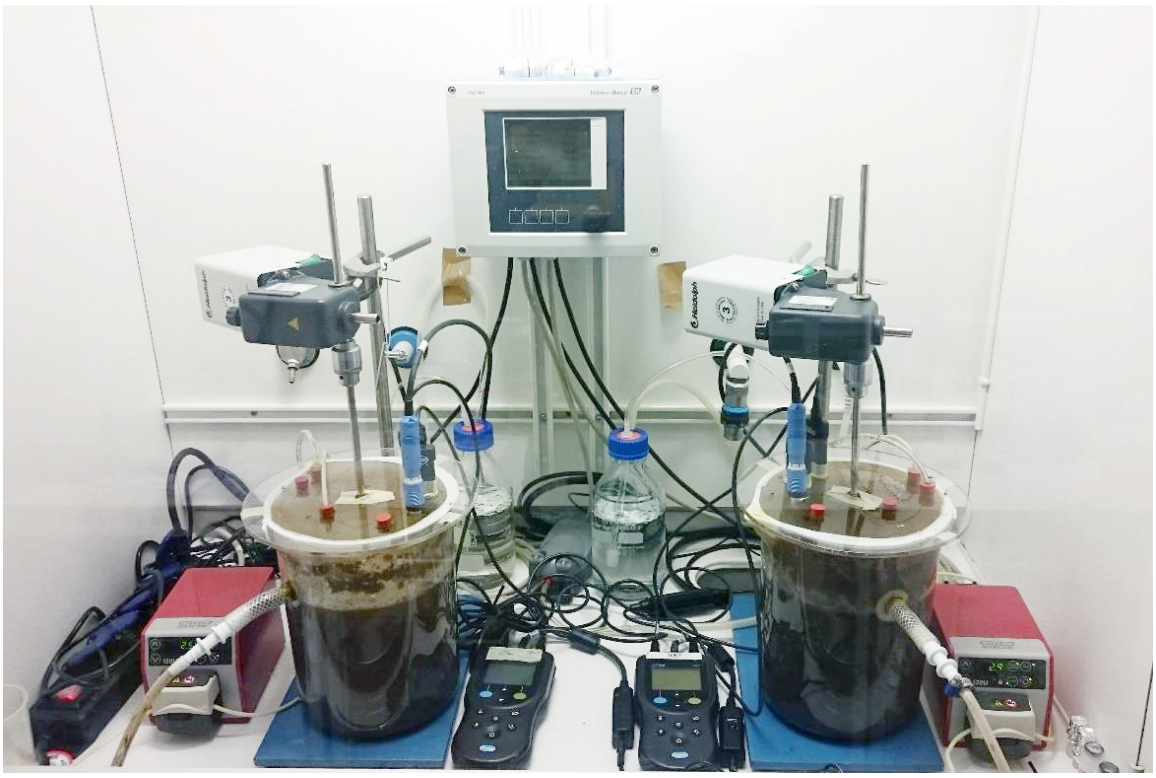
Urine nitrification

Start-up with high strength urine



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Water and Environmental Engineering
Department of Chemical Engineering
Master Thesis 2017

LUND UNIVERSITY

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Master Thesis number: 2017-08

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Department of Chemical Engineering
Lund University

June 2017

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Preface

This Master's degree project on urine nitrification was started under the initiative of Sweden Water Research and carried out at the Department of Chemical Engineering at Lund University during the period January to June 2017. The project continues on previous research on the subject performed by Marianne Olofsson and Ellen Edefell.

I would like to thank my examiner Michael Cimbritz and my head supervisor Karin Jönsson for setting the prerequisites for my degree project and my main supervisor David Gustavsson for his support, guidance and contribution of knowledge. I would also like to thank Leif Stanley for his contribution to the practical aspects of the laboratory equipment.

A special acknowledgement is dedicated to Gertrud Persson; your presence enlightens the daily work at the institution and your help in the laboratory has been indispensable.

Lastly, I wish to thank my mother for always encouraging me to aim for a higher education and supporting me when following my own path.

Lund, June 2017

Axel Olsson

Abstract

The importance of recycling nutrients becomes more important as the finite resources starts running out, and recycling of urine nutrients is an important factor in developing a sustainable society that takes advantage of nutrients already existing in the system. Urine only represents one percent of the domestic wastewater volumetric flow, but contains 80 % of the nitrogen, 50 % of the phosphorus and 60 % of the potassium of the total wastewater.

Urine nitrification with following distillation is one of the promising methods of retrieving the nutrients from urine on a large scale. The end product is a very concentrated liquid or dry fertiliser that can be used on agricultural lands and substitute the commercial fertilisers springing from finite sources and industrial nitrogen fixation.

In this study, the aim was to develop the urine nitrification process by studying the dynamics during start-up with high strength urine. Two reactors were operated simultaneously with the only difference in influent concentrations of urine; one with 25 % urine to water and the second one with 50 % urine. The inflow was controlled with pH regulated pumps.

The reactors showed similar patterns with an initial rapid increase in nitrification rate which decreased significantly after a few days of operation, probably due to the high concentrations of nitrate, ammonium and salts in both reactors. The nitrification rates increased when the pH set-point was adjusted from 6.2 to 6.3, which influenced the availability of the substrate ammonia. During changes in pH the nitrite concentrations increased with a subsequent increase of nitrous oxide concentration. The maximum nitrification rate was 0.63 g NO_3^--N m^{-2} d^{-1} with a total nitrogen concentration of approximately 4,400 mgN L^{-1} in the reactor.

In order to incorporate a fast start-up with high strength urine it is recommended to regulate the inflow pump with a pH set-point of 6.3, possibly higher, and after a sufficient nitrification rate is reached the pH set-point should be set to lower values to keep a stable balance between ammonia and nitrite oxidising bacteria. By having low nitrite accumulation nitrogen loss in form of nitrous oxide emissions can be avoided.

Keywords: Urine, nitrification, separation, stabilisation, start-up, high strength, nitrate oxidising, nitrite oxidising bacteria, nitrous oxide emissions.

Sammanfattning

Vikten av att återvinna näringsämnen blir mer betydelsefull när de ändliga resurserna börjar ta slut, och återvinning av urinens växtnäring är en viktig faktor i utvecklandet av ett hållbart samhälle som tar tillvara på redan använda näringsämnen. Urin står för endast en volymprocent av det totala avloppsvattenflödet från hushåll men innehåller 80 % av kvävet, 50 % av fosforn och 60 % av kaliumet av det som finns i avloppsvattnet.

Nitrifiering av urin följt av destillation är en av de lovande metoderna för att ta tillvara på näringsämnen i urin i större skala. Slutprodukten är en väldigt koncentrerad vätska eller ett torkat gödningsmedel som kan användas inom jordbruket och ersätta konstgödsel som härstammar från ändliga fosforkällor och industriellt fixerat kväve.

I den här studien var målet att utveckla nitrifieringsprocessen av urin genom att studera dynamiken under uppstart med hög koncentration av urin. Två reaktorer drevs samtidigt med den enda skillnaden att de hade olika startkoncentrationer av inkommande urin. Den ena reaktorn startade med 25 % urin blandat med vatten och den andra med 50 % urin. Inflödet var styrt via en pH-regulator ansluten till pumparna.

De två reaktorerna visade liknande resultat med en snabb ökning av nitrifikationshastighet vilken sjönk efter ett fåtal dagars drift, troligtvis på grund av hög koncentration av nitrat, ammonium och salter i reaktorerna. Nitrifikationshastigheterna ökade när pH-börvärdet var reglerat från 6.2 till 6.3, vilket påverkade tillgängligheten av substratet ammoniak. Vid förändring av pH ökade nitritkoncentrationerna vilket gav en efterföljande ökning av lustgaskoncentration. Den maximala nitrifikationshastigheten som uppnåddes var 0.63 g NO_3^--N m^{-2} d^{-1} med en koncentration av totalkväve på 4,400 mgN L^{-1} i reaktorn.

För att uppnå en snabb uppstart med hög koncentration av urin rekommenderas att inflödespumpen styrs med ett pH-börvärde på 6.3, alternativt högre, och när tillräckligt hög nitrifikationshastighet har uppnåtts ska börvärdet sänkas för att hålla en stabil balans mellan ammoniak- och nitritoxiderande bakterier. Genom att hålla nitritnivåerna låga kan kväveförluster i form av lustgas undvikas.

Nyckelord: Nitrifiering, urin, urinseparering, stabilisering, uppstart, koncentrerad, nitratoxiderande, nitritoxiderande bakterier, lustgas, lustgasutsläpp.

Abbreviations

AOB	Ammonia Oxidising Bacteria
DO	Dissolved Oxygen
COD	Chemical Oxygen Demand
FA	Free Ammonia
FNA	Free Nitrous Acid
HRT	Hydraulic Retention Time
IFAS	Integrated Fixed-film Activated Sludge
MABR	Membrane Aerated Biofilm Reactor
MBBR	Moving Bed Biofilm Reactor
N_2O	Nitrous oxide
NH_2OH	Hydroxylamine
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_2^--N	Nitrite nitrogen
NO_3^-	Nitrate
NO_3^--N	Nitrate nitrogen
NOB	Nitrite Oxidising Bacteria
SRT	Solids Retention Time
SS	Suspended Solids
Tot-N	Total Nitrogen
WWTP	Wastewater Treatment Plant

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1 Introduction

Modern society is dependent on the ability to provide food and pure drinking water. The food production has increased drastically since the industrial fixation of nitrogen with the Haber-Bosch process was introduced, together with the extensive use of phosphate rock for fertiliser production (Erisman et al., 2008). The phosphorus mines are finite resources of nutrients required for plant growth hence the importance of recirculating nutrients becomes larger as the availability of conventional fertiliser decreases (Larsen et al., 2013). The industrial fixation of nitrogen has approximately doubled the rate of nitrogen input into the terrestrial nitrogen cycle and the alteration has given effects such as increased concentrations of nitrous oxide (N_2O), loss of soil nutrients, acidification and accelerated loss of biological diversity (Vitousek et al., 1997), thus leading to a need to reduce the rate of nitrogen input to decrease its negative effects.

Today, most of the wastewater is handled in one stream and large volumes are sent to centralised treatment plants that generally focus on mechanical, biological and chemical treatment to remove nitrogen, phosphorus and carbon. As modern society becomes more complex the wastewater becomes more difficult to purify and the current waste handling systems are not designed to deal with these problems efficiently (Larsen and Gujer, 2001). Therefore, new approaches on wastewater handling are required.

Urine separation is one potential solution to the problems specified above, and has been used widely on smaller scale but so far it has not been established on larger scale. Urine contains most of the nutrients in domestic wastewater and makes up for less than one percent of the total wastewater volume (Maurer et al., 2006), which makes it very reasonable to divert urine from the rest of the wastewater in order to be able to reclaim these nutrients efficiently. Research has also indicated that 70-80 % of the pharmaceuticals in today's wastewater originate from urine, thus separation might be beneficial also when it comes to isolation and removal of pharmaceuticals (Larsen and Gujer, 2001).

The process of urine separation on smaller scale often involve some sort of separation at the toilet facility followed by storage and hygienisation for at least six months, or, alternatively direct use of the fresh urine as fertiliser (SSWM, 2017). A more effective system is required for the process to function on larger scale. The goal is to attain an end product that has a good fertilising capacity and is easy to transport. Stored urine is a volatile solution and stabilisation is needed to avoid nitrogen loss and bad odour (Udert et al, 2003a). Urine comes in liquid form and for transport purposes it is desirable to convert it to a smaller product easier to handle, both during transport and during the subsequent application on agricultural lands.

Nitrification of urine followed by distillation is a promising method to achieve these requirements (Udert & Wächter, 2012). Urine nitrification has previously been studied at Lund University under the supervision of Sweden Water Research (Olofsson, 2016; Edefell, 2017) and this Master's degree project will continue to explore and develop the process.

Edefell (2017) experienced nitrogen loss during her experiment and stated that this could be due to emission of N_2O. N_2O is a strong greenhouse gas which is a potential ozone depleting substance and has a Greenhouse Warming Potential (GWP) of approximately 300 units (IPCC, 2013). GWP is an index of the contribution to climate change relative to carbon dioxide when comparing the same amount of gas (ibid.). The major sources of N_2O emissions

are agriculture, fuel combustion and industry production, where agriculture stands for 75 % of the total emissions (EPA, 2017). N_2O emissions from wastewater treatment plants (WWTP) have a relatively small contribution to the overall global greenhouse gas emissions. The N_2O emissions from biological nutrient removal broadly used may however contribute significantly to the overall carbon footprint of wastewater treatment systems (Law et al., 2012). This project will therefore also focus on the role of N_2O in the process of urine nitrification.

It is desirable to have a fast start-up of the biological process in order to treat high flows of incoming urine. If the process is not fast enough the urine needs to be stored, which requires extra space and there is also a risk of loss of nitrogen through ammonia (NH_3) volatilisation.

1.1 Aim

The aim of this study was to further investigate and optimise the start-up and operation of urine nitrification based on previous research on the subject (Olofsson, 2016; Edefell, 2017; Udert et al., 2003a; Udert & Wächter, 2012; Fumasoli et al., 2016).

The two objectives of this study are to:

- Study start-ups in two equal reactors with different influent urine concentrations.
- Study dynamics of N_2O production during process disturbances in form of pH alterations.

1.2 Limitations

The study will be conducted in a research laboratory where the temperature is at an approximately constant value of 20 °C. The urine originates from the same household throughout the entire experiment. The process was operated for two months which is a too short time period for including a full start-up. Microbial analysis of the carrier biofilm is not performed during the project period but samples are collected for future analysis.

1.3 Organisation of the report

The report will start with a section on the theory and terms needed for the understanding of the subject. The experimental set up will then be described together with operational measures during the experiment. The results will be presented in the chapter that follows and discussed simultaneously. In the final chapter conclusions and recommendations for future work summarises the report. The raw data and additional figures from the project will be presented in Appendix.

2 Theory

The aim of the urine nitrification process is to convert the volatile urine to a stable solution that contains equal amounts of nitrate (NO_3^-) and ammonium (NH_4^+). This chapter will cover basic theory and terms needed to understand the process from the discharge and storage of urine to the desired product. A short update of the current research on the subject will follow in the end of the chapter.

2.1 Urine

The urine holds 80 % of the nitrogen, 50 % of the phosphorus and 60 % of the potassium of the total amount of nutrients found in household wastewater (Naturvårdsverket, 1995). The nutrient content in fresh or stored urine is small with a nitrogen content of 0.9 %, phosphorus 0.06 % and potassium 0.3 % (Maurer et al., 2006). It is therefore beneficial to concentrate the urine for transportation and storage purposes, and one of the ways to do this is by distillation preceded by nitrification.

