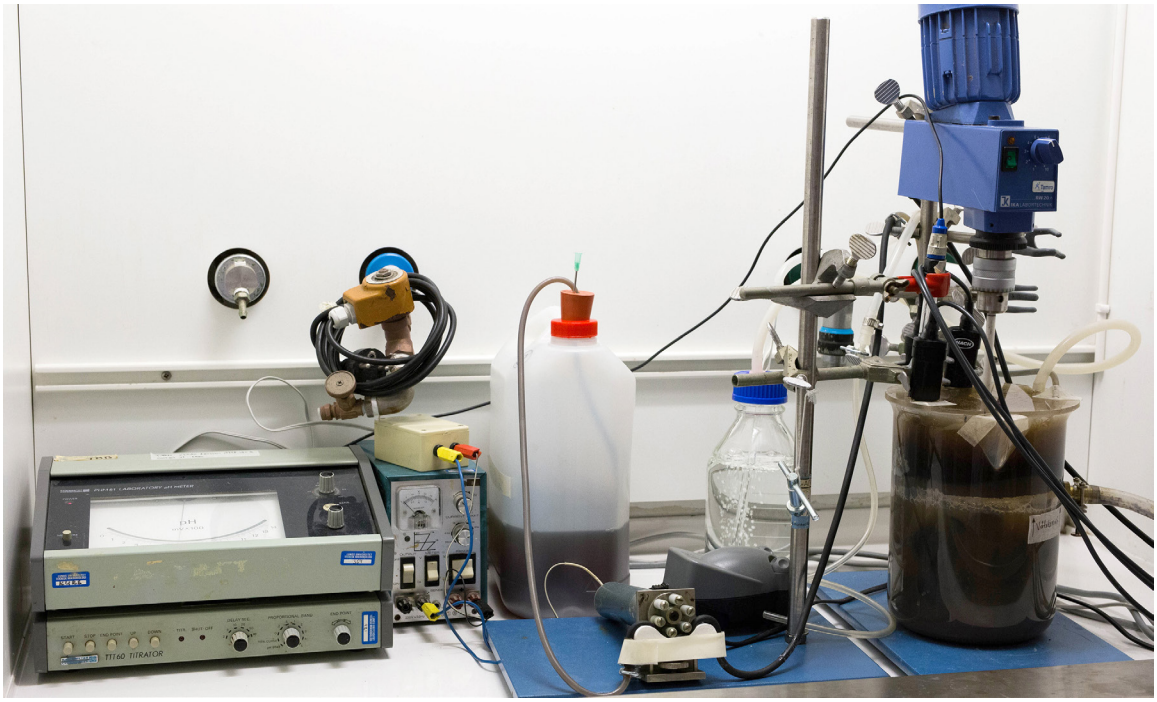
Challenges during start-up of urine nitrification in an MBBR



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Water and Environmental Engineering
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Picture on front page: Nitrification reactor. Photo by Ellen Edefell.

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Preface

This report completes my master thesis and studies in Biotechnology at Lund University. The thesis has been performed at Water and Environmental Engineering, Department of Chemical Engineering, Lund University, in collaboration with Swedish Water Research. The project aimed to further study the start-up of a nitrification reactor for stabilisation of source-separated urine.

I would like to thank some people for their support during this work. To my supervisor, David Gustavsson, at Sweden Water Research for allowing me to work in this project and supporting me along the way. To Marianne Olofsson who showed great patience with me in the early phases of the project. A special thanks to Gertrud Persson for all your support, help and for being my sounding board.

Thanks to the staff at the department for the warm welcoming and cheerful conversations. It has truly been a pleasure. Further thanks to Michael Cimbritz, my examiner, and Karin Jönsson, my supervisor, at the department for your guidance.

Furthermore, I want to thank Christer Larsson at Applied Microbiology at the Department of Chemistry, Lund University, for so generously lending me the titration equipment.

For my family, thank you for always supporting me and for the continuous discussions. The journey continues.

Lund, December 2016
Ellen Edefell

Summary

Nitrogen and phosphorous compounds are secreted by humans and treated in wastewater treatments plants. These substances are also found in agricultural fertilisers and needed for plant growth. Nutrient recycling is limited in conventional treatment systems and valuable products are lost. Urine contains most of the nutrients from humans and by separating it from wastewater it is possible to close the cycle and use as fertiliser. The overall load to existing treatment plants is simultaneously decreased which is beneficial in growing urban areas.

Urea in urine is hydrolysed to ammonia during storage. To prevent nitrogen loss due to ammonia volatilisation, separated urine needs stabilisation. One method is biological nitrification. Ammonia oxidising bacteria convert ammonia to nitrite while pH drops. Nitrite is then further oxidised to nitrate by nitrite oxidising bacteria. Limitations in alkalinity allow half of the ammonia to oxidise. The remaining half is stabilised in non-volatile ammonium when pH decrease. The treated solution contains equal parts ammonium and nitrate which are widely used in nitrogen fertilisers. However, the treated urine needs further processing to concentrate the solution to compete with existing products.

Sege Park in Malmö, Sweden, is a housing area which aims to be an example of sustainable city development by 2025. The idea is to have one house with source separation of urine and facilities for further processing. The regional water and sewage organisation, VA SYD, therefore needs to investigate and determine an appropriate method for urine treatment.

This project aimed to provide knowledge of nitrification as a stabilisation method. The start-up of a nitrification reactor for source-separated urine was studied in a bench-scale moving bed biofilm reactor, operated for two periods of 103 and 100 days respectively. The first part experienced continuous instabilities with fluctuating pH and repeated nitrite accumulations. Another start-up was initiated in the second half of the project with overall successful results. A shorter period of instabilities caused accumulation of nitrite twice at an influent nitrogen concentration of 1,390 mgN L^{-1}. The problems were overcome by lowering the load and then by exchanging the influent pump from a fixed-flow pump to pH-regulation at pH 6.2. The urine concentration could be further increased to 4,680 mgN L^{-1} nitrogen in the reactor by the end of the experimental period. The corresponding nitrification rate was 0.3 gN $m^{-2}d^{-1}$ (60 gN $m^{-3}d^{-1}$). The rate decreased while the nitrogen concentration increased. Maximum rate was 0.9 gN $m^{-2}d^{-1}$ (160 gN $m^{-3}d^{-1}$) when the reactor concentration was 2,230 mgN L^{-1}.

It seems crucial to observe and counteract process instabilities early for successful long-term operation of highly concentrated nitrification reactors. Continuous monitoring of pH and dissolved oxygen in combination with nitrite samples facilitate detection of instabilities. Reactor regulation with pH controlled influent ensure ideal conditions for well-balanced bacterial interplay and thus enhanced reactor stability.

Keywords: urine, source-separation, urine stabilisation, nitrogen recovery, nitrification, moving bed biofilm reactor, MBBR.

Sammanfattning

Kväve- och fosforföreningar som återfinns i kommunalt avloppsvatten behandlas i avloppsreningsverk. Kväve och fosfor är dessutom nödvändiga i jordbruket och viktiga komponenter i gödselmedel. Kretsloppet av näringsämnen är dock begränsat i konventionell avloppshantering. Urinen innehåller den största delen av näringsämnena i kommunalt avloppsvatten. Genom att separera urinen från spolvatten, fekalier och bad-, dusch, och diskvatten ökar möjligheterna för att sluta kretsloppet och att använda näringsämnena i jordbruket. Dessutom minskar belastningen på befintliga avloppsanläggningar vilket är gynnsamt i växande urbana områden.

Urin innehåller urea som hydrolyseras till ammoniak vid lagring. Ammoniak är lättflyktigt och för att minska kväveförlusterna via avdunstning måste urinen stabiliseras. En metod är biologisk nitrifikation, vilken sker i två steg. Ammoniakoxiderande bakterier oxiderar ammoniak till nitrit samtidigt som pH sjunker. Nitriten oxideras sedan vidare till nitrat av nitritoxiderande bakterier. Alkaliniteten i urinen begränsar nitrifikationen så att endast hälften av ammoniaken oxideras. Den kvarvarande ammoniaken stabiliseras som ammonium då pH är lägre. Den behandlade urinen innehåller lika delar ammonium och nitrat vika är mycket vanliga komponenter i kvävegödsel. För att kunna konkurrera med befintliga gödslingsprodukter bör den behandlade urinen koncentreras i en efterföljande behandling.

Det gamla sjukhusområdet Sege Park i Malmö ska på sikt bli ett område för nyskapande och hållbar stadsutveckling. Planen är att år 2025 ha minst en byggnad med urinseparering och tillhörande efterbehandling. Den regionala VA-organisationen VA SYD måste därför undersöka de olika möjligheterna för urinhantering.

Detta projekt syftar till att undersöka biologisk nitrifikation som en stabiliseringsmetod. Uppstarten av en nitrifikationsreaktor för urin studerades i labbskala i en reaktor med rörliga biofilmsbärare (MBBR) under två perioder, 103 respektive 100 dagar. Kontinuerliga driftsproblem med instabilt pH och upprepad ackumulation av nitrit under den första perioden ledde till en ny omstart. Den senare uppstarten visade stabilare resultat. En kortare period av instabilt pH ledde till två nitritackumulationer när ingående kvävekoncentration var 1390 mgN L^{-1}. Problemen korrigerades med sänkt belastning och senare genom att byta ut den ingående pumpen mot pH-reglering. Kvävekoncentrationen kunde sedan ökas ytterligare och vid slutet av experimentperioden var koncentrationen i reaktorn 4680 mgN L^{-1}. Nitrifikationshastigheten sjönk när koncentrationen ökade och var 0,3 gN $m^{-2}d^{-1}$ (60 gN $m^{-3}d^{-1}$). Den högsta nitrifikationshastigheten var 0,9 gN $m^{-2}d^{-1}$ (160 gN $m^{-3}d^{-1}$) då kvävekoncentrationen var 2230 mgN L^{-1} i reaktorn.

Det verkar kritiskt att uppmärksamma och motverka instabilitet tidigt för att upprätthålla en hållbar långsiktig drift av nitrifikationsreaktorer med höga koncentrationer. Kontinuerlig mätning av pH och löst syre samt provtagning av nitrit underlättar upptäckten av processproblem. Reaktorreglering med pH-kontrollerad urindosering möjliggör ideala förhållanden för en bra balans i bakteriekulturen och ökar därför reaktorstabiliteten.

Nyckelord: urin, urinsortering, kväveåtervinning, nitrifikation, MBBR.

List of abbreviations

AOB	Ammonia Oxidising Bacteria
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DO	Dissolved Oxygen
FA	Free Ammonia
FNA	Free Nitrous Acid
HRT	Hydraulic Retention Time
INxx	Inlet batch number xx
MABR	Membrane Aerated Biofilm Reactor
MBBR	Moving Bed Biofilm Reactor
NH4-N	Ammonium Nitrogen
Nit.	Nitrification
NO2-N	Nitrite Nitrogen
NO3-N	Nitrate Nitrogen
NOB	Nitrite Oxidising Bacteria
N-tot	Total Nitrogen
OUTxx	Outlet batch number xx
P-tot	Total Phosphorus
SRT	Solids Retention Time
SS	Suspended Solids
Std dev	Standard deviation
WWTP	Wastewater Treatment Plant

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1 Introduction

Growing cities increase the demand on municipal wastewater treatment plants (WWTP). Most of the treated compounds in wastewater originate from a small volume fraction; urine (Jönsson *et al.*, 2005). Diverting urine from wastewaters lower the nutrient load in treatment plants and allow recirculation of nutrients which may present a more sustainable waste handling (Wilsenach & van Loosdrecht, 2004; Larsen, 2015). Some agricultural fertilisers have similar composition of nitrogen and phosphorous as urine and provide a possible economic market for nutrient reuse.

Source-separation of urine in large scale present challenges for the collecting system as well as further handling. In Malmö, Sweden, the housing area Sege Park aims to showcase a sustainable city development in 2025 (Malmö Stad, 2015b). The regional water and sewage organisation, VA SYD, will develop a test bed for treatment of source-separated urine collected from at least one of the buildings (Malmö Stad, 2015a). This thesis project is part of evaluating the possibilities for urine handling. It is part of a greater project in collaboration with the research organisation Sweden Water Research, partly owned by VA SYD, called *URILAB.*

Biological nitrification followed by distillation has been proposed as a suitable treatment method for urine (Udert & Wächter, 2012). Stored urine contains high concentrations of volatile ammonia. The process stabilises the nitrogen content by forming ammonium nitrate and prevent nitrogen loss due to vaporisation (Udert *et al.*, 2003b). A pilot plant has been operated for a couple of years at Eawag Research Institute in Dübendorf, Switzerland. The start-up has shown the most challenging for the nitrification process (Udert *et al.*, 2003b; Fumasoli *et al.*, 2016; Olofsson, 2016).

1.1 Aim

This thesis continues the work of Olofsson (2016) with the aim of further evaluating operational options during start-up of a nitrification process for source-separated urine. Furthermore, action plans for avoiding process instabilities should be investigated. Lastly, the project aimed to further contribute with knowledge of the dynamics of the nitrification process in highly concentrated solutions. The report will answer the questions:

- What process instabilities can arise during start-up?
- How can the problems be handled or avoided?
- How should the start-up of a nitrification process be operated?

1.2 Limitations

The urine was supplied by one household only. This diminished the natural variations in urine composition when collected from a larger community. About three months project employment followed by about three months experimental period in the thesis defined the start-up period in this project.

2 Nutrient recovery from wastewaters

Release of untreated wastewater increases the risk of eutrophication in the recipient environment. To avoid or minimise the problem, the requirements for wastewater treatment has during the last decades greatly tightened. In general, Sweden has a high standard of nutrient removal (Statistiska centralbyrån, 2014). The three main components treated in WWTP are nitrogen, phosphorous and organic matter (Naturvårdsverket 2014).

Conventional wastewater treatment is often divided into primary and secondary treatment. The primary treatment removes particulate matter by screening and settling. Secondary treatment consists of multiple steps of biological and chemical processes and aims to remove soluble pollutants. Biological nitrogen removal is most often completed in two steps; aerobic nitrification, where ammonia is oxidised to nitrate, and anaerobic denitrification, where nitrate is reduced to nitrogen gas (Ahn, 2006). Phosphorous can be removed with biological treatment or chemical precipitation (Yeoman *et al.*, 1988). Activated sludge processes are very often used for removal of organic matter as well as nitrogen compounds. The tanks can be operated aerobic, anoxic or anaerobic modes depending on the desired process. The biomass is grown in suspended flocs. (Henze *et al.*, 2002)

2.1 Novel wastewater strategies

In recent years, some new approaches for biological nitrogen removal have been developed. The SHARON process (single reactor system for high ammonia removal over nitrite process) partly nitrifies ammonium to nitrite with less aeration than full nitrification (Hellinga *et al*., 1998). The nitrite/ammonium mix can be further processed with the ANAMMOX (anaerobic ammonium oxidation) process to form dinitrogen gas and some nitrate under anaerobic conditions (Ahn, 2006; Van Hulle *et al.*, 2010). The CANON (complete autotrophic nitrogen removal over nitrite) process is the combination of SHARON and ANAMMOX in one aerated reactor (Ahn, 2006). The dissolved oxygen concentration must be kept low. Aerobic nitrifying bacteria deplete oxygen in the outer layers of a biofilm, creating anoxic conditions in the inner layers for the anammox bacteria (Van Hulle *et al.*, 2010). In contrast to conventional denitrification, the ANAMMOX do not require additional organic carbon source (Strous *et al*., 1998), which makes it competitive on the market.

2.2 Source-separation of wastewater

Wastewater from households can be differentiated into urine (sometimes referred to as yellow water), brownwater and greywater. Greywater originates from kitchens and bathrooms while brownwater is faeces and flushwater. The term blackwater is sometimes used and refers to urine mixed with brownwater. When the population in urban areas increases the load on municipal WWTPs increases and in combination with stricter nutrient removal rates many sites needs to be upgraded to handle the loadings. Source-separation of wastewater streams provides new possibilities in terms of recycling and treatment of nutrients. Urine alone stands for 1% of the total wastewater volume but contains up to 80% of the nitrogen and 60% of the phosphorous (Jönsson *et al*., 2005). Separation of urine would lower the nutrient load on WWTPs and provide a more sustainable nutrient management (Wilsenach & van Loosdrecht, 2004; Larsen, 2015). Another resource management approach of wastewater is to treat blackwater and highly concentrated greywater from kitchens anaerobically to produce methane (CH_4) and precipitate phosphorous (Zeeman *et al.*, 2008). As well as creating

valuable products from wastewater, nutrient recycling can also decrease the carbon footprint of wastewater treatment (Larsen, 2015). Maurer, Pronk and Larsen (2006) identified seven reasons for separating and treating human urine; hygienisation, volume reduction, stabilisation, phosphorous recovery, nitrogen recovery, nutrient removal and handling of micropollutants. A single treatment step can achieve several purposes but not all. Combinations of processes can therefore provide good overall results.

Centralised wastewater treatment systems demand large quantities of water and large initial investment costs. This form of wastewater handling is not suitable in many areas of the world. Globally in 2010, diarrhea and related diseases accounted for 10% of childhood deaths (Tilley *et al.*, 2014). Diarrhea is often correlated to poor hygiene, absence of clean water and inadequate handling of human excreta. In rural and/or developing countries facing poor waste handling, source-separation of wastewater can provide many opportunities such as improved hygiene and nutrient recycling. The Blue Diversion AUTARKY project aims to develop a self-sustaining grid-free toilet for use in developing areas separating urine, faeces and water (Lienert, 2016).

2.3 Source-separated urine

Source-separation of urine is accomplished by no-mix toilets or waterless urinals. There are, and have been, a few models on the market over the years. Challenges connected to urine-diverting toilets are precipitation of phosphorous, smell and an adequate separation of urine and flush water (Udert *et al.*, 2003a; 2003c). A set of new Duravit prototypes are installed in the NEST building at Eawag Research Institute in Dübendorf, Switzerland (Etter *et al.*, 2016). The toilets have a sensor connected to a valve which opens in contact with urine. The NEST building source-separates wastewater streams and provides experimental space in the connected Water Hub for various projects.

The composition of urine varies depending on feeding habits, physical activities, body size, environmental factors and time of day. In fresh urine most of the nitrogen is in urea, 85%, while 5% is in ammonia (Udert *et al.*, 2006). Other nitrogen compounds are creatinine, amino acids and uric acid. During storage the urea is hydrolysed by bacterial urease to ammonia and bicarbonate, Equation 1, while the pH value increases to about 9 (Udert *et al.*, 2003b). After the hydrolysis, ammonia stands for 90% of the nitrogen content (Udert *et al.*, 2003b; Udert *et al.*, 2006). During storage, transportation or in direct applications as fertiliser, there is high risk of nitrogen losses due to ammonia volatilisation (Udert *et al.*, 2003b). The composition of fresh and stored urine from this project as well as in literature is shown in Table 2.1. The variations in urine composition are evident in literature. Some researchers have analysed source-separated urine from the same building at Eawag Research Institute, Dübendorf, Switzerland, during different time periods and operational conditions (Udert & Wächter, 2012; Etter *et al.*, 2013; Fumasoli *et al.*, 2016). The rather low concentrations might originate from dilution with some flush water or day time collection. This study handles mostly morning urine and is therefore more concentrated.

$$NH_2(CO)NH_2 + 2H_2O \rightarrow NH_3 + NH_4^+ + HCO_3^- \quad (1)$$

Table 2.1. Composition of source-separated urine.

		Fresh urine	Stored urine				
		Udert *et al.* (2006)[1]	**Udert *et al.* (2006)[2]**	**Etter *et al.* (2013)**	**Fumasoli *et al.* (2016)[3]**	**Udert & Wächter (2012)**	**This study**
Total nitrogen	*mgN L^{-1}*	9,200	9,200				11,000
Total ammonium	*mgN L^{-1}*	480	8,100	1,790	4,110	2,390	10,000
Urea	*mgN L^{-1}*	7,700	0				
COD	*mgO_2 L^{-1}*	10,000	10,000	2,110	3,860	4,500	9,900
Total phosphorus	*mgP L^{-1}*	740	540	108[4]	242	208	750
Potassium	*mg L^{-1}*	2,200	2,200	897	1,470	1,410	3,010
Sodium	*mg L^{-1}*	2,600	2,600	966	1,760	1,740	3,260
Chloride	*mg L^{-1}*	3,800	3,800	1,830	2,980	3,210	3,100
Calcium	*mg L^{-1}*	190	0	10		16	15.5
Magnesium	*mg L^{-1}*	100	0	<5		<5	6.64
Sulphate	*mg L^{-1}*	1,500	1,500	316		778	903
pH		6.2	9.1	8.9	9.0	8.69	9.2
Alkalinity		22 mM	490 mM				40 g L^{-1}
Electric conductivity	*mS cm^{-1}*			15.9			51.8
TIC	*mgC L^{-1}*			970	2,080	1,210	1,230
TOC	*mgC L^{-1}*			863		1,830[5]	4,700
BOD_7	*mg L^{-1}*						6,680

[1] Data collected from multiple studies
[2] Simulated values
[3] Men's urine only
[4] Phosphate, not total phosphorus
[5] Dissolved organic carbon

2.3.1 Nitrogen stabilisation

When urine is used untreated as fertiliser in agricultural soils the smell of ammonia can be noticed 24 h after application (Udert *et al.*, 2006). This is unpleasant for habitants in the surrounding area and cause nitrogen loss when ammonia volatilise. Furthermore, the hydrolysis of urea greatly increases the buffering capacity of urine and in combination with the high pH, bacterial nitrification can be inhibited causing accumulation of nitrite with negative effects in agricultural soils when untreated urine is spread on fields (Burns *et al.*, 1995). In order to improve the suitability of source-separated urine as a fertiliser the nitrogen needs to be stabilised. One approach is to add acid to lower the pH and shift the

equilibrium to form the non-volatile ammonium ion and lower the ammonia concentration. However, in hydrolysed urine the buffering capacity of bicarbonate (HCO_3^-) demand large quantities of acid to stabilise the ammonia which in turn lower the profitability of the product (Udert *et al.*, 2006).

Biological nitrification of urine is believed to be a suitable approach for nitrogen stabilisation. Bacteria convert ammonia to nitrate and simultaneously decrease the pH. The remaining ammonia is transformed to ammonium and the smell is reduced (Udert *et al*., 2003b). The process has been evaluated in several different projects and is discussed further in *4. Nitrification*. There are a few different final products that can be derived from nitrified urine. The VUNA project aims for complete nutrient recovery from source-separated human urine as well as reduced pollution of water resources (Etter *et al*., 2015). Currently there are three pilot plants running, one at Eawag, Dübendorf, Switzerland, and two in eThekwini, Durban, South Africa (Fumasoli *et al.*, 2016). The commercial fertiliser product, *AURIN Naturelle*, is produced by treating collected urine in a 120 L nitrifying moving bed bioreactor (MBBR) followed by distillation to concentrate the solution. The product contains 4.2% nitrogen at minimum in the form of ammonium nitrate, common in fertilisers (Etter, 2016). A dry powder is also possible to produce by further distillation and drying (Johansson & Hellström, 1999; Udert & Wächter 2012).

