Simultaneous Biological Nitrogen Removal and Electrochemical Phosphorus Recovery from Municipal Wastewater

by
Damian Jakub Kruk

A thesis submitted to the Faculty of Graduate Studies
In partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Civil Engineering
University of Manitoba
Winnipeg, Canada

To my loving wife Karolina.

For her patience and support; for giving me the joy of my life: two wonderful children; and for her impeccable timing.

Dziękuję.

ACKNOWLEDGEMENT

I owe my deepest gratitude to Professor Jan A. Oleszkiewicz, who has been my adviser and mentor. Professor Oleszkiewicz is a true educator with passion, who will not give you all the answers but rather guide you through the process of finding them. His advice and support was invaluable in both my academic and personal life.

My sincere thanks to Professor Richard Sparling for his devotion to bringing microbiology and wastewater process engineering closer together. I also express my gratitude to Dr. Keith Sears for providing real world engineering perspective.

Special thanks to my fellow students Tanner Devlin and Dr. Victor Wei, for all inspiring discussions and excellent comments.

I also want to thank Alan Froese for advice and help with qPCR, and Monika Niemczyk, Kamil Wiśniewski and Alessandro di Biase for all the help with maintenance of multiple reactors and sample analyses.

I am grateful to the staff of West End Water Pollution Control Center in Winnipeg for providing mixed liquor samples for the research, and Charles Bott and Andrea Nifong from Hampton Roads Sanitation District for sharing the anammox biomass seed.

Research was supported by Manitoba Government, Faculty of Graduate Studies, Lake Winnipeg Basin Initiative and Natural Science and Engineering Research Council.

ABSTRACT

The main objective of the study was to develop a partial nitrification/anammox electrically assisted struvite yield membrane bioreactor (PN/A EASY MBR) with simultaneous phosphorus recovery for treatment of municipal wastewater. The research was conducted in four separate experiments evaluating the feasibility of electrochemical phosphorus recovery, impact of electric current on nitrifying and anammox bacteria, and long term anammox MBR performance at low temperature.

The EASY method based on the magnesium dissolution from a sacrificial anode was found to be very effective in recovery of high-quality struvite from synthetic wastewater solutions and from the supernatant of fermented waste activated sludge from a wastewater treatment plant that does not practice enhanced biological phosphorus removal. Struvite purity was strongly dependent on the pH and the electric current density. Optimum pH of the 24 mM phosphorus and 46 mM ammonia solution (1:1.9 P:N ratio) was in the broad range between 7.5 and 9.3, with struvite purity exceeding 90%. Increasing the current density resulted in elevated struvite purity. No upper limits were observed in the studied current range of 0.05 to 0.2 A. Phosphorus removal rate was proportional to the current density. The highest P-removal rate achieved was 4.0 mg PO_4-P cm^{-2} h^{-1} at electric current density of 45 A m^{-2}. Initial substrate concentrations affected the rate of phosphorus removal. The precipitated struvite accumulated in the bulk liquid with significant portions attached to the anode surface from which regular detachment occurred.

Effects of short and long term exposure of nitrifying biomass to direct current were investigated using inert graphite electrodes. Both short and long term exposure kinetic tests indicated that the

activity of ammonia oxidizing bacteria (AOB) was slightly inhibited with almost linear decline, whereas the activity of nitrite oxidizing bacteria (NOB) was stimulated. The trends of relative activity changes exhibit a high correlation to the electric current applied per mass of volatile suspended solids (VSS), with 90% and 130% of base line activity for AOB and NOB at 20 mA g VSS^{-1}, respectively. Nevertheless, no accumulation of ammonia or nitrite was observed in the long term operation of sequencing batch reactor (SBR), achieving an average substrate utilization rate of 198 mg N g VSS^{-1} d^{-1} (both AOB and NOB), despite increase of current from 0 to 105 mA g VSS^{-1}. Magnesium electrodes were also used to prove the concept of electrochemical phosphorus precipitation in the same tank as the biological nitrification process. The highest achieved phosphorus removal rate was 22 g m^{-3} d^{-1} and XRD results indicated that phosphorus was bound in form of struvite. The average removal efficiency of 86±2% and effluent concentration of 1.9±0.2 mg P L^{-1} were achieved. No adverse effect of magnesium electrodes on nitrification was observed. The study proved that both biological and electrochemical processes can be applied simultaneously. However, to minimize the impact of electric current on the nitrification rate the direct current should not exceed 20 mA g VSS^{-1}.

Despite efforts of researchers and engineers in the past decade, low temperatures are one of the main obstacles to implementation in the main stream. In this study the impact of long-term exposure to a temperature of 10°C on the anammox process was investigated. The process was run in MBR configuration using an anammox biomass enriched under mesophilic conditions. Quantitative PCR was used to follow the population dynamics of 3 different strains of *Brocadia* throughout the time course. While populations fluctuated, the relative proportions of each were similar at the beginning and the end of the time course of 325 days, with *Brocadia fulgida* being the dominant species. Temperature affected the activity most strongly below 22°C, with an

estimated Arrhenius temperature coefficient θ of 1.24. Nevertheless, relatively high specific nitrogen removal rate of 115 mg N g VSS^{-1} d^{-1} was observed after 4 days of operation at 10°C. Results indicate that mesophilic biomass may be used in the mainstream process in moderate climates; however, prolonged operation in colder climates (i.e. ≤10°C) may lead to complete loss of anammox activity.

Anammox bacteria were exposed to electric current in range of 50 mA to 250 mA at the temperature of 34°C. No immediate impact of the electric current on anammox bacteria was observed. However, exposure longer than 12 days caused sudden process collapse. The test was duplicated using the electric current of 100 mA providing the same results. In both cases the process inhibition was reversible and anammox activity was recovered within a week from the process collapse.

Results of the investigation of the electric current impact on anammox activity provided evidence that simultaneous anammox and electrochemical processes is not a feasible approach. However the study provided a novel method of phosphorus recovery available also for non-EBPR plants, which can be applied in the mainstream process simultaneously with conventional nitrification. The study of anammox at low temperature generated valuable insights for development of mainstream anammox treatment.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GLOSSARY OF TERMS

AMX	Anammox bacteria
AOB	Aerobic ammonia oxidizing bacteria
AS	Activated sludge
bCOD	Biodegradable COD
BNR	Biological nutrient removal
BOD	Biochemical oxygen demand
DO	Dissolved oxygen
CD	Electric current density
COD	Chemical oxygen demand
EASY	Electrically assisted struvite yield
EBPR	Enhanced biological phosphorus removal
HRT	Hydraulic residence time
I	Electric current
IFAS	Integrated fixed-film activated sludge
MAP	Magnesium ammonium phosphate; struvite is a hexahydrate form of MAP
MBBR	Moving bed biofilm reactor
MBfR	Membrane biofilm reactor
MBR	Membrane bioreactor
ML	Mixed liquor
MLE	Modified Ludzack-Ettinger process
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
N	Nitrogen
NLR	Nitrogen loading rate
NOB	Nitrite oxidizing bacteria
NRMSE	Normalized root-mean-square error
NRR	Nitrogen removal rate
OHO	Ordinary heterotrophic organisms; heterotrophic

	organisms other than PAO capable of denitrification under anoxic conditions; term commonly used in wastewater treatment modeling
P	Phosphorus
PAO	Phosphorus accumulating organisms
PC	Primary clarifier
PE or p.e.	People equivalent
PHA	Polyhydroxyalkanoate
PS	Primary sludge
qPCR	Quantitative polymerase chain reaction
RAS	Return activated sludge
rbCOD	Rapidly degradable soluble COD
RSUR	Relative substrate utilization rate
SAA	Specific anammox activity
SC	Secondary clarifier
SEP	Specific electric power input
SRT	Solids residence time
SSUR	Specific substrate utilization rate
TKN	Total Kjeldahl nitrogen
TMP	Trans membrane pressure
TSS	Total suspended solids
U	Voltage
VSS	Volatile suspended solids
WAS	Waste activated sludge
WASSTRIP	WAS fermentation process removing P from supernatant
WWTP	Wastewater treatment plant (in this report limited to municipal utilities)

1 BACKGROUND

Nutrient removal and recovery from wastewater is an increasingly important objective of wastewater treatment in the context of increasing concern for global warming and fresh water reserves discussions (Cordell *et al.*, 2009; Schindler, 2006; Schindler *et al.*, 2012). Conventional biological and chemical nutrient removal methods have high carbon and energy footprints resulting in lower net benefit to the global ecosystem (Algeo and O'Callaghan, 2012; Heffernan *et al.*, 2011; Rosso *et al.*, 2011). This creates need for new, less energy intensive processes. This section is intended to provide the general background of the research, describing the mechanisms of conventional treatment processes and the processes taking place in the proposed system. More in depth literature review specific to each aspect of the research is presented at the beginning of Chapters 3 through to 7.

1.1 NITROGEN REMOVAL FROM MUNICIPAL WASTEWATER

Nitrogen is one of the major nutrients controlling algae and plankton growth. Thus, an increased loading of nitrogen from anthropogenic sources may cause eutrophication of water bodies, particularly in brackish and saline water (Scott *et al.*, 2011). Eutrophication results in decreased levels of dissolved oxygen (DO), increased levels of toxins produced by algae, reduced water clarity and bad odor, reducing water's recreational value and its value as a source of drinking water. Elevated concentrations of inorganic nitrogen species are directly toxic to the aquatic species (Tetra Tech, 2013; Wang *et al.*, 2017). The first nitrogen removal processes were developed and implemented as early as the 1960s, until then nitrification was discouraged as it led to the operational problem of rising sludge in the final clarifiers. Since the first three-stage or three-biomass system of separate carbon removal, followed by nitrification and then by

denitrification, the technology has evolved to one-biomass processes comprising a variety of autotrophic and heterotrophic species (Oleszkiewicz and Barnard, 2006).

Due to high operational and capital costs, physicochemical nitrogen removal methods (e.g. ion exchange columns and ammonia stripping at high pH) are generally not practiced in wastewater treatment. Due to high chemical demand they also tend to have a high carbon foot print. As such they are not considered in further discussion in this dissertation (Algeo and O'Callaghan, 2012; Oleszkiewicz *et al.*, 2015).

Biological nitrogen removal processes can be generally categorised into two main groups:

- *Conventional autotrophic/heterotrophic:* This group contains all processes based on a combined well known autotrophic nitrification (partial or complete) and heterotrophic denitrification.
- *Completely autotrophic:* All anammox based processes and autotrophic denitrification using hydrogen can be found in this group.

1.1.1 Nitrification/Denitrification

The conventional nitrification can be simplified into a two-step process. Where the first step, also known as nitrition, is the oxidation of ammonia to nitrite as per Eq. 1.1. Bacteria performing this process are referred to as ammonia oxidizing bacteria (AOB). Most common AOB genera in WWTPs are *Nitrosomonas* and *Nitrosospira* (Dytczak *et al.*, 2008; Ge *et al.*, 2015). In the second step nitrite is further oxidized to nitrate as per Eq. 1.2. Bacteria commonly responsible for this process in WWTPs are called nitrite oxidizing bacteria (NOB) and are from genera

Nitrobacter and *Nitrospira* (Kim and Kim, 2006). Both steps are reducing pH and as result alkalinity is used up as shown in the overall nitrification Eq. 1.3. It is important to remember that part of the ammonia will not be oxidized but it will be assimilated in to the new cell biomass. It is typically assumed that the biomass yield of AOB and NOB is 0.12 and 0.04 g VSS/g N, respectively (Metcalf & Eddy *et al.*, 2014). Based on this assumption the overall nitrification process accounting for the biomass synthesis can be described by Eq. 1.4. The total oxygen demand is 4.27 g O_2/g N, where 3.21 g O_2/g N would be associated with ammonia oxidation and 1.06 g O_2/g N with nitrite oxidation. The total alkalinity requirement is 7.09 g $CaCO_3$/g N (Metcalf & Eddy *et al.*, 2014).

Eq. 1.1 $$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$$

Eq. 1.2 $$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Eq. 1.3 $$NH_4^+ + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O$$

Eq. 1.4 $$NH_4(HCO_3) + 0.9852Na(HCO_3) + 0.0991CO_2 + 1.8675O_2 \rightarrow$$
$$0.01982C_5H_7NO_2\ (\text{cell biomass}) + 0.9852\ NaNO_3 + 2.9232H_2O + 1.9852CO_2$$

To complete the nitrogen removal in conventional systems, nitrate and nitirites produced by nitrifiers are later reduced to nitrogen gas. This process is called denitrification (reduction of nitrate) and denitritation (reduction of nitrite). Denitritation and denitrification are relatively common capabilities among chemoheterotrophic bacteria and archaea (Lu *et al.*, 2014). Denitrification in WWTPs is typically associated with Proteobacteria from genera *Chromobacterium*, *Comamonas*, *Flavobacterium*, *Hyphomicrobium*, *Paracoccus* and *Pseudomonas* (Lu *et al.*, 2014; Metcalf & Eddy *et al.*, 2014). Most of denitrifies are facultative aerobic bacteria, which in absence of oxygen use nitrite and nitrate as terminal electron acceptor. The theoretical stoichiometry of the process is presented in Eq. 1.5 and Eq. 1.6. The equations

indicate acetic acid as source of organic carbon only as an example. Bacteria may utilize a variety of organic substrates both from wastewater and supplemented from external sources (e.g. methanol, glycol liquid organic waste). The theoretical COD requirement using acetate would be 2.87 g COD/g N and 1.72 g COD/g N for reduction of nitrate and nitrite, respectively. However, since the process is performed by heterotrophic organisms which in general have significantly higher biomass yields than the autotrophic nitrifiers, it is important to account for the carbon requirements for the biomass synthesis. Metcalf & Eddy (2014) proposed overall denitrification stoichiometry using acetic acid as in Eq. 1.7 and Eq. 1.8. After the biomass production was accounted for, the COD requirements increased to 6.65 g COD/g N and 4.49 g COD/g N for reduction of nitrate and nitrite, respectively. Thus, the carbon requirements increased over two fold. The exact values will vary for different carbon sources due to variable biomass yields. The actual process requirements at WWTP may be even higher if there is significant oxygen transfer to the denitrification tanks, since the process can only occure under oxygen free conditions. It is typically assumed that in conventional nitrification/denitrification systems the minimum COD:TKN ratio required for nitrogen removal is 6 to 15 g COD/g N (Oleszkiewicz *et al.*, 2015; Oleszkiewicz and Barnard, 2006). Regardless the source of carbon denitrification will produce 3.57 g $CaCO_3$/g N. This is more than half of the alkalinity required for full nitrification (7.09 g $CaCO_3$/g N). Therefore the denitrification process is encouraged even in plants which are required only to nitrify (TN removal not required). This will allow for the recovery of part of the alkalinity for nitrification and reduce the load of organic carbon to the aerobic zone (reduced aeration demand) (Oleszkiewicz *et al.*, 2015).

Eq. 1.5 $$8NO_3^- + 5CH_5COOH \rightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^-$$

Eq. 1.6 $$8NO_2^- + 3CH_5COOH \rightarrow 4N_2 + 6CO_2 + 2H_2O + 8OH^-$$

Eq. 1.7 $NO_3^- + H^+ + 0.33NH_4^+ + 1.45CH_5COO^- \rightarrow 0.5N_2 + 0.33C_5H_7NO_2\ (\text{biomass}) + 0.12CO_2 + 1.6H_2O + 8OH^- + 1.12HCO_3^-$

Eq. 1.8 $NO_2^- + H^+ + 0.24NH_4^+ + 0.98CH_5COO^- \rightarrow 0.5N_2 + 0.24C_5H_7NO_2\ (\text{biomass}) + 0.008CO_2 + 1.24H_2O + 8OH^- + 0.74HCO_3^-$

The conventional nitrification and denitrification process is applied in a wide range of different process configuration such as single sludge continuous flow process, two-sludge process, sequencing batch reactor (SBR), preanoxic and postanoxic denitrification, step feed, Bardenpho-type processes in a format of suspended flocculent growth, aerobic granular sludge and combination of attached and suspended growth system. All have certain advantages under specific local conditions. For example, if the C:N ratio in the plant influent is very low and most of the carbon for denitrification has to be provided from exogenous sources (e.g. methanol) it may be more beneficial to use a two-sludge system or simultaneous nitrification-denitrification (SND) process, which will allow for optimisation of carbon usage. Development of technology is presently focused mostly on:

1. optimisation of available carbon usage,
2. reduction of aeration requirements, and
3. increase of process capacity by improved retention of biomass.

Efforts to tackle the issue of carbon availability and oxygen requirements could be categorised between: optimisation of conventional processes and development of new processes. The first category would include optimisation of aeration control, wastewater dosing point, minimization of oxygen transfer into denitrification tanks, fermentation of primary sludge (carbon recovery), etc. While these efforts may bring significant cost reductions and should be applied as the best operation practice, they are not able to bring a breakthrough in the field as they cannot go beyond

the minimum process requirements limits as described previously in this section. As such they will not be further discussed here. The other approach is based on the new processes, including partial nitritation/denitritation and whole group of completely autotrophic processes of nitrogen removal. The term "new" is used similar to "unconventional" here, though some of these processes have been practiced for decades.

1.1.2 Nitritation/Denitritation

The bioreactors using nitritation/denitritation are configured similarly to conventional nitrification/denitrification systems, providing alternating aerobic and anoxic conditions, but the operation strategy is set to inhibit growth of NOB. As a result ammonia is oxidized only to nitrite and then nitrite is reduced to nitrogen gas. This process is also referred to as the nitrite shunt. The idealized process pathway is shown in Figure 1.1. The nitritation/denitritation system, when compared to conventional system with post-anoxic zone, may reduce the energy requirements for nitrification by 25% and organic carbon requirements by 40% (Mulder *et al.*, 2006). However, if compared against the conventional system with pre-anoxic configuration (e.g. Modified Ludzack-Ettinger) and sufficient C:N ratio in the wastewater, the benefits of reduced oxygen requirements for aeration are completely offset by the increase of COD load to the aerobic zone. Thus, nitritation/denitritation is applicable only in the case of treatment of wastewater with low C:N, i.e. below 6 g COD/g N (Jimenez, 2015; Jimenez *et al.*, 2014) or as a retrofit option for post-anoxic systems.

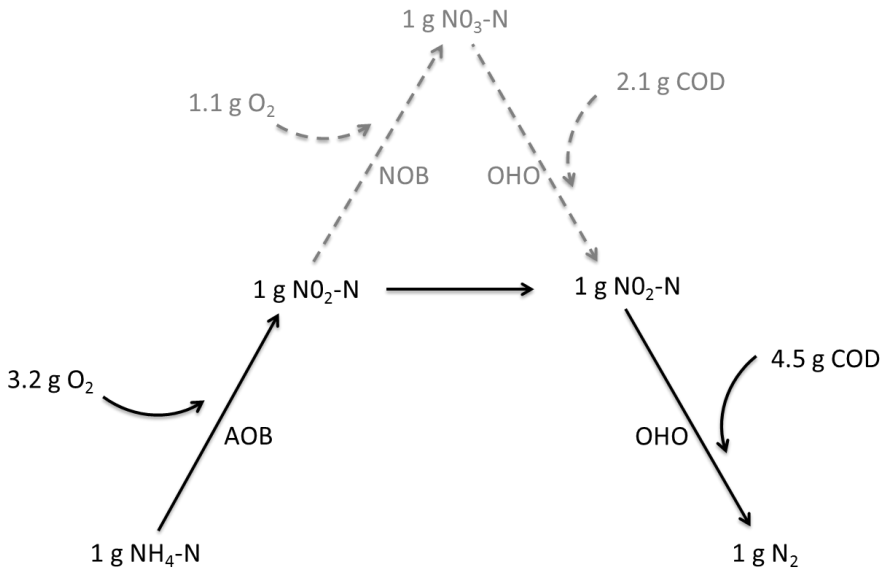


Figure 1.1: Simplified Process Pathway of Nitrite Shunt in Comparison to Conventional Nitrification/Denitrification

Developed based on Eq. 1.1, 1.2, 1.5 and 1.6. In gray process steps of nitrification and denitrification avoided when nitrite shunt used. COD demand assuming acetate as carbon source.

The main challenge and the key to nitrite shunt systems is the inhibition of growth of NOB to stop oxidation of nitrite to nitrate. There is vast literature available regarding this issue and multiple proprietary commercially available control systems, allowing enrichment of AOB with little NOB accumulation (Kim and Kim, 2006; Regmi *et al.*, 2012; Stinson *et al.*, 2014). However, most of the control strategies can be brought down to: aggressive SRT, nitrite and free ammonia inhibition and oxygen inhibition (Regmi *et al.*, 2014; Stinson *et al.*, 2014). Typically nitritation/denitritation systems will apply all of these strategies simultaneously with small variations.

The strategy of aggressive SRT is commonly used in the systems with temperatures above 17°C, and it is based on results suggesting that the relative growth rate of AOB is higher than the NOB (Hellinga *et al.*, 1998; Oleszkiewicz *et al.*, 2015; Stinson *et al.*, 2014). Thus operating at the SRT

in between minimum SRT of NOB and minimum SRT of AOB allow to selectively washout NOB from the system. The robustness of this strategy has been however, questionable, when not coupled with any of the substrate control strategies (Bunce *et al.*, 2013; Pollice *et al.*, 2002).

Another approach is to keep favourable substrate concentrations for AOB vs NOB, i.e. always keeping higher residual ammonia in the bioreactor and minimize nitrite accumulation. This can be achieved in two ways: (1) the DO in the bioreactor may be controlled at very low levels (i.e. below 0.5 mg/L) (Blackburne *et al.*, 2008) allowing denitritation to occur either simultaneously in the same reactor or using internal recycle to anoxic zone (more common in pre-anoxic continuous flow configurations) (Oleszkiewicz *et al.*, 2015); or (2) apply intermittent or cyclic aeration with higher DO during aeration periods (Stinson *et al.*, 2014) and denitritation in non-aerated periods (more common in step feed or SBR configuration) . The first approach is based on results indicating that the AOB oxygen affinity is higher than NOB (Blackburne *et al.*, 2008). However, not all researchers agree if this is true in the lower temperature range (Bunce *et al.*, 2013). The intermittent aeration with higher DO reportedly benefits also from an apparent lag in the response of NOB to changing oxygen conditions (Pollice *et al.*, 2002; Turk and Mavinic, 1986), thus it is crucial to keep high enough biomass inventory and provide sufficient carbon source to reduce the transition time between aerobic and anoxic periods (fast oxygen depletion after aeration is turned off). In both cases the maximum oxygen concentration will depend on the allowable ammonia concentration in the final effluent. The higher ammonia concentration the higher DO can be used. Successful selective NOB inhibition was reported with intermittent aeration with DO above 1.5 mg/L (Bunce *et al.*, 2013; De Clippeleir *et al.*, 2013b). Additional benefit of a higher ammonia concentration in the bioreactor is that free ammonia inhibits NOB at much lower concentrations than AOB. NOB can be inhibited at free ammonia concentrations as

low as 1 mg/L, while inhibition of AOB starts at concentration above 16 mg/L (Vadivelu *et al.*, 2007).

The success of selective wash-out of NOB is highly depended on the temperature. Most of the full scale nitritation/denitritation processes are either located in warm climates or treat only the digester liquors which have typically temperature above 25°C. Also, the relatively low effluent ammonia requirements for the main stream process, often below 3 mg/L, can be a challenge for the stability of the operation.

1.1.3 Completely Autotrophic Nitrogen Removal

Heterotrophic denitrification processes main drawbacks are: high organic carbon requirements and high sludge production. In search of new nitrogen removal process suitable for wastewater treatment, number of autotrophic denitrification processes where investigated (Celmer-Repin *et al.*, 2010; Hwang, 2010; Hwang *et al.*, 2010; Wang *et al.*, 2009; Zhou *et al.*, 2011). Autotrophic microorganisms have much smaller yields and as such require less or no organic carbon and generate less sludge. The most advanced research was conducted on denitrification using electron donors in form of sulphides, elemental sulphur and hydrogen gas. Despite some promising nitrogen removal rates at very low biomass yield, to my knowledge none of the processes went beyond laboratory scale. Most likely it was due to issues with process stability and/or issues with upscaling the system. As such these processes still are irrelevant for the full scale treatment design and operation.

The process that made a fundamental breakthrough for nitrogen removal technology allowing completely autotrophic nitrogen removal (Strous *et al.*, 1998), is anaerobic ammonia oxidation (anammox) process, discovered in the 1990's. Anammox (AMX) bacteria are capable of anaerobic ammonium oxidation to nitrogen gas utilizing nitrite as electron acceptor. Since Anammox bacteria need nitrite as one of the substrates, this process is coupled with partial aerobic nitritation. The simplified process pathway is present in Figure 1.2 in comparison to conventional nitrification and denitrification. According to Eq. 1.9 developed by Strous *et al.* (1998), 57% of the ammonium load has to be partially nitrified to provide nitrite. Although this stoichiometric equation is referred to by most researchers and engineers, recent report by Lotti *et al.* (2014b) suggests that amount of ammonium needed to be aerobically oxidized might be even lower. In practice, this translates into saving of up to 60% of energy for aeration and 90% of carbon for denitrification (Siegrist *et al.*, 2008), if compared with conventional nitrification/denitrification in post-anoxic configuration. The saving will be smaller if compared with pre-anoxic configuration where denitrification offsets part of the aeration cost. In this case aeration savings would be maximum 45% (based on the theoretical oxygen and carbon requirements, Figure 1.2). Anammox bacteria belong to the order *Brocadiales* within the *Planctomycetes* phylum (Jetten *et al.*, 2009). Most well known and most often detected in wastewater treatment systems (both full and lab scale) anammox bacteria belong to candidate genera *Kuenenia* and *Brocadia* (Harhangi *et al.*, 2012; Jetten *et al.*, 2009).

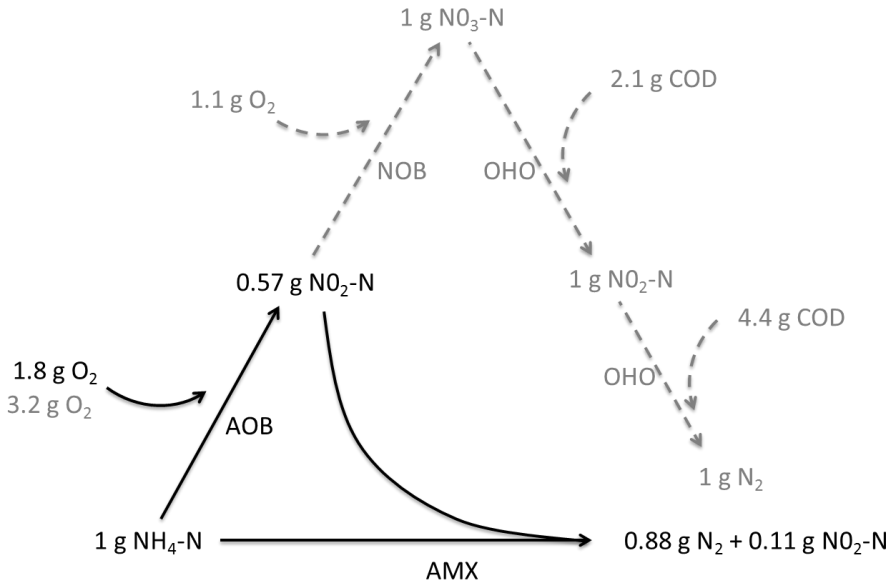


Figure 1.2 Simplified Process Pathway of Partial Nitritation/Anammox (PN/A) in Comparison to Conventional Nitrification Denitrification

Developed based on Eq. 1.1, 1.2, 1.5, 1.6 and 1.9. In gray process steps of nitrification and denitrification avoided when PN/A short-cut used. COD demand assuming acetate as carbon source.

Eq. 1.9 $$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 0.26\ NO_3^- + 1.02\ N_2 + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$$

The slow-growth characteristics of anammox bacteria led to their primary application in treatment of reject water streams, where they could proliferate at elevated temperatures (Cao *et al.*, 2013; Jetten *et al.*, 2009; Lackner *et al.*, 2014). In the last decade anammox has become the state-of-the-art process for side stream treatment, with over 100 (including industrial wastewater treatment) full scale facilities in operation (Lackner *et al.*, 2014). In recent years there has been an increased interest in applying the anammox process in the mainstream of municipal wastewater treatment plant (WWTP) (Cao *et al.*, 2013; De Clippeleir *et al.*, 2013a; Gilbert *et al.*, 2015; Hendrickx *et al.*, 2014; Hu *et al.*, 2013; Laureni *et al.*, 2015; Lotti *et al.*, 2014a; Ma *et al.*,

2013; Vázquez-Padín *et al.*, 2011), because it has been shown to be one of the most promising ways to achieve energy self-sufficiency of WWTP (Khiewwijit *et al.*, 2015).

1.2 PHOSPHORUS REMOVAL AND RECOVERY FROM MUNICIPAL WASTEWATER

Phosphorus is not directly toxic to the environment. However, it is the key limiting macroelement for the growth of algae and plankton (Scott *et al.*, 2011). Thus, its load to the environment must be controlled. At the same time the time span of phosphorus circulation in nature is significantly longer than the one of nitrogen because phosphorus does not exist in a gaseous form in a biosphere. Most of the phosphorus used by human is coming from phosphorus rich ores and these are being depleted (Mehta et al., 2015). According to some reports the "phosphorus peak" may come as early as 2033 (Cordell *et al.*, 2009). After the peak, easily accessible phosphate sources will be exhausted which will increase the prices and the demand will start to decline. In response, many regulators around the world in addition to tightening phosphorus effluent limits started promoting and/or mandating phosphorus recovery (Germany, 2016; Manitoba, 2011; Oleszkiewicz *et al.*, 2015). Nowadays a common phosphorus effluent limit is in the range 0.5 to 3 mg P/L (in Canada typically 1 mg P/L), but in some ecologically fragile areas may be as low as 0.01 mg/L (e.g. Chesapeake Bay in USA) (Chesapeake Bay Initiative, 2012; USA and Canada, 2012).

There are certain discrepancies in the literature as to the definition of removal, recovery and reuse. For the purpose of this dissertation following definitions will be used:

> *Phosphorus removal:* a process providing reduction of the load of phosphorus in the effluent of the mainstream treatment.

Phosphorus recovery: a process of phosphorus extraction either in the main stream or sidestream treatment, providing a product of quality which allows subsequent phosphorus reuse.

Phosphorus reuse: beneficial application of phosphorous products generated from waste material.

1.2.1 Phosphorus Removal

Phosphorus removal processes can be categorized in three groups: (1) biological, (2) physico-chemical, and (3) hybrid processes.

The biological phosphorus removal process (also known as enhanced biological phosphorus removal or EBPR) is based on the capability of certain microorganisms to accumulate increased amounts of phosphorus (Barnard *et al.*, 2016). All microorganisms leaving in mixed liquor will take up small amount of phosphorus which they require for synthesis of new cell biomass. Typically phosphorus will make up 2% of the biomass weight and will in small part contribute to observed phosphorus removal (Metcalf & Eddy *et al.*, 2014). The phosphorus accumulating organisms (PAO) use phosphorus not only for biomass synthesis but also to store energy in form of polyphosphates. The PAO biomass may consist even in 30% out of phosphorus (Acevedo *et al.*, 2012). Phosphorus is removed from the wastewater by wasting the sludge.

In order to allow competitive advantage for the PAO over other OHO, sludge must be introduced to alternating anaerobic and aerobic conditions (Barnard *et al.*, 2016). Under anaerobic conditions PAO will pick up VFA and store them internally in form of PHA (Schuler and Jenkins, 2003; Wentzel *et al.*, 1986). This biosynthetic process is done using energy from

transformation of the ATP to ADP, which results in a release of phosphate. Subsequently, under aerobic conditions PAO metabolize the previously stored PHA for growth and energy production. The energy is stored in form of chemical phosphate bonds – ADP is converted into ATP, which causes phosphate uptake from the solution. The uptake of phosphorus in the aerobic zone is higher than release in anaerobic zone due to synthesis of new cells with high phosphate storage, this results in overall soluble phosphorus removal (Barnard *et al.*, 2016). Since the process converts phosphorus from soluble to solid form, the total phosphorus removal will be strongly affected by the efficiency of final solids separation. The biological phosphorus removal can be applied in various process configurations. Usually these would be continuous flow tanks with an anaerobic zone followed an aerobic zone (e.g. A/O) with an anoxic zone in between, when coupled with nitrogen removal (e.g. A2O or Westbank) (Oleszkiewicz *et al.*, 2015).

Physico-chemical processes are based on the chemical precipitation of phosphates and solids separation. Typically precipitation is done using salts of iron or aluminium or lime (Cadmus Group, 2010; Kabouris *et al.*, 2009; Maher *et al.*, 2011; Oleszkiewicz *et al.*, 2015; Zhang *et al.*, 2006). Chemicals can be dosed at various points of the treatment process. Dosing in the preliminary or primary treatment will additionally reduce the load of COD to the secondary treatment but it may require additional dose of polymer to improve flocculation of solids (Cadmus Group, 2010; Oleszkiewicz *et al.*, 2015). Additionally if conventional nitrogen removal process is used in the secondary treatment, phosphorus precipitation upstream may cause phosphorus shortage for the growth of biomass in activated sludge. Chemical dosing to the secondary treatment (either bioreactor or secondary clarifier) will additionally improve sludge settleability and usually does not require flocculent dosing. In this case however, special attention must be paid to the pH and alkalinity control, since coagulation with metal salts may

cause low pH toxicity. Chemical precipitation can be also used as a tertiary treatment. This requires additional infrastructure, e.g. contact tanks and clarifiers or filters, and because of that is expensive. Tertiary treatment also provides the lowest effluent phosphorus concentration.

The electrochemical method is a variation of chemical precipitation, where metal ions are provided by electrolytic dissolution of a sacrificial anode (Holt *et al.*, 2005; Hug and Udert, 2013; Tran *et al.*, 2012; Wei *et al.*, 2012). This process was presented in laboratory scale and pilot scale using both iron and aluminum electrodes. Relatively high phosphorus removal rates were reported both for municipal wastewater and source separated urine (Hug and Udert, 2013; Wei *et al.*, 2009). The main advantage of electrochemical methods is the reduction of the bulk volume of chemicals to be used. The metal is delivered and dosed in high purity form (typically above 95% pure alloys) and the requirement for pH adjustments is significantly reduced. These methods are described in details in Chapter 3.

The hybrid approach benefits of the PAO metabolism and combines it with chemical phosphorus precipitation. The example of hybrid process is Phostrip (Figure 1.3). In this configuration the main stream process does not have to have an anaerobic zone. Instead, a part of RAS is sent to "phosphorus stripper" which is a gravity thickener. The SRT in the thickener is long enough to promote fermentation, which causes VFA production and anaerobic conditions (Barnard *et al.*, 2016). As a result, PAO release phosphate. Phosphate rich overflow is then sent to the chemical stripper and sludge is separated into WAS and RAS.

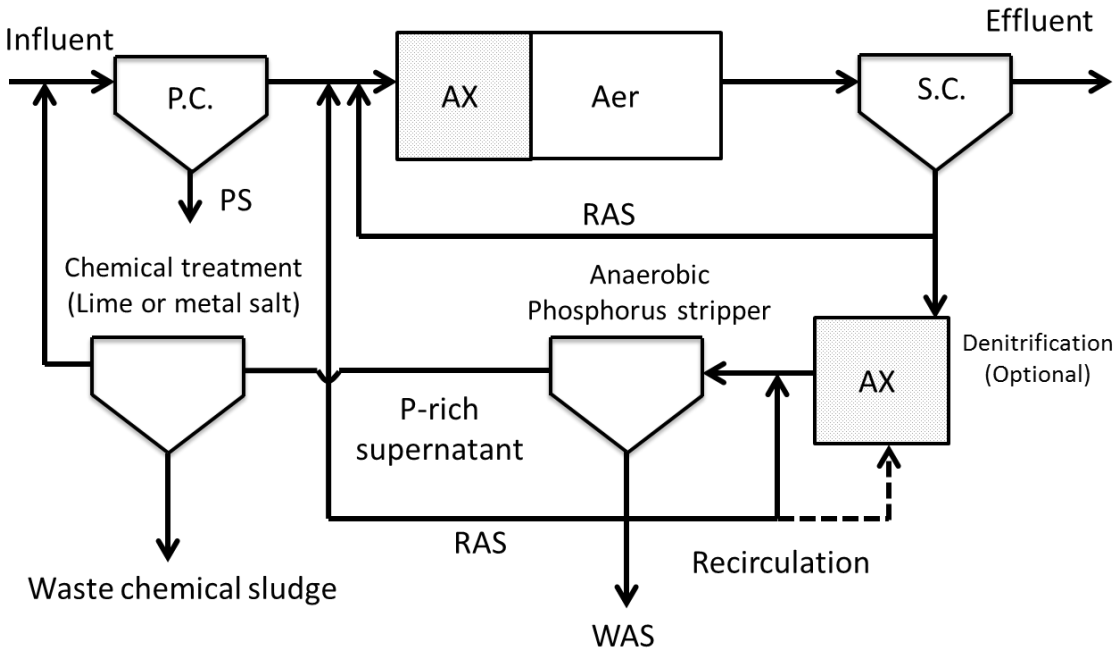


Figure 1.3: Phostrip Process Configuration in Plant with Total Nitrogen Removal

Developed after Metcalf & Eddy et al. (2014).