When urine leaves the body, it is sterile and mainly consists of the substance urea. During storage, the organic matter is degraded through microbial activity and the urea is hydrolysed. The hydrolysis release NH_3 and causes the pH to increase to about 9.2. The NH_3 is very volatile thus the nitrogen loss could be significant (Udert et al., 2006). The composition of fresh and stored urine is showed in *Table 1*.

Table 1: Average urine composition based on values from various sources presented in Udert et al. (2006).

	Unit	Fresh urine (Average)	Stored urine
Total nitrogen	$gN\ m^{-3}$	9,200	9,200
Total NH_3	$gN\ m^{-3}$	480	8,100
Ammonia NH_3	$gN\ m^{-3}$	0.3	2,700
Urea	$gN\ m^{-3}$	7,700	0
Total Phosphate	$gP\ m^{-3}$	740	540
Calcium	$g\ m^{-3}$	190	0
Magnesium	$g\ m^{-3}$	100	0
Potassium	$g\ m^{-3}$	2,200	2,200
Total Carbonate	$gC\ m^{-3}$	0	3,200
Sulphate	$gSO_4^{2-}\ m^{-3}$	1,500	1,500
Chloride	$g\ m^{-3}$	3,800	3,800
Sodium	$g\ m^{-3}$	2,600	2,600
pH	-	6.2	9.1
Alkalinity	mM	22	490
COD	$gO_2\ m^{-3}$	10,000	10,000

Urine can be used as a fertiliser and one important feature for a fertiliser is low concentration of heavy metals relative to phosphorus or nitrogen. Studies have shown that urine has low concentrations of heavy metals compared to manure and commercially available fertiliser (Maurer et al., 2006). Urine might contain many of the dissolved micropollutants derived from pharmaceuticals (Maurer et al., 2006), thus the high strength flow of separated urine could be beneficial when removing micropollutants compared to treatment of diluted wastewater in conventional treatment plants.

2.2 Nitrification

The most important groups of organisms involved in nitrification are the lithoautotrophic ammonia oxidising bacteria (AOB) and the lithoautotrophic nitrite oxidising bacteria (NOB). The lithoautotrophs rely on the oxidation of inorganic compunds as their characteristic energy source (Koops et al., 2001). They cooperate in the process where NH_3 is oxidised to NO_2^-

$$NH_3 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O$$

$$NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^-$$

and nitrite is oxidised to nitrate

$$HNO_2 + H_2O \rightarrow HNO_3 + 2H+ + 2e^-$$

During the nitrification (NH_3 oxidation) alkalinity is consumed and pH decreases.

The nitrification process is dependent on several paremeters where the most influential are; organic load, DO concentration, total ammonium nitrogen, temperature, pH, alkalinity and the previous history of the biosystem (Rusten et al., 2006).

Organisms that use organic matter for the formation of new biomass are called heterotrophs, compared to autotrophs that derive carbon for biomass production from carbon dioxide (Metcalf & Eddy, 2003, p. 564). Autotrophs need to spend more of their energy when converting carbon dioxide for cell growth which generally results in lower growth rates compared to heterotrophs (ibid.). Chemical oxygen demand (COD) is a measure of the organic content in terms of biodegradable and non-biodegradable compounds (Gray, 2004, p. 93), and can therefore be an estimation of the heterotrophic activity. COD gives a measure of the amount of organics in the water hence a lower COD indicates higher heterotrophic activity.

There is a competition between heterotrophic and nitrifying bacteria in a biofilm and often the fast growing heterotrophic bacteria occupy the outer layer where oxygen and NH_3 is more accessible, while the slow growing nitrifying bacteria can be found in the deeper layer. The nitrifiers can be negatively affected by this if the oxygen concentration is low and the oxygen cannot penetrate the outer layer. If the oxygen concentration is high, the layer of heterotrophic bacteria can serve as protection from detachment (Nogueira et al., 2002).

2.3 Nitrifying bacteria

The nitrifying bacteria are divided into two groups, NH_3 oxidisers and NO_2^- oxidisers, based on their main function in the nitrification process (Koops et al., 2001).

Ammonia oxidising bacteria

During the first part of the nitrification *Nitrosomonas* and other groups oxidise NH_3 to NO_2^- (ibid.). The most common species found in sewage treatment plants are *N. europaea* and *Nitrosococcus mobilis*, which are halotolerant or moderately halophilic (ibid.). Salt requirement is a factor that separates the different groups of AOB. The two species of *Nitrosococcus*, *N. oceani* and *N. halophilus*, are obligately halophilic and belong to the γ subclass of the Proteobacteria. The β subclass includes species that are obligately halophilic, moderately halophilic, halotolerant and salt sensitive or have no salt requirement (Koops et al., 2001).

Nitrite oxidising bacteria

During the second part of the nitrification the NO_2^- is oxidised to NO_3^- (Koops et al., 2001). In most WWTPs, *Nitrospira*-like microorganisms are the dominating NO_2^- oxidisers. Previously *Nitrobacter* was considered to be dominant (Wagner and Loy, 2002). *Nitrobacter sp.* is considered to be an r-strategist and would therefore only be able to compete in an environment with relatively high NO_2^- concentrations (Moussa et al., 2006).

2.4 Nitrification as a stabilisation method of urine

During storage the fresh urine is exposed to microbial contamination, which causes degradation of organic matter and hydrolysis of urea (Maurer et al., 2006). The hydrolysis converts urea to NH_3 and NH_4^+ and during the process the pH increases to approximately 9.2. The consequence of degradation of organic matter is bad odour, and the precipitation of various compounds can cause clogging of pipes (ibid.). Volatilisation of NH_3 has negative impacts on air quality and the loss of nitrogen that could be converted to fertiliser is also a disadvantage. By stabilising urine one can prevent the degradation of organic matter, precipitation processes and volatilisation of NH_3 (ibid.).

For a stable conversion of a urine solution it is required that NO_2^- is oxidised at the same rate as NH_3 (Udert et al., 2003a). Other factors that affect the outcomes of the nitrification process are oxygen, pH, temperature, alkalinity, substrate inhibition and limitation, product inhibition, intermediate inhibition and growth limitation by inorganic carbon or phosphate (Udert et al., 2003a).

One of the most important parameters in the nitrification process is pH which determines the acid-base equilibria of NH_3, NO_2^- and hydroxylamine (NH_2OH) (Udert et al., 2003a). The main substrate for AOB is NH_3 and by increasing the pH more NH_3 becomes available for the AOB (Suzuki et al, 1974). A sudden increase in pH can therefore cause instability in the process since NO_2^- oxidisers are unable to increase in growth rate as fast as the NH_3 oxidisers.

The desired end product of urine nitrification is a solution of ammonium nitrate with equal parts of NH_4^+ and NO_3^-.

2.5 Process parameters

There are several parameters that influence the conditions for the nitrifying bacteria thus an understanding of these parameters are required to operate a nitrifying system in a stable and efficient manner.

Temperature

The general rule is that the nitrification rate increases with temperature. In liquids between 10 and 25 °C the nitrification rate will double for every 8 to 10 °C increase in temperature (EPA, 2009). NOB can be favoured over AOB in lower temperatures (Egli et al., 2003).

According to Hellinga (1999) NOB grow faster than AOB at temperatures around 16 °C and with increasing temperature the AOB grow faster than NOB. This means that it is easier to run a stable nitrification process with limited NO_2^- accumulation at lower temperatures.

Dissolved oxygen

The nitrifying bacteria use oxygen as an electron acceptor thus they are heavily dependent on adequate oxygen supply. The growth rate of the nitrifiers starts to decline at dissolved oxygen (DO) concentrations below 3 to 4 mg/L, or even higher, and the rate decreases significantly at levels below 2 mg/L (EPA, 2009). At DO concentrations less than 0.5 mg/L *Nitrobacter* suffer more than *Nitrosomonas* which can result in NO_2^- accumulation (ibid.).

The nitrifiers need oxygen, as stated above, for the nitrification process and some species of the NO_2^- oxidisers have lower affinity to oxygen compared to the NH_3 oxidisers (Udert et al., 2003a). This means that the NOB generally needs more oxygen present for the complete oxidation to be successful. Under low oxygen conditions denitrification can occur. Denitrification is the process where NO_3^- is converted via NO_2^- to gaseous nitrogen (Gray, 2004, p. 272). NO_3^- serves as an electron acceptor instead of oxygen and a carbon source needs to be available to act as an electron donor (ibid.).

The salt concentration in a solution can affect the oxygen solubility by the so called “salting out effect”, where it has been shown that an increase in salt concentration decreases the solubility of molecular species (Moussa, 2004).

pH

The best nitrification rates can be achieved when the pH lies in the range of 6.8 and 8.0 (EPA, 2009). At lower values the rates are much slower but the benefit is that the NOB and AOB have equal production rates, thus the process becomes more stable.

AOB have affinity for NH_3 rather than NH_4^+ (Suzuki et al., 1974; Anthonisen et al., 1976; Van Hulle et al., 2007).

In the literature, NH_4^+ can be considered as substrate for biomass growth but this expression can only be used when the pH is constant since the ratio between the NH_3 and NH_4^+ concentration is thereby constant (Hellinga, 1999). The ratio changes with changing pH according to the following chemical equilibria:

$$NH_4^+ \leftrightharpoons NH_3 + H^+$$

Hellinga (1999) also states that the specific growth rate of NOB becomes higher compared to that of AOB when the pH goes below 6.6.

The concentration of NH_3 increases with increasing pH and temperature. Ionic strength can also influence the NH_3 concentration; in saline or very hard waters there is a small decrease in NH_3 concentration (Emerson et al., 1975). Emerson et al. (1975) shows that the fraction of NH_3 in an aqueous NH_3 solution at 20 °C ranges between 0.0397 % and 0.125 % when the pH ranges between 6.0 and 6.5. When the pH is 9.0 the NH_3 solution at 20 °C consists of 28.4 % NH_3. If the pH is 6.0 or lower, the NH_3 oxidation often stops except for systems where acid-tolerant AOB are present (Udert et al., 2005).

For the nitrification process to operate in a stable manner the activity of the AOB and NOB need to be in phase. Fluctuating pH is a sign of process instabilities and may be caused by changes in bacterial activity and nitrogen load. To strengthen NOB growth, thus lowering the risk of NO_2^- accumulation, it is recommended to keep a relatively low pH and temperature (Udert & Wächter, 2012; Edefell, 2017).

Hydraulic and sludge retention time

Hydraulic retention time (HRT) is a measure of the volumetric loading in a system, and expresses the time a specific volume of water spends in a reactor before being flushed out (Gray, 2004, p. 479). Sludge retention time (SRT) is a measure of the time sludge solids remain in a system and is calculated as the total amount of sludge solids divided by the rate of loss of sludge from the system (ibid., p. 478).

At longer HRT more growth is distributed in suspension than in biofilm and at short HRT a heterotrophic layer can develop on top of the nitrifying biofilm (van Benthum et al., 1997). It is desirable to have growth in biofilm on carriers since this is more space efficient than growth in suspension. Minimum growth in suspension also decreases the risk of clogging.

Conductivity and salinity

Conductivity is a measure of ions in a solution and can be an approximation of the concentration of nitrogenous ions and salt during urine nitrification. Salt has an inhibitory effect on AOB and NOB and is most likely due to ionic strength, where divalent cations have a larger impact than monovalent cations (Moussa, 2004).

Several species within the group of nitrifiers are adapted to marine environments, which could be of interest in the process of urine nitrification. The genus *Nitrosococcus* consist of two known species that have a strong salt requirement, where the optimum Sodium chloride (NaCl) concentration has been estimated to 500 mM for *N. oceani* and 700 mM for *N. Halophilus* (Koops and Pommerening-Röser, 2001).

2.6 Process inhibition

The nitrifying organisms can encounter substrate limitation and the main substrates are NH_3 for the AOB and NO_2^- for the NOB (Suzuki, 1974; Bock & Wagner, 2013). The nitrifying bacteria can also be inhibited by their products where both AOB and NOB are strongly inhibited by nitrous acid (Suzuki, 1974). The NOB are on the other hand rarely inhibited by NO_3^- (Hunik et al., 1992; Hunik et al., 1993). Compounds produced during the entire process

of nitrification, intermediates, can also have an inhibitory effect, especially NH_2OH which inhibits the growth of NOB (Udert et al., 2003a).

NO_2^- accumulation is generally explained as the result of different growth rates of the AOB and NOB. The NH_4^+ and NO_2^- concentration is however not responsible for the nitrification inhibition, rather the free ammonia (FA) and free nitrous acid (FNA) inhibit the organisms (Anthonisen et al., 1976). According to Anthonisen et al. (1976) the *Nitrosonomads* were inhibited when FA ranged between 10 and 150 mg/L and for *Nitrobacters* the range was between 0.1 and 1.0 mg/L. The nitrifying organisms started to get inhibited by FNA at concentrations between 0.22 and 2.8 mg/L.

If NO_2^- accumulation is encountered it can be hindered by switching off inflow and aeration and adding acetate. The procedure causes the NO_2^- to be removed by denitrification. The denitrification leads to a raise in pH which can be adjusted with hydrochloric acid (Udert & Wächter, 2012).

FA and FNA concentration in a system is dependent on temperature and pH, and it is therefore important to regulate these parameters in order to control and suppress inhibitory effects (Kim et al., 2006).

2.7 Start-up

The process of nitrification relies on autotrophic AOB and NOB which are slow growing compared to heterotrophic bacteria. The start-up is therefore a challenging step where it is desirable to lower the time required to establish a stable nitrifying biofilm (Young et al., 2017).

If the nitrification process is started with carriers with no or very limited biofilm it is important to have a longer start-up period with very low and gradual increase in nitrogen load. A fast start-up with virgin biofilm carriers may cause instability in the system with high NO_2^- concentrations causing process inhibition (Rusten et al., 2006).

It is possible to use biofilm carriers originating from conventional nutrient removal processes which already host a developed bacterial population. However, this inoculum might not be adapted to such high concentration of nutrients found in urine. During a start-up of a urine nitrification process it is suggested that the incoming urine solution should be highly diluted and then gradually increased in strength to give the bacterial population an opportunity to adapt to the substrate (Edefell, 2017). The nitrification rate in the process can be very slow in the beginning and then an adaptation in the biofilm culture may be experienced.

Much focus has been on biological treatment of domestic sewage water but less is known about treatment of high strength wastewater from industrial activities (Moussa, 2004). The treatment of industrial wastewater can also face the challenge of a high salt concentration. Acclimating freshwater microorganisms to relatively low saline wastewater has been the most common procedure in previous research (Cui et al., 2014).

The start-up of a marine system, with higher salinity than in freshwater, takes very long time with virgin biofilm carriers and the nitrification rate can be expected to be 60 % of a similar freshwater system when the salinity is 21-24 ‰ (Rusten et al., 2006).