Other examples of methods for nitrogen recycling are assimilation in microalgae (Muys, 2014), forward osmosis and membrane distillation (Lui *et al.*, 2016) and forward osmosis dewatering (Zhang *et al.*, 2014). Stabilisation of fresh urine can be achieved by preventing urea hydrolysis by addition of calcium hydroxide (Randall *et al*., 2016). Another approach of stabilisation is drying fresh urine on a mix of ash and lime (Dutta & Vinnerås, 2016). Urea is then also retained in the dry fertiliser product. An alternative for volume reduction is freezing urine to -14°C, the product supply 80% of the nutrients in only 25% of the volume (Lind *et al.*, 2001).

2.3.2 Nutrient precipitation

The high pH in hydrolysed urine initiates precipitation of struvite ($MgNH_4PO_4 \cdot 6H_2O$), hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) and calcium phosphate (Udert *et al.*, 2003a; 2003b). The maximum precipitation potential is almost reached when only a fraction of urea has been hydrolysed (Udert *et al.*, 2003a). In projects with separated urine or concentrated blackwater nearly all phosphorous could be recovered by adding a magnesium source (Zeeman & Kujawa-Roeleveld, 2011; Udert *et al.*, 2015). However, the process needs to be combined with other treatment methods to handle the remaining high concentrations of nitrogen and potassium (Udert *et al.*, 2015). Only 13% of the monetary fertiliser value of urine is recovered with struvite precipitation (Udert & Wächter, 2012).

2.3.3 Pathogens and pharmaceuticals

Two purposes for treating source-separated urine are hygienisation and handling of micropollutants which creates a more safe and useful product (Maurer *et al*., 2006). The alkaline pH and high urine concentrations facilitate hygienisation of urine during storage. Six months storage should be sufficient to lower the risk of transmission below an acceptable limit according to Udert *et al.* (2006). Additionally, aerobic treatment kills especially anaerobic pathogens (Udert *et al.*, 2006). If the urine solution is further processed with distillation for volume reduction, the remaining pathogens would be killed generating a hygienically safe product (Udert *et al.*, 2015).

The urine contains the majority of pharmaceuticals excreted by humans and these can have negative effects on the environment if released (Etter *et al.*, 2015). An increasing number of studies have been made on removal of micropollutants. Natural and synthetic oestrogen (estradiol, E2, and 17a-ethinylestradiol, EE2) found in contraceptives (Grandin, 2016) have been rather successfully removed in a nitrifying bioreactor while propranolol and ibuprofen remained (Escher *et al.*, 2006). The VUNA project has evaluated a number of pharmaceuticals and treatment conditions. Biological aerated nitrification was able to remove some pharmaceuticals while others are more persistent. However, in combination with powdered activated carbon (PAC) the micro-pollutant removal is more efficient. Furthermore, the ecotoxicity was reduced with PAC treatment (Etter *et al.*, 2015). The NEST project is evaluating a similar procedure with granular activated carbon (GAC) for pharmaceutical adsorption (Etter *et al.*, 2016). When all pharmaceutical residues are present in negligible concentrations in the existing fertiliser product AURIN Naturelle, the fertiliser licence can be changed to include use on agricultural land not only flowers and lawns (Etter *et al.*, 2016).

2.3.4 Fertiliser properties

Worldwide, urea stands for 54% of the market for nitrogen fertilisers (IFA, 2016b). However, in Europe ammonium nitrate and calcium ammonium nitrate are the most commonly used nitrogen fertilisers (Ahlgren *et al.*, 2008). Furthermore, farmland needs addition of phosphorous, potassium, calcium, sulphur, magnesium and numerous micronutrients to ensure proper growth (IFA, 2016a). The composition of urine includes most nutrients and several micronutrients in proportions which makes it ideal as plant fertiliser (Fureman, 2000; Etter *et al.*, 2015). When comparing urine-based fertilisers with commercial mineral fertilisers in relation to their plant uptake ability, no differences could be identified in slightly acidic soils (Bonvin *et al.*, 2015).

Heavy metal concentrations in urine are lower compared to organic and chemical fertilisers (Kirchmann & Pettersson, 2000). Additionally, urine contains rather high concentrations of sodium chloride which contribute to salinization of agricultural soils. Yet the salt to nutrient ratio is equal to manure, commonly used on farmland (Udert *et al.*, 2015).

Estimations of energy demand for production of a dry powder, urine based, fertiliser from a nitrification and distillation process compared to conventional wastewater treatment and production of nitrogen and phosphorous fertilisers in equivalent amounts showed four to five times greater primary energy demand for the urine based product (Udert & Wächter, 2012). However, if reversed osmosis was used to remove 80% of the water and the rest with vapour compression, the process was competitive with the conventional production routs energy wise. On the other hand, when distribution and production occur in the vicinity, a concentrated liquid product might be as desired a dry powder, decreasing the energy demand.

3 Nitrification

Biological nitrification is an important process step in enhanced nitrogen removal in wastewaters. Even though the process often is operated at conventional WWTPs, instabilities are common due to slow bacterial growth rates and sensitivity to changes in temperature and pH (Bock & Wagner, 2013). Treatment of source-separated urine aims it to recycle rather than removing nutrients. Nitrification prevent nitrogen loss and malodour when volatile ammonia is oxidized to nitrate while the pH decrease to about 6, stabilising the remaining ammonia in non-volatile ammonium (Fumasoli *et al.*, 2016). The oxidation to nitrate demand 2 moles of alkalinity for 1 mole ammonia and in hydrolysed urine the ratio is approximately 1:1. As a consequence about half of the total nitrogen is nitrate after treatment (Udert *et al.*, 2003b).

Nitrification takes place in two steps performed by ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB). Suzuki *et al.* (1974) showed that ammonia (NH_3) is the substrate for AOB. Nitrite is the substrate for NOB (Bock & Wagner, 2013). The process utilises the ammonia and nitrous acid equilibria (Equation 2-3). AOB oxidise ammonia to nitrite which is further oxidised by NOB to nitrate. The biological reactions can be seen in Equations 4-6. Ammonia oxidation decreases the pH value. Ammonia and nitrite oxidisers grow close together. Produced nitrite is therefore instantly oxidised further resulting in very low bulk concentrations of nitrite (Bock & Wagner, 2013). However, instabilities in the biomass can create accumulation of nitrite.

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^- \quad (2)$$

$$HNO_2 \leftrightarrow H^+ + NO_2^- \quad (3)$$

$$NH_3 + 1.5\,O_2 \rightarrow HNO_2 + H_2O \quad (4)$$

$$NO_2^- + 0.5\,O_2 \rightarrow NO_3^- \quad (5)$$

$$NH_4^+ + 2\,O_2 \rightarrow NO_3^- + 2\,H^+ + H_2O \quad (6)$$

3.1 Nitrifying bacteria

Nitrifying bacteria are lithoautotrophic organisms oxidising inorganic nitrogen compounds for energy and utilise carbon dioxide as carbon source. The AOB most often belong to the *Nitrosomonas europaea* lineage, especially in high ammonia concentrations (Fumasoli *et al.*, 2016). Other AOB genera are *Nitrospira, Nitrosococcus, Nitrosovibrio* and *Nitrosolobus* (Ahn, 2006, Bock & Wagner, 2013). When pH is low, 5.8, the more acid-tolerant *Nitrospira* is selected over *Nitrosomonas* (Fumasoli *et al.*, 2016). *Nitrobacter* is the dominating and most studied genus amongst the NOB (Ahn, 2006; Bock & Wagner, 2013). *Nitrobacter* is also better adapted to high nitrite concentrations than the NOB *Nitrospira* (Fumasoli *et al.*, 2016).

3.2 Process conditions

Various operational parameters influence the nitrification process as well as the reactor stability. Some of the more important factors are temperature, pH, oxygen and alkalinity.

3.2.1 Temperature

In conventional wastewater treatment temperatures usually range in 10-15°C and in those conditions the growth rate of *Nitrobacter* is almost twice as high as *Nitrosomonas* (Wett & Rauch, 2003). However, AOB growth rate increases more than NOB growth with rising temperatures Hellinga *et al.*, 1999). Most studies regarding nitrification of urine are conducted indoors at ambient temperatures. The maximum specific growth rate at 20°C is 1.05-1.4 d^{-1} for AOB and 0.9-1.31 d^{-1} for NOB (Munz *et al.*, 2011). Low temperatures lower the risk of nitrite accumulation.

Nitrification rates increase with temperature (Fumasoli *et al.*, 2016). In addition, the available substrate concentrations are also influenced by temperature. The free ammonia (FA) and nitrite concentrations increase with temperature (Anthonisen *et al.*, 1976).

3.2.2 pH

Nitrification rates increases with pH, especially the AOB activity (Hunik *et al.*, 1992). It can partly be explained by the available substrate concentrations in the nitrification process which are highly dependent on the pH value, FA and nitrite increases with pH (Anthonisen *et al.*, 1976). When studying pure cultures the optimal growth conditions were pH 8.1 and 7.9 for *Nitrosomonas* and *Nitrobacter* respectively (Grunditz & Dalhammar, 2001). However, these conditions are not ideal for the interplay in the nitrifying community. Hellinga and colleagues (1999) showed how AOB and NOB growth rates were equal at pH 6.6. Lowering the pH favours the NOB.

Processes with high ammonia wastewaters or urine have been operated at pH ranging from 5.8 (Fumasoli *et al.*, 2016) to 8.5 (Hunik *et al.*, 1992). Low pH selects the acid-tolerant AOB *Nitrospira* sp. When comparing two parallel reactors run at pH 5.8 and 6.2, acid-tolerant bacteria decreased the pH to 4.6 when the influent was stopped while the other reactor stopped at 5.7 (Fumasoli *et al.*, 2016). The consequences were inhibition of NOB and chemical reactions forming harmful gases. High pH on the other hand, can result in higher nitrogen losses due to heterotrophic denitrification and increases risk of nitrite accumulation (Udert & Wächter, 2012).

AOB activity is greatly reduced at pH below 6 (Udert *et al.*, 2003b; Fumasoli *et al.*, 2015). Ammonia oxidation lower the pH and the alkalinity of the urine sets the limit of oxidation (Udert *et al.*, 2003b). Aeration causes carbon dioxide stripping at low pH, reducing the available inorganic carbon to perhaps critical levels (Wett & Rauch, 2003). Another reason for the reduced activity is low substrate concentration which leads to an energy limited state of the cells. Inhibition of enzymes is believed peripheral to energy limitation for the decreased AOB activity (Fumasoli *et al.*, 2015).

3.2.3 Oxygen

Biological nitrification demand oxygen and well-tuned interactions between AOB and NOB. Critical concentrations of dissolved oxygen (DO) vary slightly depending on the microbial community, Table 3.1. Successful nitrification has been achieved with DO concentrations of 1.7 mgL^{-1} (Ruiz *et al.*, 2003) or higher.

Table 3.1. Critical dissolved oxygen (DO) concentrations for nitrification.

Reference	Successful nitrification ($mgO_2\ L^{-1}$)	Inhibited nitrification ($mgO_2\ L^{-1}$)	NOB inhibited ($mgO_2\ L^{-1}$)	AOB inhibited ($mgO_2\ L^{-1}$)
Garrido *et al.* (1997)	2.5		1-2	<1
Ruiz *et al.* (2003)	1.7		0.7	0.5
Feng *et al.* (2008)	4.29	1.43		

3.2.4 Alkalinity

Due to shortage of alkalinity in urine about half of the total nitrogen is oxidised. The product is a solution of ammonium nitrate which is common in the industry but thermally unstable. To achieve full nitrification and a more stable end-product, alkalinity needs to be dosed to the reactor (Feng *et al.*, 2008; Uhlmann, 2014). Economic profitability decreases when adding chemicals. However, the process stability might increase due to lower risk of FA inhibition. Lab-scale experiments have used potassium bicarbonate ($KHCO_3$) (Florin, 2013), sodium carbonate (Na_2CO_3) (Feng *et al.*, 2008) and sodium bicarbonate ($NaHCO_3$) (Johansson & Hellström, 1999). However, these chemicals are rather expensive. In large scale, cheaper alternatives are more likely used, e.g. powder or small stones of calcite (Florin, 2013).

3.3 Inhibition

3.3.1 Free ammonia

The substrate of AOB is inhibitory for all nitrifying bacteria. Ammonia is in equilibrium with ammonium and the FA concentration of is determined by pH, temperature, and initial ammonium concentration. The FA concentration increase with increased pH, temperature and ammonium concentration. The competitive inhibition diminishes when FA concentration decrease (Anthonisen *et al.*, 1976). There are no absolute concentrations when biological nitrification is inhibited by FA, Table 3.2. Multiple studies have showed adaptation of the biomass and transient inhibitory effects at high FA concentrations (Anthonisen *et al.*, 1976; Johansson & Hellström, 1999; Peng & Zhu, 2006).

Table 2.2. Inhibitory concentrations on biological nitrification by FA.

Reference	NOB inhibited ($mg\ L^{-1}$)	AOB inhibited ($mg\ L^{-1}$)	Comment
Anthonisen *et al.* (1976)	0.1-1.0	10-150	
Peng & Zhu (2006)[1]	4.3	>60.8	
		>8.5	Total inhibition at 24 mg L^{-1}
	>29	>29	Regained activity after concentrations of 68 mg L^{-1}
Sun *et al.* (2012)	>1		Total inhibition
Johansson & Hellström (1999)[2]	1.3-32	1.3-32	Biomass adaptation
Fureman (2000)[1]	0.1-5	7-10	
	2.2-25		Total inhibition
	24	24	Biomass adapted to 40 mg L^{-1}

[1] Review article
[2] Data from literature

3.3.2 Free nitrous acid

Another strongly inhibitory substance for nitrifying bacteria is free nitrous acid (FNA). Similarly to FA, FNA show no permanent inhibition (Anthonisen *et al.*, 1976). However, contrary to FA, FNA inhibition is non-competitive (Claros *et al*., 2013). The FNA equilibrium is affected by nitrite concentration, pH and temperature. The FNA concentration increases with decreasing pH and temperature. Nitrifying bacteria can acclimatise to high FNA concentration (Anthonisen *et al.*, 1976; Zhou *et al.*, 2011; Kouba *et al.*, 2014). However, the reported ranges for inhibition are lower and narrower for FNA than FA, 0.2-2.6 mg L^{-1} (Anthonisen *et al.*, 1976; Udert *et al.*, 2003b; Sun *et al.*, 2012). Though, other studies show inhibition at higher concentrations 0.34-8.4 mg L^{-1} (Johansson & Hellström, 1999) or 50% inhibition at 4.33 mg L^{-1} (Claros *et al.*, 2013)

3.3.3 Hydroxylamine

During ammonia oxidation the intermediate hydroxylamine (NH_2OH) is formed. Under operating conditions with low DO and high pH and ammonia concentrations, it accumulates in the system (Stüven *et al.*, 1992). Hydroxylamine is toxic to *Nitrobacter* and the inhibition is irreversible (Fureman, 2000). Strüven *et al.* (1992) proposed that NOB are inhibited by the intermediate rather than FA. In addition to the inhibitory effects, hydroxylamine can also enhance emissions of nitrous acid (N_2O), which is greenhouse gas harmful for the environment (Sabba *et al.*, 2015).

3.3.4 Salinity

Nitrifying bacteria gain little energy from oxidising nitrogen compounds which make them sensitive to high salt concentrations (Oren, 2011). Salinity increases the osmotic pressure of the cells and demands more maintenance energy which inhibits for example *Nitrosomonas europaea* (Hunik *et al.*, 1992). Nitrifiers can acclimatise to increasing salt concentrations (Campos *et al.*, 2002). *Nitrobacter* sp. and *N. europaea* are selected in high saline environments (Moussa *et al.*, 2006). However, if the inhibition is too severe addition of potassium release the osmotic pressure and the nitrification recovers (Jin *et al.*, 2007). NOB are more sensitive to salt than AOB (Jin *et al.*, 2007). Salinity concentrations of 10-15 g L^{-1} strongly decrease nitrification and at 20-25 g L^{-1} there is almost no activity (Johir *et al.*, 2013; Muys, 2014).

4 Operational options

4.1 Reactor models

Nitrification processes at WWTPs often use suspended biomass in activated sludge reactors, operated as several continuously stirred tank reactors (CSTRs) in series. The process demands low maintenance but is in turn sensitive to load variations and inhibition, and require large reactor volumes. In order to minimise the reactor volumes the biomass can be retained providing higher cell concentrations. Membrane bioreactors (MBRs) separate effluent solution and biomass with membranes. Another approach is fixed-film growth systems where the biomass is attached to carriers in a biofilm. Packed-bed bioreactors support biofilm growth on static packing material such as porous ceramic rings (Feng *et al.*, 2008) while moving bed bioreactors (MBBRs) have plastic carriers as support which are kept in suspension by mixing or aeration.

In highly concentrated solutions such as urine, biomass in fixed-film reactors are better protected and is favoured over suspended growth (Fureman, 2000). It has been showed that MBBRs have better results than MABRs and CSTRs for urine nitrification (Udert, *et al*., 2003b; Udert & Wächter, 2012). The nitrification rates were higher and nitrogen losses lower in the MBBR. Mixing in the MBBR ensures thin biofilms desirable for the nitrification process and low COD to nitrogen ratios reduce heterotrophic growth (Udert & Wächter, 2012). CSTRs are more sensitive due to higher risk for bacterial wash-out under extreme pH values (Udert *et al.*, 2003b).

4.2 Nitrifying biofilm

Attached biomass produce extracellular polymeric substances (EPS), which, fixate the microorganisms together and to the carrier material (Flemming *et al.*, 2000). Nitrifying bacteria are slow growing organisms and doubles about once a day. AOB grow slightly faster than NOB and can therefore overgrow the NOB population creating instabilities in the nitrification process, e.g. in thin biofilms where space is limited (Piculell *et al.*, 2016). It is of great importance to maintain a balance between AOB and NOB, along with the ratio between nitrifying organisms and heterotrophic bacteria. Characterisation of nitrifying biofilm show AOB clusters mostly in the outer parts while NOB are found deeper in the biofilm close to the AOB (Schramm *et al.*, 1996)

Deep in thick biofilm the pH decreases, anaerobic zones appear and the nitrifying activity is restricted due to limited substrate diffusion (Fureman, 2000; Piculell, 2016). Oxygen and ammonia is believed to penetrate biofilms up to 100-200 µm completely (Uhlmann, 2014; Alloul, 2015). However, Piculell (2016) has investigated biofilm thicknesses in a new model of carriers in relation to process performance. Z-carriers have been developed in a saddle shape to enhance mixing with grid-heights ranging from 50-500 µm. When reactors are operated with strong mixing the carriers collide and maintain the biofilm thickness to approximately the grid height. In solutions such as urine where ammonia concentrations are high and COD is low biofilm thinner than 300 µm have showed uneven growth of AOB and NOB where the NOB where outcompeted (Piculell, 2016).

Systems with highly concentrated solutions generally have low influent flow resulting in high hydraulic retention times (HRTs) in order not to overload the process. High HRT allow

growth of suspended biomass in MBBR processes, which, can increase the nitrifying capacity. In addition to biomass growth, detachment from the biofilm carriers contributes to the suspended biomass in the reactor. Evaluation of suspended solids in reactor solution determines the biomass but cannot differentiate active and dead cells.

4.3 Urine stabilisation by nitrification

There are numerous studies conducted with nitrification of source-separated urine. Processes with addition of alkalinity and full nitrification have been evaluated at varying operating conditions and results (Johansson & Hellström, 1999; Fureman, 2000; Gódia *et al.*, 2002; Feng *et al.*, 2008; Chen, 2009; Florin, 2013; Anabi, 2014; Muys, 2014; Uhlmann, 2014). At Eawag Research Institute, reactors with and without additions of chemicals have been evaluated for many years. In the last few years a pilot plant has been operated without addition of alkalinity in parallel with lab-scale experiements (Udert *et al.*, 2003b; Udert & Wächter, 2012; Etter *et al.*, 2013; Etter *et al.*, 2016; Uhlmann, 2014; Etter *et al.*, 2015; Fumasoli *et al.*, 2016). Reactors with partial nitrification have also been studied outside Eawag (Johansson & Hellström, 1999; Feng *et al.*, 2008; Sun *et al.*, 2012; Olofsson, 2016). Gathered results from some studies are presented in Table 4.1.

Processes for nitrification of source-separated urine are often affected negatively by nitrite accumulation. The literature reveals how all processes have transient or continuous problems with repressed NOB resulting in accumulation of nitrite. High nitrogen loading rates increase the stress on the biomass and the risk of instabilities. However, nitrite accumulation cannot solely be explained by high loads, Table 4.1. It is more likely pH, temperature and urine concentration have greater importance since it influence growth rate and the microbial interplay. This is favourable for reactors in larger scale, since they often are operated outside at lower temperatures in contrast to the lab scale experiments performed indoors. However, the nitrification rate decreases with temperature, which, in turn decreases the loading rate. High loading rates are desirable because it decreases the required reactor sizes.

4.4 Start-up

Urine has high concentrations of nutrients and salts which can cause inhibition and malfunction in conventional nitrifying biomass. The start-up of a process is therefore not as straight forward as nitrification processes as WWTPs. Various approaches has been reported (Udert *et al.*, 2003b; Anabi, 2009; Chen, 2009; Muys, 2014; Alloul, 2015; Fumasoli *et al.*, 2016; Olofsson, 2016). It is important to initially select a nitrifying biomass with high population diversity. Adaptation of sludge or biofilm from nitrifying WWTP is proposed to be the best approach (Moussa *et al.*, 2006; Alloul, 2015). Gradual increase of nitrogen concentration and load allow the biomass to acclimate to the composition of urine and a rather fast start-up phase (Hunik *et al.*, 1994; Gódia *et al.*, 2002; Muys, 2014). Udert and colleagues (2003b) increased the influent ammonia concentration from 1700 mg N L^{-1} to 7100 mg N L^{-1} successfully over the course of 70 days. The urine was enriched with ammonia to reach high nitrogen concentrations. However, the microbial communities from domestic WWTP often include higher heterotrophic biomass fraction than required for urine treatment. During the start-up phase the biomass are likely to have shift in composition where heterotrophs are outnumbered by nitrifying organisms (Egli *et al.*, 2003)

Table 4.1. Urine nitrification results in literature. Experiments have operated in Moving Bed Bioreactors (MBBR), Sequential Bioreactors (SBR), Stirred Tank Reactors (CSTR) and a Membrane Aerated Bioreactor (MABR).