The most important decision making aspects when deciding the phosphorus removal process type are following:

Effluent quality: While chemical precipitation, when used as tertiary treatment, will provide the lowest effluent TP concentration, even below 0.005 mg P/L (Lambert *et al.*, 2015), all three approaches can provide consistent effluent concentration below 1 mg P/L (Oleszkiewicz *et al.*, 2015).

Sludge production: Chemical precipitation may generate 30 to 40% more sludge than EBPR, assuming use of aluminium or iron salts (Oleszkiewicz *et al.*, 2015).

Operation: EBPR systems are generally more complex and require closer monitoring in order to provide consistent effluent quality. The key factors for the operation of EBPR or hybrid processes are to provide enough short chain VFA (often primary sludge fermenter is required), and minimize the nitrite and nitrate transfer to the anaerobic zone (ideally ORP <-300 mV) (Barnard *et al.*, 2016; Oleszkiewicz and Barnard, 2006). In addition dewaterability of EBPR sludge might be hindered if phosphorus is not extracted in the sidestream treatment (Benisch *et al.*, 2014).

Cost: The biological process is generally associated with lowest cost in a long term. Although it requires additional reactor volume (typically less than 10% of total bioreactor volume) it saves the cost of chemicals and sludge treatment and disposal (Oleszkiewicz *et al.*, 2015).

Phosphorus recoverability: In EBPR systems phosphorus is loosely bound as intracellular volutin granules formed of polyphosphate chains which can be easily extracted in the sidestream treatment and later recovered and reused. When phosphorus is precipitated chemically it is strongly bound with metal ions. There is still a debate if this chemical precipitate can be beneficially reused in agriculture due its very low phosphorus bioavailability (Batziaka *et al.*, 2008; Kidd *et al.*, 2007; Kresge *et al.*, 2009; Liang *et al.*, 2010; Oleszkiewicz *et al.*, 2015; Pritchard *et al.*, 2010; Viraraghavan and Ionescu, 2002).

1.2.2 Phosphorus recovery

The main steps in the phosphorus recovery are presented in Figure 1.4. First, phosphorus must be removed from the mainstream which results in concentration of phosphorus in solid form, either biomass or chemical precipitate. Depending on the concentration process used and local market

needs and regulations, product of the removal process may be directly reused, e.g. for direct land application of sludge. Most often, additional steps of phosphorus extraction from the sludge and phosphorus re-concentration are required to produce a purer final product (Crawford, 2010; Oleszkiewicz *et al.*, 2015; Stinson *et al.*, 2013).

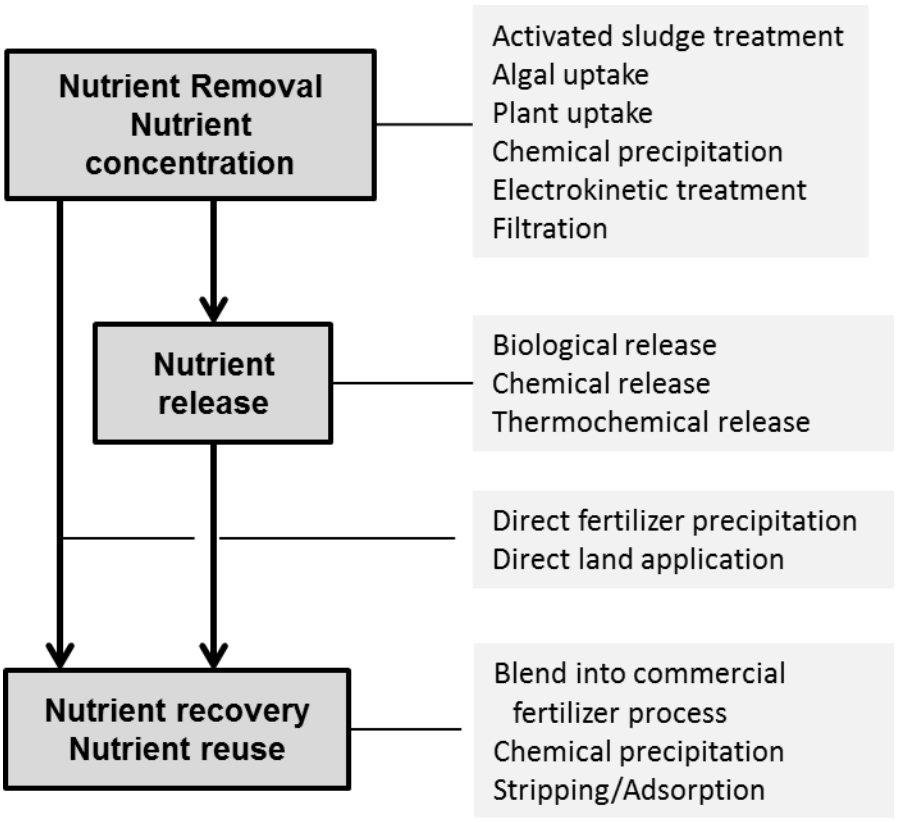


Figure 1.4: The Three Main Steps in Nutrient Recovery and Reuse

Developed after Oleszkiewicz et al. (2015).

Figure 1.5 presents phosphorus potentially available for recovery/reuse at various points of a typical WWPT with EBPR. In the case of plants with chemical precipitation in the mainstream, WAS stripping would not be viable and the phosphorus balance between biosolids and dewatering would shift towards biosolids. Based on that, ideally phosphorus should be recovered in the mainstream where the full load of phosphorus from the raw sewage is available.

Unfortunately relatively low concentration at this point (in range of 5 to 20 mg/L) prohibit economical application of available recovery methods (Britton *et al.*, 2009, 2005). Another potentially good point of recovery would be ash from sludge incineration. However, due to very strong chemical bounds in ash recovery would require high amounts of strong acids and heat (Stark, 2005; Tan and Lagerkvist, 2011; Tetra Tech, 2013). Additionally many plants practicing sludge incineration, prefer still to first digest it, which improves incineration process control but also reduces the amount of phosphorus available for recovery. Currently it is advised to store the ash in monofills, allowing future extraction if better technologies become available or phosphate rock exploration becomes too expensive (Oleszkiewicz *et al.*, 2015). In practice most of the recovery processes are designed for sludge and for sludge liquor streams.

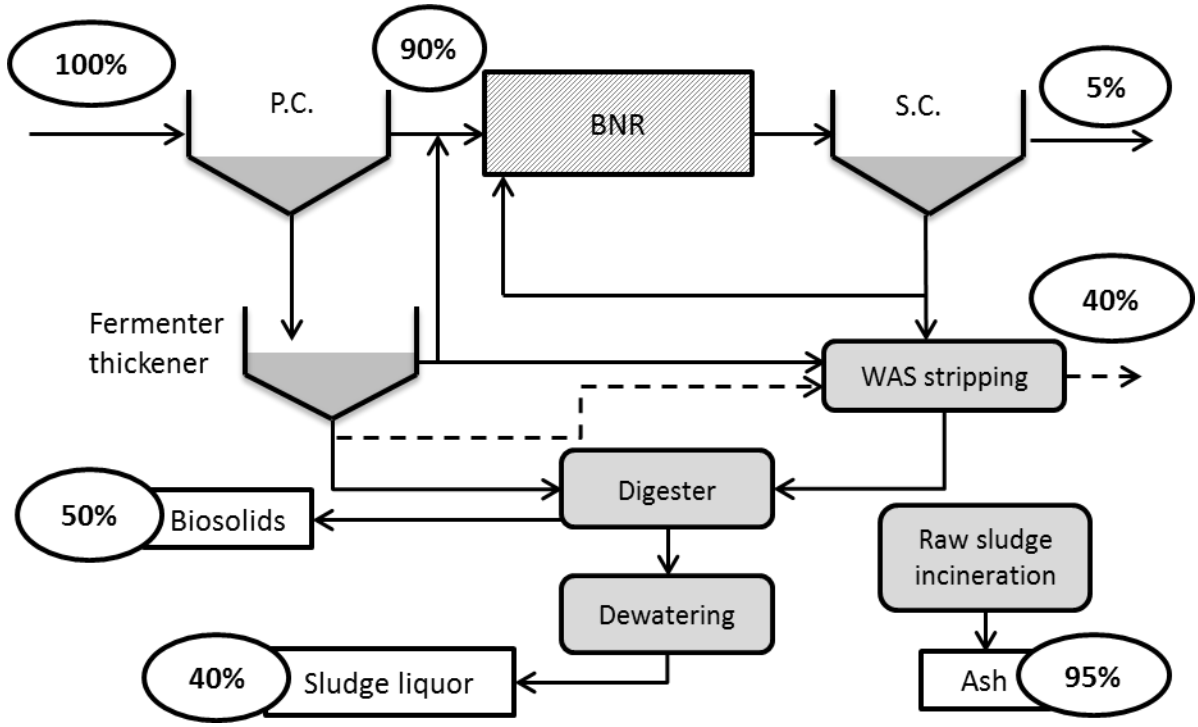


Figure 1.5: Phosphorus Available for Potential Recovery at Various Points in a Typical EBPR WWTP

Developed after Oleszkiewicz et al. (2015).

The recovery from the sidestream is essentially precipitation of phosphorus in any of the three forms: ammonium magnesium phosphate (struvite), potassium magnesium phosphate (K-struvite) and calcium phosphate (Algeo and O'Callaghan, 2012; Le Corre et al., 2009; Mehta et al., 2015; Stark, 2005). All these forms have wide range of applications in agriculture and other industries (e.g. fertilizer, additive for livestock feed, fire retardants) (Egle *et al.*, 2013; Stark, 2005). There is a number of commercially available processes (e.g. Pearl, AirPrex, Multiform Harvest, Crystalactor) (Algeo and O'Callaghan, 2012; Oleszkiewicz *et al.*, 2015). The key factors for these recovery processes are (Mehta et al., 2015; Oleszkiewicz et al., 2015):

Phosphate extraction: Typically phosphorus will be extracted from the sludge in anaerobic digestion which may be coupled with thermal hydrolysis process for improved solids destruction. However the phosphorus release during digestion will be accompanied by release of ammonia and typically alkaline pH. This may result in uncontrolled precipitation of ammonium phosphates and scaling of digesters and dewatering units. Thus, often WAS is pretreated in short retention anaerobic tanks (e.g. WASSTRIP) in order to extract the loosely bound phosphorus from the polyphosphate stored by PAO. The stream from the initial phosphorus extraction may be combined with the liquors from digested sludge or treated separately.

Control of pH: Typically pH for phosphorus precipitation needs to be maintained in the range of 7.5 to 9. The pH can be increased either by alkali dosing or CO_2 stripping with air.

Metal dosing: Usually in sidestream treatment of EBPR plant the concentration of calcium and magnesium are already relatively high because these ions are released by PAO together with phosphate. However to improve the phosphorus recovery rate additional magnesium

and calcium is dosed. Different processes may use different forms of metals, e.g. for magnesium $MgCl_2$, MgO or $MgSO_4$. Process configurations are geared towards the most efficient chemical use, minimizing required over dose.

Precipitate harvesting: Depending on the intended final application of the product, there will be different requirements for the product purity and product form. In some cases the precipitation will be performed directly in the digested sludge (e.g. AirPrex), in other cases sludge will be first dewatered and only the liquor will be used for the recovery. All technology vendors developed their own unique reactors configuration allowing them to efficiently retrieve precipitate. Most of them are variation of upflow fluidized bed reactors.

Current prices of phosphate rock are relatively low in the context of global resources to demand ratio (Algeo and O'Callaghan, 2012; Cornel and Schaum, 2009; Soil Association, 2010). At the same time extractive phosphorus recovery requires high doses of chemicals and generates an expensive product. In most cases even if process vendor guarantee product sales (e.g. Ostara for Perl product), the typical payback period is in the range 10 to 20 years (Oleszkiewicz *et al.*, 2015). Thus, there are still no economical drivers to implement recovery in standard practice. However, recovery processes proved to have also indirect benefits to the plant operation, e.g. reduced phosphorus load to the main stream treatment, reduced scaling of digesters and dewatering units (when coupled with pre-release processes), reduced scaling of equipment in the mainstream process, more stable phosphorus removal process in the mainstream. All these may generate indirect cost savings reducing the actual payback period (Khunjar *et al.*, 2013; Le Corre *et al.*, 2009; Oleszkiewicz *et al.*, 2015).

2 OBJECTIVES AND RESEARCH STRATEGY

2.1 STATEMENT OF PROBLEM

Currently practiced methods of nutrient removal from the municipal wastewater are energy and chemical intensive resulting in high carbon footprint of the treatment. In fact it might be questionable if the treatment still brings net benefit to the environment (Algeo and O'Callaghan, 2012; Heffernan *et al.*, 2011; Rosso *et al.*, 2011). The application of innovative processes such as Anammox main-stream nitrogen removal would allow energy savings and reduction of green-house gas emissions, while increasing the availability of organic carbon for biogas generation in anaerobic digestion. The Anammox process has been successfully demonstrated for treatment of concentrated and warm digested sludge liquors. Application of the Anammox biomass to main-stream wastewater treatment, subject to variable composition and temperature, poses new challenges of management of these slow-growing autotrophic bacteria. There are existing reports on the mainstream low temperature Anammox applications (mainly in bench scale) but there is still little known regarding the stability of the process in the long-term operation and microbiological population dynamics under such conditions.

The potential of the phosphorus recovery from mainstream treatment is untapped by the presently available methods. Direct recovery from the mainstream would further reduce the carbon requirements for the EBPR and/or chemicals for chemical removal, and lower the operation cost associated with scaling in the sidestream treatment.

2.2 MAIN OBJECTIVE

The main objective of this research was to assess the feasibility of new proposed treatment process for municipal wastewater which would minimize the energy demand and combine nutrient removal and nutrient recovery.

It is proposed to combine the technology of membrane bioreactor (MBR) as a single stage autotrophic ammonia removal (PN/A) with mainstream phosphorus removal/recovery using electrically assisted struvite yield (EASY) method with magnesium sacrificial electrodes (Figure 2.1). The process will be preceded by high rate activated sludge (HRAS) which will reduce load of carbon to the EASY MBR. Increased mass of waste activated sludge produced in this low SRT bioreactor will offer high biogas production. This approach will minimize energy demand (aeration) in the mainstream treatment and allow maximization of energy recovery in the sidestream treatment, bringing the nutrient removal plant closer to net carbon neutrality.

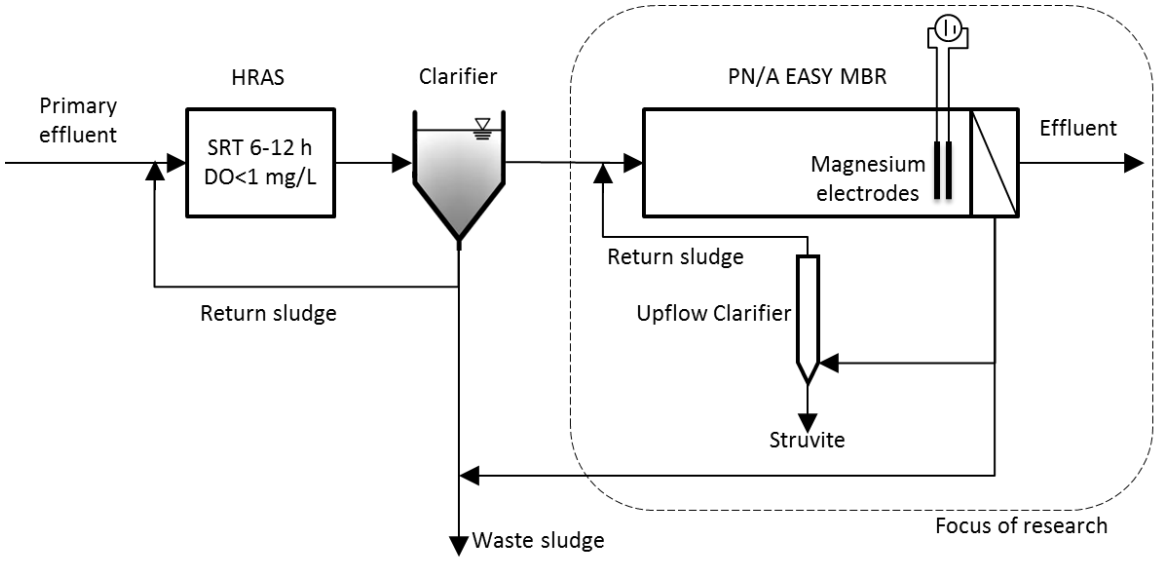


Figure 2.1: Flow Diagram of Proposed Mainstream Treatment Process

The selection of Anammox and AOB in a single stage MBR will facilitate fine tuning of SRT and control the retention of seed/seeded biomass. Alternatively, in the absence of substrate gradients (such as in biofilm systems), a more accurate control of oxygen concentration and SRT will be needed in order to ensure that nitrite oxidizing bacteria (NOB) would be outcompeted by AOB (Bunce *et al.*, 2013; Pollice *et al.*, 2002; Turk and Mavinic, 1986). Due to different temperature response of AOB and NOB, this will be particularly important at low temperature. MBRs require significant energy consumption for fouling mitigation although in recent years specific energy requirements (per m^3 of treated water) were decreasing due to improvements in module configuration and operational practice (Krzeminski *et al.*, 2012). The implementation of electrodes in a MBR was demonstrated to significantly decrease the energy consumption by extending the time between backwash cycles at least two times (Wei *et al.*, 2012, Elektorowicz et al, 2012). Application of magnesium electrodes would provide struvite precipitation within the EASY MBR; the struvite would be periodically removed through hydro-cyclone or upflow clarifier.

The proposed treatment train would be highly competitive with current state-of-the art technology due to:

- elimination of carbon source and of chemicals needed for nitrogen and phosphorus removal;
- increase of biogas production due to the low SRT first stage biological process;
- reduction of energy need for ammonia oxidation;
- reduction of energy and chemicals used for membrane maintenance through the implementation of electrode protection;

- direct recovery of phosphorus from the mainstream, which reduces scaling issues in the sidestream treatment.

2.3 SPECIFIC OBJECTIVES AND STRUCTURE OF THE PROJECT

This research was focused only on aspects of the Anammox EASY MBR part of the proposed treatment process.

Three component processes and two process conditions were identified as key for the development of a mainstream PN/A EASY MBR. These were:

Processes: nitrification; anammox; phosphorus removal/recovery

Process conditions: Electric current applied to electrodes submerged directly in the mixed liquor; low temperature (i.e. 10°C)

The project strategy was to first test independently impact of each of the key process conditions on all key processes and later to implement all of them together. Five specific research objectives were established and the research was divided into five sets of experiments. Each of the experimental sets was set-up to satisfy one of the objectives. The specific research objectives and the corresponding experimental sets are described below:

Specific Objective #1. Evaluation of suitability of phosphorus recovery in a non-EBPR system by struvite precipitation using a sacrificial magnesium anode as the sole source of magnesium. Determine the impact of solution pH and electric current on the purity of the produced struvite and the phosphorus removal ratio.

Experimental Set #1 Magnesium electrodes were used in a synthetic solution of ammonia and phosphate under a variety of conditions to assess if this method was feasible for phosphorus removal and recovery under mainstream conditions. The potential quality of product was also evaluated. This experimental set is discussed in Chapter 3.

Specific Objective #2. Assessment of the effect of electric current on nitrification, and verifying the concept of simultaneous nitrification and electrochemical struvite recovery. Assessment of the response of AOB and NOB to electric current.

Experimental Set #2 A nitrifying SBR was set up for treatment of synthetic municipal wastewater. Nitrifying biomass was exposed to the electric current, first using graphite electrodes (no coagulation effect) and later magnesium electrodes. These experiments allowed assessment of impact of electric current on the nitrifiers (both AOB and NOB) in short- and long-term operation. Feasibility of simultaneous struvite removal/recovery and biological nitrification was evaluated. This experimental set was discussed in Chapter 0.

Specific Objective #3. Assessment of short and long term impact of the low temperature prevalent in the mainstream treatment in colder climates (i.e. 10°C), on the activity of anammox biomass originating from a high temperature sidestream treatment (i.e. 34°C). Assessment of changes in the structure of the anammox population during long-term operation at low temperature.

Experimental Set #3 In this experiment anammox biomass enriched in mesophilic conditions (see Chapter 5) was exposed to gradual temperature reduction to 10°C and

over 100 day operation at this temperature. Both biomass enrichment and the experiment were conducted in MBR. This experiment was discussed in Chapter 5.4.

Specific Objective #4. Evaluation of the effect of electric current on the anammox process.

Experimental Set #4 In these experiments anammox biomass in the mesophilic MBR was exposed to the electric current. This set of experiments was discussed in Chapter 7.

Specific Objective #5. Demonstration of the concept of simultaneous partial nitritation/anammox and electrochemical phosphorus recovery under mainstream conditions.

Experimental Set #5 This experiment was planned to combine all three key process in one reactor and operate it under mainstream conditions. However, this experiment was not conducted due to negative impact of the electric current on anammox observed in experimental set #4. This experiment was also planned to test control strategies for mainstream partial nitrification.

Detailed methodology for all experiments was described in Chapters 3 to 7.

3 EASY STRUVITE RECOVERY[1]

3.1 INTRODUCTION

The objective of this set of experiments was to evaluate the suitability of phosphorus recovery in non-EBPR system by struvite precipitation using electrically assisted struvite yield (EASY) method with sacrificial magnesium anode as the sole source of magnesium. Specific objectives were to determine the impact of solution pH and electric current on purity of the produced struvite and the phosphorus removal ratio. The study was conducted in a wide range of synthetic solutions and supernatant from a non-EBPR WAS fermentation.

Struvite ($MgNH_4PO_4 \cdot 6H_2O$) deposition in pipes, on reactor walls and on submerged surfaces of devices (Ben Moussa *et al.*, 2006; Le Corre *et al.*, 2005; Suzuki *et al.*, 2007), significantly increases maintenance costs in conventional and biological nutrient removal (BNR) plants (Doyle *et al.*, 2002). Controlled struvite precipitation, may not only reduce the load of phosphorus and ammonia but also produce a valuable and marketable fertilizer. Numerous researchers have shown feasibility of the struvite production from anaerobically digested sludge dewatering liquors and from livestock manure (Doyle *et al.*, 2002; Schuiling and Andrade, 1999; Suzuki *et al.*, 2007, 2005; Zeng and Li, 2006) since they are rich in phosphorus and ammonia yet there is no mainstream struvite recovery process available. Struvite precipitates in form of stable white orthorhombic crystals (Le Corre *et al.*, 2005) - the precipitation reaction can be expressed as (Zeng and Li, 2006):

Eq. 3.1 $$Mg_2^+ + NH_4^+ + HPO_4^{2-} + 6H_2O \rightarrow MgNH_4PO_4 \downarrow \cdot 6H_2O + H^+$$

[1] Contents of this chapter was published in Kruk *et al.* (2014).

Published research indicated the most important factors affecting struvite precipitation were found to be (Ben Moussa *et al.*, 2006; Doyle *et al.*, 2002; Le Corre *et al.*, 2005; Stratful *et al.*, 2001; Zeng and Li, 2006):

- the substrates saturation depending on the concentration;
- the molar ratio Mg^{2+}:NH_4^+:PO_4^{3-} ;
- pH;
- inhibition due to presence of other ions (e.g. Ca^{2+}, K^+, CO_3^{2-}).

According to Hao *et al.* (2008) the optimal molar ratio of Mg:N:P was 1.2:3:1. The pH affects the saturation index by changing the speciation of struvite substrates and other competing precipitates, such as magnesium phosphate or magnesium carbonate. It is generally agreed that struvite precipitation in municipal wastewater and sludge dewatering liquor occurs when the pH is higher than 7.5 and it rapidly increases until pH 10.5 (Doyle *et al.*, 2002; Zeng and Li, 2006). Hao *et al.* (2008) showed that optimal pH for precipitation of high purity struvite (> 90%) was between 7.5 and 9 and dropped to 7.0 to 7.5 when Ca^{2+} ions were present. Above pH of 9, or above pH of 7.5 in the presence of calcium, the precipitation of phosphorus took place in the form of magnesium or calcium phosphates (Hao *et al.*, 2008).

The most popular method of struvite production from wastewater is chemical precipitation by dosing magnesium salts and adjusting pH with a base (Schuiling and Andrade, 1999; Suzuki *et al.*, 2007; Zeng and Li, 2006) or by stripping CO_2 using aeration (Suzuki *et al.*, 2007, 2005). Among magnesium sources most frequently used are $MgCl_2$, MgO and $MgSO_4$ (Hug and Udert,

2013). Other magnesium compounds like $Mg(OH)_2$ and $MgCO_3$ are much less suitable due to their low solubility in water (Schuiling and Andrade, 1999; Zeng and Li, 2006).

Ben Moussa *et al.* (2006) and Wang *et al.* (2010) proposed to eliminate the need for alkalinity dosing using electrolytic cell with inert anodes. In accordance with the overall reaction of oxygen reduction and hydrogen evolution (Eq. 3.2 and Eq. 3.3), hydroxide anions are produced on the cathode surface. The process was shown to increase the interfacial pH of cathode by as high as 1.5 units in comparison to the bulk solution (Ben Moussa *et al.*, 2006). Thus, struvite deposition can be done in neutral pH of bulk solution (Ben Moussa *et al.*, 2006; Wang *et al.*, 2010). Ben Moussa *et al.* (2006) reported that electrochemical methods allowed production of pure struvite. In both cases external magnesium source was dosed.

Eq. 3.2 $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$

Eq. 3.3 $2H_2O + 2e^- \rightarrow H_2\uparrow + 2OH^-$

The phosphorus removal electrodes can also be used as the source of the coagulating ions (Holt *et al.*, 2005; Hug and Udert, 2013; Tran *et al.*, 2012; Wei *et al.*, 2009). In that case besides elevating the pH, the electrodes are also used to provide iron, aluminum or magnesium cations by electrolytic dissolution. Wei *et al.* (2009) reported instantaneous ortho-phosphate removal improvement from 19% to 86% after electric current was applied to the aluminum electrodes in their electrically enhanced membrane bioreactor. The reactor was fed with synthetic municipal wastewater. The phosphorus removal mechanism was precipitation in form of $(AlOH)_3(PO_4)_2$ and $AlPO_4$ with adsorption on $Al(OH)_3$. Hug and Udert (2013) employed electrolytical magnesium dissolution to precipitate struvite from source-separated urine. Phosphorus removal rate of 3.7 mg P $cm^{-2}h^{-1}$ at an impressed current density of 55A m^{-2} was achieved in a sequencing

batch reactor process with a 2 h cycle. Struvite production cost with electrochemical magnesium dosing (4.45 € $kg_{struvite}^{-1}$, assuming no electrode was the only source of magnesium) was shown to be competitive with dosing of $MgCl_2$ and $MgSO_4$.

Song *et al.* (1997) studied anodic dissolution of magnesium in chloride and sulphate solutions. They found that the magnesium dissolution process involves intermediate Mg^+ species and suggested the following reaction sequence:

Eq. 3.4 $Mg \leftrightarrow Mg^+ + e^-$

Eq. 3.5 $Mg^+ + H_2O \rightarrow Mg^{2+} + OH^- + 0.5\ H_2$

The reaction in Eq. 3.4 was assumed to be the rate determining step. Song *et al.* (1997) found that the presence of Cl^- ions reduced passive film area, and accelerated the release of Mg^+ ions from the metal magnesium.

3.2 MATERIALS AND METHODS

Both the synthetic solution and the WAS supernatant tests were conducted in a 1 L reactor equipped with a set of two magnesium electrodes, pH probe (Accumet 13-620-108A by Fisher Scientific) and a magnetic stirrer (Figure 3.1). The electrodes were shaped as 2 mm thick rectangular plates with an active surface area of 44 cm^2 (both sides of the electrodes were used) and were made of high purity alloy AZ91HP. Direct current was supplied to electrodes by BOP 100-2D (KEPCO, USA). Water deionized in Elix® Water Purification system (Millipore, USA) with electro conductivity EC of 0.08±0.01 μS cm^{-1} was used for preparation of synthetic solutions. Conductivity measurements during tests were done with Accumet 13-620-165 conductivity electrode (Fisher Scientific) and Accumet 13-620-10 temperature electrode

connected to Accumet XL50 meter (Fisher Scientific). Conductivity was corrected to the actual solution temperature.

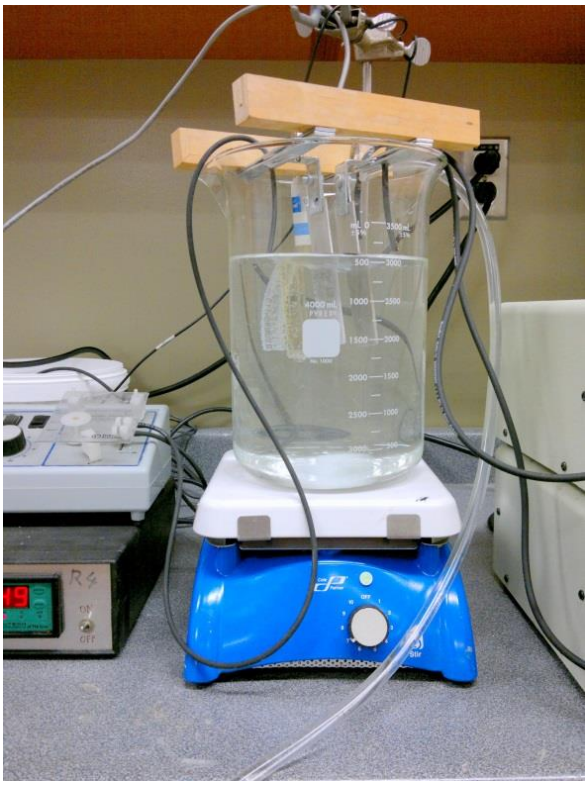


Figure 3.1: Reactor Set-Up for Electrochemical Phosphorus Recovery Batch Tests

3.2.1 Impact of pH and Electric Current on Struvite Purity and Phosphorus Removal Rate

A series of 3 h batch tests were conducted. The value of pH was continuously adjusted with 0.02N HCl solution dosed by Mini Variable-Flow Peristaltic Pump (Fisher Scientific) controlled by alpha pH200 controller (Eutech Instruments). Solution for all tests contained 24 mM $Na_2HPO_4 \cdot 7H_2O$ (743 mg P L^{-1}) and 46 mM NH_4Cl (645 mg N L^{-1}), which accounts for 1:1.9 molar ratio of P:N. Conductivity of electrolyte was adjusted to 10 mS cm^{-1} by dosing NaCl.

Four tests (T1 through T4) were run with electric current of 0.05 A and pH set points of 6.5, 7.5, 8.5 and 9.5. Three tests (T5 through T7) were run with pH set point of 7.5 and with the electric current of 0.1, 0.15 and 0.2 A.

During the tests 6 mL solution samples were taken every 30 min, to assess the nutrient removal rate. Phosphorus and ammonia in the samples were determined by flow injection analysis FIA, QuikChem Method 10-115-01-1-O and 10-107-06-1-I (QuikChem8500 by Lachat Instruments, USA).

3.2.2 Impact of Initial Substrate Concentration on Phosphorus Removal Rate

Five 3 h tests (T8-T12) were conducted at different initial ammonia and phosphorus concentrations (Table 3.1). Applied electric current and pH set point (power source and pH control as in previous tests) were the same for all five tests, 0.1 A and 7.5 respectively. As in all other tests, 6 mL grab samples were collected every 30 min, filtered and analyzed for ammonia nitrogen and ortho-phosphate using FIA.

Table 3.1: Initial Ammonia and Phosphorus Concentrations, pH Set Point and EC in Tests T8-T12

Test	Bulk pH	Current (A)	Ammonia (mg N L^{-1})	Phosphorus (mg P L^{-1})	N:P (mol:mol)
T8	7.5	0.1	490	548	1.98
T9	7.5	0.1	378	418	2.00
T10	7.5	0.1	286	313	2.02
T11	7.5	0.1	194	214	2.00
T12	7.5	0.1	98	105	2.05

An additional 7 h test (T13) was conducted to assess phosphorus removal rate at the elevated N:P concentration ratio. Initial concentrations of ammonia nitrogen and phosphorus were 482 mg N L^{-1} and 554 mg P L^{-1} (N:P molar ratio 1.92), respectively. In order to keep the ammonia concentration at a high level throughout the test, 10 mL of 30.6 g NH_4Cl L^{-1} (which accounts for 80 mg N) solution was dosed manually to the reaction beaker every two hours.

3.2.3 Phosphorus Removal from Fermented Waste Activated Sludge

Waste activated sludge was obtained from a high purity oxygen reactor in the South End Water Pollution Control Centre (SEWPCC) in Winnipeg, operating at a solids residence time of 2.5 d. The sludge was collected via return activated sludge (RAS) sampling tap. The sludge was sampled at the same time in the morning during a sludge pumping phase to ensure as much consistency as possible. Sludge was fermented for 48 to 72 h in a 4 L Nalgene batch reactors with low speed impeller mixer. After fermentation the sludge was decanted using IEC Multi centrifuge by Thermo at 6500 RPM. Sludge characteristic is presented in Table 3.2.

Three 3-hour tests of struvite precipitation were conducted on the supernatant of fermented sludge. The reactor was setup as in the previous tests. The value of pH was not controlled and the impressed current was set at 0.05, 0.1 and 0.2 A (current density CD of 11.4 A m^{-2}, 22.7 A m^{-2} and 45.4 A m^{-2}) in consecutive tests. During the tests 6 mL samples were taken every 10 to 15 min for FIA analysis of ammonia and ortho-phosphate.

Table 3.2: Characteristics of Sludge Used for Phosphorus Removal Tests

Parameter	Units	Value	Standard deviation
Raw WAS			
VS	g L^{-1}	6.94	0.20
TS	g L^{-1}	9.30	0.85
TP	mg L^{-1}	163	18
mg TP/g VS	mg g^{-1}	23.4	3.6
PO_4-P	mg L^{-1}	15.1	1.2
tCOD	g L^{-1}	10.7	0.24
sCOD	mg L^{-1}	56.5	1.50
pH		6.53	0.11
Fermented WAS supernatant			
PO_4-P	mg L^{-1}	56.1	4.2
NH_4-N	mg L^{-1}	113.8	28
pH	-	7.65	0.40
EC	mS cm^{-1}	1.8	0.2

3.2.4 Precipitate Analysis

At the end of tests T1-T7, the precipitate was harvested by filtration of treated solution on glass fiber filters (Whatman 934-AH by GE Healthcare UK Ltd) and dried at room temperature for 48 h. Due to frequent detachment of the precipitate from the surface of the anode harvested samples were a mixture of the precipitate from the bulk solution and from the anode. After homogenization of samples, X-ray diffraction (XRD) spectra were collected using a powder diffractometer D5000 (Siemens, Germany) using Cu $K\alpha_1$ radiation and operated at 40 kV and 0.4 A. The XRD analysis was not conducted on the sample from test T1 due to the insufficient amount of precipitate. Remaining samples were digested in 2% nitric acid for 24 h at 40°C. The magnesium, sodium and phosphorus content of digested samples were assessed by inductively

coupled plasma atomic emission spectroscopy analysis (Vista-MPX CCD Simultaneous ICP-OES analyzer by Varian). Digested samples after adjustment to pH of 6 were also analysed using FIA to determine the concentrations of ammonium and ortho-phosphate. Standard deviation of phosphorus results from ICP-OES and FIA analyses did not exceed 2.5%.

3.2.5 Struvite Purity Calculation

Most of the common struvite mineral impurities do not contain nitrogen, i.e. $Mg(OH)_2$, $MgHPO_4$, $Mg_3(PO_4)_2$, $MgKPO_4$, $CaHPO_4$, $Ca_5(PO_4)_3OH$ (Hao *et al.*, 2008; Zeng and Li, 2006; Le Corre *et al.*, 2005). Thus, for purity quantification it was assumed that each mole of ammonium stands for one mole of struvite. The struvite purity SP was calculated as in Eq. 3.6.

Eq. 3.6 $$SP = [NH_4\text{-}N]_{prec} \cdot [NH_4\text{-}N]_{struv}^{-1} = [NH_4\text{-}N]_{prec} \cdot (57 \text{ mg } g^{-1})^{-1}$$

Where $[NH_4\text{-}N]_{prec}$ is the measured concentration of the ammonium nitrogen in the precipitate and $[NH_4\text{-}N]_{struv}$ is the theoretical content of the nitrogen in the pure struvite (57 mg N g^{-1}).

Precipitates may also contain magnesium ammonium phosphates (MAP) with different hydration levels than struvite. Dittmarite for instance, is MAP monohydrate and its molecular weight is 37% lower than struvite and theoretical content of the nitrogen in pure dittmarite is 90 mg g^{-1}. Since many of the MAP hydrates may exist simultaneously and it is not possible to quantify all of them in the mixture (Sarkar, 1991), the assumption was made that in the ambient room temperature and humidity all MAP is hexahydrate (struvite). Even though this approach may result in purity values higher than 100%, authors find this method suitable for engineering use.

