Deammonification Process Kinetics and Inhibition Evaluation

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Abstract

A number of innovative nitrogen removal technologies have been developed to address the treatment challenges caused by stringent regulations and increasing chemical and energy cost. A major contributing factor to these challenges is the liquid stream originating from the process of dewatering anaerobically digested solids. This liquid, also knows as centrate, reject water or sludge liquor, can cause an increase of up to 25% in ammonia loading. The recently discovered anaerobic ammonia oxidation (anammox) process is a major breakthrough for treatment of these streams as it has the potential to remove up to 85% of nitrogen load without external carbon source addition. The anammox process is combined with another process that oxidizes half of the ammonia to nitrite (nitritation) in a separate reactor such as in the SHARON process, or in the same reactor such as in the DEaMmONification (DEMON) process. Despite intensive laboratory research for the last 10 years to fully understand these processes, there is still a high level of skepticism surrounding the implementation of full-scale systems. The reason for this skepticism could be due to frequent failures observed in the lab scale systems as well as reported slow bacterial growth. We think that this technology might be used more effectively in the future if process kinetics, inhibition and toxicity can be better understood.

This work focused on the DEMON process with a goal to understand the kinetics and inhibition of the system as a whole and the anammox process in particular. A DEMON pilot study was undertaken at the Alexandria Sanitation Authority (ASA) and had several study participants, including ASA, the District of Columbia Water and Sewer Authority (DCWASA), CH2M Hill Inc., Envirosim Ltd, the University of Innsbruck and Virginia Tech. We investigated the growth rate of anammox bacteria within a quasi-optimal environment. Laboratory-scale experiments were conducted to assess anaerobic ammonia oxidation inhibition by nitrite as well as aerobic ammonia oxidation inhibition by compounds present in the DEMON reactor feed, such as a defoaming agent, a sludge conditioning polymer, and residual iron from phosphorus removal practices.

The study revealed that the DEMON process can be efficiently controlled to limit nitrite accumulation capable of causing process inhibition. The target ammonium loading rate of 0.5

kg/m^3/d was reached, and no upset was noticed for a loading up to 0.80 kg/m^3/d with an HRT of 1.7 days. The ammonia removal efficiency reached an average of 76% while total nitrogen removal efficiency had an average of 52%. Most of the process upsets were caused by aerobic ammonia oxidation failure rather than anammox inhibition. Failure in ammonia oxidation affected pH control, a variable which is at the center of the DEMON process control logic. The pilot study is summarized in Chapter 3 of this Dissertation.

The low anammox maximum specific growth rate ($\mu_{max,An}$) as well as nitrite inhibition are historically reported to be the major process challenges according to the literature, but the degree to which each contributes to process problems differs widely in the literature. In this study, we estimated $\mu_{max,An}$ by using the high F:M protocol commonly used for nitrifying populations. We also studied the effect of both short term and sustained nitrite exposure on anammox activity. In this study, $\mu_{max,An}$ was estimated to be 0.017 h^{-1}. The study results also suggest that anammox bacteria can tolerate a spike of nitrite-N at concentrations as high as 400 mg/L as long as this concentration is not sustained. Sustained concentrations above 50 mg/L caused a gradual loss of activity over the long term.

Finally, the inhibition of aerobic ammonia oxidizing bacteria (AerAOB) observed in the DEMON reactor was investigated using laboratory experiments and is reported in Chapter 6. AerAOB inhibition was, in most cases, the main reason for process upset. Compounds that were suspected to be the cause of the inhibition were tested. The study noticed that a defoaming agent, polymer and ferrous iron had some inhibiting properties at the concentrations tested.

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List of Abbreviations

ΔG^o: Gibbs free energy
AerAOBs: Aerobic Ammonia Oxidizing Bacteria
AMO: Ammonia monooxygenase
AnAOBs: Anaerobic Ammonia Oxidizing Bacteria
ASA-AWTF: Alexandria Sanitation Authority- Advanced Wastewater Treatment Facility
ATP: Adenosine triphosphate
Anammox: Anaerobic Ammonia Oxidation
BABE: Bio-Augmentation Batch Enhanced
BOD: Biological oxygen demand
CANON: Completely Autotrophic Nitrogen Removal Over Nitrite
COD: Chemical Oxygen Demand
CSTR: Continuous Stirred Tank Reactor
DC WASA: District of Columbia Water and Sewer Authority
DEMON: DEaMmONification
E_0: Standard oxidation-reduction potential
EPA : Environmental Protection Agency
FISH: Fluorescent In Situ Hybridization
F:M: Food to Microorganisms ratio
FA: Free ammonia
HRT: Hydraulic Retention Time
IC_{50}: (Inhibition Concentration 50) Half maximal inhibition concentration
InNitri: Inexpensive Nitrification
K_s: Half Saturation Coefficient
Kwh: Kilowatt hour
MLSS: Mixed Liquor Suspended Solids
NAD: Nicotinamide Adenine Dinucleotide
NOBs: Nitrite oxidizing bacteria
OLAND: Oxygen-Limited Autotrophic Nitrification and Denitrification
OUR: Oxygen Uptake Rate
PLC: Programmable Logic Controller
RAS: Return Activated Sludge
R-D-N: Regeneration-Denitrification-Nitrification
rRNA: ribosomal Ribonucleic Acid
SBR: Sequencing Batch Reactor
SCADA: Supervisory Control And Data Acquisition
SHARON: Single reactor for High Activity nitrogen Removal Over Nitrite
SRT: Solids Retention Time
TAN: Total Ammonia Nitrogen
TKN: Total Kjeldahl Nitrogen
TSS/VSS: Total Suspended Solids/ Volatile Suspended Solids
WEFTEC: Water Environmental Federation Technical Exhibition and Conference
WERF: Water Environment Research Foundation
WWTP: Wastewater Treatment Plant
μ_{max}: maximum specific growth rate

CHAPTER 1:
Executive Summary

1.1. Introduction

Nitrogen removal technologies in the wastewater treatment industry are being continuously assessed and optimized in order to improve their efficiency and minimize costs. This is a response to more stringent regulations on effluent nitrogen that is discharged into the environment from wastewater treatments systems. Anaerobic ammonia oxidation (anammox) is one of the emerging microbial processes offering significant savings. In the anammox metabolism, ammonia is biologically oxidized by nitrite to dinitrogen gas by autotrophic bacteria. As a result, unlike conventional nitrification-denitrification processes, there is no need for an organic carbon source. The cost effectiveness of anaerobic ammonia oxidation-based nitrogen removal is also improved by partially nitrifying ammonia to nitrite instead of allowing it to go to nitrate, allowing approximately 40% savings on aeration. As an example, the Blue Plains Advanced Wastewater Treatment Plant in Washington DC would save $4.5 million (US) per year if side-stream anaerobic ammonia oxidation treatment is employed rather than using conventional nitrification-denitrification which requires the purchase of methanol (Constantine et al., 2005).

Despite these advantages, anaerobic ammonia oxidation is not sufficiently understood to the level needed to allow this process to be widely deployed. Some key elements that need further study include: clarifying process control strategies, characterizing process performance during steady state conditions; identifying design parameters such as specific loading rate; quantifying sludge production; and understanding the influence of environmental factors like temperature and pH. Inhibition issues also need to be clarified, especially nitrite inhibition of both the anammox process and aerobic ammonia oxidation for the case when partial ammonia oxidation is to be coupled with the anammox process in the same reactor**.**

1.2. Anammox growth rate

Despite these noted advantages, there are challenges to the implementation of anaerobic ammonia oxidation. The maximum growth rate of anaerobic ammonia oxidizing bacteria (AnAOB) was estimated to be 0.0027 h^{-1}, which is about 14 times slower than the growth rate for traditional aerobic ammonia oxidizing bacteria (AerAOBs). Despite intensive research from many different laboratories around the world over the last decade, only a very limited number of wastewater treatment plants are reported to have implemented the process on a full-scale as of 2008. The Rotterdam WWTP (Netherlands) combines separate stage ammonia oxidation to nitrite (SHARON) and anaerobic ammonia oxidation processes while the Strass WWTP (Austria) uses a single sludge system that couples these metabolisms in what is called deammonification (and for this reason, the process is referred to as DEMON). In an attempt to bring the technology to North America, the New York City Department of Environmental Protection, Blue Plains Advanced Wastewater Treatment Plant and Alexandria Sanitation Authority have joined efforts to study the feasibility of incorporating AnAOB-based processes into their respective wastewater treatment plants. The program also included Virginia Tech and the University of Innsbruck in Austria as research partners. These universities were asked to investigate some key physiological parameters of AnAOB, including estimating and understanding factors affecting the decay rate, half saturation coefficients and inhibition coefficients. This thesis reports on two facets of this effort: (i) nitrite inhibition and its mathematical expression, and (ii) quantification of AnAOB-containing DEMON biomass activity on the overall performance of the DEMON.

Anaerobic ammonia oxidation kinetics were studied after identifying the metabolic pathway in The Netherlands in 1995 (van de Graaf et al., 1996). Although research interest about and progress in understanding anaerobic ammonia oxidation metabolism and the microorganisms responsible has been significant, information on growth kinetics is quite variable in the literature. For example, in three different studies, the maximum specific growth rate was reported to range from 0.001 h^{-1} (van de Graaf et al., 1996), 0.0027 h^{-1} (Strous et al., 1998) and 0.016 h^{-1} (Isaka et al., 2005). As AnAOBs have not been isolated, all of these kinetic evaluations are for enriched mixed cultures. Furthermore, as researchers are still learning how to grow AnAOB-based cultures, the kinetics reflect suboptimal growth conditions that may be influenced by substrate

inhibition or media nutrient limitations. There is more consistency in predicting biomass yield based on thermodynamic principles. Anaerobic ammonia oxidation generates more energy (ΔG = -356 KJ/mol NH_4, (van de Graaf et al., 1995)) than aerobic ammonia oxidation (ΔG = -235 KJ/mol NH_4 (Bock and Wagner, 2002)) and the resulting growth yields reflect this difference (0.06 g cell/gNH_4^+-N and 0.04-0.13 g cell/gNH_4^+-N (Jianlong and Jing, 2004), respectively).

In this work, we attempt to overcome some of the limitations experienced when using mass balance approaches to estimate growth rate for an AnAOB-containing biomass by performing the standard growth rate estimation protocol using the F:M method, which has been successfully used to estimate ammonia oxidizing bacterial growth kinetics (Melcer et al. 2003). Our goal was to determine whether the main factor controlling growth rate in the anaerobic ammonia oxidation process can be attributed to an inherent slow growth rate, high sensitivity to substrate (nitrite) toxicity, or a combination of both. For this reason, our assessment of anaerobic ammonia oxidation growth kinetics was performed in parallel with nitrite inhibition studies. Nitrite was selected because it has been reported to be the most critical inhibitor to the anaerobic ammonia oxidation process (Dapenda-Mora et al., 2006). Using the F:M method, we estimated the anaerobic ammonia oxidation maximum specific growth rate to be 0.017 h^{-1}. Based on models used in the F:M estimate, we assume that AnAOBs constitute approximately 7% of the DEMON biomass, a percentage which was slightly lower than the mass balance calculation- based prediction (10%). However, this estimate was not confirmed using biomolecular methods. Overall, our estimate of anaerobic ammonia oxidation maximum specific growth rate is larger than van de Graaf et al. (1996) and Strous et al., (1998) but close to Isaka et al., (2005).

1.3. Nitrite inhibition

The second goal of this study was to evaluate the effect of nitrite on the anaerobic ammonia oxidation process and to provide a mathematical expression which would describe this effect. While nitrite concentration control is thought to be critical to the success of any process employing AnAOBs, studies differ on the threshold concentration above which inhibition occurs. In this study, an attempt was made to clarify the concentration range over which nitrite is inhibitory, the influence of short versus long term exposure and the impact of pH. Information

on the conditions under which nitrite is inhibitory is critical for the successful design and operation of full-scale systems that employ the anaerobic ammonia oxidation process.

In this study, the effect of nitrite on anaerobic ammonia oxidation kinetics was evaluated using fresh DEMON biomass from the pilot plant. DEMON biomass was exposed to various nitrite concentrations for a short time (nitrite was allowed to be depleted during the study) or for a long time (nitrite concentration was sustained throughout the study). The system was sealed to maintain anaerobic conditions, pH was controlled near neutral, and the temperature was maintained at 35^{o}C to reflect the conditions in the DEMON pilot.

The results show that the system was able to recover after short (3 hour) exposure to nitrite at concentrations as high as 400 mg-N/L. Overall however, the average activity decreased for nitrite concentrations above 50 mg-N/L. Long term (7 hour) exposure revealed that time of exposure is also a factor that needs to be considered to predict the effect on nitrite on AnAOB. An attempt to model these factors is presented as a first attempt toward generating tools for predicting inhibition. This result provides useful insight into full-scale use of the DEMON process.

1.4. AerAOB inhibition

The third goal of this study was developed in response to the observation that ammonia oxidation slowed down during the ASA DEMON pilot plant study and the reason for the slow down was unknown. Previous studies never observed ammonia oxidation inhibition in the DEMON process, even for cases where the process was run with a very low DO (0.3 mg/L), which is typical of this process which aims to minimize NOB growth. The pilot plant was controlled solely on pH variation, which is a reflection of alkalinity consumption caused by ammonia oxidation during aerobic periods and a combination of alkalinity generated by anaerobic ammonia oxidation and feed. Therefore, pH would decline during aerobic ammonia oxidation and increase during the non-aerated period when anaerobic ammonia oxidation was active. The DEMON pilot plant process failed multiple times when the system failed to oxidize ammonia rapidly, resulting in long aeration periods that may have enhanced the growth of nitrite oxidizing bacteria and removed a critical substrate for AnAOBs. This observation leads us to

suspect that aerobic ammonia oxidation was inhibited during the ASA-hosted DEMON pilot plant study.

The assumption in this study was that ammonia oxidation inhibition was caused by either inhibiting compounds coming directly from the feed or accumulation of such compounds in the pilot over time given the fact that the solids retention time was very high (over 25 days). Significant compounds used upstream that are capable of causing inhibition included iron compounds, a defoaming agent and a conditioning polymer. The suspected compounds were tested in the laboratory using reasonable concentrations and it was confirmed that they all imposed inhibition on aerobic ammonia oxidation. It is suspected that ferrous hydroxides hydrates are the iron compounds that are most inhibitory among the iron constituents. Both polymer and ferrous hydroxides-based ammonia oxidation inhibition could be avoided by reducing solids concentrations in the centrate. Inhibition by the defoaming agent, which was the most inhibitory of the compounds tested, might be reduced either by changing the type of defoaming agent or by applying only as much as is truly needed. The current “as needed” dosage strategy is likely to introduce the defoaming agent at a level that would be harmful to a downstream DEMON process, especially since this chemical is stable and does not seem to be retained in the solids removal process.

It is our belief that this study provides a clear understanding of the DEMON process kinetics as well as a valuable discussion about the inhibition issues to consider when designing or operating a DEMON process. The results from this study provide an optimistic point of view regarding the future of the anammox process in general and the DEMON process in particular given the process efficiency, the relatively high process resilience, and the competitive cost. The study also highlights areas where more work is needed to improve our understanding and control of the anammox process such as a clear, consistent nitritation strategy and modeling the process inhibition for design purposes.

1.5. References

Bock E., and Wagner M. (2002); Oxidation of inorganic nitrogen as energy source, *Procaryotes: an evolving electroniv resource for the microbial community, springer-verlag,* New York, NY

Constantine T., Murthy S., Bailey W., Benson L., Sadick T., Daigger G. (2005); Alternatives for treating high nitrogen liquor from advanced anaerobic digestion at the Blue Plains AWTP, *WEFTEC,* Washington DC.

Dapena-Mora A., I. Fern´andez , J.L. Campos, A. Mosquera-Corral,
R. Méndez , M. Jetten (2007); Evaluation of Activity and Inhibition effects on Anammox process by batch tests based on nitrogen gas production, *Enzyme and Microbial Technology*, 40 (4): 859-865

Isaka K., Date Y., T.Sumino, Yoshie S., Tsuneda S. (2006); Growth Characteristics of anaerobic ammonia-oxidizing bacteria in an anaerobic biological filtrated reactor, *Appl Microbiol Biotechnol*, 70 : 46-62

Jianlong W. and Jing K. (2004); Partial nitrification under limited dissolved oxygen conditions, *Process Biochemistry* 39:1223–1229

Melcer H., Stensel D., Dold P., (2003); Methods of wastewater characterization in Activated Sludge Modeling, WERF Report Series, IWA publishing

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 the slowly growing anaerobic ammonia-oxidizing microorganisms, *Appl. Microbiol Biotechnol*, 50:589-596

van De Graaf A.A., Mulder A., De Bruijn P., Jetten M.S. M., Robertson A., and Kuenen J., (1995); Anaerobic oxidation of ammonium is a biologically mediated process, *Applied and Environmental Microbiology,* 61(4):1246-1251.

CHAPTER 2:

Literature Review

2. 1. Nitrogen: Origin, Cycle and Issues

Nitrogen is by weight the most abundant element in organic matter next to carbon and oxygen. It is contained in amino acids, the building blocs of proteins. It is also found in nucleic acids and nucleotides, compounds that are vital to reproduction, differentiation and chemical energy flow in every living organism (Gaudy and Gaudy, 1980). Unfortunately, the most abundant source of nitrogen is its unreactive form, nitrogen gas (N_2). The atmosphere contains around 79% of nitrogen as N_2. There are only two natural processes that render atmospheric nitrogen available to living organisms: lightening and biological fixation. Biological fixation is extremely energy demanding, consuming 16 moles of ATP to convert one mole of N_2 to two moles of ammonia, NH_3. The process also requires 8 moles of electrons as shown by Equation 2.1 (White, 1999).

$$N_2 + 8H^+ + 16\ ATP + 8e^- \rightarrow 2NH_3 + H_2 + 16ADP + 16\ P_i \qquad (2.1)$$

A selected group of microorganisms, known as diazotrophs (or nitrogen eaters) have the capability to carry out this reaction, and most of those require symbiotic association with leguminous plants (Christiansen and Dean, 2000). As a result, it can be understood that natural nitrogen fixation would be the limiting step in vegetation growth and crop production.

The Haber-Bosch process (1910) is the industrial process that fixes N_2 to produce reactive nitrogen that is used as fertilizer for improved crop production. N_2 and hydrogen gas (H_2) are reacted over an iron catalyst (Fe) under conditions of 200 atmospheres (atm) and 450-500°C, resulting in a yield of 10-20% (Wikipedia, 2008). It is now believed that human activity (such as Haber-Bosch) fixes more atmospheric N_2 into reactive forms than all the terrestrial natural processes combined (Vitousek et al., 1997). There is a growing concern about the

consequences of this human input to the nitrogen cycle. On one hand, there is an improvement in food availability with minor elevated losses of reactive nitrogen to the environment directly. On the other hand, excessive industrial N fixation can have a serious effect on environmental health. A conceptual model of the overall public health effects of increasing human N fixation and use of atmospheric N_2 was formulated by Townsend et al. (2003). It suggests that there is a maximum point beyond which an increase in human nitrogen fixation corresponds to a decrease in net public health benefits while the air and water pollution as well as ecological feedbacks to disease increase exponentially.

Most of the reactive nitrogen resulting from the human N fixation is gradually released into the environment, rising serious environmental issues. Reactive nitrogen released to the environment from the time it is applied as fertilizer to the time the crop grown on fertilizer is consumed can be up to 96% (Galloway and Cowling, 2002). Even a significant chunk of the remaining 4% which is consumed in food ends up in the environment after nitrogen-containing compounds are metabolized. There are serious consequences of this reactive nitrogen release to the environment. Those effects can vary from stimulation of pollen production which causes an allergic response leading to hay fever, allergenic rhinitis and asthma, to methenoglobinemia (blue baby syndrome) and cancer caused by nitrate in waters as well as increase in harmful algal blooms causing disruption of ecosystems and source of nutrition (Townsend, 2003). The release of reactive nitrogen to water bodies could also lead to excessive growth of plants including phytoplankton, periphyton and macrophytes (Schnoor, 1996), a process known as eutrophication. Eutrophication is known to have troubling ecological impacts such as decrease in biodiversity, changes in species composition and dominance as well as toxic effects (Wikipedia, 2008).

2. 2. Nitrogen Removal from Environment

Nature has evolved natural processes to reconvert reactive forms of nitrogen from the environment to unreactive atmospheric nitrogen, and fortunately, these processes have been successfully engineered to speed up the reconversion rate. Ammonia, the most reduced and most frequently found form of nitrogen, is biologically oxidized to nitrate in the presence of oxygen.

Two chemolithoautotrophic genera mediate this process in two steps. In the first step, ammonia is oxidized to nitrite by Aerobic ammonia oxidizing bacteria (AerAOBs), and then nitrite is further oxidized to nitrate by nitrite oxidizing bacteria (NOBs). One would recognize that there is a thermodynamic problem in this process. The couples NO_3^-/NO_2^- (E_o = + 0.44V) and NO_2^-/NH_3 (E_o = +0.42V) are more electropositive than the couple NAD^+/NADH (Eo = -0.32 V) rendering the electrons translocation somewhat tricky. It is now accepted that nitrifiers resolve this energy difficulty by carrying out reversed electron transport to generate NAD(P)H. The reverse electron transport consists of the use of their membrane potential, Δp, for electron flow. Because of this additional energy consumption together with a small number of electrons generated in each reaction, the energy yields, and therefore the cell yields, are relatively small compared to growth on organic substrates (White, 2000). This is in agreement with experimental values for the cell yield of nitrifiers that have been reported in the literature.

There are at least five genera of nitrifiers including *Nitrosomonas, Nitrosococcus, Nitrospira, Nitrosolobus* and *Nitrovibrio*, all chemolithoautotrophs that assimilate CO_2 via the Calvin cycle. Nitrite oxidizers include *Nitrobacter*, *Nitrosococcus*, *Nitrospina* and *Nitrospira*. Ammonia oxidation happens in two steps. The first oxidation to hydroxylamine is catalyzed by ammonia monooxygenase (AMO) and nets a consumption of 2 electrons. The second oxidation to nitrite is facilitated by hydroxylamine oxydoreductase (HAO) and generates 4 electrons, two of which are reused in the first oxidation, and about 0.3 are used in reversed electron transport to generate NAD(P)H. Nitrite oxidation to nitrate requires one enzyme, nitrite oxidase.

Denitrification is the last step in active nitrogen conversion to N_2. The process results in the loss of biologically reactive nitrogen (Shapleigh, 2000). It is a four step process mediated by heterotrophs that typically use organic carbon as electron donor. Via four enzymatic reactions, nitrate (NO_3^-) is transformed stepwise to nitrite (NO_2^-), nitric oxide gas (NO) and nitrous oxide gas (N_2O) to finally yield denitrogen gas (N_2). Denitrification is important to the nitrogen cycle and includes breaking down undesirable biodegradable materials, plants materials and complex organic compounds in anoxic niches (Casella, 1996). Many bacteria denitrify, but the most commonly isolated denitrifiers are *Alcaligenes* and *Pseudomonas* species, and to a lesser extent, *Paracoccus denitrificans* (Coyne et al., 1989).

Recent developments in understanding the nitrogen cycle have focused on the use of microaerobic or anoxic ammonia oxidation by conventional AerAOBs, with internally-generated nitrite as the final electron acceptor. The existence of chemolithoautotrophs capable of carrying out this reaction was predicted some decades ago by Broda (Strous et al., 2002), based on thermodynamics. The same hypothesis was suggested by oceanographers based on the mass balance and Redfield ratio. The Redfield ratio refers to the fact that the N:C and P:C ratios are similar throughout the marine realm (Kuypers, 2005). The data in anoxic fjords and basins however suggested that an additional nitrogen sink existed in the form of ammonia if heterotrophic denitrification was the only process through which nitrogen was lost.

So far, there are two possibilities for mediating microaerobic or anoxic ammonia oxidation. The first, which is less efficient, stems from the idea that some *Nitrosomonas*-like microorganisms can nitrify and denitrify simultaneously under oxic and anoxic or microaerobic conditions, respectively (Shrestha et al., 2002; Ahn, 2006). Completely autotrophic ammonia removal mediated by *N. eutropha* has been successful using nitrogen dioxide gas (NO_2) as electron acceptor, with a maximum rate of 2 nm NH_4^+ $(min)^{-1}(mg\ protein)^{-1}$ (Jianlong and Jing, 2005). Two enzymes, nitrite reductase (NirK) and nitric oxide reductase (NorCB), were found to be expressed in *N. europaea*, while its homologues were found in other ammonia oxidizing bacteria (Beaumont et al., 2004). These enzymes are normally expressed in heterotrophic denitrifiers during denitrification. The patented process named OLAND (oxygen-limited autotrophic nitrification and denitrification) was based on this double metabolic capability of some nitrifiers (Windey et al., 2005). The basic principle of the OLAND process is based on supplying oxygen to the process such that nitrification only occurs through nitrite production and subsequently, due to a lack of oxygen as electron acceptor, nitrite is used to oxidize ammonia (Verstraete and Philips, 1998).

Analysis of *nirK* gene regulation suggested that nitrite reduction by some aerobic ammonia oxidizers is only a defense mechanism against the NO_2^- toxicity produced during nitrification, but does not support the anoxic growth of nitrifiers. The genome sequencing of *N. europaea* confirmed the presence of the two types of enzymes: nitrite reductase and nitric oxide reductase and shows the complete absence of enzyme clusters for nitrate reductases or nitrous

oxide reductases. As a result, *N. europaea* may be incapable of reducing nitrate (NO_3^-) and is not capable of producing denitrogen gas (N_2) (Chain et al., 2003). It can, however, generate significant N_2O (Henckel and Conrad, 1998). This could be a serious concern since N_2O is a greenhouse gas. Its 100-year global warming potential is about 320 times higher than that of carbon dioxide (CO_2) and has a lifetime of 120 years (Tallec et al., 2008).

The second autotrophic metabolism for oxidizing ammonia under anaerobic conditions (anaerobic autotrophic ammonia oxidation, or AnAOB) is mediated by a specific branch of Planctomycetes, known as anaerobic ammonia oxidizers (van de Graaf et al., 1995). The anammox metabolism has been maintained successfully at a lab scale in many countries and is currently used in full-scale at a very small number of wastewater treatment plants including the Strass Wastewater Treatment Plant in Austria (Wett, 2006), and the Rotterdam Wastewater Treatment Plant in the Netherlands (Gut, 2006; van der Star, 2007). Incorporating the anaerobic ammonia oxidation metabolism into treatment processes allows utilities to save up to 50% in oxygen requirement and 100% in external carbon source addition, and can reduce operational costs by 90% compared to operational costs experienced with conventional nitrogen treatment processes (Chamchoi et al., 2007).

Anaerobic ammonia oxidizers

Also called anammox bacteria, the anaerobic ammonia oxidizers are coccoidal, chemolithoautotrophs (van Niftrik, 2004) with a diameter of <1µm (Kindaichi, 2007). Based on 16S rRNA, the phylogeny of bacteria responsible for anammox has been identified as a very deep branching Planctomycetes with distinctive phenotypic properties such as the absence of any peptidoglycan in the cell wall, pits on the cell surface called “crateriforms”, reproduction by budding and internal cell compartmentalization (Strous et al., 2002). The cell compartment known as the anammoxosome is believed to be the site of anammox catabolism. The anammoxosome is made up by a lipid bilayer membrane containing unusual lipids called “ladderane” lipids that are concatenated cyclobutane moieties that are either ether and/or ester linked to the glycerol backbone or occur as free alcohols (Schouten et al., 2004). The anammoxosome also contains large quantities of hydroxylamine oxidoreductase (HAO)-like enzyme which is believed to play a key role in anaerobic ammonia oxidation (Schalk et al., 2000). This uncommon membrane may help the bacterium to limit the diffusion (Niftrik, 2004),

a function which is very critical to the survival of the anammox bacteria. For example, it is approximated that a loss of 10% of hydrazine alone through diffusion would lead to the loss of cell viability.

Three groups of anammox bacteria have been described. The fresh water species is *Candidatus "Brocadia anammoxidans"*. *T*he species found in wastewater include: *Candidatus "Brocadia Fulgida*", *Candidatus "Kuenenia stuttgartiensis*", *Candidatus "Scalindua wagneri"*, and C*andidatus "Scalindua brodae*", and the species found in marine environments is *Candidatus "Scalindua sorokinii*"(Jianlong and Jing, 2005). All the species have been extremely difficult to isolate. No pure culture has been obtained to date (Dalsgaard et al., 2005) and this complicates scientist's ability to perform a detailed study of these bacteria. Density gradient centrifugation has been so far the best isolation strategy, producing cell suspensions containing only one contaminant for 200-800 target bacteria (Jetten et al., 2001).

The species *Candidatus "Kuenenia stuttgartiensis*" and *Candidatus "Brocadia anammoxidans"* are the most studied, and the most commonly found organisms in wastewater treatment plants as well as in large scale anammox reactors (Kuenen, 2008). Both bacteria have optimal activity in the pH range from 6.4 to 8.3 and temperature range from 20 to 43^{o}C. The key physiological properties of these isolates are summarized in Table 2.1 (Strous, 1999; Jetten, 2001).

Table 2.1: Key physiological properties of anammox bacteria.

Parameters	Values
Free energy	-357 kj/mol
Biomass Yield	0.007 mol/mol C
Growth rate	0.003/h
Doubling time	10.6 d
K_s NH_4	5 µmol
K_s NO_2	>5 µmol
Temp range	20 – 43 ^{o}C
pH range	6.7 – 8.3

Despite these common features, there are significant differences between the two species. *Kuenenia stuttgartiensis* has a lower ammonia activity (26.5 nmol/min/mg protein) compared to

Brocadia anammoxidans (55 nmol/min/mg protein). On the other hand, *Kuenenia stuttgartiensis* has a higher tolerance to nitrite and is less inhibited by phosphate compared to *Brocadia anammoxidans* (Khin and Annachhatre, 2004). Both species require a certain cell density to become active but *Kuenenia stuttgartiensis* requires less cell density ($2x10^8$ cells per ml) compared to *Brocadia anammoxidans* (10^{10}-10^{11} cells/ml)(Egli et al., 2000).

The reason for the cell density requirement by anaerobic ammonia oxidizing bacteria is still obscure. It is also not clear why these bacteria tend to live in aggregates in reactor systems such as in wastewater or laboratory scale reactors. In fact, the anammox bacteria were found as single cells in their natural environment (Strous and Jetten, 2004) while they were in aggregates of 0.64 mm (average diameter) in an SBR receiving synthetic medium (Arrojo et al., 2006). In his study, Arrojo et al. (2006) also suggest a relationship between the aggregate size and activity. There has been an attempt to explain the reason for aggregation based on cell metabolism. It is possible that nitrogen monoxide and hydrazine, the intermediates of anaerobic ammonia oxidation, can be lost through the diffusion across the cell membrane, and hence, cells must form aggregates so that each cell profits the loss of its neighbor in compensation for the loss of its own compounds (Dapena-Mora et al., 2004).

Biochemistry of anammox bacteria

AnAOB use ammonium as electron donor and CO_2 as carbon source through the acetyl-CoA pathway (Strous et al., 2006). The fixation of one mole of carbon dioxide requires the oxidation of 15 moles of ammonium, according to the reaction shown in Equations 2.2 and 2.3 (Niftrik et al., 2004):

Catabolism: $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ (2.2)

Anabolism: $CO_2 + 2NO_2^- + H_2O \rightarrow CH_2O + 2NO_3^-$ (2.3)

Based on experiments, it was suggested that the complete reaction stoichiometry may be represented by equation 2.4 (Strous, 1998):

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (2.4)$$

Variations on this stoichiometry have been proposed (Wyffels, 2003). Strous et al. (1999) suggested that possible reasons for stoichiometry variation occurred when nitrite concentration in the medium varied. For example, during batch experiments, the nitrite:ammonia ratio changed from 1.3:1 at 140 mg/L NO_2-N to 4:1 at 700 mg/L NO_2-N (Strous et al., 1999). It was suggested that anammox bacteria may have generated an internal electron donor to reduce nitrite, which is reported to be a strong inhibitor of their growth.

The recent genome project provided a better understanding of the major biochemical pathways in *K. stuttgartiensis.* More than 200 genes associated with anammox catabolism and respiration were detected in the 4.2 megabase genome. Important proteins coded by the genes include the hydroxylamine oxidoreductase-like proteins which oxidize hydrazine to dinitrogen gas, the nitrite::nitrate oxidoreductase (NarGH), nitrite::nitric oxide oxidoreductase (NirS) which are both involved in denitrification. The genome also suggested the presence of a branched respiratory chain which would enable the respiration of different energy sources with different electron acceptors, confirming the possibility of anammox bacteria versatility (Strous et al., 2006). The genome study also suggests that hydroxylamine and nitric oxide are the process intermediates, rather than hydroxylamine and hydrazine as postulated previously (Jetten, 2001).

The current model for the anammox metabolic pathway suggests that nitrite is reduced to nitric oxide by the nitrite::nitric oxide oxidoreductase enzymes (*nir* genes). The intermediate nitric oxide (NO) is combined with ammonium by the hydrazine hydrolase enzyme to produce hydrazine, N_2H_4. The enzyme hydrazine dehydrogenase transfers electrons from hydrazine to ferrodoxin. The anammox bacteria get electrons from oxidation of nitrite to nitrate. Since this couple is electropositive (+0.43V), the transfer of electron to the electron transport chain uses the membrane potential Δp for reverse electron transport.

2. 3. Sidestream Nitrogen Removal

From the discussions in the previous section, it is clear that human intervention in the nitrogen fixation process could cause significant environmental imbalance that will worsen if nothing is done to prevent it. Fortunately, a good portion of the reactive nitrogen that ends up as non point source pollution is naturally converted to dinitrogen. The remaining portion ends up in

wastewater treatment facilities (at least in the industrialized world) where engineering solutions have been or are being formulated and applied to insure an optimal reactive nitrogen conversion back to dinitrogen gas. Wastewater treatment plants have to meet effluent regulations prior to discharging the wastewater effluent to water bodies. As regulations become more stringent, nutrients removal technologies are being evaluated and different strategies adopted. One of the strategies is to have a separate treatment of a high nitrogen-loaded liquid known as centrate or reject water, which is generated during the dewatering process of anaerobically digested sludge.