Reference	Process / carriers	Influent ammonia ($mgN\ L^{-1}$)	Load ($gN\ m^{-2}\ d^{-1}$)	Load ($gN\ m^{-3}\ d^{-1}$)	Nit. rate ($gN\ m^{-2}\ d^{-1}$)	Nit. rate ($gN\ m^{-3}\ d^{-1}$)	DO ($mgO_2\ L^{-1}$)	pH	Temp. (ºC)	Nitrite (%)
Udert *et al.*, 2003b	MBBR/ Kaldnes tubes	7,100[1]	3.3 NH4	750	1.7	380	3-5.2[2]	6.3	25.3±0.5	<1
	SBR	2,240		560		280[3]	<4.5[2]	6.1	24.5±0.5	51[4]
	CSTR	7,300[1]		1,580		790[3]	2.5-4[2]	6.9	30.0±0.4	50[4]
Udert & Wächter, 2012	MABR	2,390±250			0.9	134	5.5	6.2	23±2[2]	<1
	MABR	2,390±250			1.8[5]	268[5]	3.0	7.0	23±2[2]	2
Sun *et al.*, 2012	SBR	2,500		1,250			2	6.2	25	50
	MBR	1,000		500			>3	6.2	35	50
Fumasoli *et al.*, 2016	MBBR/ Kaldnes K1	1,800±140			1.0±0.2	310±50	>7	5.9±0.2	23.7±0.9	
	MBBR/ Kaldnes K1	1,790±50			2.1±0.5	640±160	>7	5.8±0.1	26.3±1	
	MBBR/ Kaldnes K1	4,100±450			0.4±0.1	120±50	>7	6.0±0.1	22.5±0.6	
Olofsson, 2016[6]	MBBR/ Z-400	1,450[7]	1.69[7]	323[7]	0.85	183	7.7-8.0	5.7	19	

[1] Ammonia enriched urine
[2] Entire experimental period
[3] Nitritation rate
[4] Desired outcome
[5] 20% nitrogen loss
[6] All data not presented in report
[7] Total nitrogen

4.5 Process instabilities and counteractions

Frequent problems in nitrification processes with high nutrient concentrations are nitrite accumulation and severe pH drop, sometimes because of equipment failure. The problems often originate from imbalances in bacterial activity due to unfavourable growth conditions or inhibition. High temperatures or high concentrations of FA or FNA are undesirable for stable operation. Systems for full nitrification where base is added possess the advantage of constantly maintaining low ammonia concentrations regardless of pH and can therefore reduce the risk of nitrite accumulation due to FA inhibition of NOB (Florin, 2013).

Nitrification reactors require regular supervision to detect and minimise damage when problems occur. Foaming is a sign of increased microbial stress and can indicate instabilities in the process (Muys, 2014). Analytical tests of nitrite and monitoring of pH and DO allow further quantification. Generally, operation at low nitrogen loads provide more stable processes due to lower impact of the growth rate dynamics (Uhlmann, 2014).

4.5.1 Fluctuations in pH and load

Sudden increase of pH or nitrogen load lead to drastic intensification of AOB activity due to more available substrate. The NOB have slower response to the changes and nitrite is easily accumulated (Udert & Wächter, 2012). Fluctuations in nitrogen load can be evened out by having sufficiently large storage tanks (Etter *et al.*, 2013). More importantly is to minimise the risk of pH variations since these rapidly influence the FA and FNA concentrations by equilibria. High pH increases the FA concentration while the FNA decrease. It is believed elevated pH is more critical to the nitrification process than pH drop (Udert & Wächter 2012). Ammonia oxidisation generally decline or stops when pH fall below 6. When the pH is raised again the nitrifying activity is resumed (Udert *et al.*, 2003b). Elevated pH can be handled with reduced nitrogen load, carbon dioxide aeration or addition or hydrochloric acid (Udert & Wächter, 2012).

Rapid changes in pH are often caused by equipment failure or drastic changes in nitrogen load. Stopped inflow results in pH drop, while stampede pumps elevate the pH (Udert & Wächter, 2012). When urine load and bacterial activity is slightly unbalanced the pH drifts. Variations can be reduced by regulating the process with pH controlled influent and hence stabilising the process (Udert *et al.*, 2003b; Udert & Wächter, 2012). Though, Uhlmann (2014) reported contradictory results. Fixed-flow operation showed greater process stability than pH regulated flow while varying the temperature. This highlights the complicated microbial dynamics in urine nitrification processes. Only AOB activity lower the pH and not NOB activity. If instabilities in the microbial community arise and AOB activity is elevated, pH regulated nitrogen load can aggravate nitrite accumulation by further increasing the load to maintain the pH set-point. In fixed-flow operation the AOB activity would instead be restrained due to limited nitrogen load. The pH could on the other hand decrease and inhibit NOB with increasing FNA concentrations.

4.5.2 Nitrite accumulation

Several studies have reported nitrite accumulation after elevated pH or load (Udert & Wächter, 2012; Etter *et al.*, 2013; Uhlmann, 2014). Load increase of 10% can result in serious nitrite accumulation (Udert *et al.*, 2015). Continuous operation at higher pH (6.2 compared to 5.8) also increased the risk of nitrite build-up (Fumasoli *et al.*, 2016).

The conditions favour AOB. Nitrite concentrations around 50 mgN L^{-1}, or higher, demand instant action to prevent further increase and inhibition of the bacteria (Udert *et al.*, 2015). Reducing the nitrogen load reduce the AOB activity and may allow the NOB to oxidise the accumulated nitrite. If NOB are inhibited by high FNA concentrations, anaerobic denitrification may remove the accumulated nitrite. Udert and Wächter (2012) stopped aeration and urine influent and added acetate to remove oxidised nitrogen when 300 mgN L^{-1} of nitrite had accumulated. In five days nitrite and nitrate had been completely removed and the process could be operated normally again successfully.

To minimise damages, it is important to monitor the nitrite concentration in the process to ensure rapid counteractions. There are UV sensors available for continuous nitrite monitoring but none are suitable for the conditions in urine nitrification processes due to the high concentrations of oxidised nitrogen (Mašić *et al.*, 2015). Manual sampling is therefore required for regular analyses. Additionally, maintaining favourable operating conditions for NOB are essential to minimise the risk of microbial imbalances and nitrite accumulations.

5 Materials and methods

Lab-scale experiments were performed in a nitrifying MBBR to investigate the start-up process for urine nitrification. The reactor had been operated previously for 57 days by Olofsson (2016) as part of her master thesis. During the following 103 days the reactor faced severe process instabilities and was terminated. A second start-up was made with carriers from a different source and some minor changes in the equipment set-up. The experimental period for the second start-up was 100 days. On day 53 the process was changed from fixed-flow operation to pH regulated inflow. Figure 5.1. shows a flow sheet of the experimental design.

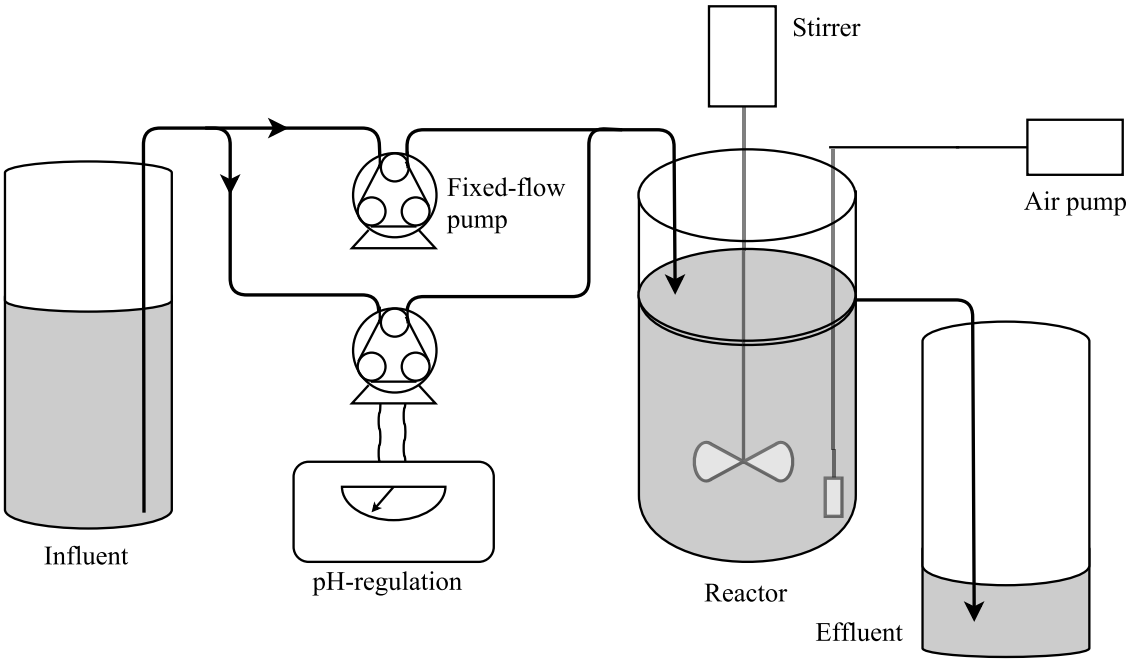

Figure 5.1. Flow sheet of the nitrification process operated with fixed-flow or pH-regulated influent.

5.1 Process equipment and set-up

The reactor for the nitrification process was a 3 L plastic beaker from VWR. Vaporisation was limited by a plastic lid. The outlet tube was placed on the side of the reactor to continuously maintain a total operating volume of 2 L, whereof 1.7 L liquid volume. Containers for inlet and outlet solutions could hold 5 or 10 L depending on the batch size. The outlet container was placed on the floor allowing gravity to facilitate outflow. The inlet container was together with most other equipment placed in a fume hood. Rubber tubes with an inner diameter of approximately 3 mm connected the containers and reactor. To further avoid vaporisation, the tubes were placed in rubber plugs on the container lids together with a needle for pressure equalisation.

During the fixed-flow period diluted urine was pumped from the inlet container by a peristaltic pump, U1-XV (S. NO. L961063), Alitea AB, Sweden. Throughout the pH regulated stage the pH was measured with EasyFerm 225 electrode from Hamilton, USA, the signal was analysed in PHM61 Laboratory pH meter and TTT60 titrator, both from Radiometer Copenhagen, Denmark, and influent was pumped with the power supply

model 0719, Mascot Electronics, Norway, and the pump GNM 2145, ENGEL GmbH, Germany. Aeration was supplied by a NEWAIR 33 air pump. The maximum air flow was 190 L h^{-1} and could be adjusted but not regulated to specific volumes. For stirring an IKA® RW16 basic was used. Biofilm support was supplied initially by 300 pieces of Z-400 carriers from Veolia Water Technologies AB. Specific surface area was approximately 190 m^2 måå$^{-3}$ in the reactor. Three probes for continuous measurements were placed through the lid of the reactor. To facilitate proper mixing a baffle was attached in the inside of the reactor covering the probes. This obstructed the carriers from getting packed in between the probes. The complete process setup is presented in Figure 5.2 and 5.3 for fixed-flow and pH regulated operation mode.

Figure 5.2. Process setup with fixed-flow influent feed. The effluent tube is placed on the right, the effluent container is not shown in the picture.

Figure 5.3. Process setup with pH-regulated influent. The effluent container is not shown in the picture.

Conductivity, DO concentration, pH and temperature were measured continuously every 30 min. The probes; conductivity (CDC401), DO (LDO101) and pH (pHC101), were from HACH LANGE AB, all logged temperature. Two HACH HQ40d multi were connected to the probes. Regularly, the probes were cleaned with distilled water.

5.1.1 Biofilm origin and carriers

The model of biofilm carriers, Z-400, are designed to maintain a thin biofilm (Piculell *et al.*, 2016). The carriers have a surface area of 1,277 mm^2 each. During the first start-up when experiments continued the work of Olofsson (2016) the carriers originated from a pilot reactor at Sjölunda WWTP in Malmö, Sweden, run by Veolia Water Technologies AB. The biofilm was used to ammonium concentrations of 10-40 mgN L^{-1} by the effluent from a high-loaded activated sludge facility. For the second start-up carriers came from a WWTP in Ulricehamn, Sweden. The nitrogen load had previously been approximately 0.8 gN $m^{-2}d^{-1}$. The nitrification rate was determined in a batch experiment at 20°C to 0.4 g $m^{-2}d^{-1}$.

5.1.2 Urine and batch preparations

One household supplied the urine for this study, mostly morning urine. It was collected 2014-2015 and stored until March 2016 indoors and since in an outdoor storage at ambient temperature in a 50 L container. During the study canisters with urine for the experiments was moved and stored at 4 °C. Dilutions during batch preparations were made with tap water, since dilution in a large scale process is likely to come from flushing or deliberate dilution.

5.1.3 Operating conditions and changed parameters

The aim of the laboratory work was to increase the nitrogen concentration in the reactor while maintaining a thriving biofilm. Initially highly diluted urine and low loads was used for start-up and re-starts. Gradually the nitrogen concentrations increased by less urine dilution. Simultaneously the load was increased and maintained between 0.7-2.7 gN $m^{-2}d^{-1}$ (130-520 gN $m^{-3}d^{-1}$) during the first start-up by regulating the inlet flow. During the second start-up the nitrogen load was regulated in relation to the trends in pH and oxygen consumption and varied from 0-1.7 g N $m^{-2}d^{-1}$ (0-320 gN $m^{-3}d^{-1}$). During the periods with fixed-flow operation the possibility to regulate the nitrogen concentration and inlet flow the nitrogen could be set rather accurately. For the duration of pH regulated inflow the nitrogen concentration and pH set-point was the operating parameters while the bacteria determined the load.

The aeration was high throughout the experiments maintaining high DO concentrations except for periods of mechanical breakdown. During the first start-up the DO was on average 7.2±1.3 mgO_2 L^{-1}, with two episodes with anoxic conditions. In the second start-up the average DO was 7.9±0.8 mgO_2 L^{-1}. These concentrations might be unnecessary high. However, it reduces the number of critical operational parameters possibly inhibiting the system. The temperature was 19.9±0.7°C and 19.8±0.5°C respectively for the start-ups. Small fluctuations were observed during working days and the temperature was stable during weekends.

5.2 Analytical methods

5.2.1 Continuous measurements

There are many aspects of the nitrification process to monitor and evaluate. By having frequent data retrieved changes in process stability could easily be detected. The pH is one of the most important parameters since it determines chemical equilibrium of the some of the

inhibitory compounds in the process as well as the bacterial activity and selectivity of organisms. Load and AOB activity determine the pH in this process. Temperature is another parameter, which affects the activity and also the solubility of compounds. Another way to monitor activity in the microbial population is the DO concentration given constant temperature and air flow. Conductivity measurements are common in the water treatment industry to monitor ionic content. In this study the ionic content is believed to be proportional to the urine concentration. The conductivity could therefore be used to monitor the increase of urine and consequently the total nitrogen content in the reactor over time.

5.2.2 Manual sampling

In addition to the continuous data, samples were taken daily during weekdays from the reactor and from every new ingoing and outgoing batch. The ingoing batch were analysed for total nitrogen, ammonium, pH and occasionally chemical oxygen demand (COD) and total phosphorus. Reactor samples were analysed to determine nitrite, nitrate, ammonium, pH and occasionally COD and total phosphorus. All concentrations were determined with test cuvettes from Hach-Lange, Berlin, Germany; Nitrite (NO_2^--N), nitrate (NO_3^--N) and ammonium (NH_4^+-N) were analysed with LCK342, LCK340 and LCK302/303 respectively. The samples were filtered before analysed (120014, Munktell, Falun, Sweden). Total nitrogen, COD and total phosphorus were analysed with LCK338, LCK714 and LCK350 respectively. Dilution of samples was made with distilled water. For manual pH measurements, a WTW pH 320 was used.

The treated outgoing solutions were used to determine suspended solids (SS). Filters (Glass microfibers filters, 691, VWR) were weight and urine samples filtered before dried for 1 h at 105°C and weight again. Each batch were analysed with triplets. Volumes of all batches and samples were noted to determine ingoing flow and vaporisation from the reactor.

5.3 Calculations

5.3.1 Flows and vaporisation

During the fixed-flow operation the flow could be regulated quite accurately by the feed pump settings. The flow was set to give a desired nitrogen load. After each batch the actual flow was calculated from volume of the used influent urine solution. When pH regulated the feed rate the average flow was determined daily by the weight difference of the ingoing container. The HRT (days) was calculated with the reactor volume 2000 mL divided by the influent flowrate. The solids retention time (SRT) (days) was calculated by the reactor volume and the effluent flowrate.

Vaporisation was calculated by the volumetric differences in ingoing and outgoing batches considering all sample volumes. Most of the volumetric losses were likely to occur due to aeration. The air was not humidified before pumped into the reactor except during the last week of the second start-up when the air bubbled through a bottle of water before entering the reactor.

5.3.2 Load and nitrification rate

Calculations were made similarly for nitrogen load and nitrification rate. The ammonium to total nitrogen ratio increased over time in the ingoing batches from approximately 0.75 to 0.99. Therefore, the nitrogen load was calculated with the total nitrogen concentration to get a more reliable result, instead of ammonium load which is used in many studies. The load can be described as the amount of nitrogen feed to the reactor per carrier area or reactor volume

and day (gN $m^{-2}d^{-1}$ or gN $m^{-3}d^{-1}$). Equations 7-8 utilises the feed flow (F), total nitrogen concentration ($C_{N\text{-}tot}$), the carrier area (A) and reactor volume (V) to determine the nitrogen load (NL_A for surface and NL_V for the volumetric load). Conversion to volumetric nitrogen load can be approximated by multiplying with a factor of 190 m^{-2}. The total area was 0.3831 m^2 for the 300 carriers. On day 50 and 53 in the second start-up 6 carriers in total was removed for future microbial analysis. The carrier area was therefore reduced to 0.3754 m^2. The nitrification rate (NR_A) was approximated with the nitrate:total nitrogen ratio in the reactor designated $R_{NO3\text{-}N/N\text{-}tot}$ in Equations 9-10.

$$NL_A = \frac{F \times C_{N-tot}}{A} \tag{7}$$

$$NL_V = \frac{F \times C_{N-tot}}{V} \tag{8}$$

$$NR_A = \frac{F \times C_{N-tot} \times R_{NO_3-N/N-tot}}{A} \tag{9}$$

$$NR_V = \frac{F \times C_{N-tot} \times R_{NO_3-N/N-tot}}{V} \tag{10}$$

5.3.3 Suspended solids

The equation to determine the SS (mg L^{-1}) concentration, Equation 11, utilises the clean filter weight (A) in mg and the weight after drying (B) as well as the sample volume (V) in mL.

$$SS = \frac{1000 \times (B - A)}{V} \tag{11}$$

5.3.4 Free ammonia and free nitrous acid concentrations

The concentration of FA was calculated by Equations 12-13 (Antonisen *et al.*, 1976; Emerson *et al.*, 1975). The total ammonium concentration denoted NH_4^+-N (mgN L^{-1}), pH and temperature (T, ºC) determine pK_a and the FA concentration (mg L^{-1}).

$$FA = \frac{17.03}{14.01} \times \frac{NH_4^+ - N}{10^{pK_a - pH} + 1} \tag{12}$$

$$pK_a = 0.09018 + \frac{2729.92}{273 + T} \tag{13}$$

The FNA concentration was calculated by Equations 14-15 (Antonisen *et al.*, 1976). The total nitrite concentration denoted NO_3^--N (mgN L^{-1}), pH and temperature (T, ºC) determine K_a and the resulting FNA concentration (mg L^{-1}).

$$FNA = \frac{46.01}{14.01} \times \frac{NO_2^- - N}{K_a \times 10^{pH}} \tag{14}$$

$$K_a = e^{-2300/(273+T)} \tag{15}$$

6 Results and Discussion

Data retrieved from continuous measurements and manual samples during the experimental period are presented in Appendix I-IV. Some additional figures are also presented in Appendix II and IV as a complement to the presented results.

6.1 Continuous operation of start-up 1

The experimental period started as a part-time project to maintain reactor operation in between the end of the lab work in Olofsson (2016) and the start of the thesis experiments. The first start-up had previously run for 57 days and continued for another 103 days. The time count was reset on day 61 to mark the transitions of projects. Shortage of time and lack of experience and knowledge in the subject resulted in an occasionally inadequate data collection. Initially, the reactor ran stabe with an influent total nitrogen concentration of 1,430 mgN L^{-1} corresponding to a 14% urine solution and a nitrogen load of 1.7 gN $m^{-2}d^{-1}$. The nitrification process produced an effluent with equal parts ammonium and nitrate and no accumulated nitrite.

For two weeks the process continued successfully. Nitrogen load had reached 2.0 gN $m^{-2}d^{-1}$ and influent nitrogen concentration of 1,700 mgN L^{-1}. However, a power-cut stopped the aeration and stirring causing the dissolved oxygen to drop. The influent was unaffected causing the pH to rise due to ceased bacterial activity. In 12 h the pH had increased from 6.4 to 8.4 and within 2 days of the power-cut the pH had reached 9.7. When the power-cut was discovered after two days, the influent was stopped while aeration and stirring was started again. The following day, 22 mgN L^{-1} nitrite had accumulated in the reactor. The nitrification was probably strongly inhibited by FA due to high pH. The process was restarted by pumping 13 L of tap water through the reactor in two hours before continued feeding with diluted urine. However, on the next day (day 19) the NOB had been repressed again causing nitrite concentrations of 109 mgN L^{-1} in the reactor. The reactor was restarted again after a few days due to shortage of time. The reactor was later restarted again with lower load and influent nitrogen concentration.

The instable operation continued with great variations in pH and DO and repeated nitrite accumulations throughout the first start-up period. The reactor was restarted 7 times in total. The reactor was emptied and filled with tap water twice during the restarts before continued feed of diluted urine again in all cases but the first. Accumulated nitrite, high pH and/or unexpected response to changed operating parameters resulted in restarts. An overview of some operating conditions is showed in Figure 6.1. Detailed graphs are presented in Appendix II.