Treatment of wastewater based on pure halophilic cultures is not efficient enough on a pilot-scale size since the metabolic pathways of the organisms are not well adjusted to complex wastewater compositions (Cui et al., 2016). A mixed culture of halophiles is therefore needed for large-scale treatment of saline wastewater (Sudarno et al., 2010). There are only a limited amount of studies that are based on inocula of halophiles from natural sources such as soil samples, saline wastewater, seawater and marine sediment (Cui et al., 2014), thus the knowledge on the use of halophilic cultures as inocula in urine nitrification is limited.

2.8 Nitrous oxide during nitrification

Factors that lead to N_2O emissions are low DO, high NO_2^- accumulation, high NH_3 load, short SRT, high salinity, toxic substances and high pH (Li *et al.*, 2015).

The N_2O released by AOB during nitrification originates from three possible pathways; nitrifier denitrification, biological NH_2OH oxidation, and chemical oxidation of NH_2OH with oxygen and NO_2^- (Li *et al.*, 2015).

In nitrifier denitrification the AOB reduce NO_2^-, derived from NH_2OH oxidation, to N_2O and N_2 (Wrage et al., 2001). Wunderlin et al. (2012) showed in their study that nitrifier denitrification by AOB was the dominant N_2O production pathway under aerobic conditions, low COD loads and NO_2^- presence.

In the second pathway NH_2OH oxidoreductase oxidise the NH_2OH to NO_2^-, and low amounts of N_2O can be generated from unstable intermediates (Kampschreur et al., 2008).

In the third pathway N_2O is released when NH_2OH is chemically oxidised with oxygen or NO_2^- (Li et al., 2015). Chemical N_2O production can be tested by adding NH_2OH to tap water and according to Wunderlin et al. (2012) low amounts of N_2O were produced under aerobic conditions and slightly increased when adding NO_2^-. The production of N_2O from NH_2OH in tap water was low compared to the production from activated sludge thus chemical N_2O production is not considered to be a dominant pathway.

When Wunderlin et al. (2012) investigated the effects of added NH4 on N_2O production, the conclusion was that NH_2OH biological oxidation and nitrifier denitrification by AOB was responsible for the N_2O emissions. Nitrifier denitrification was dominant but the role of NH_2OH oxidation grew at high NH_3 and low NO_2^- concentrations.

Li et al. (2015) conducted a study that suggested that by rising DO and pH the N_2O accumulation can be reduced. The study showed that NO_2^- production increases with increasing pH while the N_2O accumulation decreases. This indicates that an increase in N_2O production in high pH environments is not caused by high NO_2^- production. Li et al. (ibid.) further state that changes in N_2O accumulation ratios only depend on pH and DO, meaning that a high pH results in high NO_2^- production and low N_2O accumulation, and increased DO further reduces N_2O accumulation.

2.9 Previous research

Previous studies have shown promising results when it comes to nitrification and distillation of urine.

Johansson and Hellström (1999) were able to nitrify 50 % of the NH_4^+ in urine to NO_2^- and NO_3^-, where 30-70 % of the oxidised NH_4^+ was in the form of NO_2^-. The nitrifying organisms may have been inhibited by FA and FNA caused by the high NH_4^+ and NO_2^- concentration, but the authors state that an optimisation of the process would limit the NO_2^- accumulation thus increasing the nitrification rate. The total loss of nitrogen was calculated to 20-30 % and was probably due to denitrification. During drying of the nitrified urine, 10-75 % of the total nitrogen (tot-N) was lost. The loss was dependent on the initial fraction of NO_2^-, hence if the NO_2^- accumulation is kept low the nitrogen loss is expected to be low. The nitrogen loss is believed to be due to nitrogen gas formation during a reaction between NH_4^+ and NO_2^- at dry conditions (Johansson and Hellström, 1999).

Udert & Wächter (2012) concluded that biological nitrification with subsequent distillation can be a stable and efficient process for the concentration and recovery of nutrients from urine. The product from their study contained high amounts of ammonium nitrate, potassium, sulphate and phosphate. Udert & Wächter (2012) prevented instabilities and controlled the nitrification rate by implementing a pH controlled inflow pump. The authors also conclude that Moving Bed Biofilm Reactors (MBBR) are preferred over Membrane Aerated Biofilm Reactors (MABR) since the frequent biofilm erosion prevents oxygen-free layers from forming in the biofilm.

Fumasoli et al. (2016) operated a nitrification reactor for 3.5 years and reached maximum rates of 3.1 gN $m^{-2}d^{-1}$ (930 gN $m^{-3}d^{-1}$). These rates were however reached at a reactor temperature of 27.0 °C and the influent NH_3 concentration is not specified but can be assumed to be below 1,790 mgN L^{-1}. At influent NH_3 concentration of 4,100 mgN L^{-1} it was possible to nitrify urine at a rate of 0.4 gN $m^{-2}d^{-1}$ (120 gN $m^{-3}d^{-1}$) (ibid.). The temperature was then 22.5 °C. In the experiment it was possible to produce a fertiliser low in heavy metals and high in nutrients.

Olofsson (2016) operated a pilot plant reactor with manually controlled influent flow and reached a maximum nitrification rate of 0.97 gN $m^{-2}d^{-1}$ at a reactor concentration of approximately 1,150 mgN L^{-1}.

From the recent study conducted by Edefell (2017) it was possible to increase the urine concentration in the reactor to 4,680 mgN L^{-1} with a corresponding nitrification rate of 0.3 gN $m^{-2}d^{-1}$ (60 gN $m^{-3}d^{-1}$). This was possible when the influent pump was exchanged from fixed-flow to pH-regulation at a pH of 6.2. The maximum nitrification rate during the experiment was 0.9 gN $m^{-2}d^{-1}$ (160 gN $m^{-3}d^{-1}$) and occurred when the concentration in the reactor was 2,230 mgN L^{-1}. According to the results a high urine concentration decreases the nitrification rate which is also proven in other studies (Udert et al., 2003).

An estimation of nitrogen losses during the experiment was also performed by Edefell (2017). The average nitrogen loss was estimated to 12 % with a standard deviation of 5 % but the number may be exaggerated since steady state might not have been reached in some cases. Edefell (2017) believed that N_2O formation could be a cause for nitrogen loss since it can be produced by AOB in environments with high concentrations of NH_3.

3 Materials and methods

3.1 Urine solution

The urine originated from a single household and had a generous amount of time to undergo hydrolysis before usage. The urine was stored in a cooled storage room and diluted with tap water prior to use.

3.2 Experimental set-up

The lab set-up consisted of two MBBRs, each with an operating volume of 4 litres (*Figure 1*). The incoming urine solution was pumped with a 120U/DV variable speed pump (Watson-Marlow, England) which was controlled by the Liquiline CM444 transmitter (Endress+Hauser, Switzerland). The regulator sent a signal to the pump that was based on the pH in the nitrification process, measured with the pH sensor Orbisint CPS11D (Endress+Hauser, Switzerland). The reactor is continuously stirred with a Heidolph RZR 1 (Germany) operating at 120 rounds per minute. The reactors were oxygenated with aquarium pumps and the incoming air flow was hydrated in glass bottles filled with water.

The reactors were operated in room temperature (20 ± 2 °C).

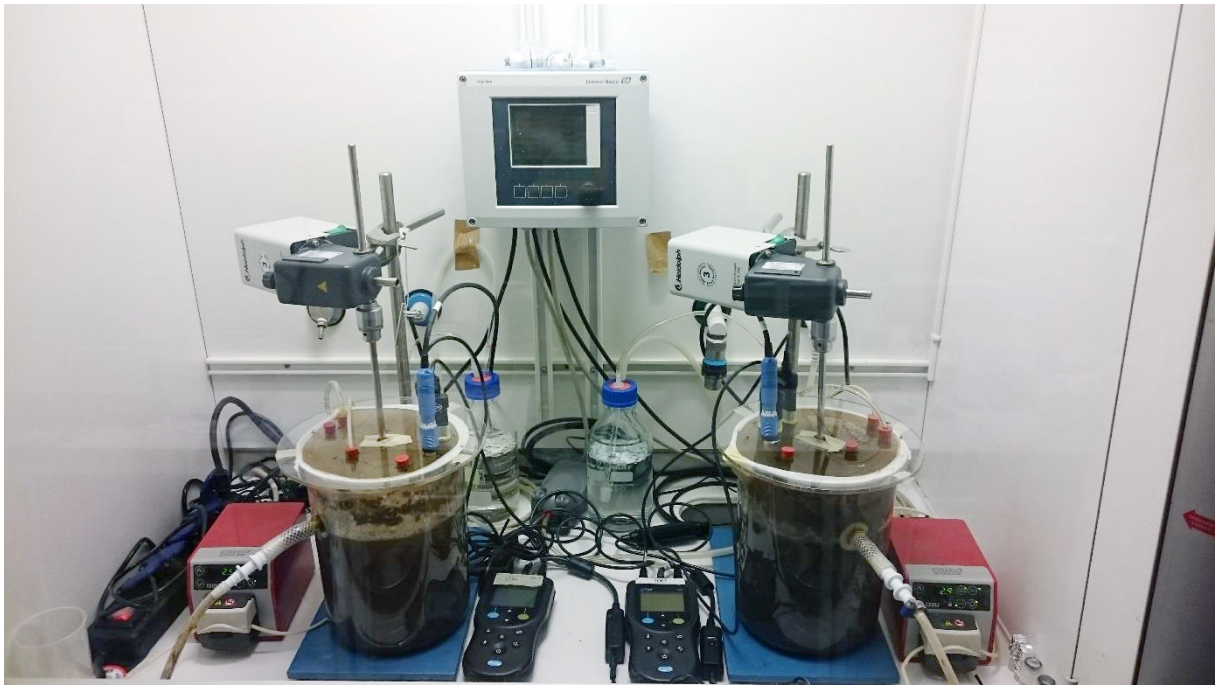

Figure 1: Urine nitrification set-up.

3.3 Continuous measurements

DO concentration was measured with the IntelliCAL™ LDO101 Standard Luminescent/Optical Dissolved Oxygen Probe (Hach, Germany) connected to the HQ40d portable meter (Hach, Germany).

Conductivity was measured with the IntelliCAL™ CDC401 Standard Conductivity Probe (Hach, Germany), also connected to a Hach HQ40d portable meter. The probe registers the

electrolytic conductivity in a solution which is a measurement of the movement of ions. The ions come from inorganic dissolved solids such as chloride, NO_3^-, sulphate and phosphate anions, and sodium, calcium, magnesium, iron and aluminium cations. The conductivity was in the experiment considered an estimation of the change in tot-N in the reactors (Fumasoli et al., 2016). The conductivity was measured in milliSiemens/centimetre (mS/cm) and to convert the unit value to gram/Litre Total Dissolved Solids (g/L TDS), mS/cm needs to be multiplied with a factor of 0.64 (Hach, 2015):

$$\frac{g}{L}\ TDS = \frac{mS}{cm} * 0.64$$

The values of conductivity measured during the experimental period dropped during certain measurements which could be due to disturbance caused by the biofilm carriers.

3.4 Analytical methods

Manual analyses of NO_2^-, NO_3^-, NH_4^+, tot-N and COD were performed continuously with LCK 342, LCK 340, LCK 303, LCK 338 and LCK 714, respectively (Hach Lange, Germany). The LCK Cuvette tests were analysed in a DR 2800™ Portable Spectrophotometer (Hach, Germany).

All the samples analysed with LCK Cuvette tests were filtered with Whatman™ Glass microfiber filters (GF/C™, diameter 55 mm, GE Healthcare Life Sciences, UK), except for tot-N which was unfiltered.

Samples for analysis of suspended solids (SS) were taken from the reactor solution. The samples were filtered through glass microfiber filters (691, VWR, France) that had been weighed before filtration, and after drying the filters for one hour at 105 °C the filters were weighed again. The difference in weight before and after filtration and drying was then used in the following formula to estimate SS:

$$SS\ \left(\frac{g}{L}\right) = \frac{1000 * (b - a)}{V}$$

a = Filter weight before filtration (g)

b = Filter weight after drying (g)

V = Sample volume (ml)

All the samples in this study have been diluted with distilled water due to the high concentrations in source-separated urine.

3.5 Nitrous oxide measurement

The N_2O in liquid phase was measured with a N_2O sensor (Unisense A/S, Denmark) connected to a PA2000 amplifier (Unisense A/S, Denmark). The data was sent to a computer

with an ADC-216 converter (Unisense A/S, Denmark) and displayed with the software SensorTrace Logger (Unisense A/S, Denmark).

Calibration of the N_2O sensor was performed frequently. The calibration required a N_2O tube with a pressure regulator. Four calibration points were used, each with different concentrations of N_2O. The concentrations were: 0 mol/L, 10 µmol/L, 15 µmol/L and 20 µmol/L. The 20 µmol/L solution was prepared by bubbling N_2O in distilled water for 30 minutes and 100 µL of the N_2O saturated water was then added to 140 mL of distilled water. The solution was stirred with a spoon to avoid loss of N_2O to the surrounding air.

3.6 Calculations

Formulas used for calculation of FA and free FNA was the following (Anthonisen, 1976):

$$FA\ as\ NH_3\ \left(\frac{mg}{L}\right) = \frac{17}{14} * \frac{NH_4^+ - N * 10^{pH}}{e^{\left(\frac{6.344}{273+℃}\right)} + 10^{pH}}$$

$$FNA\ as\ HNO_3\ \left(\frac{mg}{L}\right) = \frac{46}{14} * \frac{NO_2^- - N}{e^{\left(-\frac{2.300}{273+℃}\right)} * 10^{pH}}$$

The nitrogen load and nitrification rate was calculated according to previous studies on urine nitrification initiated by Sweden Water Research (Olofsson, 2016; Edefell, 2017). The nitrogen load was based on the tot-N concentration instead of NH_4^+ concentration since the NH_4^+ to tot-N ratio changed over time.

The nitrogen load was equal to the amount of tot-N supplied per carrier area and day according to the following formula:

$$Nitrogen\ load\ = \frac{Inflow * Total\ nitrogen}{Total\ carrier\ area}$$

The nitrification rate was approximated by using the NO_3^--N:tot-N ratio in the reactor combined with the nitrogen load:

$$Nitrification\ rate\ = \frac{Inflow * Total\ nitrogen * NO3 - N{:}\ tot - N\ ratio}{Total\ carrier\ area}$$

The total area of the biofilm carriers was adjusted when carriers were removed for microbial analysis. The microbial analysis was not performed during this project period, but the withdrawn carriers might contribute to useful results in future studies.

3.7 Biofilm carriers

The biofilm carrier type used for this study was the Kaldnes® K5 (Veolia Water Technologies), with a protected surface area of 800 m^2 m^{-3} (Veolia, 2015). In the reactors the filling degree was 50 % and the initial number of carriers 667, which gives a total surface area of 1.6 m^2.