The dewatering of anaerobically digested sludge is an important process that reduces the moisture content of biosolids for ease in handling. Potential advantages associated with the process include: a cost reduction in transport; suitability of biosolids product for further processing via incineration and landfilling; reduction in odor; and reduction in leachate production (Metcalf and Eddy, 2003). Unfortunately, the liquid phase from dewatering processes has unique characteristics that render treatment somewhat challenging. This liquid phase, which constitutes up to 97% of the sludge, has a very high ammonia concentration (up to 2,500 mg NH_3-N/L) and can increase the ammonia load of secondary influent by up to 25% (Meyer et al., 2004). For this reason, reject water treatment processes that convert this reactive nitrogen into N_2 are beneficial because they reduce the nitrogen load to the secondary process.

Reject Water Characterization and Treatment Challenges

The reject water has been characterized in many wastewater treatment plants. It is very diverse and at the same time has unique characteristics as illustrated in Table 2.2, which compares three different wastewater treatment plants.

Table 2.2: Typical examples reject water characteristics

Parameter	Units	Seoul, Korea (Gil et al., 2002)		ASA, USA (Lab data, May 2007)		Strass, Austria (Wett et al.,1998)
		Range	Average	Range	Average	
pH		7.4-8.4	8.0	7.3-7.6	7.5	11.9-12.8
Alkalinity	mg/L	875 - 2550	1379	3550-4480	4103	5000-7500
COD soluble	mg/L	87 - 1464	386	249-446	348	614 ± 27
NH3-N	mg/L	138 - 552	365	906-1289	987	1832 ± 40
TSS	mg/L	300 - 23000	8317	69-172	109	-

From these data, it is apparent that there is significant variation in reject water quality from plant to plant. The alkalinity may not be sufficient for conventional nitrification in some cases. The COD, and particularly the BOD is low, reflecting a well stabilized sludge. The COD is not sufficient to support biological denitrification without addition of an exogenous electron donor. Although a concentration range is not shown, the concentration of phosphorous in reject waters is believed to be sufficient to support biological treatment (Fillos and Diyamandoglu, 1997).

Treatment alternatives for reject waters are typically grouped into two major categories: physical-chemical and biological processes (Gaulke et al., 2004). In some cases, however, nitrogen removal is enhanced by combining both processes. Each will be discussed next.

Physical-Chemical Processes

Physical-chemical methods have the advantage of being more reliable than biological methods. Some of them also allow the recovery of ammonia for beneficial use (Gaulke et al., 2004). The most cited common physical-chemical methods for ammonia removal are each discussed next.

- Chemical Precipitation

Magnesium and phosphorous salts are added to centrate to precipitate the ammonium ion as magnesium ammonium phosphate also known as struvite (Li and Zhao, 2001). The process is very suitable when the reject water pH is about 8-10 and has high magnesium and phosphate

concentrations. It was possible to achieve up to 90% ammonia removal and 50% COD removal by chemical precipitation in a laboratory scale system (Ozturk et al., 2003).

- Air stripping

The process involves desorption of ammonia from water to air in countercurrent stripping towers at ambient temperature followed by ammonia absorption by sulfuric or nitric acid (Gaulke et al., 2004). It was possible to achieve up to 95% ammonia removal at 15^{o}C using air stripping to treat digester effluent in a laboratory scale system (Lei et al., 2007)

- Steam stripping

The process involves utilization of superheated steam instead of air stripping in order to increase the efficiency, mainly because high temperature decreases the solubility of the gas in the liquid (Horan et al., 1994). The feed stream is generally preheated and then introduced into the steam stripping tower. Ammonia is passed through the distillation column and condensed as pure ammonia, which can easily be reused.

- Ion-Exchange

Some ion-exchange materials such as natural zeolite were successfully used for ammonia removal either alone (Jorgensen and Weatherley, 2003) or together with nitrifiers growing on the exchanger medium (Miladinovic and Weatherley, 2008). The ammonia removal by ion exchange could be described by a Langmuir isotherm for low concentrations (Lin and Wu, 1996, Démir, 2002). However, no study was found that reported on performance at higher ammonia concentrations such as those expected in reject waters. Furthermore, with ion exchange, regeneration wastewater still needs to be managed.

- Reverse Osmosis

This is a pressure driven membrane solid-liquid separation process. The effluent is usually of very high quality. The resulting brine, however, has a very high dissolved solids concentration and requires further treatment prior to disposal. In the case when it is applied to ammonia removal from centrate, the brine stream is estimated to reach 8,600 mg/L NH_3-N (Gaulke et al., 2004). Ammonia retention of 93 to 97% was achieved with a pressure of 30 bars at 25^{o}C using reverse osmosis (Mourer et al., 2006). Membrane fouling was the main limiting factor of this alternative (Migliorini and Luzzo, 2004).

Biological Processes

Conventional biological nitrogen removal occurs in two steps: nitrification and denitrification. In the first step, ammonia is oxidized to nitrite, and then nitrite is oxidized to nitrate. This is an aerobic process, achieved by autotrophic bacteria that use inorganic carbon as a carbon source. Stoichiometrically, nitrification requires 4.33 grams of oxygen and 7.14 grams of alkalinity per gram of ammonia oxidized. During denitrification, nitrate or nitrite is converted to nitrogen gas by heterotrophic bacteria under anaerobic conditions. This step requires 2.85 grams of BOD (intrinsic or exogenous) and produces 3.57grams of alkalinity as $CaCO_3$. Conventional nitrogen removal has been significantly optimized to increase the efficiency and reduce the cost. Nitritation (partial nitrification to nitrite only), bioaugmentation (supplementing nitrifying biomass into the mainstream process to influence overall reactor kinetics) and anaerobic ammonia oxidation are recent developments as applied to reject water and offer significant advantages over the conventional nitrification/ denitrification approach. Each of these alternative nitrogen removal schemes will be discussed next.

Nitritation

Nitritation does not result in the removal of nitrogen, but is a component in many novel nitrogen removal processes applied to strong ammonia-ladened wastewaters. The process requires enabling ammonia oxidation to nitrite while inhibiting the second step of nitrite oxidation, according to the reaction (Bock and Wagner, 2001):

$$NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \Delta G^0 = -235kJ/mol \qquad (2.5)$$

Partial ammonia oxidation to nitrite during nitrogen removal by nitrification/denitrification may allow significant savings: (Jianlong and Ning, 2003; Bougard, 2004; Ruiz et al., 2006)

- 25% reduction in oxygen requirement resulting in significant energy saving on aeration
- 40% reduction on electron donor required for denitrification
- 40% reduction in sludge production resulting in savings by reducing the volume of secondary clarifiers
- Increase in denitrification rate by 1.5 to 2 times

Nitritation strategies

Different parameters have been used to control nitritation, including pH, dissolved oxygen (DO), temperature, SRT, free ammonia and nitric acid.

- pH

The molecular basis of the effect that pH has on the growth rate of ammonia oxidizing bacteria (AerAOB) is not completely understood. It has been suggested that the pH effect may be due to the impact on enzymatic activity since each enzyme is active only within a specific (and usually narrow) range and displays maximum activity at an optimum pH (Gaudy and Gaudy, 1980). The optimal activity of nitrifiers is typically reported to be between 7.5 and 8.5 (Féray, 2000; Ruiz et al., 2003) with an optimal value of 8 (Fillos and Diyamandoglu, 1997). However, this range is for environments where ammonia concentrations are much lower than they are in reject waters or other environments where ammonia concentrations can become quite high. For example, in natural environments, nitrification has been reported to occur at pH values as low as 4 (De Boer et al., 1991) or as high as 10 (Koops et al., 2005).

The most probable mechanism at play in how pH impacts AerAOB and NOB relates to the speciation of ammonia and nitrite. Ammonia partitions between ammonium (protonated form, NH_4^+) and free ammonia (deprotonated form, NH_3) in accordance with pH, while nitrite partitions between nitrite (deprotonated form, NO_2^-) and nitrous acid (protonated form, HNO_2). Free ammonia and nitrous acid have been known to inhibit the growth of nitrifying bacteria in general but some studies suggested that the two genera show different sensitivities towards these compounds (Anthonisen et al., 1972). Anthonisen et al. (1972) went even further to identify the zones in which selective inhibition of either NOBs or both NOBs and AerAOBs occur. The study suggests that zone 1 (NH_3>10-150 mg NH_3-N/L) marks the inhibition of both AerAOBs and NOBs by free ammonia; zone 2 (0.1 -1.0 mg NH_3-N/L < NH_3<10-150mg NH_3-N) defines where free ammonia only inhibits the NOBs while complete nitrification is possible in zone 3 (NH_3<0.1-1.0mg NH_3-N/L and HNO_2 <0.2 to 2.8 mg HNO_2-N /L. In zone 4, NOBs are inhibited by free nitrous acid (HNO_2 >0.2-2.8 HNO_2-N/L).

- DO

The optimal concentration of dissolved oxygen for nitrification has been reported to vary from 0.5 to 4 mg/L (Feray, 2000) with the Monod half saturation coefficient for DO ranging from 0.1 to 2 mg/L, although a minimum DO concentration of 2.0 mg/L has been recommended for good nitrification (Metcalf and Eddy, 2003). Some studies suggest that DO concentrations less than 2 mg/L do favor nitrite accumulation (Princic, 1998). It was also experimentally possible to inhibit nitrate production by operating in the range of 0.3 to 0.8 mg/L during the start-up of the reactor (Jenicek et al., 2004), but DO was not a reliable parameter to control afterwards. Ruiz et al. (2004) achieved nitritation for DO concentrations between 0.7 and 1.4 mg/L. Bougard (2004) reports 80% ammonia oxidation to nitrite by maintaining the DO at 0.5 mg/L while Jianlong and Jing (2004) obtained 100% ammonia oxidation to nitrite by a combination of pH (7.5), DO (1.5 mg/L) and temperature (30^{o}C) control. These results suggest that although nitritation can be achieved by controlling DO, each system might be unique and it might be necessary for each plant to carefully investigate the specific conditions that lead to nitritation prior to designing a system for a particular wastewater.

- Temperature

The optimal temperature for nitrification is between 25 and 37^{o}C, even though the process can occur between 5 and 42^{o}C in certain natural environments (Féray, 2000). The rates of biochemical reactions are temperature dependent, increasing at increased temperatures, as expressed in the famous Arrhenius expression which can be written as follows (Grady et al., 1999):

$$k_1 = k_2\theta^{(t_1 - t_2)} \text{ and } \ln\theta = C \approx 0.0015u \qquad (2.6)$$

where k_i is the temperature dependent rate coefficient associated with temperature t_i, θ is a dimensionless coefficient and u the energy coefficient.

It has been reported that the temperature coefficient for nitrite oxidizing bacteria (NOB) is smaller than it is for ammonia oxidizing bacteria (AerAOB) (Grady et al., 1999). This difference lead to the prediction that NOB can be selectively washed out under controlled

temperature together with the mean cell residence time (Gali et al., 2006). For temperatures above 20^{o}C, the minimum mean cell residence time required for ammonia oxidizers is lower than that required for nitrite oxidizers. As a result, it is possible to selectively washout the NOBs by maintaining the mean cell residence time which is high enough for AerAOBs but not for NOBs. This concept has been the basis of SHARON (Single Reactor for High Activity Ammonia Removal Over Nitrite) Process.

With an SRT of 1 day and 30^{o}C, Kempen et al. (2001) were able to obtain 90% ammonia oxidation to nitrite. The SHARON Process was developed and patented by Delft University (Netherlands). The first full-scale system was successful at the Rotterdam Dokhaven Wastewater Treatment Plant. The description of SHARON was given in Mulder et al. (2001).
Some concerns have been raised about the long-term stability of nitritation using this control strategy. There have been worries that NOBs could get acclimated to reactor conditions and start growing, oxidizing nitrite to nitrate. Fux et al. (2004) observed an increase in nitrate accumulation after 11 months of reactor operation.

Bioaugmentation Process

Bioaugmentation of nitrifying populations has been proposed as a method that can be used to overcome nitrification performance failure at wastewater treatment plants operating in cold weather. The basic concept behind the process is that a separate reactor treating reject water would have a high concentration of nitrifiers which, if added to the main stream, would increase the nitrification rate and hence eliminate the need for longer SRT during low temperature periods. There have been different proposals to implement this process. One option is to grow nitrifiers in a separate reactor and then immobilize them. It was shown that nitrifiers can be safely kept in gel beads formed by synthetic polymers (Vogelsang et al., 1999, Sievers et al., 2003). These beads could be well preserved by freeze drying and be used in case the nitrifying community collapses in the plant. Polyvinyl alcohol polymer pellets have also been used successfully to immobilize nitrifiers at a full-scale municipal wastewater treatment plant receiving animal waste (Vanotti and Hunt, 2008). A process known as PEGASUS was developed based on this idea of nitrifiers immobilization (Mikawa et al., 1996). In this process, nitrifiers were immobilized in polyethylene glycol and added to the nitrification step. The influent organic

matter was used as the electron donor for denitrification. This allowed the researchers to achieve a removal efficiency of about 86%. The use of immobilized nitrifiers has been criticized by the fact that it is a relatively expensive process (Salem et al., 2002).

Bioaugmentation was also achieved through the development of nitrifiers within the aerobic microbial granules (Shu-Fang et al., 2003). The work showed that by increasing the N/COD ratio, it is possible to increase the fraction of nitrifying bacteria in the dense microbial granule where heterotrophs, nitrifiers and denitrifiers can coexist, suggesting the possibility of a novel compact and highly efficient granules-based biological process removing both organic matter and nitrogen from wastewater.

Another approach involves accumulating nitrifying bacteria in a separate tank in a side stream like a reactor receiving centrate, and continuously recycling the excess biomass into the main treatment stream (Head and Oleszkiewcz, 2003). Nitrification enhancement via seeding biomass grown in a side stream reactor was successful in a pilot plant treating the supernatant from a digested sludge dewatering process (Plaza et al., 2001). The excess biomass from the side stream was used to seed the activated sludge process. They realized that seeding makes it possible to maintain nitrification in the main stream process at low sludge ages which would otherwise preclude nitrification. A number of reactor configurations have been developed to optimize this approach, and they include: InNitri (Inexpensive Nitrification) (Bartolomew, 2002; Kos et al., 2008); BABE (Bio-Augmentation Batch Enhanced) (Salem et al., 2004); ScanDeNi (Huijbregsen and Rosen, 2003); and R-D-N (Regeneration-Denitrification and Nitrification) (Wanner, 1998).

Bioaugmentation Challenges

Bioaugmentation success is not guaranteed (Satoh et al., 2003). Problems concerning the adaptation of inoculated microorganisms, the insufficiency of substrate, competition between introduced species with indigenous biomass, and grazing by protozoa have been given as possible reasons for the failure of this approach. Specifically, adaptation of nitrifiers from enrichment bioreactors receiving high ammonia concentrations with high temperature may be problematic in the main stream where both ammonia concentration and temperature are low. For example, Manser et al. (2005) performed a nitrifying community analysis in both streams. The

study included the analysis of the autotrophic community composition, measurement of their kinetics and examination of their competition among different groups. They concluded that the community composition in the separate ammonia-rich treatment side stream harbored a different community composition relative to nitrifiers in the main stream. As a result, the augmented nitrifiers may not be able to adapt to the conditions prevailing in the wastewater treatment plant. These conclusions contradict those of Salem et al. (2004) who used FISH to identify the nitrifiers dominant in a BABE reactor and concluded that the composition was the same as in the main stream. The failure of a bioaugmentation process has also been reported by Bouchez et al. (2000). Newly introduced bacteria almost disappeared within two days.

It is also likely that concerns about the difference in temperature can be minimized. Head and Oleszkiewicz (2003) looked at bioaugmentation limitations due to temperature change. Nitrification was sustained after cooling the biomass quickly to 10^{o}C from 20^{o}C, 25^{o}C and 30^{o}C but the nitrification decreased in all cases. The study also tested the impact of seeding with warm nitrifying centrate biomass on cold reactors (10^{o}C) operated at an SRT below the minimum necessary for nitrification. Nitrification was induced in all seeded reactors. Effluent NH_3-N concentrations were reduced to less than 5 mg/l within 26–32 days as long as seeding was continued. A seed as little as 6.7 mg VSS/l reactor volume was sufficient for full NH_3-N removal in all seeded reactors. Cessation of seeding led to rapid loss of nitrification in the cold SBR.

The Anaerobic Ammonia Oxidation Process

Since the discovery of the anaerobic ammonia oxidation metabolism, efforts have been made to engineer anammox into treatment processes with a goal to use it in full-scale systems. In general, the process allows significant savings as shown in Table 2.3.

Table 2.3: Comparison between conventional nitrification/denitrification and nitritation/anammox.

	Conventional Ni/Denitrification	Nitritation-Anammox	Units	Source
Methanol	2.3	0	kg/kg N	Jensen et al., 2007
Power	2.8	0.79	kWh/kgN	Wett, 2006
Sludge Production	0.5-1.0	0.1	kgVSS/ kgN	Vereijken and Paques, 2006
Cost	4-6.8	2.0-3.4	USD/kgN	Lai et al., 2008

The DEMON process in Strass, Austria, was started in 2003. It is a single sludge system which relies heavily on controls. The control scheme is pH-based, with a pH band of 0.01-0.02. It took almost 2.5 years to upgrade the system from a 4 L inoculum added into a 300 L reactor and, ultimately, to a 500 m^3 full-scale plant. The process achieves about 90% ammonia removal and 84% total nitrogen removal with a very low energy demand of 0.79 kwh/kg nitrogen eliminated (Wett, 2006) compared to 2.8 kwh/kg nitrogen eliminated with conventional nitrification/denitrification process.

The Rotterdam Wastewater Treatment Plant is a full-scale system that uses an anammox-based process as well. The 70 m^3 plant was started in 2002 and was initially inoculated with nitrifying sludge from the Rotterdam-Dockhaven WWTP. It thereafter received settled biomass from a 5 m^3 anammox enrichment reactor on 29 occasions (van de Star et al., 2007). The last data show that the process started improving significantly after 1300 days (3.6 years) and finally reached the design load of 7.1 $kg/m^3/d$

The Rotterdam plant includes a separate nitritation step through the SHARON process is followed by an anammox reactor (Kartal et al., 2004). Other known full-scale systems using anammox include the Waterstromen Steenderen, Netherlands, which uses a CANON process to treat potato factory liquid waste and removes about 700 kg N/day and a tannery wastewater at Hulshof with separate nitritation/anammox with the ability to remove about 150 kg N/day (Loosdrecht, 2007).

2. 4. Inhibition of Ammonia Oxidation

Aerobic ammonia oxidation inhibition

Understanding the inhibition of ammonia oxidation has been very crucial in engineering systems that rely upon ammonia oxidation for optimal performance. In the case of agriculture, immediate oxidation of fertilizer-applied ammonia by soil nitrifying bacteria to nitrite or nitrate can initiate environmentally and economically deleterious effects by releasing those nitrification byproducts into the groundwater through leaching (Banuls et al., 2001). Several studies indicated that a significant number of compounds interfere with ammonia oxidation, whether it is

aerobic or anaerobic. Some of these inhibitors include: trichloroethylene (Hyman et al., 1995); heavy metal ions including Pb^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, and Ag^{+} (Quastel and Scholefield, 1951); nitrapyrin (Powell and Prosser, 1986); acetylene (Hyman and Arp, 1992); and allysulfide (Juliette et al., 1993). It has been suggested that many compounds inhibit AMO solely by interacting as a substrate at the active site(s) of the enzyme (competitive inhibition), while other substrates oxidized by AMO can lead to the generation of reactive intermediates that can disrupt ammonia oxidation by permanently damaging intracellular proteins (Juliette et al., 1993). Inhibition was also suggested to originate from the formation of complexes which interfere with the transport mechanism of the electron acceptor (Coby and Picardal, 2005).

Anaerobic ammonia oxidation inhibition

Anammox inhibition has been reported to be a major process limiting factor. Nitrite is most often reported as the primary inhibitor of concern because it also serves as a substrate. Concentrations over 100 mg/L completely inhibited anaerobic ammonia oxidation in a sequencing batch reactor (Strous et al., 1999). This inhibition could be relieved by adding a small concentration of hydrazine. The inhibition concentration may be site specific and may depend on the species. Dapena-Mora et al. (2007) reported concentrations as high as 350 mg NO_2^--N/L causing only 50% inhibition (IC_{50}). Oxygen as low as 0.064 mg/L inhibited anammox activity as well, but unlike the nitrite inhibition which is non-reversible, oxygen inhibition was stopped whenever the oxygen was removed from the system. Acetylene and phosphate strongly inhibited anammox activity (Khin and Annachhatre, 2004) while acetate and sulfide increased it (Dapena-Mora, 2007). It is obvious that the success of the anammox process in the future will require a clear understanding of the kinetics and factors influencing process inhibition / toxicity in order to reduce process upsets and failures.

2.5. References

Ahn Y.(2006); Sustainable nitrogen elimination biotechnologies: a review, *Process Biochemistry* 41: 1709-1723

Arrojo B., Mosquera-Corral, Campos J.L., Mendez R. (2006), Effects of mechanical stress on anammox granules in a sequencing batch reactor (SBR), *J. Biotechnol*, 123(4):453-463

Antonisen A, Loehr R, Prakasam T, Srinath E (1976); Inhibition of nitrification by ammonia and Nitrous acid, *Journal of Water Pollution Control Federation*, 48(5): 835-851

Banuls J., Quinones A., Primo-Millo E., Legaz F., (2001); A new nitrification inhibitor (DMPP) improves the nitrogen fertilizer efficiency n citrus-growing systems, *Food security and sustainability of agro-systems through basic and applied research*, 92: 762-763

Bartholomew R. (2002), PA Department of Environmental Protection, Innovative Technology Section.(www.dep.state.pa.us/dep/deputate/watermgt/wsm/WSM_TAO/InnovTech/ProjReviews/InNitri.htm).

Bougard D. (2004); Traitement Biologique d'Effluents Azotés avec l'ârret de la nitrification au stade nitrite, *These de Doctorat*, Ecole Nationale Superieure de Montpellier, France.

Beaumont H. J.E., Lens S.I., Reijnders W.N.M., Westerhoff H.V., Spanning R.J.M. (2004); Expression of nitrite reductase in *Nitrosomonas europaea* involves NsrR, a novel nitrite-sensitive transcription repressor, *Molecular Microbiology* (2004) (1) 148-158

Bock, E. and Wagner, M. (2006); Oxidation of inorganic nitrogen as energy source, *Procaryotes: Ecophysiology and Biochemistry*, *Springer* New York, pp 475-495

Bouchez T., Patureau D., Dabert P., Juretschko S., Dore J., Delgenes P., Moletta R.and Wagner M.(2000); Ecological study of a bioaugmentation failure, *Environmental Microbiology* 2(2), 179-190

Casella S, Payne W.J., (1996); Potential of denitrifiers for soil environment protection, *FEMS Microbiol. Lett.* 140:1-8

Chain P. Lamerdin J., Larimer F., Regala W., Lao V., Land M., Hauser L., Hooper A., Klotz M., Norton J., Sayavedra L., Arciero D., Hommes N., Whittaker M., Arp D., (2003); Complete Genome Sequence of the Ammonia-Oxidizing Bacterium and Obligate Chemolithoautotroph *Nitrosomonas europaea*, *Journal of Bacteriology*, pp 2759-2773

Chamchoi N.and Nitisoravut S. (2007); Anammox enrichment from different conventional sludges, *Chemosphere* 66 (11): 2225-2232

Christiansen J. and Dean D., (2000); Nitrogenase – from Agriculture to Molecular Medecine, *Biotech Times*, Vol 6, No 2.

Coby A., Picardal F., (2005); Inhibition of NO3- and NO2- Reduction by Microbial Fe (III) Reduction: Evidence of a Reaction between NO2- and cell Surface-Bound Fe2+, *Applied and Environmental Microbiology*, 71(9); 5267-5274

Coyne M., Arunakumari A., Averill B., Tiedje J.,(1989); Imminological Identification and Distribution of Dissimilatory Heme cd1 and Nonheme Copper Nitrite Reductase in Denitrifying Bacteria, *Applied and Environmental Microbiology*, 55(11):2924-2931

Dalsgaard T.Thamdrup B., Canfield D.E (2005), Anaerobic ammonia oxidation (anammox) in the marine environment, *Research in Microbiology* 156: 457-464

Dapena-Mora A, Campos J.L, Mosquera-Corral A., Jetten M.S.M., Mendez R.,(2004); Stability of anammox process in a gaslift reactor and SBR, *Journal of Biotechnology* 110: 159-170

Dapena-Mora A., I. Fern´andez , J.L. Campos, A. Mosquera-Corral, R. Méndez , M. Jetten (2007); Evaluation of Activity and Inhibition effects on Anammox process by batch tests based on nitrogen gas production, *Enzyme and Microbial Technology*, 40 (4): 859-865

De Boer W., Gunnewiek K.P.J.A., Veenhuis M., Bock E., Laanbroek H.J., (1991); Nitrification at Low pH by aggregated Chemolithotrophic Bacteria, *Applied env.microb*. 57(12: 3600-3604

Demir A., Gunay A., Debik E., (2002); Ammonium removal from aqueous solution by ion-exchange using packed bed natural zeolite, *Water SA*, 28(3):329-336

Egli K. (2003); On the use of anammox in treating ammonium-rich wastewater, PhD Dissertation, Swiss Federal Institute of Technology, Zurich, Suitzland

Egli K., Fanger U., Alvarez P., Siegrist H., van der Meer J., Zehnder A. (2001); Enrichment and characterization of anammox bacterium from a rotating biological contactor treating ammonium rich leachate, *Arch Microbiol*, 175:198-207

Einsle O and Kroneck M.H.P.(2004); Structural basis of denitrification, *Biol. Chem*, Vol 385: 875-883

Féray C. (2000), Nitrification en sediment d'eau douce: Incidence de rejets de station d'épuration sur la dynamique des communautés nitrifiantes, Thèse de Doctorat, Université Claude-Bernard, Lyon 1, France

Fillos J. and Diyamandogly V (1997), Characterization and Treatment of the New York City Sludge Dewatering Centrate, City College of CUNY

Fux C., Huang D., Monti A.and Siegrist H.(2004); Difficulties in maintaining long-term partial nitritation of ammonium-rich sludge digester liquids in a moving-bed biofilm reactor (MBBR), *Water Science and Technology* Vol 49 No 11–12 pp 53–60

Gallow JN and Cowling E.B., (2002); Reactive nitrogen and the world: 200 years of change, *Ambio* 31:64-71

Gaudy A. and Gaudy E.(1980); Microbiology for Environmental Scientists and Engineers, McGraw Hill, Inc, p 8.

Head M.and Oleszkiewicz (2003); Bioaugmentation for nitrification at cold temperatures, *Water Research*, 38:523-530

Hellinga, C., Schellen, A.A.J.C., Mulder, J.W., van Loosdrecht, M.C.M. and Heijnen, J.J. (1998). The SHARON-process; an innovative method for nitrogen removal from ammonium rich waste water. *Wat. Sci. Tech.* **37**(9), 135–142

Henckel T.and Conrad R.,(1998); Characterization of microbial NO production and N_2O production and CH_4 oxidation initiated by aeration of anoxic rice field soil, *Biogeochemistry*, 40:17-36

Horan N.J., Lowe P., Stentiford E.I, (1994); Nutrient removal from wastewaters: Proceedings from the European conference, pp 114.
http://en.wikipedia.org/wiki/Haber_process

Hyman M. R., Aro D.J., C_2H_2 and CO2-labelling studies of de novo synthesis of polypeptides by Nitrosomonas europaea during recovery from acetylene and light inactivation of ammonia monooxygenase, *J. Biol. Chem.* 267:1534-1545

Hyman M.R., Russell S.A., Ely R.L., Williamson K.J., Arp D.J.,(1995); Inhibition, Inactivation and recovery of ammonia-oxidizing activity in cometabolism of trichloroethylene by *Nitrosomonas europaea, Appl Environ. Microbiology*, 61(4) 1480-1487

Janus H.M, Van der Roest H.F. (1997); Don't reject the idea of treating reject water. *Water Science and Technology*, 34(3-4), 87-94

Jensen M, Thandrup B., Dalsgaard T. (2007), Effects of specific inhibitiors and denitrification in marine sediments, *Appl. Environ. Microbiology*, 73 (10):3151-3158

Kuypers M., Lavik G., Woebken D., Schmid M., Fuchs B., Amann R., Joegensen B., Jetten M.C.M. (2005); Massive nitrogen loss from the Banguela upwelling system through anaerobic ammonium oxidation, *PNAS*, 102(18) 6483

Manser R., Gujen W. and Siegrist H. (2006); Decay Processes in nitrifying bacteria in biological wastewater treatment systems, *Water Research* 40: 2416-2426

Maurer M., Pronk W., Larsen T.A., (2006); Treatment processes for source-separated urine, *Water Reseach* 40:3151-3166

Migliolini G., Luzzo E., 2004; Seawater reverse osmosis plant using the pressure exchanger for energy recovery: a calculation model. *Desalination* 165, 289-298

Mulder R., (2007); Future Perspectives of Anaerobic Industrial Wastewater Treatment, LeAF 10 year anniversary, Netherlands.

Gaulke L., Alli R., Ecklebe K., Xi Y. (2004); Reducing the methanol requirement for the LOTT biological nutrient removal wastewater treatment plant, *Water Innovative Methods Inter Nationally* (WIMIN), Design Project, Olympia, Washington.

Gali A, Dosta J., van Loosdrecht, Mata-Alvarez J., (2007); Two ways to achieve an anammox influent from real reject water treatment at lab-scale: Partial SBR nitrification and SHARON process, *Process Biochemistry*, 42: 715-720

Gil K., Choi E., Yun Z., Lee J., Ha J., Park J., (2002); The nomographic design approach to recycled water treatment by the nitritation process, *Water Science and Technology* 46 (11–12): 85–92

Grady L.C.P., Daigger T.G, Henry C. Lim H.C. (1999); Biological Wastewater Treatment, 2ed, Marcel Dekker

Gut L. (2006); Assessment of partial nitrification/anammox system for nitrogen removal, Licenciate Thesis, KTH, Department of Land and Water Resources Engineering, Stockholm, Sweden.

Isaka K., Date Y., Sumino T., Yoshie S., Tsuneda S. (2006); Growth Characteristics of anaerobic ammonia-oxidizing bacteria in an anaerobic biological filtrated reactor, *Appl Microbiol Biotechnol*, 70 : 46-62

Jenicek P., Svehla P., Zabranska J. and Dohanyos M., Factors affecting nitrogen removal by nitritation/denitritation, Water *Science and Technology* Vol 49 No 5–6 pp 73–79

Jetten S., Wagner M., Fuerst J., van Loosdrecht M., Kuenen G.and Strous M. (2001); Microbiology and application of anaerobic ammonia oxidation (‘anammox’) process, *Current Opinion in Biotechnology* 12:283–288

Jetten M., Strous M., van de Pas-Schoonen K., Schalk J. , van Dongen U., van de Graaf A., Logemann S., Muyzer G., van Loosdrecht M., Kuenen G. (1999):, Anaerobic Oxidation of Ammonia, *FEMS Microbiology Reviews*, 22: 421-437

Jianlong W. and Jing K. (2005); The characteristics of anaerobic ammonia oxidation (ANAMMOX) by a granular sludge from an EGSB reactor, *Process Biochemistry* 40:1973–1978

Jianlong W.and Ning Y. (2004); Partial nitrification under limited dissolved oxygen conditions, *Process Biochemistry* 39: 1223–1229

Jorgensen T.C. and Weatherley L.R. (2003); Ammonia removal from wastewater by ion exchange in the presence of organic contaminants, *Water Research*, 37(8): 1723-1728

Juliette L., Hyman M., Arp D., (1993); Mechanism-based Inactivation of Ammonia Monooxygenase in Nitrosomonas europaea by Allysulfide, Applied and *Environmental Microbiology*, 59(11), 3728-3735

Kartal B., Koleva M., Arsov R., van der Star W., Jetten M., Strous M. (2006); Adaptation of a fleshwater anammox population to high salinity wastewater, *Journal of Biotechnology* 126: 546-553

Khin T. and Annachhatre P.(2004); Novel Microbial Nitrogen Removal Processes, *Biotechnology advances* 22: 519-532

Kindaichi T., Tsushima I., Ogasawara Y., Shimokawa M., Ozaki N., Satoh H., Okabe S., (2007); In situ activity and special organization of anaerobic ammonium-oxidizing (Anammox) bacteria in Biofilms, *Applied and Environmental Microbiology*, 73(15):4931-4939.

Koops H.P., Purkhold, U., Pummerening-Roser, A., Timmerman G., Wagner M., (2005), The Lithoautrophic ammonia oxidizing-bacteria. In *The Procaryotes: An evolving electronic resources for the Micrbiological community*, Springer, New York.

Kos P., Head M., Oleszkiewicz J., Warakomski A, (2008); Demonstration of low temperature nitrification with a short SRT, , www.m2ttech.com/docs/innitri_weftc.pdf

Kuenen G. (2008), Anammox bacteria: from discovery to application, *Nature Reviews*, 6:320-325

Lai E., Schaar H., Solley D., Semple J., Keller J.(2008); Novel Nitrogen removal processes using nitritation and anammox processes at laboratory and pilot scale, Advanced Wastewater Management Center, University of Queensland, Australia

Lei X., Sugiura N., Feng C., Maekawa T., (2007); Pretreatment of anaerobic digestion effluent with ammonia stripping and biogas purification, *Journal of Hazards Materials*, 145:391-397

Li X.Z.and Zhao Q,L.,(2001); Efficiency of biological treatment affected by high strength of ammonium-nitrogen in leachate and chemical precipitation of ammonium-nitrogen as pretreatment, *Chemosphere* 44:37-43

Lin S. and Wu C., (1996); Ammonia removal from aqueous solution by ion exchange, *Ind. Eng. Chem. Res.*, 35(2): 553-558

Manser R (2005); Population dynamics and kinetics of nitrifying bacteria in membrane and conventional activated sludge plants, Thesis, Swiss Federal Institute of Technology, Switzerland.

Metcalf and Eddy (2003); Wastewater Engineering: treatment and reuse, 4th ed, pp 1558-1559

Meyer S. and Wilderer A.; Reject Water: Treating of Process Water in Large Wastewater Treatment Plants in Germany, a Case Study, Journal of Environmental Science and Health, *Part A—Toxic/Hazardous Substances and Environmental Engineering* Vol. A39, No. 7, pp. 1645–1654, 2004

Mikawa K., Emori H., Takeshima T., Ishiyama E., Tanaka K., (1996); High rate and compact two-stage post denitrification process with single stage denitrificaction, *Water Science and Technology*, 34(1-2): 467-474

Miladinovic N., Weatherley L.R.,(2008), Intensification of ammonia removal in a combined ion-exchange and nitrification column, *Chemical Engineering Journal*, 135:15-24

Mulder J.W., van Loosdrecht M.C.M., Hellinga C. and van Kempen R.(2001);
Full-scale application of the SHARON process for treatment of rejection water of digested sludge dewatering, *Water Science and Technology* Vol 43 No 11 pp 127–134

Niftrik L., Fuerst J.A., Damste J.S.S., Kuenen J.G., Jetten M.S.M., Strous M., (2004), The anammoxosome: an intracytoplasmic compartment in anammox bacteria, *FEMS Microbiology letters*, 233: 7-13

Ozturk I., Altinbas M., Koyuncu I., Arikan O., Gomez-Yangin C., (2003); Advanced physico-chemical treatment experiences on young municipal landfill leachates, *Waste Management* 23: 441-446.