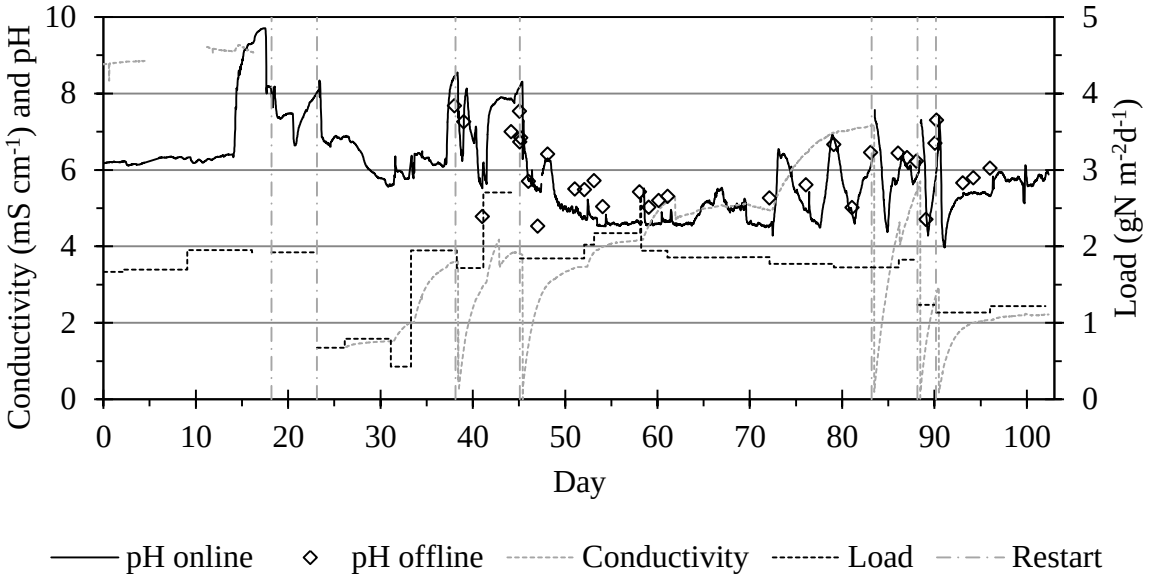

Figure 6.1. Overview of the instable reactor conditions in first start-up 1. Data from continuous measurements, manual pH and nitrogen load. Detailed figures are presented in Appendix II. Note that pH online is used to observe variations over time while pH offline is considered accurate results.

Additionally, observations after a few weeks showed deviating pH readings for the continuous reactor probe and the manual electrode, even though proper calibration. It was believed the harsh environment in the urine solution influenced the online probe negatively. However, it was kept to display the trend over time.

The influent nitrogen concentration was decreased by 32% on average (std dev 34%) after the restarts to reduce the stress on the bacteria. Simultaneously, the nitrogen load was lowered on average 24% (std dev 19%), also to allow NOB to recover without making too great load changes. The operating condition before and after each restart is displayed in table 6.1.

Table 6.1. Operating conditions before and after restarts in the first start-up. FA and FNA concentrations varied greatly due to rapid pH variations.

Reactor breakdown						**Restart**	
Day	**Influent N-tot (mg N L^{-1})**	**Load (gN $m^{-2}d^{-1}$)**	**NO_2^--N (mg N L^{-1})**	**FA (mg L^{-1})**	**FNA (mg L^{-1})**	**Influent N-tot (mg N L^{-1})**	**Load (gN $m^{-2}d^{-1}$)**
17	1650	1.92	22	703.14[1]	0.0[1]	1650	1.92
23	1704	1.92	108.8[1]	1.24[1, 2]	0.0[1, 2]	216	0.67
38	580	1.95	56.3	4.91	0.01	580	1.95
45	822	2.71	96.8	4.11	0.02	522	1.84
83	1042	1.72	187.6			1042	1.72
88	1130	1.83	289.6	0.33	1.44	461	1.32
90	461	1.23	112	0.36	0.19	280	1.14
102	297	1.22	178.4	0.04[1]	2.05[1]		

[1] Calculated from online pH results
[2] Measured on day 19

The radical changes in operating conditions caused by the restarts might have contributed to continuous fluctuations in pH and DO. Additionally, the consumption of alkalinity in the nitrification process results in a poor buffering capacity in the reactor. After the restart on day 45 the nitrogen concentration was increased slowly and the load changes kept small, the biofilm recovered to some extent and stable operation was possible for one month. Though, the pH ranged from 5.0-5.7 which is considered critically low. When the influent total nitrogen concentration was increased by 30% to 1,070 mgN L^{-1} the pH started changing rapidly despite somewhat lower load. It clearly highlighted the sensitivity of the biofilm for changes in those conditions.

Nitrite was repeatedly accumulating in the reactor, always in combination with rapid pH increase, Figure 6.2. However, not all pH shifts resulted in nitrite accumulation. Due to inadequate data sampling it is difficult to determine whether FA or FNA inhibition repressed the NOB activity causing the nitrite to accumulate. More frequent samples of ammonium, nitrite and pH would make it easier to draw conclusions. In addition, restarts made it difficult to predict or approximate the reactor conditions in the initial phase of the nitrite accumulations. One exception is the first accumulation after the power-cut two weeks into the experiments. The process had previously run steadily with a slowly increasing ammonium concentration. If approximating the ammonium concentration to 840 mgN L^{-1}, a minor pH increase could inhibit the NOB activity by the rising FA concentration. It can explain the build-up of nitrite. AOB was probably inhibited later. The free ammonia concentration probably exceeded 150 mg L^{-1} within the first day and continued to increase as long as the pH increased, up to about 680 mg L^{-1} in the reactor. Extreme FA concentration is believed to cause total inhibition of the nitrification process.

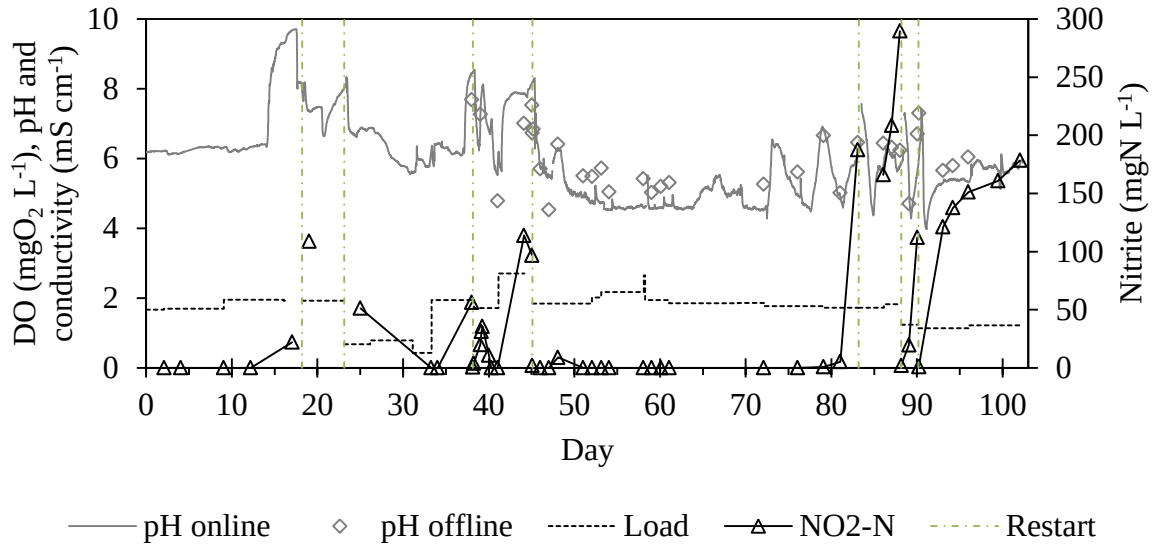

Figure 6.2. Nitrite was accumulating after some rapid changes in pH value. Note that pH online is used to observe variations over time while pH offline is considered accurate results.

In the second half of the start-up the pH oscillated greatly and nitrite accumulated repeatedly. On day 89 the FNA concentration likely passed 3.3 mg L^{-1}. Nitrite accumulated while the nitrate concentration dropped indicating severe inhibition of NOB while the AOB stayed more unaffected.

Despite instable process conditions the reactor managed to remove accumulated nitrite three times without major changes of operation parameters. The recoveries appeared a couple of

days after restarts of the process, days 23, 37 and 45. Still, the process conditions remained unsteady or harmful for the biofilm. The persistent problems lead to a decision to restart the process with new carriers and some minor setup changes.

6.2 Initial phase of start-up 2

The reactor was cleaned and some minor changes done to improve mixing before the second start-up was initiated. Carriers with nitrifying biofilm from the WWTP in Ulricehamn replaced the carriers from start-up 1. Initially, the influent was 100 times diluted urine with a total nitrogen concentration of 101 mgN L^{-1} and nitrogen load of 0.4 gN $m^{-2}d^{-1}$. The intention was to repeat the operating procedure in Olofsson (2016) in a slightly slower manner to allow adaptation of the biofilm to the changing environment. In eight days the pH had stabilised at approximately 6.4 while the load and influent nitrogen concentration had increased to 1.7 gN $m^{-2}d^{-1}$ and 520 mgN L^{-1} respectively. The operating conditions during the initial phase are presented in Figure 6.3. The desired operating pH value was 6.2 but even when the load was lower the pH did not decrease. Over the course of 37 days the total nitrogen concentration in the reactor reached 1,220 mg L^{-1}. The nitrification process was successful from the start. The nitrite concentration in the bulk never exceeded 1.5 mgN L^{-1}.

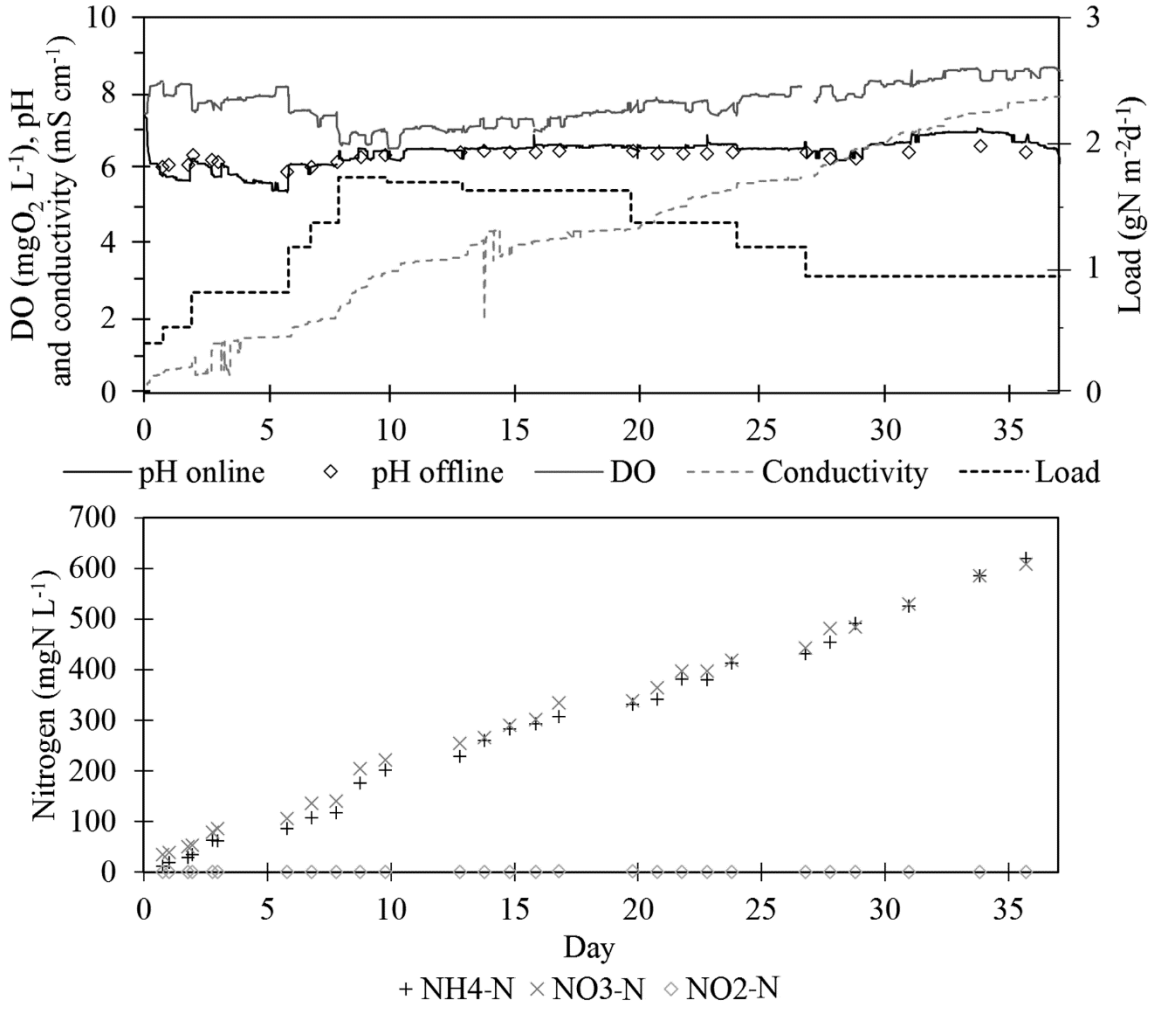

Figure 6.3. The initial 37 days of the second start-up. Above, data from continuous measurements and nitrogen load. Below, ammonium, nitrate, and nitrite concentrations.

6.3 Process instabilities in start-up 2

Between days 35-39 pH steadily declined due to an increased AOB activity. The influent nitrogen concentration was increased to 1,390 mgN L^{-1} (14% urine) on day 37. The load was by mistake lower than the previous batch which resulted in further pH decline. Ammonia oxidation stopped at pH 5.1 and pH then increased while the bacteria recuperated. Nitrite was accumulating when AOB activity excelled NOB activity. When the nitrite concentration was 121 mgN L^{-1} the load was reduced by 66%. The influent flow was low and the pump was operated in the lower range of the capacity which made it difficult to predict the flow. Overnight the flow was unintentionally too high resulting in pH of 7.6 by the morning. Even though, the nitrite concentration decreased slightly. In the following two days the influent was shut off for some hours during daytime to allow the pH to fall. Graphic display of the reactor conditions is shown in Figure 6.4. The lower load and overall pH decrease further oxidised the remaining nitrite. In two days there was no accumulation of nitrite (day 43).

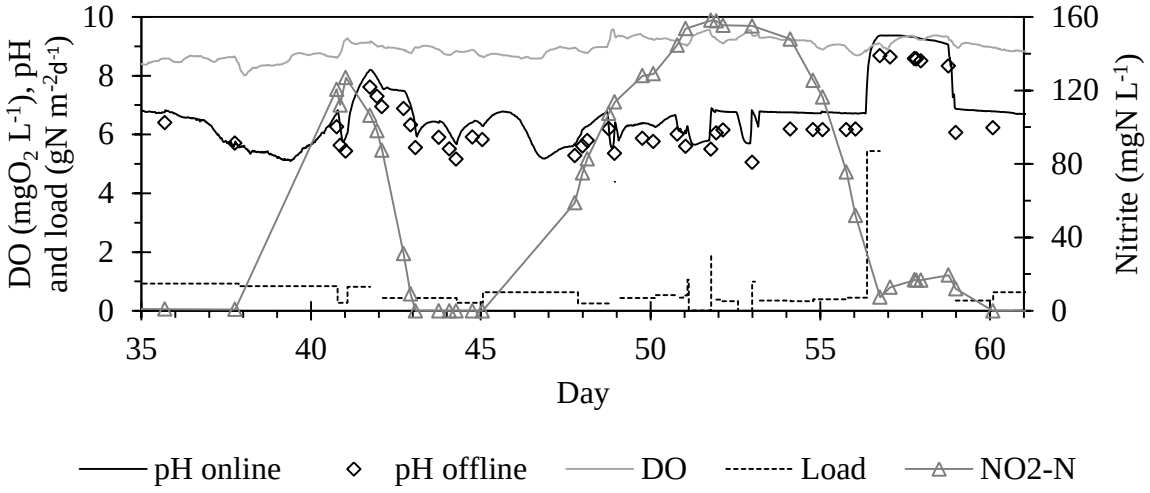

Figure 6.4. Process instabilities during the second start-up with nitrite accumulations and pH variations. The influent was turned off in short periods in between day 41-52 and entirely between day 56-59. Note that pH online is used to see the trends and variations over time while pH offline is considered accurate results.

Stable pH is the result of a balance between influent flow and AOB activity. The changes in activity was difficult to predict and in combination with the unpredictable influent pump, impossible to reach and maintain stable operation. During a weekend without supervision the pH dropped to approximately 5.1 again (day 46). The load had been increased and AOB activity increased rapidly once more, causing nitrite to build-up. In a few days the nitrite concentration reached 158 mgN L^{-1} (day 51).

The process regulation was changed in order to improve process stability. On day 51, the influent pump was replaced with a titration equipment. The influent flow was regulated to maintain a pH of 6.2 instead of maintaining fixed-flow feed. The inaccurate readings of the online pH-probe were evident, despite of regular calibration, Figure 6.4. Poor choice of settings made the influent stop twice and the pH dropped immediately due to ammonia oxidation, see day 52-53 in Figure 6.4., before the error was corrected. The accumulated nitrite started to decrease and in five days only 7.4 mgN L^{-1} nitrite was present in the reactor. In day 56 another equipment failure caused the influent to be pumped continuously and the pH was raised to 8.7 before the pump was shut off. In two days the nitrification process was

strongly inhibited. Nitrite increased to 19.4 mgN L^{-1} and pH sunk to 8.3 indicating some activity for ammonia oxidisers. Hydrochloric acid (1M) was added to the reactor to reduce the pH to 6.2 over the course of 5 h on day 59. The nitrifying activity was resumed instantly and the nitrite diminished within two days.

6.3.1 Inhibition

During stable pH conditions the FA concentration was kept below 1 mg L^{-1} and no detectable bulk concentration of nitrite made the FNA levels minimal. However, drastic and rapid changes in pH and minor variations in temperature caused the equilibria to shift and the FA and FNA concentrations varied, Figure 6.5. Biofilm instabilities caused nitrite to build-up on day 40 and 47 after pH dropped due to increased AOB activity. No samples were taken during the days prior to the accumulations which make it impossible to determine the exact start of build-up and current process conditions. The data showed accumulated nitrite (concentrations over 1.5 mg N L^{-1}) when FNA was higher than 0.6 mg L^{-1}. However, on day 54 NOB managed to decrease the accumulated nitrite concentration despite of 0.9 mg L^{-1} in FNA. It is therefore likely FNA concentration below 0.9 mg L^{-1} do not inhibit the bacteria severely. It is more likely the nitrite accumulated due to slower NOB activity increase than AOB. The fluctuating pH could also have greater negative impact on NOB.

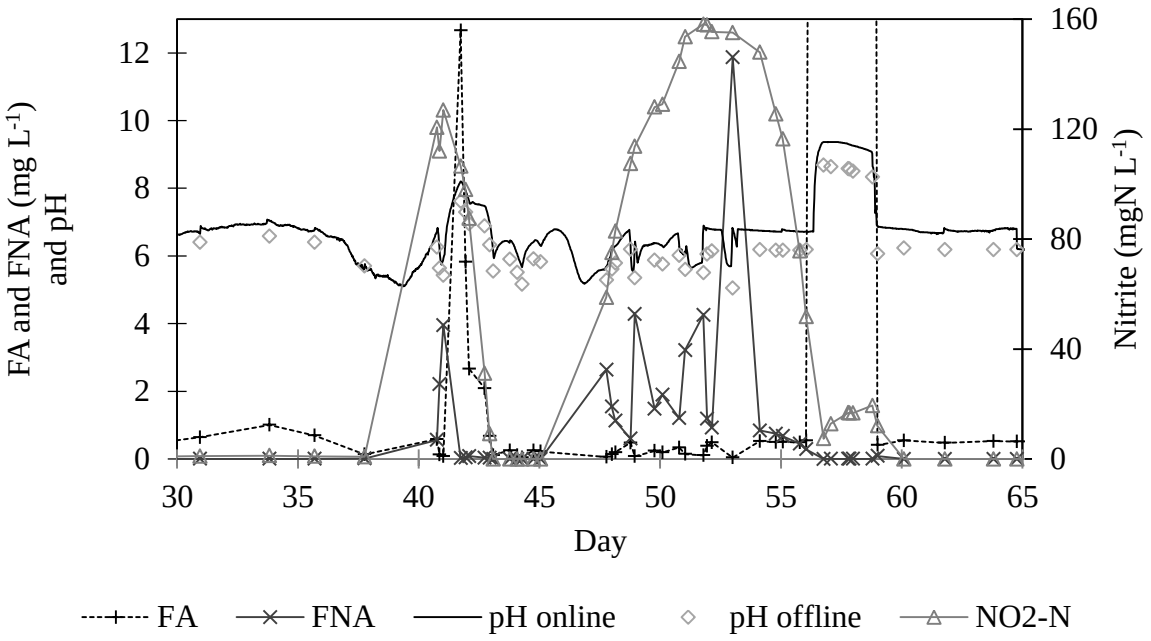

Figure 6.5. FA, FNA and nitrite concentrations during the second start-up. The slow gradual increase of FA is due to increasing nitrogen concentration in the reactor while the fast changes are results of pH and temperature changes. The FA concentration during day 56-58 was 66-151 mg L^{-1}, details are shown in Figure 6.6. Note that pH online is used to see the trends and variations over time while pH offline is considered accurate results.

During the first nitrite accumulation, on day 41, pH quickly rose from 5.4 to 7.6 and subsequently the FA concentration reached 12.7 mg L^{-1} before decreasing again. Nonetheless, the nitrite concentration decreased from 127 to 107 mgN L^{-1}. However, the high FA concentration was maintained for a very short time and could still have inhibited NOB activity. Nitrite oxidation continued steadily when the FA was 5.8 mg L^{-1}. Free ammonia concentrations of 5.8 mg L^{-1} did not considerably inhibit NOB. It is likely inhibition appear at higher concentrations. The nitrification process was strongly inhibited after the pump

malfunctioned on day 56 and FA reached 151 mg L^{-1}. For two days the influent was shut off while aeration allowed the nitrification to continue. The pH declined slightly from 8.7 to 8.3 and the resulting FA concentration decreased to 65.6 mg L^{-1}. Although the AOB activity was strongly affected, the nitrite concentration increased from 7.4 to 19.4 mg N L^{-1} indicating some activity, Figure 6.6. The high FA concentrations are known to inhibit nitrifying bacteria (Anthonisen *et al.*, 1976; Peng & Zhu, 2006; Sun *et al.*, 2012). But the repressed activity might also originate from high pH level and general enzymatic inhibition.

