trains became more apparent when the numbers of different AOB and NOB species were studied. Before the bioaugmentation started, there were eight nitrifying species in each reactor as a maximum. When the bioaugmentation was running, the average number of species in the non-bioaugmented Reference Train decreased. This is in concordance with Urakawa et al. (2008), who found that the diversity of AOB decreased with a lowered temperature. Simultaneously, the opposite pattern was found in the Augmented Train with increasing numbers of different nitrifying species (also in the SBR), even during the coldest season. Both AOB and NOB species increased. This is in concordance with Gatti et al. (2015) and Smith et al. (2008), who observed that bioaugmentation enhances the microbial diversity. The increased number of nitrifying species in the Augmented Train and the SBR is the result of an interaction from bioaugmentation, boosting each other in a win-win system. Furthermore, Naeem & Li (1997) stated that biodiversity enhances the reliability of an ecosystem. If so, bioaugmentation can be claimed to provide a more sustainable composition of nitrifiers.

The average relative abundance of AOB and NOB in the three different reactors during the bioaugmentation period are presented in Figure 6.2. As expected, the highest abundance of nitrifiers are found in the SBR, caused by the high nitrogen concentration in the digester supernatant. When comparing the Augmented Train to the Reference Train, both AOB and NOB are more abundant in the Augmented Train. The abundance of AOB and NOB are 32% and 17% higher in the Augmented Train, respectively. The abundance of nitrifiers is on average 25% higher in the Augmented Train for the whole bioaugmentation period of four months. This is in concordance with differences in average nitrification rates for the two trains (**Paper III**). An increment of nitrifier abundance in the mainstream process was also observed by Gatti et al. (2015) as a consequence of bioaugmentation: the abundance of AOB and NOB increased from 4 to 8% and from 2 to 9%, respectively, which is higher than was observed in the frame of this work.

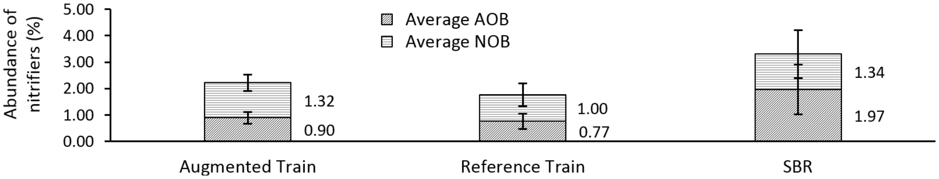

Figure 6.2. Average abundance of AOB and NOB in all three reactors during the bioaugmentation period (weeks 3–19). From **Paper IV**. Printed with permission IWA Publishing.

The distribution of NOB species during the experiment revealed that the bioaugmentation impacted both the Augmented Train and the SBR. During the first period of bioaugmentation, the abundance of *Nitrospira* was dominating in all three reactors. After a few weeks the abundance of *Nitrotoga* increased in the Reference Train. Meanwhile, the abundance of *Nitrotoga* in the Augmented Train and the SBR was still very low. A few weeks later, the abundance of *Nitrotoga* increased simultaneously in the Augmented Train and the SBR. This delay of the *Nitrotoga* entering the Augmented Train and the SBR might be explained by the fact that *Nitrospira* is favored by the higher temperature in the SBR and is thereby retained in the system. When *Nitrotoga* finally starts growing in the augmented system its abundance in the Augmented Train and the SBR increase in the same pace.

The fact that the type of nitrifiers was influenced by bioaugmentation was also shown by Salem et al. (2004): *Nitrosomonas* were found only in the sidestream plant and the bioaugmented train in the mainstream process, not in the reference train in the mainstream process.

7 CONCLUSIONS

The work included in this research project investigates different risks and possibilities in treating digester supernatant by conventional nitrification-denitrification in an SBR. The risks are related to efforts to obtain a lower operational cost by reducing the aeration in the reactors and decreasing the dosing of external carbon. The possibilities include the improved operation of the mainstream process by interconnecting to the sidestream reactor.

It is possible to find certain thresholds for when nitrous oxide production is obviously increased with regard to the oxygen concentration in the reactor and the dosing of external carbon. In order to avoid obviously increased emissions of nitrous oxide, the oxygen concentration during nitrification should not fall below 1.0–1.5 mg/L and the dosing of external carbon should not be lower than 3.5 kg COD/kg TN in the influent.

As long as the formation of nitrous oxide is moderate, it is possible to build a model for the simulation of nitrous oxide production with a reasonably good prediction of the emissions from a full-scale reactor.

It is shown that treatment of digester supernatant in combination with bioaugmentation is a feasible way of boosting the nitrifiers in the mainstream process. This implies a higher capacity for nitrogen removal for the whole WWTP or decreased energy consumption due to a decreased SRT in the mainstream process. The most important parameters for the magnitude of bioaugmentation are the temperature in the mainstream process and the flow rate ratio of RAS/digester supernatant to the sidestream reactor. Furthermore, it is demonstrated that the microbial composition is affected and the diversity of nitrifiers is increased in the mainstream process. In addition, the diversity of nitrifiers also increased in the sidestream reactor during bioaugmentation.

8 FUTURE STUDIES

There are still gaps in the knowledge of what is triggering the formation of nitrous oxide in the treatment of digester supernatant. Further work is needed on how to optimize the control strategies. Moreover, because many of the methods to mitigate production of nitrous oxide are hard to combine with a low operation cost, more effort is needed to find the narrow ranges where the nitrite oxide emissions are low but the operation costs still could be decreased.

Further work is also required to develop simulation models that can better predict emissions of nitrous oxide. This is essential in order to enable an easily approachable way to evaluate different low-emission control strategies.

A better knowledge and understanding of how to optimize the nitrification in the mainstream process by bioaugmentation is needed. Further work is necessary to find the optimal flow rate ratio of RAS/digester supernatant to the sidestream process. Also, the understanding of how the increased diversity of nitrifiers affects the mainstream process during bioaugmentation must be improved.

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Sometimes in life doors open that were not expected to open, although longed for. After 10 years working at Veolia Water Technologies – VA Ingenjörerna as a consulting engineer and project manager, I was ready for new challenges. But instead of looking for a job at another employer, I spoke with managers within the company and everything was arranged for me to start as a PhD student at Lund University, with Jes la Cour Jansen as my supervisor.

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