Plaza E., Trela J., Hultman B., (2001); Impact of seeding with nitrifying bacteria on nitrification process efficiency, *Water Science and Technology*, 45(1): 155-164

Powell S., Prosser J., Inhibition of ammonium oxidation by nitrapyrin in soil and liquid culture (1986), *Applied and Environmental Microbiology*, 52(4), 782-787

Princic A., Mahne I., Megusar F., Paul E.A, Tiedje J.M. (1998); Effects of pH and Oxygen and Ammonium Concentration on the community Structure of Nitrifying Bacteria from Wastewater, *Applied and Environmental Microbiology*, 1998, p. 3584-3590

Quastel J.H., Scholefield P.G., (1951); Biochemistry of Nitrification in Soil, *Microbiol. Mol Biol. Rev*., 15 (1), pp 1-53

Rosén B.and Huijbregsen C. (2003); The ScanDeNi process could turn an existing under-performing activated sludge plant into an asset, *Water Science and Technology*, Vol 47, No 11, pp 31-36

Ruiz G., Jeison D., Rubilar O., Ciudad G., Chamy R. (2006): Nitrification-Denitrification via Nitrite accumulation for nitrogen removal from wastewaters, *Bioresource Technology* 97: 330–335

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

Salem S., Berends D., van der Roest H., van der Kuij R.and van Loosdrecht M. (2004): Full-scale application of BABE technology, *Water Science and Technology*, 50(7): 87-96

Salem S. Berends D.J.G., Heijnen J.J., van Loosdrecht M.C.M., (2003); Bio-augmentation by nitrification with return sludge, *Water Research* 37:1794-1804

Satoh H. Okabe S., Yamaguchi Y., Watanabe, Y.(2003); Evaluation of the impact of bioaugmentation and biosimulation by in situ hybridization and microelectrode, *Water Research*, 37:2206-2216

Schalk J., de Vries S., Kuenen J., Jetten M., (2000); Involvment of a novel hydroxylamine oxidoreductase in anaerobic ammonium oxidation, *Biochemistry* 39: 5405-5412.

Schnoor J., (1996); Environmental Modeling: Fate and Transport of Pollutants in Water, Air and Soil, Environmental Science and Technology, A Wiley-interscience Publication, John Wiley & Sons, Inc, New York, NY

Schouten S., Strous M., Kuypers M., Rijpstra I., Baas M., Schubert C., Jetten M., Damsté J., (2004); Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing bacteria, *Applied and environmental microbiology*, 70(6): 3785-3788

Shrestha N.K., Hadano S., Kamachi T., Okura I. (2002), Denitrogen production from ammonia by Nitrosomonas europeae, *Applied catalysis A*: General 237:33-39

SliekersA. O., Derwort N., Gomez J.L., Strous M., Kuenen J.G., Jetten M.S.M.,(2002); Completely autotrophic nitrogen removal over nitrite in one single reactor, *Water Research* 36:2475-2482

Sievers M., Vorlop K.D., Hahne J., Schlieker M.and Schäfer S. (2003); Pre-nitrification by encapsulated nitrifiers – a possibility for self-sufficient energy operation of domestic WWTPs, *Water Science and Technology*, Vol 47, No 11, pp 173-180

Shu-Fang Y., Tay J.H, Liu Y., (2003); A novel granular sludge sequencing batch reactor for removal of organic and nitrogen from wastewater, *J. of Biotechnology*, 106(1)77-86

Strous M. and Jetten M.(2004); Anaerobic oxidation of methane and ammonium, *Annu. Rev. Microbiol.* 58:99–117

Strous M., Kuenen G., Fuerst J., Wagner M., Jetten M. (2002): The anammox case-A new experimental manifesto for microbiological eco-physiology, *Antonie van Leeuwenhoek* 81: 693-702

Strous M. (1999), Key physiology of anaerobic ammonia oxidation, *Applied Environmental Microbiology*, 65: 3243-3245

Strous M., Pelletier E., Mangenot S., Rattei T., Lehner A., Taylor M. W., Horn M., Daims H., Bartol-Mavel D., Wincker P., Barbe V., Fonknechten N., Vallenet D., Segurens B., Schenowitz-Truong C., Médigue C., Collingro A., Snel B., Dutilh B., Camp H., van der Drift C., Cirpus I., van de Pas-Schoonen K., Harhangi H., van Niftrik L., Schmid M., Keltjens J., van de Vossenberg J., Kartal B., Meier H., Frishman D., Huynen M. A., Hans-Werner Mewes J. Weissenbach, Jetten M., Wagner M. and Le Paslier D., *(2006*); Deciphering the evolution and metabolism of an anammox bacterium from a community genome, *Nature*, , 440(7085): 790-794

TallecG., Garnier J., Billen G., Gousailles M., (2008); Nitrous oxide emissions from denitrifying activated sludge of urban wastewater treatment plants, under anoxia and low oxygenation, *Bioresources Technology* 99: 2200-2209

Third K.A., Paxman J., Schmid M., Strous M., Jetten M.S.M., Cord-Ruwisch R., (2005), Treatment of nitrogen-rich wastewater using partial nitrification and anammox in the CANON process, *Water Science and Technology*, 52(4):47-54

Townsend A., Howarth R., Bazzaz F.A., Booth M., Cleveland C., Collinge S., Dobson A., Epstein P., Holand E., Keeney D., Mallin M., Rogers C., Wayne P., Wolfe A., (2003); Human health effect of a changing global nitrogen cycle, *Front Ecol Environ*, 1(5): 240-246

Van De Graaf A., Mulder A., De Bruijn P., Jetten S. M., Robertson A., and Kuenen J., (1995); Anaerobic oxidation of ammonium is a biologically mediated process, *Applied and Environmental Microbiology,* 61(4):1246-1251.

Van der Star W., Abma W., Blommers D., Mulder J,W., Tokutomi T., Strous M., Picioreanu C., van Loosdrecht M., (2007); Startup of reactors for anoxic ammonium oxidation: Experiences from first full-scale anammox reactor in Rotterdam, *Water Research* 41: 4149-4169

Van Loosdrecht M. (2007), Innovative N-Removal Processes, Delft University Technology, I-learning course.

Vanotti M., Ammonia removal from swine wastewater using immobilized nitrifiers, US Department of Agriculture, URL: www.ramiran.net/doc98/FIN-ORAL/VANOTTI.pdf

Verstraete W., Philips S., (1998); Nitrification-denitrification processes and technologies in new contexts, *Environ. Pollut.* 102:712-726

Vitousek P., Aber J., Howarth R., Likens G., Matson P, Schinder D., Schesinger W., Tilman D., (1997); Technical Report: Human Alteration of the Global Nitrogen Cycle: Sources and consequences, *Ecological Applications*, 7(3): 737-750

Vogelsang C., Gollembiewski K., Ostgaard K.(1999); effect of preservation techniques on the regeneration of gel entrapped nitrifying sludge, *Water Research*, 33 (1), pp 164-168

Vereijken T., Paques B.V, (2006); ANAMMOX® demonstration on full-scale in Rotterdam, The Netherlands, Local Action 1782, Topic Session FT3-04, Netherlands

Wett B., Rostek R., Rauch W., Ingerle K.(1998), pH-controlled reject water treatment, Water *Science and Technology*, 37(12):165-172

Wett B. (2006); Solved Scaling problems for implementing Deamonification of Reject Water, *Sci. Technol*., 53 (12):121-128.

White D. (1999); The physiology and Biochemistry of Prokaryotes, 2nd Ed, Oxford University Press,

Windey K., Bo I., Verstraete W. (2005); Oxygen-Limited autotrophic nitrification-denitrification (OLAND) in a rotating biological contactor treating high- salinity wastewater, *Water Research* 39 (18): 4512-4520

Wyffels S (2003); Identification and quantification of nitrogen removal in a rotating biological contactor by 15N tracer techniques, *Water Research*, 37(2003) 1252-1259

CHAPTER 3:

Operations and Process Control of the Deammonification (DEMON) Process as a Sidestream Option for Nutrient Removal

3.1. Introduction

Current regulations challenge wastewater treatment plants in nitrogen sensitive regions (typically, in coastal or estuarine watersheds) to remove nitrogen to low levels to avoid negative impacts on the aquatic ecosystem. In order to meet these regulations, wastewater treatment plants that were originally designed for BOD removal are being upgraded to include nitrogen removal processes that meet increasingly stringent total nitrogen limits. The demand for high removal efficiencies coupled with high operational costs for achieving those efficiencies solely through mainstream processes have motivated the search for new strategies that help achieve high N removal efficiencies plant-wide while minimizing operational costs. Separate treatment of internally generated recycle streams from anaerobic digesters, which have high nitrogen content, is one new strategy that offers promise. This strategy could be very beneficial for plants such as the Alexandria Sanitation Authority (ASA) Advanced Wastewater Treatment Facility (AWTF) where the ammonia-rich centrate liquid stream is recycled to the biological reactor basins, thereby increasing the nitrogen load to the mainstream process.

The Alexandria Sanitation Authority Advanced Wastewater Treatment Facility (ASA-AWTF) has a separate solids process that produces Class A biosolids. The process includes a Kruger Bio Pasteur process for pasteurization followed by anaerobic digestion for sludge stabilization prior to land application. The centrate from the dewatering process has an average ammonia concentration of 1,000 mg N/L and contributes up to 20% of the total nitrogen load to the mainstream process at the facility. Biological nitrogen removal is achieved using a single-sludge step-feed nitrogen removal process with methanol addition to enhance denitrification. ASA-AWTF has consistently achieved its target annual average total nitrogen limit of 8 mg/L since the facility upgrade in 2003 by using conventional nitrification/denitrification in the

mainstream of the treatment system; however, sidestream treatment of the centrate could be an economical option to achieve the current total N limit and recent changes in regulations which require ASA to reduce the total nitrogen concentration in their effluent to 3 mg/L by the year 2011.

Treatment processes have been deployed to control high strength internal recycle wastewaters by using partial nitrification of ammonia to nitrite (nitritation) coupled with either heterotrophic denitrification from nitrite to N_2 (heretofore called denitritation) or anaerobic ammonia oxidation (nitrite plus ammonia to N_2 and nitrate) (van der Star et al., 2007). Many of these processes have proven to be beneficial when used for sidestream treatment at utilities (Mulder et al., 2001; Wett, 2006; van der Star et al., 2007). In some cases, nitritation and denitritation, or nitritation and anaerobic ammonia oxidation have been combined into a single reactor and achieved using a single sludge system (Fux et al., 2006, Lai et al., 2004; Nielsen et al., 2005; Third et al., 2005).

ASA, in collaboration with other partners including DC WASA, University of Innsbruck, Virginia Tech, Envirosim and CH2M Hill, evaluated the feasibility of two side stream treatment options: the DEamMONification (DEMON) process (nitritation coupled with anaerobic ammonia oxidation using pH control in a single sludge SBR: (Wett et al., 2006) and nitritation/denitritation of the centrate. Both side stream treatment options decrease the total nitrogen and ammonia load to the mainstream portion of the plant and would reduce operating costs associated with methanol and caustic soda addition and energy for aeration in comparison to conventional nitrification/denitrification. DEMON has the greatest potential for reducing operating costs for chemical and energy operating costs through use of the anammox process. The AWTF currently spends about $1 million each year on methanol alone for conventional nitrification/ denitrification in the mainstream process. By employing ammonia removal from the centrate using a sidestream treatment process, operators can reduce and possibly eliminate the methanol and caustic feeds to the mainstream biological reactor basins.

The DEMON process embodies nitritation and anaerobic ammonia oxidation, also referred to as the anammox process. The stoichiometry of the DEMON process can be described by the equations:

$$2.34\,NH_4^+ + 1.8\,O_2 + 2.66\,HCO_3^- \rightarrow 0.024\,C_5H_7NO_2 + NH_4^+ + 1.32\,NO_2^- + 2.54\,CO_2 + 3.94\,H_2O$$

for nitritation (assuming a biomass yield of 0.14 g VSS/g NH_3-N), and

$$NH_4^+ + 0.066\,CO_2 + 0.066\,H^+ + 1.32\,NO_2^- \rightarrow 0.066\,CH_2O_{0.5}N_{0.15} + 1.02\,N_2 + 0.26\,NO_3^- + 1.96\,H_2O$$

for anaerobic ammonia oxidation (assuming a biomass yield of 0.07 g protein/g NH_4-N). The anammox process is facilitated by chemolithoautotrophic anaerobic ammonia oxidizing bacteria (AnAOB) belonging to the order of planctomycetes that use nitrite as the terminal electron acceptor (Strous et al., 1999).

The nitritation process is performed by aerobic ammonia oxidizing bacteria (AerAOBs) and can be optimized through appropriate regulation of key variables, including the temperature, solids retention time (SRT), pH, dissolved oxygen (DO) concentration, substrate (ammonia) concentrations and load, operational and aeration pattern and through use of selective inhibitors (Peng and Zhu, 2006). Selection of AerAOBs by temperature (Kempen et al., 2001) takes advantage of their faster growth rate at high temperatures compared to nitrite oxidizing bacteria (NOBs). The SRT can be used to selectively wash out NOBs given the fact that AerAOBs typically grow faster than NOBs (Hellinga et al., 1999). The inhibition of nitrite oxidation by free ammonia and nitrous acid (Abeling and Seyfried, 1992, Pamprun et al., 2000, Jenicek et al., 2004), which are the toxic forms of their acid/base couples, is based on the relatively high tolerance of AerAOBs for these toxic forms compared to NOBs (Anthonisen et al., 1976) and is usually regulated by selecting an appropriate pH (Akerman, 2005). Finally, DO control takes advantage of the slow growth of NOBs under low DO concentrations due to their lower affinity for O_2 as a terminal electron acceptor compared to AerAOBs (Hanaki et al., 1991, Jianlong and Ning, 2004). In a full-scale system in Strass, Austria, nitritation has been successfully deployed using low DO at limited aeration intervals as the only control strategy, making the control system relatively simple to manage (Wett et al., 2006).

Nitritation provides significant savings relative to complete nitrification (ammonia oxidation to nitrate) because stoichiometry tells us that it demands 25% less oxygen and produces 40% less sludge resulting in savings on secondary clarifiers volume requirement. Furthermore, when coupled with heterotrophic denitrification, 40% less organic electron donor is consumed relative to complete denitrification. If well controlled, nitritation can be managed so that sufficient ammonia remains and enough nitrite is produced to meet the molar ratio required for autotrophic denitrification (anaerobic ammonia oxidation) using anammox metabolism. The combination of these two processes in DEMON results overall in 60% less oxygen and 100% less organic electron donor relative to conventional nitrification/denitrification. Alternating in a single-sludge between nitritation and anaerobic ammonia oxidation requires careful process control because the AnAOB are reversibly inhibited by dissolved oxygen and are irreversibly inhibited by nitrite (Strous, 1997). Although Strous et al. (1999) reported that nitrite inhibition of AnAOB is irreversible, the nitrite inhibition study reported here suggested that short term nitrite exposure effects are reversible (see Chapter 5).

The objective of this study was to determine the reliability and efficiency of the DEMON process in treating ammonia-rich centrate. The specific objectives were to:

- Observe the performance of the DEMON pilot plant during steady loading conditions in comparison to the performance of the pilot plant when operated as a nitritation/denitritation process.
- Provide ASA operational experience with the DEMON monitoring and control system to assist with any future implementation, including design, construction, start-up and operation of a full-scale side-stream treatment process, and
- Investigate the impact of relevant design parameters (e.g. SRT, HRT, specific loading and sludge production) on performance to inform future designs.

3.2. Pilot Study Description

Pilot Plant Setup

The pilot plant reactor was a plexiglass cylinder 4 m (13 ft) long and 25 cm (9.8 inches) in diameter. This configuration allowed oxygen transfer efficiency in the pilot plant to mimic a full-scale DEMON system located in Strass, Austria. The pilot plant configuration also

minimized uncontrolled oxygen intrusion which improved DO control. The reactor was fed ASA centrate collected from the pipe that conveyed centrate from the holding tank to the biological reactor basins in the mainstream process. Centrate used to feed the pilot plant was stored in two- 270 gal feed tanks; one tank was actively used at a time while the second tank served as a storage tank. Centrate was never stored longer than 8 days. Pilot plant effluent was collected in a 70 gal tank. Sufficient mixing was applied in the effluent tank before sampling to ensure a representative sample was collected. Both the feed tank and pilot reactor were equipped with heating mechanisms and were insulated to minimize heat loss during the winter. Figure 3.1 shows the pilot reactor before and after insulation.

Figure 3.1 Pilot reactor with a feed tank and a spare holding tank before insulation (right) and after insulation (left). The un-insulated tank served as a holding tank for centrate.

Inoculation and Start Up

The study was organized into two phases. During Phase 1, the reactor was operated as a nitritation/denitritation system. During this phase, the equipment associated with the pilot plant system was tested, including the PLC programming and nitritation process control. During Phase

2, the pilot plant was operated using the DEMON process. During Phase 1, 107 L of return activated sludge (RAS) from ASA was collected and used for start up. Methanol was added to the centrate to supplement COD to be used during denitritation. The methanol:ammonia ratio was held at 1:3 (COD:N). Phosphoric acid was also added to the centrate to ensure that P was not limiting. The reactor was operated as a sequencing batch reactor (SBR) with 3 cycles in a day, 6 hours fill/react, 1.7 hours settle and 0.3 hours of decant. Controlled intermittent aeration was provided through a fine-bubble membrane diffuser and was the main mixing mechanism. There were two ways to control aeration based on the apparent oxygen uptake rate. During low oxygen uptake rate periods, the blower was set to go on and off during nitritation periods. The on and off periods were set by the operator to sustain the required DO. During high oxygen uptake rate periods, aeration was totally controlled by the pH setpoints, with the blower turning on until the pH reached the low set point. In addition to the air mixing, a recirculation pump was installed and allowed to re-circulate up to 162 L/hr to enhance mixing, especially during anoxic periods. The mass loading was increased gradually from 0.152 kg NH_3-N/m^3/d (with an HRT of 5.3 days based on a flow rate of 23 L/d), and was meant to be increased to reach the design mass load (per unit reactor volume) of 1.00 kg NH_3-N/m^3/d with an HRT of 1 day, by a weekly hydraulic loading increase of 20 mL/min. The pilot was run in the nitritation/denitrification mode for a period of 5 months during the autumn and winter.

Once Phase 1 was complete, the reactor was prepared for Phase 2 by temporarily stopping the pilot for two days to allow residual methanol to be depleted. This was important because methanol has been reported to inhibit the anammox metabolism (Jensen et al., 2007). Phase 2 was started by blending 20 L of settled solids collected from the DEMON process in Strass, Austria and about 90 L of existing sludge used during Phase 1. The DEMON sludge from Strass was kept at 4^{o}C for 30 days prior to inoculation. No additional chemicals were added to the centrate prior to feeding to the reactor during this phase. Centrate was fed continuously to the reactor during the 6 hour fill and react period of the cycle. During this period, aeration was provided to maintain the DO within the upper and lower setpoints, and the aeration period was controlled by the pH. The control strategy is detailed in the following section. The initial hydraulic load was also set at 23 L/day; however, the centrate was diluted in half for the first few days to reduce the ammonia concentration and minimize the chance of imposing a shock load to

the biomass. The mass load was increased gradually by reducing the amount of dilution until no dilution was used. The target loading was 0.50 kg NH_3-N/m^3/d with an HRT of 1.7 days.

Control Variables

A supervisory control and data acquisition (SCADA) interface was used to provide operational control and data collection for the pilot plant (Figure 3.2). Aeration in the pilot plant was controlled by pH during both Phases 1 and 2. During the aerobic period, nitritation by AerAOBs decreased the pH to the lower pH setpoint specified by the operator. Once the setpoint was achieved, aeration ended, dissolved oxygen was consumed, and the non-aerobic process commenced; both denitritation (Phase 1) and anammox (Phase 2) reduce acid and, therefore, have the impact of increasing pH. In order to avoid nitrite accumulation in the reactor, nitritation was limited to a reasonable time period above which the controls would freeze and stop the pilot operation to minimize the potential negative effect of nitrite. Nitratation (oxidation of nitrite to nitrate) was suppressed during both phases by operating at low DO concentrations (below 0.7 mg/L) and high temperature (up to 37 ^oC). The purpose of operating the pilot reactor at 35°C was to potentially eliminate the need for a heat exchanger in the design of the full-scale system. Two months after starting Phase 2, it was suggested that process stability could be reached quickly if the pilot was run at optimal temperatures. Based on past studies that found 35^oC to be the optimal temperature for *Nitrosomonas* (Grunditz and Dalhammar, 2001), the pilot temperature was held at 35^oC. During Phase 2, the DO concentration during aerobic periods was reduced to 0.3 mg/L to prevent the accumulation of nitrite. Later it was allowed to go up to 0.5 mg/L to counteract the possible inhibition of AerAOB by DO limitation. It was important to maintain the minimum liquid level at 2.7 m to minimize CO_2 stripping because CO_2 was the source of carbon for the autotrophic biomass. A typical SCADA output captured during a typical operating day for Phase 2 is shown in Figure 3.3.

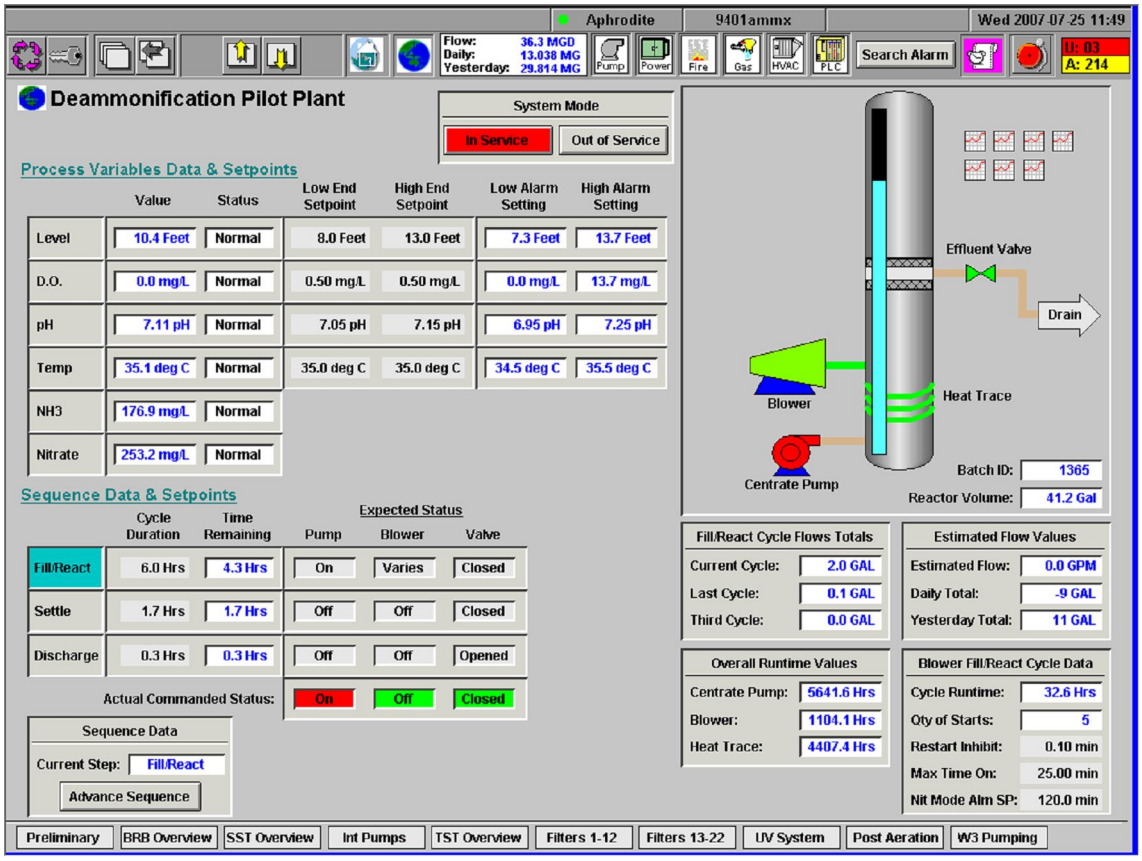

Figure 3.2 The Supervisory Control and Data Acquisition (SCADA) interface for the pilot plant reactor.

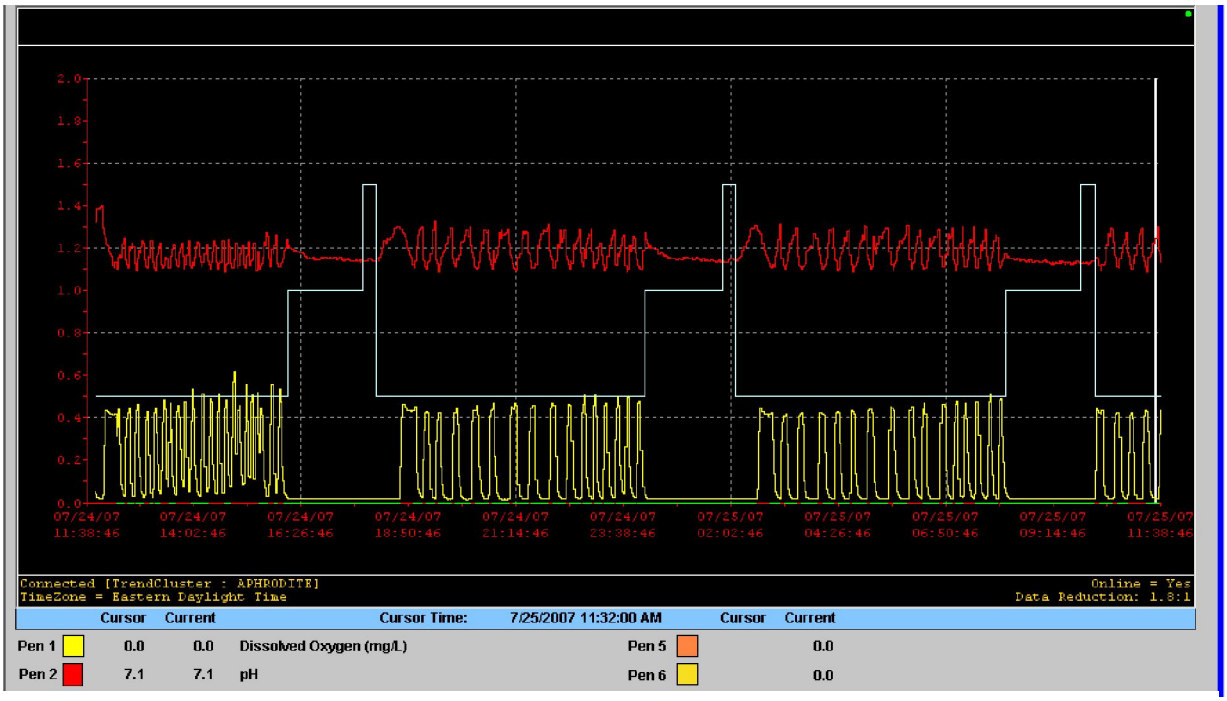

Figure 3.3 DO (yellow) and pH (red) profiles with time. Note that the y-axis is DO for this screen shot. The white line indicates the SBR cycles (the lowest level corresponds to the fill/react period the next highest line indicates the settle period, and the highest line represents the decant period).

Sample Collection and Analysis

Samples were collected five days per week from the feed and effluent tanks by gently mixing for 2 to 3 minutes prior to sampling to ensure a representative sample was collected. Influent and effluent samples were analyzed for ammonia, nitrite, nitrate, total suspended solids (TSS), volatile suspended solids (VSS), chemical oxygen demand (COD), orthophosphate, total Kjeldahl nitrogen (TKN) and alkalinity. Biomass samples were collected from the sampling port located in the middle of the reactor while the reactor was in aeration mode and were analyzed for TSS and VSS (Standard Methods). Grab samples were filtered through a 0.45 µm pore size glass microfiber filter for analysis of ammonia (method EPA 350.1), nitrite (EPA 353.2), NO_x (NO_2^- +NO_3^-) (EPA 353.3) orthophosphorous (EPA 365.1), and soluble COD (HACH, method 8000). TKN samples were digested and analyzed according to method EPA 351.2 protocol. Samples for total COD were not filtered prior to analysis. A semi-automated colorimeter (Astoria Analyzer, Clackamas, OR) was used for these grab samples except COD samples for which a spectrophotometer (HACH, DR/4000, Loveland, CO) was used. Alkalinity was measured by acid titration using 0.2 N sulfuric acid. In addition to grab sample analyses, inline measurements were taken in the reactor and captured by the SCADA system. The pH was measured inline by means of a SensoLyt® 700 IQ which uses a combination electrode for potentiometric measurement. DO was measured by a Trioximatic® 700 IQ, a membrane-covered amperometric sensor with a potentiostatically operated 3-electrode system. Both nitrate and ammonia were measured by a VARION®^{Plus} 700 IQ ion sensitive electrode combined with a reference electrode. All three inline sensors also simultaneously measured the temperature, and were provided by WTW GmbH, Weilheim, Germany.

Free ammonia (FA) inhibition played an important role in interpreting the data during this pilot plant study. Free ammonia was calculated based on total ammonia nitrogen available in the system, the pH and the temperature, using an equilibrium-based relationship:

$$[NH_3] = \frac{[TAN]}{1 + \frac{10^{-pH}}{k_{e,T}}} \qquad \text{[equation 1]}$$

where [TAN] is the total ammonia nitrogen concentration (Anthonisen et al., 1976).
and $k_{e.T}$, is the temperature dependent equilibrium constant defined by the relationship

$k_{e,T} = e^{\frac{-6344}{T+273}}$ where T is the degrees in centigrades.

3.3. Results and Discussion

Phase 1 - Nitritation/Denitritation

It was desirable to maintain the nitrite and nitrate concentrations at a low level in the effluent from the pilot plant during Phase 1. Although low nitrate does not prove that denitritation (via nitrite) is occurring, it is a necessary condition consistent with denitritation. Reasonably low nitrite concentrations are also desirable because such a condition would indicate that free ammonia inhibition is not overwhelming the system. During the Phase 1 pilot plant study, such ideal conditions proved difficult to maintain. The nitritation/denitritation process showed an initial increase in nitrate concentration in the reactor effluent, despite the tight control of DO to a maximum of 0.7 mg/L for the first 20 days. After 20 days, the system HRT was gradually decreased from 5.3 days to 2 days by increasing the hydraulic loading from 23.4 to 81.9. Simultaneously, the ammonia mass loading was increased from 0.15 kg/m^3/d to 0.5 kg/m^3/d. As observed in Figure 3.4, the system responded quickly. Nitrate decreased and nitrite increased, suggesting that ammonia loading to the reactor may play a significant role in achieving nitritation control. Higher ammonia loads may result in higher levels of free ammonia (FA), which can selectively inhibit NOBs for FA concentrations above 1.0 mg-N/L and below 10 mg-N/L (Antonisen et al., 1976). Indeed, during the start up period from days 23 to 28 when nitrite buildup occurred and residual nitrate decreased, the FA concentration in the reactor ranged from 4.6 mg NH_3-N /L to 5.3 mg NH_3-N /L and averaged 4.9 $\pm$ 0.84 mg NH_3-N/L. The relationship between nitrite/nitrate concentrations with free ammonia concentration is illustrated in Figure 3.5. Nitrate accumulation was not hindered by free ammonia concentrations as high as 4 mg-N/L but nitrite build up stopped for concentrations above 4.5 mg N/L. This concentration is much higher than the inhibiting concentration suggested by Kim et al. (2005) who reported serious inhibition of nitrite oxidizers with only 0.1 mg/L of FA, but falls within the range of FA concentrations to nitrite oxidizers only (0.1 -1.0 mg NH_3-N/L < NH_3<10-150 mg NH_3-N/L) proposed by Anthonisen et al. (1976). This study suggests that the best strategy to control nitritation would be to start with a high FA concentration (>4.5 mg/L) to selectively inhibit NOBs.

A second period of process instability started at the end of January (day 110) and coincided with the addition of a new batch of centrate. Process control was lost due to an inability to decrease the pH during the aeration period, even when the mass loading was decreased from 0.64 kg/m^3/d to 0.23 kg/m^3/d (hydraulic loading decreased from 93 L/d to 35 L/d), suggesting AerAOB inhibition given that the pilot alkalinity did not change (data not shown) . In order to address this process control difficulty, approximately 148 g of the pilot sludge was replaced by the same mass of RAS to overcome the ammonia oxidation slow down. The introduction of new RAS caused nitrate accumulation (Figure 3.5), probably because of the nitrite oxidizers introduced in the pilot with the RAS.