3.3 RESULTS AND DISCUSSION

3.3.1 Impact of pH and Electric Current on Struvite Purity and Phosphorus Removal Rate

XRD spectra of precipitates from tests T2-T7, presented in Figure 3.2, demonstrated high similarity in the position of peaks and relative peak values to spectrum of struvite standard. That indicated high purity of produced struvite. The molar ratios N:P:Mg in precipitate presented in Table 3.3 were calculated based on results of ICP and FIA analysis conducted on digested precipitate samples. Molar concentration of N was lower than P and Mg in all samples. This is in agreement with expectations, since most of struvite impurities, e.g. $MgHPO_4$, $Mg_3(PO_4)_2$, $Mg(OH)_2$, and when other ions are present e.g. $MgKPO_4$, $CaHPO_4$, $Ca_5(PO_4)_3OH$, do not contain ammonium ions (Hao *et al.*, 2008; Le Corre *et al.*, 2005; Zeng and Li, 2006). Thus, for purity quantification it was assumed that each mole of ammonium stands for one mole of struvite.

Table 3.3 Molar Ratios N:P:Mg in Precipitates from Tests T1 to T7

Test	bulk pH	current (A)	Molar ratio in precipitate				
			N	:	P	:	Mg
T1	6.5	0.05	1	:	1.15	:	2.36
T2	7.5	0.05	1	:	1.16	:	1.44
T3	8.5	0.05	1	:	1.11	:	1.28
T4	9.5	0.05	1	:	1.14	:	1.57
T2	7.5	0.05	1	:	1.16	:	1.44
T5	7.5	0.10	1	:	1.12	:	1.29
T6	7.5	0.15	1	:	1.09	:	1.30
T7	7.5	0.20	1	:	1.05	:	1.12

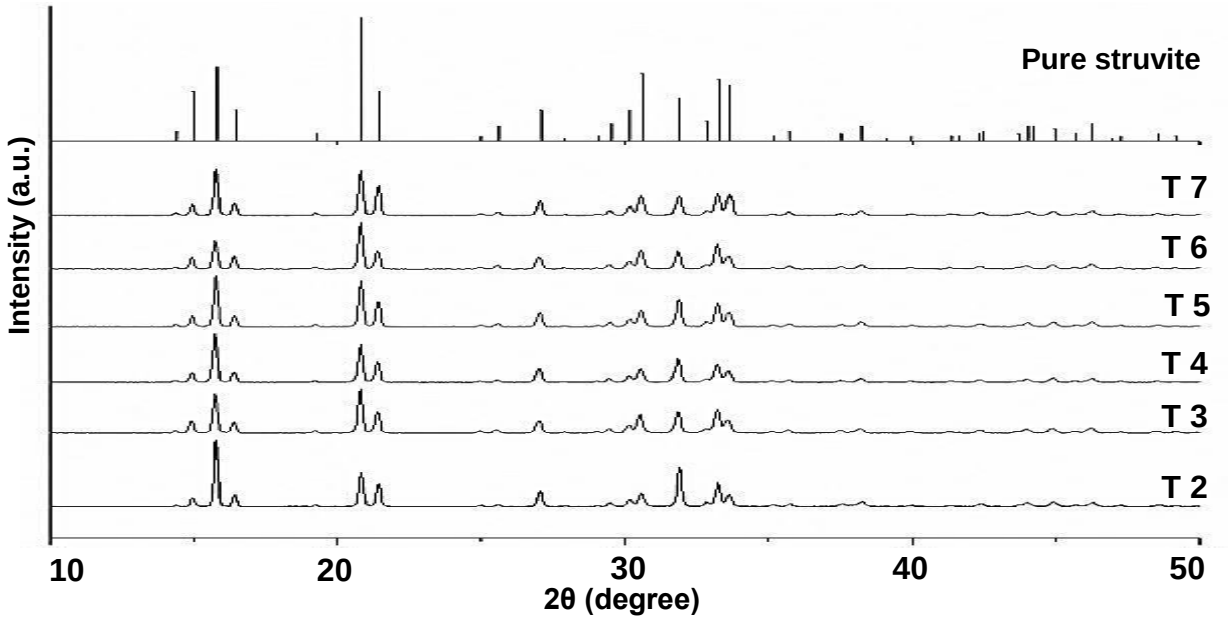


Figure 3.2: XRD Spectra for Precipitate Produced in Tests T2-T7 and Spectrum for Pure Struvite ($MgNH_4PO_4 \cdot 6H_2O$) According to International Centre for Diffraction Data

Purities calculated for tests T1-T4, where the current was constant at 0.05 A and the pH was changed in consecutive steps from 6.5 to 9.5 (Figure 3.3), indicate that optimum pH for struvite precipitation is in the vicinity of 8.5. High struvite purity above 90% was achieved in whole range of pH from 7.5 to 9.3. Visual MINTEQ software was used to calculate saturation indexes of possible precipitates at various pH. Out of all possible precipitates only two, $MgHPO_4 \cdot 3H_2O$ and $Mg_3(PO_4)_2$, had saturation index close to struvite in the pH range of 6.5 to 9.5 (Figure 3.4). There are few reports of struvite precipitation at pH below 7 (Doyle *et al.*, 2002; Zeng and Li, 2006), however in this study, even at pH of 6.5 the purity at 76% was still relatively high. The increase of pH from 6.5 to 7.5 resulted in an over three-fold increase of phosphorus removal rate, from 0.25 to 0.82 mg PO_4-P cm^{-2} h^{-1} (Figure 3.3). The increase is related to significantly decreased saturation index of struvite at lower pH. Figure 3.4 shows that at pH 6.5 the struvite is almost in the equilibrium with the solution. Further increase of pH resulted in only a 5% increase

of P removal rate and reached a plateau at 0.86 mg PO_4-P cm^{-2} h^{-1} at pH of 8.5. Thus, increasing the solution pH above 8.5 was not beneficial for the quantity, or for the quality, of produced struvite.

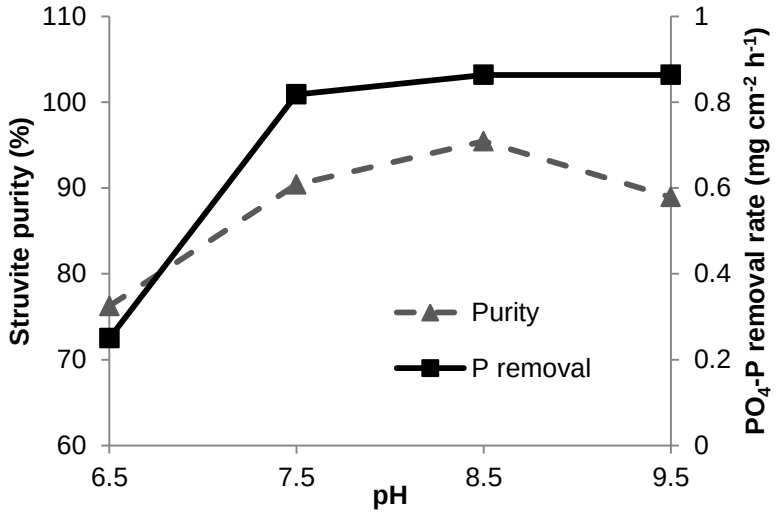


Figure 3.3: Struvite Purity and PO_4-P Removal Rate per Surface Area of the Electrode as a Function of Bulk Solution pH at 0.05 A.

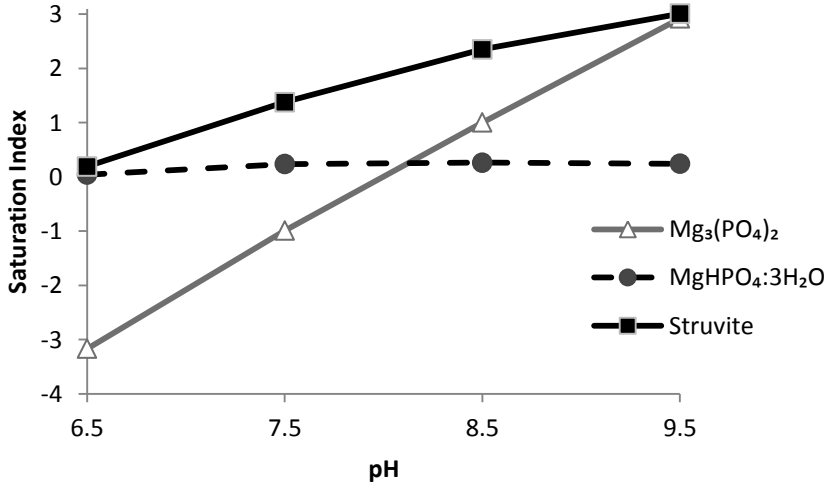


Figure 3.4: Saturation Index of Most Probable Struvite Impurities at Various pH: Results of Visual MINTEQ Model

Note: At initial concentration of NH_4^+, PO_4^{-3} and Mg^{+2} of 46mM, 24mM and 2.8 mM , respectively.

It was shown that struvite purity increased with increased values of applied electric current (Figure 3.5). The current increase from 0.05 to 0. 2 A resulted in a 13% increase of struvite purity at pH of 7.5. According to the Faraday's laws of electrolysis, mass of magnesium released from the anode is proportional to the delivered charge. Theoretical magnesium release can be calculated using the following equation.

Eq. 3.7 $m_t = A \cdot I \cdot t \cdot (n \cdot F)^{-1}$

Where m_t is theoretical magnesium release (g), A is atomic weight of magnesium (24.3 g mol^{-1}), I is electric current (A), t is time elapsed (s), n is magnesium valence (2) and F is Faraday constant (96,485 C mol^{-1}). However observed magnesium release can be even higher due to: (a) the loss of metal by spalling (detachment of metal chunks), (b) self-corrosion, (c) the formation of meta-stable monovalent magnesium ions and (d) charge wastage due to hydrogen evolution (Andrei *et al.*, 2003; Kim *et al.*, 2000). Hug and Udert (2013) reported observed magnesium release to be up to 220% higher than theoretical. Stratful *et al.* (2001) identified magnesium concentration as the main factor limiting struvite precipitation. Thus, correlation between the purity and the current observed in the present study may be explained by the aforementioned higher magnesium release rate at higher current, which elevated the Mg:P molar concentration ratio in the vicinity of the anode.

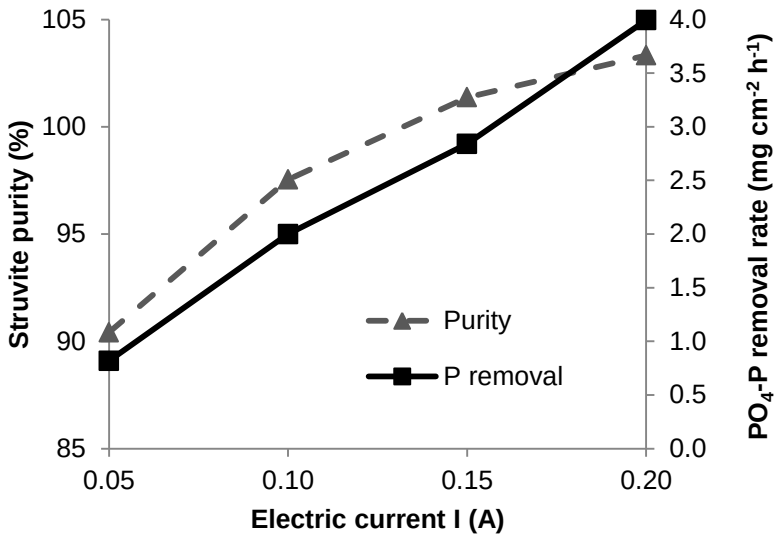


Figure 3.5: Struvite Purity and PO_4-P Removal Rate per Surface Area of the Electrode as a Function of Electric Current at pH of 7.5

Note: Struvite purities exceeding 100% are explained in Section 3.2.5

The phosphorus removal rate was established to be proportional to the electric current in the studied current range (Figure 3.5). Phosphorus removal rate from the synthetic solution of 4.0 mg PO_4-P cm^{-2} h^{-1} was achieved at a current density of 45.5 A m^{-2}, which is comparable with 3.7 mg PO_4-P cm^{-2} h^{-1} at 55 A m^{-2} reported by Hug and Udert (2013). Based on the results it seems that the higher the current the better is the overall system efficiency. However, to establish an optimum operation current two major factors should be considered: (a) local electric power and struvite prices and (b) impact of current density on biomass if coupled with biological treatment. Wei *et al.* (2011) reported that electric current density of 6.2 A m^{-2} does not significantly affect biomass viability for at least 4 hours. They did find though that the current density of 24.7 A m^{-2} decreased live cell count by 29%.

Cycles of deposition crust buildup and self-detachment on the surface of the anode were observed. Cycle time decreased when impressed current increased. On the surface of the cathode only a thin, white film-like layer was deposited. Therefore, no electrodes scraping was required.

3.3.2 Impact of Initial Substrate Concentration on Phosphorus Removal Rate

Tests T8 to T12 (results in Figure 3.6), indicate that the phosphorus removal rate decreased with initial concentrations of ammonia and phosphorus in the solution. Phosphorus removal rate deteriorated from 2.04 mg PO_4-P cm^{-2} h^{-1} in test T8 to 0.57 mg PO_4-P cm^{-2} h^{-1} in test T12 (Figure 3.7). This is in agreement with the expected decrease of struvite precipitation due to lower supersaturation of solution.

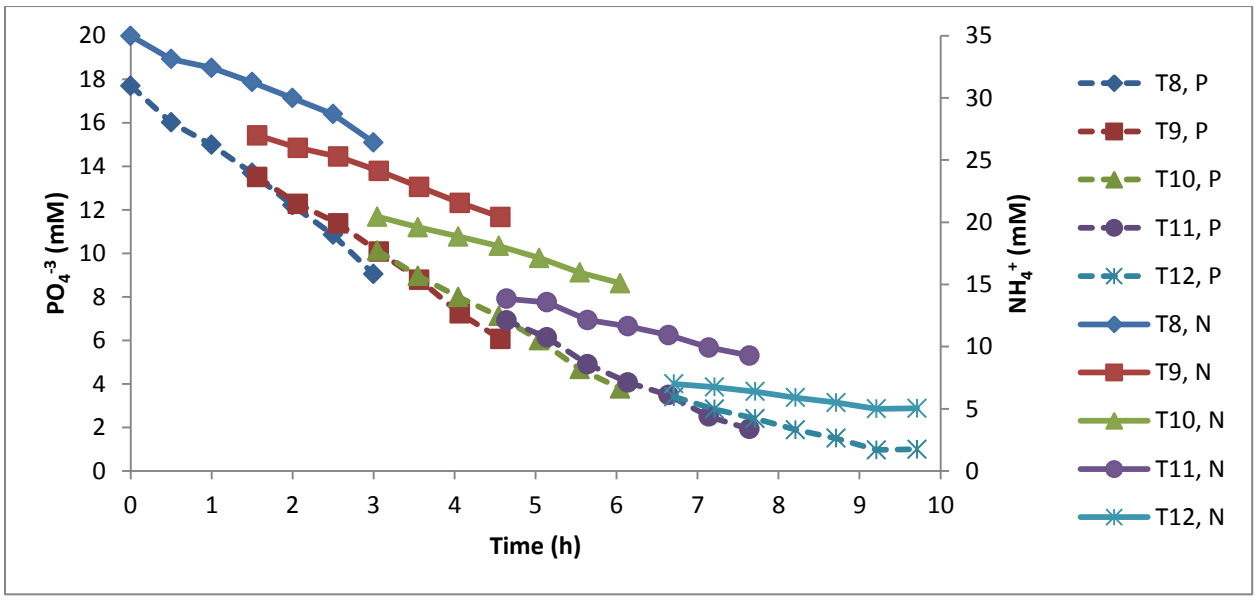


Figure 3.6: Combined Profiles of Ammonia and Phosphate Concentrations for Tests T8-T12

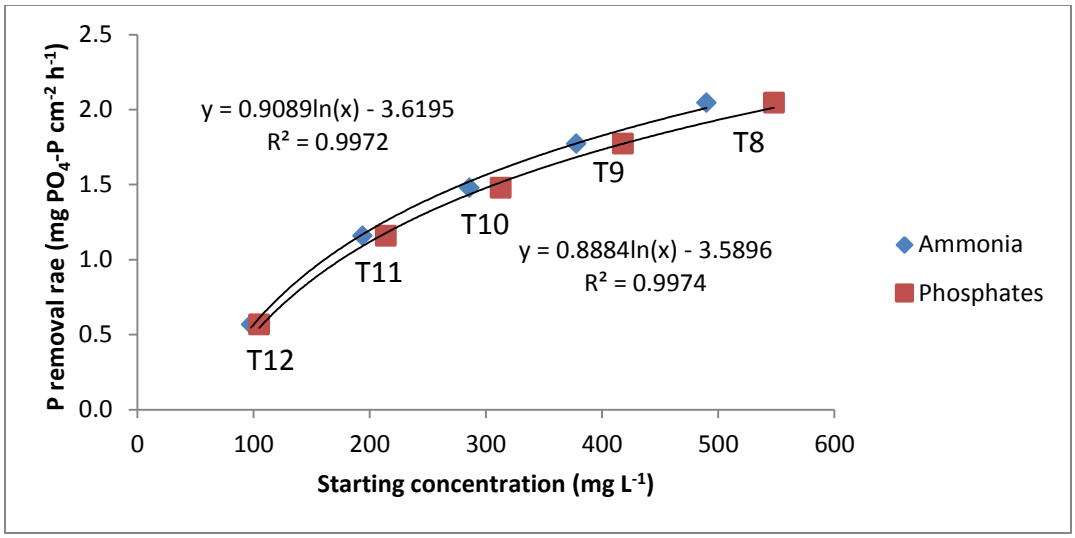


Figure 3.7: Phosphorus Removal Rate at Various Initial Concentrations of Ammonia and Phosphate

Tests T8-T12 were conducted with initial N:P mass concentration ratio close to 1. In practice, the mass concentration of phosphorus (PO_4-P) is much lower than mass concentration of ammonium nitrogen. Thus, concentration of ammonium nitrogen in test T13 was kept at elevated level throughout the test by dosing ammonium chloride.

Results presented in Figure 3.8 indicate almost constant removal rate of phosphorus (linear decline of phosphate concentration) throughout the test duration, despite decreasing phosphate concentration. The overall average removal rate achieved in the test was 1.70 mg PO_4-P cm^{-2} h^{-1}. The average ammonia concentration in the test was 401 mg N L^{-1}. Estimated removal rate based on tests T8 to T12 at this ammonia concentration is 1.83 mg PO_4-P cm^{-2} h^{-1}, which is only 8% higher than observed in test T13. This means that the concentration of ammonia has stronger impact on the phosphorus removal rate than the concentration of phosphorus in tested range, and decrease of phosphorus removal rate due to lower concentration of phosphorus can be partially offset by higher N:P molar concentration ratio.

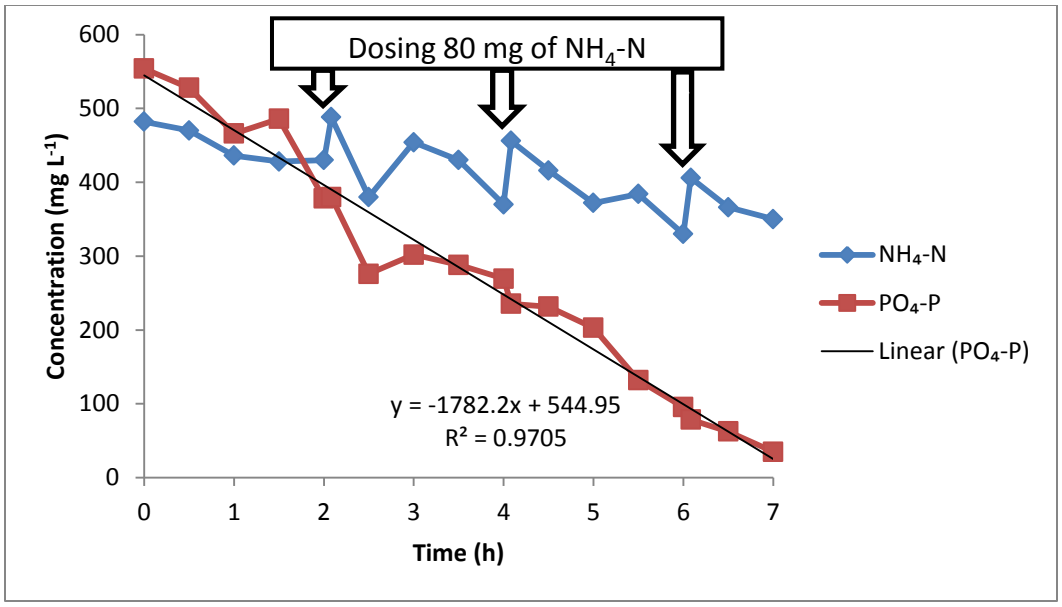


Figure 3.8: Ammonia and Phosphorus Concentrations Profiles for Test T13

3.3.3 Phosphorus Removal from Fermented Waste Activated Sludge

Tests performed on SEWPCC WAS fermented for 2-days and for 3-days showed high phosphorus removal rates, strongly dependent on applied electric current (EC). At 0.2 A the maximum removal rate was 3.95 mg PO_4-P cm^{-2} h^{-1}, and at 0.05 A 1.45 mg PO_4-P cm^{-2} h^{-1}. The results are comparable to those achieved in tests with synthetic solutions 4.00 mg PO_4-P cm^{-2} h^{-1} in T7 (pH of 7.5 and EC of 0.2 A) and 0.82 mg PO_4-P cm^{-2} h^{-1} in T2 (pH of 7.5 and EC of 0.05 A). The presented method was capable of reducing phosphorus to low levels, i.e. 1.3 mg PO_4-P L^{-1} at applied current of 0.2 A or 2.4 mg PO_4-P L^{-1} at 0.05 A. That translated to P removal efficiencies in the range of 95 to 98% at relatively low initial P concentrations of 56 mg PO_4-P L^{-1}. For comparison, fluidized bed struvite precipitation processes have been shown to successfully remove only 70% of P from digester supernatant at concentrations of 40 mg PO_4-P L^{-1} and achieve up to 90% removal at PO_4 concentrations of 70 mg P L^{-1} with sufficient Mg addition and

pH control (Britton *et al.*, 2005). In this research, even at the lowest tested electric current, almost complete removal of soluble P required no more than 1.75 h (Figure 3.9).

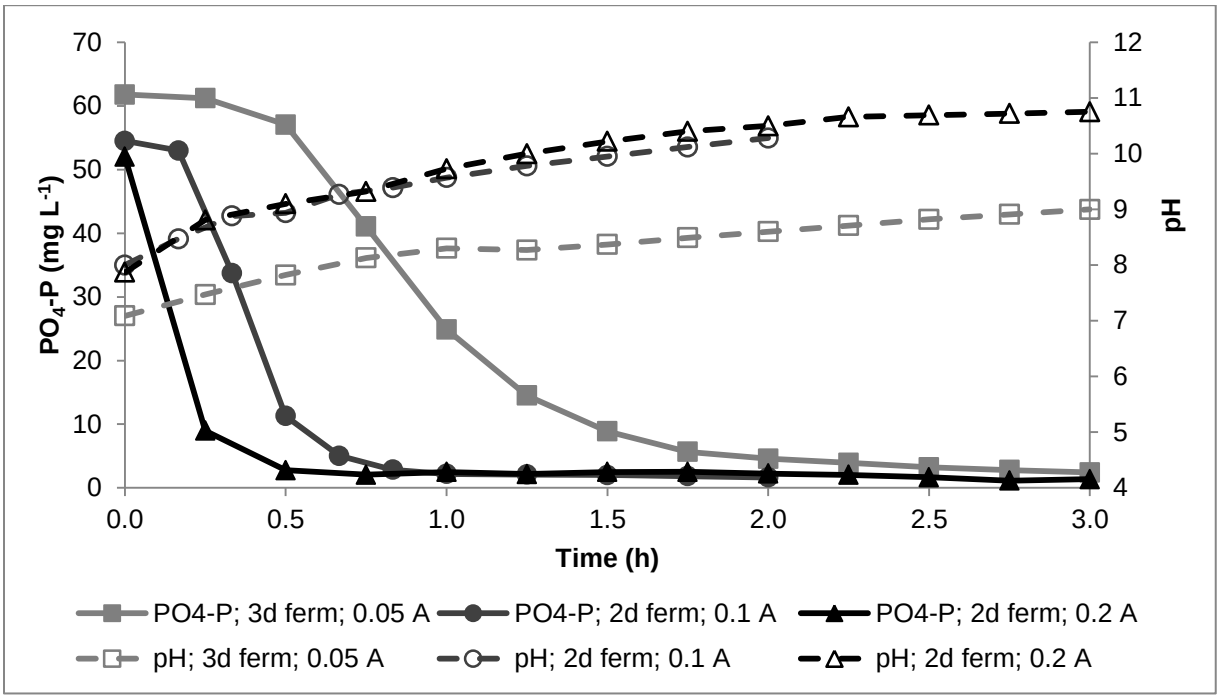


Figure 3.9: Soluble P and pH Profiles In Struvite Precipitation Tests with Fermented Sludge Supernatant

Note: Electric current was set to 0.05, 0.1 and 0.2 A in consecutive tests; pH was not controlled; two tests conducted on sludge fermented for 2d and one test on sludge fermented for 3d.

In addition, phosphorus and ammonia were removed in molar ratio close to 1:1. That suggests precipitation of high-purity struvite. Results from tests with the highest initial ammonia concentration (153 mg L^{-1}) are presented in Figure 3.10.

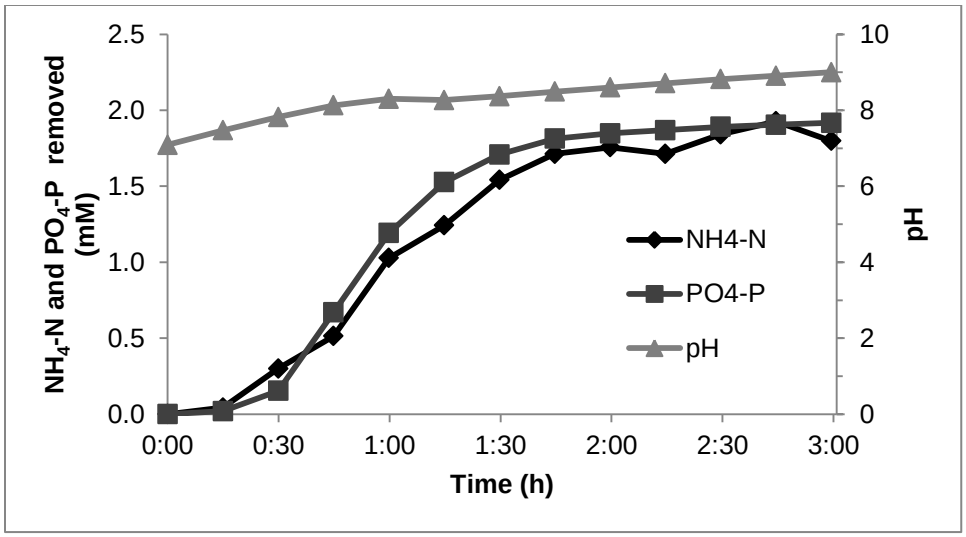


Figure 3.10: Soluble P And Ammonia N Removed In Struvite Precipitation Test

Note: WAS after 3d fermentation; pH was not controlled; electric current 0.05 A.

3.4 CONCLUSIONS

- Although struvite was produced in the whole pH range (6.5-9.5) studied, the highest struvite purity (above 90%) was obtained at pH 8.5 in the test with synthetic solution. The increase of applied electric current resulted in an increase of struvite purity and in proportional increase of phosphorus removal.
- High phosphorus removal rate of 4.0 mg PO_4-P cm^{-2} h^{-1} was attained at electric current density of 45 A m^{-2}. The rate depended strongly on initial concentration of ammonia and phosphorus in the solution, decreasing when concentrations decreased. The impact of low phosphorus concentration may be offset by increasing the N:P molar concentration ratio.
- Since the proposed method does not require any chemical dosing, does not have any harmful by-products and can produce high purity struvite at relatively low pH of 7.5, it can provide an alternative to chemical and biological phosphorus removal processes in water and wastewater

treatment systems. Unlike traditional chemical coagulation or precipitation with aluminium sacrificial anodes, this method allowed direct recovery as struvite.

- Electrolytic magnesium dissolution was shown to be an effective method of high-purity struvite precipitation and phosphorus removal from fermented waste activated sludge supernatant, which achieves removal efficiency of 98% within 2 h.

4 IMPACT OF ELECTRIC CURRENT ON NITRIFIERS

4.1 INTRODUCTION

In the proposed PN/A EASY MBR process nitrifying biomass will be exposed to the electric current. The objective of set of experiments presented in this chapter was to assess the effect of electric current on nitrification, and prove the concept of simultaneous process of nitrification and electrochemical struvite recovery. A comparison of AOB and NOB response to the electric current was conducted to identify the potential impact on the selective cultivation of AOB over NOB that is required for the partial nitritation process.

There is a number of emerging technologies involving direct electric current (DC) application directly in the mixed liquor (ML) in bioreactors in wastewater treatment (Huang *et al.*, 2014; Mustacchi *et al.*, 2005; Rodziewicz *et al.*, 2011; Wang *et al.*, 2013; Wei *et al.*, 2011; Zeyoudi *et al.*, 2015; Zhang *et al.*, 2012; Zurzolo *et al.*, 2013). The purpose for current (I) impression and the material which electrodes are made of may vary significantly. One approach is to use aluminum or iron sacrificial electrodes for phosphorus precipitation and/or to reduce membrane fouling in electrically enhanced membrane bioreactors (EMBR) (Ibeid *et al.*, 2013; Stafford *et al.*, 2014; Wang *et al.*, 2013; Wei *et al.*, 2011). Electric current causes oxidation of anode and release of metal cations (i.e. Fe^{+3}, Al^{+3}). The cations in the solution undergo similar processes as in conventional chemical coagulation and chemical phosphorus precipitation (Wei *et al.*, 2009), improving clarification and filterability of ML and reducing phosphorus effluent concentration. Kruk *et al.* (2014) and Hug and Udert (2013) used the same approach but employed magnesium electrodes. When magnesium electrodes were used in solutions containing ammonia and phosphate, magnesium cations released on the surface of anode Eq. 3.4 and Eq. 3.5 formed

magnesium ammonium phosphate precipitate (struvite; Eq. 3.1). Struvite is a value added product, it can be used as a high quality slow release fertilizer (Gaterell *et al.*, 2000). Electrochemical struvite precipitation has never been applied in the same reactor together with activated sludge process.

Other applications of electric current involve use of inert or graphite electrodes in bioelectrical reactors (BER) to provide either directly or indirectly electron donors and acceptors for bacterial metabolism (Goel and Flora, 2005; Li *et al.*, 2001; Thrash and Coates, 2008; Zhang *et al.*, 2012). Extensive research was conducted on use of BERs for simultaneous nitrification and autotrophic denitrification (Mousavi *et al.*, 2012; Thrash and Coates, 2008). In their configuration nitrifying biomass was provided with oxygen produced due to water electrolysis on the anode (Eq. 4.1), and the autotrophic denitrifiers were using hydrogen gas produced due to hydrogen evolution on the cathode surface (Eq. 4.2). Most of that research however focussed on hydrogen-driven denitrification and provided very little insight into activity of nitrification bacteria.

Eq. 4.1 Anode: $4OH^- \rightarrow O_2 \uparrow + 2H_2O + 4e^-$

Eq. 4.2 Cathode: $2H_2O + 2e^- \rightarrow H_2 \uparrow + 2OH^-$

Although, many studies were conducted in which biomass was exposed to the electric current, it is still not clear what impact current has on the nitrification biomass and more specifically on ammonia oxidizing organisms (AOB) and nitrite oxidizing organisms (NOB). Wei *et al.* (2011) reported 29% drop in live bacteria count in ML at current density of 25 A m^{-2}. Li *et al.* (2001) observed 20% decrease of ammonia removal rate at 15 A m^{-2}. Activity of NOB was not tested. Shin *et al.* (2011) used inert anode to supply oxygen for nitrification. Low NOB activity and positive correlation of AOB activity to applied voltage (U) were reported. Huang *et al.* (2014)

observed improvement of structure of aerobic granules, no effect on ammonia removal rate, and reduced inhibition of NOB at elevated free ammonia concentration. Others (Rodziewicz *et al.*, 2011; Wang *et al.*, 2013) did not observe any effects of current on nitrification activity.

There are several mechanisms of how direct current may affect activity of microorganisms in aquatic environment. Electromagnetic field may cause change of bacteria cell surface charge and lead to morphological changes or even cell lysis (Ibeid *et al.*, 2013; Qiao *et al.*, 2014; Wei *et al.*, 2011; Wouters and Smelt, 1997). Products of redox reactions occurring on both electrodes surfaces may stimulate bacteria serving as the electron carriers in their metabolic pathways (e.g. oxygen, hydrogen, quinones, phenazines, etc.). However, electrochemical reactions depending on the potential of the electrodes and pH may also produce strong disinfecting agents such as Cl_2 and H_2O_2 (Thrash and Coates, 2008; Wei *et al.*, 2011).

4.2 MATERIALS AND METHODS

4.2.1 Effects of Short Term Exposure to Electric Current

The experiments were conducted in order to test initial response of nitrifiers to the wide range of electric current. At this stage relatively inert, graphite electrodes were used in order to avoid any potential impact of magnesium metal on nitrification.

The experimental series consisted of six batch tests, three tests for ammonium oxidizing bacteria (T1-T3) and three tests for nitrite oxidizing bacteria (T4-T6). In each test, 5 reactors (R1 to R5) were set-up with voltage applied in the range of 0 to 19 V (Table 4.1). Test T1 to T3 and T4 to T6 were conducted in the same process conditions (pH, temperature, DO, initial alkalinity and

substrate concentration), to prove the statistical significance of the results. All tests were conducted at ambient room temperature. The temperature in the reactors was 22±0.5°C.

Table 4.1: Electric Current Applied During Short and Long Term Exposure Experiments

Experiment	Parameter	Unit	Value				
Reactors			R1	R2	R3	R4	R5
Short exposure	T1, T2, T3	U	VDC	0	5	10	15	19
		I	mA	0	54 ± 1	158 ± 7	273 ± 12	385 ± 7
		CD	A m^{-2}	0	2.6 ± 0.1	7.6 ± 0.3	13.0 ± 0.6	18.4 ± 0.3
		SEP	W m^{-3}	0	77 ± 2	452 ± 19	1169 ± 50	2092 ± 38
	T4, T5, T6	U	VDC	0	5	10	15	19
		I	mA	0	51 ± 3	142 ± 8	241 ± 13	314 ± 2
		CD	A m^{-2}	0	2.5 ± 0.1	6.8 ± 0.4	11.5 ± 0.6	15.0 ± 0.1
		SEP	W m^{-3}	0	74 ± 4	406 ± 22	1033 ± 56	1703 ± 9
Time period				days 0-200	days 201-249	days 250-285		
Long exposure		U	VDC	0	5	5		
		I	mA	0	100 ± 15	200 ± 26		
		CD	A m^{-2}	0	16.7 ± 2.5	55.6 ± 7.2		
		SEP	W m^{-3}	0	143 ± 21	286 ± 4		

Note: U voltage; I electric current; CD electric current density; SEP specific electric power input

The reactors were 3.5 L glass beakers (Figure 4.1 and Figure 4.2). Electrodes were made of fine grain rectangular graphite sheets, 0.5 mm thick (Tgon 820 by Laird Technologies) with active surface area of 209 cm^2, and were fixed to reactors' walls with an adhesive. The distance between the electrodes was 22 cm. The DC electric current was impressed with four PS503A power supplies (Tektronics) working in constant voltage mode. The voltage of the power supply was set-up at the beginning of each test and controlled several times during the tests using portable multimeters DM6400 (Gardner Bender). The current was constantly measured with four

DM502A multimeters (Tektronics). The pH in the reactors was measured with alpha pH200 meter (Eutech Instruments) and manually adjusted by dosing 1N HCl and 1N $NaHCO_3$ to keep it in the range of 7.5 to 8.0. The pH reading was made every 10 to 15 min, and during the reading power supply was turned off. The DO in the reactors was measured with portable Star Plus DO meter with RDO probe (Thermo Scientific). Constant aeration was provided with diaphragm air pumps and aeration stones to keep the DO level above 5.5 mg L^{-1}. Reactor content was stirred with magnetic stirrers.