The carriers were collected from an integrated fixed-film activated sludge (IFAS) reactor at Sundet WWTP in Växjö. The treatment plant is located in the midlands of the county of Småland and receives wastewater from the urban centre and the peripheral districts of the city of Växjö and from the town of Gemla (Växjö municipality, 2015). The biofilm on the carriers are adapted to concentrations of NH_3 and salinity much lower than those present in a urine nitrification system, which means that it needs time to adapt to the new conditions.

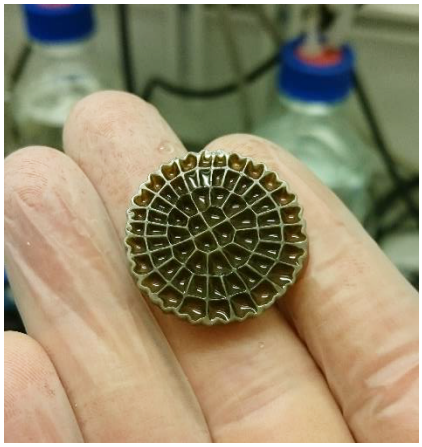

Figure 2: Biofilm carrier of the type K5, Veolia Water Technologies.

3.8 Sources of error

During the manual analyses the samples are diluted with distilled water since the concentrations of different substances in the urine and in the reactors can be very high. Large dilutions may result in larger insecurity regarding the obtained measured values. The influent and effluent flow is measured as a mean value of the flow during a day or even longer. The real nitrification rates may therefore differ from the values found in this report. The liquid volume in the reactors may also be higher or lower than 4 litres due to growth at the outlets. The stirrers also disrupt the plane liquid surface.

4 Results and discussion

4.1 Initial phase of the start-up

The process was started by adding biofilm carriers to the reactors containing a solution of 1% urine and 99 % tap water, i.e. 40 mL of urine and 3,960 mL of tap water for each of the reactors. As the nitrifying bacteria started to grow, the pH dropped and when the pH went below the set-point, initially set to 6.2, the pumps started to pump in the urine solution. The nitrogen load and nitrification rates for the reactors are displayed in *Figure 3* and *Figure 4*.

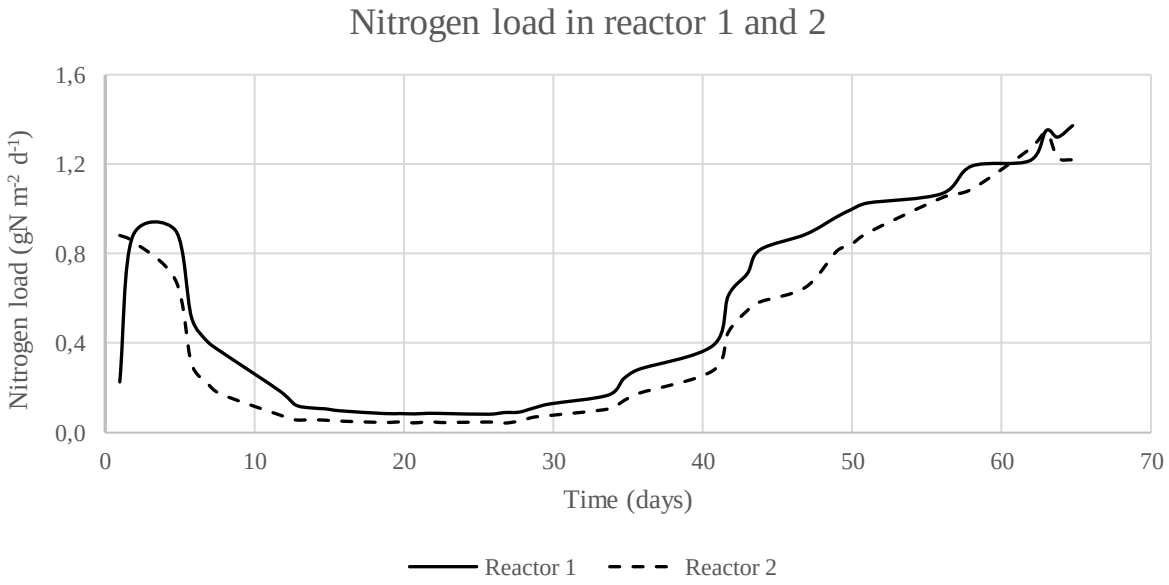

Figure 3: Nitrogen load in reactor 1 and reactor 2.

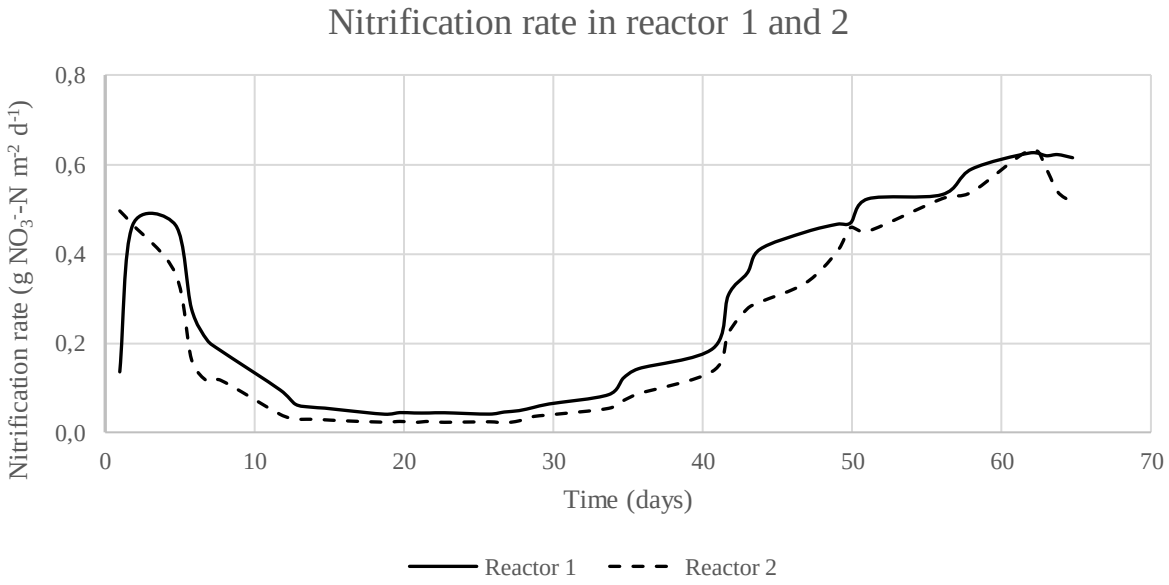

Figure 4: Nitrification rate in reactor 1 and reactor 2.

The nitrification rate followed the nitrogen load and the rate was approximately half of the load. The nitrification rate in reactor 1 increased fast and reached a peak at day 5 where the nitrogen load was around 0.9 gN m^{-2} d^{-1} and the nitrification rate 0.45 g NO_3^--N m^{-2} d^{-1}. After this day the rate started to decrease steadily and at day 13 the declining trend levelled out. After a decision to increase the pH set-point the nitrification rate increased from day 27 until the end of the experimental period. The pH set-point adjustment will be further processed in sequent subchapters.

In reactor 2 the same patterns as in reactor 1 can be seen. The nitrogen load and nitrification rate reached peak values after one or two days where the maximum load was almost 0.9 gN m^{-2} d^{-1} and the nitrification rate 0.5 g NO_3^--N m^{-2} d^{-1}. There was a steady decrease until day 12 and then the declining trend levelled out. After the pH set-point adjustment a steady increase in nitrification rate was observed until the final days of the experimental period.

The maximum nitrification rate for both reactors was 0.63 g NO_3^--N m^{-2} d^{-1} and occurred around day 62.

The conductivity was an estimation of tot-N in the reactors and a comparison between tot-N concentration and conductivity is showed in *Figure 11* in *Appendix I*. The relation between tot-N concentration and nitrification rate is shown below in *Figure 5a and Figure 5b*.

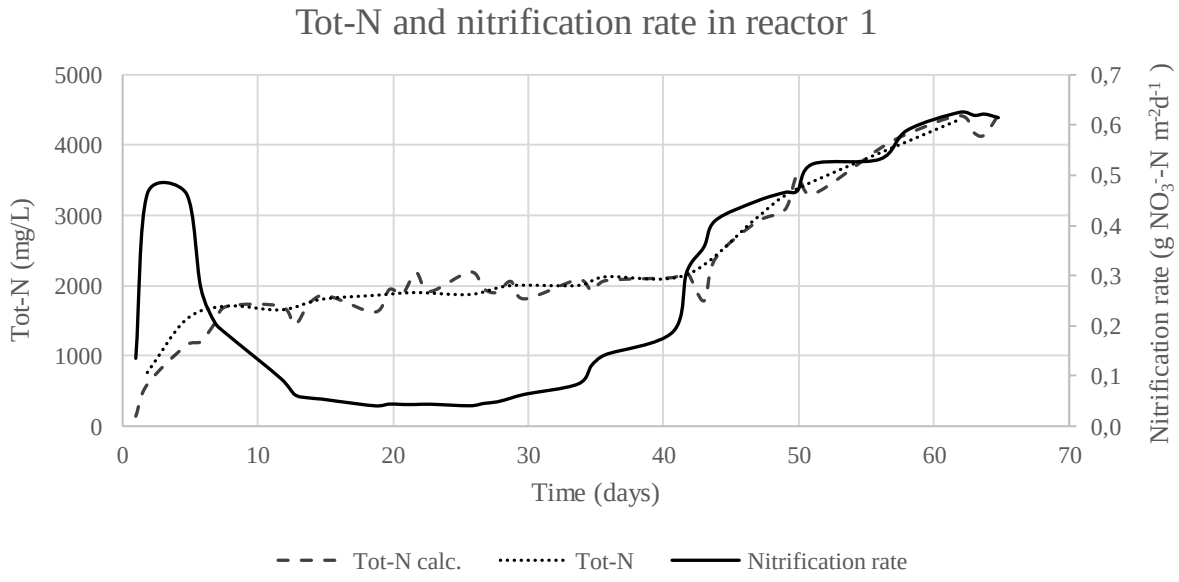

Figure 5a: Relationship between tot-N and nitrification rate in reactor 1. Tot-N calc. is a summary of NO_2^-, NO_3^- and NH_4^+ concentrations.

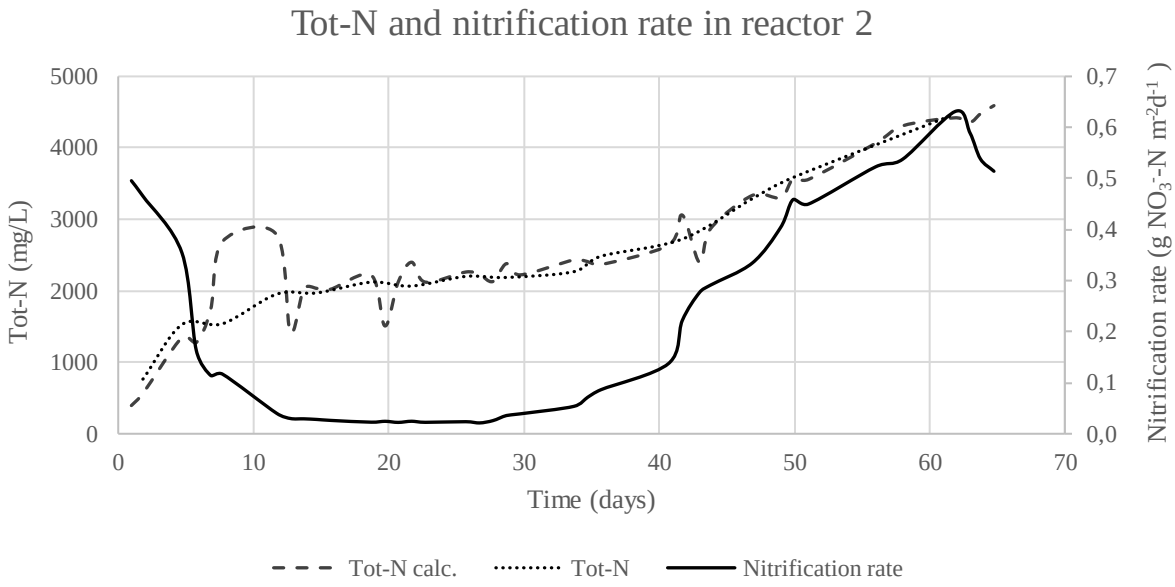

Figure 5b: Relationship between tot-N and nitrification rate in reactor 1. Tot-N calc. is a summary of NO_2^-, NO_3^- and NH_4^+ concentrations.

A similar pattern can be seen in both of the reactors when comparing tot-N and nitrification rate. The *tot-N calculated* is a summary of NO_2^-, NO_3^- and NH_4^+ concentrations in a solution. The measurement of tot-N on unfiltered samples is in these graphs a good indicator of potential measuring errors of the nitrogenous ions in a solution.

In reactor 1 the nitrification rate decreased after day 5 where the tot-N in the reactor was around 1,500 mgN L^{-1}. After that the tot-N only increased slightly until day 27 where the activity in the reactor increased with a subsequent increase in tot-N.

In reactor 2 the nitrification rate decreased after day 2 and the tot-N was at this point approximately was 600 mgN L^{-1}. Just as in reactor 1, reactor 2 experienced a significant increase in nitrification rate after the pH set-point adjustment on day 27 with a subsequent increase in tot-N concentration.

The carriers with biofilm originated from a municipal WWTP in Växjö and the microbial population was, as mentioned previously, probably adapted to wastewater with significantly lower concentration of NH_3 and salt. The rapid increase in nitrification rates during the initial days might be due to the extreme abundance of substrate for the nitrifiers.

The nitrification rates decreased drastically after a few days which could be explained by oxygen limitation of the nitrifying organisms. Nitrifying and heterotrophic bacteria can coexist in a biofilm and an active heterotrophic population can be maintained even when the presence of organic compounds is limited (Bassin et al., 2012). Species of the nitrifying bacteria have the capability to produce organic substrates that can be used by heterotrophs (ibid.). It is therefore possible that the rapid increase in nitrifying activity supported growth of heterotrophic bacteria and contributed to a development of a heterotrophic layer.

A heterotrophic layer on the carrier biofilm can cause an increased oxygen mass transfer resistance inhibiting the nitrification process (Nogueira et al., 2002). The nitrifiers' production of organic substrate might however not have been sufficient to support growth of a thick heterotrophic layer.

A possible proof for significant heterotrophic activity could on the other hand be that the oxygen consumption was extremely high during this period compared to later in the experiment when similar nitrification rates were observed. In *Figure 6a* and *Figure 6b* below the relationship between DO, pH and nitrification rates can be seen.