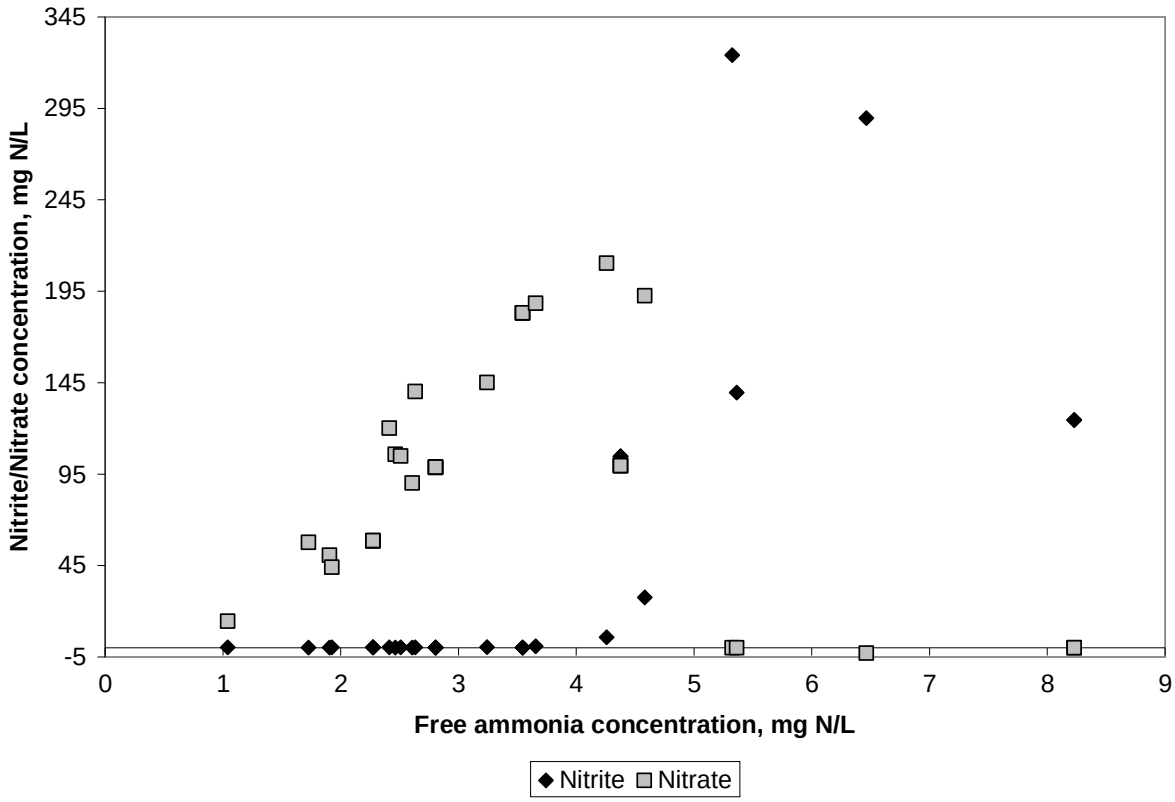

Figure 3.4 Nitrite and nitrate concentration as a function of free ammonia concentration for the first 40 days of the pilot operation during Phase 1. FA was calculated using the relationship suggested by Anthonisen et al. (1976).

Overall, the system achieved about 85% ammonia removal and 75% total nitrogen removal, on average, during Phase 1 (Figure 3.6). However, due to problematic performance, the mass loading to the system was never increased to the target loading of 1.0 kg/m^3/d, although the target HRT of 1 day was achieved.

The main reason for this difficulty was the apparent inhibition of nitritation due to a significant slow down in AerAOB growth. This inhibition was directly observed from the slow rate of pH decrease during the nitritation phase. During normal operation, the pH decreased from high point to low point within 5 to 30 minutes for the pilot scale system. An appropriate time of 30 minutes was also reported by Wett (personal communication) to be a reasonable time interval for the full-scale system at STRASS. Because of the metabolic slowdown by AerAOBs, the reactor could not consume the alkalinity provided by the centrate in the time frame of the SBR reaction cycle. The fact that no significant change in reactor alkalinity was observed during the upset reinforce the possibility of AerAOB inhibition. The inhibition resulted in pH being an insensitive control parameter for the system**.** An in-depth investigation of the cause of this inhibition is detailed in Chapter 6. The fact that the target HRT of 1.0 day was achieved despite an inability to achieve the mass loading design goal suggests that the ammonia mass loading, rather than hydraulic loading, is the more important design parameter for the nitritation/denitrification process.

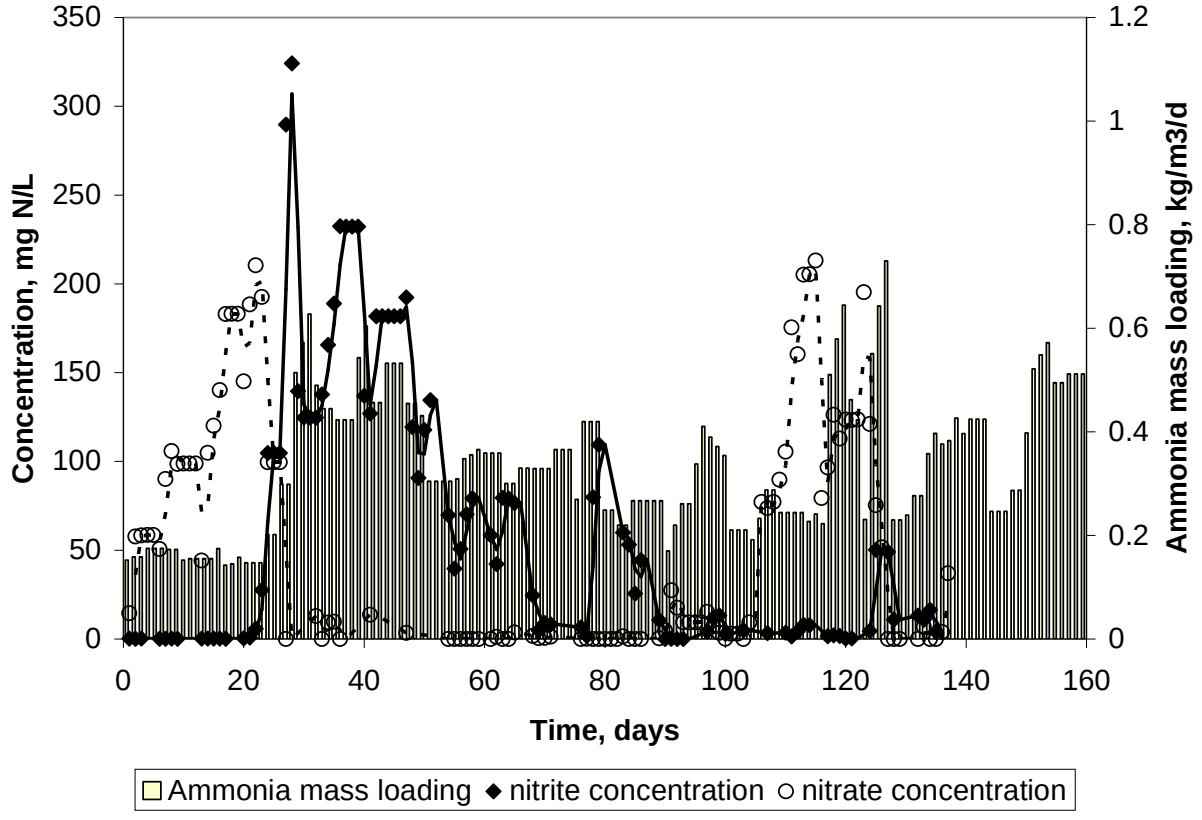

Figure 3.5 Nitrite and nitrate concentration profiles (left Y axis) and mass loading (vertical bars, right Y-axis) during Phase 1 of the pilot plant study.

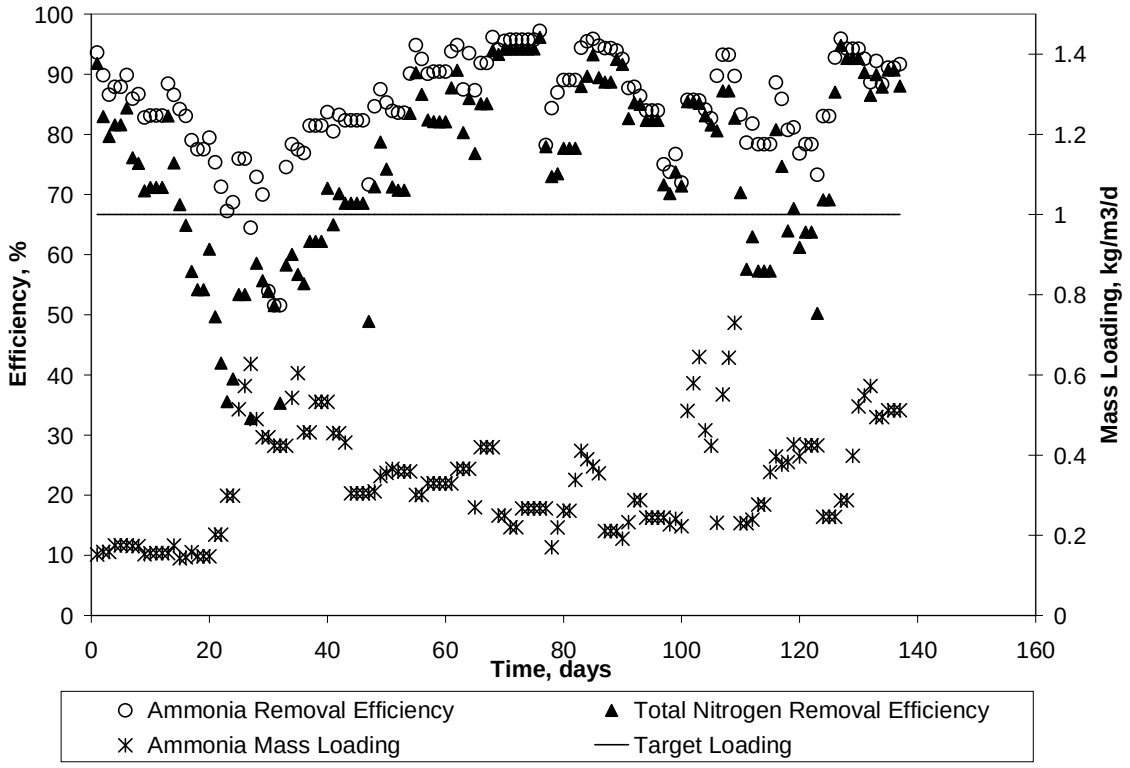

Figure 3.6 Ammonia load and removal efficiency as a function of time during Phase 1.

It is also worth noting that the nitritation/denitritation process held the alkalinity level in the reactor to a range of 200-400 mg/L as $CaCO_3$ (data not shown). Values below this range caused the pH to drop too fast, resulting in nitritation periods that were too short. Values above this range caused very long aeration periods, which could lead to the accumulation of nitrite to a level thought to cause inhibition or toxicity. This balance of alkalinity was especially important during Phase 2 because AnAOB are believed to be particularly sensitive to high nitrite levels.

Phase 2 – the Nitritation/Anammox (DEMON) Process

The main goal during this phase was to achieve stable process performance with 85% total nitrogen removal under the hydraulic loading rate of 108 L/d and a mass loading rate of 0.5 kg NH_3-N/m^3-day. A secondary goal was to apply a mass load to the system above this level to see how far the process could be stressed before experiencing failure. The process was started March 2007 with a very low loading rate of 0.08 kg NH_3-N/m^3-day. The loading was increased whenever possible by 10 mL/min increments (4.3 mg of ammonia /L/hr) in the centrate feed rate. The increase was assessed based on the stability of the process and was maintained as long as the

process control strategy remained functional, regardless of the process efficiency. The mass loading is presented in Figure 3.7 and shows a sustained increase in loading up to more than the design loading until day 99, 2007. Mass loadings were even above the target loading for a large portion of the experimental time frame. Figure 3.7 shows an upset at day 100 that required a sudden decrease in loading. The upset occurred when the system failed to nitrify adequately and, hence, a significant disturbance in pH control occurred. This was the same type of upset observed during the first phase of the study. The dissolved oxygen data (Figure 3.8) also show a decrease in oxygen uptake rate as well as the inability of the system to deplete the DO sufficiently to create anaerobic periods that could support the anammox process.

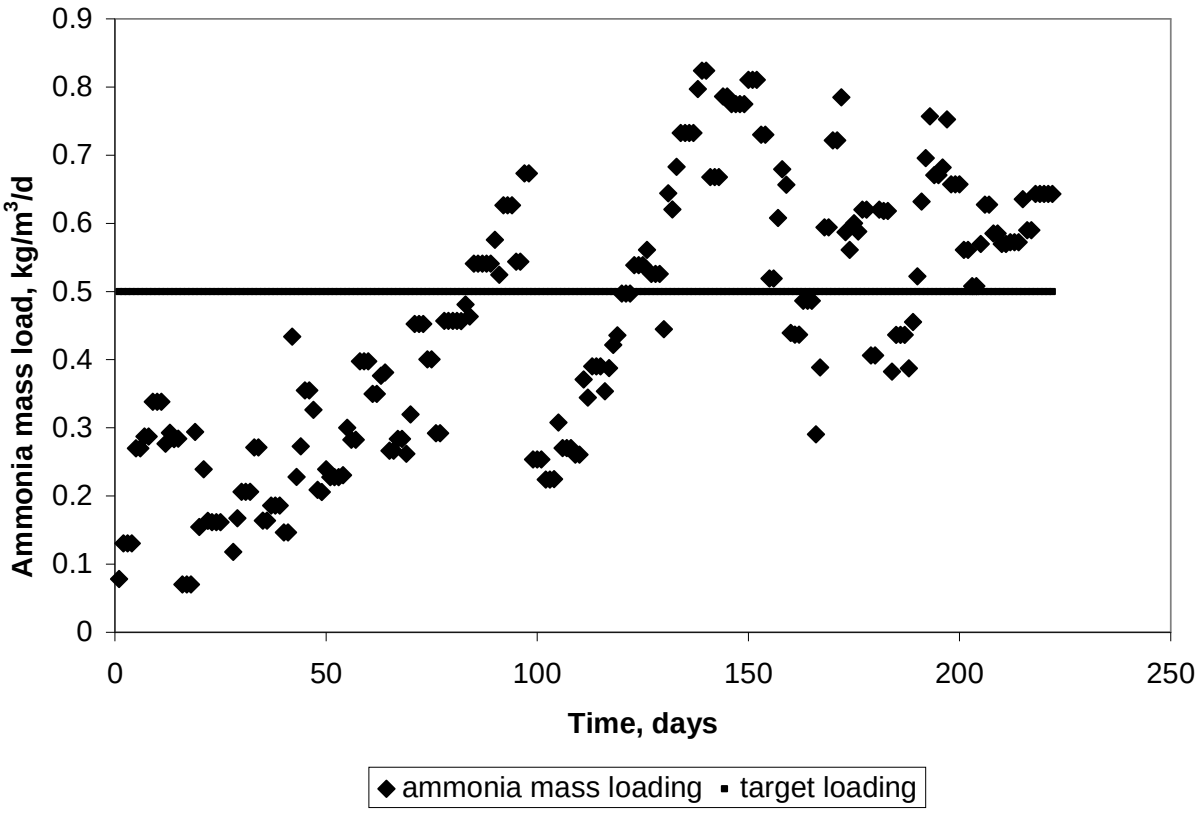

Figure 3.7 DEMON pilot plant mass loading and target mass loading over time (Phase 2). Decreases in loading were due to decisions to recover from an upset or to decreases in feed ammonia concentrations.

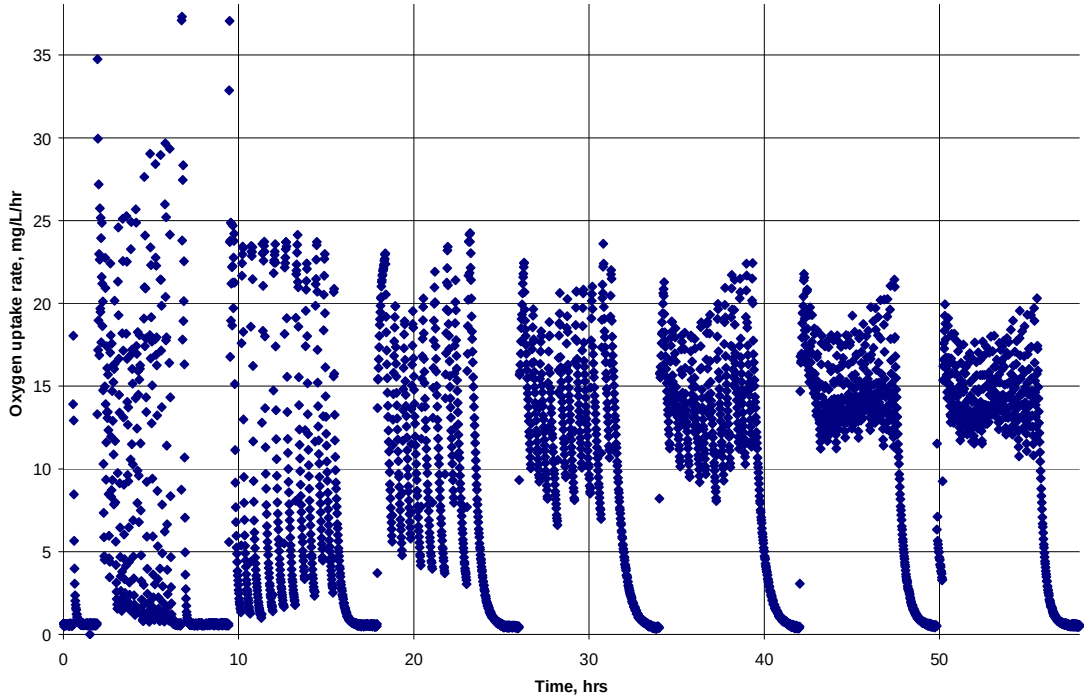

Figure 3.8 Oxygen uptake rate profiles prior to process upset. The oxygen uptake rates at time t (OUR_{ti}) were calculated using the formula $OUR_{t_i} = \frac{DO_{t_{i+1}} - DO_{t_i}}{t_{i+1} - t_i}$

The ammonia removal process efficiency was 78±7% on average while the total N removal efficiency was 56±14, substantially lower than the target removal efficiency of 85% (Figure 3.9). A substantial loss in N removal efficiency occurred after the first 30 days. This was about two weeks after the reactor had experienced a significant failure due to loss of DEMON sludge when a tubing connection broke. The reactor was completely emptied and reseeded with 103 L of fresh RAS and 10 L of DEMON sludge that was stored under refrigeration for 3 months. An interesting performance trend that started 2 weeks after the system was reseeded was the distinct difference in total N removal and ammonia removal that occurred from day 30 onward (Figure 3.9). This corresponded with a significant and sustained increase in nitrate in the effluent (Figure 3.10). Therefore, it appears that the DEMON sludge had limited nitritation capacity from that point forward. The introduction of fresh RAS to the system with NOBs was also a potential boost to the NOB population, which correlates with the sustained increase in nitrate in the effluent for the remainder of the study. Finally, the system failure resulted in a significant reduction in the stability of the nitritation control, which only exacerbated the apparent reestablishment of NOBs in the system. The slow down of AerAOB activity extended

the duration of aeration periods and allowed NOBs to reestablish. As a consequence, nitrate accumulated. The fact that NOBs managed still to compete even for DO as low as 0.3 mg/L suggests that DO alone was not sufficient to control nitritation.

The nitritation control problem is also illustrated in Figure 3.10 which shows a very wide margin between measured and predicted nitrate concentrations in the effluent starting at day 55, after an upset. The results confirm that dissolved oxygen might not constitute a sufficient nitritation control strategy, similar to what was observed in the first phase. It is important to note that the data show that the gap between measured and calculated nitrate based on anammox stoichiometry was closing with time.

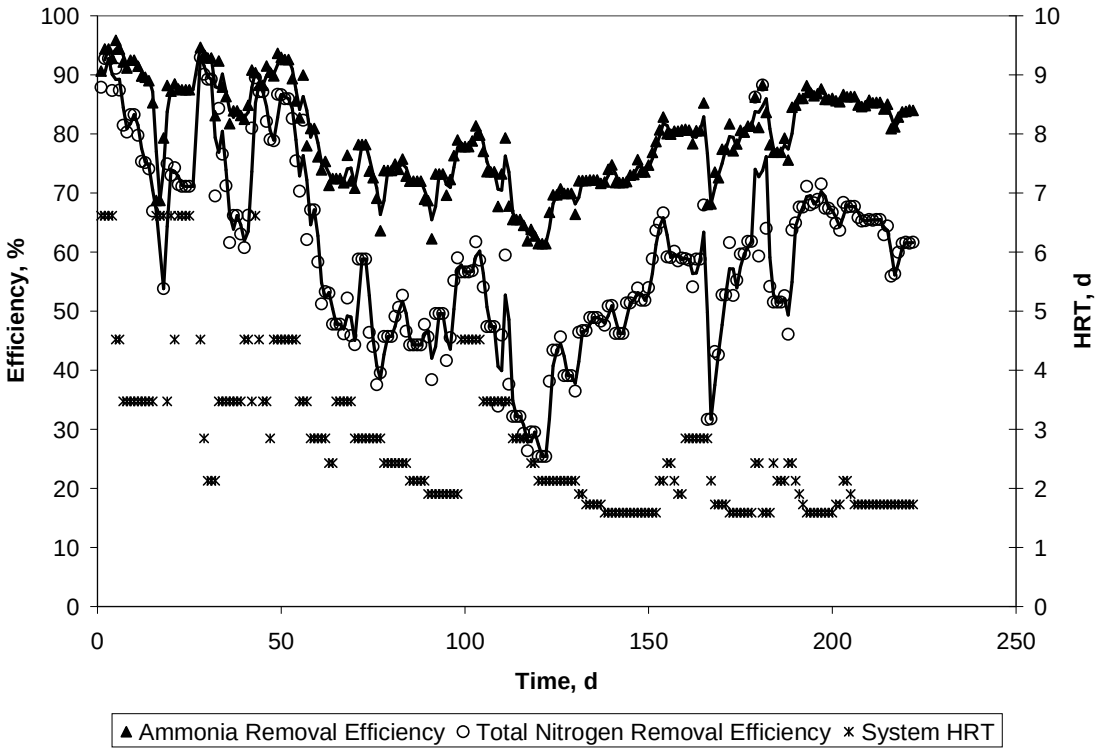

Figure 3.9 Process efficiency (left axis) and HRT (right axis) as a function of time during Phase 2.

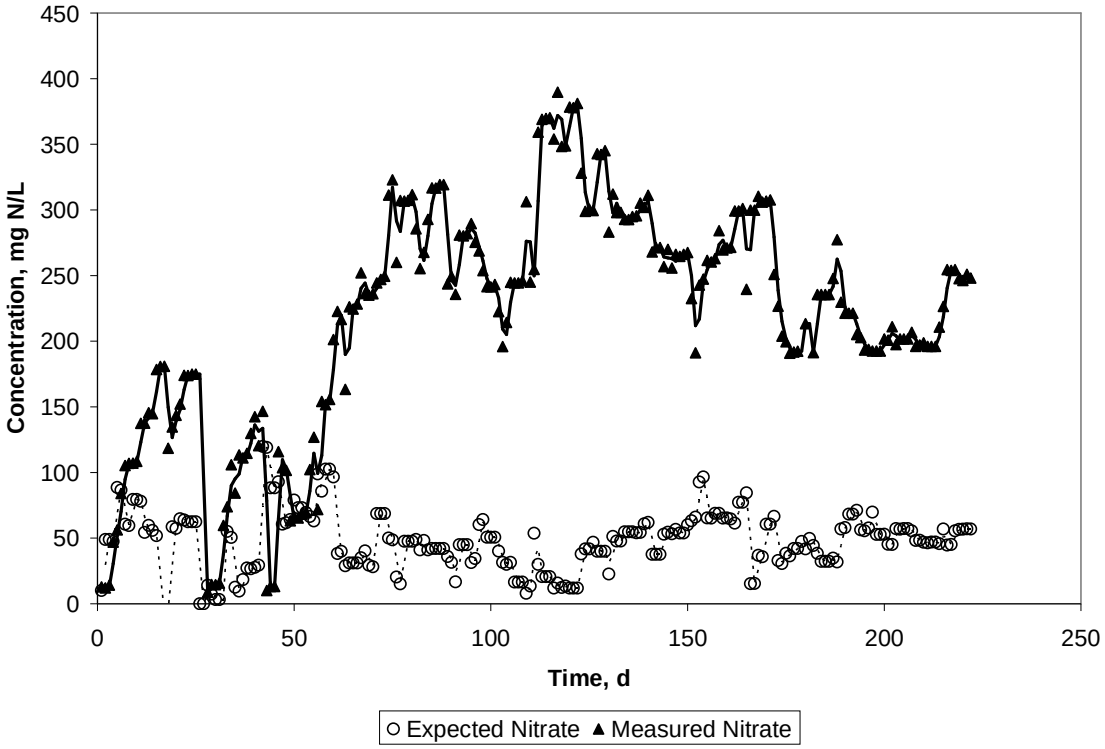

Figure 3.10 Measured nitrate profiles versus expected nitrate concentrations based on DEMON stoichiometry, which predicts that 1.32 moles of ammonia are oxidized to nitrite through nitritation for every 2.32 moles of ammonia metabolized, and 0.26 mole of ammonia ends up as nitrate due to anammox. The calculation also assumed that soluble COD lost in the process was used for denitrification.

Although the design mass load was targeted to be 0.5 kg/m^3/d, it is clear that the system could tolerate more loading. The average ammonia removal of 76% did not change much with mass loading even when the mass loading was increased by 170% of the design load (Figure 3.11).

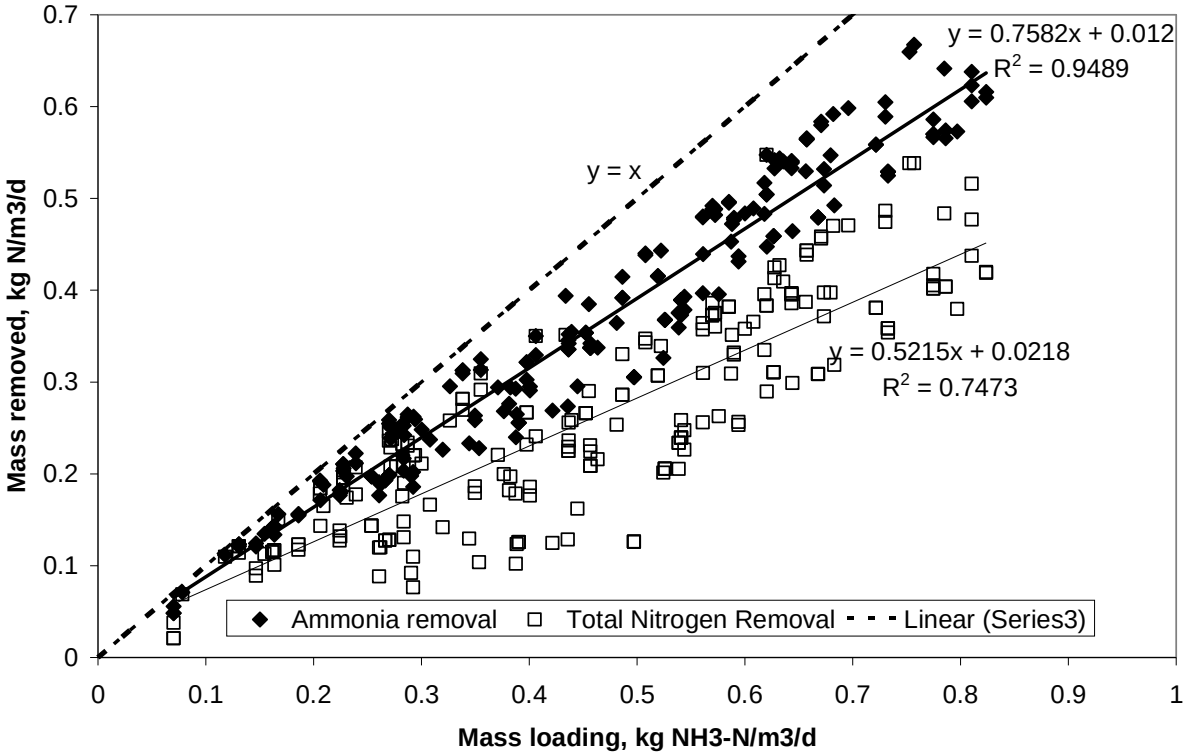

Figure 3.11 Mass of ammonia removed and total mass of nitrogen removed vs mass of ammonia loaded (Phase 2).

In general, both the mass and volumetric loading objectives of Phase 2 were met despite lower efficiency compared to the theoretically possible maximum removal of 85% based on the DEMON stoichiometry or about 90% removal if influent COD is considered to be available for denitrification. The trends suggest that this difference could be reconciled over time. The nitrogen conversion was much higher than what was reported previously for similar systems such as CANON. Third et al. (2005) reported mass loadings of 0.08 kg/m^3/d, while Sliekers et al. (2002) reported 0.16 kg/m^3/d for the same system. Both studies were conducted with an SBR configuration. DEMON process optimization could result in elimination of ammonia return to the mainstream process at ASA and eliminate any need for additional methanol to be added to the mainstream process at ASA given that the treatment plant influent BOD is sufficient to achieve influent total nitrogen removal. Furthermore, a significant reduction in chemicals required for alkalinity adjustment in the mainstream process would be realized. The main drawback of the DEMON process is based on the fact that the process is new (the oldest plant in Strass is only about 4 years old), implying that some studies are still needed, especially to fully understand the causes of possible process upset.

Control System Recommendations

As a response to the periodic process upsets, the control strategy was modified to avoid significant damage which would require restarting the process. During process upset, the SCADA was programmed to take the reactor out-of-service if the nitritation period that was too long (value to be specified by the operator) or if the denitritation period was too short. Although the program response to take the reactor out of service was a good strategy to avoid unfortunate events such as nitrite accumulation, there was a significant loss of AerAOB activity if the reactor was held idle too long, as indicated by the decrease in oxygen uptake rate of up to 90% in some cases. The current scheme that was used to address the control problem in the case of upset event is summarized in Figure 3.12.

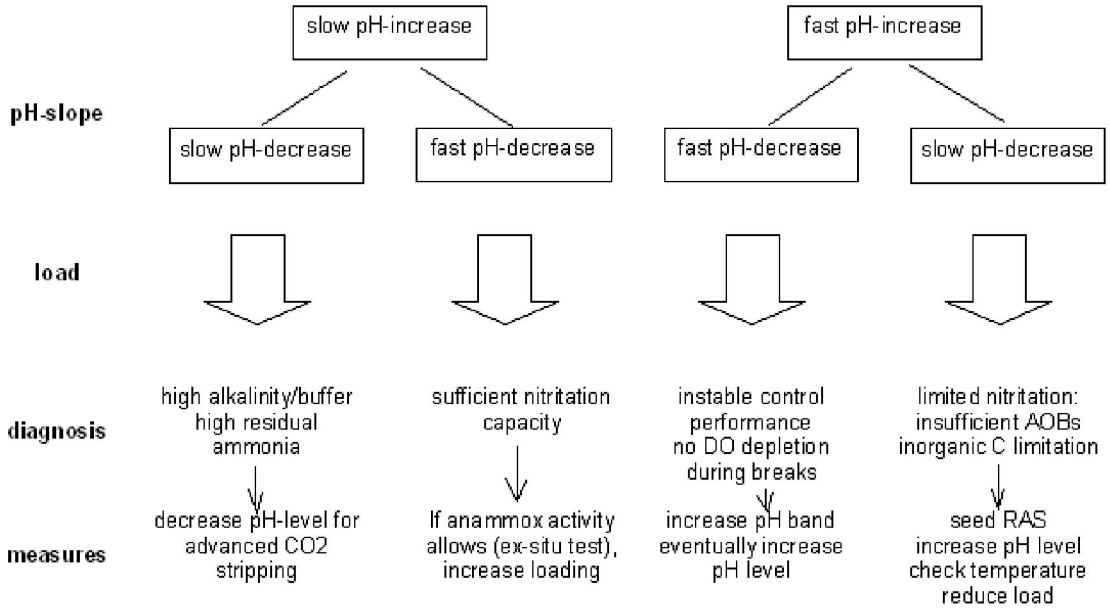

Figure 3.12 Pilot control strategy in the case of process upset. The control scheme was used during both phases of the study.

From an operational point of view, the DEMON process could be improved by providing more flexibility in its control strategy. Although the pH-centered control appears the best strategy for the process, there should be a provision to allow the operator to choose aeration and DO-centered control over the pH-centered control in some circumstances. This could help significantly when there is a significant imbalance between the aerobic period and anoxic periods caused by a decrease or increase in buffering capacity, for example. For this demonstration, periods of longer

aeration and shorter anoxic phase were frequent and favored an increase in nitrate. The control should also allow the feed to be stopped without shutting down the whole process in case the aeration period becomes too long. The current control strategy, in order to avoid the risk on nitrite accumulation, limited the aeration time above which all the inputs and outputs became inoperable, and the reactor was taken out of service frequently. This resulted in serious difficulties in regaining control. Stopping the feed while the reactor is in service would force the pH to drop and start the anoxic period.

3.4. Conclusion

The ASA DEMON Process Pilot Study revealed that centrate can be treated in a sidestream successfully without any chemical requirement. The process was resilient to loading up to 170% of the pilot design loading of 0.5 kgN/m^3/d without any sign of shock or reduced performance due to loading. However, frequent performance instabilities did not allow us to demonstrate that this loading could be stably sustained. The process mass loading seems to be a more critical design parameter than volumetric loading. Nitration control with oxygen alone was not sufficient to completely inhibit NOBs in this system, and dual inhibition methods are proposed. Because higher nitrate was produced than expected, process efficiency was lower than was theoretically possible based on DEMON stoichiometry. Because of some process upsets following purported inhibition of AerAOBs, more understanding about AerAOB kinetics under DEMON process conditions could improve the overall design and operation of the DEMON system.

3.5. Acknowledgment

We acknowledge DC WASA and ASA for funding this project. We are also grateful to ASA staff for their commitment to the day-to-day tasks to manage the pilot including pilot maintenance, collection and analysis of samples. We extend our special thanks to Maureen O'Shaughnessy, Jim Sizemore, Bernhard Wett, Imre Takacs, Dwight Houweling, Paula Senjines, and Karen Pallansch for their contribution to this work.