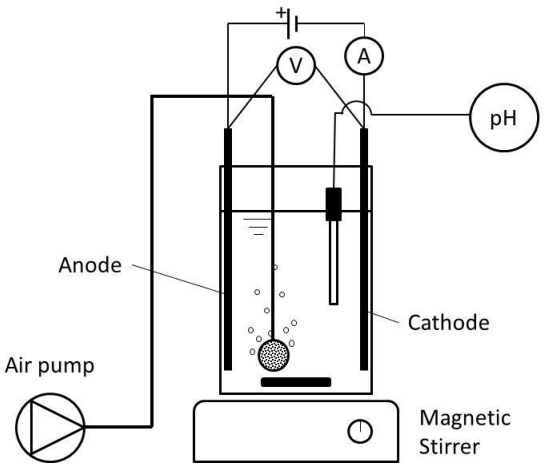


Figure 4.1: Schematic Reactor Setup for Short-Term Nitrifiers Exposure to Electric Current

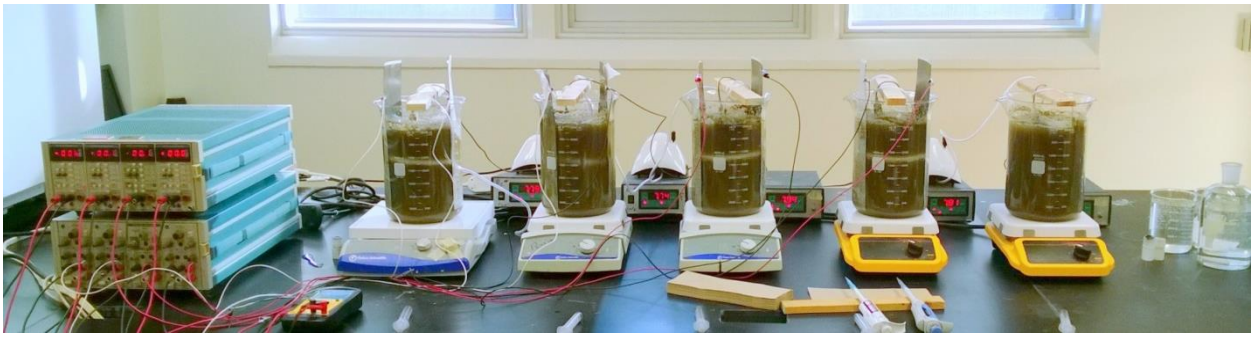


Figure 4.2: Picture of Experimental Set-Up for Short-Term Nitrifiers Exposure to Electric Current

The biomass used in the experiment was mixed liquor (ML) from Winnipeg West End Water Pollution Control Centre (WEWPCC). The WEWPCC employs West Bank biological nutrient removal process. Fresh ML batch grabbed directly from the end of the aeration zone was used for each testing day. Before the ML was transferred to the reactors for test runs it was aerated for at least 1.5 h in the laboratory. At that time samples for suspended solids where taken. The alkalinity of ML was adjusted to 400 mg $CaCO_3$ L^{-1} dosing $NaHCO_3$. The ML was continuously stirred while it was transferred to the reactors to assure homogenous biomass concentration in all reactors.

In all reactors ammonia and nitrite were spiked, at the beginning of activity tests of AOB and NOB, respectively. Aqueous solutions of NH_4Cl and $NaNO_2$ were dosed to provide 40 mg L^{-1} of ammonia nitrogen and 15 mg L^{-1} nitrite nitrogen, respectively. Samples (9 mL) for soluble nitrogen speciation analysis were taken every 30 min during AOB activity tests and every 15 min in NOB tests. All samples were filtered directly after sampling through grade 1 cellulose filter papers (Whatman, 1001-125).

4.2.2 Long Term Effects of Electric Current and Simultaneous Struvite Precipitation

The second phase of the study was conducted to assess the effects of electric current on nitrifiers in the long term operation. Inert graphite electrodes were still used in the first stage of the experiment to avoid any potential interference of magnesium metal. Later the electrodes were replaced with magnesium to prove the concept of simultaneous nitrification and struvite recovery. The test was conducted at ambient room temperature. The temperature in the reactor was 22±0.5°C.

The experiment was conducted in one of the reactors used in short term exposure tests. The reactor was additionally equipped with feed, effluent and sludge wasting peristaltic pumps and pH controller (Figure 4.3). Also, the magnetic mixer was replaced with an overhead stirrer. The reactor was operated in SBR mode with 8 h cycle consisting of: 7 h 10 min aeration including four 15 min feeding periods; 40 min settling; 5 min decantation; and 5 min idle. At the beginning of the experiment, the reactor was seeded with a fresh batch of biomass from the WEWPCC. Synthetic feed with high total nitrogen (TN) of 90±8 mg L^{-1} was used (detailed feed stock is presented in Table 4.2). The feed contained beef and yeast extracts, providing C:N ratio of 4:10. The minimal amount of bioavailable carbon was provided to sustain small population of heterotrophic microorganisms to improve sludge settleability. The HRT and SRT were maintained at 18.7 h and 10 d, respectively. The pH was adjusted to 7.6±0.2 using 0.1 N $NaHCO_3$. After 200 days of start-up and stabilization period 100 mA (5 V) was applied to the reactor through a pair of graphite plates (12 cm by 5 cm immersed dimensions) installed in the center of the reactor with 3 cm gap. The current was ON only during aeration period in 5 min ON and 5 min OFF cycle. After next 49 days the electric current was increased to 200 mA. Two days later, on day 252, the electrodes were replaced with magnesium electrodes (12 cm by 3 cm

immersed dimensions) to start the process of phosphorus recovery. Electric current passing through the magnesium anode oxidized it providing magnesium cations for phosphorus precipitation as struvite. That electrochemical phosphorus recovery method was described in detail in Chapter 3. The gap between electrodes had to be adjusted to keep the voltage constant. Detailed process conditions are presented in Table 4.1. Polarity of cell was switched every day to allow uniform electrode depletion. Replacement of electrodes to magnesium promoted accumulation of precipitate in the reactor. At that time upflow sedimentation column was installed (Figure 4.3). During the aeration phase the ML was recirculated through the column with upflow velocity of 1.2 m min^{-1}. Material with settling velocity higher than 1.2 m min^{-1} accumulated and was manually extracted from the bottom of the column.

Table 4.2: Feed Composition for Long Term Exposure Experiment

Component	Concentration in feed, mg L^{-1}
KH_2PO4	6 to 20
K_2HPO4	28 to 85
Beef Extract	50
Yeast Extract	50
$MnSO_4 \cdot H_2O$	1
$MgSO_4$	40
$FeSO_4 \cdot 7H_2O$	15
NH_4Cl	320
$CaCl_2$	180
$NaHCO_3$	600

Feed was prepared with tap water.

Throughout the experiment three times per week samples of feed, effluent and wasted sludge were taken for analyses. Regular *in situ* kinetic tests were conducted to assess the maximum

specific substrate utilization rate ($SSUR_{max}$). The tests were conducted without electric current at the end of regular cycle just before a decantation period. For the duration of the test the aeration was turned back ON and the nitrite and ammonia were spiked for NOB and AOB activity test, respectively.

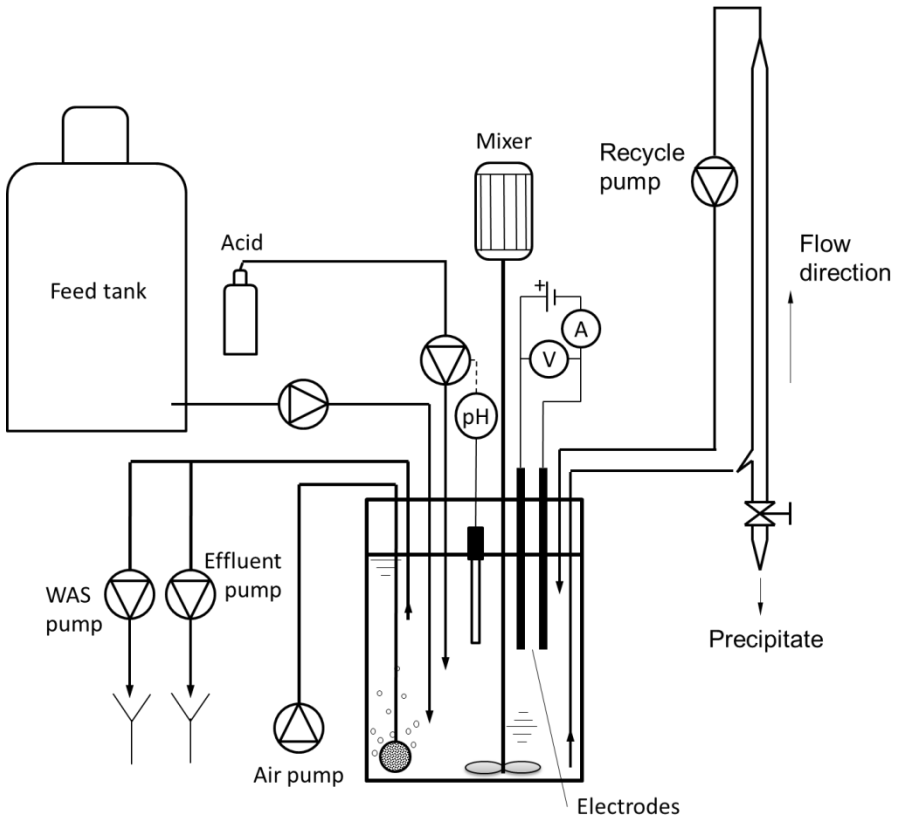


Figure 4.3: Experimental Set-Up for the Long-Term Experiment with an Upflow Sedimentation Column

4.2.3 Activity Indicators

The main indicator of nitrifiers' activity was the $SSUR_{max}$ calculated from the results of kinetic tests as grams of nitrogen removed per day per grams of volatile suspended solids (g N g VSS^{-1} d^{-1}). In addition, during the long term exposure experiment, specific substrate utilization rate

(SSUR) was calculated based on the substrate mass balance in the regular operation of the reactor (g N g VSS^{-1} d^{-1}).

Relative substrate utilization rate (RSUR) was calculated as a ratio of $SSUR_{max}$ at the relevant voltage to the rate in the control reactor (U = 0 V) in the same short term exposure test. RSUR was expressed in %.

4.2.4 Analysis

Concentration of ammonia, nitrite and nitrate nitrogen species in wet samples were measured using flow injection analysis FIA, QuikChem Method 10-115-01-1-O and 10-107-06-1-I (QuikChem8500 by Lachat Instruments). Suspended solids concentration and alkalinity analysis were performed as outlined by Standard Methods (Eaton and Franson, 2005).

X-ray diffraction (XRD) analysis of precipitate generated in the last period of long term exposure experiment was conducted as previously described in section 3.2.

4.3 RESULTS

4.3.1 Effects of Short Term Exposure to Electric Current

Results of short term exposure tests were very consistent, with a standard error in the range of 0.2 to 7.9% (Figure 4.6). The average $SSUR_{max}$ in control reactors was 131±6 mg NH_4-N g VSS^{-1} d^{-1} and 154±11 mg NO_2-N g VSS^{-1} d^{-1} in AOB and NOB tests, respectively (Figure 4.4 and Figure 4.5). Increased current caused almost linear decrease of AOB activity, resulting in a relative ammonia removal rate of 78±6% at 385 mA (19 V) (Figure 4.6). Conversely the NOB population was stimulated by the electric current. Relative nitrite removal rate reached maximum

of 130±9% at 142 mA (10 VDC). Despite descending trend for current greater than 142 mA, relative NOB activity did not decrease below 100% in the studied range.

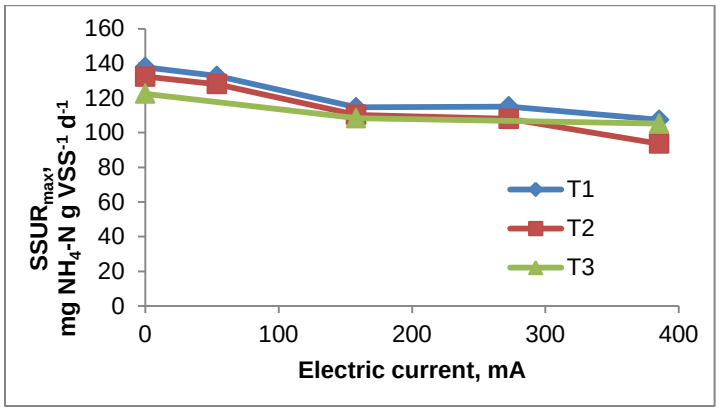


Figure 4.4: Effect of Short Term Exposure to Electric Current on $SSUR_{max}$ of AOB

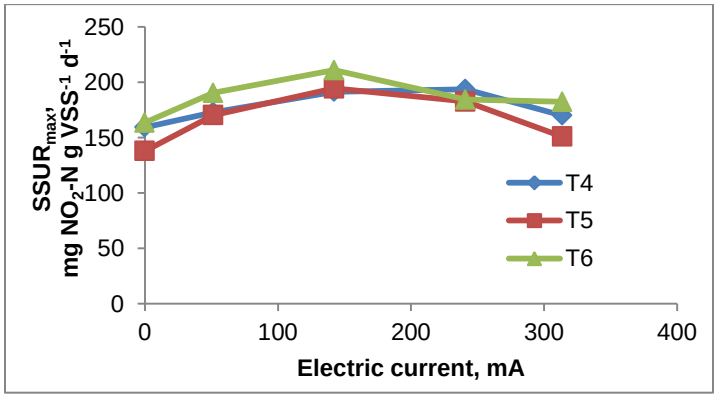


Figure 4.5 Effect of Short Term Exposure to Electric Current on $SSUR_{max}$ of NOB

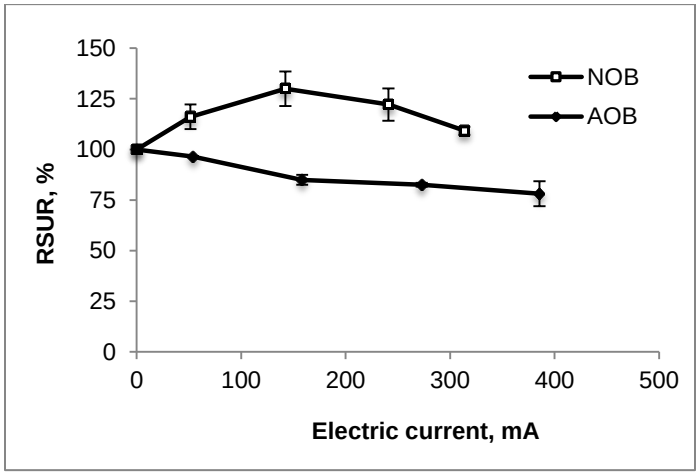


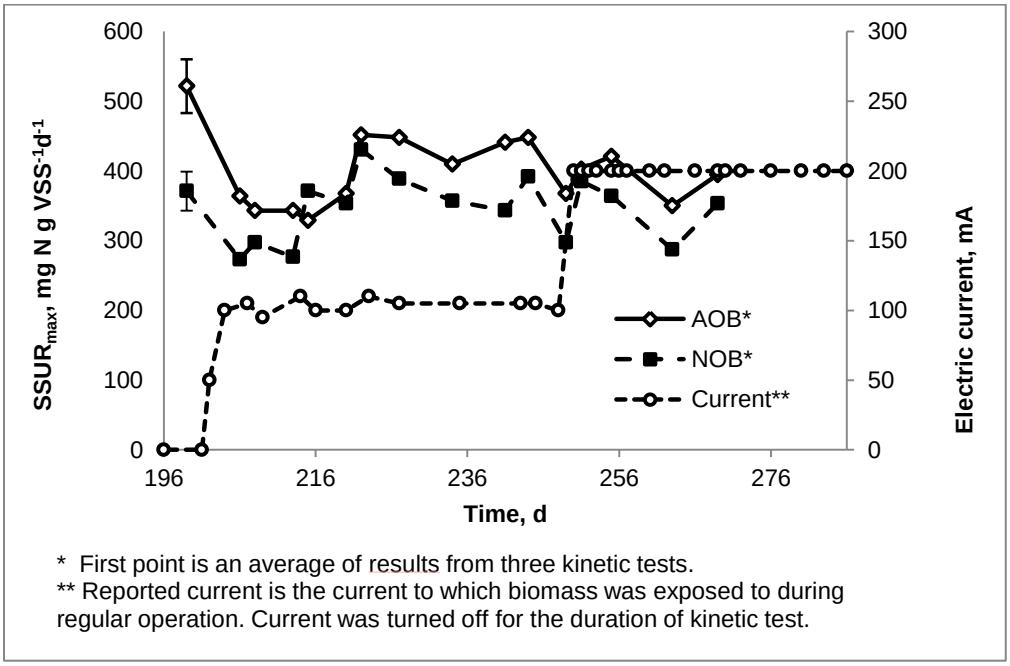
Figure 4.6 Effect of Short Term Exposure to Electric Current on Relative Substrate Utilization Rate

4.3.2 Long Term Effects of Electric Current

The start-up and stabilization period of the long term exposure experiment lasted 200 days. At the end of this period activity of AOB and NOB biomass was 522±38 mg NH_4-N g VSS^{-1} d^{-1} and 371±28 mg NO_2-N g VSS^{-1} d^{-1}, respectively (Figure 4.7). The feed used in this experiment had very low C:N ratio (4:10) in order to reduce heterotrophic growth to minimize potential shielding of nitrifiers. As a result mixed liquor VSS (MLVSS) was relatively low at 540±42 mg L^{-1} (2050±217 mg L^{-1} on average in fresh ML used in short term exposure tests), which together with much brighter yellowish colour and high $SSUR_{max}$ may indicate high autotrophic nitrifiers enrichment.

Electric current of 100 mA (5 VDC) was introduced on day 201 and was applied in a 5 min ON and 5 min OFF cycle. Introduction of the current caused initially decrease of the activity of both nitrifying populations (Figure 4.7). The $SSUR_{max}$ of AOB and NOB dropped by 36% and 26%, respectively. Following the inhibition period, 15 days after the current was first applied NOB activity fully recovered. The inclining trend continued to reach the maximum at 421 mg NO_2-N g VSS^{-1} d^{-1} (113% of the baseline activity), 22 days after electric current was introduced. In following days $SSUR_{max}$ declined slightly eventually stabilizing at the level close to that from before current was introduced. No notable change of the activity of NOB was observed even when the electric current was increased to 200 mA (5 VDC) on day 250. The average $SSUR_{max}$ after the initial decline and recovery was 360±39 mg NO_2-N g VSS^{-1} d^{-1} (97% of the baseline activity). The beginning of the AOB recovery trend was observed 20 days after the current was introduced (5 days later than NOB). However, AOB had not recovered fully. The average $SSUR_{max}$ of AOB after the recovery was 419±53 mg NH_4-N g VSS^{-1} d^{-1} (80% of the baseline activity). The increase of the electric current to 200 mA resulted in further decline of AOB

activity. However, at that time no sudden drop and recovery period was observed as when the current was first introduced. The average $SSUR_{max}$ at 200mA was 387±28 mg NH_4-N g VSS^{-1} d^{-1} (74% of the baseline activity). Despite the decrease in the $SSUR_{max,}$ no accumulation of ammonia or nitrite occurred in the regular operation of the SBR (Figure 4.8). The SSUR calculated based on nitrogen mass balance was in the range of 160 to 230 mg N g VSS^{-1} d^{-1} for both AOB and NOB and was a function of the nitrogen load.



* First point is an average of results from three kinetic tests.
** Reported current is the current to which biomass was exposed to during regular operation. Current was turned off for the duration of kinetic test.

Figure 4.7: Effect of Long Term Exposure to Electric Current on SSUR

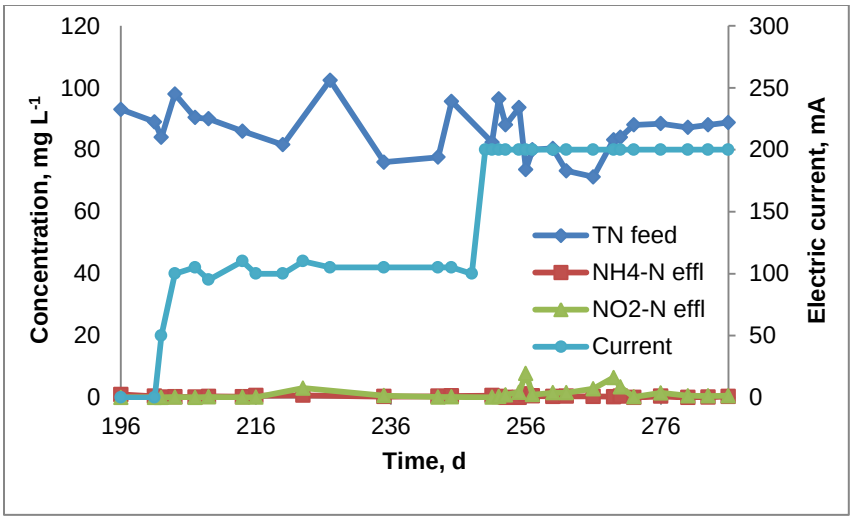


Figure 4.8: The Concentration of Ammonia and Nitrite in Effluent from SBR in Regular Operation During Long Term Exposure Experiment

A number of attempts were made to find the correlation between results from long and short term exposure experiments. The best fit gave tying the RSUR to electric current applied per mass of MLVSS in the reactor (I:MLVSS; mA g VSS^{-1}). The decrease of AOB activity was approximated using exponential function with normalized root-mean-square deviation (NRMSE) of 0.11. The changes in NOB activity were modeled separately with linear function for the activity increase (0 to 20 mA g VSS^{-1}) and with logarithmic function for activity decline (≥20 mA g VSS^{-1}) with NRMSE of 0.07 and 0.08, respectively.

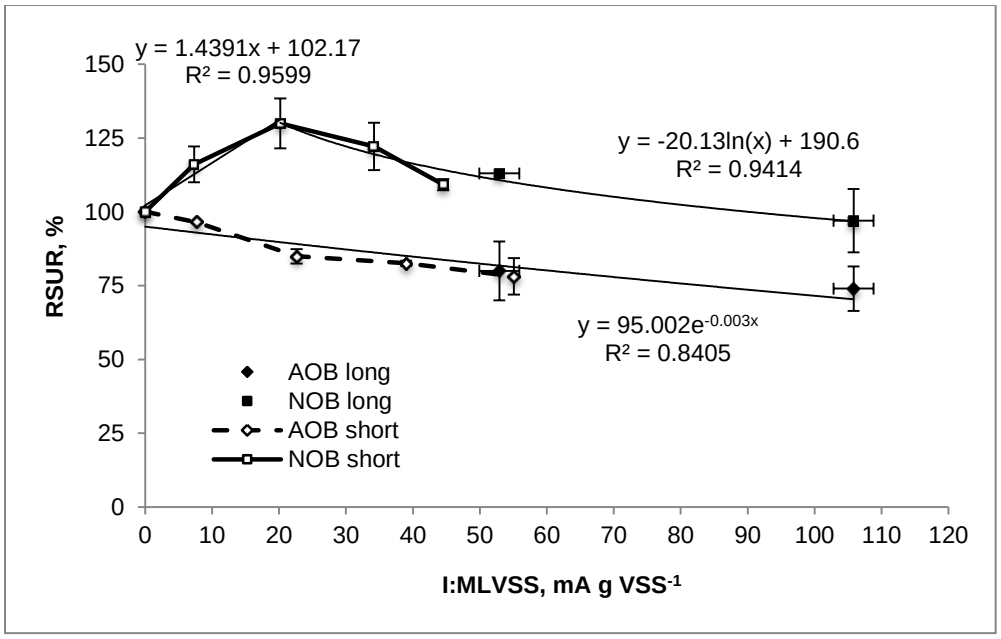


Figure 4.9: Correlation of RSUR and Electric Current to MLVSS Ratio

Results from long- and short-term experiments included.

4.3.3 Phosphorus Precipitation

Neither AOB nor NOB were affected negatively by change of electrodes from graphite to magnesium on day 252. The lower $SSUR_{max}$ values on day 263 coincided with lower loading of nitrogen to the SBR between days 261 and 266 (Figure 4.7 and Figure 4.8). The removal of phosphorus, before magnesium electrodes were introduced, was on average 2.1±0.6 g P m^{-3} d^{-1} with an average concentration in the effluent of 5.2±0.4 mg L^{-1} (Figure 4.10). On the day when electrodes were changed the phosphorus concentration in the feed was increased from 6.5 to 20.5 mg L^{-1}. This resulted in the effluent concentration of 14 mg L^{-1}. Following the initial spike, the effluent concentration declined and dropped to 2.2 mg L^{-1} after 5 days. At that time in order to check if lower phosphorus effluent concentrations are attainable without changes in electric current, the concentration of phosphorus in the feed was reduced to 14.5 mg L^{-1}. This change, however, did not provide much improvement, with an average effluent concentration of 1.9±0.2

mg L^{-1} thereafter. The highest phosphorus removal rate achieved was 22.0 g P m^{-3} d^{-1} on day 256. The average efficiency of phosphorus removal after effluent concentration stabilized was 86±2%.

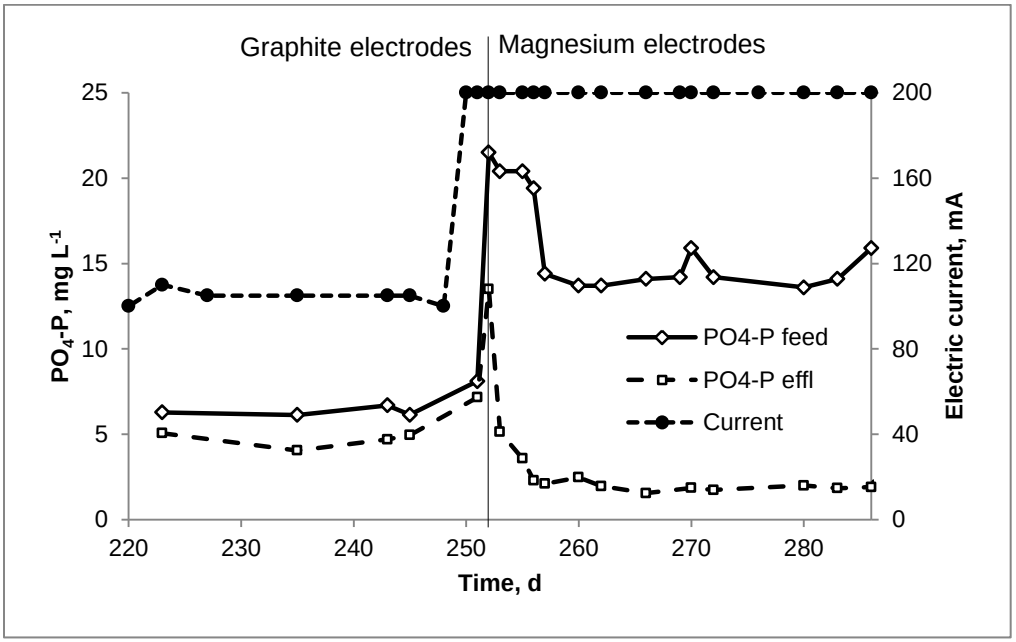


Figure 4.10: Phosphorus Removal Using Magnesium Electrodes in the SBR

After magnesium electrodes were introduced accumulation of precipitate in the reactor was observed. The precipitate was formed in big (>2cm) flake like aggregates on the surface of the electrodes. The aggregates regularly fell off the electrodes when thickness exceeded approximately 1 mm (dimensions not measured). The size of the aggregates was later reduced due to intensive stirring of the reactor. On day 254 upflow sedimentation column was installed and portion of the precipitate was regularly harvested. Figure 4.11 presents the comparison of precipitate harvested in this study to products of two leading struvite recovery technologies, Ostara Pearl an AirPrex. XRD results indicated that struvite was present in all harvested samples but brucite (magnesium hydroxide) was the dominant mineral. Struvite was also the only

detected mineral containing phosphate. Example of XRD spectra for combined sample is presented in Figure 4.12.

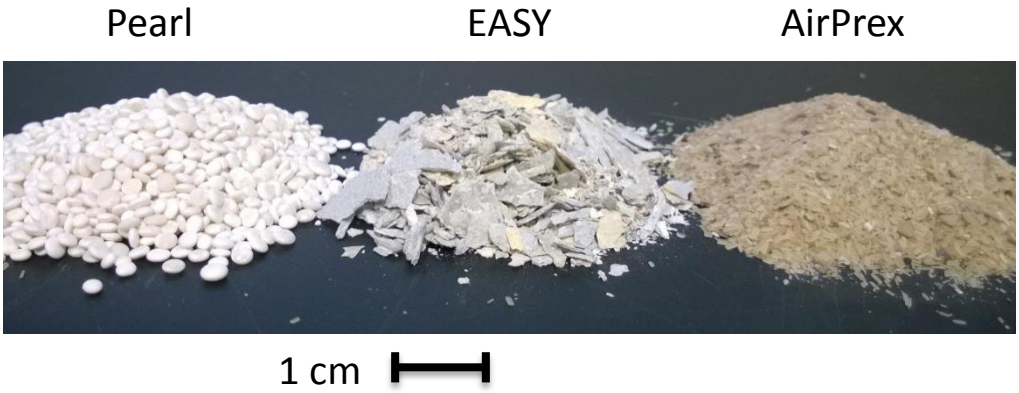


Figure 4.11: Comparison of Struvite Products Generated in this Study and Two Leading Commercial Technologies, Pearl and Airprex

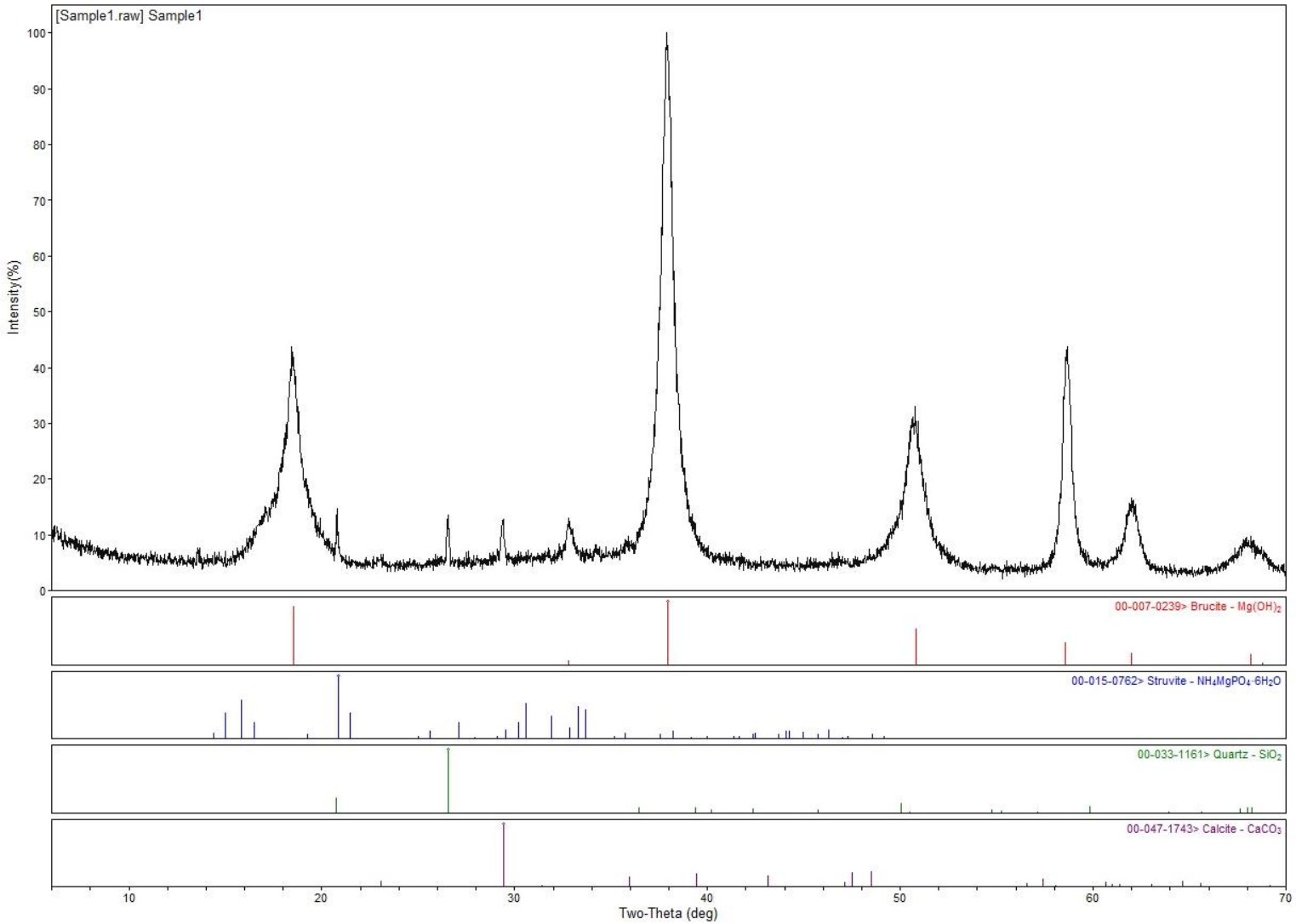


Figure 4.12: XRD Spectra of a Combined Sample of Precipitate and Reference Spectra of Most Probable Constituents

Reference spectra from International Centre for Diffraction Data.

4.4 DISCUSSION

The literature is inconclusive about inhibition and stimulation effect of the electric current on the nitrifying biomass. Li *et al.* (2001) found that the ammonia removal rate in the biofilm decreased by 20% at 15 A m^{-2}. Shin *et al.* (2011) reported positive correlation of ammonia removal rate with cathode potential (240% increase between 1.3 and 2 V; I or CD not reported). Rodziewicz *et al.* (2011) did not observe any change in nitrification efficiency between 0.2 and 1.5 A m^{-2} (U not reported). The main difference between aforementioned studies is the DO concentration in the bulk of the reactor volume. Study reporting nitrification stimulation was conducted under low DO conditions and electrodes were used to actually produce oxygen due to water hydrolysis. The higher the electric current the more oxygen was provided to the bacteria, hence better substrate removal rate. In studies in which electric current inhibited biomass or no change was observed, the DO was not a limiting factor. It may suggest that electric current indeed affects negatively the nitrifiers' activity; however, the impact is weak enough that at low currents may be not noticeable or even overcome by increased oxygen supply. Results of this study are only partially in agreement with this hypothesis, because while AOB activity was slightly inhibited by the electric current (almost linear negative correlation) the NOB activity was stimulated for I:MLVSS below approximately 90 mA g VSS^{-1}. According to preferential sequence of redox reactions of nitrogen species presented in Table 4.3, the reduction of nitrite to nitrogen gas is the most electrochemically favourable of nitrogen reactions. This may result in overestimation of the $SSUR_{max}$ for NOB. However, the kinetic tests during long term exposure experiment were conducted with current turned OFF and still the RSUR of NOB reached the maximum of 113% confirming the initial results of short term exposure tests. The specific trend of NOB activity changes with steep incline with maximum at 20 mA g VSS^{-1} and gradual decline thereafter,

suggesting that there are at least two counteracting mechanisms affecting NOB activity, and the NOB may be inhibited at higher currents. Huang *et al.* (2014) also observed positive impact of electric current on NOB. In their study application of less than 0.35 mA g VSS^{-1} (indirect estimation) eliminated inhibition of NOB even at free ammonia level of 18 mg L^{-1} (almost complete nitrite accumulation in the control reactor).

Table 4.3: Nitrogen Redox Reactions in Declining Order or Standard Potential

Half reaction		E_H^0, V	Eq. number
Reduction			
$O_2(aq) + 4H^+ + 4e^- \rightarrow$	$2H_2O$	1.272	Eq. 4.3
$2NO_3^- + 12H^+ + 10e^- \rightarrow$	$N_2(aq) + 6H_2O$	1.225	Eq. 4.4
$NO_2^- + 8H^+ + 6e^- \rightarrow$	$NH_4^+ + 2H_2O$	0.897	Eq. 4.5
$NO_3^- + 10H^+ + 8e^- \rightarrow$	$NH_4^+ + 3H_2O$	0.881	Eq. 4.6
$NO_3^- + 2H^+ + 2e^- \rightarrow$	$NO_2^- + H_2O$	0.854	Eq. 4.7
Oxidation			
$2NH_4^+ \rightarrow$	$N_2 + 8H^+ + 6e^-$	0.28	Eq. 4.8

It is important to note that despite the observed decline of the AOB activity in kinetic tests, there was no accumulation of ammonia in the SBR during the long term operation experiment, even when it was operated at a current of 105 mA g VSS^{-1}. Nitrifiers were clearly limited with substrate in the regular operation (the minimum $SSUR_{max}$ of AOB of 343 mg N g VSS^{-1} d^{-1} vs maximum SSUR of 230 mg N g VSS^{-1} d^{-1}). Thus, the absence of noticeable nitrification inhibition in the SBR might be explained by the inherent overcapacity of the system. Based on the results it was found safe to apply electric current of up to 20 mA g VSS^{-1} to nitrifying biomass, which led to only moderate (~10%) reduction in the $SSUR_{max}$ in studied range of VSS

and pH. Also, the application of electric current in aerated reactors will counteract NOB out-selection for partial nitrification processes.

The strong correlation between nitrifiers activity and the I:MLVSS excluded potential impact of electrodes change from graphite to magnesium. The results suggest that in the tested conditions it is the electric charge per mass of biomass what will determine the bacteria activity rather than the electromagnetic field. Thus, observed inhibition and stimulation of activity of nitrifiers in studied voltage range would be most probably caused by direct impact on metabolic processes in opposition to changes to morphology or physical cell rupture. Nevertheless, at this stage further detailed research would be required to explore the exact mechanisms behind observed changes in activity.