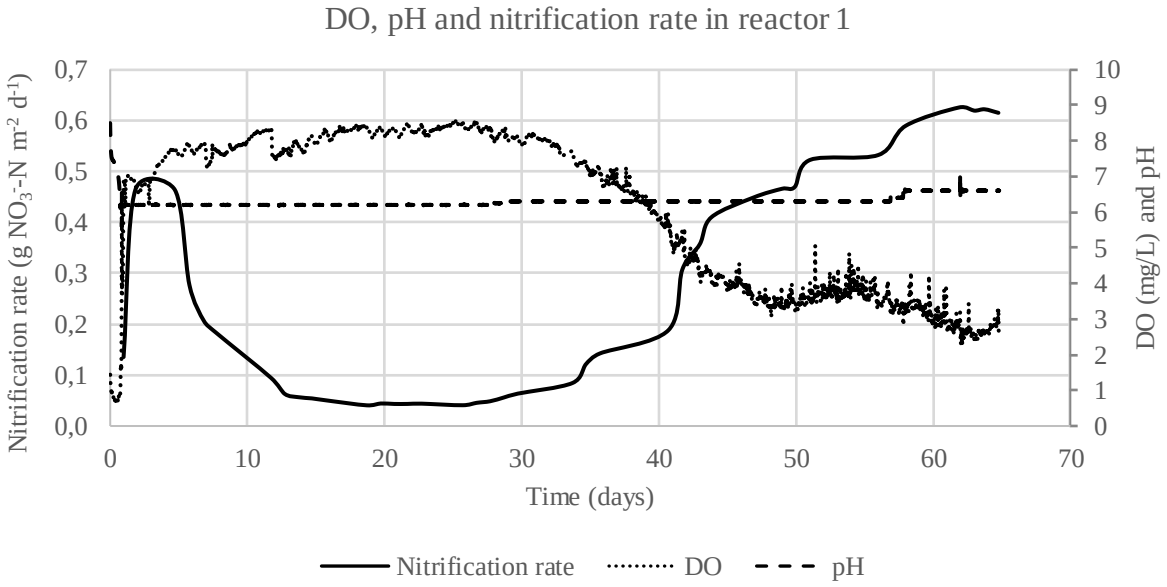

Figure 6a: Relationship between DO, pH and nitrification rate in reactor 1. Between day 1 and day 12 extra oxygen pumps were installed to avoid low DO concentrations.

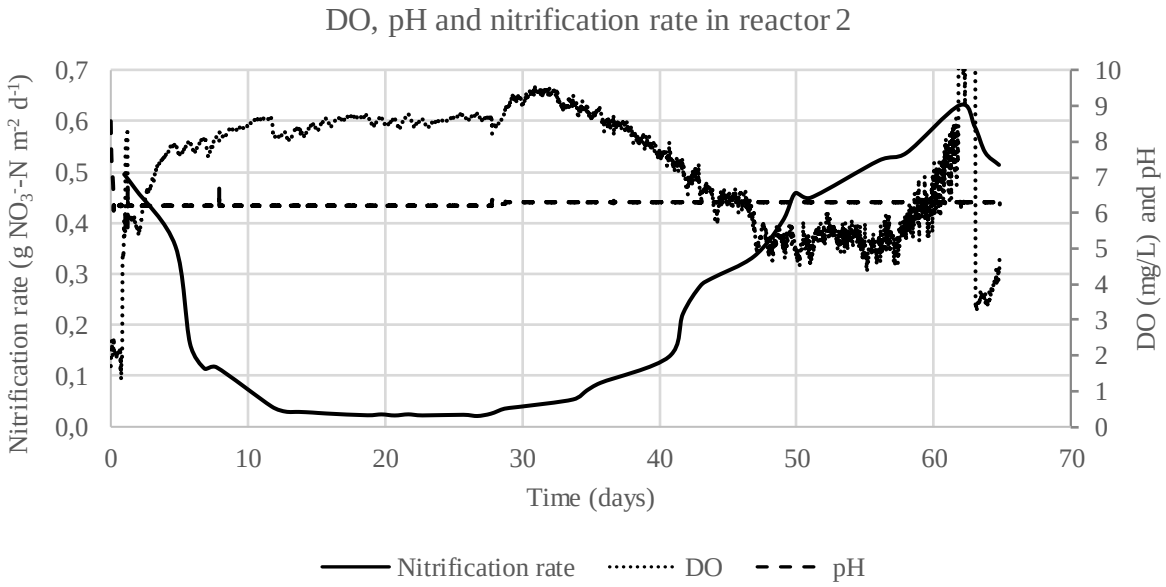

Figure 6b: Relationship between DO, pH and nitrification rate in reactor 2. Between day 1 and day 12 extra oxygen pumps were installed to avoid low DO concentrations. The data from the oxygen sensor in reactor 2 is not completely reliable since the sensor tip collapsed and was replaced on day 63.

During the first day the DO levels were critically low, and two extra oxygen pumps were installed to keep sufficient DO levels. After 12 days the extra pumps were removed due to the decreased activity in the reactors. The DO consumption increased after the pH set-point adjustment on day 27 due to the increased activity. The oxygen consumption in the beginning of the period is most likely much higher than in the end due to the extra oxygen pumps installed. The data from the oxygen sensor in reactor 2 is not completely reliable due to a collapse of the sensor tip. It was replaced on day 63, and it is possible that the old sensor tip started to malfunction already on day 28. A comparison between the sensors in the different reactors can be seen in *Figure 12* in *Appendix I*. The DO in reactor 2 was probably similar as in reactor 1 during the entire experimental period, if only slightly higher.

Another limiting factor for the nitrifying bacteria can be salinity and ionic strength. Conductivity is estimated to be a measure of the salt content and the ionic strength in the solution and the relationship between conductivity and nitrification rates in the reactors are shown in *Figure 7a* and *Figure 7b*.

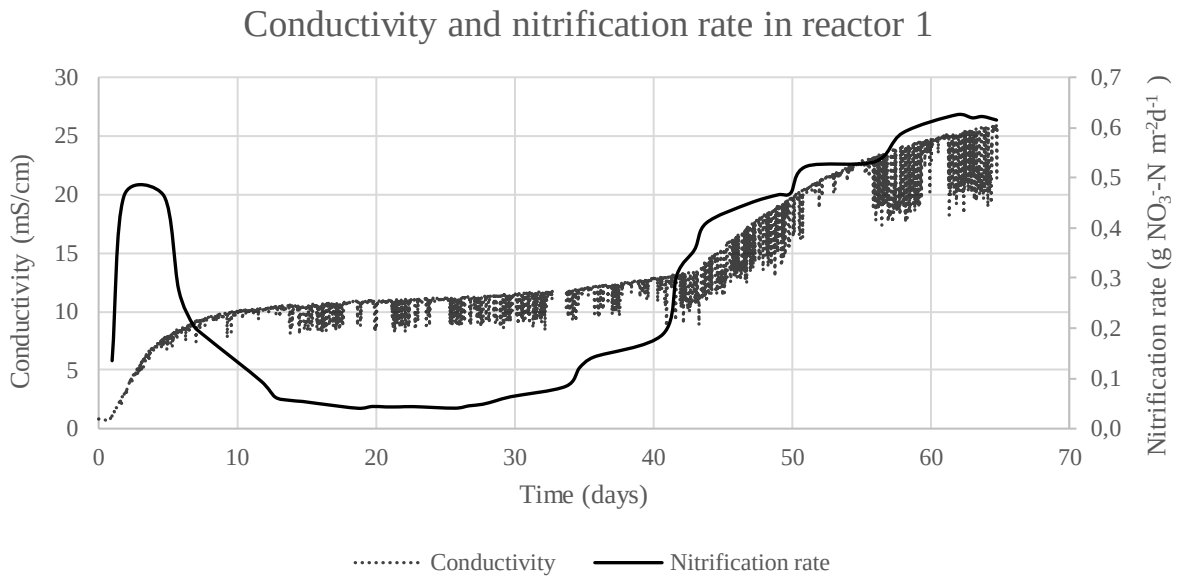

Figure 7a: Relationship between conductivity and nitrification rate in reactor 1.

In reactor 1 the conductivity increased as the nitrifying capacity improved and when the nitrification rate decreased after day 5 the conductivity only experienced a slight increase. At the nitrifying peak on day 5 the conductivity was measured to 8 mS cm^{-1}. After the peak the conductivity stabilised at vales slightly above 10 mS cm^{-1}. The conductivity started to increase significantly after improved nitrification activity due to a pH set-point adjustment on day 27.

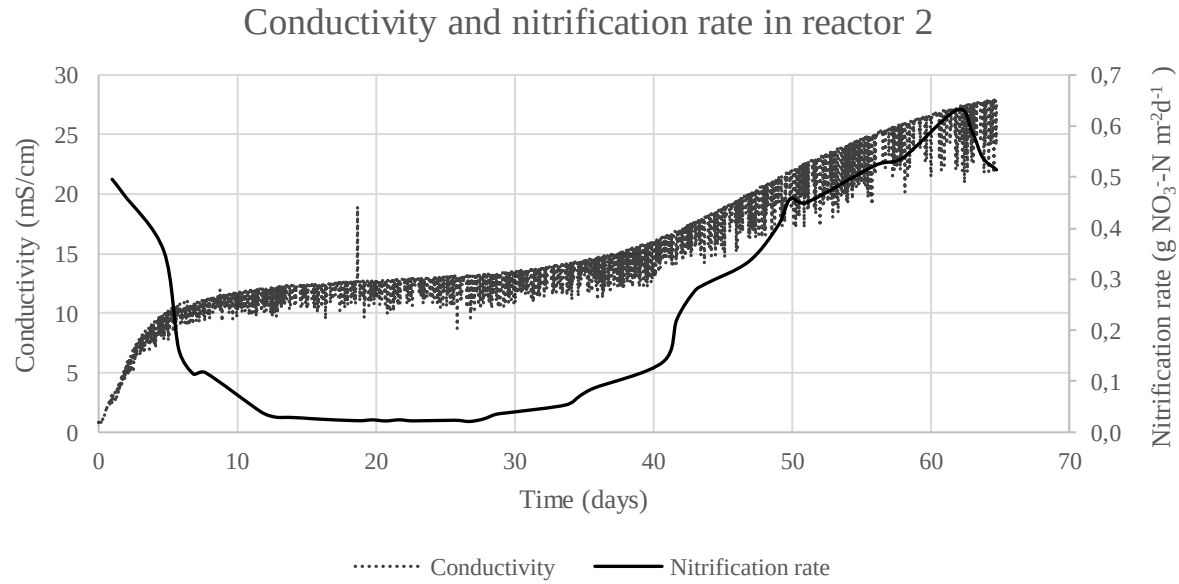

Figure 7b: Relationship between conductivity and nitrification rate in reactor 2.

In reactor 2 a similar pattern can be seen as in reactor 1. The nitrifying activity increased very rapidly and a declining activity was observed during day 1-2. At this point the conductivity was approximately 5 mS cm^{-1} and stabilised between 10 and 15 mS cm^{-1} during the period of low nitrification rate. Conductivity increased as a consequence of the pH set-point adjustment on day 27.

Hunik et al. (1993) shows a strong correlation between NOB inhibition and NO_3^- concentration. With higher NO_3^- concentration the NOB inhibition increased and the effect became even more prominent when lowering the pH to 6.5. The authors show that the NH_3 oxidisers are even more sensitive to high salt concentrations than the NO_2^- oxidisers. Hunik et al. (1992) states that substrate inhibition does not have a significant impact on *N. europaea* but the osmotic pressure in high strength waste streams can, however, have a significant inhibitory effect. The nitrification rates in reactor 1 and reactor 2 started to decrease when the conductivity was 8 mS cm^{-1} and 5 mS cm^{-1}, respectively. Due to this difference in the relationship between nitrification rate and conductivity in the two reactors it is difficult to draw a clear conclusion regarding the inhibitory effect of increased ionic strength. It is however probable that the ionic strength contributes to the inhibition of the nitrifiers, together with the formation of a heterotrophic layer on the biofilm.

Fumasoli et al. (2016) experienced an increase in urine inflow rate during the first 35 days of a start-up at pH 6.2. The inflow rate thereafter decreased and it was suggested that the increasing salt concentration reduced the AOB and NOB activity. The total concentration of NO_3^--N and NH_4^+-N at the peak inflow rate was around 2000 mgN L^{-1}. The peak inflow rate in this study was reached after 5 days in reactor 1 and 1-2 days in reactor 2 and the total concentration of NO_3^--N and NH_4^+-N were approximately 1,150 mgN L^{-1} and 550 mgN L^{-1}, respectively. Fumasoli et al. (2016) did the start-up with more diluted urine and a pattern can be seen when comparing with this study; Fumasoli et al. (2016) reached a higher concentration of NO_3^--N and NH_4^+-N before the AOB and NOB activity decreased compared to this study where the activity decreased at lower concentrations. In reactor 2 the start-up was performed with 50 % urine to water and the peak inflow rate was reached faster than in reactor 1 that started with an inflow of 25 % urine. An explanation to this pattern is that nitrifying organisms adapt to high ion strength and salinity better when they are exposed to a low and gradual increase in nitrogen load (Rusten et al., 2006).

There is a risk of severe inhibition of NOB at high NO_3^- concentrations and this is a major challenge during nitrification of urine and other high strength waste streams, hence Hunik et al. (1993) recommends nitrification combined with denitrification as a promising alternative. However, by implementing denitrification the nitrogen will be lost through nitrogen gas together with the opportunity to recycle nutrients in form of fertiliser. A more sustainable solution would thus be to develop a process that tolerated higher salt concentrations. A possibility is then to take advantage of microbial populations that are adapted to marine environments. This would not only be beneficial for urine nitrification, but also for the use of seawater in domestic systems.

4.2 Increase of pH set-point

From day 13 until day 27 the load was very low in reactor 1 (see *Figure 3*), hence it was decided to raise the pH with the hope to speed up the nitrification rate. The pH was raised to 6.25 and the NO_2^- concentrations did not increase significantly, thus after one day the pH was further increased to 6.3. After the pH set-point adjustment a steady increase in nitrogen load

and nitrification rate was monitored until day 62 where NO_2^- started to accumulate. The NO_2^--N concentrations in the reactors are displayed in *Figure 13* in *Appendix I*. NO_2^- started to accumulate after a calibration of the pH sensors regulating the inflow pumps. The real pH was slightly below the pH value showed on the meter and after the calibration the real pH was slightly above the indicated, which lead to imbalance in the system. The load and nitrification rates hereafter declined. The pH set-point adjustment in reactor 2 led to similar results as in reactor 1. The nitrogen load and nitrification rate steadily increased and continued to increase until the calibration of the pH sensors on day 62 (*Figure 3* and *Figure 4*).