3.6. References

Abeling U and Seyfried C.F.,(1992), Anaerobic-Aerobic Treatment of High Strength ammonium wastewater-nitrogen Removal via Nitrite, *Water Science and Technology,* 26(5-6) 1007-1015

Akerman A., (2005); Feasibility of nitrate-shunt (nitritation) on landfill leachate, Master's Thesis, Department of Water and Environmental Engineering, Lunds Universitet

Anthonisen A C., Loher R C., Prakasam T.B., Srinath E G., (1976);, Inhibition of nitrification by ammonia and Nitrous acid, *Journal of Water Pollution Control Federation,* 1976 48(5) 835-851

Dapena-Mora A., Fernandez I., Campos J.L., Mosquera Corral A., Mendez R ans Jetten M.S.M., 2007; evaluation of activity and inhibition effects on anammox process by batch tests based on nitrogen gas production, *Enzyme and Microbial Technology*, 40(5):859-865

Fux C., Velten S., Carozzi V., Solley D., Keller J. (2006), Efficient and stable nitritation and denitrification of ammoniu-rich sludge dewatering liquor using an SBR with continuous loading, *Water Research,* 40 (18):2765-2775

Jenicek P., Svehla P., Zabranska J. and Dohanyos M.(2004), Factors affecting nitrogen removal by nitritation/denitritation, *Water Science and Technology Vol* 49 No 5–6 pp 73–79

Jensen M.M., Thamdrup B., Dalsgaard T. (2007), Effects of Specific Inhibitors on Anammox and Denitrification in Marine Sediment, *App. Environ. Microbiol.,* 73(10):3151-3158

Jianlong W. and Ning Y., (2004); Partial nitrification under limited dissolved oxygen conditions, *Process Biochemistry*, 39: 1223-1229

Kempen R., Mulder J., Uijterlinde C., Loosdrecht M., (2001); Overview: full-scale experience of the SHARON® process for treatment of rejection water of digested sludge dewatering, *Water Science and Technology* 2001, Vol 44 No 1 pp 145–152

Kim D., Ahn D., Lee D., (2005); Effects of free ammonia and dissolved oxygen on nitrification and nitrite accumulation in a biofilm airlift reactor, *Korean Journal of Chemical Engineering*, 22(1), 85-90

Lai E, Senkpiel S., Solley D., Keller J., (2004); Nitrogen removal of high strength wastewater via nitritation/denitrification using a sequencing batch reactor, *Water Science and technology*, 50(10): 27-33

Mulder J.W, van Loodsdrecht M.C.M., Hellinga C., van Kempen R., (2001); Full-scale application of rejection water of digested sludge dewatering, *Water Science and Technology*, 43(11):127-134

Musabyimana, M., Love, N. G., Bott, C. B., and Murthy, S. (in preparation) Evaluating and modeling the nitrite effect on anaerobic ammonia oxidation using DEMON biomass. *Water Research*.

Musabyimana, M., Love, N. G., Bott, C. B. and Murthy, S. (in preparation) Ammonia oxidation inhibition in the DEMON process. *Water Environment Research*.

Nielsen M., Bollman A., Sliekers O., Jetten M., Schmid M., Strous M., Schmidt I., Larsen H., Nielsen P.L., Revsbech N.P., (2005); Kinetics, Diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor, *FEMS Mibrobiology Ecology*, 51:247-256

Pambrun V, Sperandio M, Paul E. (2004);Treatment of Nitrogen and Phosphorous in Highly Concentrated Effluent in SBR and SBBR Processes.. *Wastewater Treatment for Nutrient Removal and Reuse*. Bangkok, Thailand

Peng Y. and Zhu G., (2006); Biological nitrogen removal with nitrification and denitrification via nitrite pathway, *App Microbiol Biotechnol*, 73:15-26

Third K. A., Paxman J., Schmid M., Strous M., Jetten M.S.M., Cord-Ruwisch R.,(2005) Treatment of nitrogen-rich wastewater using partial nitrification and anammox in the CANON process, *Water Science and Technology* 52(4):47-54

Third K. A., Paxman J., Schmid M., Strous M., Jetten M.S.M., Cord-Ruwisch R.,(2005) Enrichment of anammox from activated sludge and its application in the CANON process, *Microbial Ecology*, 49(2):236-244

Strous M., Kuenen, J.G., Jetten, M.S.M. (1999) Key physiology of anaerobic ammonia oxidation, *Applied Environmental Microbiology*, 65, 3243-3245

Peng Y. and Zhu G., (2006); Biological nitrogen removal with nitrification and denitrification via nitrite pathway, *App Microbiol Biotechnol*, 73:15-26

Sliekers O.A., Derwort N., Campos J.L., Strous M., Kuenen J., Jetten M., (2002), Completely autotrophic nitrogen removal over nitrite in one single reactor, *Water Research* 36:2475-2482

van der Star, W. R. L., W. R. Abma, D. Blommers, J.-W. Mulder, T. Tokutomi, M. Strous, C. Picioreanu, M. C. M. van Loosdrecht. (2007) Startup of reactors for anoxic ammonia oxidation: experiences from the first full-scale anammox reactor in Rotterdam. *Water Research*, 41:4149-4163.

Wett B. (2006); Solved up-scaling problems for implementing deammonification of rejection water, *Water Sci. Technol* 53 (2006), pp 121-128

Wett B., Murthy S., Takacs I., Hell M., Bowden G., Deur A., O'Shaughnessy M., (2006); Key parameters for control of Deammonification Process, WEFTEC, Nitrogen Removal, Baltimore

CHAPTER 4:
Estimating the maximum specific growth rate of anaerobic ammonia oxidizing bacteria from a single sludge N removal system

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For submission to *Water Environment Research*

4.1. Abstract

The high F:M protocol was used to estimate the maximum specific growth rate of anaerobic ammonia oxidizing (anammox) bacteria. This is the first study, to our knowledge, that used the high F:M protocol to estimate anammox bacteria growth rates. The inoculum was collected from a deammonification (DEMON) pilot reactor combining both nitritation and anaerobic ammonia oxidation metabilisms. The maximum specific growth rate was estimated to be 0.0167 hr^{-1}. The values estimated by the WERF (2003) model are close to those estimated by the Baranyi, McKellar (1997) and Hills and Wright (1994) models. Although this value might seem high compared to most reported growth rates for anammox bacteria (doubling time of 11 days), there are both lab-and-full-scale studies that have reported values consistent with this estimate. We suggest that difficulties encountered while maintaining anaerobic ammonia oxidizing processes are more likely dependent on our limited understanding of inhibition and toxicity issues in engineered environments rather than exceedingly low maximum specific growth rates.

4.2. Introduction

The anaerobic ammonia oxidation process is an emerging cost effective alternative for converting reactive nitrogen to denitrogen gas by anaerobically oxidizing ammonia with nitrite as final electron acceptor. The microorganisms responsible for this process (AnAOBs) were identified as a deep branching Planctomycetes (Schimidt et al., 2003). These organisms have been detected in many ecosystems ranging from wastewater treatment plants to arctic ice (Schmidt al, 2005) and could be responsible for up to 50% of the loss of inorganic nitrogen in the oceans (Kartal et al., 2007). AnAOBs are completely chemolithoautotrophic (van de Graaf et al, 1995). This autotrophic nature makes the anammox process very attractive as a component of biological nitrogen removal technologies. Unlike the conventional nitrification-denitrification process, the anammox process does not require any organic carbon source. Also the energy requirement is reduced by more than 50% when anammox process is used in lieu of conventional nitrification-denitrification (Wett, 2006).

A number of studies have focused on this process over the last decade to understand not only the microbiology and biochemistry of the anammox process, but also how best to engineer this process for nitrogen removal. As a result of these studies, a limited number of full-scale treatment plants are fully operational. The wastewater treatment process in Strass, Austria (Wett, 2006), combines the anammox process with nitritation in a single reactor, using a process known as DEaMmmONification (DEMON) at the treatment plant in Rotterdam, The Netherlands (van der Star, 2007), the ammonia oxidation process to nitrite (SHARON process) is separated from the anammox process. Other known treatment plants using anammox in the nitrogen removal process include the Hattingen wastewater treatment plant in Germany (Rosenwinkel and Cornelius, 2005), the Waterstromen Steenderen treatment plant in The Netherlands and the tannery wastewater plant of Hulshof in The Netherlands (Loosdrecht, 2007).

Despite achieving some breakthroughs in understanding the process, there are key physiological parameters like the maximum specific growth rate (μ_{max}) that have not been fully characterized for AnAOB. There is no doubt that the μ_{max} is the most critical parameter in the modeling and design of nitrogen removal systems, as it plays a dominant role in determining the minimum sludge age ($\theta_{c,min}$)(Sozen et al., 1996). For the specific case of AnAOBs, this

parameter becomes particularly important given a significant number of reported difficulties in starting the anammox-based systems both at the laboratory and full-scale. The start up time for laboratory-scale systems have been reported to range from 14 to 350 days (van Hulles, 2005), while the full-scale system took 3 to 4 years (Wett, 2006; van der Star, 2007). It is therefore clear that estimating μ_{max} for AnAOBs ($\mu_{max,An}$) could help in determining whether the longer start up times are related to low μ_{max} or simply to sub-optimal environmental conditions.

Despite the importance of the maximum specific growth rate in process design, the measurement of this parameter is not straightforward. Microbiologists have routinely used turbidity measurements, viable cell counts or dry weight and protein to estimate bacterial growth rates (White, 2000). These parameters are however of limited use in wastewater treatment processes where one encounters a complex media with a diverse consortia of microorganisms. Consequently, different approaches have been suggested to estimate the value of μ_{max} in activated sludge; in general, they involve using activity measurements such substrate consumption or product formation rates (Sozen, 1996; Hunnik et al., 1990).

There have been some attempts to estimate $\mu_{max,An}$ using data from either laboratory-scale systems or full-scale systems. Van de Graaf et al. (1996) performed a mass balance during the period of highest substrate uptake rate on his laboratory-scale system and reported a $\mu_{max, An}$ of 0.001 h^{-1}. Strous et al. (1998) used the same mass balance approach with an enriched lab-scale system and estimated $\mu_{max, An}$ to be 0.0027 h^{-1}. The difference in estimates may be due to a higher degree of enrichment in the second study than in the first, or the inactivation of part of the population due to limited mass transfer in biomass granules (Strous et al., 1998). Isaka et al. (2005), however, suggested that previous estimations have in fact significantly underestimated the anammox growth rate. In their study, cell concentration was determined using fluorescent in situ hybridization (FISH). By coupling this with measured substrate uptake rate, $\mu_{max, An}$ was estimated to be 0.016 h^{-1}.

In the present study, we estimated the $\mu_{max, An}$ using a protocol known as the high F:M method, which was developed for aerobic nitrifying bacteria as reported in detail by Melcer et al. (2003) and is referred to herein as the WERF model. In the high F:M method, a relatively small

mass of active biomass solids are exposed to optimal growth conditions and substrate utilization is monitored over time. The model assumes that the decay rate and yield coefficients are constant. The procedure was modified from the situation it was originally developed for (aerobic nitrification) by considering nitrite consumption rate in the model, and $\mu_{max,\ An}$ was estimated. The estimate is compared to that obtained by other models.

4.3. Material and Methods

Batch experiments set up

Medium

Centrate from the Alexandria Sanitation Authority Advanced Wastewater Treatment Facility was filtered through 0.45 μm pore size membrane GN-6 matricel membrane (Ann Arbor, MI). Filtrate (0.5 L) was added to 3.5 L of de-ionized water in a 6 L bioreactor (Bellco Biotechnology, Vineyard, NJ) equipped with a magnetic stirring mechanism. The temperature was adjusted and controlled to 35^{o}C by means of a water bath. Ammonia concentration was checked and adjusted to above 200 mg-N/L to avoid substrate limitations. Nitrite was added in the form of sodium nitrite (Fisher, Fair Lawn, NJ) to reach an initial concentration of 100-120 mg N/L except in one experiment where this concentration was increased to 180 mg N/L. The pH was adjusted and controlled to 7.30-7.40 by means of an external pH control loop using 2N HCl solution. This loop consisted of an in-line pH probe through which the reactor content was pumped and re-circulated. A pH controller (Cole-palmer, IL) automatically adjusted the pH once the measurement indicated that the pH was above the range by activating a small peristaltic acid pump (Cole-palmer, IL).

Inoculum

A fresh biomass sample was collected from the ASA DEMON pilot reactor (Chapter 3). Samples were analyzed for TSS and the volume containing 120 mg of solids was added to the batch reactor to achieve an initial biomass concentration of about 30 mg TSS/L. The solids in this calculated volume were allowed to settle, the supernatant was discarded and solids were inoculated into the reactor. The system was then purged with nitrogen gas for 5 minutes to remove dissolved oxygen, the system was closed (airtight), and gentle mixing was applied The

reactor was allowed to run for at least one hour before sampling to allow residual oxygen tobe scavenged.

Sampling and Analysis

Samples were collected every 10 to 14 hours from the beginning of the experiment until nitrite was depleted. Samples were collected from the sampling port located in the external pH loop, filtered through a 0.45 µm GN-6 matricel membrane (Ann Arbor, MI), and kept at 4^{o}C. They were then analyzed for ammonia (method EPA 350.1), nitrate + nitrite (method EPA 353.2) and nitrite (EPA 353.2) by using semi-automated colorimetry (Astoria Analyzer, Clackamas, OR).

Estimating growth rate

In this study, we adopted the model suggested by Sozen et al. (1996) and recommended by WERF (Melcer et al., 2003). In order to calculate the maximum growth rate, we assumed that the decrease in nitrite concentration is the best indicator of AnAOB growth. A mathematical expression was used to correlate nitrite consumption to biomass production (Metcalf and Eddy, 2003).

$$r_{su} = -\frac{\mu_{\max,\,An} X_{An} S}{Y_{An}(K_s + S)} \qquad (4.1)$$

r_{su} = the rate of substrate utilization (mg/L/hr)

Y_{An} = the yield coefficient of anammox biomass (mg COD/mg N)

X_{An} = the biomass concentration of anammox biomass (mg/L)

$\mu_{max,An}$ = growth rate of anammox biomass (h^{-1})

S = substrate concentration at any time (in this case nitrite) (mg/L)

K_s = half-saturation coefficient (mg/L)

It is assumed that for a high F:M ratio, the half saturation coefficient Ks becomes irrelevant because $S >> K_s$ and $\mu_{max,\,An} \sim \mu_{An}$. Therefore, the expression can be simplified to

$$r_{su} = -\frac{\mu_{\max,\,An} X_{An}}{Y_{An}} \qquad (4.2)$$

The biomass mass balance equation can also be expressed as

$$\frac{dX_{An}}{dt} = (\mu_{An} - b_{An})X_{An} \quad (4.3a)$$

where b_{An} is the decay rate (hr)

X_{An} is the biomass concentration (mg/L)

Under optimal conditions, the growth rate μ_{An} becomes $\mu_{max,\ An}$ and Equation 4.3a becomes

$$\frac{dX_{An}}{dt} = (\mu_{max,\ An} - b_{An})X_{An} \qquad (4.3b)$$

Equations (4.2) and (4.3b) were use to derive the general expression given in Equation 4.4 (derivation is provided in Appendix C). AnAOB yield and decay rate must be assumed to perform the calculation for μ_{An}. The values used are 0.114 g COD/g N and 0.0008h^{-1} respectively, which are the default values specified in the simulation package BioWin (EnviroSim, Ltd), and confirmed by van Hulle (2004).

$$[NO_2^-]_t = [NO_2^-]_0 - \frac{\mu_{max,An} X_{An,o}}{Y_{An}(\mu_{max,An} - b_{An})}[e^{(\mu_{max,An} - b_{An})t} - 1] \qquad (4.4)$$

where

$[NO_2^-]_t$: Nitrite concentration at time t (mg/L)

$[NO_2^-]_0$: Initial nitrite concentration (mg/L)

t: time (hr)

$X_{An,o}$ is the AnAOB biomass concentrations at time 0 (mg/L)

Other terms are defined with equation 4.1

The estimation of the growth rate was done by solving a minimization problem with an objective function that was the residual sum of squares (RSS) of measured nitrite values (x_i) and calculated nitrite values (x) according to equation 4.4. The $\mu_{max,\ An}$ was changed to fit a dataset of nitrite versus time.

Validation of experimental trials and statistical analysis

In total, nine anammox growth experiments were conducted. Despite a careful control of the key parameters such as pH and temperature, three experiments completely failed to consume nitrite over a period of 5 days, probably as a result of inadequate mixing. Mixing was provided using a magnetic stirring mechanism which was susceptible to intermittent mixing. In two of the successful experiments, an insufficient number of data points were collected to consider the estimation significantly valid, although these experiments provided results very similar to the four valid measurements. The Chi-square goodness of fit was a statistic used to test association of data, such as measured and calculated data in our case.

4.4. Results and Discussion

Nitrite consumption and nitrate generation data

The nitrite consumption data reflect typical microbial growth kinetics in batch reactors, characterized by a lag phase of growth with no or very little nitrite consumption, followed by exponential growth indicated by rapid nitrate consumption and a declining phase of growth corresponding to nitrite exhaustion. All the three phases lasted between 4 and 6 days. Nitrite was consumed while nitrate was produced as predicted by the anammox process. Given that we had a closed system and the system pH tended to increase (acid was used to stabilize the pH), we believe that nitrate came solely from anammox metabolism. Both nitrite consumption and nitrate production are illustrated in Figure 4.1.

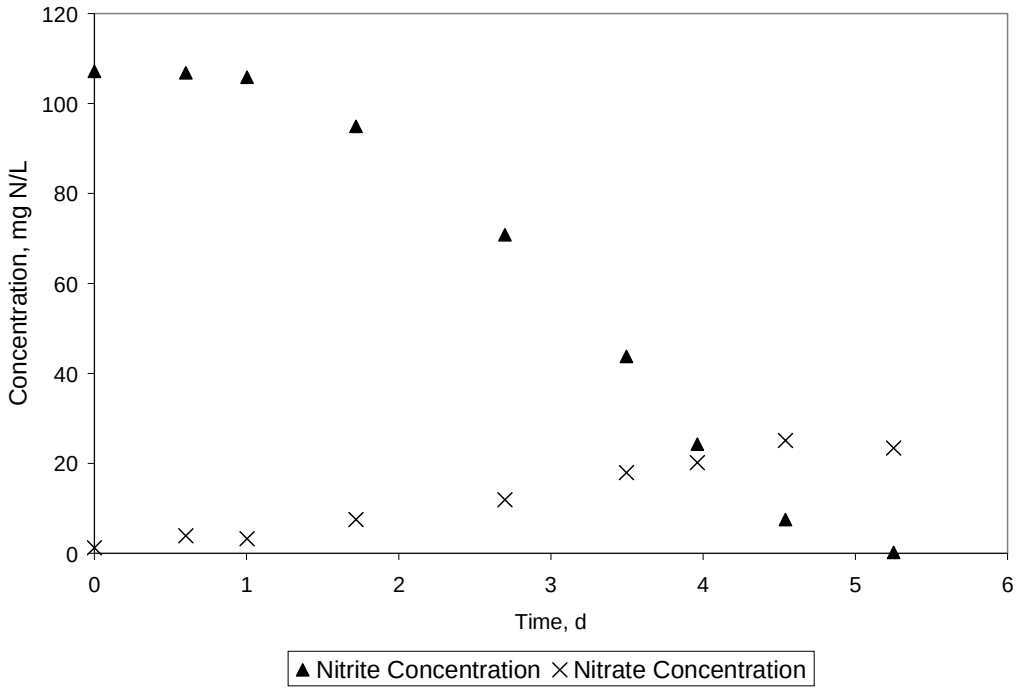

Figure 4.1: Example of nitrite uptake and nitrate production over time during a high F:M experiment to estimate anammox maximum specific growth rate.

Maximum specific growth rate estimation

Figure 4.2 provides a comparison between measured nitrite concentrations and those calculated using the growth model proposed by WERF (Melcer et al., 2002). The statistical analysis indicates that all data pass the Chi-square goodness of fit test with a level of significance of 0.10 (90% confidence interval). Indeed, the stoichiometric ratio of nitrate formed per unit nitrite consumed was 0.19 (Figure 4.3) close to the expected ratio of 0.20 from the anammox metabolism. Furthermore, the coe-xistance of nitrate generation and nitrite consumption is evidence that heterotrophic denitrification did not occur to a significant degree during the batch tests. The ratio of ammonia consumption to nitrite consumption was 1.65 (Figure 4.4). This ration is slightly higher than the hypothetical anammox stoichiometric ratio of 1.32 most likely because, unlike nitrite and nitrate, a portion of ammonia can be lost in the gas phase. Ammonia was not recommended for use in the growth rate calculation within the high F:M protocol eventually because of this difficulty.

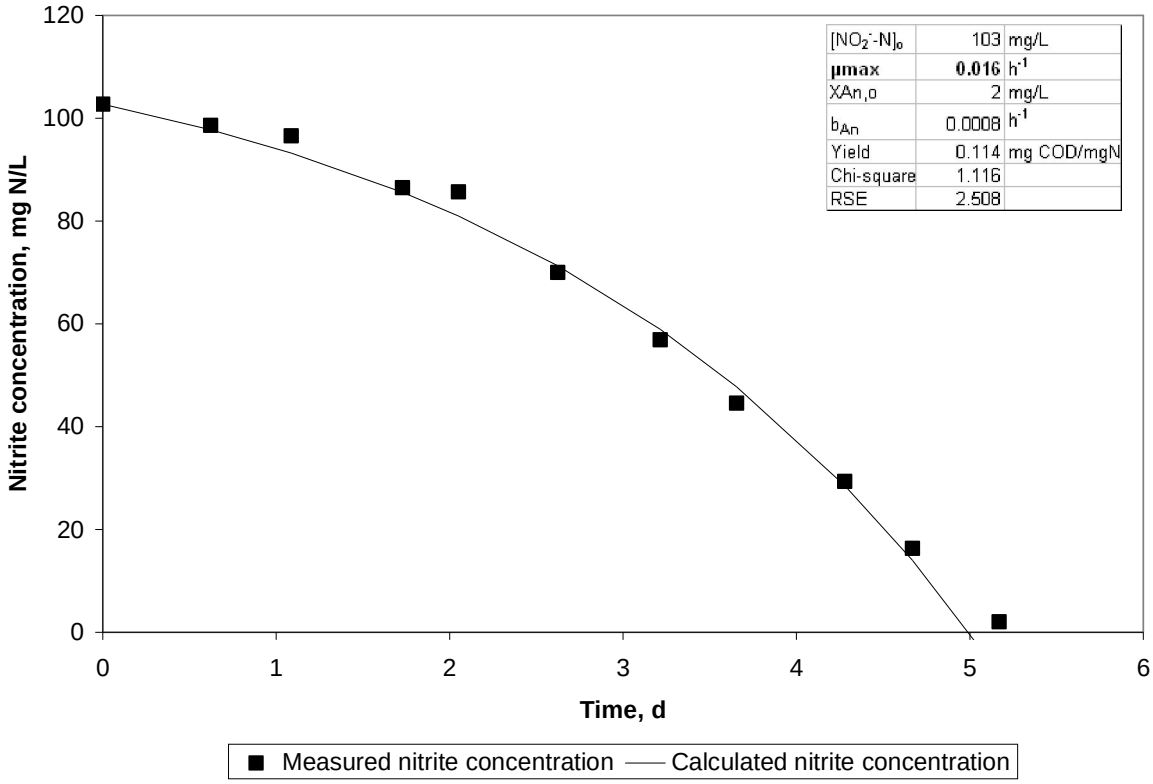

Figure 4.2: Measured and modeled nitrite concentration using WERF growth model. The growth rate, µmax, and initial anammox biomass $X_{An,o}$ concentration were estimated by the model.

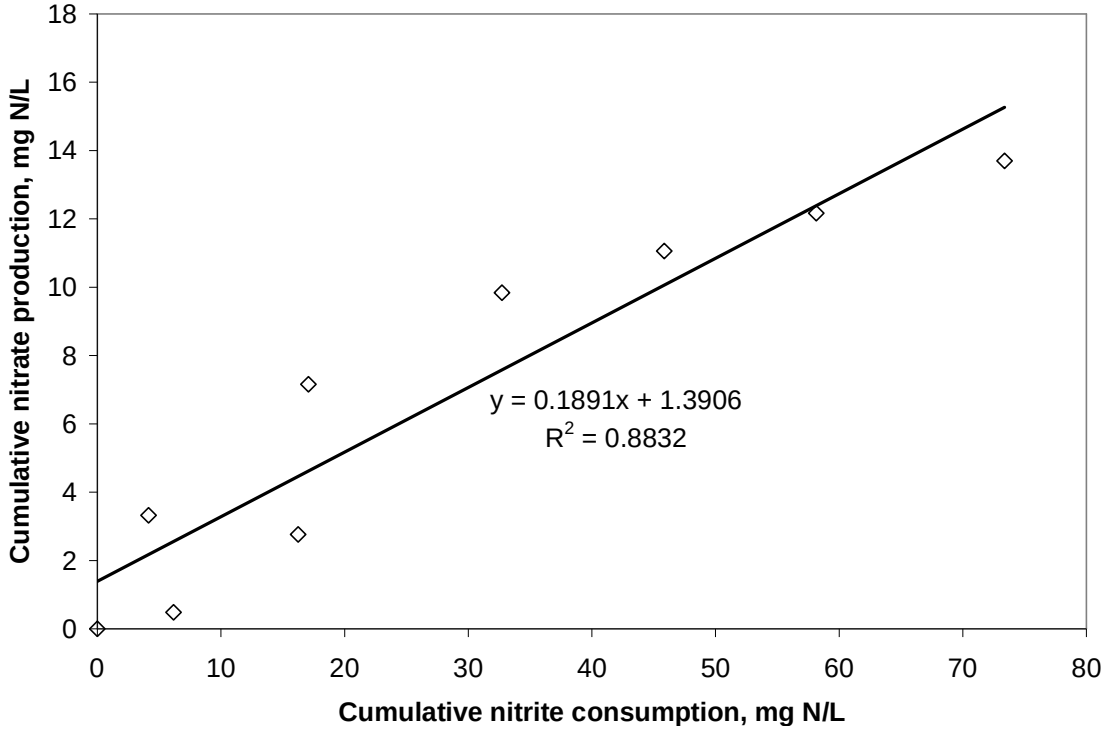

Figure 4.3: Example of nitrite consumption versus nitrate production. The anammox stoichiometry predicts the ratio nitrite consumed:nitrate produced to be 1:0.20.

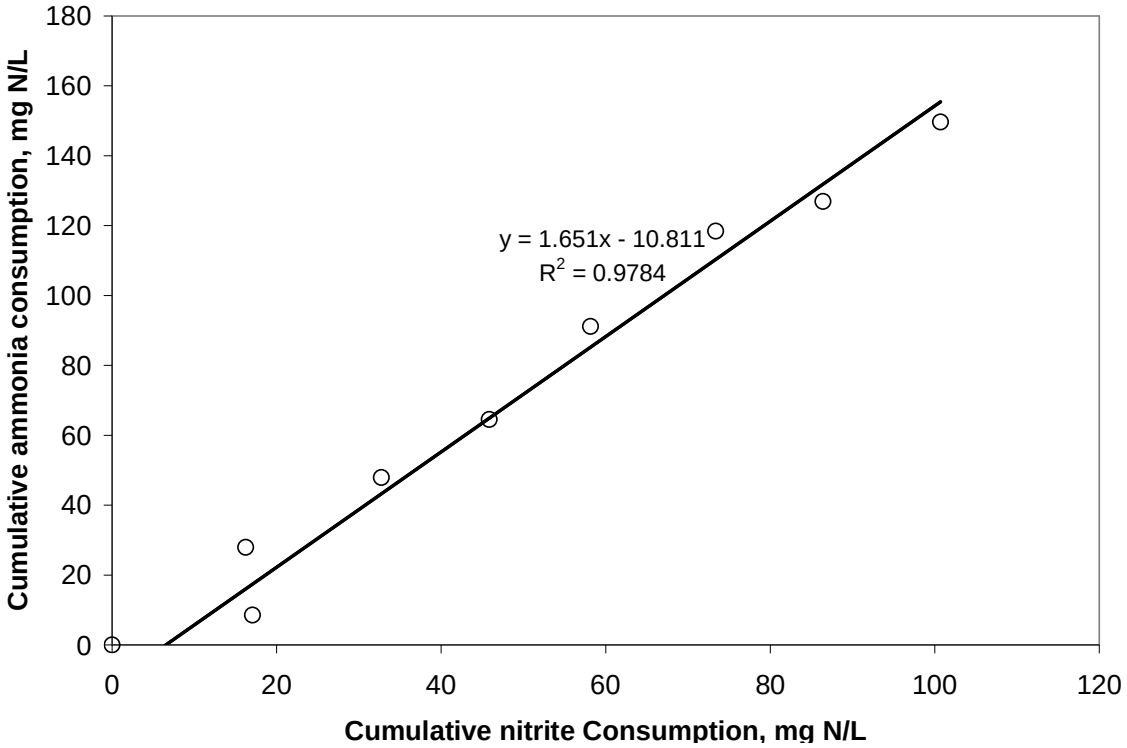

Figure 4.4: Example of ammonia consumption versus nitrite consumption. The anammox stoichiometry predicts that ammonia and nitrite are consumed in the ratio of 1:132 respectively.

The maximum specific growth rate values estimated using the WERF model are summarized in Table 4.1 showing the estimated maximum growth rate and initial anammox biomass. The analysis assumed a yield coefficient and decay rate of 0.114 mg COD/mg N and 0.0008h^{-1} , respectively and as described above.

Table 4.1: Estimated values of maximum specific growth rate ($\mu_{max,An}$) and initial biomass concentration

Experiment #	μ_{max} (h^{-1})	$X_{An,\,o}$ (mg/L)	$[NO_2^-\text{-}N]_o$, mg/L
1	0.016	2.0	103.
2	0.010	3.3	184.
3	0.018	2.0	128.
4	0.017	2.6	113

With the exception of experiment #2 where the initial nitrite concentration was significantly higher, this work suggested that the AnAOB growth rate is in the range of 0.016-0.018 h^{-1}. A nitrite inhibition study was completed (Chapter 5) and results suggest that nitrite concentrations as high as 100 mg/L can cause anammox activity to decrease by about 11% relative to

uninhibited activity at 50mg/L NO_2^-N, but decreases by as much as 43% when nitrite concentrations are 200 mg N/L. Therefore, the lower maximum growth rate obtained for the initial nitrite concentration in the F:M batch test at 184 mg N/L was due to nitrite inhibition in the first days of the experiment. Interestingly, this inhibition caused an overall 43% decrease in $\mu_{max, An}$ compared to estimates from the case when initial nitrite concentrations near 100 mg N/L were used. The F:M essay results paralleled findings in the inhibition study.

The average of the maximum specific growth rate values estimated from the three valid experiments is 0.017 h^{-1}. This value is much higher than the values suggested previously by van de Graaf et al. (1996) and Strous et al. (1998), but are in agreement with the estimated value of 0.016 h^{-1} reported by Isaka et al., 2005. None of these previous studies used the F:M approach used here. The study by Isaka et al (2005) was performed on a fixed bed lab reactor and the AnAOB concentration was measured directly using FISH.

In addition to these studies, AnAOB kinetics parameters were estimated using the full-scale anammox system in Rotterdam (van der Star et al., 2007).

Two procedures were used to estimate $\mu_{max, An.}$ With one approach, a mass balance was performed on the treatment system and yielded an estimate of 0.014h^{-1}, a value which is only 15% lower than the average found in the current study. The second estimation approach involved using quantitative PCR (qPCR) to estimate growth based on cell abundance as determined by the number of 16S rDNA copies associated with anammox bacteria. Using this method, $\mu_{max, An}$ was estimated to be 0.0025h^{-1}, a value close to that suggested by Strous et al. (1998). Although the authors of the Rotterdam study suggested that using qPCR was probably more accurate could than the mass balance-based kinetic estimate, one has to keep in mind that these two estimates were evaluated at different periods of time, and a comparison of the data from the two different protocols in this specific case would be hard. Moreover, previous protocols based on activity measurements are extensively used to estimate $\mu_{max, An}$ values used for design purposes and have not been proven inaccurate.

Effect of Initial AnAOB Biomass Concentration

The initial AnAOB concentration was estimated to be about 2 to 3 mg/L using the F:M test. Given that initial TSS concentration was around 30 mg/L, the anammox population in the DEMON pilot reactor was approximately 7% to 10 % of the total suspended solids in the reactor. This is similar to typical estimates for the approximate proportion of nitrifiers in a conventional nitrifying activated sludge reactor. Models performed on data from the DEMON pilot plant system at Alexandria, Virginia, estimated the AnAOB population to be 10-14% of the total population (Wett, 2006). This assumption assumes that no nitrite oxidizers were present, an assumption which might not hold given that the nitrate concentration observed in the reactor was substantially higher than that expected based on predictions from Stoichiometry.

Estimating the anammox bacteria maximum specific growth rate using common growth models used in microbiology

The growth rates estimates using common growth models used in microbiology compare very well with the WERF model. Comparing all models by looking at both the estimated maximum specific growth rate values and the residual squared error as a measure of model fitness to data shows that all models give similar predicted results, although the WERF model estimates values are slightly higher. These models are hardly distinguishable graphically. A comparison table is provided as Table 4.2.

Table 4.2: Comparison of the WERF model prediction with other growth model predictions.

	Model				
	Symbol	Baranyi	Hills and Wright	McKellar	WERF
Initial nitrite concentration (mg/L)	$[NO_2^-]_o$	103	103	103	103
Maximum specifix growth rate (h^{-1})	μ_{max}	0.009	0.010	0.010	0.016
Initial biomass concentration (mg/L)	$X_{An,o}$	4	4	3	2
Yield (mg COD/mg N)	Y_{An}	0.114	0.114	0.114	0.114
Decay (h^{-1})	b_{An}	0.0008	0.0008	0.0008	0.0008
Residual Standard Error	RSE	3.235	2.815	2.656	2.508

Inhibition versus toxicity

The basic assumption in high the F:M protocol for estimating μ_{max} is that the decay rate is constant, and that all organisms present grow at rates near the maximum during the test. This assumption fails to acknowledge the fact that toxic substances might instead accelerate the decay rate while the growth rate stays the same, rather than slowing down the growth keeping the decay rate the same. Given that the mechanism for nitrite inhibition on AnAOBs is still unknown (inhibitor or toxin?) we attempted to use the WERF growth model to assess the two possibilities:

1) For the case when the initial nitrite concentration started at 184 mg N/L, the decay rate stayed the same and the growth rate decreased (inhibition of growth). If the decay rate r is assumed to be 0.00075 h^{-1} as in the initial F:M estimates, then $\mu_{max,An}$ is estimated to be 0.01 h^{-1} (RSE=5.9).

2) For the case when initial nitrite concentration started at 184 mg/L, the decay rate increased and the growth rate stayed the same (toxicity that results in accelerated decay but growth is not affected) The maximum growth was assumed to be 0.017 h^{-1} and the decay rate was estimated to be 0.0075 h^{-1} (RSE= 5.9).

It turns out that both alternatives have the same residual error, so the fitting does not favor any option other the other. Notice that if the decay rate is variable, it would be a more sensitive indicator of the kinetic impact of the nitrite compared to the growth rate. In the case when nitrite increased from 100 mg/L to 184 mg/L, the growth rate would decrease by 43 % if the decay remained constant. If, on the other hand, the decay increased with an increase in nitrite concentration while the growth rate remained constant, the decay rate would increase by 90%.