The small phosphorus removal (2.1±0.6 g P m^{-3} d^{-1}) before magnesium electrodes were introduced may be attributed to conventional assimilation by growing biomass (Metcalf & Eddy *et al.*, 2014). When electrodes were changed to magnesium phosphorus removal drastically increased due to the process of electrochemical precipitation with magnesium. The maximum rate of 22.0 g P m^{-3} d^{-1} is comparable with other electrochemical studies (Hug and Udert, 2013; Chapter 3) and enhanced biological phosphorus removal (Metcalf & Eddy *et al.*, 2014). Electric current of 200 mA was chosen mainly to test nitrification activity under wide range of currents. Following the calculation method presented in section 3.3.1 the theoretical magnesium dose at this current delivered over 30 times more magnesium needed for the phosphorus recovery as struvite (calculated at 20.5 mg P L^{-1} in the feed). This provided ample capacity for overall removal rate; the actual removal rate could be higher at higher phosphorus loading. However, magnesium requirements for struvite precipitation strongly depend on the initial concentration of

substrates (phosphorus and ammonium), and the intermittent feeding mode designed to avoid inhibition of nitrifiers with high concentration of ammonia in the feed caused very low concentration of phosphorus in the reactor (3.4±0.6 mg L^{-1} during feeding period). As a result it was impossible to reduce the effluent phosphorus concentration below 1.9 mg L^{-1}, even when phosphorus load was reduced and overdose rate increased to 42. The high overdose ratio at low initial phosphorus concentrations explains also high brucite ($Mg(OH)_2$) content in the precipitate as indicated by XRD analysis. However, struvite was present as the sole mineral containing phosphorus in all samples. It was shown in Chapter 3 that high purity struvite precipitate can be achieved at lower electric current. For the scale-up of the process presented here the feeding mode would have to be optimized to accommodate improved process conditions for electrochemical struvite precipitation. This would provide lower final effluent concentration and precipitate with higher content of struvite.

4.5 CONCLUSIONS

- Electrochemical phosphorus recovery can be applied simultaneously in the same reactor with biological nitrification process.
- In the conditions defined in this study, an impact of the electric current on the nitrifying biomass was strongly correlated to the electric current applied per mass of MLVSS. NOB population was stimulated at I:MLVSS below 90 mA g VSS^{-1}. The maximum observed specific substrate utilization rate (SSUR) in short term exposure tests was 200±8 mg NO_2-N g VSS^{-1} d^{-1} at 20 mA g VSS^{-1}, which was 30% increase in comparison to the control reactor.
- The overall nitrification rate was limited by AOB activity, which showed moderate, almost linear decline in the response to increasing electric current. The SSUR of AOB at 20 mA g VSS^{-1} in short term exposure tests was 114±6 mg NH_4-N g VSS^{-1} d^{-1}, which was 10% decrease in comparison to the control reactor. Thus, although results from long term operation did not indicate any reduction of nitrification efficiency in the nutrient limited reactor, it is recommended not to exceed a current of 20 mA g VSS^{-1}.
- Results suggest that inhibition/stimulation in studied voltage range (0 to 19 VDC) was caused by direct impact of electric charge on the metabolism of the microorganisms rather than by electromagnetic field affecting their morphology. The exact mechanisms of how electric current effects nitrification activity requires further exploration.

5 ANAMMOX PROCESS START-UP AND BIOMASS ENRICHMENT

5.1 INTRODUCTION

This chapter describes the reactor set-up and the procedure of anammox biomass enrichment which was later used in the experiments evaluating impact of low temperature (Chapter 5.4) and electric current (Chapter 7). The biomass enrichment was conducted using membrane bioreactor (MBR1) in sidestream conditions (i.e. temperature of 34°C and high substrate concentration). The generated biomass was later split equally between MBR1 and MBR2. At this stage both MBRs were operated in sidestream conditions. When both reactors stabilized, MBR1 was used in experiment #4 (see Chapter 7) and MBR2 was used in experiment #3 for testing of anammox process under low temperature conditions (see Chapter 5.4). A diagram of anammox biomass seed is presented in Figure 5.1.

The initial anammox seed originated from the full scale sidestream SBR at the York River plant in the Hamptons Road Sanitation District (HRSD) in Virginia Beach, VA. The HRSD commissioned granular PN/A process (DEMON) in fall 2012. The seed for this research was sent in late June 2013 when the anammox process at York River Plant was fully established. The designed capacity of the plant was 1000 kg/d of TKN load, with estimated ammonia removal efficiency of 90%. The designed flow of centrate was 2.8 ML/d (71,500 gal/d). The plant was operating with three SBR cycles per day at influent temperature in the range of 33 to 35°C. More details about the York Plant design and commissioning can be found in Nifong et al. (2013).

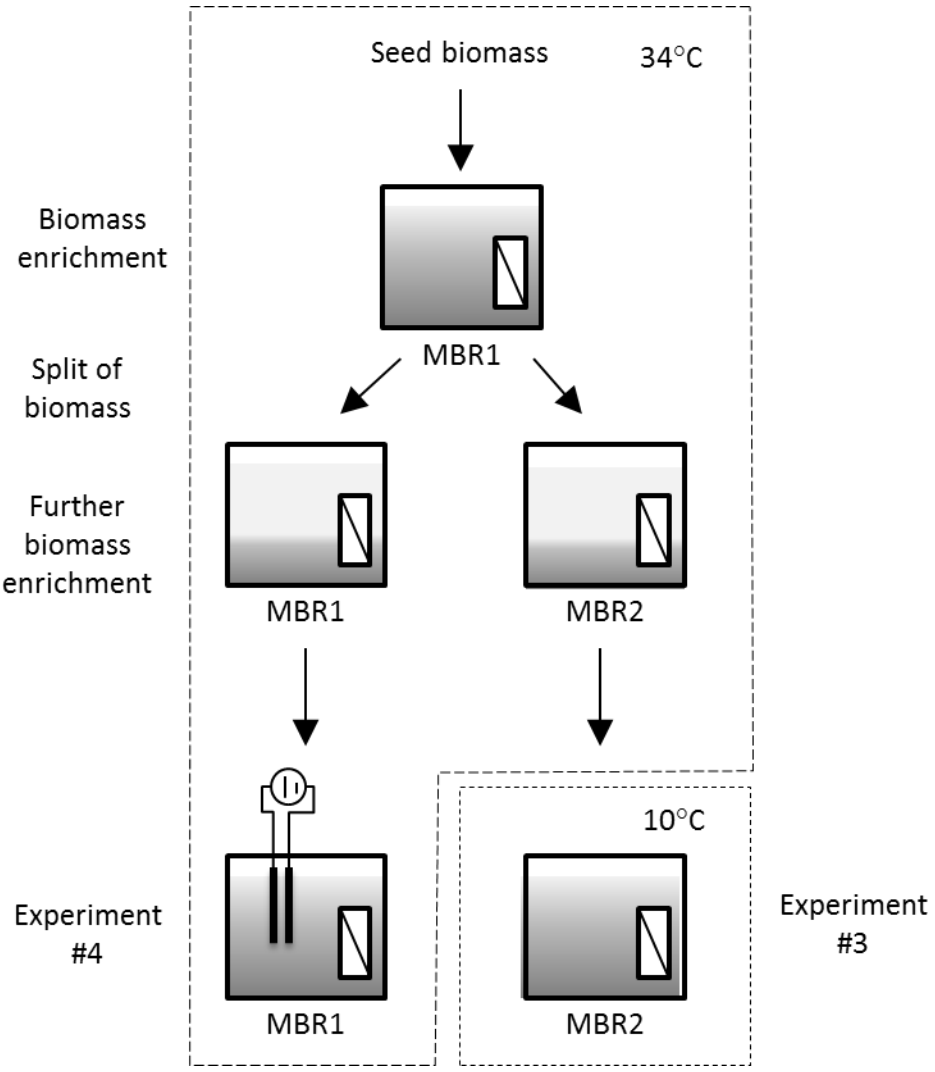


Figure 5.1: Anammox Biomass Enrichment for Experiments #3 and #4

5.2 METHODOLOGY

5.2.1 Reactor Setup

A ZW-1 membrane (GE, Canada) was used. The reactor was operated in a continuous mode. The permeate was withdrawn using a peristaltic digital metering pump (P/S 1300 series by Thermo Scientific, USA). Transmembrane pressure (TMP; Cebar ceramic pressure transmitter by Endress+Hauser, Germany), dissolved oxygen (DO; WQ-FDO optical DO transmitter by Global Water, Germany), and pH (Orbipore digital electrode by Endress+Hauser, Germany) were continuously monitored and logged using a multichannel controller with online access (Liquiline 444 by Endress+Hauser, Germany). The membrane was sparged with nitrogen gas as needed to maintain the TMP below 30 kPa and to assure anoxic conditions in the reactor. Measured DO was below the detection limit of 0.05 mg L^{-1} throughout the study period. The pH was maintained at 7.3 by dosing 0.1N HCl acid using peristaltic pump (Masterflex C/L by Cole Parmer, USA) actuated by the multichannel controller. The reactor was fed by gravity using a float valve. Solids residence time (SRT) was controlled by wasting part of the mixed liquor five times per day using, peristaltic pump (Masterflex C/L by Cole Parmer, USA).

When TMP rose above 40 kPa, the membrane was cleaned. All inputs and outputs from the reactor were stopped and the reactor was opened to replace the membrane. The membrane was cleaned according to the manufacturer's maintenance guide. The membrane was first soaked in hypochlorite solution (200 mg/L) and later in citric acid (5 mg/L). There were two membrane modules used in rotation, one was being cleaned while the other was in operation.

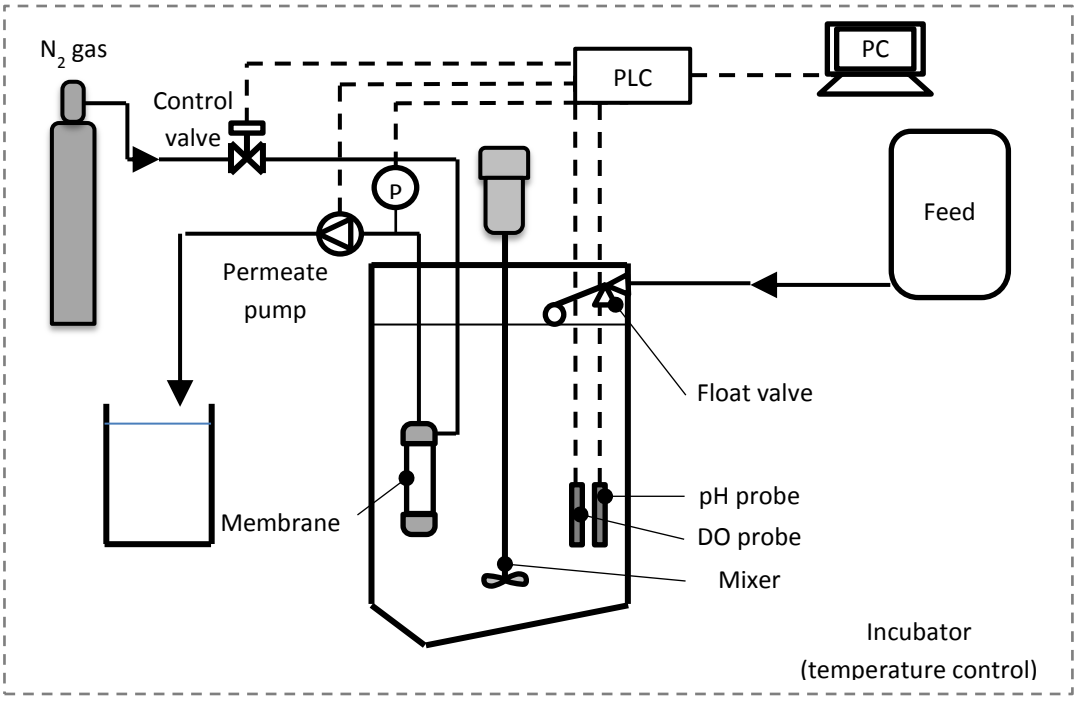


Figure 5.2: The Experimental Set-Up Of Anammox MBR1 At 34°C

The reactor was seeded with 4 L of concentrated anammox biomass from the York River plant in Virginia. The initial MLSS in the reactor was 776 mgTSS/L. There was no biomass wasting during the process start-up and during most of the biomass enrichment period. Wasting was introduced in later stages of the biomass enrichment – after part of the biomass was taken to seed the MBR2 for low temperature experiment. The resulting SRT changes are presented in Table 5.1.

5.2.2 Feed

The reactor was fed with synthetic feed. The source of ammonia and nitrite was ammonium chloride and sodium nitrite, respectively. Ammonia and nitrite were provided initially in a 1:1 ratio (g N: g N). The ratio was later gradually increased to reach 1:1.3 at the end of start-up period, and it was kept at this level throughout the enrichment period. The total nitrogen (TN)

concentration in the feed was adjusted as needed based on the current state of the process (details in Table 5.1). Micronutrients solutions were also added with minor changes according to Graaf *et al.* (1996); 1mL of each solution presented in Table 5.2 was added per liter of feed. Alkalinity (900 mg $CaCO_3$ L^{-1}) in the form of sodium bicarbonate was added as a source of mineral carbon for anammox growth. The feed was deoxygenated by sparging with N_2 gas and was prepared fresh every 1 to 3 days. The feed tank was located in one environmental chamber with the reactor.

Until day 355 the feed flow rate was 7.9 L/d. The flow rate was gradually increased to 15 L/d between day 355 and 367. This was done to increase the flux through the membrane from relatively low (subcritical) 168 to 320 $L/m^2 \cdot d$, which is closer to a typical flux for MBRs treating wastewater. A typical full scale plant design flux is in the range of 650 to 1000 $L/m^2 d$. However, due to less efficient scouring (much shorter fibers) and usually much higher dissolved solids in artificial feed, the laboratory scale membrane module is not expected to perform nearly as well as the full scale membranes. Concentration of the feed remained constant, as a result the load of nitrogen was increased from an average of 500 to 935 $g/m^3 \cdot d$. The changes of SRT, flow, feed concentration, and loads are presented in Table 5.1.

Table 5.1: Process Conditions In MBR1

Day	Feed					Flux	SRT	Comments
	Ammonia	Nitrite	TN load	Nitrite/ Ammonia	Flow			
	mg N/L	mg N/L	g/m^3·d	N:N	L/d	L/m^2·d	d	
0 to 89	100 to 800	100 to 1000	56 to 508	1:1 to 1:1.3	7.9	168	Infinite	Process start-up
90	764±79	984±84	499±45	1.3	7.9			Arbitrary end of start-up period
172								MBR2 seeding
312								Electrodes installed
323							23.3	Sludge wasting started
355								Beginning of flow rate increase
367	771±30	972±33	935±30	1.3	15	320		New constant flow rate
407								End of start-up period/Electric current first introduced

Table 5.2: Composition of Mineral Solutions Used For Preparation of Feed

Component	Concentration in the mineral solution (as compound), g L^{-1}
Solution A:	
KH_2PO_4	27.200
EDTA	15.000
$ZnSO_4 \cdot 7H_2O$	0.430
$MnSO_4 \cdot H_2O$	0.990
$CoCl_2 \cdot 6H_2O$	0.240
$CuSO_4 \cdot 5H_2O$	0.250
$Na_2MoO_4 \cdot 2H_2O$	0.220
$NiCl_2 \cdot 6H_2O$	0.190
$NaSeO_4 \cdot 10H_2O$	0.210
H_3BO_4	0.014
Solution B:	
EDTA	5
$MgSO_4$	147
$FeSO_4 \cdot 7H_2O$	15
Solution C:	
$CaCl_2 \cdot 2H_2O$	180

5.2.3 Analyses

The nitrate, nitrite and ammonium analyses were made using a QuikChem 8500 flow injector analyser (Lachat, USA) employing the following QuikChem methods: 10-107-04-1-A (nitrate/nitrite in range of 0.2 to 25 mg N L^{-1}) and 10-107-06-1-I (ammonium in range 0.1 to 25 mg N L^{-1}). All other analyses were conducted according to Standard Methods (Eaton and Franson, 2005).

5.2.4 Anammox Activity Quantification

The level of anammox activity was assessed based on three different parameters. The first parameter, the nitrogen removal rate (NRR) (Eq. 5.1) was a volumetric rate of ammonium and nitrite removal calculated based on the difference of the influent and effluent concentration in a continuous operation of the reactor (mg N L^{-1} d^{-1}). The second parameter was the specific anammox activity (SAA) (Eq. 5.2) expressed as ammonium and nitrite nitrogen removal rate per mass of volatile suspended solids (mg N g VSS^{-1} d^{-1}). Since the reactor was completely mixed and was operated to maintain relatively low concentrations of ammonia and nitrite in the effluent, anammox process might have been inhibited by the substrate loading rate and/or low substrate concentration. To assess the true anammox activity maximum specific anammox activity (SAA_{max}) (Eq. 5.3) was calculated based on the results of *in situ* batch tests at the temperatures the reactor was currently operating at (the same kinetic tests were conducted in low temperature experiment). At the time of the test the reactor was operated in the batch mode as opposed to the regular continuous operation mode. Before each kinetic test, the permeate pump and feed were stopped. Concentrations of ammonium, nitrite and alkalinity in mixed liquor (ML) were analysed and adjusted by adding a mix of ammonium chloride and sodium nitrite, to a level of 50 mg N L^{-1}. The length of the tests and the sampling periods were determined based on the speed of nitrogen depletion. In all tests at least 6 samples per test were taken.

Eq. 5.1 $$NRR = \frac{([NO_2{-}N]_{in} + [NH_4{-}N]_{in} - [NO_2{-}N]_{out} - [NH_4{-}N]_{out}) \cdot Flow}{\text{reactor volume}} \quad \left(\frac{\text{mg N}}{\text{L·d}}\right)$$

Eq. 5.2 $$SAA = \frac{([NO_2{-}N]_{in} + [NH_4{-}N]_{in} - [NO_2{-}N]_{out} - [NH_4{-}N]_{out}) \cdot Flow}{\text{reactor volume} \cdot [VSS]} \quad \left(\frac{\text{mg N}}{\text{g VSS·d}}\right)$$

Eq. 5.3 $$SAA_{max} = \frac{([NO_2{-}N]_{t1} + [NH_4{-}N]_{t1} - [NO_2{-}N]_{t2} - [NH_4{-}N]_{t2})}{[VSS] \cdot (t_2 - t_1)} \quad \left(\frac{\text{mg N}}{\text{g VSS·d}}\right)$$

Where:

$[NH_4\text{-}N]_{in/out}$ – concentration of ammonia in the feed or in the permeate;

$[NO_2\text{-}N]_{in/out}$ – concentration of nitrite in the feed or in the permeate;

$[NH_4\text{-}N]_{t1/t2}$ – concentration of ammonia at time t_1 or t_2 of the test;

$[NO_2\text{-}N]_{t1/t2}$ – concentration of nitrite at time t_1 or t_2 of the test;

[VSS] – concentration of VSS in the reactor;

t_1 or t_2 – time count from the beginning of a test.

5.3 RESULTS AND DISCUSSION

After bioreactor inoculation the feed rate was kept relatively low to avoid inhibition of the biomass with free ammonia. Initially nitrite was removed at a much faster pace than ammonia (Figure 5.3). This was most likely due to heterotrophic denitrification utilizing organic carbon generated due to biomass decay. As a result the anammox bacteria had to compete for nitrite with heterotrophic denitrifiers. After 6 days of operation the ammonia in the effluent started to decline, this is probably when most of the accumulated bCOD from decay was used and there were more nitrite available for anammox. A significant ammonia drop in the effluent was observed after day 15. This is when concentration of ammonia and nitrite started to be gradually increased. After 90 days of operation the feed load reached 506 $g/m^3 \cdot d$ achieving 85% removal efficiency and 490 $g/m^3 \cdot d$ NRR. The feed in concentration after day 90 remained relatively constant till the end of the experiments in MBR1 (with exception of temporary process upsets described further in this section). The NRR after 90 days of start-up was a little higher than half of the designed NRR for the York River DEAMON plant which was 900 $g/m^3 \cdot d$. However this was only due to lower biomass concentration. The SAA achieved in the MBR1 was 515 mg N/g VSS·d and the SAA at York River plant shortly before seed was sent was 408 mg N/g VSS·d (SAA estimated based on the values read from Figure 10 in Nifong et al., 2013).

Although biomass was not wasted during the first 40 days, solids concentration declined from 700 mg VSS/L to 520 mg VSS/L (Figure 5.4).This is likely the result of heterotrophic activity feeding on old dead biomass. After this period, accumulation of biomass was observed. On day 171 MLVSS reached 2030 mg/L. The next day biomass was split equally between MBR1 and the new twin MBR2 (used for the low temperature experiment). The volume in both bioreactors after the biomass transfer was topped up using mineral solution prepared similarly as regular

feed but without ammonium chloride and sodium nitrite. To prevent substrate accumulation in the rector, the feed concentration was reduced by 50% (Figure 5.3) for the next six days. After this short period, normal feeding was reintroduced despite the fact that it was not enough time to rebuild the biomass. There was very slow substrate accumulation in the effluent. This means that the actual maximum specific anammox activity must have been much higher than the previously provided nitrogen load. The reactor was substrate limited. However, the initially ignored slow accumulation trend created a snowball effect and when left over the weekend, resulted in ammonia and nitrite concentration in the effluent of 108 and 135 mg/L. The calculated free ammonia in the reactor was 1.8 mg/L which is close to the suggested inhibition level of 2 mg/L for anammox (Jaroszynski, 2012). The feed to the reactor was stopped for one day, in order to recover the anammox activity. This was enough to regain fully nitrogen removal efficiency.

The sudden drop of solids concentration in the reactor was a result of biomass wash, on day 280. On day 279 sodium bicarbonate was mistakenly replaced in the feed with an unknown substance, which resulted in a complete process inhibition. In order to prevent permanent damage to the process, the biomass was settled and 26 L of liquid from the reactor was decanted. The reactor was topped up with the mineral solution prepared similarly to regular feed but without ammonia and nitrite. Feed to the reactor was reduced by 85% for 7 days and gradually increased back to the regular concentration over a period of 15 days. Full nitrogen removal was recovered.

The SRT control was established on day 323. This was followed by a gradual increase of the nitrogen load between day 355 and 367. There was 50 days after the process changes before the evaluation of the impact of the electric current commenced.

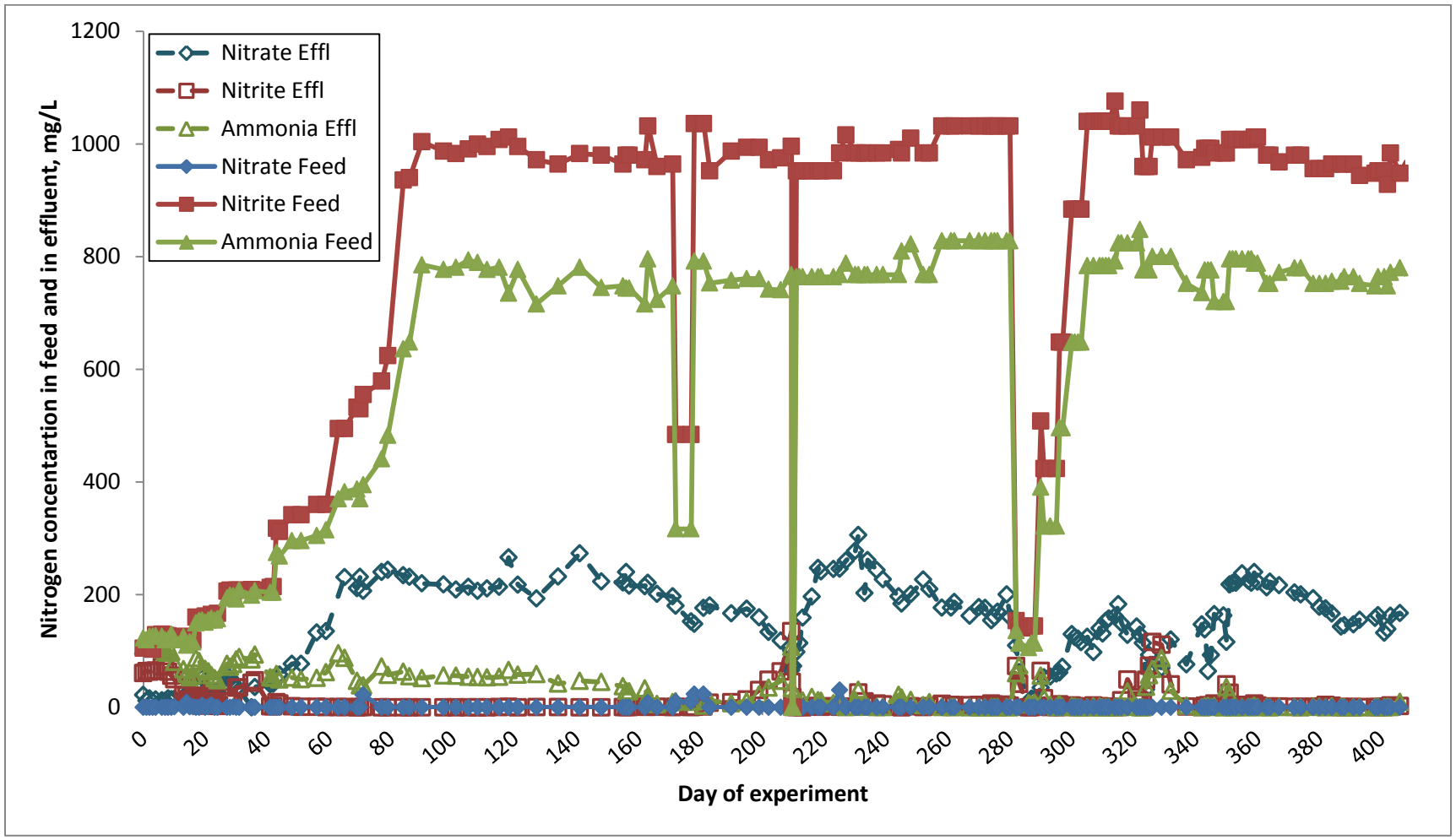


Figure 5.3: MBR1 Nitrogen Feed and Effluent During Process Start-Up

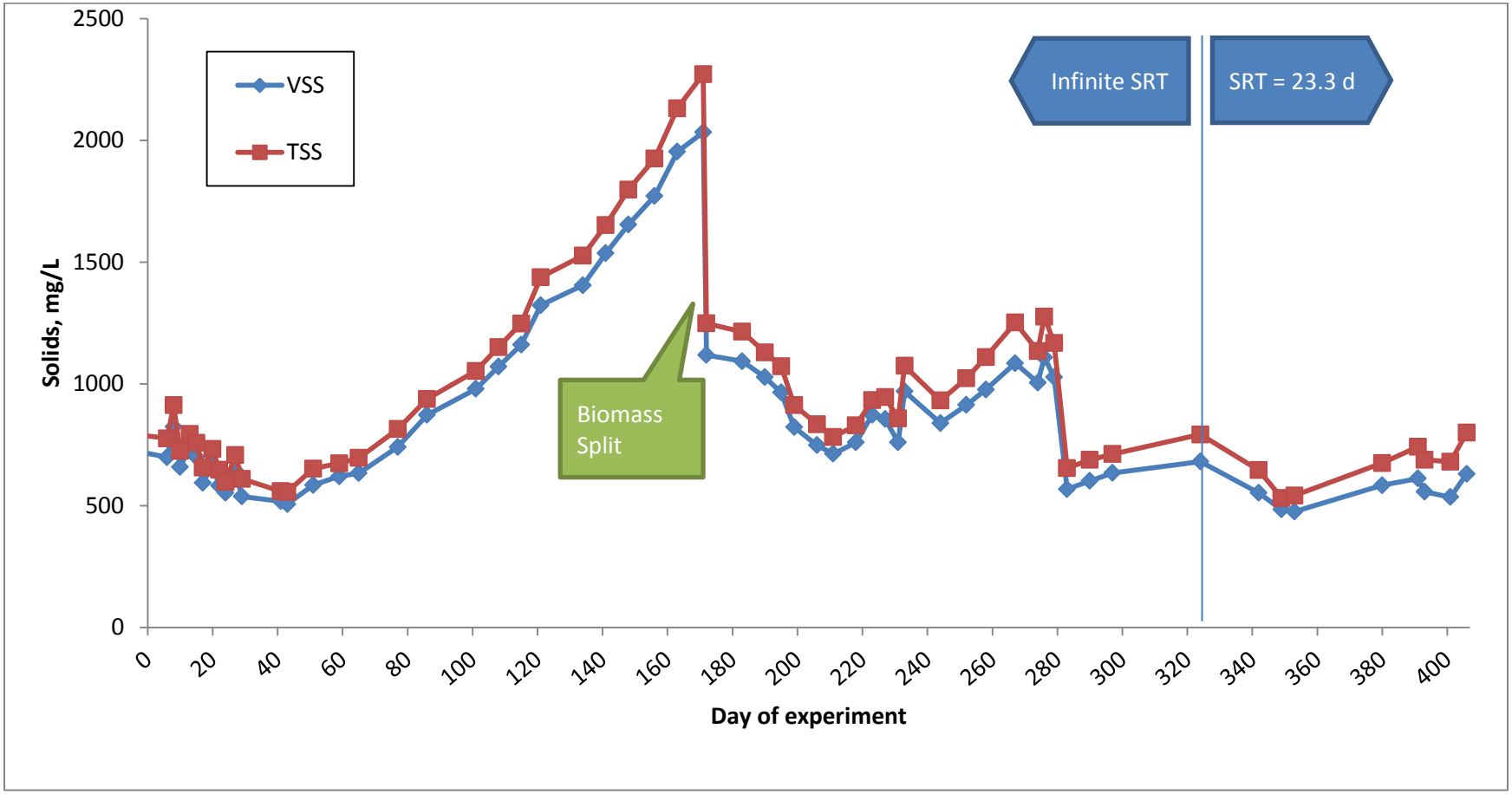


Figure 5.4: MBR1 Mixed Liquor Solids During Process Start-Up

5.4 CONCLUSIONS

- After 90 days from inoculation of MBR1 the biomass achieved SAA of 515 mg N/g VSS·d which was higher than SAA of the biomass at the seeding plant.
- The process indicated to be substrate limited achieving high removal efficiency 85%.
- Anammox process can be readily recovered after period of high substrate inhibition, when substrate concentration is decreased. In this case biomass was washed with the mineral solution.

6 LOW TEMPERATURE ANAMMOX

6.1 INTRODUCTION

The purpose of this study was to assess the impact of low temperature prevalent in mainstream treatment in colder climates (i.e. 10°C), on the activity of anammox biomass enriched at high sidestream temperature (i.e. 34°C). The other objective was to assess any changes in the structure of anammox population during long-term operation at low temperature.

One of the main limitations of biological nutrient removal (BNR) systems is the high energy demand of aeration for aerobic nitrification and the insufficient organic carbon available in the wastewater for denitrification (Oleszkiewicz *et al.*, 2015). The anaerobic ammonia oxidation (anammox) process, discovered in the 1990's, has made a fundamental breakthrough for nitrogen removal technology allowing completely autotrophic nitrogen removal (Strous *et al.*, 1998). Anammox bacteria are capable of anaerobic ammonium oxidation to nitrogen gas utilizing nitrite as electron acceptor. Only about half of the ammonium load has to be partially oxidized in aerobic conditions to provide nitrite. As a result, up to 60% of energy for aeration and 90% of carbon for denitrification can be saved (Siegrist *et al.*, 2008).

The slow-growth characteristics of anammox bacteria led to their primary application in treatment of reject water streams, where they could proliferate at elevated temperatures (Cao *et al.*, 2013; Jetten *et al.*, 2009; Lackner *et al.*, 2014). In the last decade anammox has become the state-of-the-art process for side stream treatment, with over 100 (including industrial wastewater treatment) full scale facilities in operation (Lackner *et al.*, 2014). In recent years there has been an increased interest in applying the anammox process in the mainstream of municipal

wastewater treatment plant (WWTP) (Cao *et al.*, 2013; De Clippeleir *et al.*, 2013a; Gilbert *et al.*, 2015; Hendrickx *et al.*, 2014; Hu *et al.*, 2013; Laureni *et al.*, 2015; Lotti *et al.*, 2014a; Ma *et al.*, 2013; Vázquez-Padín *et al.*, 2011), because it has been shown to be one of the most promising ways to achieve energy self-sufficiency of WWTP (Khiewwijit *et al.*, 2015).

In the mainstream process, anammox bacteria would have to work at much lower substrate concentrations (20 to 80 mg NH_4-N L^{-1} in wastewater versus 400 to 2000 mg NH_4-N L^{-1} in reject water) and lower temperatures. Low substrate concentration should not be an issue thanks to the high affinity of anammox bacteria for both nitrite and ammonium (half-saturation constant below 0.1 mg N L^{-1}; Strous *et al.*, 1999). However, despite observations of anammox activity at very low temperatures (even below 1°C) in marine sediments or in soil (Hu *et al.*, 2011; Lam and Kuypers, 2011), so far temperature proves to be the key obstacle for anammox in engineered wastewater treatment processes. Temperature reduction slows down the already slow-growing bacteria and additionally the impact seems to be even stronger below 20°C (Lotti *et al.*, 2015). In some cases prolonged operation below 12°C resulted in loss of ammonia removal and temperature increase was required for process to recover (Laureni *et al.*, 2016; Lotti *et al.*, 2014a). It was shown that granular and attached growth biomass is generally more resilient to lower temperatures (Gilbert *et al.*, 2015; Lotti *et al.*, 2015). Reported removal rates vary significantly, depending on the biomass aggregation state and the process conditions (either nitritation/anammox or anammox; and feed concentration) they were found to be in the range of 0.01 to 0.15 g N g VSS^{-1} d^{-1} at 10 to 15°C and between 0.15 to 0.90 g N g VSS^{-1} d^{-1} at 18 to 20°C (De Clippeleir *et al.*, 2013a; Gilbert *et al.*, 2015; Hendrickx *et al.*, 2014; Hu *et al.*, 2013; Laureni *et al.*, 2016, 2015; Lotti *et al.*, 2014a; Ma *et al.*, 2013; Vázquez-Padín *et al.*, 2011). Considering that potentially the easiest way to start-up the mainstream anammox process would be to utilize

biomass from existing sidestream rectors as seed, it is vital to learn specifically how the anammox bacteria cultivated in the sidestream conditions will perform in the mainstream. Thus far most of the studies on low temperature anammox were conducted with biomass cultivated at 20 to 22°C. In the two studies with biomass cultivated at 30°C the temperature was dropped only to approximately 12.5°C (Laureni *et al.*, 2015) and 16°C (Ma *et al.*, 2013).

Anammox bacteria belong to the order *Brocadiales* within the *Planctomycetes* phylum (Jetten *et al.*, 2009). Most well known and most often detected in wastewater treatment systems (both full and lab scale) anammox bacteria belong to candidate genera *Kuenenia* and *Brocadia* (Harhangi *et al.*, 2012; Jetten *et al.*, 2009). In low temperature systems particularly *B. fulgida* is often determined as the dominant anammox species (Hendrickx *et al.*, 2014; Hu *et al.*, 2013; Laureni *et al.*, 2015; Lotti *et al.*, 2014a). However, most often the level of anammox enrichment is tracked using methods targeting the general anammox population and the dominant species are identified only at the end of a study, giving a limited picture of the anammox population dynamics. It is possible that the dominant species is not the best suited species for the operating conditions if its relative count in the population is in decline. Thus, knowledge of the dynamics of the population structure may potentially allow to identify and enrich the true anammox bacteria best suited for operation at lower temperatures.

6.2 MATERIAL AND METHODS

6.2.1 Reactor Set-Up and Operation

The study was conducted in a 28 L membrane bioreactor (MBR2) (Figure 6.1). The reactor set-up was the same as the MBR1 operated at 34°C, except MBR2 was placed in fridge size incubator. The detailed reactor description and biomass enrichment is described in Chapter 5.