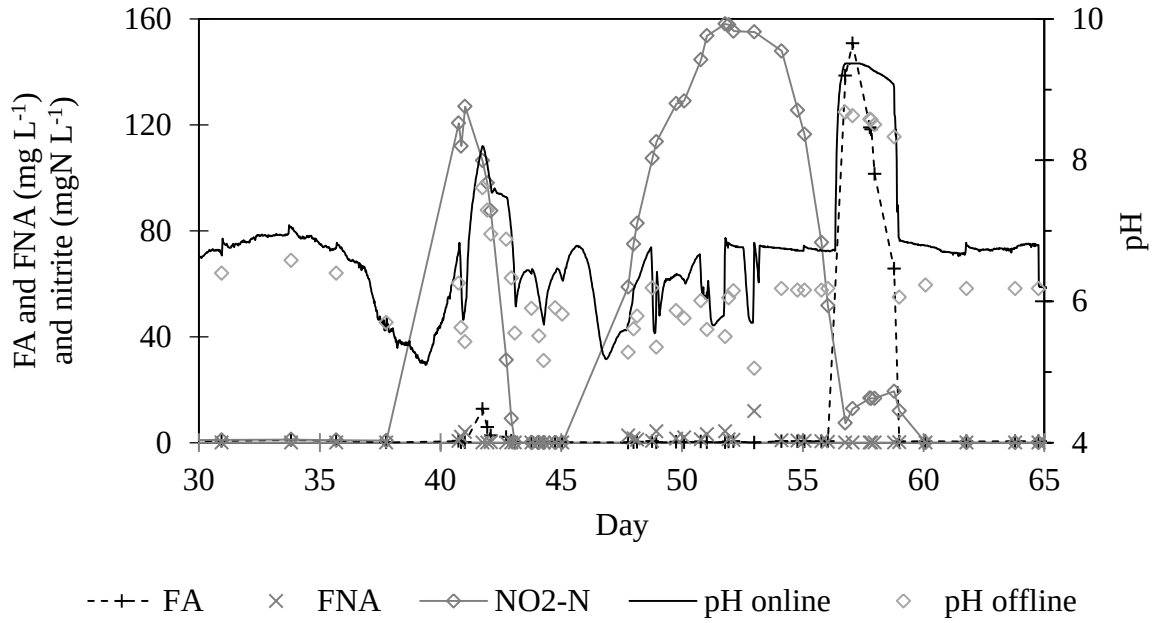

Figure 6.6. FA, FNA and nitrite concentrations in the second start-up. Changes in pH generate the variations in FA and FNA concentrations. Note that pH online is used to observe variations over time while pH offline is considered accurate results.

6.4 Process stability with pH-regulation in start-up 2

Exchanging the fixed-flow pump to a titration equipment allowed the biofilm to recover and the process stabilised with an operating pH set-point of 6.2, Figure 6.7. Between the days 60-100 the reactor operated successfully with steadily increasing concentrations of equal parts ammonium and nitrate in the effluent, nitrite remained below 1 mgN L^{-1}. The influent nitrogen concentration was further increased. Previously the urine concentration was amplified in steps of 2-3%. However, the new mode of operation allowed greater changes in influent concentration because the load was automatically regulated. During the instabilities the influent was a 14% urine solution. Increments were conducted twice to 25% and 40% urine solutions on days 63 and 77 respectively. The influent nitrogen concentrations were 2,360 mg L^{-1} and 4,100 mg L^{-1}. Concentrated urine ensured low volumetric flows and a reasonably slow concentration increase in the reactor allowing adaptation of the biofilm. By the end of the experimental period nitrate had reached 2,370 mgN L^{-1} and ammonium 2,320 mgN L^{-1} in the reactor. The total nitrogen concentration in the effluent was higher than the influent as a consequence of water losses via evaporation.

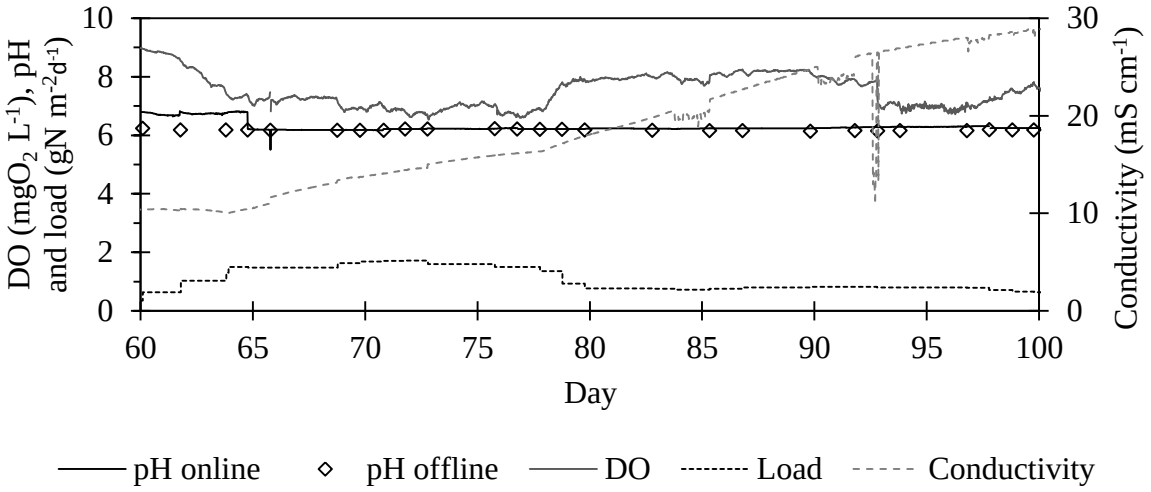

Figure 6.7. Data from continuous measurement and nitrogen load during day 60-100 with pH-regulated influent.

Measurements of pH continued to differ between the online and manual probes. A new online probe was installed on day 64, see Figure 6.7. The environment in the nitrification reactor seems rather harsh for the probes. Over time the results slowly started drifting for the online probe again. Within a month calibration was needed. Drift in readings from the pH electrode in the titration equipment was not observed during the short experimental period. However, long-time reactor operation demands regular supervision to maintain correct pH readings.

6.5 Nitrogen load and nitrification rates in start-up 2

For the first 51 days the reactor was operated with manually regulated fixed-flow influent. During that period the maximum nitrogen load was 1.7 gN $m^{-2}d^{-1}$ (330 gN $m^{-3}d^{-1}$). It was lowered with the intention to lower the pH from 6.4 to 6.2. However, the pH remained unchanged until day 30. The maximum nitrification rate during the first part of the start-up was 0.9 gN $m^{-2}d^{-1}$ (180 gN $m^{-3}d^{-1}$). The nitrification rate is about half of the nitrogen load and correlate with the ratio of ammonium and nitrate. The nitrification rates and nitrogen load throughout the experimental period can be seen in Figure 6.8.

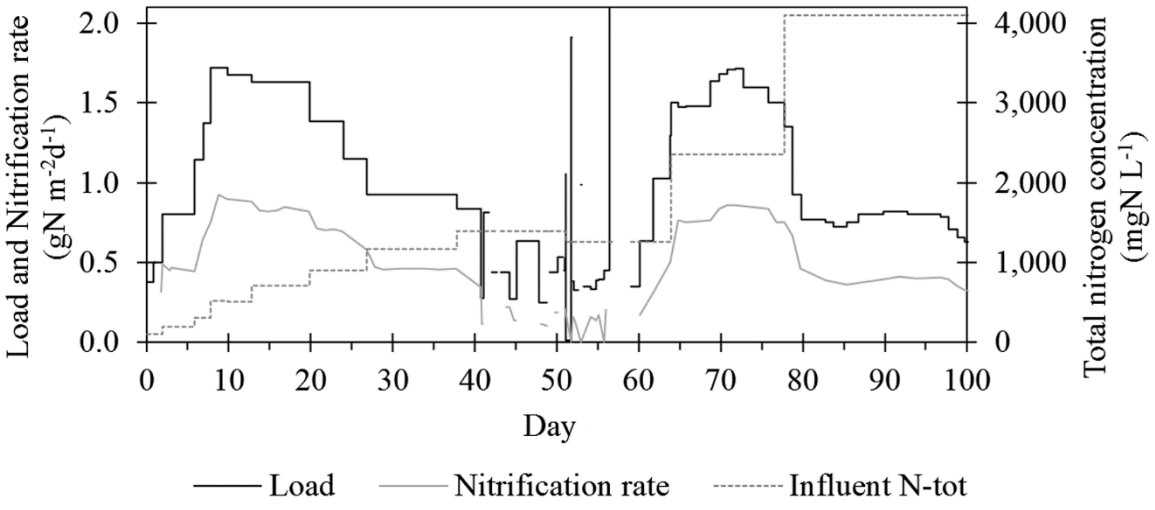

Figure 6.8. Nitrification rates, nitrogen load and influent nitrogen concentration during the second start-up. On day 54 the load was 5.4 gN $m^{-2}d^{-1}$ overnight due to equipment failure.

In the second half of the start-up the nitrogen load was regulated continuously based on AOB activity. After the process had recovered from the process instabilities the load increased along with the urine concentration. After twelve days with continuously stable pH the nitrogen load reached its maximum, 1.7 gN $m^{-2}d^{-1}$ (320 gN $m^{-3}d^{-1}$). The nitrification rate was 0.9 gN $m^{-2}d^{-1}$ (160 gN $m^{-3}d^{-1}$) documented while the total nitrogen concentration was 2,230 mgN L^{-1}. Higher nitrogen concentrations resulted in a decreased nitrogen load. By the end of the experiments the sum of ammonium, nitrate and nitrite concentrations was 4,680 mgN L^{-1} in the reactor and the load 0.6 gN $m^{-2}d^{-1}$ (120 gN $m^{-3}d^{-1}$). The nitrification rate was 0.3 gN $m^{-2}d^{-1}$ (60 gN $m^{-3}d^{-1}$). The highest peak in nitrogen load (day 56) in Figure 6.8. was not intentional. Equipment malfunctioned and excessive influent was pumped to the reactor overnight.

The nitrification rate showed a declining trend while the urine concentration increased. This pattern has also been observed in other studies (Table 4.1 in section 4.3. Urine stabilisation by nitrification). Udert and Wächter (2012) showed nitrification rates similar to the highest reported rates in this study when operated with comparable conditions (influent ammonia 2,390 mgN L^{-1}, pH 6.2, nitrification rate 0.9 gN $m^{-2}d^{-1}$ respectively 134 gN $m^{-3}d^{-1}$). The final nitrogen concentration and nitrification rate in this study are similar to the highest in Fumasoli *et al.* (2016). They reached a nitrification rate of 0.4±0.1 gN $m^{-2}d^{-1}$ corresponding to 120±50 gN $m^{-3}d^{-1}$. The results are in line with this study. The differences in volumetric rates demonstrate a low filling rate with the Z-400 carriers in this study.

Long-time adaptation to highly concentrated urine might increase the nitrification rate and improve the prospects of the process. However, in this study the urine is still diluted and the nitrogen concentration will be increased further in subsequent experiments conducted by Sweden Water Research. Whether the reactor can operate successfully with ingoing nitrogen concentrations about 10,000 mgN L^{-1} and the resulting nitrification rates, will be determined in the future. Udert and colleagues (2003b) have run a nitrification reactor with flush water diluted urine spiked with ammonia, the influent reached up to 7,300 mgN L^{-1}. However, the influence of the combination of high salinity and nitrogen in highly concentrated urine on the nitrifying bacteria is not yet known.

6.6 Nitrified urine solution in start-up 2

Although the nitrification rate was rather low during the last 20 days of operation the nitrate concentration increased steadily to the end of the experiments, Figure 6.9. The nitrate:ammonium ratio was on average 1.1±0.3, excluding the periods with nitrite accumulation (day 40-43 and 47-59). Nitrate concentration was as expected higher than ammonium due to the COD:N-tot ratio (average 1.1) which supplied alkalinity to oxidise more than 50% of the ammonium. Nitrate reached 2,370 mgN L^{-1} and ammonium 2,320 mgN L^{-1} by the end of the experiments.

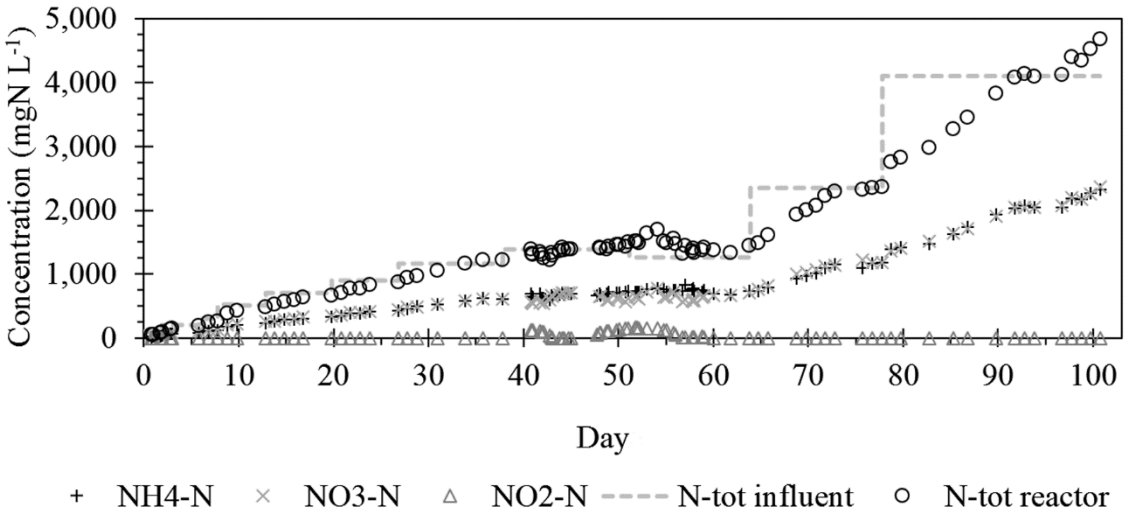

Figure 6.9. Total nitrogen, ammonium, nitrate and nitrite concentrations during the second start-up.

The total nitrogen concentration in the reactor exceeded the influent concentration on day 92. Heavy aeration with dry air caused evaporation of water and volume loss. The air flow was constant during the experiment and the overall volumetric influent flow decreased. Daily evaporation generally increased over time, Figure 6.10. The experiments were conducted during the autumn when humidity in the air indoors normally tends to decline. As a result, more water evaporates to saturate the air passing through the reactor. In combination with very low influent flows the percentile evaporation of the influent volume reached 83% between days 77-89. This caused the nitrogen compounds to concentrate in the reactor. In order to reduce the volume losses a humidifier was connected to the aeration on day 93. The results were evident, the evaporation decreased from 67% to 45%. Ammonium and nitrate concentrations in the reactor ceased to increase for a couple of days. However, the long-term consequence of the humidifier cannot yet be concluded. The evaporation increased again during the final days of the experiments.

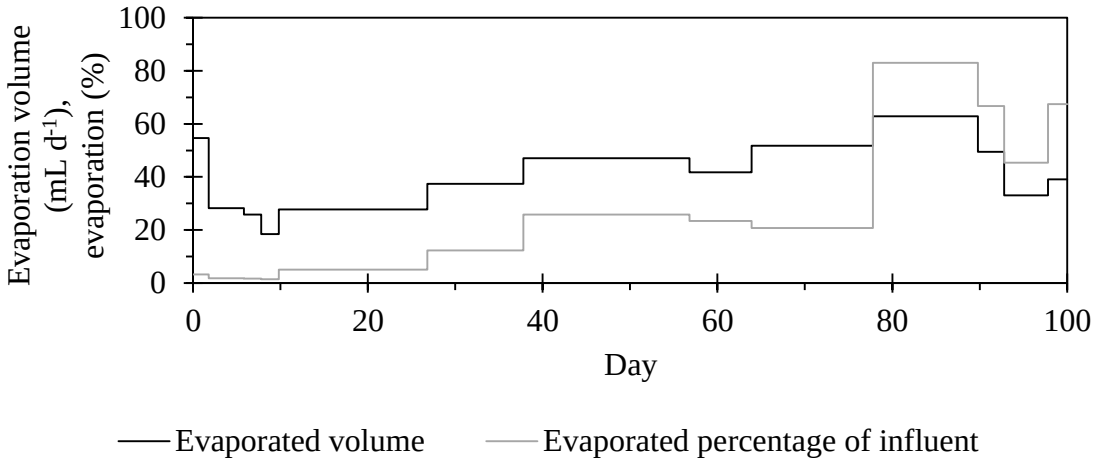

Figure 6.10. Volumetric and percentile evaporation from the reactor during start-up 2.

Nitrogen losses in the process could be estimated on seven occasions during the second start-up. Nitrogen balances were corrected for volumetric losses due to evaporation. The average nitrogen loss was estimated to 12% (std dev 5%). The trend showed a clear increase over

time, figure presented in Appendix IV. However, the nature of the start-up process makes it difficult to estimate the losses. Increasing influent concentration create dynamic concentrations in the reactor. The ingoing batches were exchanged when the reactor concentration had stabilised slightly. In some cases the reactor concentration might not have reached steady state and correlate correctly to the influent concentration. The nitrogen losses could therefore be somewhat exaggerated.

It is difficult to predict the origin of the nitrogen loss in the process. Denitrification to nitrogen gas is often discussed and requires anaerobic conditions and COD. High DO concentrations and thin biofilm make anaerobic zones very thin. Formation of nitrous oxide, N_2O, is a slightly more feasible cause. It can be produced by AOB in environments with highly concentrated ammonia. However, diverting ammonia oxidation from production of nitrite to nitrous oxide is facilitated by low DO concentrations (Sabba *et al.*, 2015). Further experiments can determine the biofilm conditions and nitrous oxide concentrations.

Removal of COD was only possible to calculate from three batches and the results differed greatly. During stable process operation before and after the nitrite accumulations the COD removal reached 77% and 87% respectively. The high pH in days 56-58 probably affected the COD reducing heterotrophic bacteria negatively as well as the nitrifying bacteria. When stable operation was regained the COD reduction was calculated to merely 29% on day 63.

On the final day of the experiments, day 100, the reactor solution contained 4,680 mgN L^{-1} nitrogen compounds, 2,020 mgO_2 L^{-1} COD and 290 mgP L^{-1} total phosphorus. Ammonium and nitrate was still increasing.

6.7 Suspended solids and hydraulic retention times

In order to maintain relatively stable nitrogen load in the reactor the volumetric inflow of urine was reduced as the influent concentration increased. Subsequently, the HRT increased from approximately 1 day at the start to maximum 35 days by the end of the experiments, apart from brief periods during the instabilities (day 37-60). The results are shown in Figure 6.11. The long retention times allow growth and accumulation of suspended biomass.

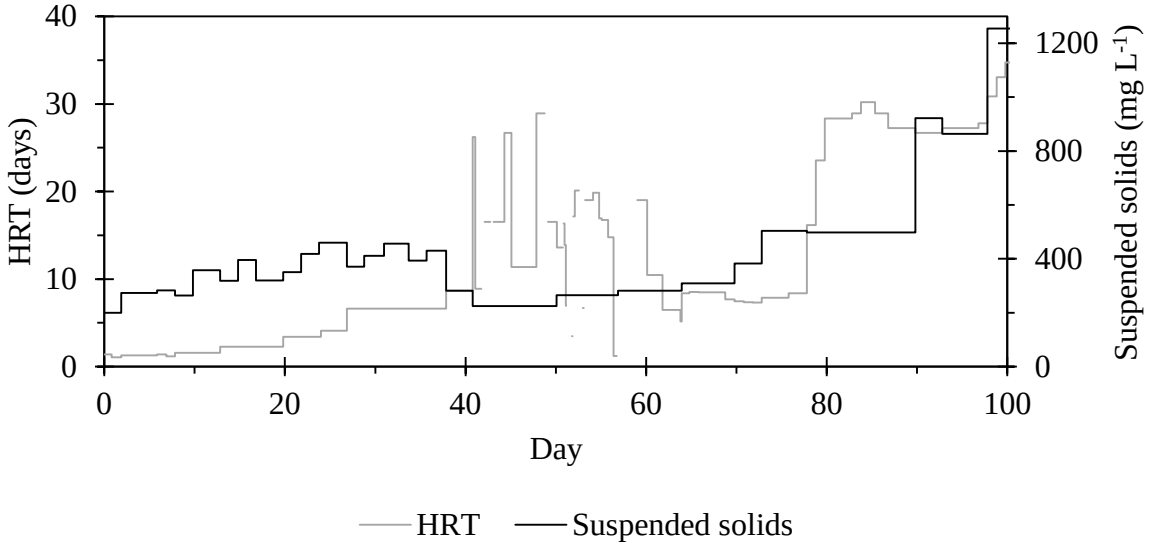

Figure 6.11. Hydraulic retention times and suspended solids during the second start-up.

Suspended solids in undiluted urine were approximately 340 mg L^{-1} and solutions containing 1-40% urine were used in the experiments. Thus the influent solids ranged between 3-140 mg L^{-1}. Generally the nitrification process has a low sludge production. However, the effluent showed high concentrations of suspended solids, 200-1,250 mg L^{-1} throughout the experiments. In the first 37 days the excess suspended solids is believed to originate from cell detachment from the biofilm. Initially the biofilm was thick and completely covered the carriers. Fifty days later only a thin biofilm remained on the carriers. The biofilm was developed in a WWTP with higher COD:nitrogen ratio compared to the urine reactor. The population of heterotrophic COD reducing bacteria were therefore expected to decline initially. It enabled a biofilm shift and allowed a boost in AOB activity and probably caused the first nitrite accumulation. The accumulating solids in the second half of the experiment is probably a combination of microbial growth and reduced effluent due to evaporation. The low influent flow and evaporation lead to an extremely low effluent flow. The SRT ranged between 53-198 days during the last 23 days of experiments. Previously the SRT was kept lower than 18 days.

The HRT allowed accumulation of suspended growth and the risk of clogging was severe. More interesting is the potential nitrifying capacity in suspension. Future analysis of bacterial activity in the biofilm and suspended biomass separately could give an indication of where the nitrification takes place. The nitrification rate would probably decrease if the suspended biomass was removed. Nevertheless, continuously accumulating biomass does not facilitate long-term process stability. To avoid clogging the suspended biomass will probably have to be reduced at some point without damaging the process too much.

6.8 Further reflections

Nitrification of highly concentrated solutions such as urine is challenging for the biofilm as well as operators. The first start-up was operated with little experience and knowledge in the subject. In combination with little supervision of the process, the reactor suffered greatly from pH instabilities and nitrite accumulations. Some of the operational mistakes were avoided in the second start-up. One typical example was prioritisation of instabilities. Initially removing accumulated nitrite was considered more important than maintaining pH stable. However, since nitrite builds up by repressed NOB activity, it is more important to sustain optimal bacterial conditions to allow maximal oxidation of nitrite. In the second start-up the emphasis was to uphold a suitable pH level.