4.5. Conclusion

The maximum specific growth rate of AnAOB is estimated to be 0.017 d^{-1}. Difficulties reported with operating the anammox processes are more likely to be related to our limited understanding of the inhibition and toxicity issues affecting AnAOB rather than the slow maximum specific growth rate. The high F:M protocol is an appropriate method to estimate the $\mu_{max, An}$ given the repeatability of the results. This relatively high growth rate values (compared to

0.0027 h^{-1} reported commonly) suggests that the focus of future studies of the anammox process should be directed to better understanding inhibition and toxicity issues rather than assuming that the process is limited by the anammox cell doubling time. This finding could improve the design of systems that combine nitritation and anammox metabolisms such as in CANON or DEMON.

4.6. Acknowledgement

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4.7. References

Gunthert F.W., Wastewater Research in Germany: New Technologies? University of Federal Armed Forces, *Institute of Hydroscience*, Munich, Germany

Gut L. (2006), Assessment of partial nitrification/anammox system for nitrogen removal, Licentiate Thesis, KTH Land and Water Resources Engineering, Sweden

Hills B.P., Wright K.M., (1994), A new model for bacterial growth in heterogeneous systems, J. *Theor. Biol.* 168:31-41

Hunnik J.H., Hamelers H.V.M. and Koster I.W (1990), Growth rate inhition of Acestoclastic Methanogens by ammonia and pH in Poultry Manure Digestion, *Biological Wastes* 32:285-297

Isaka K., Date Y., T.Sumino, Yoshie S., Tsuneda S. (2006); Growth Characteristics of anaerobic ammonia-oxidizing bacteria in an anaerobic biological filtrated reactor, *Appl Microbiol Biotechnol*, 70 : 46-62

Kartal B., Rattray J., van Niftrik L., van de Vossenberg J., Schmidt M., Webb R., Schouten S., Fuerst J., Sinninghe Damste J., Jetten M., Strous M., (2007) *Candidatus* "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria, *Systematic and applied microbiology*, 30(1):39-49

McKellar R., (1997), Modeling microbial responses in food, *CRC Series in Contemporary Food Science*, CRS press, pp 29-30

Melcer H., Stensel D., Dold P., (2003); Methods of wastewater characterization in Activated Sludge Modeling, *WERF Report Series,* IWA publishing

Rosenwinkel K., Cornelius A., (2005); Deammonification in the moving-bed process for the treatment of wastewater with high ammonia content, *Chemical Engineering and Technology*, 28(1) 49-52

Schmidt M.C., Maas B., Dapena-Mora A., van de Passchoonen J., Kartal B., van de Vossenberg J., van Niftrik L., Schimidt I., Cirpus I., Kuenen J.G., Wagner M., Sinninghe Damste J.S., Kuypers M., Revbech N.P. Mendez R., Jetten M.S.M., Strous M., (2005); Biomarkers for in situ detection of anaerobic ammonium-oxidizing (anammox) bacteria, *Appl. Environ. Mibrobiol*. 71: 1677-1684.

Schmidt M., Walsh K., Webb R., Rijpstra R., van de Passchoonen K.T., Verbruggen M.J., Hill T., Moffett B., Fuerst J., Schouten S., Damste J.S., Harris J., Shaw P., Jetten M., Strous M., (2003), Candidatus "*Scalindua brodae* sp. nov., Candidatus "Scalindua wagneri, sp. , nov., two new species of anaerobic ammonium oxidizing bacteria. *Syst. Appl. Microbiol*. 26, 529-538.

Sozen S., Orphon D.and H.A. San (1996), A new approach for the evaluation of the maximum growth specific growth rate in nitrification, *Wat. Res.* Vol 30, No.7 pp 1661-1669.

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 the slowly growing anaerobic ammonia-oxidizing microorganisms, Appl. *Microbiol Biotechnol*, 50:589-596.

Strous M., Pelletier E., Mangenot S., Rattei T., Lehner A., Taylor M. W., Horn M., Daims H., Bartol-Mavel D., Wincker P., Barbe V., Fonknechten N., Vallenet D., Segurens B., Schenowitz-Truong C., Médigue C., Collingro A., Snel B., Dutilh B., Camp H., van der Drift C., Cirpus I., van de Pas-Schoonen K., Harhangi H., van Niftrik L., Schmid M., Keltjens J., van de Vossenberg J., Kartal B., Meier H., Frishman D., Huynen M. A., Hans-Werner Mewes J. Weissenbach, Jetten M., Wagner M. and Le Paslier D., *(2006*); Deciphering the evolution and metabolism of an anammox bacterium from a community genome, *Nature*, , 440(7085): 790-794.

Wett B., Murthy S., Takas I, Hell M., Bowden G., Deur A., O'Shoughnessy M. (2006), Key parameters for control of Deammonification process, Biological Nitrogen Removal, State the Art, Baltimore, MD

Wett B. (2006); Solved Scaling problems for implementing Deamonification of Reject Water, *Sci. Technol*., 53 (12):121-128.

White D., The physiology and biochemistry of Procaryotes (1996), 2nd ed., Oxford University Press, New York.

Van de Graaf A.A., de Bruijin P., Robertson L.A., Jetten M.C.M., Kuenen J.G. (1996); Autotrophic ammonia-oxidizing microorganisms in a fluidized bed reactor, *Microbiology* 142:2187-2196.

Van de Graaf A., Mulder A., BruijnP., Jetten M., Roberton L., Kuenen G., (1995); Anaerobic Oxidation of Ammonium is a Biologically Mediated process, *Appl. Environ*. Microbiol. 61(4):1246-1251.

Van Hulle S., Modelling, simulation and optimization of autotrophic nitrogen removal processes, Doctoral Thesis, Gent University, Academic Year 2004-2005.

Van der Star W., Abma W., Blommers D., Mulder J,W., Tokutomi T., Strous M., Picioreanu C., van Loosdrecht M., (2007); Startup of reactors for anoxic ammonium oxidation: Experiences from first full-scale anammox reactor in Rotterdam, *Water Research* 41: 4149-4169.

Van Hulle S.W.H., Maertens J., De Pauw D.J.W., Vanrolleghem P.A., (2004); Using parameter sensitivity analysis of the canon biofilm process: what to measure, where to measure, and under which conditions?, *Young Researchers*, 59-66.

Wilson P., Wilson D., Brocklehust T., Coleman H., Mitchell G., Waspe C., Jukes S. and Robins M., Batch growth of Salmonella typhimurium LT2: Stoichiometry and factors leading to cessation of growth, *International Journal of Food Microbiology*, 89(2-3): 195-203.

Yu C., Davidson V., Yang S., (2006); A neural network approach to predict survival/ death and growth/no-growth interfaces for *Escherichia coli* O157:H7, *Food Microbiology*, 23(6); 552-560.

CHAPTER 5:
Evaluating the impact of nitrite concentration on anaerobic ammonia oxidation in a DEMON process

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5.1. Abstract

The effect of nitrite concentration on anaerobic ammonia oxidizing bacteria (AnAOB) contained within biomass from a deammonification (DEMON) reactor was evaluated using short-term batch (nitrite concentration decreasing with time) and long-term fed-batch (nitrite concentration sustained over time) experiments. The results show that short-term batch tests underestimated the impact of nitrite when present at concentrations that were inhibitory because the nitrite concentration decreased during the test and the inhibition was reversible. During fed-batch experiments, inhibition was more pronounced over extended time periods for nitrite concentrations higher than 50 mg/L; the biomass did not reflect inhibition at 50 mg/L nitrite-N or below. These results show that AnAOB contained within a DEMON biomass may be more tolerant to nitrite than previously found using enriched AnAOB cultures.

5.2. Introduction

Biological **an**aerobic **amm**onia **ox**idation (also referred to as anammox) is a microbiologically-mediated metabolism that has been identified in nature only recently and is believed to be a significant metabolic contributor to the global nitrogen cycle (Pilcher, 2005). It is now generally accepted that up to 50% of nitrogen in the aquatic environment is cycled through this pathway (Hamersley et al., 2007, Kuypers et al., 2005). The mediating anaerobic

ammonia oxidizing bacteria (AnAOB) are chemolithoautotrophs (Jetten et al., 1999); therefore, nitrogen can be anaerobically converted to gaseous products without use of an external organic energy source. Deployment of this metabolism through wastewater treatment technologies that are focused on nitrogen removal allows utilities to save up to 60% in oxygen requirement and 100% in external organic energy source addition, and can reduce operational costs significantly relative to conventional nitrogen treatment processes (Jetten et al., 2001).

Ever since anammox was identified to be a significant progress in global nitrogen cycling, a limited number of full-scale treatment systems that incorporate the anammox metabolism have been successfully deployed in Europe (Wett et al., 2006; Gunthert, 2006; Gut, 2006); however, factors associated with the processes that impact performance are still being determined. One key variable that has been debated in the literature is the degree to which nitrite inhibits AnAOB in these treatment processes, and how to quantitatively predict that inhibition.

Anammox stoichiometry predicts that 1.32 moles of nitrite is reduced for every one mole of ammonia oxidized (van de Graaf, 1996). Therefore, nitrite needs to either be present in the feed stream or generated by oxidizing part of the ammonia while retaining an appropriate fraction of the ammonia in the waste stream in order to achieve the desired stoichiometry. To do this, partial oxidation of ammonia to nitrite (nitritation) via aerobic ammonia oxidizing bacteria (AerAOB) is often employed in the anammox-based treatment process. Nitritation can be performed by AerAOB either in a separate reactor (e.g., Hellinga et al., 1998; Mulder et al., 2001) or in a single sludge configuration where AerAOB and AnAOB coexist, such as in the CANON (Nielsen et al., 2005; Third et al., 2005) or DEaMMONification (DEMON) (Wett, 2006) processes. Although nitrite is a substrate for AnAOB, high nitrite concentrations are believed to have a negative effect on anammox metabolism, complicating the balance one needs to achieve in anammox-based process technologies. This effect may be due to inhibition (reducing the rate of catabolism), toxicity (damaging the cell and possibly causing cell death), or both. However, the nitrite concentration reported to slow anammox performance has varied widely in the literature. Jetten et al. (1999) reported that the anammox process was inhibited by 280 mg nitrite-N/L. Other studies suggested that concentrations as low as 5 mg N/L are detrimental to the process (Wett et al., 2007).

The nitrite inhibition mechanism is not well understood. One of the assumptions is that acidification of nitrite generates reactive nitrogen species (RNS) that have cytotoxic properties according to the reactions (Nakaki et al, 1990):

$$NO_2^- + H^+ \leftrightarrow HNO_2 \quad (5.1)$$

$$2HNO_2 \leftrightarrow H_2O + N_2O_3 \quad (5.2)$$

$$N_2O_3 \leftrightarrow NO^{\cdot} + NO_2^{\cdot} \quad (5.3)$$

The final radicals formed by this series of reactions inhibit respiratory chain enzymes through inactivation of iron-sulphur complexes, and disrupt DNA replication by inhibiting ribonucleotide reductase. Another assumption is that the toxicity is accomplished via the formation of peroxynitrite ($ONOO^-$) in the presence of superoxide (O_2^-) (Dykhuizen, 1998) or the formation of metal-nitrosyl complexes when these RNS species interact with bacterial enzymes. For example, it was proven that nitrite causes loss of activity to ammonia monooxygenase, AMO (Stein et al, 1998), probably because of similar complexation with RNS.

These assumptions are still hypothetical, but they suggest that nitrite destroys the cells and hence the effect should be expressed in the decay term rather than inhibition term. Other studies alluded to substrate inhibition to characterize the effect of nitrite on anammox bacteria (Strous, 1999).

The objective of this study was to investigate the effect of nitrite concentration on AnAOB performance using biomass from a DEMON pilot plant-scale reactor. Both short term and long term exposures were assessed. The results of this study can assist operators and designers in formulating strategies that help to avoid failures in full-scale systems that rely upon the anammox metabolism.

5.3. Materials and Methods

Cultures

All experiments were performed using biomass collected from the DEMON pilot plant-scale reactor maintained at the Alexandria Sanitation Authority (ASA) Wastewater Treatment Facility. Information about design, operation and performance of the pilot plant can be found in Chapter 3.

Short-term exposure to nitrite: batch experiments

A fresh DEMON biomass sample was collected and transferred into four 4 L containers, except for one experiment where the sludge from the Strass full-scale DEMON plant was blended with the ASA pilot DEMON sludge in the ratio of 1:3 (volume:volume). The biomass was allowed to settle for at least 30 minutes, the supernatant was discarded, and the biomass was decanted from each container and introduced into a 6 L covered cylindrical bioreactor (Bellco Glass, Vineland, NJ). Each reactor was filled to 4 L with 8x diluted (with tap water)) centrate from the ASA biosolids management system to provide essential nutrients to the bacteria. The temperature was maintained at 35^{o}C using a water bath. Oxygen was removed from the biomass by bubbling nitrogen gas for 5 minutes prior to sealing the vessel with a rubber stopper; this setup provided sufficient headspace to accommodate gas generation and air intrusion was assumed to be minimal because the vessel was under positive gas pressure during most of the experiment. Gentle mixing was applied throughout the experiment. The pH was maintained between 7.30 and 7.40 with an external pH control system consisting of a recirculation pump (Masterflex® L/S® Easy-Load® II, Cole-Parmer, Barrington, IL), a mixing port for injecting 6 N HCl, an inline pH meter installed in the recirculation channel, a pH controller (pH 550 pH/ORP Monitor, Eutech Instruments, Veron Hills, IL) and an acidity pump connected to the pH controller. The bioreactors were spiked with target nitrite concentrations of 50, 100, 200 and 400 mg/L as nitrite-N (two experiments each) and 10 and 300 mg/L nitrite-N (one experiment each). Each nitrite spike was accompanied by an ammonia spike at a molar ratio of 1:1 to prevent ammonia limitation. Nitrite was supplied as sodium nitrite (Fisher Scientific, Fair Lawn, NJ) while ammonia was supplied as ammonium chloride (Fisher Scientific, Fair Lawn, NJ). Samples were collected every 20 to 30 minutes for the first 2 hours except for the 10 mg/L nitrite-N experiment where the rate was determined by collecting 7 samples over 30 minutes after which nitrite was completely consumed.

Samples were filtered through 0.45 µm membrane filters (Fisher Scientific, Fair Lawn, NJ), acidified to pH<2 and kept at 4°C until analyzed (within two to four days). Each sample was analyzed for ammonia (EPA method 350.1 or HACH salycilate method 10031), nitrite (EPA method 353.2 or HACH diazotization method 10019), $NO_2^-+NO_3^-$ (designated NO_X, using EPA method 353.3) or nitrate (HACH cadmium reduction method 8039).

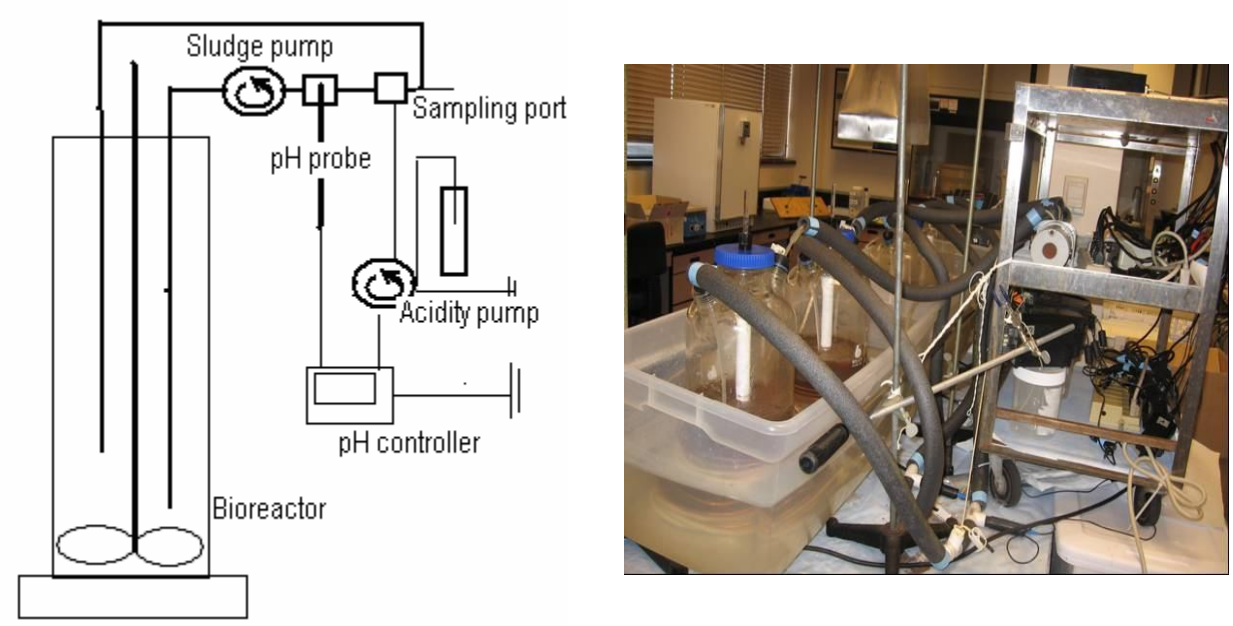

Figure 5.1. Setup for short term batch experiments. Left: a schematic of the reactor setup. Right: a photograph of four batch reactors during operation.

Activity was calculated for the short-term batch experiments as

$$\text{Activity}_{\text{BATCH}} = \frac{(C_t - C_i)}{t} \qquad (5.4)$$

t = the time period over which the activity was being measured

C_t = the concentration of nitrite in the reactor at time t

C_i = the concentration of nitrite in the reactor at the time associated with the initiation of the activity measurement period

Activity recovery experiment

To investigate if short-term nitrite exposure resulted in a long-term effect on AnAOBs, DEMON biomass was subjected to an initial nitrite-N concentration of 400 mg/L and evaluated

according to the description for short-term batch experiments above. Once nitrite was depleted from that experiment, the biomass was allowed to settle, supernatant was decanted, and biomass was resuspended in 8x diluted ASA centrate spiked with 50 mg/L nitrite-N. Samples were collected to measure the activity. This experiment was used to determine the time period over which biomass recovered from a nitrite shock of 400 mg/L. This experiment was conducted once.

Long-term exposure to nitrite: fed-batch experiments

In order to understand the effect of nitrite exposure time on the activity of AnAOB, nitrite was continuously fed into reactors to sustain the nitrite level in the reactor for the duration of the experiment (ranged from 8 to 27 hours) while impacting reactor volume minimally (fed-batch experiments). In this study, long-term experiments refer to those experiments where samples were collected for a period of 8 hours or more. Sustained nitrite concentration implies that a small volume of nitrite was continuously added to the reactor to maintain the nitrite concentration to a given concentration range. The experimental set up was the same as for the short-term experiment. A fresh DEMON biomass sample was collected and transferred into four 4 L containers. The biomass was allowed to settle for at least 30 minutes, the supernatant was discarded, and the biomass was decanted from each container and introduced into a 6 L covered cylindrical bioreactor (Bellco Glass, Vineland, NJ). Each reactor was filled to 4 L with 8x diluted centrate from the ASA biosolids management system to provide essential nutrients to the bacteria. The temperature was maintained at 35^{o}C using a water bath. Oxygen was removed from the biomass by bubbling nitrogen gas for 5 minutes prior to sealing the vessel with a rubber stopper. Gentle mixing was applied throughout the experiment. The pH was maintained between 7.30 and 7.40 with an external pH control. Once the temperature was stabilized and oxygen was purged from the biomass, each reactor was spiked with nitrite and ammonia in an approximate ratio of 1:1 to reach a target starting concentration. In order to maintain the target nitrite concentration throughout the experiment, a concentrated nitrite (28 M) and ammonia (28 M) solution (ratio 1:1) was continuously fed into the reactor. Samples were collected hourly and either stored for nitrite, nitrate and ammonia analysis to be performed later, or processed immediately for nitrite using the HACH diazotization method. The flow rate needed to achieve the target nitrite concentration was calculated and adjusted regularly based on the rapid, hourly nitrite measurements. Stored samples were analyzed by either the HACH or EPA methods

described in the short-term batch experiment section. In total, four experiments were conducted with an average nitrite concentration ranging from 2.6 mg/L to 200 mg N/L.

Anammox activity was calculated based on a mass balance by using equation 5.5:

$$\text{Activity}_{\text{FED-BATCH}} = \frac{\sum_{t=0}^{t}\left(\frac{C_F \times V_F}{V}\right) - (C_t - C_i)}{t} \qquad (5.5)$$

where:

C_F = the nitrite concentration in the feed (mass/volume)

V_F = the volume of feed pumped into the reactor for the time period over which the activity was being calculated

V = the reactor volume

t = the time period over which the activity was being measured

C_t = the concentration of nitrite in the reactor at time t (mass/volume)

C_i = the concentration of nitrite in the reactor at the time associated with the initiation of the activity measurement period (mass/volume)

One can see that the first term in the numerator of the equation represents the mass of nitrite fed into the system over the activity measurement period per unit reactor volume. The second term in the numerator considers the degree to which the nitrite solution pump kept pace with activity. If this term is positive, then the nitrite feed pump was adding nitrite faster than the culture's nitrite consumption activity and it was necessary to subtract the amount of nitrite accumulated over the activity measurement period from the mass added over that same period. If this term is negative, then the nitrite feed pump was not keeping pace with the culture's nitrite consumption activity and it was necessary to add the amount of nitrite consumed (obtained from the nitrite reserve present at time zero) to the mass added over the same period. Activity was typically calculated every hour.

The effect of pH on nitrite inhibition

Batch reactors were prepared as described for the short-term batch experiments above. Each reactor was spiked with nitrite to reach an initial concentration of 50 mg N/L. The pH was controlled as shown in Table 5.1. Samples were collected every 20 minutes for two hours, filtered through a 0.45 μm glass fiber filter, preserved according to Standard Methods, and analyzed for nitrite, ammonia and nitrate using the protocols described previously. The average activity was calculated using the slope of the line fitting the concentration datapoints over the two hour experiment. The activity measured at the different pH ranges was used to assess whether pH has an effect on nitrite inhibition of AnAOB. Experiments for each pH condition were performed in duplicate.

Table 5.1. pH setpoints used in the experiment designed to evaluate the impact of pH on nitrite inhibition of AnAOB.

Reactor No.	Low pH setpoint	High pH setpoint
1	6.75	6.85
2	7.15	7.25
3	7.55	7.65
4	7.95	8.05

Modeling the effect of nitrite on anammox process

Two approaches were considered in an attempt to describe the effect of nitrite on AnAOB with a mathematical expression. In the first approach, nitrite was assumed to be a substrate inhibitor whose effect could be described by substrate inhibition models. Three models were identified (Haldane, Eduards' (1) and (2), and Luong) and evaluated relative to the data to assess their suitability to describe the nitrite inhibition effect observed. The mathematical expressions of each substrate inhibition model are given in Table 5.2. It is important to note that none of these models considers the impact of exposure time on inhibition; therefore, a second approach was taken that considers time as a variable and is introduced in the Results and Discussion section because its development considers the results from experiments. . Model parameters were estimated using the Solve procedure in Excel version 2002 which minimizes residual sum of squares to optimize the parameter set for each model. The suitability of each mathematical

expression was evaluated by comparing the predicted concentrations from each mathematical expression with measured values from lab scale experiments.

Table 5.2: Common substrate inhibition models

Name	Expression	Source
Haldane (Andrews)	$r = \frac{r_{\max} S}{K_s + S + \frac{S^2}{K_{IH}}}$ [5.6]	Luiz et al. (2003) Carrera et al. (2004) Jubany et al. (2005) Grady et al. (1999)
Edwards	$r = r_{\max} \exp(-\frac{S}{K_{IE}}) - \exp(-\frac{S}{K_S})$ (I) [5.7] $r = \frac{r_{\max} S}{K_s + S} \exp(-\frac{S}{K_{IA}})$ (II) [5.8]	Carrera et al. (2003)
Luong	$r = \frac{r_{\max} S}{K_s + S} \exp(1 - (\frac{S}{Sm}))^n$ [5.9]	Carrera et al. (2003)
S*ymbols*: *S*: substrate concentration (nitrite in our application) (mg/L) *r*: substrate uptake rate (mg/L/h) r_{max}: maximum rate of substrate uptake (mg/L/hr) *Ks*: Half saturation coefficient (mg/L) K_I: Haldane inhibition coefficient (mg/L) K_{IE}: Edwards inhibition coefficient (mg/L) K_{IA}: Aiba inhibition coefficient (mg/L) *n*: Luong coefficient (dimensionless) S_m: substrate concentration above which net growth ceases (mg/L)		

5.4. Results and Discussion

AnAOB are quickly inhibited by nitrite and the inhibition is reversible

The results show that short-term exposure to nitrite-N concentrations above 50 mg/L have a negative effect on the activity of AnAOBs over two hours of exposure (Figure 5.2).

AnAOB activity declined up to 88% for the case when the highest initial nitrite concentration (400 mg/L as N) was applied. One problem with using the short-term batch experimental protocol to assess long term impacts of nitrite on AnAOB activity is the fact that nitrite continued to decrease over time in these experiments (see Appendix F). Indeed, as nitrite concentration decreased, AnAOB activity increased (Figure 5.3), implying that short-term nitrite inhibition was reversible.

An activity recovery experiment was performed to confirm that short-term nitrite inhibition was partially recoverable after nitrite stress was eliminated. The activity of the DEMON biomass exposed to 50 mg/L nitrite-N after being exposed for 3 hours to 400 mg/L nitrite-N was 28.5 mg-N/L/h after 2 hours. This is an increase of 2.5 times the activity measured after 2 hours of exposure to an initial concentration of 400 mg/L nitrite-N. An inhibition experiment was conducted at the same time with an initial nitrite concentration of 100 mg/L as N and resulted in a 2 hour activity measurement of 23.7 mg NO_2^--N/L/h. When the biomass was exposed to an initial concentration of 50 mg/L nitrite-N for 2 hours without previously being exposed to 400 mg/L nitrite-N, the activity was 26 mg NO_2^--N/L/h, a very similar value (conducted at a different time than the recovery experiment). Overall, these results show that the biomass recovered almost completely from the high nitrite exposure under short-term batch conditions, and that short term (1-3 hours) nitrite inhibition is reversible.

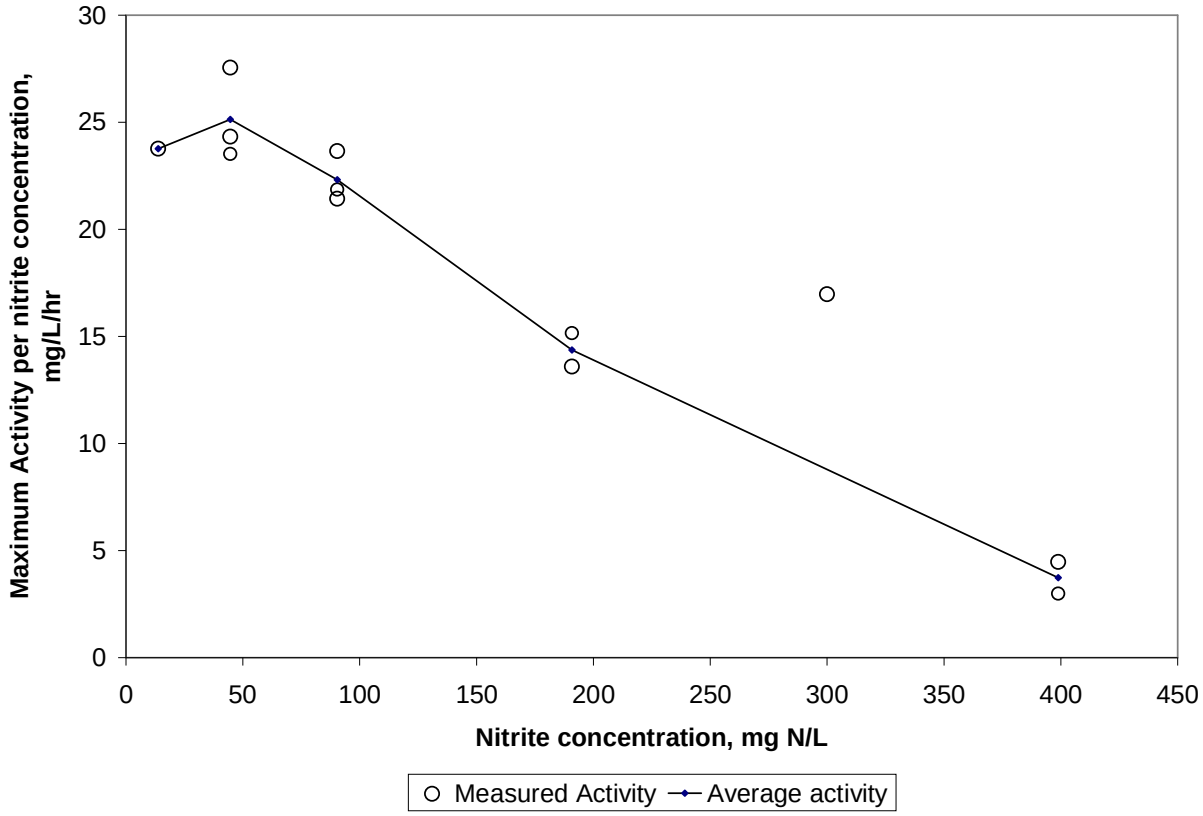

Figure 5. 2. AnAOB activity in DEMON biomass as a function of nitrite concentration as determined with the short-term batch experiment protocol. Measured activities are given as symbols while the average activity trend is shown with a line.

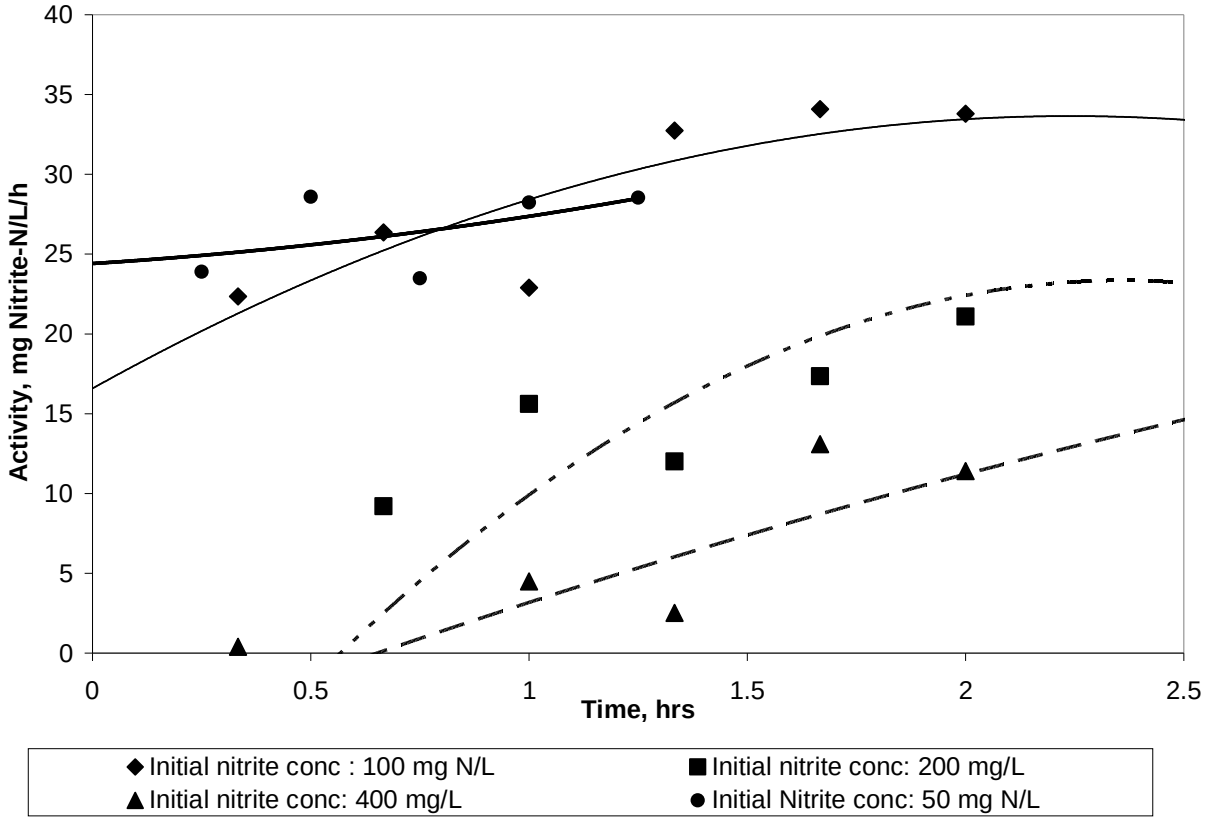

Figure 5.3 Response by AnAOB-containing DEMON biomass to nitrite loads over time as nitrite was consumed using short-term batch tests. Anaerobic conditions were maintained throughout the test.

Previous studies suggested that nitrite inhibition of AnAOBs is not reversible (Strous et al., 1999) unless trace amounts of hydroxylamine (>0.7 mg/L) or hydrazine (> 1.4 mg/L) are present. Hydrazine is known to be the intermediate in the anammox metabolism and the gain or loss of this intermediate means gain or loss of 15 catabolic cycles for the cell (Niftrik, 2004). Our attempt to measure the hydrazine using the p-Dimethylaminobenzhaldehyde method was unsuccessful because of nitrite interference. This study suggests that the short-term effect of nitrite exposure to AnAOBs in a DEMON biomass is fully recoverable in situations when the nitrite concentration is not sustained (i.e., nitrite concentration decreases during the short-term tests).

Nitrite inhibition increases over time under sustained nitrite exposure greater than 50 mg/L as N

Unlike the short-term batch experiments where AnAOB activity increased with time as nitrite concentration decreased, the long-term nitrite exposure studies revealed that exposure to constant nitrite concentrations over long time resulted in a decrease in AnAOB activity in DEMON biomass over time. An example is illustrated in Figure 5.4 for the case when nitrite-N concentration was kept close to 100 mg N/L (98.4 ± 10.8 mg N/L) for eight hours. The AnAOB activity decreased over the time course of the experiment by 58%. For the case when 200 mg/L of nitrite-N was maintained over the course of the experiment, activity decreased by 66% (Figure 5.5). Similar losses in activity did not occur for nitrite concentrations of 50 and 5 mg/L as N (Figure 5.5). Therefore, the threshold sustained concentration that results in a detrimental impact on anaerobic ammonia oxidation activity is between 50 and 100 mg/L nitrite-N for the DEMON biomass used in this study.