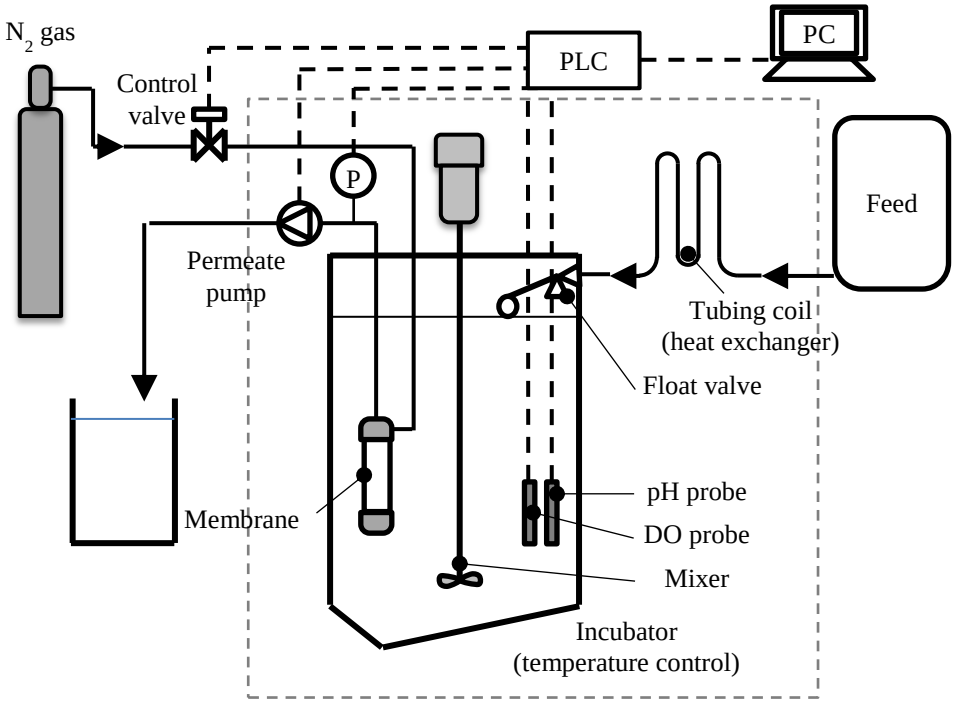


Figure 6.1: The Experimental Set-Up for Low Temperature Anammox Study

The synthetic feed used in this study contained ammonium chloride and sodium nitrite, in a 1:1.3 ratio of NH_4-N to NO_2-N. The total nitrogen (TN) concentration in the feed varied between 1755 and 42 mg N L^{-1} (Table 6.1). Micronutrients solutions was also added with minor changes according to Graaf *et al.* (1996); 1mL of each solution presented in Table 5.2 was added per liter of feed. Alkalinity (900 mg $CaCO_3$ L^{-1}) in form of sodium bicarbonate was added as source of mineral carbon for anammox growth. Feed was deoxygenated by sparging with N_2 gas and was

prepared fresh every 1 to 3 days. Feed was stored at ambient temperature (i.e. 21±2°C). The feed line inside the incubator was extended and formed a heat exchanger allowing equalization of feed temperature to the temperature of reactor.

Table 6.1: Process Conditions Throughout Experimental Period

Day	Temperature, °C	TN feed, mg L^{-1}	Volumetric nitrogen load (VNL), mg L^{-1} d^{-1}	HRT, d
1-105	34	1755±40	500±11	3.5
105-137	34 ↓ to 5.7	1755↓ to 415	500↓ to 120	3.5
138-144	11	0	0	∞ (feed stopped)
145-239	10	45±20	15±7	3
240-289	10 ↑ to 20	42±4	14±1	3
290-317	20	47±1	16±1	3
318-325	20	50±1	17±1	2.6

6.2.2 Activity Measurement

Anammox activity quantification methods are described in section 5.2.4.

6.2.3 Temperature Coefficient

The temperature coefficient θ was estimated based on the slope of a linear regression of modified Arrhenius plot (ln (SAA) vs temperature in °C) according to the Eq. 6.1.

Eq. 6.1 $\theta = e^{a}$,

where a is the slope of a linear regression.

6.2.4 Analyses

Nitrate, nitrite and ammonium analyses were made using a QuikChem 8500 flow injector analyser (Lachat, USA) employing the following QuikChem methods: 10-107-04-1-A (nitrate/nitrite in range of 0.2 to 20 mg N L^{-1}) and 10-107-06-1-I (ammonium in range 0.1 to 30 mg N L^{-1}). All other analyses were conducted according to Standard Methods (Eaton and Franson, 2005). Volumetric nitrogen loading rate (VNL) is the sum of ammonium and nitrite loads per volume of the reactor (mg N L^{-1} d^{-1}).

6.2.5 DNA Isolation

Five biomass grab samples were taken along the experiment for quantitative polymerase chain reaction (qPCR) analysis. DNA isolation was performed following a slightly modified version of the protocol described by Harhangi *et al.* (2012): 0.5 mL of sample was resuspended in 0.5 mL cell lysis solution containing 5% sodium dodecyl sulfate, 120 mM sodium phosphate (monobasic), 350 mM sodium chloride, pH 8.0. Cell lysis was performed by adding resuspended sample to tube half-full of beads, adding 0.5 mL of phenol/chloroform/isoamyl alcohol solution, and bead beating for 1 minute. No protein precipitation was performed. DNA precipitation was performed by adding 2.5 volumes of ice-cold absolute ethanol and incubating at -20°C for 1 hour. DNA quality and quantity were measured using NanoDrop analysis (Thermo Scientific, USA).

6.2.6 Quantitative PCR

Primers targeting the 16S rRNA gene of all bacteria and primers targeting the hydrazine synthase gene (*hzsA*) of all anammox bacteria were taken from the previous studies (Harhangi *et al.*, 2012;

Muyzer *et al.*, 1993). Species-specific primers were designed by Alan Froese (at the time MSc student in The Department of Microbiology at the University of Manitoba) after aligning the 16S rRNA genes from all the anammox species available, obtained from NCBI, using MEGA 6.06, and then validated using NCBI Primer BLAST against the database of Candidatus *Brocadiaceae* sequences. Quantitative PCR (qPCR) amplifications were performed in a total reaction volume of 20 µL containing 10 µL of SsoAdvanced Universal SYBR Green Supermix (Bio-Rad), 0.2 µL of forward primer (20 µM), 0.2 µL of reverse primer (20 µM), 2 µL of 10-fold diluted template DNA, and Milli-Q water. Amplification was accomplished using a CFX Connect Real-Time PCR Detection System (Bio-Rad). The thermal profile used was 95°C for 3 min, 40 cycles of denaturation (95°C for 10 s), primer annealing (10 s), and extension (72°C for 30 s), followed by melt curve cycling at 0.5°C intervals between 55 and 95°C. Primer sequences and annealing temperatures used are shown in Table 6.2. Absolute quantification of targets was performed using qPCR CopyCount (DNASoftware, Ann Arbor, USA). Cell counts (cells per mL) were estimated by assuming an average of 1.7 16S rRNA genes per genome for anammox bacteria and an average of 4.2 16S rRNA genes per genome for all other bacteria (Větrovský and Baldrian, 2013).

Table 6.2: Primer Sequences and Annealing Temperatures used for qPCR

Primer	Sequence (5'-3')	Target gene	Target organism	Annealing temp, °C	Reference
16S-F	CCTACGGGAGGCWGCAG[1]	16S rRNA	All bacteria	65	Muyzer *et al.* (1993)
16S-R	TATTACCGCGGCTGCTG[1]				
HZ-F	WTYGGKTATCARTATGTAG	hzsA	All anammox bacteria	55	Harhangi *et al.* (2012)
HZ-R	AAABGGYGAATCATARTGGC				
BA-F	AAGGGATGCTAAACTGTAAA	16S rRNA	*Brocadia anammoxidans*	55	This study
BA-R	AATCTGAACTGGGATTGGT				
BC-F	CGAACGAGGGAGCATT	16S rRNA	*B. caroliniensis*	N/A[2]	This study
BC-R	ACGTTGTTATTCTCATACTCG				
BF-F	CTATTGCTGCTATTGGTAGTA	16S rRNA	*B. fulgida*	61	This study
BF-R	AGCCTTAGTCAAACAAAGAT				
B4-F	GCATTGATAACCTACCTCCA	16S rRNA	*Brocadia* sp. 40	61	This study
B4-R	GCCTTAGTCAAGCAAGAAC				
KS-F	GGAATAACTGCGTTTCGAG	16S rRNA	*Kuenenia stuttgartiensis*	N/A[2]	This study
KS-R	CAAGTGCTTTTGCACCTAT				

[1] These primers were slightly modified from their referenced versions to better match the 16S rRNA sequences for all anammox bacteria

[2] No temperature listed due to complete lack of any signal at any temperature tested

6.3 RESULTS

6.3.1 Long-Term Operation at Low Temperature

After 105 days of the process start-up period at 34°C, effluent ammonium and nitrite averaged 7±6 mg N L^{-1} and 16±16 mg N L^{-1}, respectively. SAA measured 7 times in last two weeks of operation at 34°C was steady and averaged 611±16 mg N g VSS^{-1} d^{-1}. Total nitrogen removal efficiency in the period between day 47 and 105 was on average 88±2%, which was close to the empirical maximum efficiency of 88.8% (Strous *et al.*, 1998). For the first 35 days reactor was operated with the SRT of 24 d. Later sludge wasting was stopped in order to build biomass inventory for the rest of the experiment.

Starting on day 105 the temperature was gradually reduced over 24 days in four increments, 6°C each. Results presented in Figure 6.2 indicate relatively small activity reduction after first two steps of the temperature reduction. Six days after the temperature was decreased to 22°C, SAA was still at 534 mg N g VSS^{-1} d^{-1}. Further drop of the temperature to 16°C brought much more pronounced activity reduction with SAA of 113 mg N g VSS^{-1} d^{-1}. The first kinetic tests at the temperature of 10°C showed SAA to be 115 and 40 mg N g VSS^{-1} d^{-1} four and six days after the temperature reduction, respectively. Due to technical difficulties on day 137 the temperature dropped to 5.7°C. The temperature in the reactor was restored to 10°C after less than 12 hours. After that incident the activity of the biomass was lost. In an effort to recover the activity, feed to the reactor was stopped for 6 days. Concentration of the ammonium and nitrite in the reactor at the time was 42 and 52 mg N L^{-1}, respectively. During the 6 day period only alkalinity was added to maintain 450 mg $CaCO_3$ L^{-1} in the reactor. Biomass in the reactor was washed after six days with 24 L of micronutrient media, to be sure that there is no inhibition caused by

intermediate products such as hydrazine reported by Carvajal-Arroyo *et al.* (2013). The feed was reinstated with average TN concentration of 45 mg N L^{-1} (VNL of 15 mg N L^{-1} d^{-1}). For the next 26 days there was no measurable activity recorded. On day 170 slow recovery was observed which was followed by increase of concentration of TN in the feed to 66 mg N L^{-1} (VNL of 22 mg N L^{-1} d^{-1}). However, after NRR reached 20 mg N L^{-1} d^{-1} on day 191, activity started to decline again to be lost completely on day 205. Activity was observed again on day 230. However, the NRR was very low with 3±1 mg N L^{-1} d^{-1} on average in 10 day period. Thus, the temperature was gradually increased to 13°C on day 240, to 15°C on day 254 and finally to 20°C on day 290. This promoted recovery of the anammox activity – 6.4 mg N $gVSS^{-1}$ d^{-1} on day 249 (13°C), 15 mg N $gVSS^{-1}$ d^{-1} on day 264 (15°C) to 74 mg N $gVSS^{-1}$ d^{-1} on day 318 (20°C).

Temperature coefficient (θ) was estimated using SAA results. Distribution of the data in plot of ln(SAA) vs temperature showed that the value of the coefficient was not constant throughout the tested temperature range (Figure 6.3). In fact, based on the best fit of linear regression, θ for temperature in the range of 22 to 34°C, and 10 to 22°C was estimated as 1.01 (R^2=0.42 and normalized root-mean-square error NRMSE=0.51) and 1.24 (R^2=0.99 and NRMSE=0.04), respectively. Despite the relatively high NRMSE for the higher temperature range, it is clear that the temperature had lesser impact on the activity at temperatures above 22°C. Furthermore, the temperature coefficient was also distinctly different for the period of temperature increase with an estimated value of 1.31 (R^2=0.93 and NRMSE=0.05).

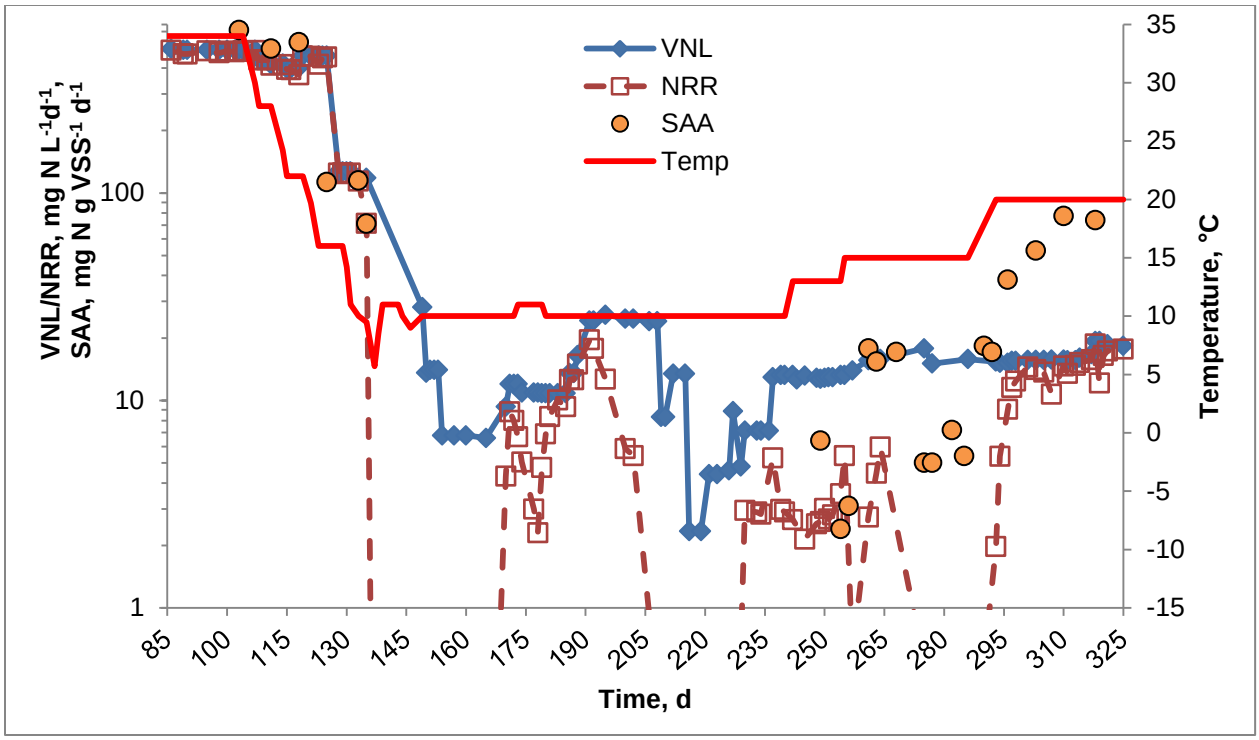


Figure 6.2: Specific Anammox Activity (SAA), Nitrogen Removal Rate (NRR) and Volumetric Nitrogen Loading (VNL) During Reduced Temperature Period

SAA values from in situ batch tests, NRR calculated from continues operation results.

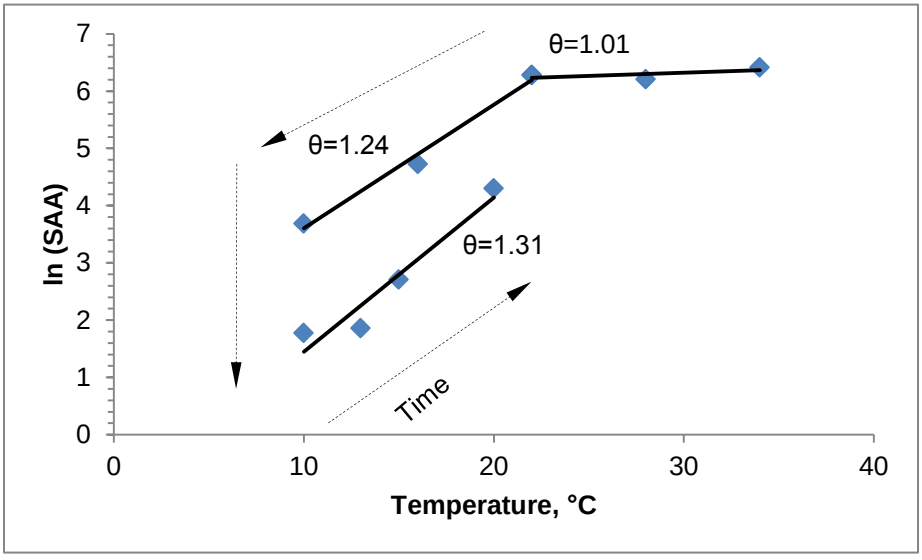


Figure 6.3: Modified Arrhenius Plot for the Specific Anammox Activity (SAA) During Temperature Decrease and Increase Periods

SAA at 10°C for the temperature increase period was calculated based on NRR and MLVSS.

High removal efficiency of 88±2% was observed through the period between day 105 and 136, when the temperature was reduced from 34 to 10°C. On day 137 it was completely lost along with the loss of the activity (Figure 6.2). From day 249 onward, an increasing efficiency trend was observed together with a gradual recovery of activity. The efficiency was recovered to 75% after the temperature was increased to 20°C.

Nitrite to ammonium removal molar ratio (NO_2:NH_4) and nitrate produced to ammonium removed molar ratio (NO_3:NH_4) were relatively steady throughout the start-up and temperature reduction period (days 1 to 137) averaging 1.31±0.08 and 0.28±0.09, respectively (Figure 6.4).

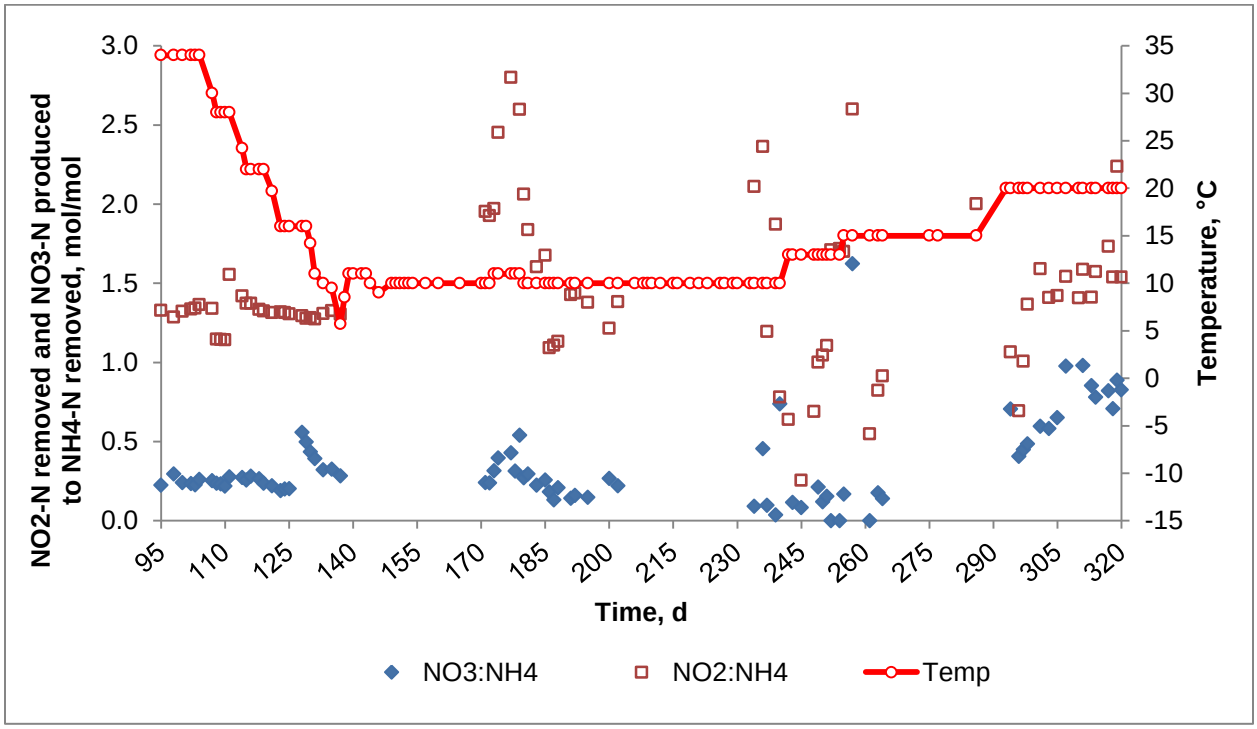


Figure 6.4: Nitrite Removed and Nitrate Produced to Ammonium Removed Molar Ratios

During the recovery period at 10°C (days 170 to 205) NO_2:NH_4 and NO_3:NH_4 ratios presented very steep increase in first 7 days reaching 2.80 and 0.54, respectively. After that, they equally fast decreased to 1.30±0.14 and 0.18±0.05. When anammox activity started to recover at 10°C and during the temperature increase both ratios demonstrated significant variations. They stabilized after 20 days of operation at 20°C at 1.51±0.26 and 0.74±0.11 for NO_2:NH_4 and NO_3:NH_4, respectively.

The increasing trend of MLVSS at 34°C was caused by operation at infinite SRT. The MLVSS continued to grow despite the decrease of the temperature until day 125 when it reached a plateau at 990 mg L^{-1} (Figure 6.5). However, soon after the temperature was reduced to 10°C the amount of VSS in the reactor started to decline. The trend continued thereafter down to 560 mg L^{-1} on day 229. Following day 230 the decline slowed down and continued at much slower pace until day 254 going down to 540 mg L^{-1}. At that time SRT control was re-established (SRT=280 d).

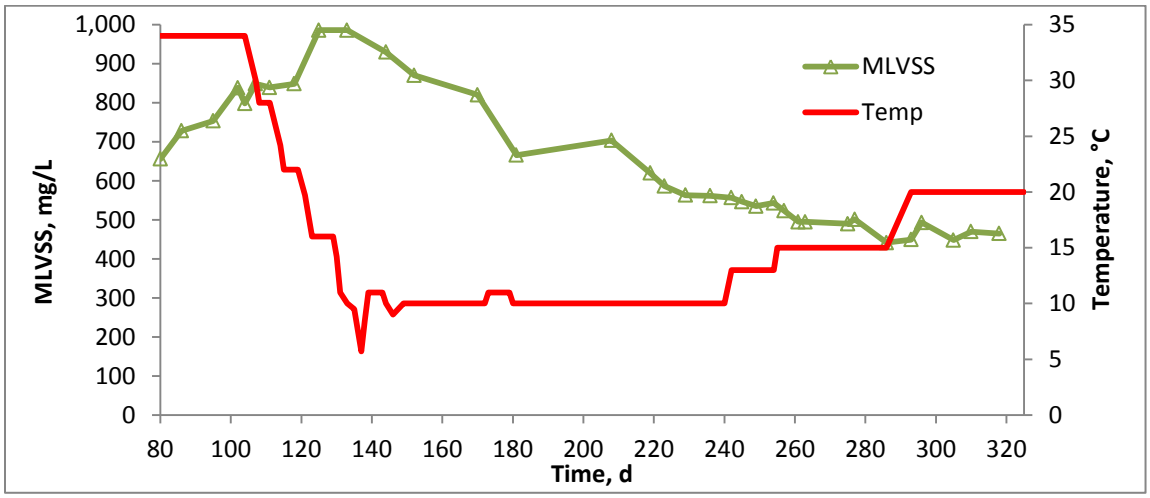


Figure 6.5: MLVSS Changes During Reduced Temperature Period

6.3.2 qPCR Analysis

The population of anammox organisms and total population of bacteria were measured at key points during the time course: population at 34^{o}C (day 104), population during the inactive phase at 10^{o}C (day 223), population during the temperature increase phase (days 261 and 294), and finally, populations after recovery at 20^{o}C (day 325). The total cell count of bacteria based on the general probe targeting 16S rRNA followed the same declining trends of MLVSS and total anammox cell count (Figure 6.6 and Figure 6.7). Of the 5 anammox species specific probes used, 16S rRNA for *B. caroliniensis* and *K. stuttgartensis* were not detected by qPCR. Comparison of cell count of the *B. anammoxidans*, *B. sp. 40*, *B. fulgida* and the total anammox bacteria (based on hzsA) suggests that most probably only these three species comprised the whole anammox population (Figure 6.7). Of the 3 strains, *B. anammoxidans* was shown to be the most temperature resilient anammox species that was monitored, while the *Brocadia* sp. 40 showed the largest fluctuation (Figure 6.8). Its population remained at constant levels until the final stages when it decreased at 20°C. Both populations that hybridized with the *B. sp. 40* and *B. fulgida* probes were steady after the temperature was dropped, but decreased when the temperature was initially increased. However, further increasing of the temperature to 20°C resulted in expansion. On the basis of the primers used, the results suggest that *B. fulgida* was the dominant anammox species for most of the time points measured (Figure 6.7). The different measured populations of anammox bacteria showed that the relative proportion of each were similar at the beginning and end of the time course of 325 days.

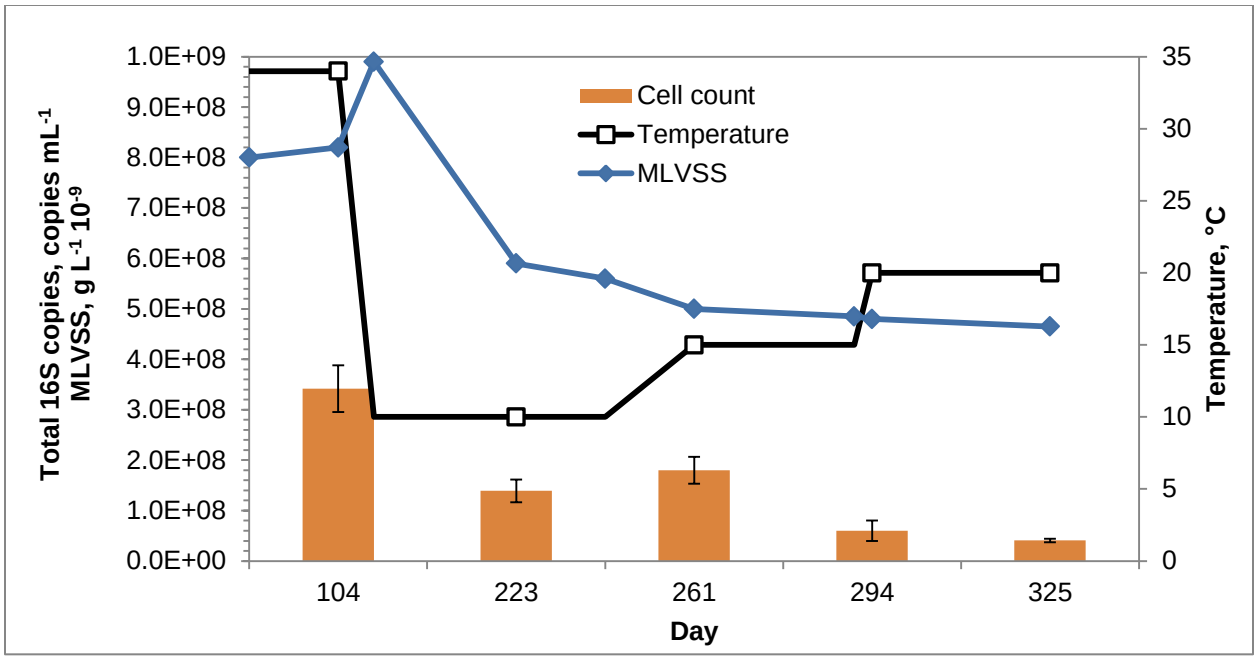


Figure 6.6: Total 16S Copies Count in Perspective of Volatile Suspended Solids.

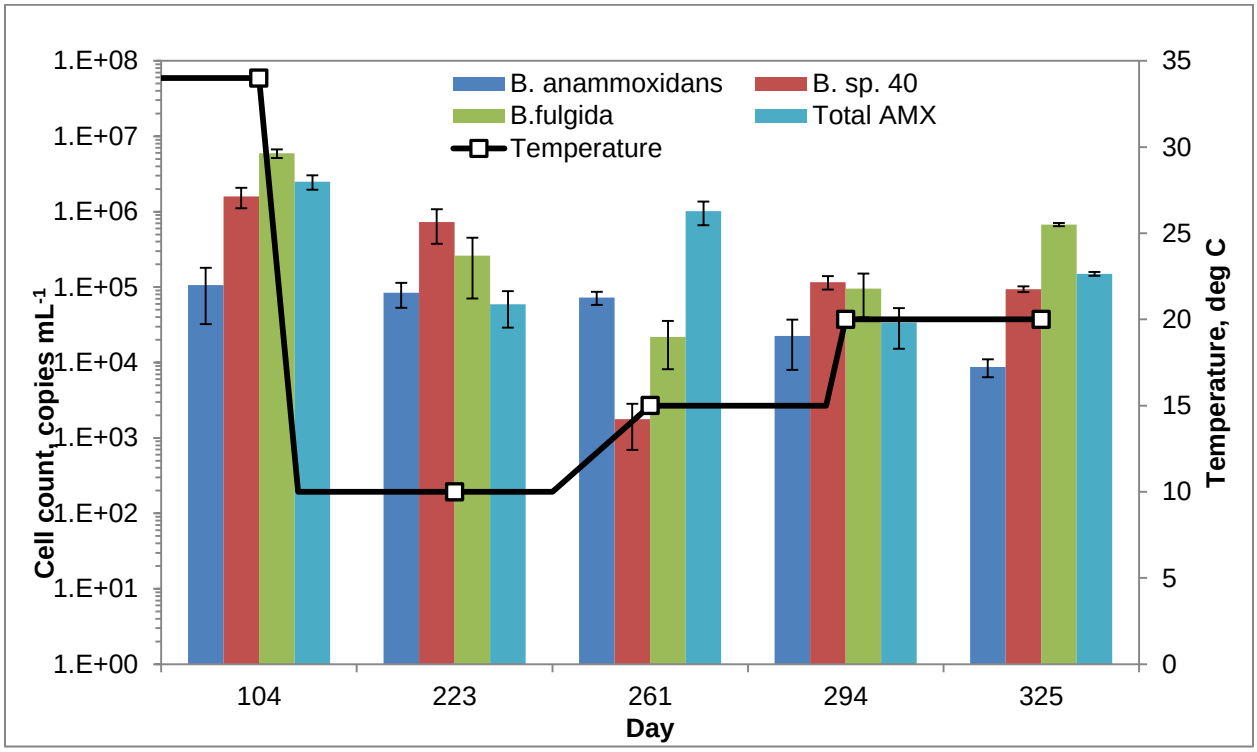


Figure 6.7 Cell Count Of Three Detected Anammox Species And Total Anammox Population Based On Primers Targeting Hydrazine Synthase.

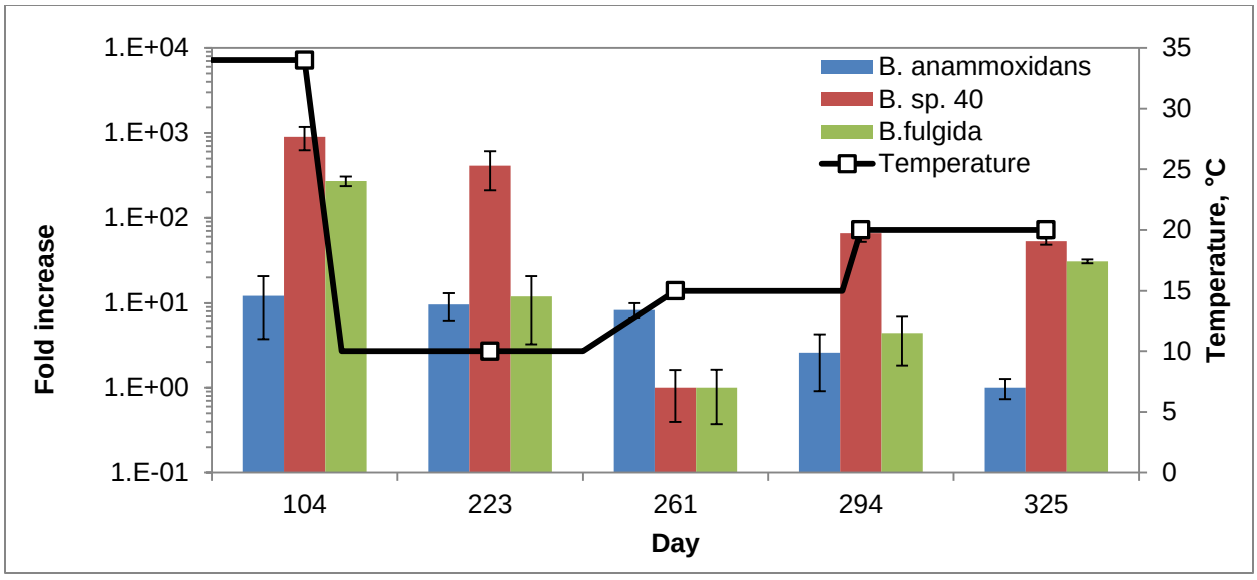


Figure 6.8: Fold Change in the Population of Specific Anammox Species Assessed in qPCR Analysis

6.4 DISCUSSION

6.4.1 Impact of Low Temperature on Anammox Activity

Rapid temperature decrease (average 1°C d^{-1}) strongly affected the anammox activity. Impact of the temperature was not, however, linear and was particularly noticeable below 22°C, with θ of 1.24 when the temperature was in regress and 1.31 when the temperature was in increase. The variable θ may have a significant impact on modeling of anammox process, since commonly used approach of constant temperature coefficient with high reference temperature (i.e. 30 °C) will either overestimate the activity at low temperatures or underestimate in the mid-range (i.e. 15 to 25°C).

The more pronounced drop of the activity below 22°C was expected considering that the biomass originated from a mesophilic reactor and it is in agreement with previous reports (De Clippeleir *et al.*, 2013a; Gilbert *et al.*, 2015; Hendrickx *et al.*, 2014; Hu *et al.*, 2013; Laureni *et*

al., 2015; Lotti *et al.*, 2014a; Ma *et al.*, 2013; Vázquez-Padín *et al.*, 2011). SAA of 40 mg N g VSS^{-1} d^{-1} measured after six days of operation at 10°C is in the middle of the range reported in the literature, range from below 10 to 70 mg N g VSS^{-1} d^{-1} at 10°C (Table 6.3). This might be result of the small-floc nature of biomass cultivated in MBR which, according to Gilbert *et al.* (2015), should be more affected by temperature than large bacteria agglomerates, i.e. granules or biofilms. Lotti *et al.* (2015) also reported highest loss of activity at 10°C (over 95% normalized to 30°C) of anammox biomass originating from MBR in comparison to five different anammox sludges.

Observed anammox behavior at 10°C is consistent with an "unrestrainable decrease in anammox activity" during prolonged operation at that temperature reported by Lotti *et al.* (2014), and "dramatic decrease in activity" reported by Laureni *et al.* (2016). Although high SAA after six days at 10°C was promising, it must be noted that it was in steep decline, losing almost 40% over two days (SAA two day earlier was 115 mg N g VSS^{-1} d^{-1}). MLVSS stopped increasing at that time despite absence of sludge wasting, an observation also reported by (Laureni *et al.*, 2015), who observed the MLVSS decrease below 12.5°C. Thus, although the unintended temperature drop to 5.7°C clearly expedited the anammox activity regress, it is not clear if that was the sole cause of complete loss of activity. After that event, it took over two month of continuous operation to partially recover the process and again after the NRR reached 20 mg N L^{-1} d^{-1} the activity started to decline to be completely lost. This time it took 25 days to see any activity and the NRR reached only 3±1 mg N L^{-1} d^{-1}. A notable anammox recovery was observed with a gradual increase of the temperature. The SAA reached 74 mg N g VSS^{-1} d^{-1} at 20°C with concentration of TN in the feed of 50 mg L^{-1}. This corresponded to a 75.6% TN removal efficiency and concentrations of ammonium and nitrite in the effluent below 1 mg L^{-1}.

Two most common causes of anammox inhibition in the literature are substrate inhibition with nitrite and free ammonia (Jaroszynski *et al.*, 2012; Lotti *et al.*, 2012). Lotti *et al.* (2012) used concentration of 50 mg NO_2-N/L in control tests for maximum specific anammox activity (at 30°C), assuming this concentration as non-inhibiting. Jaroszynski *et al.* (2012) presented that even much higher concentrations of nitrite (up to 120 mg NO_2-N/L) are not inhibiting (at 34°C) as long as free ammonia is below 2 mg NH_3-N/L. In this experiment the maximum concentration of nitrite and free ammonia after the temperature dropped to 5.7°C was respectively 40 mg NO_2-N/L and 0.1 mg NH_3-N/L. Thus, nitrite and free ammonia inhibition in this experiment are unlikely.

Relative to aerobic nitrification, high anammox SAA was observed at 16°C. The four day lag in the anammox activity observed after the temperature change from 16 to 10°C indicates that anammox might be suitable for mainstream implementation in moderate climates, where the temperature of raw wastewater rarely drops below 16°C. Mainstream operation may be also possible in colder climates with bioaugmentation from warmer sidestream treatment. Further research is needed to fully understand the physiological mechanisms behind the loss of activity during prolonged operation below 16°C.