The significantly increasing nitrification rates after the change of pH set-point could be a sign of substrate limitation among the AOB, as previously stated. When the pH is raised more substrate becomes available for the AOB (Suzuki et al., 1974). Another possibility is also that the bacteria just started to adapt to the rough conditions and the raise in pH gave the organisms a boost.

In previous studies (Udert et al., 2003a) the pH was initially controlled to values between 7.0 and 7.8 and when the nitrification was strong enough the pH was decreased to below 7.0. In their study Udert et al. (ibid.) found that NH_3 oxidation stopped at pH values far below 6, but did not have a theory about why that was the case. The results from their study might however not be of larger relevance for this study since it is a big step in the process between a pH of below 6 and a pH of 6.2. It is therefore more probable that the increased substrate availability at pH 6.3 is the reason for the increased nitrification rate. As discussed previously, a heterotrophic layer on top of the nitrifying biofilm combined with the ionic strength in the reactors could have prevented substrate from reaching the nitrifiers.

4.3 Hydraulic and sludge retention time

After the adjustment of pH set-points the HRT decreased significantly in both of the reactors. The relationship between HRT, nitrification rate and SS in reactor 1 and reactor 2 is displayed in *Figure 8a and Figure 8b*, respectively.

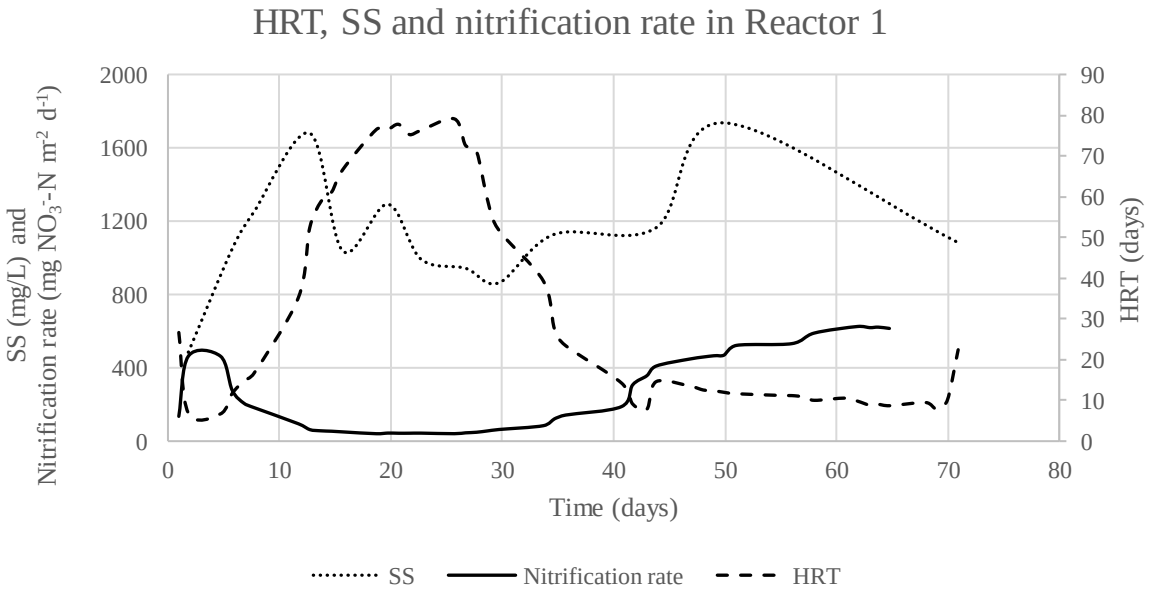

Figure 8a: Relationship between HRT, SS and nitrification rate in reactor 1.

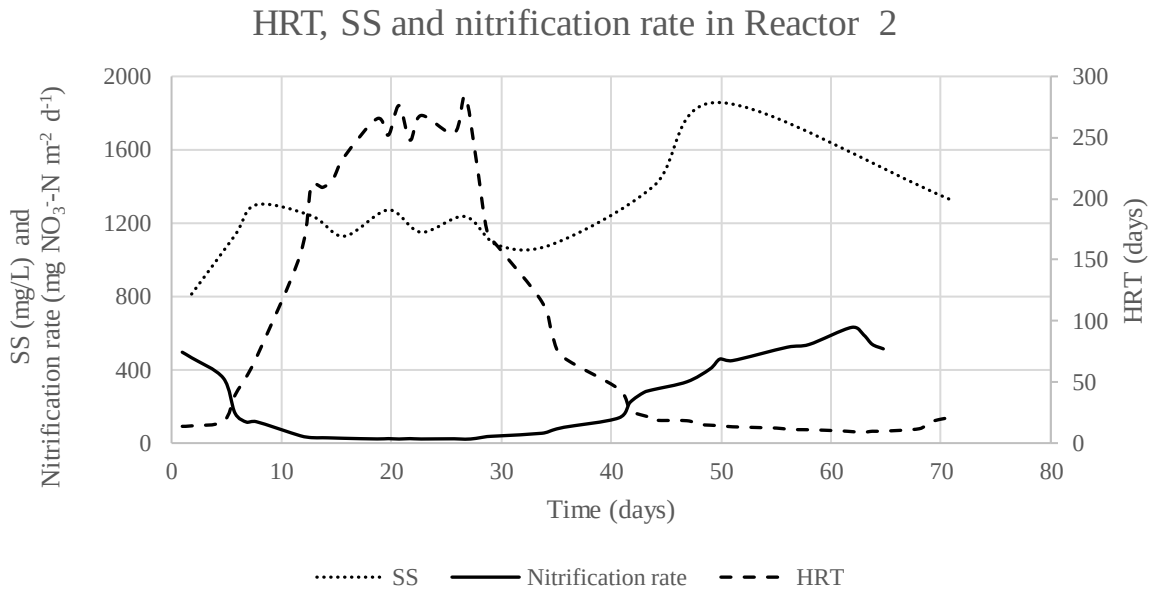

Figure 8b: Relationship between HRT, SS and nitrification rate in reactor 2.

In the two reactors the same patterns can be observed. An increased nitrification rate is accompanied with a lower HRT and in general the SS increased when nitrification rate increased.

The lower HRT could be due to the increased nitrification with subsequent increased flow rate. One possibility is also that the HRT influenced the nitrification rate after the initial increase in NH_3 availability, leading to a combined effect on the nitrification rate.

The HRT may have more impact on the suspended biomass compared to biofilm on carriers. The SS was roughly at a constant level as the HRT increased in both of the reactors, which indicates that the suspended organisms did not benefit from the prolonged HRT. The SS did however increase when the HRT decreased due to the increased activity in the reactors.

It is very uncertain to draw any conclusions of the dynamics between the biofilm and the suspended biomass in the reactors since data of SS was collected less frequently in the end of the experimental period due to lack of time. The severe NO_2^- accumulation after the pH-calibration on day 62 could however have a connection to the HRT. As the pH was slightly increased the AOB speeded up the process consequently lowering the HRT even further. This could have caused a wash out of the suspended NOB, lowering the NO_2^- oxidising capacity of the system. Probably, the suspended biomass had a significant impact on the nitrifying capacity of the reactors.

In Udert et al. (2003a) the SRT for the SS during nitritation was at least 4.8 days and calculations according to model of Hellinga et al. (1999) showed that the minimum SRT for NOB growth was 4.9 days at a temperature of 30 °C. The NOB was therefore believed to be inhibited by short SRT, and also by NH_2OH and high salt concentrations (Udert et al, 2003a). The minimum SRT in that study was valid for a reactor with no biofilm carriers. If there is competition between carrier biofilm and suspended biomass, the organisms in the biofilm will

consume a certain fraction of the available substrate, which means that a minimum SRT of 4.9 days might not be entirely valid for this study. The temperature was also considerably different. Hence, it might be possible that the minimum SRT for this study is higher, even close to 8.6 days, which was the lowest SRT experienced.

Suspended biomass can also be affected by salinity. Bassin et al. (2012) observed a reduction of SS by up to 45 % when increasing the salinity to 20 g NaCl L^{-1}. According to the authors elevated salt concentrations are known to increase water density, which might cause washout of small sludge flocs. There are several parameters that can influence the growth of suspended biomass and it is possible that the increasing concentration of NO_3^- and NH_4^+ ions, which has similar effects as salinity, had a negative impact on the suspended biomass activity.

4.4 Nitrite levels

The NO_2^--N levels were kept low through the experiment except when the pH set-points were altered on day 27, where the NO_2^--N levels went up for a short while and subsequently stabilised at values around 1 mg L^{-1}. The NO_2^- levels also increased, severely, after a calibration of the pH sensors at day 62. The NO_2^--N concentrations related to the pH are showed in *Figure 9a* and *Figure 9b*.

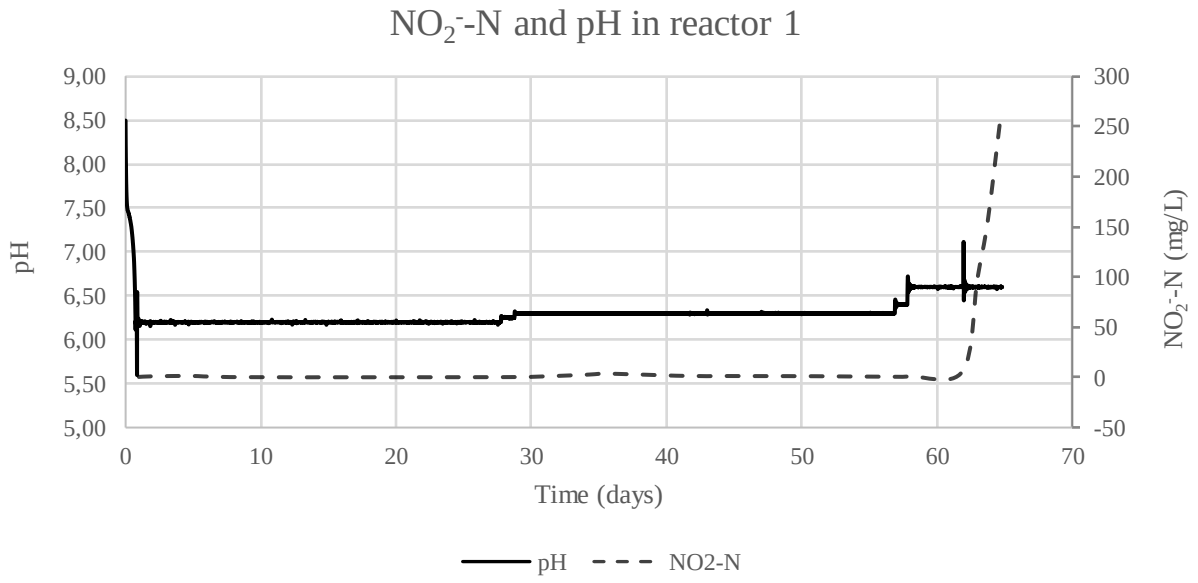

Figure 9a: NO_2^--N concentration related to the pH in reactor 1. The increase in NO_2^--N occurred after the calibration of pH sensors on day 62.

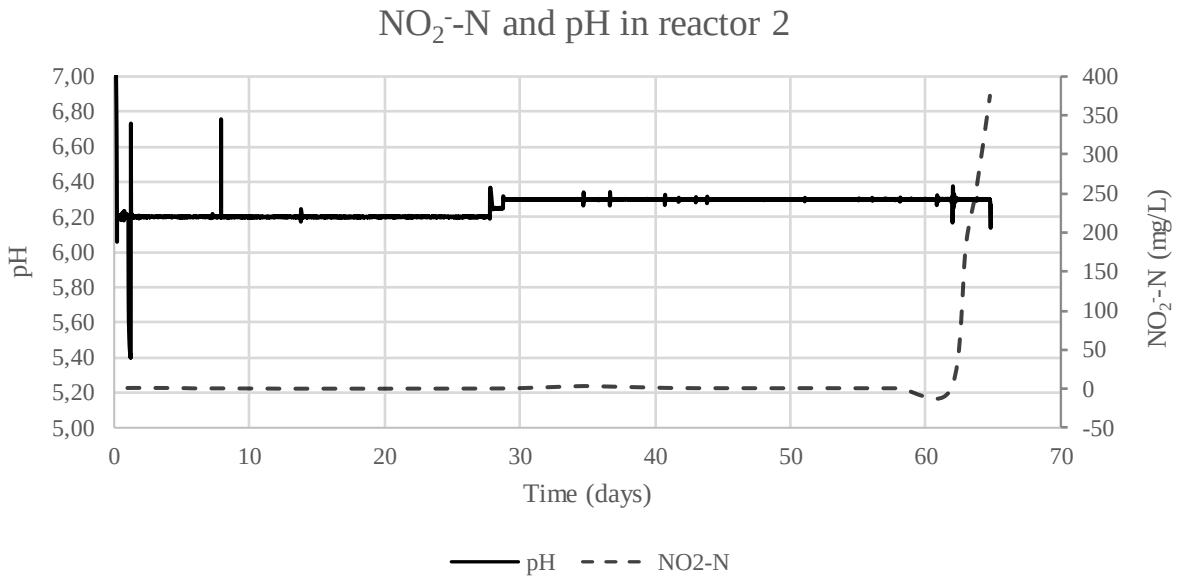

Figure 9b: NO_2^--N concentration related to the pH in reactor 2. The increase in NO_2^--N occurred after the calibration of pH sensors on day 62.

Udert and Wächter (2012) experienced higher concentrations of NO_2^--N at the pH interval 6.1-6.3. The concentrations ranged from 5-13 mgN L^{-1} with a standard deviation of 1-3. The oxygen levels in Udert's and Wächter's study was between 5.5 and 5.0 mgO_2 L^{-1} for the relevant pH interval which is significantly higher than in this study, where the oxygen levels was around 3 mg L^{-1}.

The low nitrification rates in the middle of the experimental period were probably not caused by NO_2^- since the concentrations were very low. NO_2^- is the base in the equilibrium between NO_2^- and nitrous acid (Hunik et al., 1993), which means that low NO_2^- levels gives low nitrous acid concentrations. Nitrous acid can be inhibitory for the AOB (Hunik et al., 1992), and the low levels of NO_2^- thus eliminate the possibility that nitrous acid inhibited the NH_3 oxidation.

The results showed that a rapid increase in NO_2^- occurred after the pH sensor calibration. In *Figure 9a* and *Figure 9b* it can be seen that the change in pH was relatively large after the calibration and this could be evidence for the extreme sensitivity of pH in urine nitrification processes.

4.5 Nitrous oxide

The N_2O concentration in reactor 1 was measured between day 56 and day 64 in the experimental period. The results are showed in *Figure 10*.