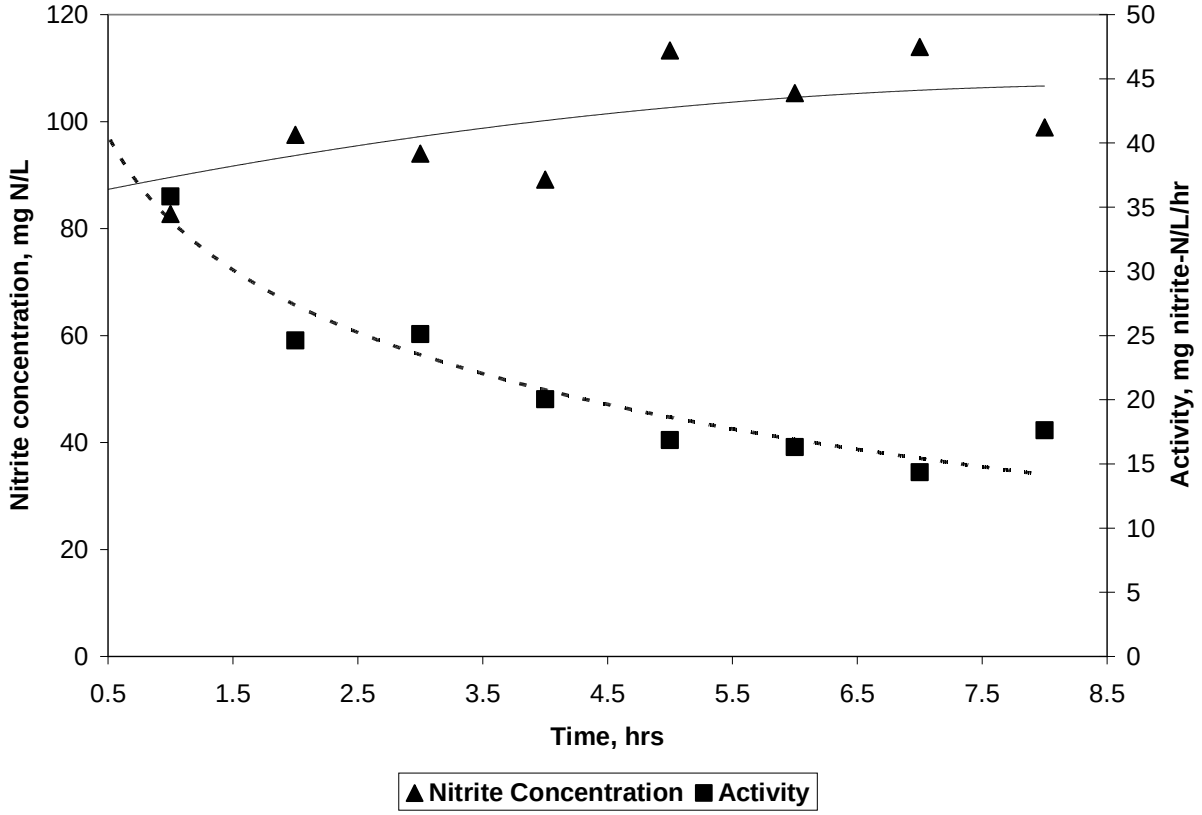

Figure 5.4: AnAOB activity and sustained nitrite concentration as a function of time during long-term fed batch experiments.

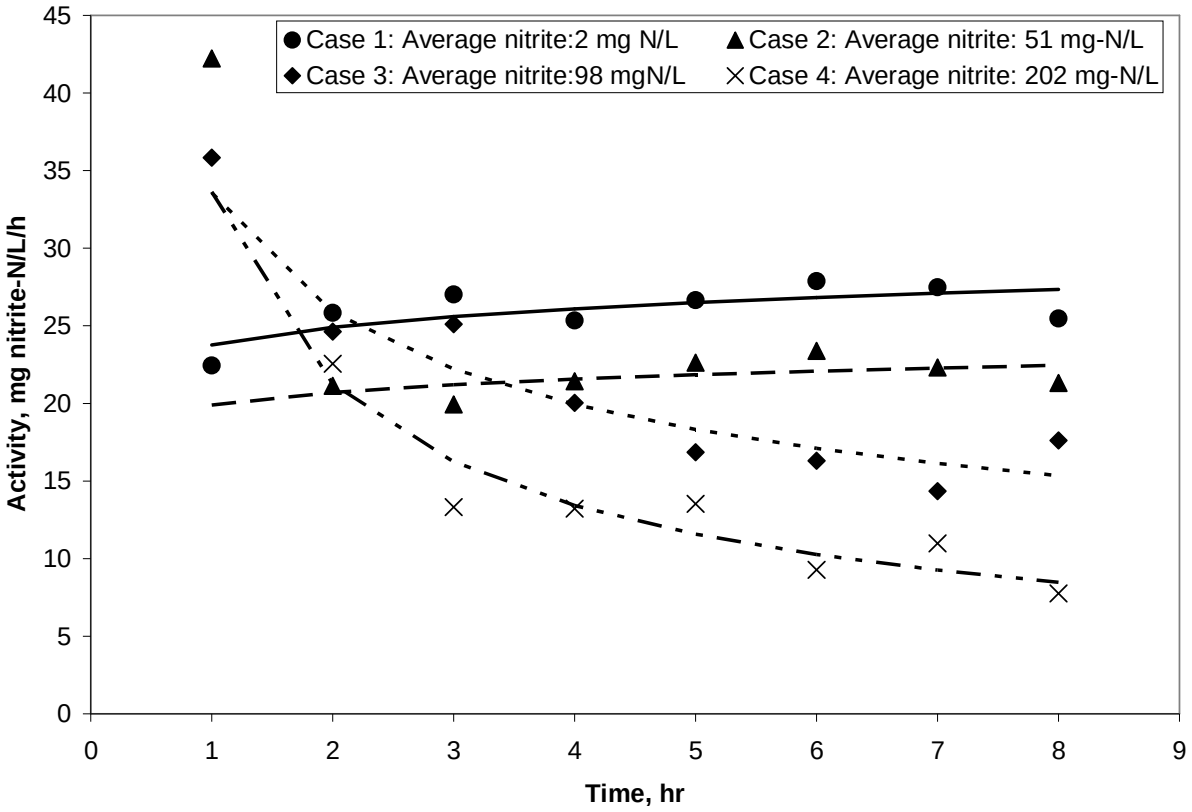

Figure 5.5: Effect of sustained nitrite concentration on AnAOB activity in a long-term, fed batch experiment. Nitrite concentrations were maintained at the average reported in the legend for the duration of the experiment. The lines indicate the calculated values using the expression in Equation 5.10.

An optimum pH range exists for AnAOB activity in the presence of nitrite

Results from the pH experiment indicate that growth of DEMON-derived AnAOB is more favorable under neutral conditions when 50 mg/L nitrite-N is present. No activity was detected when pH was held between 6.75 to 6.85, while maximum activities were observed when the pH was held at 7.2 and 7.6, indicating that the AnAOB bacteria in the DEMON biomass are neutrophiles. The reason of this preference might relate to a combination of factors related to the optimal pH condition for anammox enzymes and the possibility that nitrous acid toxicity is a factor at lower pHs (O'Learry and Solberg, 1976). Hydrazine hydrolase, one of the two key enzymes in anammox metabolism, is known to have an optimal pH range between 7.5 and 8.00 but is known to be stable between 6 and 9 (Shimanura et al., 2007), suggesting that it might contribute to the nitrite toxicity at low pH observed here, but that it is not the only factor because it does not explain the loss in activity at pH 8. Another key enzyme, hydrazine hydrolase, is less well studied and its activity as a function of pH is not known; therefore, its role in defining an

optimum functional pH range is not known. In summary, it is clear that pH does play a role in defining the impact of nitrite on AnAOB activity in DEMON biomass, but the optimal pH range for AnAOB activity is rather wide (at least from pH 7.2 to 7.6). Fortunately, this pH range is within the optimum pH ranged used to generate nitrite by AerAOB, which coexist with AnAOB in DEMON biomass systems.

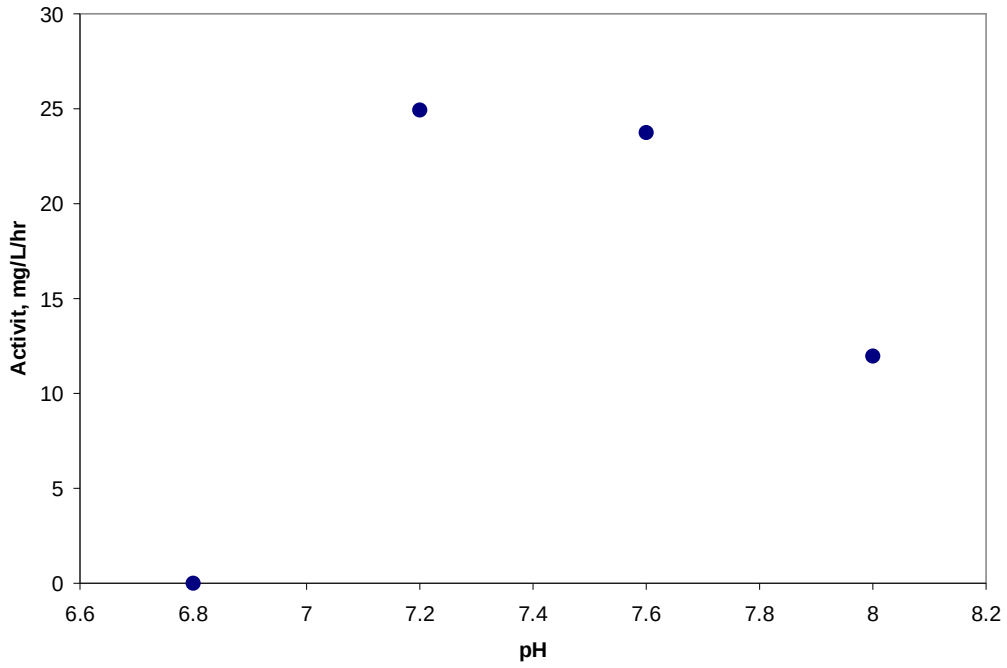

Figure 5.6: Effect of pH on AnAOB activity when exposed to 50 mg/L nitrite-N using a short-term batch test.

Nitrite inhibition in DEMON biomass is less severe than reported previously

Both the long-term fed batch and short-term batch experimental results suggest that DEMON biomass-based AnAOB can tolerate much higher nitrite concentrations than previously reported. According to past studies, nitrite concentrations as high as 100 mg/L-N completely stopped anammox activity (Strous, 1999) while 5 mg nitrite-N/L was reported to cause significant activity loss (Wett et al., 2007). In these studies, the culture used was from an anammox lab-scale enrichment reactor and full-scale DEMON reactor, respectively. The results presented here are consistent with Jetten at al. (1999) who reported that nitrite concentrations up to 280 mg/L N cause 100 percent inhibition; however, the current study showed that this concentration did not completely stop anammox activity. The data from this study suggests that

the concentration that causes 50% inhibition (IC50) is about 240 mg N/L. This is lower than the concentration reported by Dapena-Mora et al. (2007) with an IC_{50} for nitrite of 350 mgN/L.

Based on an upset that occurred with the DEMON pilot plant, it may be concluded that long term (more than 7 hours) nitrite exposure at concentrations of 200 mg/L nitrite-N is catastrophic for the system. Specifically, an accidental build-up of nitrite to 200 mg/L as N that lasted in the reactor system for at least 48 hours resulted in a significant reduction in AnAOB activity. A week later, AnAOB activity was completely lost and attempts to reactivate the process were unsuccessful. It is unclear if the failure was solely due to unrecoverable inhibition of AnAOBs or to other operational problems that occurred because of the perturbation. Nevertheless, this suggests that even if a given nitrite concentration may not completely inhibit AnAOB, the imbalance that occurs in the single sludge system as a result of the metabolic slow down can become confounded when recovery is attempted with a highly automated system. Further work needs to be done to determine how to recover from perturbations most effectively.

Substrate Inhibition Models can predict nitrite inhibition

Of the substrate inhibition models assessed against the short-term batch inhibition data collected in this study, the Eduards' (2) model seem to best fit the data (Figure 5.5). First, the standard error and Chi squared values were lowest for Eduards' (2), although the fits were quite good for all the substrate inhibition models assessed (see Appendix F). In contrast, Strous et al. (1999) found that the Luong model best described the nitrite inhibition demonstrated in their study. This study shows that even though the Luong model fit our data quite well (RSE = 0.93 compared to 0.66 for Eduards (2)), the estimated value for S_m in the Luong mdoel was 0.56 mg NO_2^-N. This parameter reflects the nitrite concentration above which anammox growth ceases, and the model estimate was orders of magnitude below nitrite concentrations tested in this study where AnAOB activity was not eliminated. Therefore, the Luong model was rejected based on the fact that this parameter was found to be unrealistic. Furthermore, the Monod half- saturation coefficient for nitrite (Ks_{NO2}) as a substrate was estimated to be 4.6 mg-N/L, which is higher than that reported by Strous et al. (1999). In their study, Strous et al. (1999) suggested that the ks_{NO2} for anammox is less than 0.1 mg/L. This might be due to differences in the diffusional limitation experienced in a biomass mixture. Our estimate from the growth rate experiment using high F:M

protocol (see Chapter 4) predict that the DEMON biomass contained about 7% of the AnAOB as opposed to the system run by Strous et al. (1999) where 80% of the population was AnAOB.

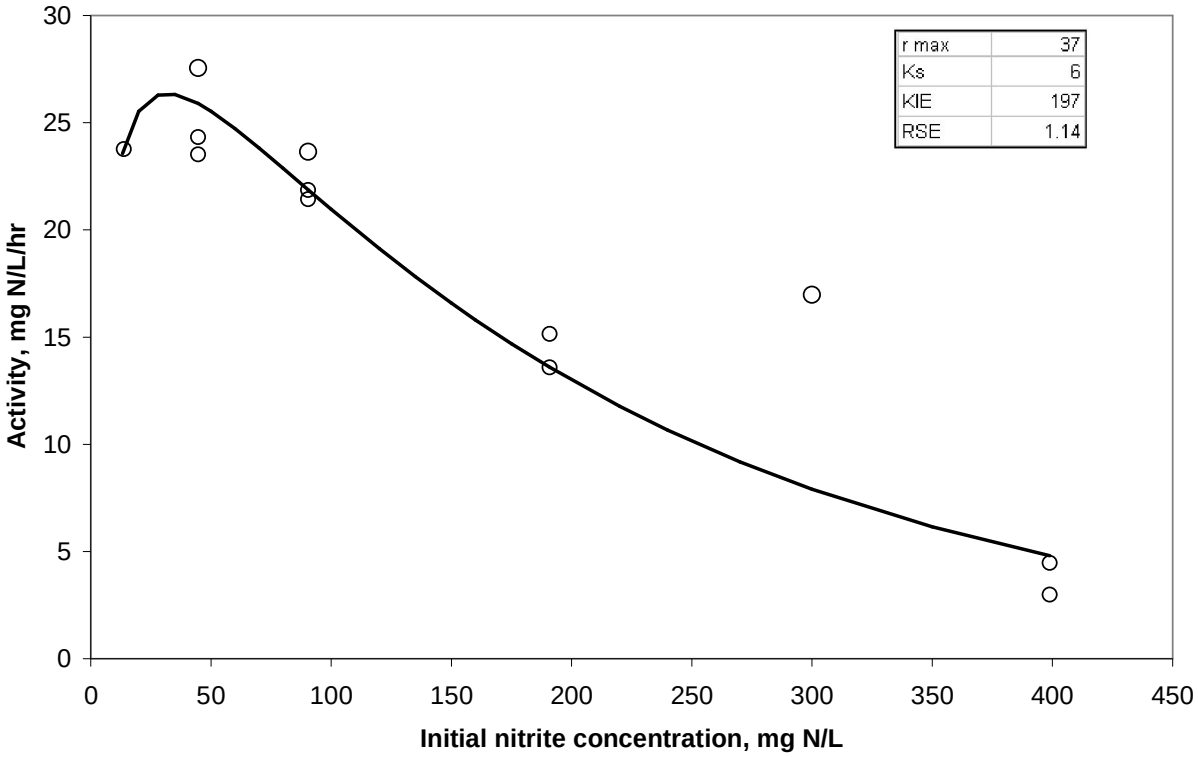

Figure 5.7: Eduards (2) model fit to activity versus nitrite exposure data collected from short-term batch experiment inhibition experiments.

Table 5.3: Coefficient values generated through the model fitting process. All parameters are defined in Table 5.2. RSE is the Residual standard error.

	Haldane	Edward (1)	Edward (2)	Luong
Parameter				
r_{max}(mg/L/h)	54.7	32.3	37.0	36.8
K_s(mg/L)	14.7	8.4	6.2	6.2
K_{IH}(mg/L)	60.6	N/A	N/A	N/A
K_{IE}(mg/L)	N/A	217.9	N/A	N/A
K_{IA}(mg/L)	N/A	N/A	197.00	N/A
S_m(mg/L)	N/A	N/A	N/A	0.6
n	N/A	N/A	N/A	0.003
RSE	3.1	1.6	1.1	1.6
$\chi 2$	1.213	0.195	0.080	0.176

Although the Eduards (2) model predicted inhibition responses in this study from the short-term experiments when nitrite exposure was relatively constant for 2 hours, we also found in this work

that inhibition patterns were different when nitrite exposure occurred over long time periods. For modeling flow through reactors where long term inhibitory nitrite exposure is possible, it is important to capture the time effect on inhibition. To do this, we considered injury-mortality theory and resulting models that have been used to predict microbial activity upon exposure to a harmful chemical over time. According to this theory, a fraction of a microbial community under stress (chemical, temperature or any other source of stress) becomes injured and can either survive or die (may become non-viable). A sustained stress will likely increase the extent of community injury and death in a way that can modeled. We established a model based on the Weibullian model (Corradini and Peleg, 2007) (which failed to fit our data). In our model derivation, we hypothesized that for a constant nitrite concentration, the nitrite uptake activity (rate) can be approximated by the expression:

$$r(C,t) = \theta\, r_{max}\, t^{n(C)} \qquad \text{Equation (5.10)}$$

where:

$r(C,t)$ = activity (mg/L/hr) at a given concentration C at any time t

θ = correction coefficient which reflects variables associated with reactor conditions such as mixing, small variations in temperature etc. (dimensionless)

r_{max} = maximum activity assuming an uninhibited DEMON sludge (mg/L/hr)

t = time (hours)

$n(C)$ = concentration dependent coefficient (dimensionless)

This expression was used to fit the time-based data in Figure 5.5 using Solver in Excel. The parameter estimates vary with time, as shown in Table 5.4. Of special note is the n(C) coefficient which decreased as nitrite concentration increased (see Table 5.4). This suggests that above this concentration, nitrite concentration causes a decrease in activity for long time exposures. A second data set was evaluated with this model and showed similar predictive power (Appendix F).

Table 5.4: Model coefficients determined through the fitting process

Average Nitrite (mg-N/L)	2.6	51.5	98.4	202
θ	0.68	0.57	0.96	0.96
n	0.07	0.06	-0.38	-0.66
RSE	1.29	0.95	2.05	1.92
r_{max} (mg [NO_2^--N]/L/h)	35	35	35	35

5.5. Conclusion

Nitrite affects AnAOB metabolism in biomass from a DEMON process, and the degree of inhibition depends on the nitrite concentration as well as the time of sustained exposure. The inhibition was found to be rapidly reversible, although the reversibility may not be 100%. When DEMON-based AnAOB cultures were exposed to both short-term and sustained nitrite concentrations, inhibition was observed for nitrite concentrations higher than 50 mg N/L. Inhibition was also found to be a function of pH, and it was determined that the optimal pH range for DEMON-based AnAOB metabolism was between 7.2 and 7.7. This study showed that a DEMON culture capable of anammox metabolism could tolerate much higher nitrite concentrations than was reported in past studies and may be a function of the microbial ecology or overall concentration of AnAOB present in the biomass.

5.6. Acknowledgment

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5.7. References

Carrera J., Jubany I., Carvallo L., Chamyb R., Lafuente J., (2004); Kinetic models for nitrification inhibition by ammonium and nitrite in a suspended and immobilised biomass systems, *Process Biochemistry*, 39: 1159-1165

Chamchoi N. and Nitisoravut S. (2007); Anammox enrichment from different conventional sludges, *Chemosphere* 66 (11): 2225-2232

Corradini M, Peleg M. (2007); A Weibullian model for microbial injury and mortality, *International Journal of Food Microbiology*, 119:319-328

Dapena-Mora A., Fern´andez I., Campos J.L., Mosquera-Corral A., R. Méndez , M. Jetten (2007); Evaluation of Activity and Inhibition effects on Anammox process by batch tests based on nitrogen gas production, *Enzyme and Microbial Technology*, 40 (4): 859-865

Dykhuizen R S, Fraser A, McKenzie H, Golden M, Leifert C and Benjamin N (1998); Helicobacter pylori is killed by nitrite under acidic conditions, *Gut* 42: 334-337.

Grady, G. Gaigger, H. Lim, 1999; Biological Wastewater Treatment, *Marcel Dekkel*,

Gunthert F.W., Wastewater Research in Germany: New Technologies, University of Federal Armed Forces, Institute of Hydroscience, Munich, Germany

Gut L. (2006), Assessment of partial nitrification/anammox system for nitrogen removal, Licentiate Thesis, *KTH Land and Water Resources Engineering*, Sweden

Gut L., D. Jeison, R. Chamy, (2003); Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration, *Water Research* 37: 1371-1377

Hamersley M. R., Lavik G., Woebken D., Rattray J., Lam P., Hopmans E.C., Kruger S., Graco M., Gutiérrez D., Kuypers M.(2007); Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone, *Limnology and Oceanography*, 52 (3): 923-933

Hellinga C., Schellen A.J.C., Mulder J.W., Van Loosdrecht M.C.M., Heijen J.J, (1998), The SHARON process: an innovative method for nitrogen removal from ammonium-rich wastewater, *Water Sci Technol*, 37(9):135-142

Imajo U., Tokutomi T, Furukawa K. (2004); Granulation of Anammox microorganisms in up-flow reactors, *Water Sci Technol*. Vol 49, No 6, pp 155-163

Jetten S.M., Strous M., Pas-Schoonen K., Schalk J., van Dongen G.J.M., van de Graaf A., Logemann S., Muyzer G., van Loosdrecht M., Kuenen G.(1999), The anaerobic oxidation of ammonia, *FEMS Microbiology Reviews* 22: 421-437.
Jetten M., Wagner M., Fuerst J., Loosdrecht M., Kuenen G., Strous M., (2001); Microbiology and application of the anaerobic ammonium oxidation (‘anammox’) process, *Current Opinion in Biotechnology*, 12:283-288

Jubany I., Baeza J., Carrera J. _, Lafuente J., (2005); Respirometric calibration and validation of a biological nitrite oxidation model including biomass growth and substrate inhibition, *Water Research*, 39,:4574-4585

Khin T., Annachhatre A.P. (2004), Novel microbial nitrogen removal processes, *Biotechnology Advances*, 22: 519-532

Kuypers M., Lavik G., Woebken D., Schimid M., Fuchs B., Amann R., Jorgensen J., Jetten M.(2005), Massive nitrogen loss from Benguela upwelling system through anaerobic ammonia oxidation, *Proceedings of the National Academy of Sciences of the United States of America* (PNAS), 102:6478-6583

Mulder J.W., Van Loosdrecht M.C.M., Helliga C, Van Kempen R. (2001), Full-scale application of the SHARON process for treatment of rejection water of digested sludge dewatering. *Water Sci Technol*, 43(11): 127-134

Nielsen M., Bollmann A., Sliekers O., Jetten M., Schimid M., Strous M, Schmidt I., Larsen L.H., Nielsen L.P., Revesbech N.P. (2005), Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor, *FEMS Microbiology Ecology*, 51: 247-256

O'Learly V.and Solberg M., (1976); Effect of sodium nitrite inhibition on intracellular thiol groups and on the activity of certain glycolytic enzymes in Clostridium perfringens, *Appl. Environm Microbiology*, 31(2):208-212.

Peleg M., (2002), Modeling and simulation of microbial survival during treatment with dissipating lethal chemical agent, *Food research International*, 35:327-336

Pilcher H. (2005); Microbiology: Pipe Dreams, *Nature* 437: 1227-1228

Shimanura M., Nishiyama T., Shigetomo H., Toyomoto T., Kawahara Y., Furukawa K., Fujii T., (2007); Isolation of a multiheme protein with features of hydrazine-oxidizing enzyme from an anaerobic ammonium-oxidizing enrichment culture, *Appl. Environ. Microbial*., 73(4):1065-1-72

Sliekers O., N. Derwort, Gomez C., StrousM., Kuenen J., Jetten M. (2002), Completelt autotrophic nitrogen removal over nitrite in one single reactor, *Water Research*, 26(10): 2475-2481

Stein L. and Arp D. J. (1998); Loss of Ammonia Monooxygenase Activity in *Nitrosomonas europaea* upon Exposure to Nitrite, *Applied Environmental Microbiology*, 64: 4098-4102

Strous M. (1999):, Key Physiology of Anaerobic Ammonia Oxidation, *Applied and Environmental Microbiology*, 65: 3248-3250

Third K.A., Paxman J., Schimidt M., Strous M., Jetten M.S.M., R. Cord-Ruwisch,(2005); Treatment of Nitrogen-rich wastewater using partial nitrification and Anammox in the CANON process, *Water Sci and Technology*, Vol 52 (4), 47-54

van de Graaf A., de Bruijn P., Robertson L., Jetten M., Kuenen G., (1996), Autotrophic growth of anaerobic ammonium-oxidizing micro-organismsin a fluidized bed reactor, *Microbiology* 142:2187-2196

Warakomski A., Van Kampen R., Kos P., (2006), Microbiology/Biochemistry of nitrogen cycle, Innovative process applications, *Environmental Science and Technology*, Vol 1, 234-240

Wett, B. (2006), Solved up-scaling problems for implementing deammonification of reject water, *Water Sci. Technol*., 53(12):121-128

Wett B., Murthy S., Takacs I., Bowden G., Deur A., O'SHaughnessy M. (2007); Key Parameters for Control of Demon deammonification process, WEFTEC, Nitrogen Removal, Baltimor

CHAPTER 6:

Ammonia oxidation inhibition in the DEMON Process

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6.1. Abstract

The Deammonification (DEMON) pilot centrate treatment process at the Alexandria Sanitation Authority wastewater treatment facility was challenged by an inability to maintain a stable balance between partial ammonia oxidation to nitrite (nitritation) and anaerobic ammonia oxidation (anammox). In this case, it appears that system performance was more a function of nitritation process upset as opposed to problems with anammox activity. This study demonstrated that aerobic ammonia oxidizing bacteria (AerAOB) were inhibited in the DEMON process and that the inhibition could be attributed to the input of compound such as iron hydroxides, defoaming agents, and residual cationic polyelectrolytes (polymers) or breakdown products thereof used in the dewatering process. Nitritation inhibition was evaluated by a combination of respirometry and ammonia oxidation rate assays. The results suggest that each compound mentioned above exhibits AerAOB inhibition potential at the concentrations tested.

6.2. Introduction

Novel nitrogen removal technologies are evolving as discharge standards become more and more stringent. The recently discovered anaerobic ammonia oxidation (anammox) process is a breakthrough in nitrogen removal. It is a very suitable alternative for sidestream treatment for centrate or reject water with high ammonia concentrations. The anammox process is a

completely chemolithoautotrophic process whereby ammonia is oxidized to nitrogen gas by nitrite in the absence of oxygen (van de Graaf et al., 1996) according to equation 6.1:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (6.1)$$

If well controlled, nitrite can be produced from partial ammonia oxidation. The anammox process can then be combined with the partial ammonia oxidation either in the same reactor such as in the deammonification (DEMON) process (Wett , 2006; Wett et al., 2007) or in a separate reactor such as SHARON process (Moulder et al., 2001). It has been suggested that, irrespective of the process configuration used, a combination of partial ammonia oxidation and anammox can a chieve a stable nitrogen removal of at least 80% (Jardin et al., 2006).

The DEMON process uses a single mixed biomass in an SBR configuration with controlled intermittent aeration that allows part of the influent ammonia to be oxidized to nitrite during aerobic periods by aerobic ammonia oxidizing bacteria (AerAOB) and anammox activity during anoxic periods. Nitritation control is achieved by controlling the reactor DO concentration to a low value around 0.3 mg/L and the high temperature in the range of 30-40°C. The SRT is normally high enough to avoid AnAOB wash out, but should be low enough to exclude as much as possible aerobic nitrite oxidizing bacteria. Aerobic and anoxic periods are determined by upper and lower pH setpoints. During the anoxic period, the addition of alkalinity from the feed and from the activity of AnAOB causes the pH to increase to the upper pH set point, signaling the end of the anoxic period and start of aerobic period. During the aerobic period, AerAOB activity consumes alkalinity causing the pH to decrease to the low set point, which signals the end of the aerobic period. The pH control strategy has worked successfully in a full-scale system in Strass, Austria, where about 84% of nitrogen removal efficiency was obtained (a 50 day average from December 2004 to January 2005). This control strategy was also generally successful during a pilot study done in Alexandria, Virginia (Chapter 3).

Although the overall pilot plant performance was satisfactory, there were incidents during he study when ammonia oxidation appeared to be inhibited. Ammonia inhibition impacts negatively the overall process by affecting the control logic. Ammonia inhibition slows down the

ammonia oxidation rate and hence the pH decrease, meaning that the aeration period is extended if no other parameter is changed. Longer aeration periods can impact the process by stimulating NOB growth and selection, and can reduce the time available for annamox activity. Increase in NOBs results in increased effluent nitrate concentrations and a decrease in nitrogen removal efficiency.

From operational point of view, ammonia oxidation inhibition forced a reduction in loading as indicated in Figure3.7.

The origin of inhibition was not identified during the study. It was hypothesized that three factors may have led to the upsets observed. Possible causes include very high temperature (our original temperature was 37^{o}C while the Strass full-scale DEMON system is operated at 30^{o}C) and the presence of chemicals in the feed. Among those suspected chemicals were ferric iron used for phosphorus removal in the liquid process, residual cationic dewatering polymers present in the centrate or byproducts thereof, and antifoaming agents used for foam control in the anaerobic digesters. It is also possible that the impact of these potentially inhibitory compounds is enhanced by the accumulation with time of those compounds in the DEMON reactor, given the high process solid retention time (more than 25 days).

Ferric chloride is added to the liquid treatment process at the ASA WWTP to provide chemical phosphorus removal. The hydrate ferric oxides (with coordinate orthophosphate) are removed from the process in both primary clarifiers and waste activated sludge and transferred to the anaerobic digestion process. It is well established that under anaerobic conditions, Fe^{3+} can be reduced to Fe^{2+} by iron reducing bacteria in the presence of organic material in the digester (Stumm and Morgan, 1970) some of which is mobile and could end up in the centrate. Fe^{2+} can have a negative effect on ammonia oxidation especially if nitrite is present in the system. Both nitrite and nitrate have been documented to play a significant role in the fate of iron in activated sludge. It has been reported that in the presence of nitrite, sorbed ferrous ions react with nitrite ions, and this leads to the production of a solid iron hydroxide according to equation 6.2:

$$Fe^{2+} + \frac{1}{2}NO_2^- + \frac{9}{4}H_2O \rightarrow Fe(OH)_3 + \frac{1}{2}N_2O + \frac{3}{2}H^+ \qquad (6.2)$$

The hydroxide forms a coating on the cell and then physically blocks the transport of substrates (Cooper et al., 2003; Coby et al., 2005). Other studies report the abiotic reactivity of Fe(II) with nitrite to produce ferric iron and nitric oxide (NO) which is toxic to microorganisms (Cleemput and Samater, 1996). The iron analysis during the pilot plant study revealed that revealed that the total iron content was about 150 mg/L or 6% of the reactor solids.

Other potential inhibiting chemicals used upstream in the solids handling process included cationic dewatering polymers and defoaming agents. ASA applies cationic polymer at an average rate of 17 lbs of polymer per tone of biosolids. There are reports of polymer inhibition to biological processes. Application of polymer prior to anaerobic digestion resulted in methane gas production reduction (Mirlohi, 2006). Cationic polymer has also been associated with municipal wastewater whole effluent toxicity (Rowland et al., 2000).

Defoaming agents constitute another category of chemicals used in upstream treatment processes that might end up in the centrate. ASA uses a silicone emulsion manufactured by Dow Corning Corporation. Although defoaming solutions are effective for controlling foam in wastewater treatment processes, little is know about their fate, their ecological impact and their effects during wastewater treatment (Material Safety Data Sheet, Dow Corning Corp).

The objective of this study was to evaluate the effect of these potential inhibiting compounds on the activity of AerAOB in the DEMON process. Understanding their effect would be expected to help optimize the sidestream centrate treatment options in general and the DEMON process in particular.

6.3. Materials and Method

Abiotic reaction between iron species and NOx

The possibility of abiotic reactions between iron species (ferric or ferrous iron) was investigated individually with both nitrite and nitrate to determine whether the products from the reaction are detrimental to ammonia oxidation or not. Three flasks, 250 mL each, were filled with 3.6 mM solution of Fe^{2+} (ferrous ammonium sulfate, $Fe(SO_4)_2(NH_3)_2.6H_2O$ being the source of Fe^{2+}). This concentration was close to the total iron concentration measured in the

reactor, assuming the worse case scenario when all iron is in the ferrous form. The pH was adjusted to 7.4 using sodium hydroxide. The first flask was used as a control; the second flask was spiked with sodium nitrite and the third flask with potassium nitrate to make a 7 mM solution of nitrite and a 7 mM solution of nitrate respectively. This concentration was also chosen assuming the worse case scenario when nitrite could accumulate in the reactor. The flasks were placed in an incubator at 35^{o}C, and gentle mixing was applied with a magnetic stirring mechanism. Samples were collected at intervals of 0, 10, 40, 80,120 minutes and analyzed for nitrite (Diazotization Method) and nitrate (cadmium reduction method) using a UV/visible light spectrophotometer (HACH, DR/4000, Loveland, Co). The experiment was repeated using a 3.6 mM solution of Fe^{3+} (with Ferric Chloride, $FeCl_3.7H_2O$ as source of Fe^{3+}). The pH was checked at the end of the experiment but was not controlled throughout the experiment. The experiment was conducted two times.