Table 6.3: Reference List of Lab Scale Anammox Reactors Operated at Temperature ≤16°C

Author	Study temp., °C	Enrichment temp., °C	Low temp. operation period, d	Including nitritation?	Form of biomass aggregation[j]	Operation mode	Dominant anammox species	TN Influent conc. mg N L^{-1}	Effluent NH_4^+ (NO_2^-) conc. mg N L^{-1}	TN rem. efficiency, %	NRR, mg N $L^{-1}d^{-1}$	SAA, mg N g VSS^{-1} d^{-1}	Comment
This study	10	34	4	no	S	SBR	B. fulgida and B. sp. 40	415	5.3 (8.9)	88	106	115	Temperature was reduced over 24 days in 4 steps, 6°C per step. After 7 days at 10°C temperature dropped to 5.7°C and activity was lost.
			6					415	47 (61)	30	36	40	
Ma *et al.* (2013)	16	30	63	no	G	UASB	NR	51	<10 (<5)	50	2280	470	Seed from municipal WWTP.
Vazquez-Padin *et al.* (2011)	15	20	100	yes	G	SBR	NR	175	NR	29[c]	200	130[d]	
De Clippeleir *et al.*(2013)	14	29	31	yes	B	RBC	NR	70	7 (15)	42	529	NR	
Hu *et al.* (2013)	12	25f	>200	yes	NR	SBR	B. fulgida	70	<20[a] (<0.1)	92	NR	36[e]	Temperature reduced at 1.2°C per day. DO in the reactor <0.05 mg O_2 L^{-1}.
Laureni *et al.* (2016)	15	29	>150	yes	B	MBBR-SBR	Ca. Brocadia[n]	22	1.8 (<0.2)	74	30	103	Reactors fed with aerobically pretreated municipal wastewater
					B + S	hMBBR-SBR[l]		22	2.1 (<0.2)	63	26	138	
	11 to 17[m]	29	26	yes	B	MBBR-SBR		22	>16	NR	<20[o]	NR	
Laureni *et al.* (2015)	12.5[k]	29	<30	no	S[h]	SBR	B. fulgida	130	5 to 20[i]	NR	46	42[d]	Reactor fed with aerobically pretreated municipal wastewater
Lotti *et al.* (2014)	15	22	>300[a]	yes	G	SBR	B. fulgida	160	10 to 70 (10 to 30)[a]	73	400	150	After ca. 200 days temperature was increased to 20C for 20 days. Continuous decline of the activity observed.
	10	22	>100[a]	yes	G	SBR	B. fulgida	130	10 to 30 (5 to 30)[a]	47	200	70	
Gilbert *et al.* (2015)	10	20	1[g]	yes	S	SBR	NR	50	6	NR	<10[a]	<10	Temperature reduced at 0.5°C per week.
	10	20	1[g]	yes	G	SBR	NR	50	6	NR	<10[a]	<10	
	10	20	1[g]	yes	B	MBBR	NR	50	6	NR	20[a]	<10	
Hendrickx *et al.* (2014)	10	10	722	no	S	CSTR/SBR MBR[b]	B. fulgida	61	NR	81[c]	27	30 to 44	Seed from municipal WWTPs.

Notes:
NR – not reported; UASB – upflow anaerobic sludge blanket; SBR – sequencing bioreactor; RBC – rotating biocontactor; MBBR – moving bed biofilm reactor; CSTR – completely stirred-tank reactor; MBR – membrane bioreactor;
a Read from graph
b Reactor operated in continuous mode with membrane in the last phase and in SBR mode during enrichment phase.
c Calculated from reported values of total nitrogen removal and loading rate
d Calculated from reported values of total nitrogen removal rate and reactor biomass concentration
e Specific activity in ex situ test at 10°C calculated considering 0.6 g-protein g-VSS-1
f Reactor was operated at 25C for 124 days directly before temperature was reduced to 12 C. However, the reactor was seeded with biomass enriched at 30 C.
g Results after 20 weeks of slow gradual temperature decrease.
h In later period biofilm was developed on walls on the reactor in addition to suspended biomass,
i TN concentration in the reactor
j G - granules; B - biofilm; S - suspended biomass
k The temperature based on the figure 2 (Laureni et al., 2015) was fluctuating between 12 and 15°C.
l Hybrid system including attached growth on plastic media and suspended growth.
m The reactor was operated at 17°C and the temperature was suddenly dropped to 11°C for 26 days.
n Applied methodology targeted only the Candidatus Brocadia genera related bacteria. Results were not species specific but indicated that the Ca. Brocadia related was the dominant anammox organisms.
o Sudden decrease of the temperature resulted in the sharp decrease of the activity. Single point value was reported after the temperature decrease.

6.4.2 Population Dynamics Measured By qPCR

The quantitative PCR data indicated that a minimum of 3 different strains from the genus *Brocadia* (*B. fulgida*, *Brocadia sp. 40* and *B. anammoxidans*) were detected in the reactor. The anammox bacteria count dropped on average 10-fold after ca. 100 day of operation at 10°C and another 10-fold during the initial steps temperature increase before it started to recover at 20°C. This is in agreement with Hu *et al.* (2013) who observed 100-fold decrease in the copy numbers

of anammox bacteria after they decreased temperature from 25 to 12°C. The population proportions did not change significantly from the initial mesophilic mixture even after nearly 100 days at 10°C. While there appeared to be some fluctuations in the populations when the temperature was increasing, the proportion of these organisms appeared to stabilize to similar proportions as the original sample once the system reached its new steady state at 20°C. With high probability, the dominant species was *B. fulgida*. Despite the fact its apparent optimum is between 20 and 30°C, this bacterium was reported to be a dominant species also in other low temperature studies (Hu *et al.*, 2013; Laureni *et al.*, 2015; Lotti *et al.*, 2014). Hendrickx *et al.* (2014) detected bacteria similar to the genus *Brocadia* (along with strains from the genus *Kuenenia* and *Scalindua*) in the mainstream of WWTP at the end of winter period. This indicates the resilience of these mesophilic organisms through the time period where the temperature is too cold for proliferation.

Although it is expected to observe some heterotrophic microorganisms growing on the decay of anammox bacteria at a long SRT, the low anammox cell counts in comparison to general bacteria population is likely an underestimation. This may be due to lower efficiency of DNA extraction from anammox bacteria. Thus, we cannot draw any conclusion as to the level of anammox enrichment based on the direct comparison of total cell count and total anammox cell count. However, since it is safe to assume that closely related bacteria (the same genus) have similar DNA extraction efficiency, the observations of the population structure are valid.

6.5 CONCLUSIONS

- Anammox process was strongly affected by the temperature and the rate of impact of temperature was increasing below 22°C. In this study temperature coefficient was found to be 1.01 and 1.24 for the temperature ranges of 22 to 34°C and 10 to 22°C, respectively.
- Relatively high activity of 113 mg N g VSS^{-1} d^{-1} at 16°C, lag in response to further temperature decrease and high TN removal efficiency above 88% indicate that even mesophilic-enriched anammox biomass is suitable for main stream operation in moderate climates and possibly in colder climates with bioaugmentation from the side stream.
- Nevertheless, anammox activity was completely stopped after short exposure to temperature below 6°C. No significant process recovery was observed during further operation at 10°C for over 100 days. To start recovery of anammox activity it was required to increase the temperature to 20°C.
- Despite decrease in abundance, no change in anammox population structure was observed. *B. fulgida* was most probably the dominant species in this study. This, in context of existing literature, indicates that *B. fulgida* may be a species worthy of a more detailed investigation of the anammox metabolism in mainstream conditions.

7 IMPACT OF ELECTRIC CURRENT ON ANAMMOX ACTIVITY

7.1 BACKGROUND

In the proposed PN/A EASY MBR process anammox biomass will be exposed to the electric current. The objective of this set of experiment was to evaluate the impact of an electric current on anammox process. The secondary objective was to assess the interaction of soluble nitrogen species with electrodes and its impact on the estimation of nitrogen removal rates in anammox systems.

The impact of an electric current on the anammox bacteria is the part of the research for which there is the least background literature available. The two major reasons for that might be: (1) anammox is still a relatively new process and (2) currently there are no processes implemented in full scale involving application of an electric current directly to ML in wastewater treatment. Available reports can be grouped into two categories: (1) no direct contact of electrodes with biomass (impact of magnetic or electromagnetic field), and (2) electrodes submerged in the bacteria suspension (impact of an electric current, electric field and metals released from electrodes).

Qiao *et al.* (2014) conducted a short and long term exposure experiment with electrodes placed outside of a reactor. It was found that anammox was stimulated up to 25% with electric field strength of 2V/cm applied for 20 minutes. However, longer exposure (i.e. 24 h) to an electric field in the range of 1 to 4 V/cm resulted in inhibition. The inhibition effect was fully reversible. In the long term (continuous flow and continuous exposure) decrease of activity at 1 V/cm and 2 V/cm, and collapse of activity at 4 V/cm.

Liu *et al.* (2008) examined the impact of a magnetic field on the anammox activity in short term exposure tests. Fields between 0 and 100 mT resulted in increased activity. For reference, a typical fridge magnet generates a magnetic field of 5 to 10 mT. The maximum measured at 75 mT was a 50% increase. Field strength above 120 mT caused inhibition. Long term operation at 60 mT shortened the process start-up by 25% and increased activity by 30%.

Zhang *et al.* (2012) conducted a long term experiment using iron sacrificial anode placed directly in the bioreactor. Good process performance was reported with voltage up to 0.8 V. Process start-up in a reactor with electrodes was 24% shorter than in the control reactor. However, voltage increased to 1 V caused immediate process collapse. Increased concentration of Heme C was reported for the reactor with electrodes.

Yin *et al.* (2015) conducted a 95 day study using carbon fiber felt electrodes submerged in the bioreactor. The applied voltage ranged from 0.01 to 0.32 V (current not reported). The optimum was found to be at 0.08 V, which resulted in a 13% increase of nitrogen removal efficiency. It was found that an electric current improved biomass agglomeration and promoted attached growth on electrodes.

7.2 METHODOLOGY

The impact of a direct current on the anammox activity was examined in two consecutive experiments. The first experiment consisted of a series of *ex situ* batch tests and the second experiment was a continuous *in situ* test. Both experiments were conducted using biomass from MBR1.

7.2.1 Ex Situ Batch Tests Experiment

The batch tests were initially intended to provide information on the short term response of anammox biomass to the wide range of electric currents allowing establishing appropriate current range for the long term in situ tests. However, due to interference of redox reactions of the anammox substrates and products caused by the current, the results were ambiguous and this experiment was stopped after four tests.

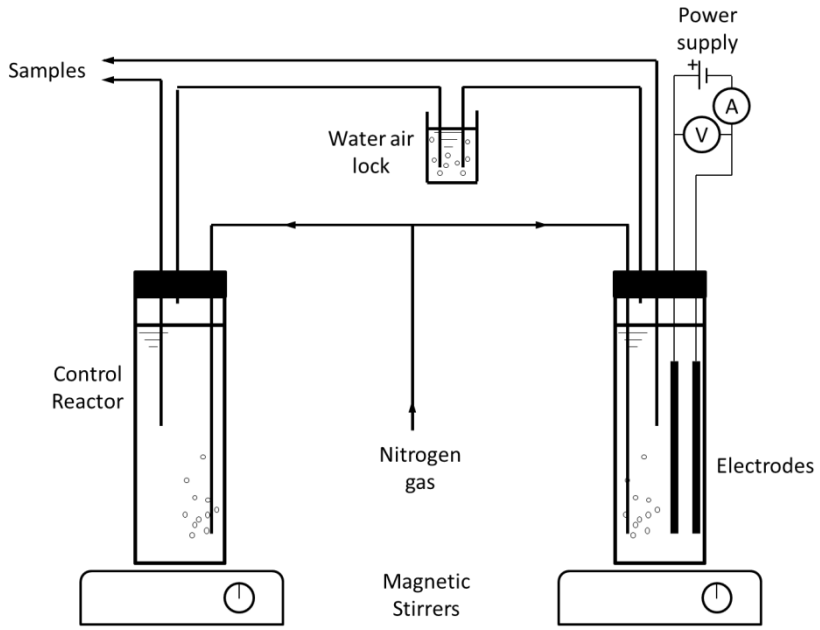


Figure 7.1 Experimental Set-Up for Ex Situ Tests of Anammox Activity with Electric Current

The tests were conducted in the 34°C walk-in incubator (the same in which MBR1 was located) using two 1 L glass bottles as reactors. The reactors were sealed with rubber stoppers and the content was mixed using magnetic stirrers (Figure 7.1). Each reactor was equipped with a sampling port, a port for sparging with nitrogen gas and a head space pressure release with water lock. The water lock was setup to maintain 10 mmH_2O pressure in the headspace. One of the reactors was additionally equipped with two electrodes. The electrodes were made of fine grain

graphite plates (submerged dimensions: 10 mm by 3 mm by 120 mm) mounted on brass rods. Graphite electrodes were chosen as they are simple to handle and relatively inexpensive in comparison to titanium or platinum. No reports have been found of anammox inhibition by graphite. Each test was conducted in parallel in both reactors (reactor without electrodes was considered as a control). The four tests were run with process conditions as described in Table 7.1.

Table 7.1: Conditions of Batch Ex Situ Tests of Electric Current Impact on Anammox

Parameter	Unit	Test reactor				Control reactor			
		T1	T2	T3	T4	T1	T2	T3	T4
Biomass	Yes/No	Y	Y	N	N	Y	Y	N	N
Biomass washed	Yes/No	N	Y	na	na	N	Y	na	na
El. Current	mA	50	50	50	50	0	0	0	0
Voltage	V	2.4	3.1	3.3	3.7	0	0	0	0
Initial Ammonia	mg N/L	32 (25)	28 (25)	28	28	32 (25)	28 (25)	28	28
Initial Nitrite	mg N/L	26 (25)	27 (25)	29	0	26 (25)	27 (25)	28	0
Initial Nitrate	mg N/L	290 (0)	19 (10)	32	0	272 (0)	19 (10)	32	0
Initial pH	-	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1

Note: Concentrations presented in brackets were added at the beginning of a test. The difference between total concentration and supplemented concertation originated from the biomass matrix.
na – not applicable

The bacteria suspension (750 mL) for tests T1 and T2 was taken fresh each time from MBR1. Additionally, for test T2 biomass was washed with the mineral solution (see section 0); To wash the biomass, the bacteria suspension was allowed to settle for 30 minutes in the reactor bottles. Next, 550 mL of the supernatant was decanted and replaced with the mineral solution. These steps were repeated twice. In tests T3 and T4 no biomass was used, instead reactors were filled with 750 mL of mineral solution. Before each test, both reactors were vigorously mixed and

sparged with nitrogen gas for 20 minutes to remove dissolved oxygen. The sparging time was established in preliminary tests and was sufficient to provide DO concentrations below a detection limit of 0.05 mg/L. Later, the nitrogen gas flow was reduced to below 5 cm^3. This was enough to maintain the positive pressure in the reactors' head space. Next, ammonia, nitrate, nitrite, alkalinity and a pH buffer were added through the sampling port. Ammonia, nitrite and nitrate were added in the form of concentrated solution of ammonium chloride, sodium nitrate and sodium nitrite. The solution was prepared for each test separately. A concentration of the solution was calculated such that 20 mL of it would bring the concentration in the reactor to a concentration as presented in Table 7.1. The alkalinity was supplemented by adding 10 mL of solution sodium bicarbonate, increasing alkalinity in the reactor by 320 mg $CaCO_3$/L. HEPES 0.8 M (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid) solution was added as a pH buffer. In primary tests, the buffer dose of 20 mL (20 mM final concertation in the rector) was established as the optimal to reduce pH change without process inhibition. The alkalinity and the pH buffer were also added for the tests without biomass (T3 and T4). Thus, the total liquid volume in the reactor at the beginning of each test was 800 mL. The first sample was taken from the reactor immediately after vigorous sparging was stopped and all solutions were added. The second sample was taken ten minutes later. Following the second sample, samples were taken every 15 to 20 minutes for up to 3 hours. The volume of each sample was 9 mL. These samples were analyzed for ammonia, nitrate and nitrite (see section 5.2.3). At the end of test, two 100 mL samples were taken from each reactor for analysis of VSS and TSS (see section 5.2.3). The SAA was calculated, as described in section 5.2.4.

7.2.2 In Situ Continuous Flow Experiment

This experiment was conducted in the 28 L MBR1 (see section 5.2.1 for details of the reactor setup) operated in continuous mode at 34°C. On day 222 of MBR1 operation, electrodes were installed in the reactor. Since the ultimate objective of this project was to establish the feasibility of electrochemical struvite precipitation in the same reactor with anammox, in this experiment it was decided to use magnesium electrodes. Identical magnesium electrodes (12 cm by 3 cm immersed dimensions) and power source were used as in the experiment involving conventional nitrification (see Chapter 0). The electrodes were spaced 2.5 cm apart.

The reactor was fed with synthetic feed at a rate of 935 g TN/m^3·d. The ammonia and nitrite concentration in the feed was 771 mg N/L and 972 mg N/L, respectively. The flow rate was 15 L/d, which translates to 320 L/m^2·d membrane flux. SRT in the reactor during this experiment was 23.3 d. A detailed feed description is in section 0.

The electric current was first introduced on day 318 (almost 2 full SRT cycles after the last process change). Direct current was applied in 5 minutes ON and 5 minutes OFF cycles. Polarity on the electrodes was switched once per week to avoid an uneven ware of electrodes.

Samples of the feed and permeate were taken regularly and analyzed for ammonia, nitrate and nitrite. Periodically, suspended solids in the reactor, phosphate and alkalinity were also measured. The analyses methods are described in section 5.2.3.

Based on the mass balance of nitrogen in the reactor, NRR and SAA were calculated as described in section 5.2.4. Note that the generated nitrate was not included in the calculations. The reactor was operated to maintain low concentrations of ammonia and nitrite (i.e. below 5

mg/L). Thus, anammox activity in continuous operation might have been limited by the substrate load and low concentration. In order to assess the changes in the maximum specific nitrogen removal rate (SAA_{max}), *in situ* batch test were conducted starting on day 305 every 2 to 5 days. During a batch test, the feed line was closed and the permeate pump was off. At the beginning of a batch test, ammonia was spiked to 60-70 mg N/L and nitrite was spiked to 35-45 mg N/L (concentration as measured in the reactor; concentrations varied depending on the initial concentration in the reactor). Alkalinity was also increased to 750 mg $CaCO_3$/L. No pH buffers (other than sodium bicarbonate) were added and the pH control pump was left turned on. To avoid the impact of redox reactions of nitrogen species caused by passing current, the power supply to the electrodes was turned off during batch tests. The time between sampling and the time of the entire test was adjusted based on the expected activity of the biomass, and varied between 10 to 40 minutes and 2 to 4 hours, respectively. A sample of biomass was taken at the end of each test for suspended solids analysis.

7.3 RESULTS AND DISCUSSION

7.3.1 Ex Situ Batch Tests

The results of the batch tests are presented in Figure 7.2. SAA activity observed in the control reactor in tests T1 and T2 was 782 and 1064 mg N/g VSS·d, respectively. Both results are in the expected range of anammox activity at 35°C of 500 to 2,000 mg N/g VSS·d. Higher SAA in test T2 may indicate that there might be some inhibitors present in the bacteria matrix taken directly from the MBR1, which were removed by washing the biomass with mineral solution before the test (in T1 biomass was not washed).

Although in test T1, initially ammonia removal in the reactor with electrodes was similar to the control reactor (control reactor removal rate was on average 3% higher), the final concentration in the reactor was higher. The nitrite decrease in the reactor with electrodes was faster than in the control reactor in the first part of the test but slowed down towards the end of the test. Since the biomass matrix was not washed for this test, the initial concentration of nitrate was high (in the vicinity of 300 mg N/L) and the dilution factor of 15 did not allow the accurate quantification of the change of nitrate concentration. The final NO_2:NH_4 removed in the control reactor and the reactor with electrodes was 1.33 and 1.46, respectively.

For the next test, T2, the bacteria matrix was washed to reduce the initial nitrite concentration. Trends observed in test T1 were magnified in test T2. The ammonia removal rate with the electric current was significantly slower from the beginning of the test, at the same time the nitrite removal was faster in the first 90 minutes and it slowed down again in the last part of the test. The final NO_2:NH_4 removed in the reactor with electrodes was at 2.24 (control at 1.33). Furthermore, the concentration of nitrate was increasing much faster in the reactor with electrodes and at the end of the test, the ratio of nitrate produced to ammonia removed was 0.90 (0.25 in the control reactor).

The results of the first two tests were ambiguous in terms of anammox activity in the reactor with electrodes, because the faster removal rate of nitrite and the faster production of nitrate would indicate higher SAA but it was contradicted with the slower removal rate of ammonia. Moreover, higher than expected NO_2:NH_4 (theoretical for anammox 1.32) and NO_3:NH_4 (theoretical for anammox 0.26) ratios indicated that anammox is not the only process transforming nitrogen in the reactor. Two additional tests were conducted to observe the nitrogen

reactions without biomass. In a test with solution containing ammonia, nitrite and nitrate (test T3); significant nitrite removal was observed, accompanied by an increase of ammonia and nitrate concentrations. The test with only ammonia in the solution, the electric current resulted in the removal of ammonia.

Nitrate, nitrite and ammonia can be relatively easily reduced or oxidised on the surface of electrode according to the following equations (Benjamin, 2015; Bunce and Bejan, 2011; Sawyer, 1994). Reactions described in Table 4.3 will run from right to left (reduction) on cathode and from left to right (oxidation) on an anode surface.

In addition to the direct interaction with the electrodes in tested conditions, ammonia can also be removed due to indirect oxidation generated on anode hypochlorous acid as shown in Eq. 7.1 and Eq. 7.2 (Bunce and Bejan, 2011).

Eq. 7.1 $\mathrm{OCl^- + H^+ - 2e^- \leftrightharpoons Cl^- + H_2O}$

Eq. 7.2 $\mathrm{2NH_3 + 3HOCl \rightarrow N_2 + 3HCl + 3H_2O}$

Although there is ample amount of literature available on the electrochemical reactions in nitrate, nitrite and ammonia solutions, there is still a debate as to the exact mechanisms of some of the processes (Mook *et al.*, 2012). The fact that in current studies, all three forms of nitrogen are present in the solution at the same time and a mixture of minerals added for biomass growth, only increases to the complexity of the problem. An estimation of the bioactivity in these conditions based on the mass balance of ammonia and nitrite would be questionable. It was decided to proceed directly to the continuous flow tests in a bigger scale bioreactor.

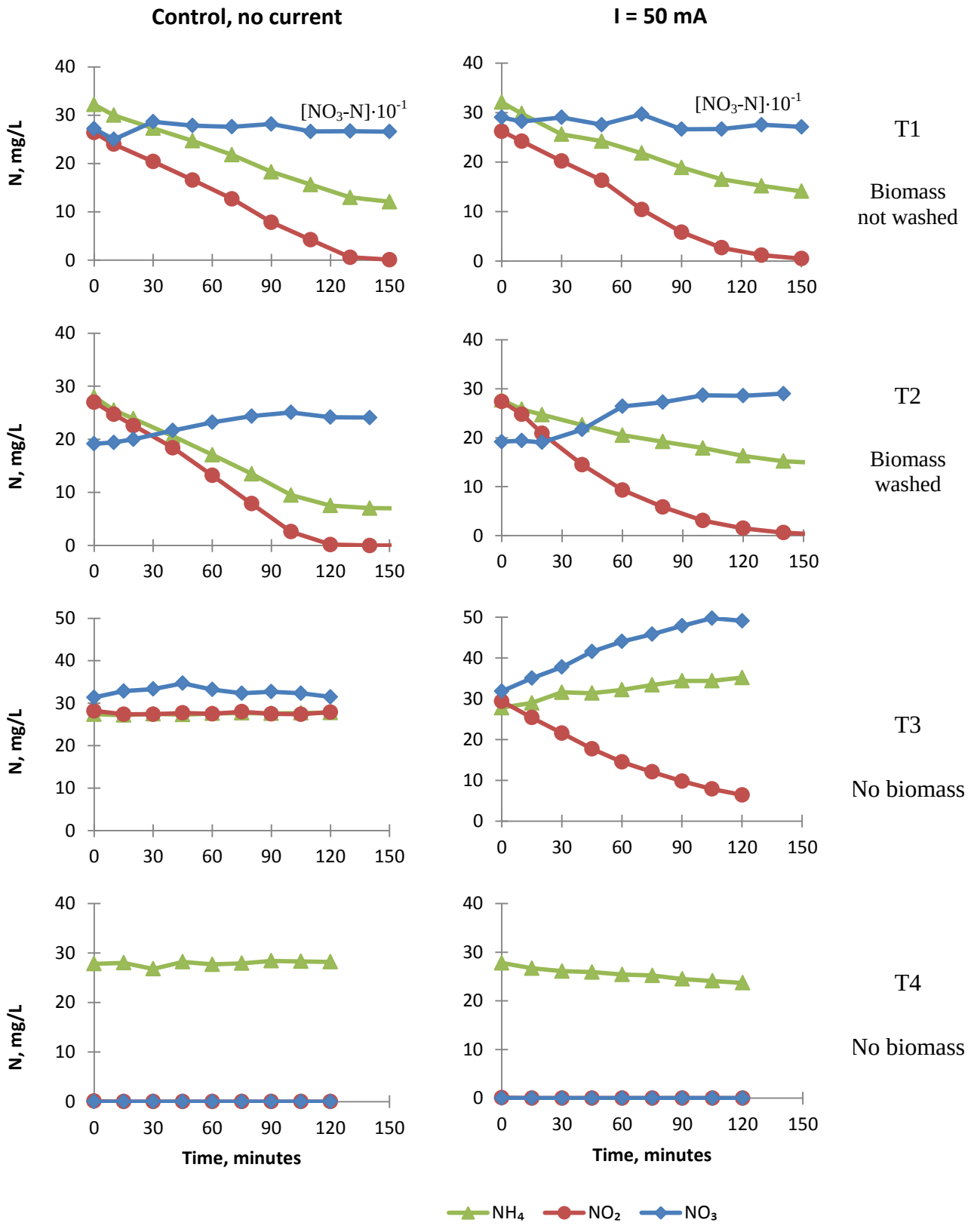


Figure 7.2 Impact of Electric Current on Anammox Activity in Ex Situ Batch Tests

7.3.2 In Situ Continuous Flow Experiment

Electrodes were installed in the tank on day 312 and no inhibition due to magnesium electrodes presence was observed.

The SAA_{max} before current was introduced was on average 1950±21 mg/g·d. After the electric current was turned ON on day 408, neither ammonia accumulation nor SAA_{max} decline were observed during the first 12 days with a current of 50 mA and following 6 days at 100 mA (in this test current was applied in 5 min ON and 5 min OFF cycles). The first symptoms of anammox inhibition were manifested on day 426 when the electric current was increased to 150 mA. The SAA_{max} dropped to 1083 mg/g·d. However, since there was still not accumulation of ammonia observed in the daily effluent sample (SAA_{max} was still higher than the loading rate) and the SAA_{max} test results were known the following day, the current was further increased to 250 mA. Over the next three days, ammonia started to slowly accumulate in the reactor, accounting for the remainder of ammonia from *in situ* kinetic tests. On day 427, the accumulation of ammonia in the effluent increased by 10 mg/L (measured 35 mg/L, but 25 mg/L was the remainder from the kinetic test) and by day 429, it reached 19 mg/L (measured at 62 mg/L). At the same time the SAA_{max} was measured at 1070 mg/g·d. On day 430, since nearly 50% of activity was lost, a decision was made to turn OFF the electric current to avoid further escalation of ammonia accumulation and complete the loss activity. No changes to the loading rate were made at this time.

Within 6 days, the effluent ammonia dropped back to its normal range below 5 mg/L. After the electric current was turned off ,a partial recovery of SAA_{max} was observed. The average SAA_{max}

between days 433 and 457 was 1382±90 mg/g·d, which is 71% of the activity measured before electric current was first applied.

In the first run with the electric current, symptoms of inhibition were observed only after the current was increased above 100 mA, it was decided to repeat the test at 100 mA. The second test was started on day 458, nearly 30 days after the first test was stopped, full activity was not recovered but operation was stable. Similar to the first test, no signs of process inhibition were initially observed. No substrate accumulation was observed until day 473 (two weeks after current was turned ON). Beginning on day 473 ammonia and nitrite accumulation propagated fast, reaching to 450 mg/L and 400 mg/L of ammonia and nitrite concentration in the effluent on day 475. The SAA_{max} measured that day dropped to 520 mg/g·d, which is 38% of the activity measured before the second test was commenced. The electric current was turned OFF again.

After the second test was stopped, the accumulated ammonia was too high for the process to recover. Therefore, the biomass in the reactor was washed. The permeate pump, feed flow and mixer were stopped. The biomass was allowed to settle for 1 hour, then 20 L of the supernatant was decanted. The liquid volume was replaced with a deionized water, sparged with nitrogen gas to remove DO. For next 10 days, the reactor nitrogen load was reduced to 120 mg/g·m^3 (8 times reduction of feed concentration). On day 486, the effluent concentration of nitrite and ammonia were below 1 mg/L and the normal nitrogen loading rate was reintroduced. No further ammonia or nitrite accumulation was observed until end of the study. The wash procedure resulted in fast activity recovery. SAA_{max} measured on day 392 was already at the level from before the seconded test (1303 mg/g·d) and on day 490, it exceeded the average SAA_{max} from before the first test reaching 2290 mg/g·d.

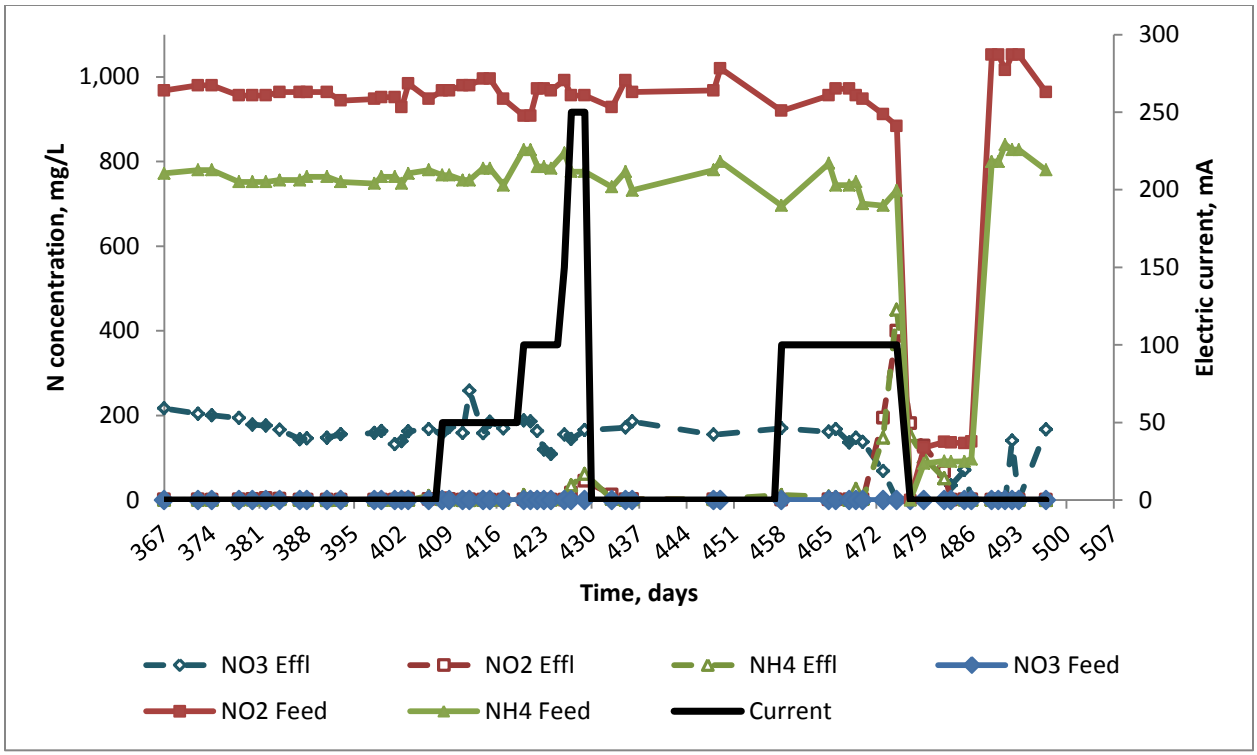
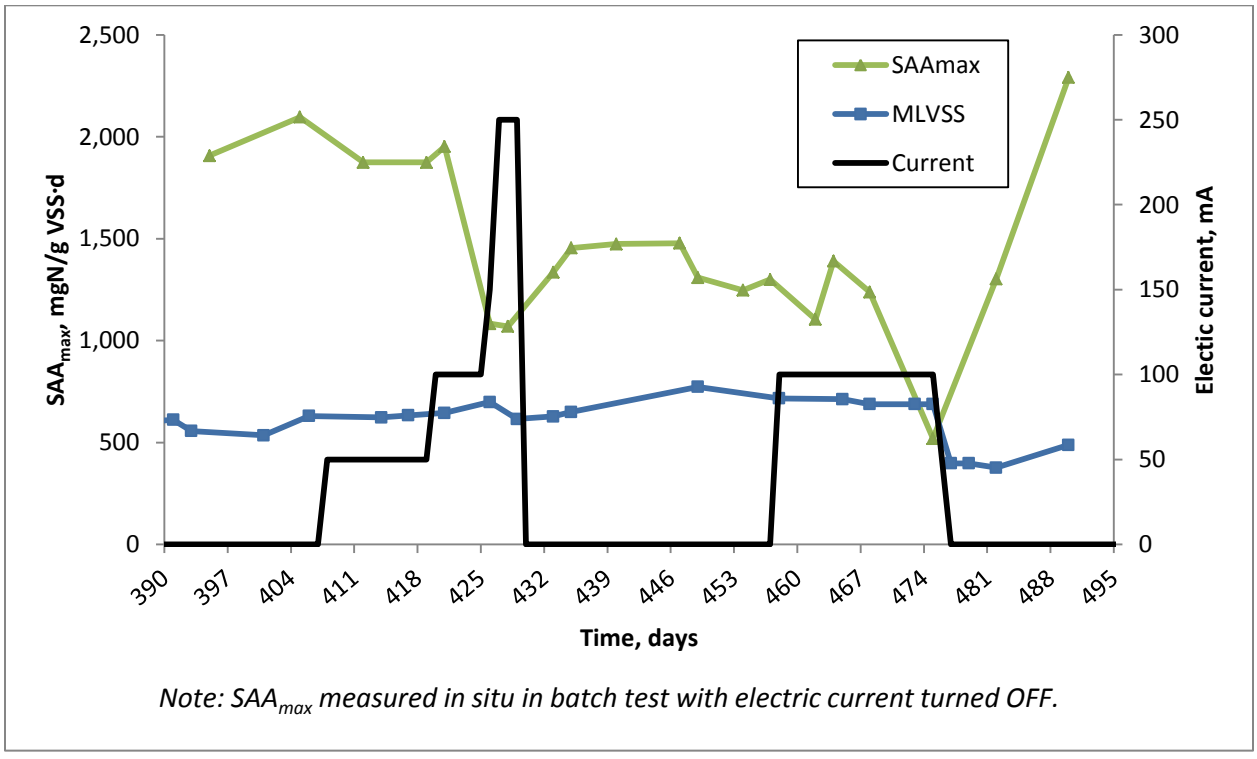


Figure 7.3: MBR1 Effluent Concentrations During the In Situ Continuous Flow Experiment with Electric Current



Note: SAA_{max} measured in situ in batch test with electric current turned OFF.

Figure 7.4: SAA_{max} During Continuous Flow Experiment

After initial negative results, the experiment was not continued. Thus, the exact cause of inhibition can only be hypothesized. Based on the observed pattern of activity changes, it is possible that the electric current may affected the permeability of intracytoplasmic and/or anammoxasome membrane, hindering transport of elements required for enzyme production. The bacteria would function normally until their internal resources were exhausted. This could explain why there is a lag between exposure of the biomass to the electric current and the decrease of activity. In this case there would be no factor which would directly affect the viability of cells (e.g. cell lysis). Thus, once the electric current was taken away and inhibitory concentrations of substrates were reduced, bacteria normal functionality came back. This would be in line with reversible inhibition effect reported by Qiao *et al.* (2014).

7.3.3 Conclusions

- The anammox bacteria are strongly inhibited by electric current in long term operation. Even relatively low electric current of 100 mA applied intermittently in 28 L tank did cause complete failure of process.
- Nitrogen redox reactions with electrodes may significantly affect results of test conducted in small volume anoxic batch reactors. Thus, bacterial activity in such test should be evaluated using methods other than methods based on the mass balance of nitrogen.

8 OVERVIEW AND CONCLUSIONS

8.1 FINAL CONCLUSIONS

The main objective of this study was to develop mainstream partial nitritation/anammox EASY MBR for simultaneous nitrogen removal and phosphorus recovery. In course of the work the idea of coupling anammox process with electrochemical struvite precipitation was found to be not feasible (see Chapter 7). Still, the work resulted in development of EASY method for phosphorus recovery applicable for mainstream and sidestream treatment even at non-EBPR plants, and further insights in development of mainstream anammox.

Following are the specific conclusions from the work.

- The study proved that phosphorus can be recovered electrochemically using magnesium sacrificial anodes (the EASY method). The method found to be efficient under mainstream and sidestream conditions, producing high quality struvite product (see Chapter 3).
- EASY method of phosphorus recovery may be successfully applied in non-EBPR plants and could be applied simultaneously with biological nitrification (see Chapter 0).
- Anammox bacteria were strongly affected by low temperatures, especially below 22°C. Relatively high specific nitrogen removal rate of 113 mgN $gVSS^{-1}$ d^{-1} at 16°C makes anammox a feasible option for mainstream treatment in moderate climates (see Chapter 5.4).
- Extremely low temperatures (i.e. below 6°C) may cause immediate anammox inhibition. Process recovery at temperatures below 20°C may take over 100 days, which is too long for practical application (see Chapter 5.4).