Fixed-flow influent in the nitrification process is a suitable way to regulate the process given proper bacterial interplay. The load is set by the operator and it is possible to gradually push the biofilm to reach a desired load. The drawback arise when the ammonia oxidising activity increase. The influent has to be changed manually to compensate for the pH decrease. This has proven difficult in the experiments. First of all, the process needs close supervision to observe changes in the pH trends and take action. The true challenge is then to determine appropriate flow changes not to cause overload and further pH fluctuations. The best way to overcome the problems is to have pH regulated feed instead of fixed-flow. The process set-up itself compensate for variations in AOB activity. The balance in the microbial community is also likely to improve with pH-regulation. NOB are generally more sensitive to FA and FNA than AOB (Anthonisen *et al.*, 1976; Peng & Zhu, 2006). Fluctuations in pH are therefore more likely to have negative effects on NOB. Ensuring constant pH hence strengthen the bacterial interplay.

Both start-ups suffered from malfunctioning equipment which resulted in extreme pH increase. In the first start-up this initiated the persistent operating instabilities. It was impossible to maintain pH afterwards and nitrite was accumulating repeatedly. The problems could probably be avoided with pH-regulation as in the second start-up. The results also indicate that stable pH is more important than stable nitrogen load to maintain a functioning nitrification process.

In the second start-up, an increased AOB activity caused a drastic drop in pH and later accumulation of nitrite in the reactor. The influent nitrogen concentration was 1,390 mgN L^{-1}. The proportion of heterotrophic COD reducing bacteria was probably decreasing initially in the experiments when the substrate was limited. A shift in the biofilm population might have triggered the increase of AOB activity and accumulation of nitrite. Interestingly, Olofsson (2016) experienced nitrite at a similar influent nitrogen concentration, 1,220 mgN L^{-1}. Olofsson initiated the experimental work for the first start-up so the results come from the same reactor. The biofilm originated from an environment with low COD. Indications of increased AOB activity and a biofilm shift was not as prominent as in this study. However, future microbial analyses of collected carriers can perhaps shed some insight to if a biofilm shift contributed to the nitrite accumulation.

In the first start-up, the common pattern before accumulated nitrite was an elevated pH while the two nitrite accumulations in the second start-up showed the opposite trend. Over a weekend without supervision the pH dropped to 5.1 before the AOB activity was suppressed and the pH increased again. Frequent samples could have narrowed down the starting point and the exact operating conditions causing accumulations in both start-ups. Supervision would also prevent the pH from drifting too far. If elevated AOB activity initiated the nitrite accumulation, it is possible FNA caused further inhibition of NOB while the pH decreased. Yet, without frequent samples it is not possible to draw conclusions.

During the approximately 40 days of successful pH-regulation the reactor did not experience accumulating nitrite. However, if unbalances in the biofilm would occur and nitrite started accumulating the process set-up does not allow manual corrections of the load. It will still be determined by the AOB. Hopefully, the NOB activity will increase and manage to oxidise the nitrite over time. If there is a risk of inhibition by FA and FNA the pH set-point could be changed slightly to improve the conditions. Another alternative could be to turn off the influent and let the NOB recover and remove nitrite as long as the pH does not decrease too far. Allowing anaerobic denitrification by switching off the aeration as well is also an alternative (Udert & Wächter, 2012).

During the experiments, heavy aeration maintained high DO concentrations. Periodically even close to saturation. It was intentional to minimise the risk of oxygen limitations and hence reduce the number possible causes for process instabilities. However, the concentrations are not realistic if the process would be operated in larger scale. High DO makes the aeration ineffective and rather costly in pilot and full scale reactors. Somewhat lower DO might not have a negative effect on the nitrification.

7 Conclusion

Fluctuating pH is a significant sign of process instabilities in urine nitrification processes. Drastic changes in bacterial activity or nitrogen load can cause rapid disruptions in pH and lead to accumulation of nitrite. Though, the true initial cause of nitrite build-up has not been identified. The reactor never experienced accumulating nitrite and stable pH simultaneously.

Strategies for removing accumulated nitrite depend on the conditions in the reactor. Decreased nitrogen load and increased aeration is generally known to reduce accumulated nitrite. However, it is important to maintain favourable pH to minimise the risk of FA or FNA inhibition. This is of great importance in fixed-flow process regulation. If the reactor has pH regulated influent, the approach should be to await. The NOB activity is likely to catch up and further oxidise the accumulated nitrite over time.

Another approach to reduce nitrite was suggested by Udert and Wächter (2012), is to turn off influent and aeration and add acetate and hydrochloric acid and reduce the nitrogen concentration via denitrification. If a nitrifying reactor has reached an unhealthy high pH level, addition of hydrochloric acid can reduce it in an effective way without damaging the process. A somewhat more drastic action is to empty the reactor solution and refill with tap water. But it should be considered the last option since it is not applicable in larger scale operation which is the long-term aim for the project. Additionally, the suspended nitrifying capacity is lost if the reactor is emptied.

It is important to have favourable growth conditions for NOB to facilitate optimal biofilm interplay. Relatively low temperature and pH is desirable to strengthen NOB growth compared with AOB. However, the nitrification rate is lower in cold temperatures so a compromise has to be found to ensure process stability. Process control via pH-regulation was proven efficient for maintaining stable operating conditions. Regular supervision of the reactor allows rapid counteractions if abnormalities arise. Reliable equipment is also important to minimise the risk of mechanical breakdown causing instabilities in the nitrification process.

Continuous recording of pH, DO and conductivity provide an opportunity to monitor changes in trends and stability. In the future additional continuous measurements of nitrite could contribute to a complete online monitoring system which would reduce the manual process supervision greatly.

When designing a new reactor start-up, the biofilm should originate from a WWTP to supply a functioning biofilm with a diverse population. The urine should be highly diluted initially and increased slowly to maintain a low HRT and washout of detached biomass. Once stable operation is obtained the influent concentration can be increased in steps of 10-20%. Low volumetric flow allow the reactor concentration to increase slowly despite high influent concentration.

8 Future work

The experimental work in this thesis project initiated a reactor start-up and reached a urine concentration of 40%. Further experiments are needed to potentially use undiluted influent in the future. The impact of the suspended biomass should be evaluated and some action will probably have to be taken if the suspended solids continue to increase to avoid clogging in tubes. It might be beneficial to increase the biofilm surface area by introducing more carriers. It can improve the low volumetric nitrification rates and influent flow. Carriers have been removed from the reactor a few times and are stored at -20ºC. Microbial analysis of the carriers might highlight some additional information regarding the biofilm conditions in stable versus instable process conditions. Further experiments are also needed to determine cause of the nitrogen losses. Initially the production of nitrous oxide could be measured and samples taken in order to calculate the nitrogen mass balance and how it changes over time.

In order to improve and facilitate the process supervision an online monitoring system with an alarm function should be considered. The continuous data for pH, DO and conductivity can be sent automatically to a database and accessed remotely. Additionally, an alarm can be sent by email to an operator if the results differ from a set range. This could facilitate rapid counteractions to process instabilities. Continuous measurements of nitrite would be beneficial to determine the start and cause for nitrite accumulations. However, until an online measurement probe for nitrite is available manual samples are still needed to determine nitrite concentrations in the reactor. Work in the lab is still needed even if some data and trends can be monitored from an office but the overall work load could be reduced.

9 References

Ahlgren, S., Baky, A., Bernesson, S., Nordberg, Å., Norén, O. and Hansson, P-A., 2008. Ammonium nitrate fertiliser production based on biomass – Environmental effects from a life cycle perspective. *Bioresource Technology*, 99, pp. 8034-8041.

Ahn, Y.-H., 2006. Sustainable nitrogen elimination biotechnologies: A review. *Process Biochemistry*, 41, pp. 1709-1721.

Alloul, A., 2015. *Effect of urine dilution on the biofilm formation in a MBMBR setup*. MSc thesis. University of Ghent, Belgium.

Anabi, M.A.N., 2009. *Nitrification of human urine using a Membrane Aerated Biofilm Reactor (MABR)*. MSc thesis. UNESCO-IHE, the Netherlands.

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

Bock, E. and Wagner, M., 2013. Oxidation of inorganic nitrogen compounds as an energy source. In: E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt and F. Thompson, eds. 2013. *The Prokaryotes*, *Prokaryotic and Biochemistry*. Berlin: Springer, 2013. pp. 83-118. ISBN: 978-3-642-30141-4.

Bonvin, C., Etter, B., Udert, K.M., Frossard, E., Nanzer, S., Tamburini, F. and Oberson, A., 2015. Plant uptake of phosphorous and nitrogen recycled from synthetic source-separated urine. *AMBIO*, 44(Suppl. 2), pp. S217-S227.

Burns, L.C., Stevens, R.J., Smith R.V. and Cooper, J.E., 1995. The occurrence and possible source of nitrite in a grazed, fertilized grassland soil. *Soil Biology and Biochemistry*, 27(1), pp. 47-59.

Campos, J.L., Mosquera-Corral, A., Sánchez, M., Méndez, R. and Lema, J.M., 2002. Nitrification in saline wastewater with high ammonia concentration in an activated sludge unit. *Water research*, 36, pp 2555-2560.

Chen, Y., 2009. *Full nitrification of human urine in a sequencing batch reactor*. Thesis. The Hong Kong University of Science and Technology. Hong Kong

Claros, J., Jiménez, E., Aguado, D., Ferrer, J., Seco, A. and Serralta, J., 2013. Effect of pH and HNO2 concentration on the activity of ammonia-oxidizing bacteria in a partial nitritation reactor. *Water Science and Technology*, 67(11), pp. 2587-2594.

Dutta, S. and Vinnerås, B., 2016. Fertilizer from dried human urine added to ash and lime – a potential product from eco-sanitation system. *Water Science and Technology*, 74(6), pp. 1436-1445.

Egli, K., Langer, C., Siegrist, H-R., Zehnder, A.J.B., Wagner, M. and van det Meer, J.R., 2003. Community analysis of ammonia and nitrite oxidizers during start-up of nitritation reactors. *Applied and Environmental Microbiology*, 69(6), pp. 3213-3222.

Emerson, K., Russo, R.C., Lund, R.E. and Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. *Journal of the Fisheries Research Board of Canada*, 32, pp. 2379-2383.

Escher, B., Pronk, W., Suter, M.J-F. and Maurer, M., 2006. Monitoring the removal efficiency of pharmaceuticals and hormones in different treatment processes of source-separated urine with bioassays. *Environmental Science & Technology*, 40(16), pp. 5095-5101.

Etter, B., Hug, A., and Udert, K. M., 2013. Total nutrient recovery from urine – operation of a pilot-scale nitrification reactor. In: *WEF/IWA Conference on Nutrient Removal and Recovery*. Vancouver, Canada, 28-31 July 2013.

Etter, B., Udert, K. M and Gounden, T., (editors) 2015. VUNA Final Report. Eawag, Dübendorf, Switzerland.

Etter, B, (contact), 2016. *AURIN Naturelle*. [pdf] Available at: http://www.eawag.ch/fileadmin/Domain1/Abteilungen/eng/projekte/vuna/doc/500_mL_Etikette_-_web.pdf> [Accessed 20161020]

Etter, B., Wittmer, A., Ward, B.J., Udert, K. M., Strande, L., Larsen, T.A. and Morgenroth, E., 2016. Water Hub @ NEST: A living lab to test innovative wastewater treatment solutions. In: *13th IWA Specialized Conference on Small Water and Wastewater Systems & 5th IWA Specialized Conference on Resources-Oriented Sanitation*. Athens, Greece, 14-16 September 2016.

Feng, D., Wu, Z. and Xu, S., 2008. Nitrification of human urine for its stabilization and nutrient recycling. *Bioresource Technology*, 99, pp. 6299-6304.

Flemming, H-C., Wingender, J., Griebe, T. and Mayer, C., 2000. Physico-Chemical Properties of Biofilms. In: Evans, L.V., *Biofilms: Recient Advances in their Study and Control*. CRC Press, p. 20. ISBN 978-9058230935.

Florin, A., 2013. *Full nitrification of urine by adding a base*. MSc thesis. Swiss Fedefal Institute of Technology Zürich (ETHZ), Switzerland.

Fumasoli, A., Morgenroth, E. and Udert, K.M., 2015. Modeling the low pH limit of *Nitrosomonas eutropha* in high-strength nitrogen wastewaters. *Water Research*, 83, pp. 161-170.

Fumasoli, A., Etter, B., Sterkele, B., Morgenroth, E. and Udert, K.M., 2016. Operating a pilot-scale nitrification/distillation plant for complete nutrient recovery from urine. *Water Science & Technology*, 73(1), pp. 215-222.

Fureman, H., 2000. *Nitrifikation av humanurin* (In Swedish). MSc thesis. Luleå University of Technology, Sweden.

Garrido, J.M., van Bentum, W.A.J., van Loosdrecht, M.C.M. and Heijnen, J.J., 1997. Influence of Dissolved Oxygen Concentration on Nitrite Accumulation in a Biofilm Airlift Suspension Reactor. *Biotechnology and Bioengineering*, 53, pp. 168-178.

Gódia, F., Albiol, J., Montesinos, J.L., Pérez, J., Creus, N., Cabello, F., Mengual, X., Montras, A. and Lasseur, Ch., 2002. MELISSA: a loop of interconnected bioreactors to develop life support in Space. *Journal of Biotechnology*, 99, pp. 319-330.

Grandin, C., 2016. *Riskanalys: Faror och risker med p-piller bestående av Ethinylestradiol (EE2) och Estradiol (E2) i Helge å.* Bachelor thesis. Lund University, Sweden.

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

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M. and Heijnen, J.J., 1998. The Sharon process: an innovative method for nitrogen removal from ammonium-rich waste water. *Water Science and Technology*, 37(9), pp. 135-142.

Hellinga. C., van Loosdrecht, M.C.M. and Heijnen, J.J., 1999. Model based design of a novel process for nitrogen removal from concentrated flows. *Mathematical and Computer Modelling of Dynamical Systems*, 5(4), pp. 351-371.

Henze, M., Harremoë, P., la Cour Jansen, J. and Arvin, E., 2002. Wastewater treatment: biological and chemical processes. (3rd, ed.). Berlin Heidelberg: Springer-Verlag.

Hunik, J.H., Meiler, H.J.G. and Tramper, T., 1992. Kinetics of *Nitrosomonas europaea* at Extreme Substrate, Product and Salt Concentrations. *Applied Microbial Biotechnology*, 37, pp. 802-807.

Hunik, J.H., Tramper, T. and Wijffels, R.H., 1994. A strategy to scale up nitrification processes with immobilized cells of *Nitrosomonas europaea* and *Nitrobacter agilis*. *Bioprocess Engineering*, 11, pp. 73-82.

IFA, International Fertilizer Association, 2016a. *Humans, animal and plants need 16 nutrients to survive*. [online] Available at: <http://www.fertilizer.org/En/Knowledge_Resources/About_Fertilizers/About_Fertilizers_Home_Page.aspx?WebsiteKey=411e9724-4bda-422f-abfc-8152ed74f306&New_ContentCollectionOrganizerCommon=2#New_ContentCollectionOrganizerCommon> [Accessed 20160928]

IFA, International Fertilizer Association, 2016b. *World nitrogen fertilizer consumption*. [online] Available at: <http://www.fertilizer.org/En/Knowledge_Resources/About_Fertilizers/About_Fertilizers_Home_Page.aspx?WebsiteKey=411e9724-4bda-422f-abfc-8152ed74f306&New_ContentCollectionOrganizerCommon=2#New_ContentCollectionOrganizerCommon> [Accessed 20160928]

Jin, R-C., Zheng, P., Mahmood, Q. and Hu, B-L., 2007. Osmotic stress on nitrification in an airlift bioreactor. *Journal of Hazardous Materials*, 146, pp. 148-154.

Johansson, E. and Hellström, D., 1999. Nitrification in combination with drying as a method for treatment and volume reduction of stored human urine. In: Johansson, E. *Urine Separating Wastewater Systems: Design Experiences and Nitrogen Conservation*. Licentiate thesis. Luleå University of Technology, Sweden.

Johir, M.A.H., Vigneswaran, S., Kandasamy, J., BenAim, R. and Grasmick, A., 2013. Effect of salt concentration on membrane bioreactor (MBR) performances: Detailed organic characterization. *Desalination*, 322, pp 13-20.

Jönsson, H., Baky, A., Jeppsson, U., Hellström, D. and Kärrman, E., (2005). *Composition of urine, faeces, greywater and biowaste – for utilization in the URWARE model*. Report 2005:6. Urban Water, Chalmers, Sweden. Available at: <http://www.urbanwater.se/en/en/research-programme>

Kirchmann, H. and Pettersson, S., 2000. Human urine – Chemical composition and fertilizer use efficiency. *Fertilizer Research*, 40, pp. 149-154.

Kouba, V., Catrysse, M., Stryjova, H., Jonatova, I., Volcke, E.I.P., Svehla, P. and Bartacek, J., 2014. The impact of influent total ammonium nitrogen concentration on nitrite-oxidizing bacteria inhibition in moving bed biofilm reactor. *Water Science and Technology*, 69(6), pp. 1227–1233.

Larsen, T.A., 2015. CO_2-neutral wastewater treatment plants or robust, climate friendly wastewater management? A system perspective, *Water Research*, 87, pp. 513-521.

Lienert, J., 2016. *Blue Diversion AUTARKY – Wastewater Treatment off the Grid*. [Online] Available at: <http://www.eawag.ch/en/research/humanwelfare/wastewater/projekte/autarky/> [Accessed 20161020]

Lind, B-B., Ban, Z. and Bydén, S., 2001. Volume reduction and concentration of nutrients in human urine. *Ecological Engineering*, 16, pp. 561-566.

Liu, Q., Liu, C., Zhao, L., Ma, W., Liu, H. and Ma, J., 2016. Integrated forward osmosis-membrane distillation process for human urine treatment. *Water Research*, 91, pp. 45–54.

Malmö Stad, 2015a. *Hållbarhetsstrategi för Sege Park* (In Swedish). [pdf] Available at: <http://malmo.se/download/18.4bef0e6a14c5b4a1bc94d98f/1429257009296/5.+H%C3%A5llbarhetsstrategi++f%C3%B6r+Sege+Park.pdf#search=%27h%C3%83%C2%A5llbarhetsstrategi+f%C3%83%C2%B6r+sege+park%27> [Accessed 20161020]

Malmö Stad, 2015b. *Pp 6047, Sege Park* (In Swedish). [online] Available at: < http://malmo.se/Stadsplanering--trafik/Stadsplanering--visioner/Planprogram/Pp-6047-Sege-Park--.html> [Accessed 20161020]

Mašić, A., Santos, A.T.L., Etter, B., Udert, K.M. and Villez, K., 2015. Estimation of nitrite in source-separated nitrified urine with UV spectrophotometry. *Water Research*, 85, pp. 244-254.

Maurer, M., Pronk, W. and Larsen, T.A., 2006. Treatment processes for source-separated urine. *Water Research*, 40, pp. 3151–3166.

Moussa, M.S., Sumanasekera, D.U., Ibrahim, S.H., Lubberding, H.J., Hooijmans, C.M., Gijzen, H.J. and van Loosdrecht, M.C.M., 2006. Long term effects of salt on activity, population structure and floc characteristics in enriched bacterial cultures of nitrifiers. *Water research*, 40, pp, 1377-1388.

Munz, G., Lubello, C. and Oleszkiewicz, J.A., 2011. Factors affecting the growth rates of ammonium and nitrite oxidizing bacteria. *Chemosphere*, 83, pp. 720-725.

Muys, M., 2014. *Optimization of sustainable nitrification strategies for source separated urine*. MSc thesis. University of Ghent, Belgium.

Naturvårdsverket, 2014. *Rening av avloppsvatten i Sverige 2014* (In Swedish). [pdf]] Available at: <http://www.naturvardsverket.se/Documents/publikationer6400/978-91-620-8728-9.pdf?pid=14480> [Accessed 20161017]

Olofsson, M., 2016. *Stabilization of urine by nitrification in a Moving Bed Biofilm Reactor*. MSc thesis. Lund University, Sweden.

Oren, 2011. Thermodynamics limits to microbial life at high salt concentrations. *Environmental Microbiology*, 13(8), pp. 1908-1923.

Peng, Y. and Zhu, G., 2006. Biological nitrogen removal with nitrification and denitrification via nitrite pathway. *Applied Microbial Biotechnology*, 73, pp. 15-26.

Piculell, M., 2016. *New Dimensions of Moving Bed Biofilm Carriers*. PhD. Lund University. Sweden.

Piculell, M., Welander, P., Jönsson, K. and Welander, T., 2016. Evaluating the effect of biofilm thickness on nitrification in moving bed biofilm reactors. *Environmental Technology*, 37(6), pp. 732-743.

Randall, D. G., Krähenbühl, M., Köpping, I., Larsen, T.A. and Udert, K.M., 2016. A novel approach for stabilizing fresh urine by calcium hydroxide addition. *Water Research*, 95, pp. 361-369.

Ruiz, G., Jeison, D. and Chamy, R., 2003. Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Research*, 37, pp. 1371-1377.

Sabba, F., Picioreanu, C., Pérez, J. and Nerenberg, R., 2015. Hydroxylamine diffusion can enhance N_2O emissions in nitrifying biofilms: a modeling study. *Environmental Science & Technology*, 49(3), pp. 1486-1494.

Schramm, A., Larsen, L. H., Revsbech, N. P., Ramsing, N. B., Amann, R. and Schleifer, K.-H., 1996, Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. *Applied and Environmental Microbiology*, 62(12), pp. 4641–4647.

Statistiska centralbyrån, 2014. *Utsläpp till vatten och slamproduktion 2014*. (MI22 SM 1601). (In Swedish). [pdf] Available at:<http://www.svensktvatten.se/globalassets/fakta-om-vatten/avloppsfakta/scb-statistiskt-meddelande-sm_mi22sm1601-2014.pdf> [Accessed 20161003].

Strous, M., Heijnen, J.J., Kuenen, J.G. and Jetten, M.S.M., 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. *Applied Microbiology and Biotechnology*, 55(5), pp. 589-596.

Stüven, R., Vollmer, M. and Bock, E., 1992. The impact of organic matter on nitric oxide formation by *Nitrosomonaseuropaea*. *Archives of Microbiology*, 158, pp. 439–443.

Sun, F.Y., Dong, W.Y., Shao, M.F., Li, J. and Peng, L.Y., 2012. Stabilization of source-separated urine by biological nitrification process: treatment performance and nitrite accumulation. *Water Science & Technology*, 66(7), pp. 1491-1497.

Suzuki, I., Dular, U. and Kwok, S.C., 1974. Ammonia or ammonium ion as substrate for oxidation by *Nitrosomonas europaea* cells end extracts. *Journal of Bacteriology*, 120(1), pp. 556-558.

Tilley, E., Strande, L., Lüthi, C., Mosler, H-J., Udert, K.M., Gebauer, H. and Hering, J.G., 2014. Looking beyond technology: an integrated approach to water, sanitation and hygiene in low income countries. *Environmental Science and Technology*, 48, pp. 9965-9970.

Udert, K.M., Larsen, T.A., and Gujer, W., 2003a. Estimating the precipitation potential in urine-collecting systems. *Water Research*, 37, pp. 2667-2677.