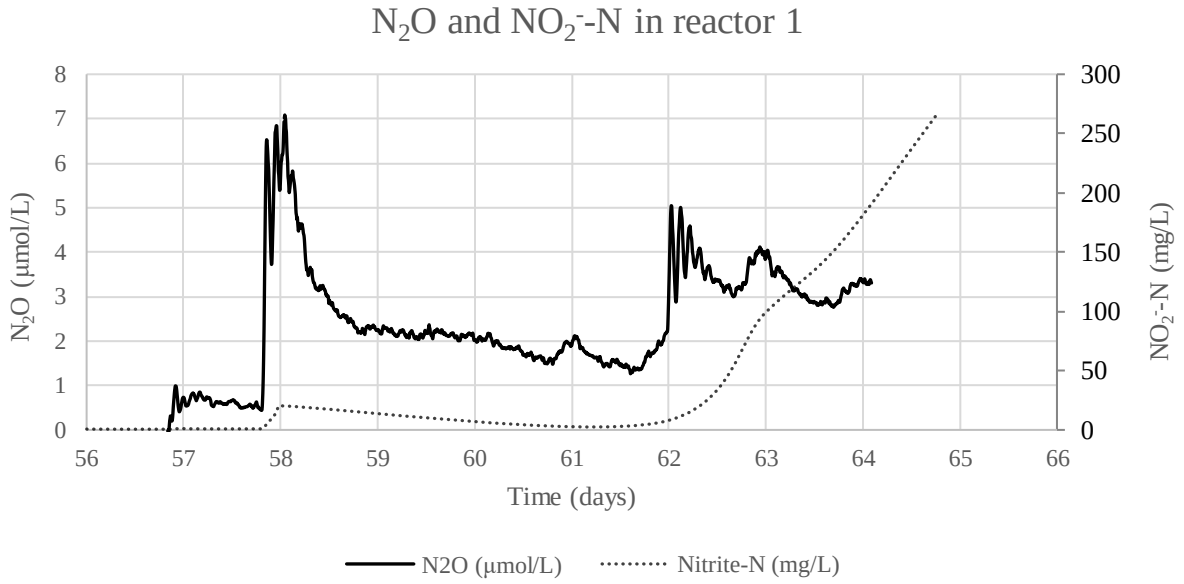

Figure 10: Relationship between N_2O and NO_2^--N in reactor 2. The pH set-point was increased from 6.3 to 6.4 on day 56 and increased further to 6.6 around day 58. A calibration of the pH sensor was performed on day 62.

The initial concentration of N_2O was very low, almost undetectable. When the reactor, on day 56, was targeted with a process disturbance in form of a raise in pH from 6.3 to 6.4, the NO_2^--N concentration increased from approximately 0.6 mg/L to 1 mg/L and a subsequent increase in N_2O concentration can be seen in *Figure 10*. When the pH was raised to 6.6 around day 58 the NO_2^--N concentration peaked at a value of 20 mg/L where a peak in N_2O concentration could also be observed. The NO_2^--N hereafter decreased together with the N_2O until day 62 where a calibration of the pH sensor was performed. The NO_2^--N level increased drastically after the calibration, where the set-point probably changed from slightly below 6.6 to slightly above 6.6. The N_2O concentration also increased during the NO_2^--N accumulation but stabilised within a day and decreased slightly even though the NO_2^--N continued to accumulate.

N_2O can be produced from three possible pathways as previously mentioned. Since the N_2O concentration followed the NO_2^- accumulation fairly well in the initial stage it can be assumed that the nitrifier denitrification pathway is mostly responsible for the N_2O production. The N_2O concentration reached peak values when there was a change in NO_2^- concentration and decreased soon after the peaks. The NO_2^- concentration was in turn connected to changes in pH (*Figure 9a*), which lead to increased activity of AOB. The N_2O peaks can be explained by the slower reaction of NOB towards the increased substrate concentration. As the NOB started to utilise the NO_2^-, the N_2O concentration decreased.

The NO_2^- accumulated drastically after a change in pH on day 62 and the N_2O concentration decreased after the initial peak even though the NO_2^- continued to accumulate. The AOB that utilize NO_2^- for N_2O production are inhibited by high concentrations of nitrous acid (Suzuki, 1974), and that might be the reason why N_2O did not continue to accumulate together with the NO_2^-.

5 Conclusions

The dynamics between different species involved in the nitrification of urine is very complex and difficult to anticipate. Start-ups with high strength urine subjects the microbial culture of extreme conditions and the adaptation to the new environment might take some time. This study indicated that nitrifying organisms are more sensitive to nitrogenous ions and salts with stronger concentrations of influent urine. An increase in pH however showed that that the higher availability of the substrate NH_3 possibly can prevent inhibition of AOB and NOB.

The urine nitrification process is largely influenced by pH and this study indicated that larger changes in pH can imbalance the system and cause NO_2^- accumulation. During NO_2^- accumulation an initial, significant increase of N_2O was observed which was thereafter followed by a declining N_2O concentration.

Even though the reactors were started with different dilutions of urine, the same maximum nitrification rates were reached. The maximum rates were 0.63 g NO_3^--N m^{-2} d^{-1} which occurred around day 62 when the tot-N concentrations were approximately 4,400 mgN L^{-1}.

The dynamics in start-ups with high strength urine needs to be further studied. There is however a potential in starting with stronger urine and in the future it might be possible to achieve fast start-ups at larger treatment plants, thus avoiding the need to store large amounts of incoming urine. In this study it was indicated that a stable process with low pH fluctuation produces low amounts of N_2O. If modern society were to convert from the combined sewer systems to separation of urine, the stabilisation of urine through nitrification followed by distillation have the potential to lower the N_2O emissions originating from wastewater treatment systems.

6 Future work

For future work with start-ups with high strength urine it is recommended that the pH set-point initially is set to a value of 6.3 or higher and thereafter lowered to pH 6.2 when desired nitrification rate is reached. The pH adjustments should be performed in small steps of 0.05 pH units. The different start-up strategies did not differ significantly thus it might be of interest to compare start-ups with even stronger urine solutions. A possible way of improving the start-up process would be to use inoculum with marine origin and compare with freshwater inoculum.

This study only covers a very limited part of the N_2O emissions from urine nitrification and future studies could involve measurements during start-up and during longer periods of operation.

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Appendix I – Additional figures

The conductivity was estimated to be an indicator of the concentration of nitrogenous ions in the reactors and *Figure 11* shows the realtionship between conductiviy and tot-N.

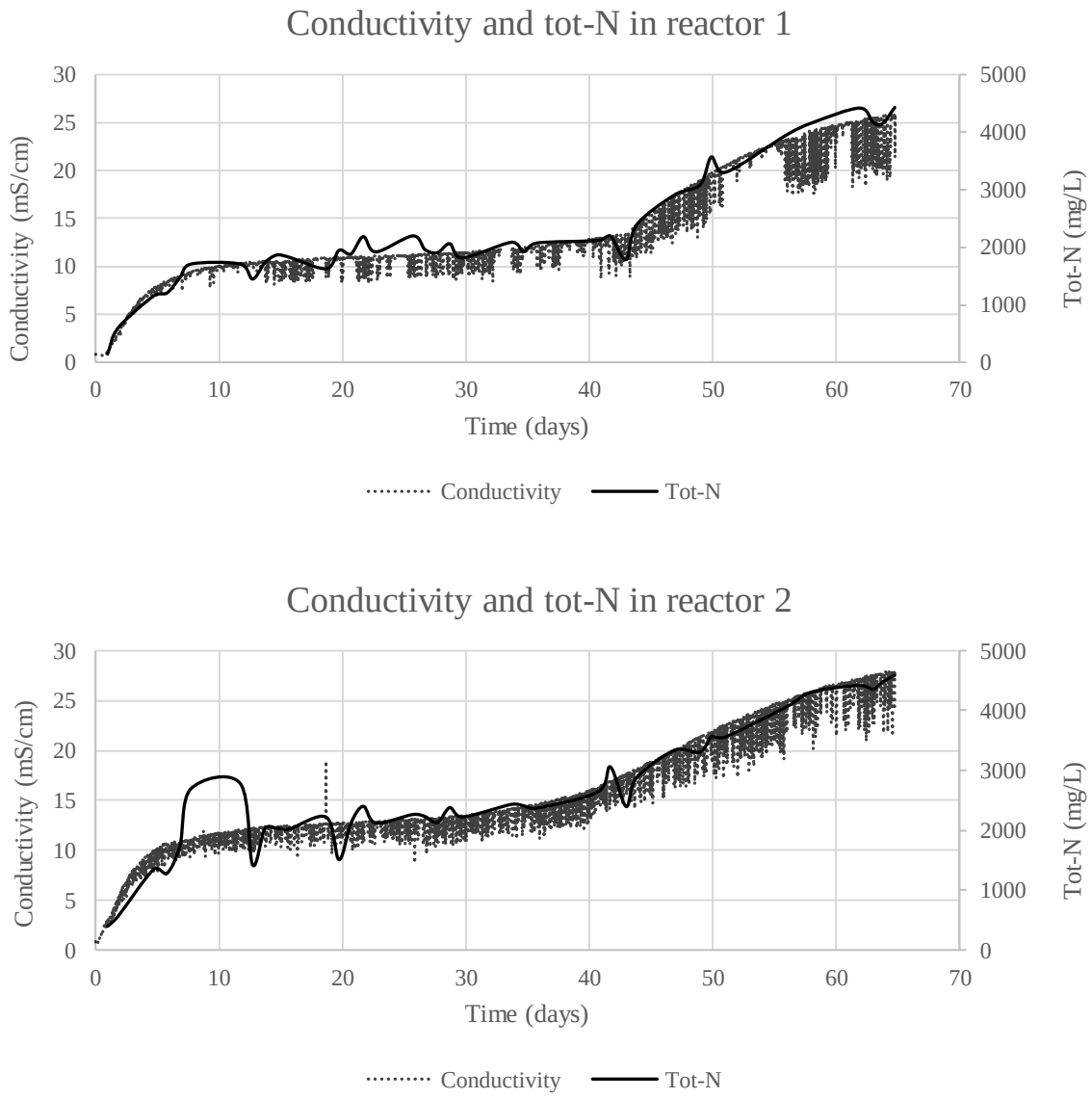

Figure 11: Conductivity related to tot-N in reactor 1 and reactor 2. The upper part of the conductivity graphs follows a smooth line and corresponds well to the tot-N in the reactors. Deviations from the conductivity could be due to measuring errors.

The conductivity dropped regularly during of the measurements which gives the graphs in *Figure 12* their special appearance. This is believed to be caused by disturbance by the carriers during the measurements. The upper part of the conductivity graphs does however follow a smooth line and corresponds well to the tot-N in the reactors. Deviations from the conductivity graph were most probably caused by errors during measurement of nitrogenous ions. The analysis required large dilution of the reactor solution and the dilution procedure is a potential source of errors.

A comparison between DO concentrations in reactor 1 and reactor 2 is shown in *Figure 12*. The slight increase in DO around day 30 in reactor 2 might be due to oxygen sensor malfunction. The oxygen sensor tip was replaced on day 63.

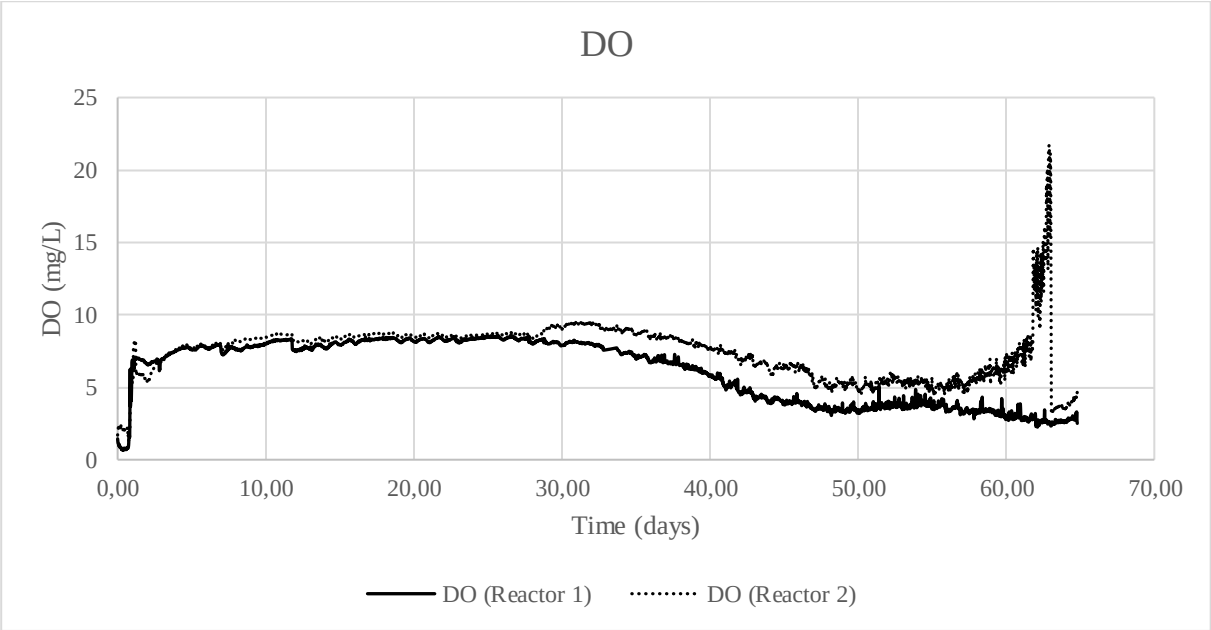

Figure 12: Shows the concentration of DO in both of the reactors. After 28 days DO in reactor 2 is slightly higher than in reactor 1. It is possible that these values are false since a malfunction of the oxygen sensor was detected around day 60. The oxygen sensor tip was replaced on day 63 and measured DO in reactor 2 was hereafter close to the values in reactor 1.

The NO_2^--N concentrations in both of the reactors are shown in *Figure 13*. A zoom in of the graphs are used to register changes at low concentrations. Fluctuations can be seen in the beginning of the start-up and later when pH in the reactors was altered.