- Despite decrease in abundance during the long process low activity caused by extreme cold inhibition, no change in anammox population structure was observed. *B. fulgida* was most probably the dominant species. This, in context of existing literature indicates that *B. fulgida* may be a species worthy of a more detailed investigation of the anammox metabolism in mainstream conditions (see Chapter 5.4).
- Although initially observed response of anammox biomass to exposure to electric current was neutral, the long term exposure cause reversible but severe process inhibition (see Chapter 7).
- Anammox inhibition caused by high ammonia accumulation was easily reversible with biomass washing (see Chapter 5 and 7).

8.2 ENGINEERING SIGNIFICANCE

8.2.1 Electrochemical Phosphorus Recovery

Typically phosphorus is recovered in form of struvite, which requires at least 1:1:1 molar ratio of ammonium, magnesium and phosphate, and pH in range of 8 to 9. In wastewater treatment usually magnesium and pH are the limiting factors. Thus, phosphorus recovery requires dosing of magnesium compound (e.g. $MgCl_2$), alkali for pH increase during the production and acid for equipment maintenance. Hug and Udert (2013) showed that if only the cost of the magnesium source were accounted for, the EASY method would be 35% cheaper than struvite precipitation with $MgCl_2$. The electrochemical method does not require pH adjustment. Electrochemical methods could be also applied in streams with much lower concentrations of phosphorus and with lower pH in the bulk volume, thus allowing much stricter control of the precipitation process – less scaling of equipment. In result the amount of acid needed for equipment

maintenance would be significantly lower. The magnesium source is also much more concentrated. Technical grade anhydrous $MgCl_2$ will consist only in approximately 25% of magnesium, whereas magnesium electrodes may contain up to 95% of magnesium (accounting for alloy impurities and structural support). The lower chemical requirements not only reduces the direct cost of chemicals but also affects storage and transportation, which may be very important for remote locations.

Although phosphorus recovery requirement in remote locations may presently seem to be rather unlikely in near future, the phosphorus removal requirements may be introduced soon. Phosphorus precipitation with magnesium electrodes could be an alternative to EBPR or conventional chemical precipitation. Biological phosphorus removal is a relatively complex process which requires qualified full time operators and may also result in increased scaling problems in sludge management system. Conventional precipitation will require large amounts of chemicals which often would be delivered using winter roads, ships or aircrafts. A typical community of 1000 people, assuming 2 g P per capita per day, only 70% phosphorus to be precipitated and ferric overdose ratio of 2.5, would require 18 t of ferric (37% w/v) per year. In comparison the same community would require less than 500 kg of magnesium electrodes (95% w/w). Additionally to ease operation of the electrochemical process and lower cost of chemical transportation, recovered product could be locally used as a high quality fertilizer, further reducing cost of transportation.

The important benefit of the electrochemical method is that it can be applied simultaneously with conventional biological nitrogen removal process. This allows potentially simple retrofit option.

Electrodes could be installed for example in the ML channels upstream from clarifiers and the product could be harvested from RAS.

8.2.2 Mainstream Anammox

This research provides steppingstones for further development of mainstream anammox. It was shown that the anammox biomass enriched in the sidestream treatment has high potential for application in the mainstream treatment in moderate climates. However, in case of process upset, the recovery at low temperature may not be practically feasible. This suggests that it is imperative for plants planning to implement mainstream anammox, to first develop sidestream anammox treatment and use it as seed at start-up of the mainstream process and in case of process upsets.

Temperature coefficient (Arrhenius) for the anammox growth rate was estimated. It was shown that the coefficient is not constant. The anammox bacteria were affected stronger by the temperature below 22°C. This information is very important for process modeling and design.

The insights in the anammox population dynamics during long term exposure to low temperature may be invaluable in further research searching for the most viable mainstream anammox species. In turn this may result in development of new process controls promoting growth of specific “mainstream anammox” bacteria.

8.3 RECOMMENDATIONS FOR FUTURE WORK

- Pilot study of the electrochemical phosphorus recovery would allow evaluating scalability of the method. The critical aspects would have to be investigated are:

    a. *Construction of the electrodes.* In lab scale study the electrodes did not have any inner structural core in result when electrodes got too thin they had tendency to break. This would be considered wasted material. Inner core made of metal less corrosive than magnesium would provide needed support and allow full utilization of magnesium. Construction of electrodes would also have to allow high magnesium packing density. The more magnesium could be placed at once in the system the less often electrodes would have to be replaced.

    b. *Electrodes cleaning*. In this study no significant biofilm build up was observed on electrodes. However, in pilot or full scale there is potential for biofilm growth on electrodes if operated lower electric currents or if electrodes will would be activated in sequence. Thus cleaning methods would have to be evaluated. Cleaning could be potentially done mechanically (e.g. water jets, air sparging) or electrically. Cycling the polarity electrodes together with short burst of increased electric current showed promising results in conducted not presented here side tests.

    c. *Product extraction*. In this study struvite was separated from the ML using upflow clarifier. However, other options should be also explored, e.g. sieves or hydrocyclones.

    d. *Power supply and power distribution safety*. Although relatively small voltage is required for magnesium release, the process rate is driven by the electric current. For safety reasons the individual electrode should work at low currents (e.g. below 1 A),

however the total current passing through the system might need to be much higher. For the 1000 capita community from example in previous section the system would require approximately 110 A.

- This study indicated that the effect of the temperature on anammox bacteria is not linear. The process may be affected much stronger at lower temperatures. Current process design relies strongly on mathematical models. Models however are only as reliable as the process parameters inputs are. One of the key parameters for the mainstream anammox process is the temperature growth rate coefficient (Arrhenius). This study provided estimation of the temperature coefficients at different temperatures but these were based on the results attained from not stabilized system. While the presented values may be correct and be significant values for microbiologist, the process design engineers are rather interested in temperature coefficients after process stabilisation. The potential difference between these values may occur when coefficient is estimated for the whole functional population (in this case anammox) and the population structure will shift due to long term exposure to the new temperature. Thus, further long term studies at low temperature using anammox seed from different sources would build stronger reference frame for model developers.

9 REFERENCES

Acevedo, B., Oehmen, A., Carvalho, G., Seco, A., Borrás, L., Barat, R. (2012). Metabolic shift of polyphosphate-accumulating organisms with different levels of polyphosphate storage. *Water Res.* **46**, 1889–1900.

Algeo, T., O'Callaghan, P. (2012). Nutrient Removal and Recovery Market and Technology Overview. Vancouver, BC.

Andrei, M., di Gabriele, F., Bonora, P.L., Scantlebury, D. (2003). Corrosion behaviour of magnesium sacrificial anodes in tap water. *Mater. Corros.* **54**, 5–11.

Barnard, J.L., Dunlap, P., Steichen, M. (2016). Rethinking the Mechanisms of Biological Phosphorus Removal, in: Proceedings of IWA/WEF Nutrient Removal and Recovery Conference. Denver, CO.

Batziaka, V., Fytianos, K., Voudrias, E. (2008). Leaching of nitrogen, phosphorus, TOC and COD from the biosolids of the municipal wastewater treatment plant of Thessaloniki. *Environ. Monit. Assess.* **140**, 331–8.

Ben Moussa, S., Maurin, G., Gabrielli, C., Ben Amor, M. (2006). Electrochemical Precipitation of Struvite. *Electrochem. Solid-State Lett.* **9**, 97–101.

Benisch, M., Schauer, P., Neethling, J.B. (2014). Improving Dewaterability of Digested Sludge from EBPR Facilities, in: WEF Residual and Biosolids Conference. Austin, TX.

Benjamin, M. (2015). Water Chemistry, 2nd ed. Waveland Press, Long Grove, IL.

Blackburne, R., Yuan, Z., Keller, J. (2008). Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. *Biodegradation* **19**, 303–312.

Britton, A., Koch, F., Mavinic, D., Adnan, A., Oldham, W., Udala, B. (2005). Pilot-scale struvite recovery from anaerobic digester supernatant at an enhanced biological phosphorus removal wastewater treatment plant. *J. Environ. Eng. Sci.* **4**, 265–277.

Britton, A., Prasad, R., Balzer, B., Cubbage, L. (2009). Pilot testing and economic evaluation of struvite recovery from dewatering centrate at HRSD's Nansemond WWTP, in: Ashley, K., Mavinic, D., Koch, F. (Eds.), International Conference on Nutrient Recovery from Wastewater Streams. IWA Publishing, Vancouver, BC, Canada, pp. 193–202.

Bunce, N.J., Bejan, D. (2011). Mechanism of electrochemical oxidation of ammonia. *Electrochim. Acta* **56**, 8085–8093.

Bunce, R.W., Miller, M.W., Regmi, P., Hingley, D.M., Kinnear, D., Bott, C.B. (2013). Modification of a B-stage MLE to Take Advantage of SND and Nitrite Shunt in an A/B Process Pilot Study, in: WEF/IWA Nutrient Removal and Recovery. Vancouver, BC.

Cadmus Group (2010). EPA Manual: Nutrient Control Design Manual. Watertown, MA.

Cao, Y., Kwok, B., Hin Yong, W., Chye Chua, S., Long Wah, Y., Ghani, Y. (2013). Mainstream Partial Nitritation–ANAMMOX Nitrogen Removal in the Largest Full- Scale Activated Sludge Process in Singapore: process analysis, in: WEF/IWA Nutrient Removal and Recovery. Vancouver, BC.

Carvajal-Arroyo, J.M., Sun, W., Sierra-Alvarez, R., Field, J. a. (2013). Inhibition of anaerobic ammonium oxidizing (anammox) enrichment cultures by substrates, metabolites and common wastewater constituents. *Chemosphere* **91**, 22–27.

Celmer-Repin, D., Hwang, J.H., Cicek, N., Oleszkiewicz, J. a (2010). Autotrophic nitrogen-removing biofilms on porous and non-porous membranes. *Environ. Technol.* **31**, 1391–1401.

Chesapeake Bay Initiative (2012). Bay Action Plan [WWW Document]. URL http://www.bayactionplan.com/ (accessed 10.30.13).

Cordell, D., Neset, T., Drangert, J.-O., White, S. (2009). Preferred future phosphorus scenarios: A framework for meeting long-term phosphorus needs for global food demand., in: International Conference on Nutrient Recovery from Wastewater Streams. Vancouver, BC.

Cornel, P., Schaum, C. (2009). Phosphorus recovery from wastewater: needs, technologies and costs. *Water Sci. Technol.* **59**, 1069–76.

Crawford, G. (2010). Technology Roadmap for Sustainable Wastewater Treatment Plants in a Carbon-Constrained World. Alexandria, VA.

De Clippeleir, H., Vlaeminck, S.E., De Wilde, F., Daeninck, K., Mosquera, M., Boeckx, P., Verstraete, W., Boon, N. (2013a). One-stage partial nitritation/anammox at 15°C on pretreated sewage: feasibility demonstration at lab-scale. *Appl. Microbiol. Biotechnol.* **97**, 10199–10210.

De Clippeleir, H., Vlaeminck, S.E., De Wilde, F., Jordaens, R., Courtens, E., Boeckx, P., Verstraete, W., Boon, N. (2013b). Mainstream Sewage Treatment with Partial Nitrification-Anammox: Potential Rolle of NO Production, in: WEF/IWA Nutrient Removal and Recovery. Vancouver, BC.

Doyle, J., Oldring, K., Churchley, J., Parsons, S. (2002). Struvite formation and the fouling propensity of different materials. *Water Res.* **36**, 3971–8.

Dytczak, M.A., Londry, K.L., Oleszkiewicz, J.A. (2008). Activated sludge operational regime has significant impact on the type of nitrifying community and its nitrification rates. *Water Res.* **42**, 2320–2328.

Eaton, A.D., Franson, M.A.H. (2005). Standard Methods for the Examination of Water and Wastewater, 21st ed. APHA, AWWA, WEF.

Egle, L., Rechberger, H., Zessner, M. (2013). Integrated assessment of Phosphorus recycling technologies from waste water, in: WEF/IWA Nutrient Removal and Recovery. Vancouver, BC, BC, Canada.

Gaterell, M., Gay, R., Wilson, R., Gochin, R., Lester, J. (2000). An economic and environmental evaluation of the opportunities for substituting phosphorus recovered from wastewater treatment works in existing UK fertiliser markets. *Environ. Technol.* **21**, 1067–1084.

Ge, S., Wang, S., Yang, X., Qiu, S., Li, B., Peng, Y. (2015). Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: A review. *Chemosphere* **140**, 85–98.

Germany (2016). Ordinance reorganising sewage sludge recovery (Sewage Sludge Ordinance).

Gilbert, E.M., Agrawal, S., Schwartz, T., Horn, H., Lackner, S. (2015). Comparing different Reactor Configurations for Partial Nitritation/Anammox at low Temperatures. *Water Res.* **81**, 92–100.

Goel, R., Flora, J. (2005). Stimulating biological nitrification via electrolytic oxygenation. *J. Environ. Eng.* **131**, 1607–1613.

Graaf, A.A. Van De, Bruijn, P. De, Robertson, L.A., Jetten, M.M., Kuenen, J.G. (1996). Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiology* 2187–2196.

Hao, X.-D., Wang, C.-C., Lan, L., van Loosdrecht, M.C.M. (2008). Struvite formation, analytical methods and effects of pH and Ca2+. *Water Sci. Technol.* **58**, 1687–92.

Harhangi, H.R., Le Roy, M., van Alen, T., Hu, B.L., Groen, J., Kartal, B., Tringe, S.G., Quan, Z.X., Jetten, M.S.M., Op den Camp, H.J.M. (2012). Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. *Appl. Environ. Microbiol.* **78**, 752–758.

Heffernan, B., Blanc, J., Lubbe, J. Van Der (2011). Comparison of greenhouse gas emissions from UASB based and conventional primary clarifier based sewage treatment plants, in: 11th IWA Specialised Conference on Design, Operation and Economics of Large Wastewater Treatment Plants. Budapest, Hungary, pp. 83–92.

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

Hendrickx, T.L.G., Kampman, C., Zeeman, G., Temmink, H., Hu, Z., Kartal, B., Buisman, C.J.N. (2014). High specific activity for anammox bacteria enriched from activated sludge at 10°C. *Bioresour. Technol.* **163**, 214–21.

Holt, P., Barton, G., Mitchell, C. (2005). The future for electrocoagulation as a localised water treatment technology. *Chemosphere* **59**, 355–367.

Hu, B.L., Rush, D., Biezen, E. Van Der, Zheng, P., Mullekom, M. Van, Schouten, S., Sinninghe Damsté, J.S., Smolders, A.J.P., Jetten, M.S.M., Kartal, B. (2011). New anaerobic, ammonium-oxidizing community enriched from peat soil. *Appl. Environ. Microbiol.* **77**, 966–971.

Hu, Z., Lotti, T., de Kreuk, M., Kleerebezem, R., van Loosdrecht, M., Kruit, J., Jetten, M.S.M., Kartal, B. (2013). Nitrogen removal by a nitritation-anammox bioreactor at low temperature. *Appl. Environ. Microbiol.* **79**, 2807–12.

Huang, W., Wang, W., Shi, W., Lei, Z., Zhang, Z., Chen, R., Zhou, B. (2014). Use low direct current electric field to augment nitrification and structural stability of aerobic granular sludge when treating low COD/NH4-N wastewater. *Bioresour. Technol.* **171**, 139–144.

Hug, A., Udert, K.M. (2013). Struvite precipitation from urine with electrochemical magnesium dosage. *Water Res.* **47**, 289–99.

Hwang, J. (2010). Two Stage Membrane Biofilm Reactors for Nitrification and Hydrogenotrophic Denitrification. University of Manitoba.

Hwang, J.H., Cicek, N., Oleszkiewicz, J. a (2010). Achieving biofilm control in a membrane biofilm reactor removing total nitrogen. *Water Res.* **44**, 2283–91.

Ibeid, S., Elektorowicz, M., Oleszkiewicz, J.A. (2013). Novel electrokinetic approach reduces membrane fouling. *Water Res.* **47**, 6358–66.

Jaroszynski, L.W. (2012). The Influence of Nitrite and Free Ammonia on Nitrogen Removal Rates in Anoxic Ammonium Oxidation Reactors. University of Manitoba.

Jaroszynski, L.W., Cicek, N., Sparling, R., Oleszkiewicz, J. a (2012). Impact of free ammonia on anammox rates (anoxic ammonium oxidation) in a moving bed biofilm reactor. *Chemosphere* **88**, 188–95.

Jetten, M.S.M., Niftrik, L. Van, Strous, M., Kartal, B., Keltjens, J.T., Op den Camp, H.J.M. (2009). Biochemistry and molecular biology of anammox bacteria. *Crit. Rev. Biochem. Mol. Biol.* **44**, 65–84.

Jimenez, J. (2015). Innovations in nitrogen and phosphorus removal, in: RMWEA 1st Annual Innovative Wastewater Technologies Seminar.

Jimenez, J., Wise, G., Burger, G., Du, W., Dold, P. (2014). Mainstream nitrite - shunt with biological phosphorus removal at the city of st. Petersburg Southwest WRF, in: WEFTEC. New Orleans, LA, pp. 696–711.

Kabouris, J.C., Engelmann, M., Dulaney, J., Narayanan, B., Gillette, R. a., Todd, a. C. (2009). EBPR with Struvite Recovery to Reduce Chemical Consumption and Increase Nutrient Removal Reliability. *Proc. Water Environ. Fed.* **2009**, 149–165.

Khiewwijit, R., Temmink, H., Rijnaarts, H., Keesman, K.J. (2015). Energy and nutrient recovery for municipal wastewater treatment: How to design a feasible plant layout? *Environ. Model. Softw.* **68**, 156–165.

Khunjar, W.O., Pitt, P., Latimer, R., Vadiveloo, E., Bilyk, K., Nguyen, V., Cubbage, L., Balzer, B., Baumler, R., Bott, C.B., Harris, R., Porter, R., Elmendorf, H., Richards, T., Fergen, R., Moncholi, M., Lobdell, S. (2013). Extractive Nutrient Recovery is a Mature Option for Managing Phosphorus in Sidestreams. *Florida Water Resour. J.* 38–42.

Kidd, P.S., Domínguez-Rodríguez, M.J., Díez, J., Monterroso, C. (2007). Bioavailability and plant accumulation of heavy metals and phosphorus in agricultural soils amended by long-term application of sewage sludge. *Chemosphere* **66**, 1458–1467.

Kim, D.J., Kim, S.H. (2006). Effect of nitrite concentration on the distribution and competition of nitrite-oxidizing bacteria in nitratation reactor systems and their kinetic characteristics. *Water Res.* **40**, 887–894.

Kim, J., Joo, J., Koo, S. (2000). Development of high-driving potential and high-efficiency Mg-based sacrificial anodes for cathodic protection. *J. Mater. Sci. Lett.* **19**, 477–479.

Kresge, R., Barbeau, D., Bishop, R., Bowers, K., Leaf, W. (2009). Phosphorus Recycling for a Sustainable Future, in: WEFTEC. pp. 4275–4294.

Kruk, D.J., Elektorowicz, M., Oleszkiewicz, J.A. (2014). Struvite precipitation and phosphorus removal using magnesium sacrificial anode. *Chemosphere* **101**, 28–33.

Krzeminski, P., van der Graaf, J.H.J.M., van Lier, J.B. (2012). Specific energy consumption of membrane bioreactor (MBR) for sewage treatment. *Water Sci. Technol.* **65**, 380–392.

Lackner, S., Gilbert, E.M., Vlaeminck, S.E., Joss, A., Horn, H., van Loosdrecht, M.C.M. (2014). Full-scale partial nitritation/anammox experiences - An application survey. *Water Res.* **55**, 292–303.

Lam, P., Kuypers, M.M.M. (2011). Microbial Nitrogen Cycling Processes in Oxygen Minimum Zones. *Ann. Rev. Mar. Sci.* **3**, 317–345.

Lambert, R., Auer, M., Effler, S., Greene, M., Downer, B., Kuczynski, A. (2015). Onondaga to Ontario: Management of bioavailable phosphorus in municipal wastewaters for control of Cladophora. *J. Gt. Lekes Res.* **41**, 1106–1113.

Laureni, M., Falås, P., Robin, O., Wick, A., Weissbrodt, D.G., Nielsen, J.L., Ternes, T.A., Morgenroth, E., Joss, A. (2016). Mainstream partial nitritation and anammox: long-term process stability and effluent quality at low temperatures. *Water Res.* **101**, 628–639.

Laureni, M., Weissbrodt, D.G., Szivák, I., Robin, O., Nielsen, J.L., Morgenroth, E., Joss, A. (2015). Activity and growth of anammox biomass on aerobically pre-treated municipal wastewater. *Water Res.* **80**, 325–336.

Le Corre, K., Valsami-Jones, E., Hobbs, P., Parsons, S. (2009). Phosphorus Recovery from

Wastewater by Struvite Crystallization: A Review. *Crit. Rev. Environ. Sci. Technol.* **39**, 433–477.

Le Corre, K., Valsami-Jones, E., Hobbs, P., Parsons, S. (2005). Impact of calcium on struvite crystal size, shape and purity. *J. Cryst. Growth* **283**, 514–522.

Li, X.-G., Cao, H.-B., Wu, J.-C., Yu, K.-T. (2001). Inhibition of the metabolism of nitrifying bacteria by direct electric current. *Biotechnol. Lett.* **23**, 705–709.

Liang, Z., Peng, X., Wang, J., Luan, Z., Liu, Z., Wang, Y. (2010). Immobilization of phosphorus in sewage sludge using inorganic amendments. *Environ. Earth Sci.* **63**, 221–228.

Liu, S., Yang, F., Meng, F., Chen, H., Gong, Z. (2008). Enhanced anammox consortium activity for nitrogen removal: Impacts of static magnetic field. *J. Biotechnol.* **138**, 96–102.

Lotti, T., Kleerebezem, R., Hu, Z., Kartal, B., Jetten, M.S.M., van Loosdrecht, M.C.M. (2014a). Simultaneous partial nitritation and anammox at low temperature with granular sludge. *Water Res.* **66C**, 111–121.

Lotti, T., Kleerebezem, R., Lubello, C., van Loosdrecht, M.C.M. (2014b). Physiological and kinetic characterization of a suspended cell anammox culture. *Water Res.* **60C**, 1–14.

Lotti, T., Kleerebezem, R., van Loosdrecht, M.C.M. (2015). Effect of temperature change on anammox activity. *Biotechnol. Bioeng.* **112**, 98–103.

Lotti, T., van der Star, W.R.L., Kleerebezem, R., Lubello, C., van Loosdrecht, M.C.M. (2012).

The effect of nitrite inhibition on the anammox process. *Water Res.* **46**, 2559–69.

Lu, H., Chandran, K., Stensel, D. (2014). Microbial ecology of denitrification in biological wastewater treatment. *Water Res.* **64**, 237–254.

Ma, B., Peng, Y., Zhang, S., Wang, J., Gan, Y., Chang, J., Wang, S., Wang, S., Zhu, G. (2013). Performance of anammox UASB reactor treating low strength wastewater under moderate and low temperatures. *Bioresour. Technol.* **129**, 606–11.

Maher, C., Brown, G., Picard, E. (2011). Phosphorus Removal by Alum Addition with a Treatment Goal of 0.02 mg/L; 30 Years of Experience at the Upper Blue Sanitation District, in: WEFTEC. pp. 2085–2096.

Manitoba (2011). The Save Lake Winnipeg Act, S.M. 2011, c. 36. Winnipeg, MB.

Mehta, C.M., Khunjar, W.O., Nguyen, V., Tait, S., Batstone, D.J. (2015). Technologies to Recover Nutrients from Waste Streams: A Critical Review. *Crit. Rev. Environ. Sci. Technol.* **45**, 385–427.

Metcalf & Eddy, I., Tchobanoglous, G., Stensel, H.D., Tsuchihashi, R., Burton, F.L. (2014). Wastewater Engineering: Treatment and Resource Recovery, 5th ed. McGraw-Hill.

Mook, W.T., Chakrabarti, M.H., Aroua, M.K., Khan, G.M.A., Ali, B.S., Islam, M.S., Abu Hassan, M.A. (2012). Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. *Desalination* **285**, 1–13.

Mousavi, S., Ibrahim, S., Aroua, M.K., Ghafari, S. (2012). Development of nitrate elimination by autohydrogenotrophic bacteria in bio-electrochemical reactors - A review. *Biochem. Eng. J.* **67**, 251–264.

Mulder, J., Duin, J.O.., Goverde, J., van Veldhuizem, H.M., van Kempen, R., Roeleved, P. (2006). Full-Scale Experience with the Sharon Process Through the Eyes of the Operators, in: WEFTEC. Dallas, TX, pp. 5256–5270.

Mustacchi, R., Knowles, C.J., Li, H., Dalrymple, I., Sunderland, G., Skibar, W., Jackman, S. a. (2005). Enhanced biotransformations and product recovery in a membrane bioreactor through application of a direct electric current. *Biotechnol. Bioeng.* **89**, 18–23.

Muyzer, G., de Waal, E., Uitterlinden, A. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Apply Environ. Microbiol.* **59**, 695–700.

Nifong, A., Nelson, A., Johnson, C., Bott, C.B. (2013). Performance of a Full-Scale Sidestream DEMON® Deammonification Installation, in: WEF/IWA Nutrient Removal and Recovery. Vancouver, BC.

Oleszkiewicz, J., Barnard, J. (2006). Nutrient removal technology in North America and the European Union: A review. *Water Qual. Res. J. Canada* **41**, 449–462.

Oleszkiewicz, J., Kruk, D.J., Devlin, T., Lashkarizadeh, M., Yuan, Q. (2015). Options for Improved Nutrient Removal and Recovery from Municipal Wastewater in the Canadian Context. Waterloo, ON.

Pollice, A., Tandoi, V., Lestingi, C. (2002). Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. *Water Res.* **36**, 2541–2546.

Pritchard, D.L., Penney, N., McLaughlin, M.J., Rigby, H., Schwarz, K. (2010). Land application of sewage sludge (biosolids) in Australia: risks to the environment and food crops. *Water Sci. Technol.* **62**, 48–57.

Qiao, S., Yin, X., Zhou, J., Furukawa, K. (2014). Inhibition and recovery of continuous electric field application on the activity of anammox biomass. *Biodegradation* **25**, 505–13.

Regmi, P., Miller, M.W., Bunce, R., Hingley, D.M., Kinnear, D., Murthy, S., Bott, C.B. (2012). Nitrogen Removal 3.0: A Pilot Study to Evaluate the Feasibility of Mainstream Deammonification, in: WEFTEC. Water Environment Federation, New Orleans, LA, pp. 1994–2017.

Regmi, P., Miller, M.W., Holgate, B., Bunce, R., Park, H., Chandran, K., Wett, B., Murthy, S., Bott, C.B. (2014). Control of aeration, aerobic SRT and COD input for mainstream nitritation/denitritation. *Water Res.* **57**, 162–171.

Rodziewicz, J., Filipkowska, U., Dziadkiewicz, E. (2011). Electrolytically aided denitrification on a rotating biological contactor. *Environ. Technol.* **32**, 93–102.

Rosso, D., Lothman, S.E., Jeung, M.K., Pitt, P., Gellner, W.J., Stone, A.L., Howard, D. (2011). Oxygen transfer and uptake, nutrient removal, and energy footprint of parallel full-scale IFAS and activated sludge processes. *Water Res.* **45**, 5987–5996.

Sawyer, C. (1994). Chemistry for environmental engineering, 4th ed. McGraw-Hill.

Schindler, D.W. (2006). Recent advances in the understanding and management of eutrophication. *Limnol. Oceanogr.* **51**, 356–363.

Schindler, D.W., Hecky, R.E., McCullough, G.K. (2012). The rapid eutrophication of Lake Winnipeg: Greening under global change. *J. Great Lakes Res.* **38**, 6–13.

Schuiling, R.D., Andrade, A. (1999). Recovery of Struvite from Calf Manure. *Environ. Technol.* **20**, 765–768.

Schuler, A.J., Jenkins, D. (2003). Enhanced Biological Phosphorus Removal from Wastewater by Biomass with Different Phosphorus Contents, Part III: Anaerobic Sources of Reducing Equivalents. *Water Environ. Res.* **75**.

Scott, K.J., McCullough, G., Stainton, M., Ayles, B., Hann, B. (2011). Lake Winnipeg - State of the Science: What is the Scientific Basis for Understanding and Protecting Lake Winnipeg? Winnipeg.

Shin, S., Choi, S.S., Yoo, Y.J. (2011). Partial nitrification using an electrolytic aerating bioreactor with ammonia-oxidizing bacteria-dominant activated sludge. *Biotechnol. Lett.* **33**, 699–703.

Siegrist, H., Salzgeber, D., Eugster, J., Joss, a. (2008). Anammox brings WWTP closer to energy autarky due to increased biogas production and reduced aeration energy for N-removal. *Water Sci. Technol.* **57**, 383–388.

Soil Association (2010). A rock and a hard place: Peak Phosphorus and the Threat to Our Food Security. Bristol UK.

Song, G., Atrens, A., John, D., Wu, X. (1997). The anodic dissolution of magnesium in chloride and sulphate solutions. *Corros. Sci.* **39**, 1981–2004.

Stafford, B., Dotro, G., Vale, P., Jefferson, B., Jarvis, P. (2014). Removal of phosphorus from trickling filter effluent by electrocoagulation. *Environ. Technol.* **35**, 3139–46.

Stark, K. (2005). PhD Thesis: Phosphorus Release and Recovery from Treated Sewage Sludge. KTH.

Stinson, B., Murthy, S., Al-Omari, A., Bott, C., De Clippeleir, H. (2014). Removing the Primary Barriers to Successful Nitrite Shunt and Mainstream Deammonification. Presentation for Americam Academy of Environmental Engineers and Scientists.

Stinson, B., Murthy, S., Bott, C., Wett, B., Al-Omari, A., Bowden, G., Mokhyerie, Y., De Clippeleir, H. (2013). Roadmap Toward Energy Neutrality & Chemical Optimization at Enhanced Nutrient Removal Facilities, in: WEF/IWA Nutrient Removal and Recovery. Vancouver, BC.

Stratful, I., Scrimshaw, M.D., Lester, J.N. (2001). Conditions influencing the precipitation of magnesium ammonium phosphate. *Water Res.* **35**, 4191–9.

Strous, M., Heijnen, J.J., Kuenen, J.G., Jetten, M.S.M. (1998). The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing

microorganisms. *Appl. Microbiol. Biotechnol.* **50**, 589–596.

Strous, M., Kuenen, J.G., Jetten, M.S.M. (1999). Key Physiology of Anaerobic Ammonium Oxidation Key Physiology of Anaerobic Ammonium Oxidation. *Appl. Environ. Microbiol.* **65**, 3248.

Suzuki, K., Tanaka, Y., Kuroda, K., Hanajima, D., Fukumoto, Y. (2005). Recovery of phosphorous from swine wastewater through crystallization. *Bioresour. Technol.* **96**, 1544–50.

Suzuki, K., Tanaka, Y., Kuroda, K., Hanajima, D., Fukumoto, Y., Yasuda, T., Waki, M. (2007). Removal and recovery of phosphorous from swine wastewater by demonstration crystallization reactor and struvite accumulation device. *Bioresour. Technol.* **98**, 1573–8.

Tan, Z., Lagerkvist, A. (2011). Phosphorus recovery from the biomass ash: A review. *Renew. Sustain. Energy Rev.* **15**, 3588–3602.

Tetra Tech (2013). EPA Report: Emerging technologies for wastewater treatment and in-plant wet weather managment. Fairfax, VA.

Thrash, J., Coates, J. (2008). Review: direct and indirect electrical stimulation of microbial metabolism. *Environ. Sci. Technol.* **42**, 3921–3931.

Tran, N., Drogui, P., Blais, J.-F., Mercier, G. (2012). Phosphorus removal from spiked municipal wastewater using either electrochemical coagulation or chemical coagulation as tertiary treatment. *Sep. Purif. Technol.* **95**, 16–25.

Turk, O., Mavinic, D.S. (1986). Preliminary assessment of a shortcut in nitrogen removal from wastewater. *Can. J. Civ. Eng.* **13**, 600–605.

USA, Canada (2012). The Great Lakes Water Quality Protocol [WWW Document]. URL http://www.epa.gov/glnpo/glwqa/20120907-Canada-USA_GLWQA_FINAL.pdf (accessed 10.30.13).

Vadivelu, V., Keller, J., Yuan, Z. (2007). Free ammonia and free nitrous acid inhibition on the anabolic and catabolic processes of Nitrosomonas and Nitrobacter. *Water Sci. Technol.* **56**, 89–97.

Vázquez-Padín, J.R., Fernández, I., Morales, N., Campos, J.L., Mosquera-Corral, A., Méndez, R. (2011). Autotrophic nitrogen removal at low temperature. *Water Sci. Technol.* **63**, 1282–8.

Větrovský, T., Baldrian, P. (2013). The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. *PLoS One* **8**.

Viraraghavan, T., Ionescu, M. (2002). Land application of phosphorus-laden sludge: a feasibility analysis. *J. Environ. Manage.* **64**, 171–177.

Wang, C.-C., Hao, X.-D., Guo, G.-S., van Loosdrecht, M.C.M. (2010). Formation of pure struvite at neutral pH by electrochemical deposition. *Chem. Eng. J.* **159**, 280–283.

Wang, H.-J., Xiao, X.-C., Wang, H.-Z., Li, Y., Yu, Q., Liang, X.-M., Feng, W.-S., Shao, J.-C., Rybicki, M., Jungmann, D., Jeppesen, E. (2017). Effects of high ammonia concentrations on three cyprinid fish: Acute and whole-ecosystem chronic tests. *Sci. Total Environ.* **598**,

900–909.

Wang, J., Lu, H., Chen, G.-H., Lau, G.N., Tsang, W.L., van Loosdrecht, M.C.M. (2009). A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. *Water Res.* **43**, 2363–2372.

Wang, Y.K., Li, W.W., Sheng, G.P., Shi, B.J., Yu, H.Q. (2013). In-situ utilization of generated electricity in an electrochemical membrane bioreactor to mitigate membrane fouling. *Water Res.* **47**, 5794–5800.

Wei, V., Elektorowicz, M., Oleszkiewicz, J. a (2012). Electrically enhanced MBR system for total nutrient removal in remote northern applications. *Water Sci. Technol.* **65**, 737–42.

Wei, V., Elektorowicz, M., Oleszkiewicz, J. a (2011). Influence of electric current on bacterial viability in wastewater treatment. *Water Res.* **45**, 5058–5062.

Wei, V., Oleszkiewicz, J., Elektorowicz, M. (2009). Nutrient removal in an electrically enhanced membrane bioreactor. *Water Sci. Technol.* **60**, 3159–63.

Wentzel, M.C., Lotter, L., Loewenthal, R.E., Marais, G. (1986). Metabolic behaviour of Acinetobacter spp. in enhanced biological phosphorus removal - a biochemical model. *Water SA* **12**, 209–224.

Wouters, P., Smelt, J.P.M. (1997). Inactivation of microorganisms with pulsed electric fields: Potential for food preservation. *Food Biotechnol.* **11**, 193–229.

Yin, X., Qiao, S., Zhou, J., Quan, X. (2015). Using three-bio-electrode reactor to enhance the activity of anammox biomass. *Bioresour. Technol.* **196**, 376–382.

Zeng, L., Li, X. (2006). Nutrient removal from anaerobically digested cattle manure by struvite precipitation. *J. Environ. Eng. Sci.* **5**, 285–294.

Zeyoudi, M., Altenaiji, E., Ozer, L.Y., Ahmed, I., Yousef, A.F., Hasan, S.W. (2015). Impact of continuous and intermittent supply of electric field on the function and microbial community of wastewater treatment electro-bioreactors. *Electrochim. Acta* **181**, 271–279.

Zhang, H., Jain, J.S., Brand, M., Perkovich, B., Lai, K., Carmody, S., Urgun-Demirtas, M., Pagilla, K. (2006). Full scale test on chemical P removal during a step feed BNR study at John E. Egan Water Reclamation Plant, in: WEFTEC. Dallas, TX, pp. 5176–5184.

Zhang, J., Zhang, Y., Li, Y., Zhang, L., Qiao, S., Yang, F., Quan, X. (2012). Enhancement of nitrogen removal in a novel anammox reactor packed with Fe electrode. *Bioresour. Technol.* **114**, 102–8.

Zhou, W., Sun, Y., Wu, B., Zhang, Y., Huang, M., Miyanaga, T., Zhang, Z. (2011). Autotrophic denitrification for nitrate and nitrite removal using sulfur-limestone. *J. Environ. Sci.* **23**, 1761–1769.

Zurzolo, F., Kruk, D., Oleszkiewicz, J. (2013). Phosphorus recovery and VFA generation from waste activated sludge alone and co-thickened with primary sludge, in: WEFTEC. Chicago, IL.