Udert, K. M., Fux, C., Münster, M., Larsen, T. A., Siegrist, H. and Gujer, W., 2003b. Nitrification and autotrophic denitrification of source-separated urine. *Water Science and Technology*, 48(1), pp. 119–130.

Udert, K. M., Larsen, T.A., Biebow, M. and Gujer, W., 2003c. Urea hydrolysis and precipitation dynamics in a urine-collecting system. *Water Research*, 37(11), pp. 2571–2582.

Udert, K.M., Larsen, T.A., and Gujer, W., 2006. Fate of major compounds in source-separated urine. *Water Science & Technology*, 54(11), pp. 413-420.

Udert, K.M. and Wächter, M., 2012. Complete nutrient recovery from source-separated urine by nitrification and distillation. *Water Research*, 46, pp. 453–464.

Udert, K. M., Buckley, C. A., Wächter, M., McArdell, C. S., Kohn, T., Strande, L., Zöllig, H., Fumasoli, A., Oberson, A. and Etter, B., 2015. Technologies for the treatment of source-separated urine in the eThekwini Municipality. *Water SA*, 41(2), pp. 212–221.

Uhlmann, C., 2014. *Dynamics of complete and partial nitrification of Source-separated urine*. MSc thesis. Swiss Federal Institute of Technology Zurich, Switzerland.

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

Wett, B. and Rauch, W., 2003. The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. *Water Research*, 37, pp. 1100-1110.

Wilsenach J.A. and van Loosdrecht M.C.M., 2004. Effects of separate urine collection on advanced nutrient removal processes. *Environmental Science and Technology*, 38(4), pp. 1208-1215.

Yeoman, S., Stephenson, T., Lester, J.N. and Perry, R., 1988. The removal of phosphorous during wastewater treatment: A review. *Environmental Pollution*, 49(3), pp. 183-233.

Zeeman, G., Kujawa, K., de Mes, T., Hernandez, L., de Graaff, M., Abu-Ghunmi, L., Mels, A., Meulman, B., Temmink, H., Buisman, C., van Lier, J. and Lettinga, G., (2008) Anaerobic treatment as a core References 85 technology for energy, nutrients and water recovery from source-separated domestic waste(water). *Water Science and Technology*, 57(8), pp. 1207-1212.

Zeeman, G. and Kujawa-Roeleveld, K., (2011). Resource recovery from source separated domestic waste(water) streams; full scale results. *Water Science and Technology*, 64(10), pp. 1987-1992.

Zhang, J., She, Q., Chang, V. W. C., Tang, C. Y. and Webster, R. D., 2014. Mining nutrients (N, K, P) from urban source-separated urine by forward osmosis dewatering. *Environmental Science and Technology*, 48(6), pp. 3386–3394.

Zhou, Y., Oehmen, A., Lim, M., Valdivelu, V. and Ng, W.J., 2011. The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants. *Water Research*, 45, pp. 4672-4682.

10 Appendices

Appendix I: Experimental data from manual analyses during start-up 1.

Appendix II: Continuous measurement data, random pH results and calculated nitrogen load for start-up 1.

Appendix III: Experimental data from manual analyses during start-up 2.

Appendix IV: Continuous measurement data, random pH results and calculated nitrogen load for start-up 2.

Appendix V: Popular science summary (Swedish).

Appendix I

Table A1.1. Operational times and volumes for the inlet and outlet batches during the first start-up. Restarts are marked with a thick line. 'S' refers to the total sample volume taken.

Influent				**Effluent**			
Batch no.	**Time period from (day)**	**Time period to (day)**	**Volume (mL)**	**Batch no.**	**Time period from (day)**	**Time period to (day)**	**Volume (mL)**
1	0.00	2.08					
2	2.08	9.06	3,000	**1**	2.08	4.04	778
				2	4.04	9.06	1,992
				S	2.08	9.06	8
3	9.06	16.06	3,070	**3**	9.06	12.20	
				4	12.20	16.06	
4	16.06	16.36					
4	18.20	23.04					
5	23.12	26.14	3,593	**5**	23.12	24.04	1,093
				6	24.04	26.14	2,495
6	26.14	31.11		**7**	26.14	31.11	6,550
7	31.11	33.32	2,562	**8**	31.11	33.32	2,480
				S	31.11	33.32	5
8	33.32	38.06	6,215	**9**	33.32	38.04	5,780
				S	33.32	38.04	10
8	38.13	38.25	175				
9	38.25	41.15	3,873	**10**	38.25	40.03	2,240
				11	40.03	41.15	1,427
				S	38.25	41.15	44
10	41.15	44.17	3,950	**12**	41.15	44.17	3,930
				S	41.15	44.17	20
11	45.08	52.06	9,401	**13**	45.08	46.02	1,370
				14	46.02	48.07	2,670
				15	48.07	51.03	3,920
				16	51.03	52.06	1,325
				S	45.08	52.06	45
12	52.06	53.13	1,675	**17**	52.06	53.13	1,570
13	53.13	58.11	6,742	**18**	53.13	58.11	6,560
				S	52.06	58.11	36
14	58.11	58.24	165	**19**	58.24	61.06	2,510
	58.24	61.06	2,675				
				S	58.24	61.06	10
15	61.06	69.12	6,920	**20**	61.06	69.12	6,130
				S	61.06	69.12	5
16	69.12	72.12	2,575	**21**	69.12	72.12	2,340
				S	69.12	72.12	23
17	72.12	79.10	4,419	**22**	72.12	74.06	1,185
				23	74.06	76.07	1,200
				24	76.07	79.10	1,685
				S	72.12	79.10	25
18	79.10	83.15	2,572	**25**	79.10	81.03	1,180
				26	81.03	83.05	1,145
				S	79.10	83.05	8
18	83.19	86.14	1,874	**27**	83.19	86.14	
19	86.14	88.13	1,247	**28**	86.14	88.13	1,190
				S	83.19	88.13	20
20	88.16	90.10	8,942	**29**	88.16	90.10	8,639
				S	88.16	90.10	16
21	90.17	96.00	4,821	**30**	90.17	96.00	4,700
				S	90.17	96.00	18
22	96.01	102.03					

Table A1.2. Primary results from manual analysis of substance concentrations in the influent batches during start-up 1. Letters in brackets indicate the storage container origin.

Batch no. cont.	N-tot (mgN L^{-1})	NH_4^+-N (mgN L^{-1})	COD (mgO_2 L^{-1})	P-tot (mgP L^{-1})	pH	Concentration (% urine)
1 (A)	1,430	1,376	1,585	110	9.1	14
2 (A)	1,510	1,068	1,625	106	9.1	14
3 (A)	1,708	134	1,580	94	9.0	14
4 (A)	1,704	1,336	1,650	135	9.1	14
5 (A)	216	179	235	17	8.9	2
6 (A)	230	185	245	18	9.0	2
7 (B)	320[1]	280	346		9.0	3
8 (B)	534	472	580	27	8.9	5
9 (C)	513	446	556	40	9.0	5
10 (C)	791	651	822	50	9.0	7
11 (B+C)	522	417	564	36	9.1	5
12 (C)	489	372	462	30	9.1	5
13 (C+D)	615	535	672	43	9.1	6
14 (D)	783	711	870	56	9.2	8
15 (D)	822[2]	750	894		9.2	8
16 (D)	824	763	915	57	9.2	8
17 (D)	1,072	1,013	1,197	78[1]	9.2	11
18 (D)	1,042	925	1,200	78[1]	9.2	11
19 (E)	1,130	960	1,208	78[1]	9.2	11
20 (E)	461	390	500	29	9.2	5
21 (E)	280	246	302		9.1	3
22 (E)	327	289	397			3

[1] Approximated values

Table A1.3. Primary data from manual analysis of substances in the reactor for the first start-up. The drawn lines indicate restarts of the reactor.

Day	NO_2^--N (mgN L^{-1})	NO_3^--N (mgN L^{-1})	NH_4^+-N (mgN L^{-1})	pH offline	pH online	Temp (°C)
2.08	<0.6		740[1]		6.2	19.4
4.06	<0.6		760[1]		6.2	19.6
9.06	<0.6		810[1]		6.2	19.9
12.20	<0.6		840[1]		6.3	20.3
17.04	22		884[1]		9.7	19.2
19.04	109	52	115		7.4	19.0
25.03	51				6.9	19.8
33.27	<0.6		140		6.2	21.7
34.02	<0.6		155		6.4	20.0
38.02	56		236	7.7	8.6	19.0
38.12	0.6				7.6	19.9
38.26	4		0[1]		6.9	19.8
39.02	20		110[1]	7.3	8.1	19.5
39.14	31		130[1]		7.8	19.5
39.25	36	102	136[1]		7.4	19.8
40.01	11	159	177[1]		7.1	19.8
40.28	<0.6	206	185[1]		5.9	19.7
41.03	<0.6	167	206	4.8	5.7	19.7
44.13	114		251[1]	7.0	7.8	19.9
45.04	97	233	266	7.5	8.3	19.6
45.08	2	10	2	6.7	6.3	19.2
45.19				6.8	8.9	19.9
46.02	<0.6	138	110	5.7	5.7	19.7
47.03	<0.6	201	181	4.5	5.4[2]	19.9
48.08	9	217	200	6.4	6.2	20.3
51.03	<0.6	256	190	5.5	4.9	19.7
52.07	<0.6	265	238	5.5	4.7	20
53.13	<0.6	260	226	5.7[3]	4.7	20.2
54.06	<0.6	310	268	5.0	4.5	20.4
58.03	<0.6	307	252	5.4	4.6	20.2
59.03	<0.6	345	318	5.0	4.7	20.3
60.11	<0.6	378	333	5.2	4.6	20
61.09	<0.6	382	367	5.3	4.8	20.1
72.12	<0.6	378	384	5.3	4.6	20.1
76.07	<0.6	510	389	5.6	5.0	19.9
79.10	0.8			6.7	6.6	19.6
81.05	5			5.0	4.6	20.2
83.07	188			6.5	6.4	20.2
86.06	166	212	315	6.4	6.2	20.7
87.04	209		376	6.3	6.1	20.5
88.02	290		388	6.2	6.0	19.9
88.16	2				7.2	20.4
89.06	20	323	107	4.7	4.5	20.2
90.03	112	116	158	6.7	6.4	19.5
90.22	1			7.3	7.3	20.3
93.05	121	35	113	5.7	5.4	19.4
94.20	138	44	123	5.8	5.4	19.2
95.99	151	62	128	6.0	5.5	19.6
99.44	161	48	129		5.1	19.3
102.03	178	54	129		5.9	18.7

[1] Approximated value
[2] Online pH probe calibrated
[3] New offline pH probe

Appendix II

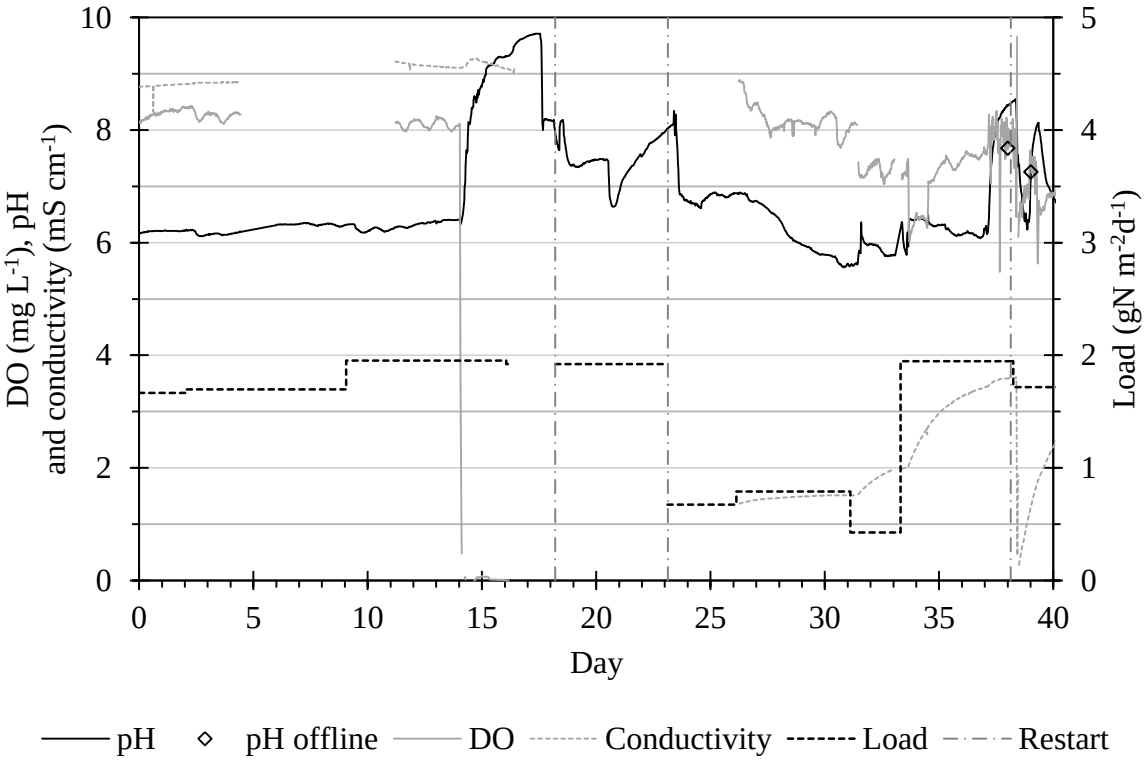

Figure A2.1. Continuous measurement data of dissolved oxygen, pH and conductivity as well as random pH measurements and calculated nitrogen load during the first 40 days in start-up 1.

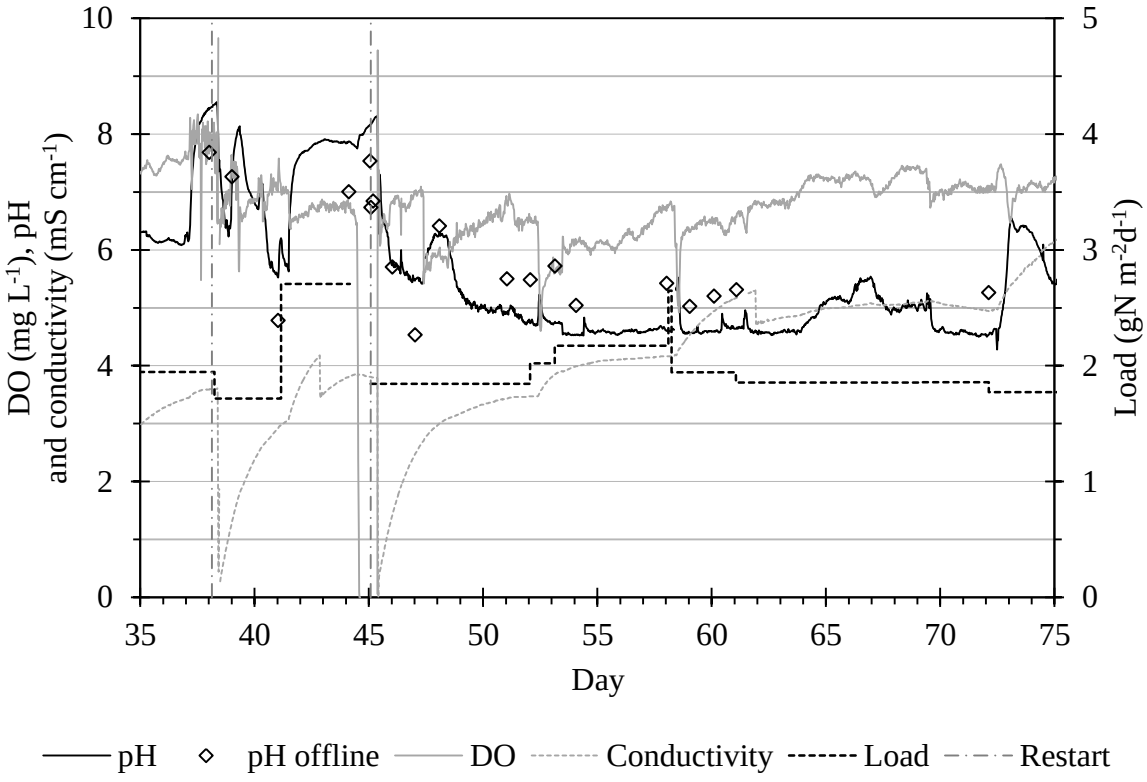

Figure A2.2. Continuous measurement data of dissolved oxygen, pH and conductivity as well as random pH measurements and calculated nitrogen load in day 35-75 in the first start-up.

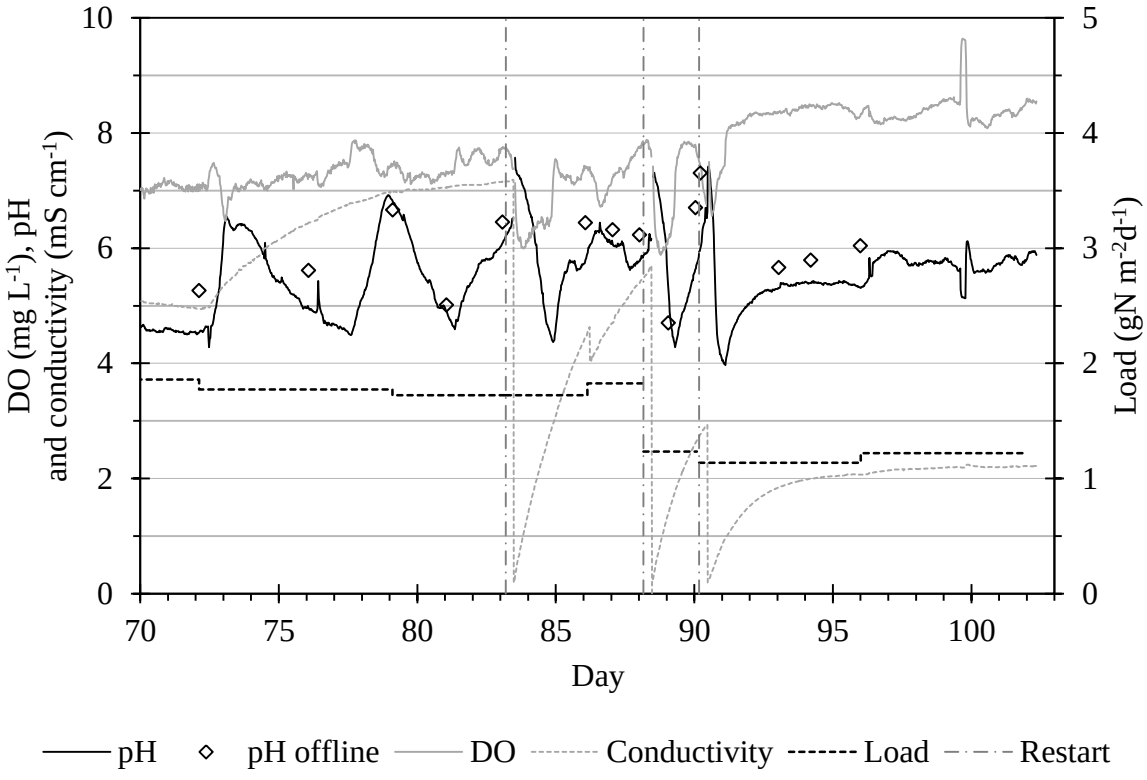

Figure A2.3. Continuous measurement data of dissolved oxygen, pH and conductivity as well as random pH measurements and calculated nitrogen load in day 70-103 in the first start-up.

Appendix III

Table A3.1. Operational times and volumes for the inlet and outlet batches during the second start-up. 'S' refers to the total sample volume taken from the reactor.

Influent				**Effluent**			
Batch no.	**Time period from (day)**	**Time period to (day)**	**Volume (mL)**	**Batch no.**	**Time period from (day)**	**Time period to (day)**	**Volume (mL)**
1	0.00	0.81	1,157	**1**	0.00	1.86	3,040
	0.81	1.86	2,000	**S**	0.00	1.86	15
2	1.86	5.84	6,220	**2**	1.86	5.84	6,065
				S	1.86	5.84	23
3	5.84	6.87	1,467	**3**	5.84	7.82	3,020
	6.87	7.82	1,618	**S**	5.84	7.82	14
4	7.82	9.82	2,549	**4**	7.82	9.82	2,485
				S	7.82	9.82	27
5	9.82	12.82	3,762	**5**	9.82	12.82	
				S	9.82	12.82	10
6	12.82	19.82	6,114	**6**	12.82	14.81	1,680
				7	14.81	16.79	1,650
				8	16.79	19.82	2,587
				S	12.82	19.82	41
7	19.82	23.98	2,462	**9**	19.82	21.79	1,090
	23.98	26.85	1,395	**10**	21.79	23.79	1,090
				11	23.79	26.85	1,450
				S	19.82	26.85	32
8	26.85	37.86	3,357	**12**	26.85	28.79	510
				13	28.79	30.98	600
				14	30.98	33.72	740
				15	33.72	35.69	517
				16	35.69	37.86	510
				S	26.85	37.86	68
9	37.86	40.78	915	**17**	37.86	40.78	805
	40.78	41.07	22	**18**	40.78	50.08	1,049
	41.07	41.74	150	**S**	37.86	50.08	100
	41.74	42.11	0				
	42.11	42.76					
	42.76	43.10	0				
	43.10	44.28	222				
	44.28	45.06	148				
[1]	45.06	47.86	630				
	47.86	48.77	249				
	48.77	48.95	0				
	48.95	48.96	15				
	48.96	49.10	0				
	49.10	50.12	123				
	50.12	50.78	96				
	50.78	50.86	0				
	50.86	50.96	13				
	50.96	51.09	18				
	51.09	51.13	11				
10	51.13	51.79	3	**19**	50.08	56.88	851
	51.79	51.84	0	**20**	56.88	63.93	780
[2]	51.94	52.11	20	**S**	51.13	63.93	110
	52.11	52.57	47				
	52.57	53.00	0				
	53.00	53.07	23				
	53.07	53.22	0				
	53.22	54.12	94				

Influent				Effluent			
Batch no. cont.	**Time period from (day)**	**Time period to (day)**	**Volume (mL)**	**Batch no.**	**Time period from (day)**	**Time period to (day)**	**Volume (mL)**
10 cont.	54.80	55.08	34				
	55.08	55.79	85				
	55.79	56.05	36				
	56.05	56.38	45				
	56.38	56.76	622				
	56.76	59.00	0				
	59.00	60.09	115				
	60.09	61.79	325				
	61.79	63.81	626				
	63.81	63.93	44				
11	63.93	64.79	206	**21**	63.93	69.80	1,054
	64.79	65.79	235	**22**	69.80	72.81	647
	65.79	68.78	706	**23**	72.81	77.78	1,001
	68.78	69.80	266	**S**	63.93	77.78	45
	69.80	70.82	275				
	70.82	71.80	266				
	71.80	72.79	272				
	72.79	75.77	758				
	75.77	77.78	480				
12	77.78	78.78	123	**24**	77.78	89.82	127
	78.78	79.77	84	**S**	77.78	89.82	28
	79.77	82.79	213				
	82.79	83.78	69				
	83.78	85.33	102				
	85.33	86.81	101				
	86.81	89.82	220				
	89.82	91.81	148	**25**	89.83	92.79	70
	91.81	92.79	73	**S**	89.83	92.79	4
	92.79	93.82	74	**26**	92.79	97.78	188
	93.82	96.79	219	**S**	92.79	97.78	12
	96.79	97.78	71				
	97.78	98.81	67	**27**	97.78	103.90	98
	98.81	99.78	58	**S**	97.78	103.90	18
	99.78	100.78	58				
	100.78	103.90	174				

[1] Inlet and outlet containers was continuously weighted to determine the volumes. Previously the volumes had been analysed directly causing greater error range.
[2] Process regulation by pH regulated inflow of urine.