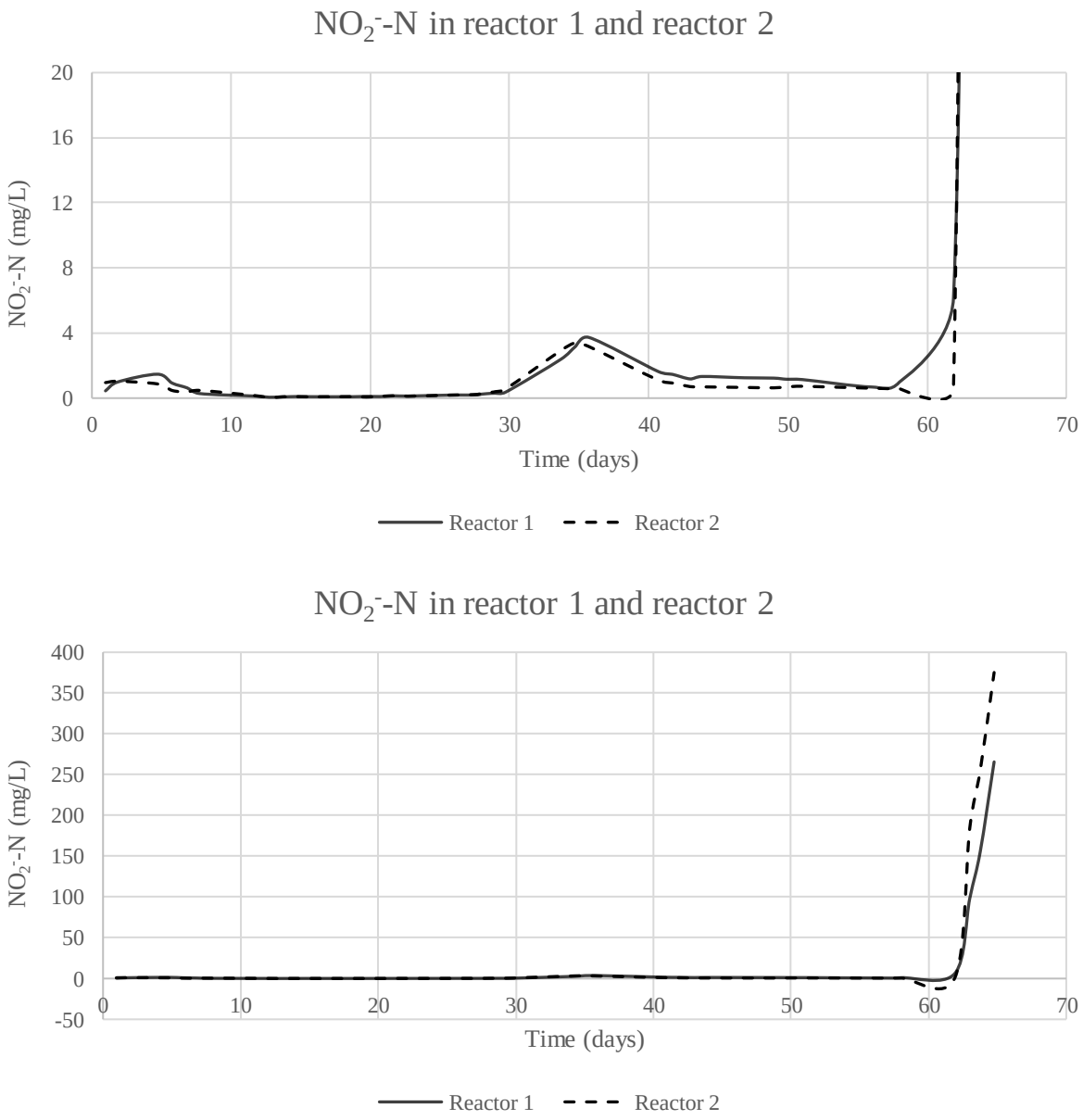

Figure 13: Shows NO_2^--N concentrations in reactor 1 and reactor 2. The upper diagram shows a zoom in of the graphs and the lower diagram shows an overview.

Appendix II - Raw data

Data from manual analyses – Reactor 1

Day	NO_2^--N (mg/l)	NO_3^--N (mg/l)	NH_4^+-N (mg/L)	Tot-N calc. (mg/L)	Tot-N (mg/L)
1,0	0,45	85	57	142	
1,8	0,98	315	280	596	765
4,7	1,47	592	560	1153	1520
5,8	0,92	646	555	1202	
6,8	0,63	742	726	1469	
7,7	0,28	860	846	1706	1710
11,7	0,13	875	832	1707	1656
12,7	0,06	732	714	1446	
13,7	0,08	882	849	1731	
14,7	0,10	948	917	1865	1806
15,7	0,09	942	883	1825	
18,7	0,09	774	852	1626	1863
19,7	0,11	1008	937	1945	
20,7	0,10	972	911	1883	
21,7	0,14	1092	1092	2184	1902
22,7	0,13	984	934	1918	
25,7	0,19	1086	1110	2196	1875
26,7	0,19	990	966	1956	
27,7	0,20	1008	896	1905	
28,7	0,29	1044	1016	2061	2001
29,7	0,36	900	912	1812	
33,7	2,39	1044	1039	2086	2004
34,7	3,14	972	946	1921	
35,7	3,73	1044	1024	2071	2127
40,7	1,63	1020	1094	2116	
41,7	1,46	1100	1093	2195	2157
43,0	1,18	895	888	1784	
43,8	1,33	1195	1188	2384	
46,9	1,25	1470	1436	2907	2965
49,0	1,23	1488	1594	3083	
49,9	1,16	1680	1886	3567	3380
51,0	1,14	1680	1620	3301	
56,0	0,68	1968	1984	3953	
58,0	0,982	2056	2100	4157	
61,9	6,18	2272	2140	4418	4365
63,0	98,40	1912	2160	4170	
63,8	156,00	1952	2040	4148	
64,8	265,60	1984	2180	4430	

Data from manual analyses – Reactor 2

Day	NO_2^--N (mg/l)	NO_3^--N (mg/l)	NH_4^+-N (mg/L)	Tot-N calc. (mg/L)	Tot-N (mg/L)
1,0	0,95	222	222	395	
1,8	1,04	307	307	565	765
4,7	0,87	702	702	1348	1528
5,8	0,47	676	676	1276	
6,8	0,39	898	898	1738	
7,7	0,48	1815	1815	2691	1539
11,7	0,16	1425	1425	2785	1953
12,7	0,03	744	744	1424	
13,7	0,08	1026	1026	2025	
14,7	0,09	1020	1020	2034	1971
15,7	0,08	1026	1026	2022	
18,7	0,09	1104	1104	2218	2121
19,7	0,07	768	768	1508	
20,7	0,11	1080	1080	2121	
21,7	0,14	1206	1206	2400	2067
22,7	0,12	1056	1056	2122	
25,7	0,18	1122	1122	2263	2202
26,7	0,20	1110	1110	2230	
27,7	0,23	1056	1056	2130	
28,7	0,36	1218	1218	2380	2187
29,7	0,56	1134	1134	2223	
33,7	2,93	1230	1230	2433	2268
34,7	3,39	1206	1206	2409	
35,7	3,17	1164	1164	2367	2484
40,7	1,10	1308	1308	2641	
41,7	0,93	1510	1510	3059	2724
43,0	0,71	1215	1215	2398	
43,8	0,69	1435	1435	2876	
46,9	0,66	1715	1715	3336	3290
49,0	0,63	1644	1644	3305	
49,9	0,69	1950	1950	3565	3585
51,0	0,73	1792	1792	3559	
56,0	0,61	2024	2024	4065	
58,0	0,59	2128	2128	4309	
61,9	0,66	2200	2200	4421	4475
63,0	184,80	1912	1912	4357	
63,8	255,60	1960	1960	4476	
64,8	375,20	1936	1936	4591	

Weight of incoming and outgoing plastic cans – Reactor 1

	Day	Vikt, In (g)	Vikt, Ut (g)
Batch 1	0,0	4159	205
	1,0	4004	431
	1,8	3558	838
	4,8	1793	2319
	5,8	1447	2587
	6,8	1179	2799
	7,7	970	2966
Batch 2	7,9	4200	169
	11,7	3763	530
	12,7	3686	586
	13,7	3618	638
	14,7	3554	717
	15,7	3494	772
	18,7	3337	860
	19,7	3284	861
	20,7	3234	872
	21,7	3181	876
	22,7	3128	876
	22,8	3124	1195
	25,7	2977	1313
	26,7	2922	1362
	27,7	2866	1412
	28,7	2798	1473
	29,7	2720	1510
	33,7	2315	1868
	34,7	2170	1997
	35,7	1999	2170
	40,7	602	3562
Batch 3	40,7	4210	205
	41,7	3763	634
	43,0	3113	1249
Batch 4	43,0	4225	172
	43,8	3994	413
	46,9	3079	1233
	48,0	2716	1597
	49,0	2407	1968
	49,9	2099	2209
	51,0	1706	2564
Batch 5	51,0	4221	205

	Day	Vikt, In (g)	Vikt, Ut (g)
	55,0	2793	1682
	56,0	2444	1975
	57,0	2058	2323
	58,0	1672	2641
Batch 6	58,0	4229	170
	60,8	3160	1348
	61,9	2717	1749
	63,0	2221	2246
	63,8	1881	2555
	64,8	1417	3031
Batch 7	64,8	3739	204
	68,0	2285	1591
	68,9	1875,4	2054
	69,9	1431,1	2459,3
	70,9	1291,1	2636,8

Weight of incoming and outgoing plastic cans – Reactor 2

	Day	Vikt, In (g)	Vikt, Ut (g)
Batch 1	0,0	4178	178
	1,0	3875	607
	1,8	3656	614
	4,8	2988	997
	5,8	2884	1243
	6,8	2811	1244
	7,7	2761	1244
	11,7	2658	1248
	12,7	2639	1270
	13,7	2620	1272
	14,7	2602	1272
	15,7	2585	1276
	18,7	2540	1302
	19,7	2524	1309
	20,7	2510	1312
	21,7	2494	1313
	22,7	2479	1343
	25,7	2432	1385
	26,7	2418	1392
	27,7	2401	1400
	28,7	2378	1419

	Day	Vikt, In (g)	Vikt, Ut (g)
	29,7	2353	1422
	33,7	2216	1503
	34,7	2170	1514
	35,7	2113	1549
	40,7	1663	1980
	41,7	1515	2099
	43,0	1290	2400
	43,8	1131	2470
	44,0	1091	2491
Batch 2	44,0	4225	170
	46,9	3593	849
	48,0	3297	1120
	49,0	3036	1374
	49,9	2776	1621
	51,0	2433	1926
	55,0	1127	3242
	56,0	783	3580
Batch 3	56,0	4228	183
	57,0	3846	581
	58,0	3492	859
	60,8	2365	2018
	61,9	1902	2490
	63,0	1408	2916
	63,8	1091	3234
	64,8	678	3653
Batch 4	64,8	3731	173
	68,0	2613	1274
	68,9	2394,6	1491,4
	69,9	2187,9	1691
	70,9	1998,8	1895,4

Batch concentrations – Reactor 1

	NH_4^--N (mg/L)	Tot-N (mg/L)	Tot-P (mg/L)	COD (mg/L)
Batch 0 (Concentrated urine)	8270	9675	652	10950
Batch 1	2067,5	2418,75	163	2737,5
Batch 2	1752	2610	167,7	2720
Batch 3	1922	2470	176,2	2370
Batch 4	3810	4710	354	5190

Batch concentrations – Reactor 2

	NH_4^--N (mg/L)	Tot-N (mg/L)	Tot-P (mg/L)	COD (mg/L)
Batch 0 (Concentrated urine)	8270	9675	652	10950
Batch 1	4135	4837,5	326	5475
Batch 2	3630	4700	354	5410

Comment: The batches of urine used in the end of the experimental period was not analysed due to lack of time.

Appendix III – Popular scientific summary

Kriget om gödningsmedel – Bakteriernas återkomst

Många av näringsämnena som används som gödning av våra åkrar återfinns i maten vi äter och senare också i avloppsvattnet vi skickar iväg. Att avskilja urinen från resten av avloppsvattnet är en lovande metod för att återanvända dessa näringsämnen. För att det här ska vara möjligt krävs det dock att nya metoder för hantering av urin utvecklas.

Axel Olsson

Populärvetenskaplig sammanfattning av masteruppsatsen:

Urine nitrification – Start-up with high strength urine

På dagens avloppsreningsverk används i stor grad bakterier för att bli av med kväve i avloppsvattnet och på så sätt minska risken för övergödning och algblomning i våra sjöar, vattendrag och havsområden. Den biologiska reningsmetod som är vanligast idag leder dock till att en stor del av näringsämnet kväve går förlorat upp i luften i form av kvävgas tillsammans med en viss del lustgas. Lustgas är en farlig växthusgas som ger 300 gånger värre växthuseffekt än koldioxid.

Att skicka användbara näringsämnen upp i luften känns onödigt, så för att ta bättre tillvara på dem kan man stabilisera och torka urin för att sedan kunna sprida ett utmärkt gödningsmedel på våra åkrar.

I denna studie har urin omvandlats av bakterier. Nyckeln kallas nitrifikation och går ut på att bakterierna äter ammoniak och andas syre för att överleva, och lämnar efter sig ämnen som vi kan gödsla våra åkrar med. Ammoniak är en flyktig gas som finns i lagrad urin och den försvinner lätt upp i luften om obearbetad urin skulle transporteras och spridas på åkrar.

Projektet gick ut på att starta nitrifikationsprocessen med starkt koncentrerat urin. Tidigare har man startat processen genom att späda urinen med mycket vatten för att sedan öka styrkan successivt. Detta har gjorts för att vänja bakterierna vid de nya levnadsförhållandena, problemet är dock att en sådan uppstart tar lång tid. Vid ett reningsverk är det önskvärt att kunna behandla den inkommande urinen direkt, och en snabb uppstart är då av yttersta vikt. Detta är ett sätt att spara på lagringsutrymme och slippa vänta på ”morgontrötta” bakterier, och risken för ammoniakförluster skulle också minska. I studien var målet också att undersöka lustgasutsläpp då den har en betydande miljöpåverkan.

I undersökningen jobbade bakterierna på bra i början, men efter drygt en vecka sjönk de i arbetstakt. I väntan på att de skulle återhämta sig bestämde vi oss för att höja pH-värdet i reaktorerna. pH är en väldigt betydande faktor i processen och styr många samband, bland annat hur mycket mat (ammoniak) som är tillgänglig för bakterierna. Efter höjningen av pH ökade arbetstakten markant. pH-nivån visade sig också påverka mängden lustgas som producerades i processen och resultaten indikerade på att lustgasutsläpp kan minimeras om pH-nivån är stabil och hålls på relativt låg nivå.

Än är uppstart med starkt koncentrerad urin i en utvecklingsfas och i framtida forskning behöver beroendet av pH-nivån undersökas närmre. Kanske är du personen som för stafettpinnen vidare? I så fall går det bra att börja så smått hemma och allt det nödvändiga du behöver för att tillverka en egen hemmareaktor är följande: två plastdunkar, spann, elvisp, akvariepump, slangar och ett gäng plastkorkar från exempelvis PET-flaskor eller mjölkpaket som bakterierna kan växa på. Och urin såklart. Hemmareaktorn är troligtvis inte lika effektiv som reaktorn i den här studien, så förvänta dig inga snabba resultat!