Table A3.2. Primary data of analysed substances in the influent batches during start-up 2. Letters in brackets indicate the storage container origin.

Batch no.	N-tot ($mgN\ L^{-1}$)	NH_4^+-N ($mgN\ L^{-1}$)	COD ($mgO_2\ L^{-1}$)	P-tot ($mgP\ L^{-1}$)	pH	Concentration (% urine)
1 (E)	101	82			9.1	1
2 (E)	196	161			9.1	2
3 (E)	308	230			9.2	3
4 (E)	520	436			9.2	5
5 (E)	507	436	548		9.2	5
6 (E)	711	582			9.2	7
7 (F)	898	734	987		9.2	9
8 (F)	1,172	1,080		76	9.2	12
9 (F)	1,390	1,052	1,480		9.2	14
10 (F)	1,257	1,250	1,470	83	9.1	14
11 (F)	2,356	2,200	2,670		9.2	25
12 (F)	3,440	3,140	4,420		9.1	40

Table A3.3. Primary data of anlysed substances in the reactor in the second start-up.

Day	NO_2^--N (mgN L^{-1})	NO_3^--N (mgN L^{-1})	NH_4^+-N (mgN L^{-1})	pH offline	pH online	Temp (°C)	COD (mgO_2 L^{-1})	P-tot (mgP L^{-1})
0.77	<0.6	34	12	6.0	5.	19.9		
1.04	<0.6	40	19	6.1	5.8	20.4		
1.78	<0.6	50	29	6.	5.6	19.8		
1.96	<0.6	54	35	6.3	6.3	20.2		
2.77	<0.6	80	63	6.2	5.9	19.8		
3.00	<0.6	86	62	6.2	5.8	20.3		
5.78	<0.6	106	86	5.9	5.4	19.9		
6.80	<0.6	137	108	6.0	5.9	20.1		
7.80	<0.6	141	117	6.2	6.1	20.0		
8.77	0.7	204	175	6.3	6.3	20.3		
9.79	0.8	222	202	6.3	6.4	20.3		
12.79	0.9	255	229	6.4	6.4	20.1		
13.77	1.1	266	260	6.4	6.6	19.6		
14.80	1.1	290	284	6.4	6.5	20.0		
15.85	1.3	302	293	6.4	6.6	20.4		
16.80	1.3	335	308	6.4	6.7	20.0		
19.79	1.5	338	332	6.4	6.5	19.8	482	
20.78	1.2	365	342	6.4	6.5	19.8		
21.77	1.1	398	381	6.4	6.5	20.6		
22.78	1.3	398	381	6.4	6.5	20.1		
23.78	1.2	419	413	6.4	6.7	20.7		
26.78	1.2	443	432	6.4	6.5	19.7		
27.78	0.9	482	455	6.2	6.5	20.0		
28.78	0.9	484	492	6.2	6.4	21.0		
30.95	1.0	530	525	6.4	6.7	20.3		
33.82	1.2	586	585	6.6	7.0	19.3		
35.69	1.0	608	620	6.4	6.8	19.1		
37.76	0.8	614	609	5.7	5.7	18.9	490	75
40.76	120	574	689	6.3	6.7	19.7		
40.86	112	553	653	5.6	6.2	20.0		
41.02	127	539	642	5.4	5.9	20.2		
41.74	107	552	688	7.6	8.2	19.5		
41.94	98	587	623	7.3	7.9	20.2		
42.09	88	540	626	7.0	7.6	20.1		
42.73	31	600	597	6.9	7.5	19.6		
42.93	9.1	660	659	6.3	7.0	20.1		
43.08	<0.6	651	642	5.6	6.0	20.4		
43.77	<0.6	684	673	5.9	6.4	19.9		
44.08	<0.6	711	709	5.5	6.1	20.5		
44.28	<0.6	696	676	5.2	5.7	19.9		
44.76	<0.6	705	689	5.9	6.4	19.3		
45.05	<0.6	702	689	5.8	6.3	20.3		
47.77	59		666	5.3	5.7	19.5		
48.00	75	657	692	5.6	6.0	20.1		
48.14	83	651	671	5.8	6.3	20.1		
48.77	107	590	698	6.2	6.8	19.1		
48.94	114	595	722	5.4	5.6	20.0		
49.77	128	626	711	5.9	6.4	19.3		
50.09	129	621	706	5.8	6.3	19.9		
50.79	145	562	720	6.0	6.7	19.6		
51.03	154	617	736	5.6	6.1	20.4		
51.78	158	646	716	5.5	5.8	19.6		
51.94	159	638	722	6.1	6.6	19.8		
52.14	155	609	716	6.2	6.8	20.2		
53.00	155	716	766	5.1	5.1	19.3		
54.12	148	776	779	6.2	6.8	19.2		
54.79	126	637	756	6.2	6.7	19.3		

Day cont.	NO_2^--N (mgN L^{-1})	NO_3^--N (mgN L^{-1})	NH_4^+-N (mgN L^{-1})	pH offline	pH online	Temp (°C)	COD (mgO_2 L^{-1})	P-tot (mgP L^{-1})
55.07	116	642	727	6.2	6.7	20.3		
55.77	76	727	750	6.2	6.7	19.2		
56.04	52	670	753	6.2	6.7	20.0		
56.76	7.4	570	736	8.7	9.4	19.7		
57.06	13	598	830	8.6	9.4	20.7		
57.78	17	576	765	8.6	9.3	19.8		
57.84	17	612	771	8.6	9.3	19.9		
57.97	17	579	738	8.5	9.3	20.4		
58.78	19	632	718	8.3	9.1	19.6		
59.00	12	656	748	6.1	7.4	20.0		
60.09	<0.6	670	700	6.2	6.8	19.5		
61.78	<0.6	660	675	6.2	6.7	19.7		
63.80	<0.6	708	738	6.2	6.7	19.9	1500	
64.76	<0.6	756	732	6.2	6.8	19.7		
65.79	<0.6	825	792	6.2	6.2	19.6		
68.76	<0.6	999	936	6.2	6.2	19.5		
69.77	<0.6	1,026	975	6.2	6.2	20.1		
70.82	<0.6	1,064	1,012	6.2	6.2	19.6		
71.78	<0.6	1,124	1,104	6.2	6.2	20.0		
72.78	<0.6	1,144	1,152	6.2	6.2	19.8		
75.76	<0.6	1,220	1,104	6.2	6.2	19.6	1055	
76.77	<0.6	1,180	1,168	6.2	6.3	20.0		
77.77	<0.6	1,184	1,180	6.2	6.2	19.3		
78.76	<0.6	1,368	1,388	6.2	6.2	19.1		
79.77	<0.6	1,405	1,420	6.2	6.2	19.4		
82.78	<0.6	1,510	1,475	6.2	6.2	18.7		
85.32	0.7	1,638	1,626	6.2	6.2	19.4		
86.80	0.6	1,710	1,740	6.2	6.2	19.1		
89.81	0.7	1,912	1,932	6.1	6.2	19.2		
91.80	0.8	2,040	2,034	6.2	6.3	19.8		
92.81	0.8	2,051	2,079	6.1	6.3	19.6		
93.81	0.8	2,051	2,044	6.2	6.3	19.6		
96.79	0.8	2,072	2,044	6.2	6.3	19.7		
97.78	0.8	2,205	2,191	6.2	6.3	19.7		
98.80	0.8	2,177	2,170	6.2	6.3	20.1		
99.77	0.9	2,254	2,268	6.2	6.3	19.6		
100.78	0.8	2,366	2,317	6.2	6.3	19.7	2016	286

Table A3.4. Primary data of suspended solids in the effluent batches during the second start-up period.

Batch no.	Sample 1 (mg L^{-1})	Sample 2 (mg L^{-1})	Sample 3 (mg L^{-1})	Average (mg L^{-1})
1	210	190	165	200
2	290	280	250	273
3	270	280	300	283
4	250	260	280	263
5	367	340	367	358
6	347	280	327	318
7	447	400	340	396
8	287	320	353	320
9	367	347	340	351
10	420	400	433	418
11	473	427	480	460
12	367	373	373	371
13	413	400	420	411
14	500	450	420	457
15	400	390	390	393
16	450	420	420	430
17	287	267	293	282
18	213	240	220	224
19	253	267	273	264
20	327	273	247	282
21	333	307	287	309
22	327	433	387	382
23	513	513	487	504
24	493	500	500	498
25	980	967	820	922
26	1027	733	833	864
27	1233	1280	1250	1254

Appendix IV

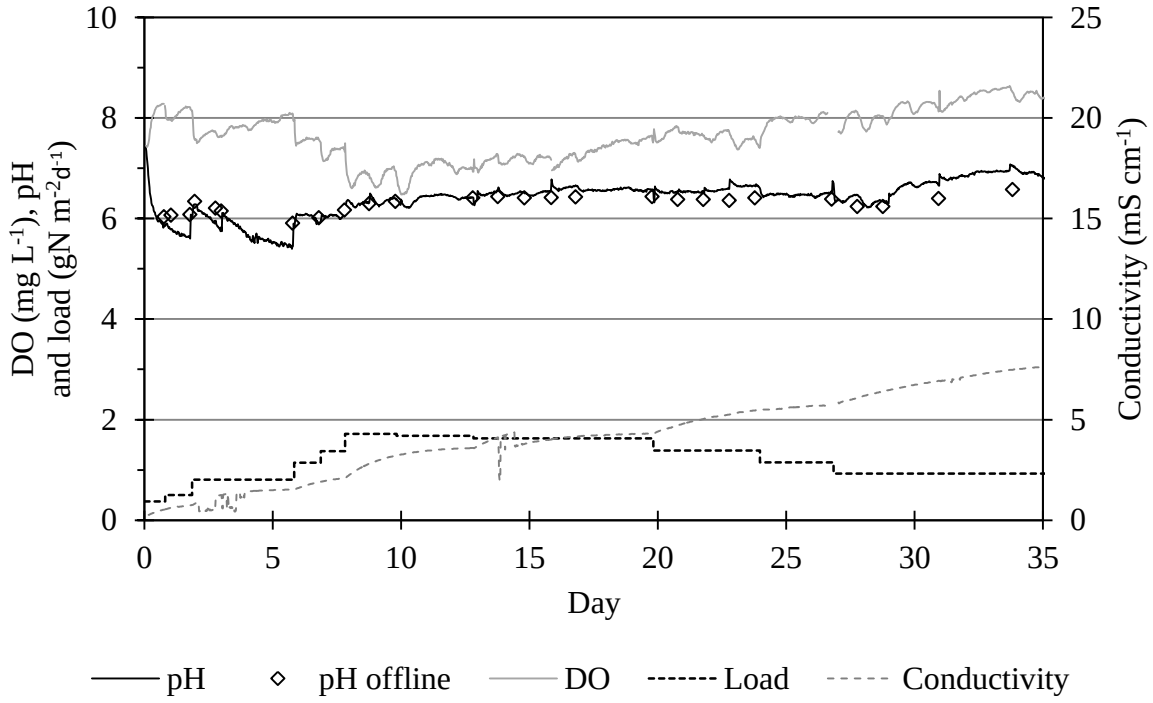

Figure 1. Continuous measurement data of DO, pH and conductivity as well as random pH measurements and calculated nitrogen load in day 0-35 in the second start-up.

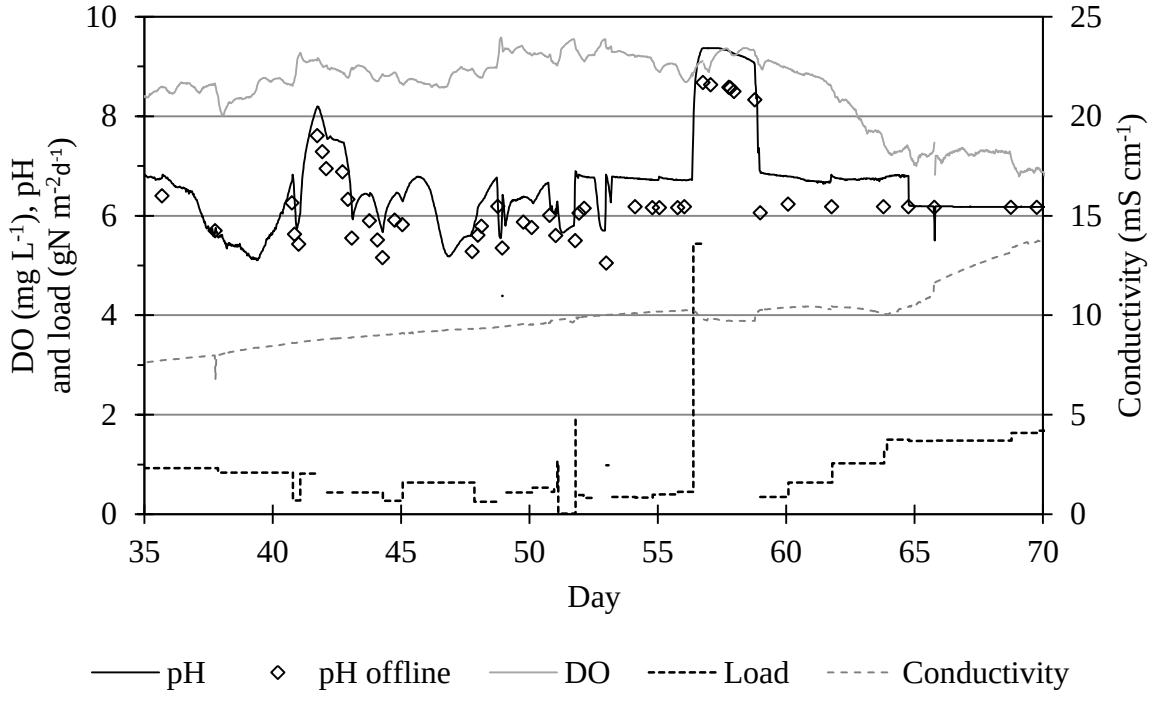

Figure A4.2. Continuous measurement data of DO, pH and conductivity as well as random pH measurements and calculated nitrogen load in day 35-70 in the second start-up.

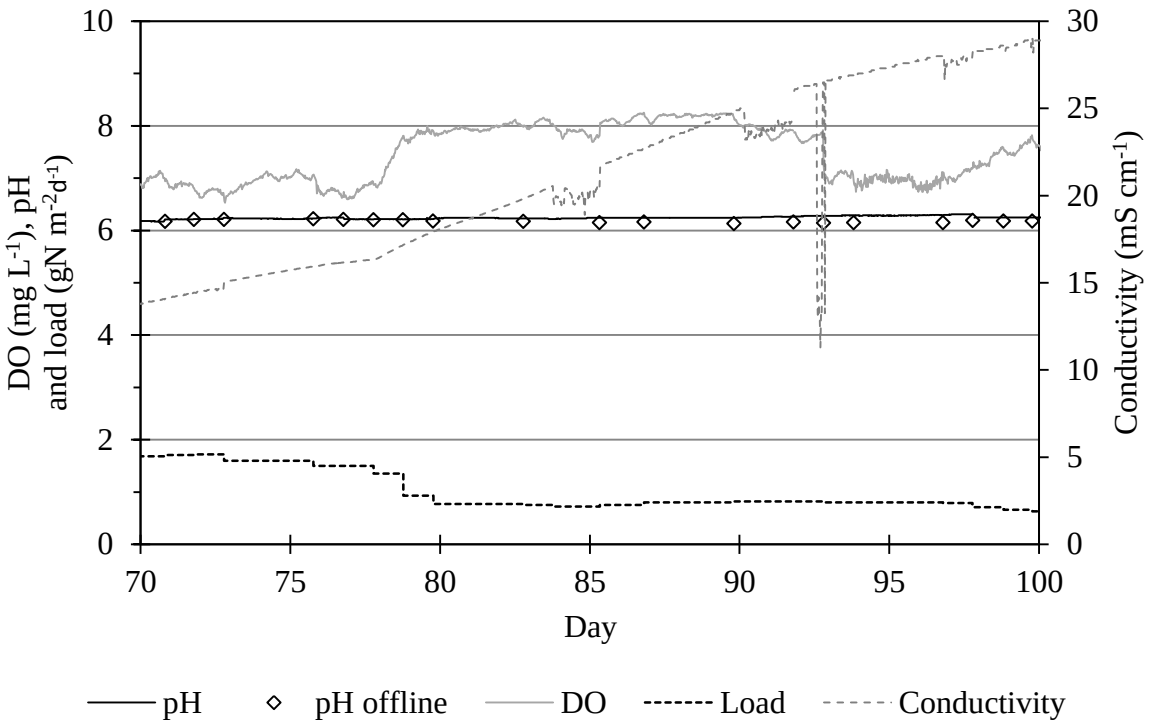

Figure A4.3. Continuous measurement data of DO, pH and conductivity as well as random pH measurements and calculated nitrogen load in day 70-135 in the second start-up.

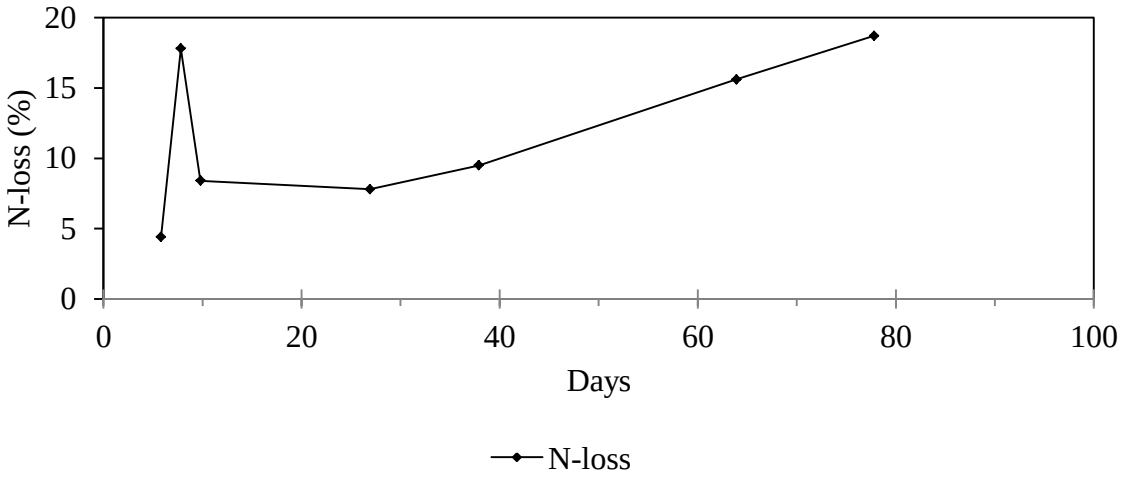

Figure A4.4. Nitrogen loss over time during the second start-up.

Appendix V

Avloppets gula guld kan sluta naturens kretslopp

Dagens jordbruk kräver tillskott av växtnäringsämnen genom gödsling. Många av dessa ämnen återfinns i avloppsvatten. Med dagens avloppshantering är möjligheterna ganska små att återanvända näringen på åkrarna. Urinsortering skapar nya vägar att sluta naturens kretslopp.

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Challenges during start-up of urine nitrification in an MBBR

Urbana områden växer och med det höjs belastningen på kommunala avloppsreningsverk. På sikt måste många verk byggas ut för att matcha behovet och möta kraven på rening. I avloppsvatten är det urinen som står för den största delen av näringsämnena. Genom att separera urinen från resterade avlopp i urinseparerande toaletter kan belastningen minska på de befintliga anläggningarna. Dessutom skapas möjligheter för vidare behandling av urinen för att kunna sluta naturens kretslopp av växtnäring.

Urin innehåller kväveföreningen urea som snabbt bryts ner till ammoniak då den lämnar kroppen. Ammoniak luktar fränt och avdunstar lätt, vilket minskar möjligheterna för en optimal kväverecirkulering. Men genom behandling kan kvävet i urinen stabiliseras så att den inte avdunstar och istället kan återföras till åkermark. I denna studien har nitrifikation studerats som en stabiliseringsmetod. Biologisk nitrifikation utnyttjar bakterier för att omvandla ammoniak till nitrat. Organismerna finns naturligt i jord och används i konventionell avloppsrening, då koncentrerade i slam eller på plastbärare.

Denna studien har undersökt uppstarten av en nitrifikationsreaktor i labbskala, med mikroorganismer på bärare från ett avloppsreningsverk. Koncentrationerna av näringsämnen är betydligt högre i urin än i kommunalt avloppsvatten. Urinen var därför kraftigt utspädd med vatten i början av uppstarten och koncentrationen höjdes långsamt för att vänja bakterierna vid de nya förhållandena.

Försöken visade att det är möjligt att behandla ganska koncentrerad urin. Men uppstarten tar tid och processen är långsam. Urinen skapar en ganska extrem miljö för mikroorganismerna. Dessutom är systemet känsligt för variationer, vid flertalet tillfällen varierade pH kraftigt och mellanprodukten nitrit ackumulerades i reaktorn. Höga nitritkoncentrationer kan ha förödande konsekvenser i processen. Genom att göra en del ändringar i regleringen av reaktorn kunde processtabiliteten öka.

I framtiden är planen att ett system för urinsortering och efterföljande behandling ska installeras i området Sege Park i Malmö. Området ska visa förslag på hur hållbar stadsutveckling kan se ut. Innan dess måste processen undersökas mer för att klargöra att nitrifikation är vägen att gå. På sikt kan det finnas möjligheter att installera liknande system i nybyggda områden eller i samband med stambyte. På det sättet minskar belastningen på de kommunala avloppsreningsverken.

En del förändringar i livsstil och infrastruktur krävs för att skapa en hållbar stadsutveckling. Genom att ta tillvara avloppets gula guld ökar möjligheterna att sluta naturens kretslopp.