Investigating biological ammonia oxidation inhibition

Respirometry was used to investigate the effect of iron on ammonia oxidation rate using a Challenge 4-position respirometer. This is a static liquid, mobile gas respirometer that measures the input rate of pure O_2 into bottles as demanded by any oxygen consuming process (chemical or biological) in the liquid. The measurement is based on pressure drop created in the headspace when oxygen is consumed by the process. Samples were collected from the RAS line in Alexandria Sanitation Authority, and distributed into 4 respirometer vessels with 500 ml volume each. Figure 2 summarizes the content of each vessel. The temperature was controlled to 35^{o}C by means of a water bath. To increase the alkalinity, 5 mL of 50 mg/ml $NaHCO_3$ solution was added to each vessel. The pH was then adjusted to 7.4-7.60 by means of a 6 N sodium hydroxide solution and 85% phosphoric acid solution. Each vessel was aerated for 3 minutes. A magnetic stirrer was introduced into the vessel, a CO_2 adsorption tube containing 30% KOH solution was placed in the tube and then the vessel was closed and mixed at about 550 rpm. Oxygen uptake rate data was automatically logged into Microsoft Excel© by means of data acquisition software. The data logging interval was two minutes. The experiment was conducted four times.

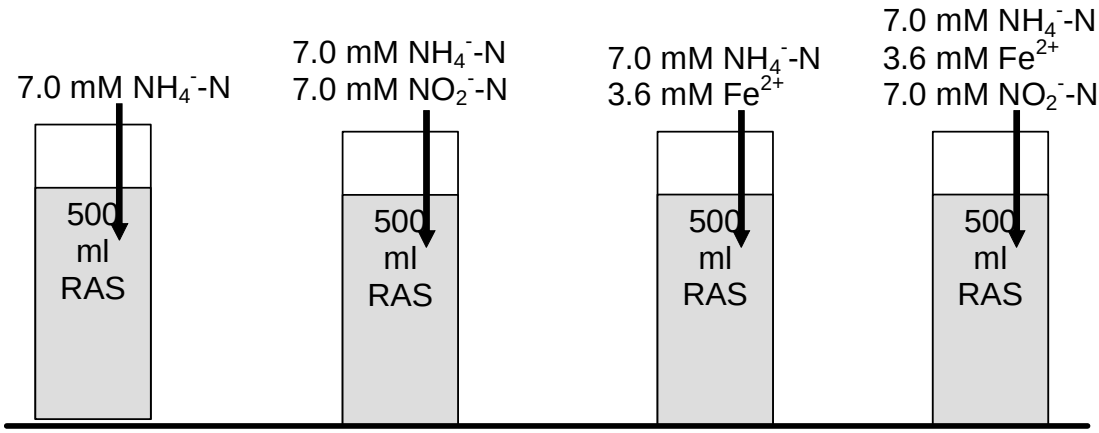

Figure 6.1: Respirometry vessels content prior to starting the experiment.

Evaluation of polymer and defoaming agent inhibition

To test for inhibition caused by the defoaming agent, return activated sludge samples were collected from the ASA plant and 2 L were placed into each of 3x5 L reactors. The first reactor was filled with plant effluent water spiked with ammonia to reach approximately 500 mg/L-N in the form of ammonium chloride. The second reactor was filled with 2 L of centrate and the third reactor with 2 L of plant effluent 50 mL of concentrated defoaming agent (Antifoarm 1410, Dow Corning Corp., Midland, Mi) used in ASA and about 500 mg/L of ammonia in the form of ammonium chloride. Continuous aeration was supplied to provide both air and mixing. A rotameter was used to ensure that each reactor received the same air flow rate, and a DO meter was frequently used to ensure appropriate conditions were maintained in the range of 3.0-4.0 mg/L. The pH was also checked and kept between 7.0 and 7.8. Samples were collected every 30 minutes for the first 2 hours and after one hour for the last sample. Samples were filtered through 0.45 µm GN-6 matricel membrane (Ann Arbor, Mi) and analyzed for ammonia using EPA 350.1 method. The selection of an ammonia concentration close to 500 mg/L was used to determine whether centrate itself had some inhibiting compounds compared to the plant effluent. The probable ammonia inhibition at these concentrations was not an issue since the control which served as a reference was exposed to the same conditions. The experiment was condicted once.

To test the polymer, 50 mL of neat polymer (Clarifloc SE-890 polymer, Polydyne Inc., Riceboro, GA) was introduced into a reactor containing 2 L of RAS and 2 L of plant effluent in a 5 L reactor. The reactor was spiked with about 50 mg/L of ammonia. The experiment also included a control reactor maintained under the same conditions as the control reactor described for the defoaming agent except that it did not contain the polymer. Samples were collected every 30 minutes for a period of two hours, filtered through 0.45 µm GN-6 matricel membranes (Ann Arbor, Mi) and analyzed for ammonia by EPA method 350.1. The experiment was condicted once.

Oxygen uptake rate (OUR) measurement

OUR was used to assess the impact of low DO in the DEMON reactor. DEMON sludge (500 mL) was collected from the DEMON reactor and diluted 8X with tap water. A portion (300 mL) of the diluted sludge was introduced into a BOD bottle aerated to saturation, then closed by inserting an airtight DO probe (YSI 5010). The probe mixer was started and DO was recorded by the DO meter (YSI 5100) every 30 seconds until the DO reading stabilized. This experiment was run three times.

6.4. Results and Discussion

Abiotic reaction between iron species and NOx

Ferrous iron very likely reacted instantaneously with nitrite as indicted by a sudden change in color and release of bubbles (Table 6.1, Figure 6.2). The gas composition released through the bubbles was not identified but is believed to be either N_2O according to previous studies (Cooper et al., 2003) or NO (Cleemput and Samater, 1996). Analysis of nitrie and nitrate-nitrogen revealed a decrease in nitrite and NOx, also suggesting the reduction of NO_2 to N_2O or NO (Figure 6.3). The experiment suggests that at neutral pH, ferrous iron instantly becomes ferrous hydroxide which is characteristic of the green color. The green color however gets darker with time, probably because of further oxidation to ferric oxides with the input of dissolved oxygen. Under limited dissolved oxygen conditions, it has been reported that ferrous hydroxide reacts with oxygen to generate Fe_3O_4 (Baudisch and Welo, 1925) which is blackish. This implies that the combination of ferrous hydroxide and ferric oxide might be the cause of the precipitate with a blackish green color. At high oxygen concentrations, ferrous iron gets oxidized to Fe^{3+},

which then forms red colored solids characteristic of hydrated ferric oxides – $Fe(OH)_3$, FeOOH, etc. Nitrate does not seem to have an impact on the ferrous or ferric chemistry in water based on the fact that no significant change in nitrate was noticed. However, there have been reports that the reaction between ferrous and nitrate is possible at neutral pH (Philips et al., 2003). The presence of nitrite causes the generation of a yellow precipitate from ferrous ions, which might be characteristic of hydrated yellow hydroxides (Baudisch, 1925). The nature and characterization of this reaction together with the characterization of this precipitate is beyond the scope of this work. However, the reaction between ferrous ion and nitrite suggests that these abiotic reactions could have been occurring in the DEMON pilot reactor.

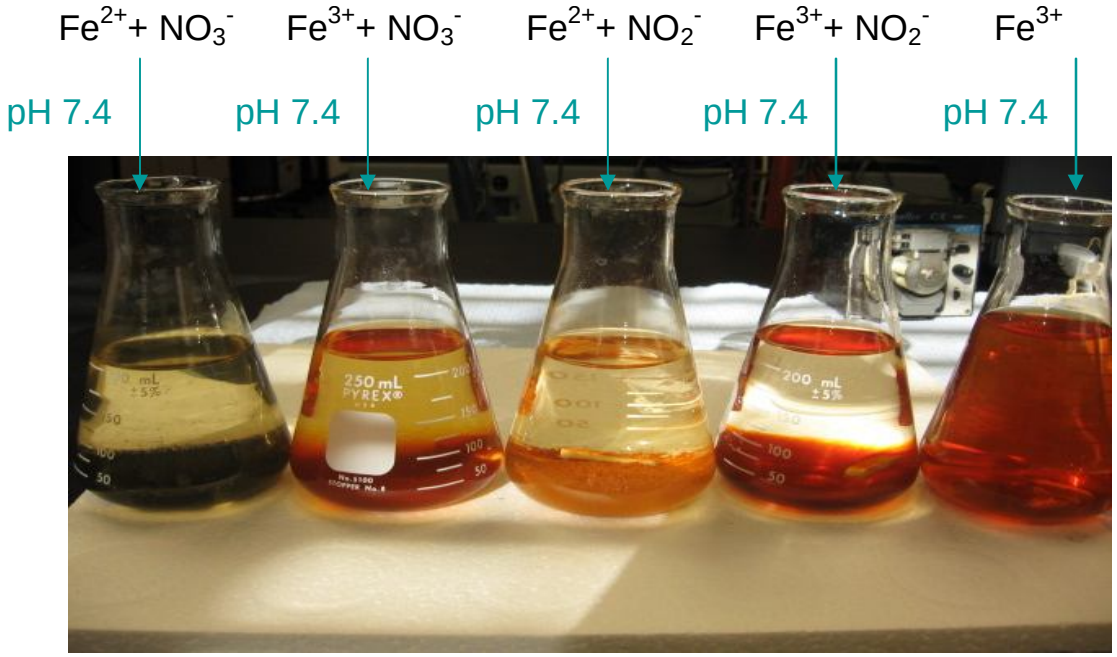

Figure 6.2: Color development of the precipitates during the ferric/ferrous reaction essay with nitrite/nitrate. The picture was taken about two hours after chemicals were mixed. Notice that 2nd and 4th flasks' precipitates have the same color. The last flask displays ferric ion in solution with very few precipitates.

Table 6.1: Ferric/ferrous reaction essay with nitrite/nitrate ions; the chemicals were gently mixed in a flask with 250 ml de-ionized water and incubated at 35oC

Content	Color of precipitate	Rate of reaction	Comment
$Fe^{2+} + NO_3^-$	Blackish green	Instantaneous	The color change was due to pH adjustment, rather than NO_3^-addition
$Fe^{3+} + NO_3^-$	Orange	Slow	
$Fe^{2+} + NO_2^-$	Yellow	Instantaneous	Observed bubbles
$Fe^{3+} + NO_2^-$	Orange	Relatively quick	

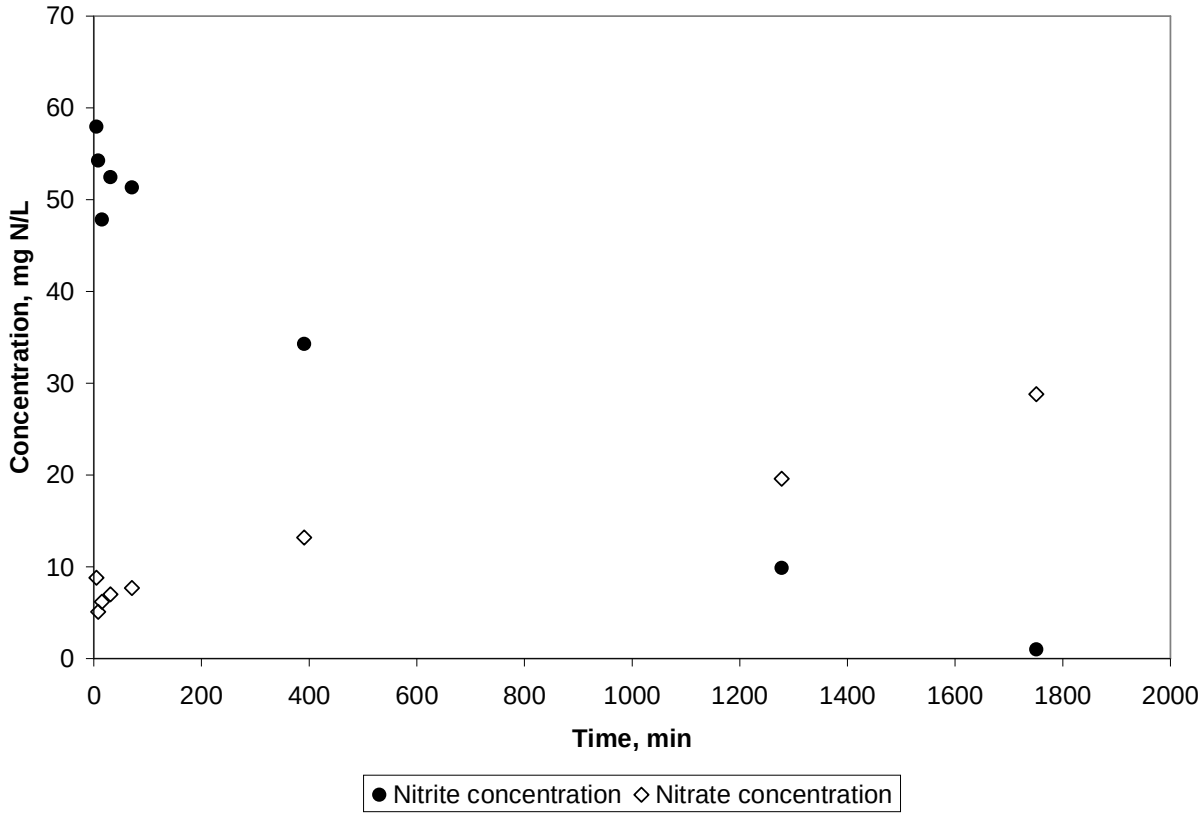

Figure 6.3: Nitrite and Nitrate profiles during abiotic nitrite-ferrous reaction, indicating net nitrogen loss. The pH dropped from 7.6 to 6.2 during this experience.

<u>Potential impact of abiotic ferrous reactions with nitrite on the environment</u>:

The importance of nitrogen removal under these abiotic conditions, or chemodenitrification, is certainly hard to quantify in the activated sludge. While nitrite and ferrous are readily available for reaction in de-ionized water, ferrous iron is short lived at the neutral pHs found in biological systems. However, chemodenitrification has been reported in natural systems especially in soils (Kappelmeyer et al., 2003). Chemodenitrification was also

reported to play a significant role in NO and N_2O generation in the atmosphere (Anderson and Levine, 1986). Given the growing concerns about greenhouse gases however, the authors encourage further studies.

Biological ammonia oxidation inhibition in the presence of ferrous iron and nitrite

According to the respirometry data (Figure 6.4), the oxygen uptake rate was lowest when nitrite and ferrous iron were both supplied to the biomass at the same time, suggesting that their reaction could have an inhibiting effect. The control which contained both nitrite and ammonia revealed that part of the inhibition that was detected occurred under the control conditions but this inhibition decreased with time, more likely because nitrite was oxidized to nitrate by NOB. It was not determined whether this ferrous/nitrite inhibition originated from membrane transport interference by ferrous hydroxides resulting from the chemical reaction between nitrite and ferrous according to Cooper et al. (2003) or whether other mechanisms were involved in the inhibition process. Regardless of the mechanism involved, one could argue that in the DEMON reactor with a total iron content of around 2.7 mM, such inhibition is possible. It has been reported that the presence of 3 mM of iron inhibits *Chlorella vulgaris* (Chapman and Dean, 1982) while 6 mM total iron completely inhibited the growth of this single-celled algae. The mechanism of this inhibition was not discussed.

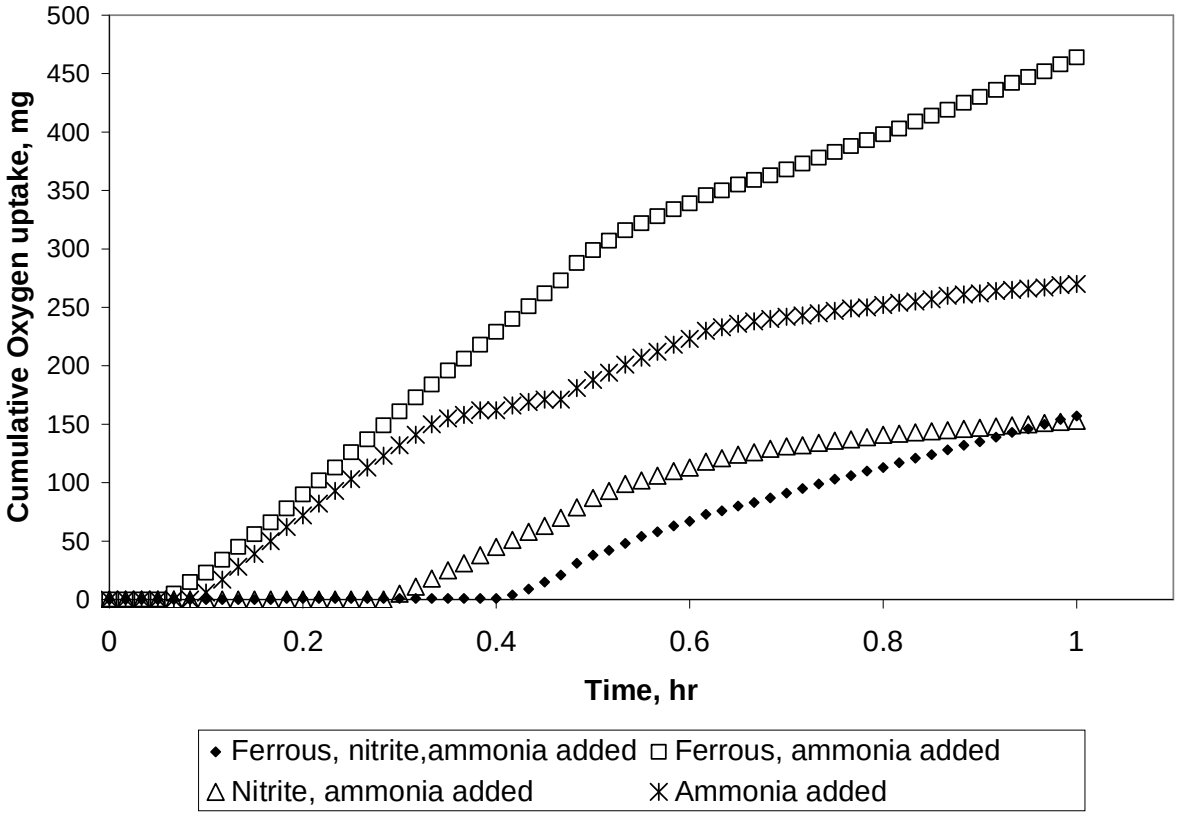

Figure 6.4: Effect of ferrous iron interaction with nitrite to oxygen uptake with RAS from ASA as the source of biomass. Ferrous and nitrite were in concentrations of 150 mg/L and 100 mg/L respectively.

Defoaming Agent Inhibition

The defoaming agent was also identified as a potential inhibitor to the process. The experiment shows that the defoaming agent dose of 1.25% neat (volume to volume) completely inhibited ammonia oxidation as shown in Figure 6.5. Ammonia oxidation was better with plant effluent spiked with ammonia than with centrate. This is an indication that centrate might contain inhibiting compounds and the defoaming agent is certainly one of them. There are two observations that appear to strongly indicate that the defoaming agent inhibited to the DEMON process. First, the defoaming agent seems to prevail through the solid-liquid separation process. Its milky color can still be seen after filtering a sample containing the defoaming agent through a 0.45 μm pore size filter. The implication is that most of the defoaming agent ends up in the centrate and hence in the DEMON reactor. Second, the fact that the defoaming agent is applied "as needed" suggests the possibility that in case of high foam formation, the operator might apply a dose which could reach the inhibition or toxicity level. Given the fact the major

deammonification process upsets happened following a new batch of centrate, it wouldn't be very surprising to correlate these upsets to defoaming agent applications.

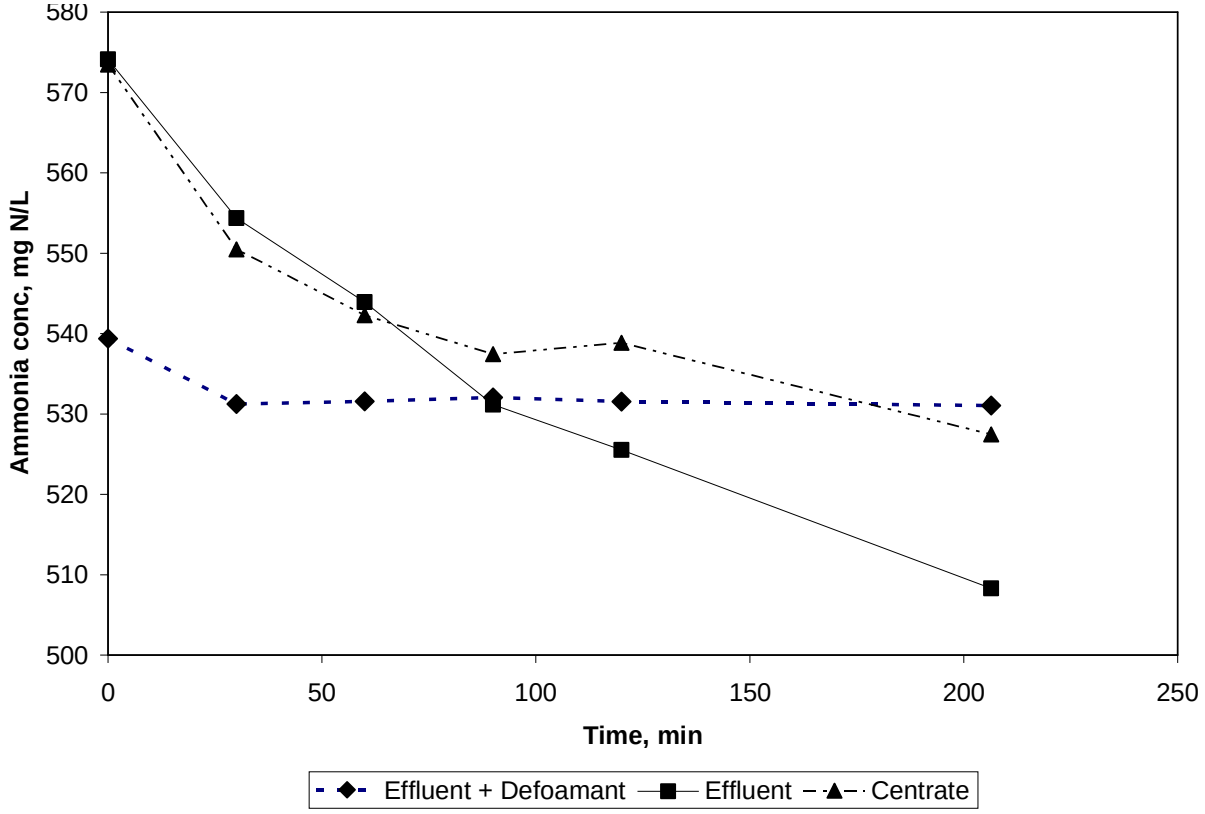

Figure 6.5: Defoaming agent effect to ammonia oxidation. The defoaming agent (Antifoam 1410) content was 1.25%. The centrate is supposed to contain the defoaming agent as well given that it is used upsetream of the centrate production process and given that it (defoaming agent) is stable.

Ammonia oxidation inhibition by polymer

The role of the polymer in ammonia oxidation inhibition was very hard to investigate, mainly because it is hard to predict how much polymer prevails in the solids separation process and ends up in the centrate to be a potential inhibition to the deammonification process. Application of polymer in a system having ability to oxidize ammonia biologically resulted in a decrease in ammonia oxidation rate (Figure 6.6).

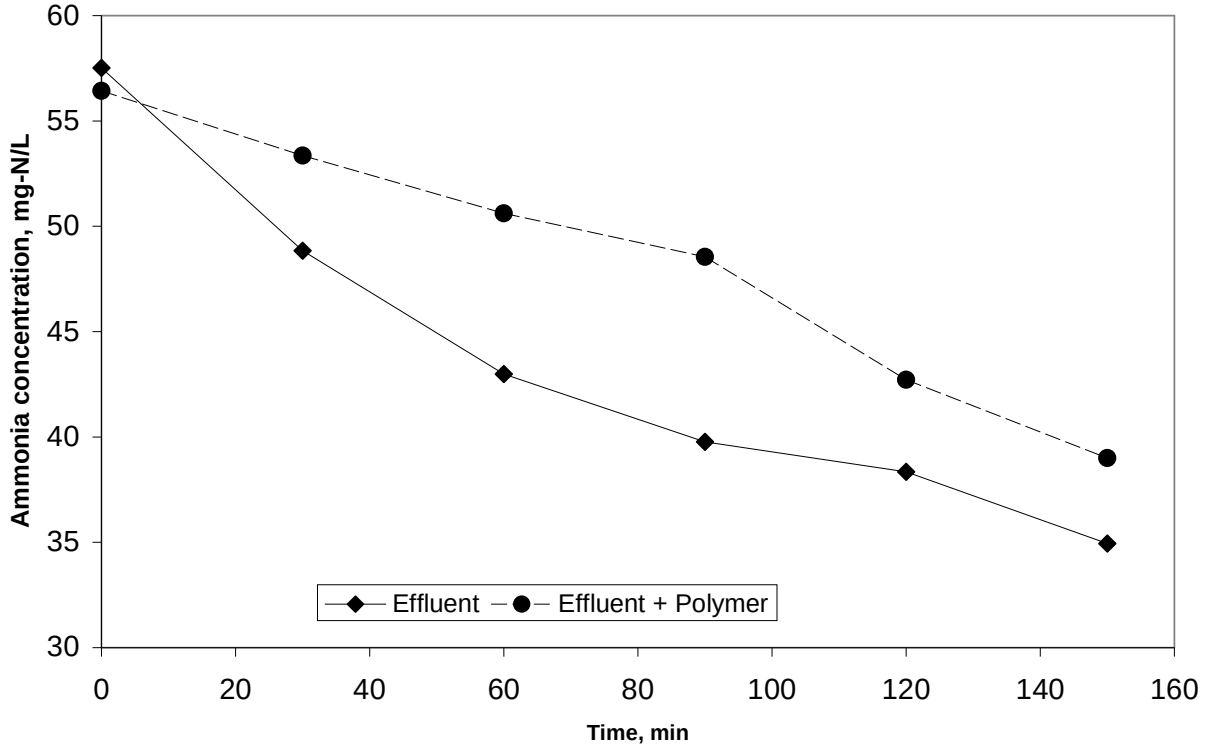

Figure 6.6: Impact of polymer in ammonia oxidation. The polymer (CLARIFLOC SE-890) content was 1.25%. The experiment was run at room temperature

There is lack of information about the polymer inhibition mechanism. There are suggestions that polymer inhibition comes indirectly from an increase in viscosity to affect the mixing efficiency, and hence, the accessibility of food and nutrients to microorganisms (Mirlohi, 2006). It is also possible that polymer by-products generated through decomposition cause inhibition or toxicity. The experiment in this study did not consider the second alternative, but Mirlohi (2006) reported that inhibition to anaerobic methane production started 9 days after polymer application, implying this possibility. Attempts to have a fresh centrate feed applied to the deammonification process by reducing the centrate storage from 7 days to 3 days were intended to eliminate the possible formation of polymer by-products, but no major change was noticed with regard to process performance (data not shown). It is believed that enhanced solids removal in centrate can reduce this inhibition potential since most polymers are embedded in solids.

Impact of low oxygen level in the DEMON reactor

Another significant factor that might have contributed to system vulnerability to ammonia oxidation inhibition was certainly the low oxygen level. With an average dissolved oxygen level of 0.4 mg/L, the nitrification rate decreased significantly as observed during the experiment. A study of oxygen uptake rate predicted a decrease in respiration by about 42% when DO decreases from 0.8 mg/L to 0.4 mg/L, and about 50% when DO decreases from 2 mg/L to 0.4 mg/L (Figure 6.7). The half saturation coefficient for DO (Ks) was estimated to be 0.5 mg/L based on the OUR data, which is comparable to other nitrifying systems (Stenstrom and Poduska, 1980). The oxygen uptake rate data have helped adopt the strategy to deal with ammonia inhibition concerns by increasing the DO levels to counteract their inhibition effect by increased the microbial respiration at higher DO.

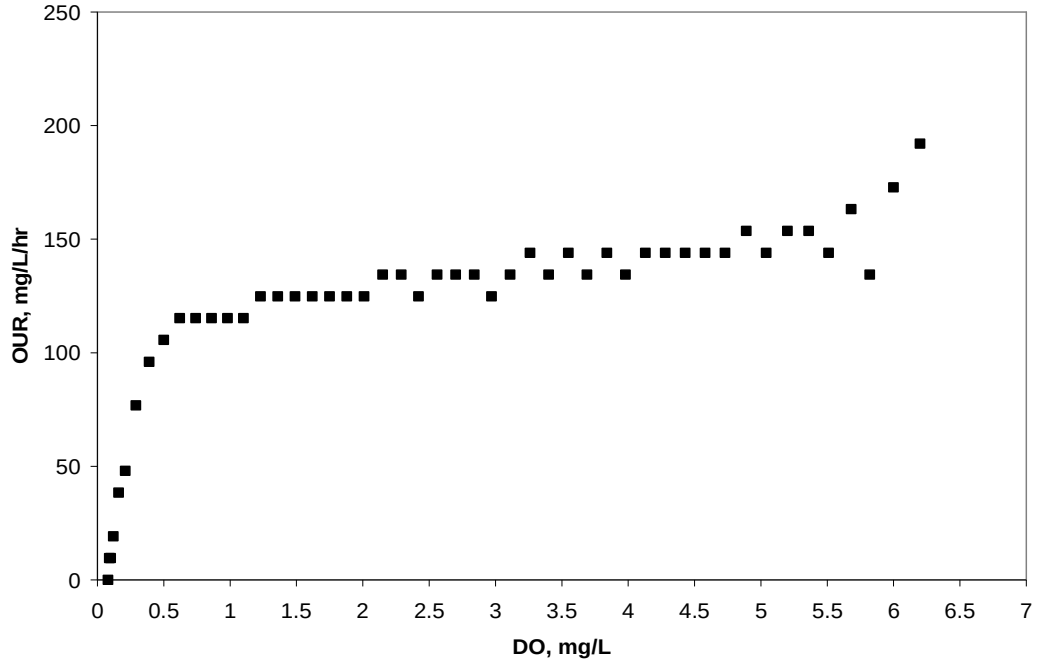

Figure 6.7: Oxygen uptake rate as a function of dissolved oxygen (DO). DO measured by means of YSI meter and stirring probe in a BOD bottle. DO was recorded at intervals of 30 seconds.

6.5. Conclusion

Aerobic ammonia oxidation inhibition in the deammonification process has different variables in play rather than a single compound. Accumulation of potential inhibiting compounds are either generated through the wastewater treatment process or added to enhance the process upstream prior to generating the centrate which is the sole feed to the deammonification process. Potential inhibitors include ferrous iron (when nitrite is also present) and residual cationic

polymer (or breakdown products) and defoaming agents left in the centrate after dewatering.. Enhanced solids removal from centrate and choosing the right defoaming agent could potentially reduce the AerAOB inhibition issues in the deammonification process.

6.6. Acknowledgement

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6.7. References

Anderson I.C. and Levine J. (1986), Relative rates of nitric oxide and nitrous oxide production by nitrifiers, denitrifiers and nitrate respirers, *Applied and Environmental Microbiology*, 51(5): 938-945

Baudisch O. and Welo L.A. (1925), On the aging of ferrous hydroxide and ferrous carbonate, *Journal of Biological Chemistry*, pp 753-770

Chapman R., Dean A., (1982), Action of Iron-complexes on Klebsiella 'pneumoniae (Klebsiella aerogenes), Folia Microbiology, 27:295-302

Cleemput V., Samater A.H. (1996), Nitrite in soils: accumulation and role in the formation of gaseous N. compounds. *Fertilizer Res*. 30(5), 1228-1236.
Coby A., Picardal F., Inhibition of NO_3^- and NO_2^- reduction by microbial Fe(III) reduction: evidence of a reaction between NO_2^- and cell surface-bound Fe^{2+}, *Applied and Environmental Microbiology*, 71(9):5267-5274

Cooper D.C., F. Picardal, A. Schimmelmann and A.J. Coby (2003), Chemical and biological interactions during nitrate and goethite reduction by Shewanella putrefaciens 200. *Appl. Environ. Microbiol*. 69:3517-3525

De Freitas J.M. and Meneghini R., (2001), iron and its sensitive balance in the cell, *Mutation Research* 475, 153-159
Hamersley M.R., Lavik G., Woebken D. (2007), Anaerobic ammonium oxidation in the Peruvian oxygen minimum zone, *Limnol. Oceanography*, 52(3), 923-933

Kappelmeyer U., Kuschk P., Stottmeister U., (2003); Model experiments on the influence of artificial humic compounds on chemodenitrification, *Water, Air and Soil Pollution*, 147(1-4):317-330

Jardin N., Thole D., Wett B., (2008), Treatment of Sludge Return Liquors: Experiences from Operation of Full-scale Plants, WEFTEC.

Jefferson B, Burgess J.E., Pichon A., Harkness J. and Judd S. J., Nutrient addition to enhance biological treatment of grey water, *Water Research* vol 35, No 11, 2702-2711

Mulder J.W., van Loosdrecht M.C.M., Hellinga C., van Kempen R. (2001), Full-scale application of the SHARON treatment of rejection water of digested sludge dewatering, *Water Science and Technology*, Vol 43, No 1, pp 127-134

Mirlohi S., "Hold the Polymer Please", Presentation at Water Environmental Association, Virginia, May 3, 2006

Nielsen J.L., Nielsen P.H., 1998, Microbial nitrate-dependent oxidation of ferrous iron in activated sludge, *Environ. Sci. Technol*., 32, 3556-3561

Philips S., Rabaey K. and Verstraete W. (2003), Impact of iron salts on activated sludge and interaction with nitrite or nitrate, *Bioresource Technology* 88, 229-239

Stenstrom M., Poduska R., (1980); The effect of dissolved oxygen concentration on nitrification, *Water Research*, 14:643-649

Rowland C., Burton G.A., Morrison M.S. (2000), Implication of polymer toxicity in a municipal wastewater effluent, *Environmental Toxicology and Chemistry*, Vol 19(8) pp:2136-2139

Stumm W., and Morgan J.J (1970); Aquatic Chemistry, *Wisley Interscience*, New York

van de Graaf A., Bruijn P., Robertson L., Jetten M., Kuenen G., (1996); Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor, *Microbiology*, 142, 2187-2196

CHAPTER 7:
Engineering Significance

There is no doubt that there is concern about the rising prices of organic carbon sources that are used in conventional nitrification-denitrification processes. Stricter nitrogen removal regulations in the future also imply that the cost increase will continue into the future. Anaerobic ammonia oxidation-based treatment processes are promising solutions to this problem because they eliminate 100% of exogenous organic carbon source demand and 50% of energy demand due to reduced aeration. However, despite a high level of research on this novel technology over the last decade, there is still skepticism over implementing the technology at full-scale. This is justified by the limited number of full-scale systems that utilize the anaerobic ammonia oxidation metabolism into the process scheme so far. This is probably due to the fact that the wastewater industry needs more assurances about the reliability, stability and performance of the process. This study addressed some of these wastewater industry concerns.

The DEMON process pilot study that was operated on dewatering centrate at the Alexandria Sanitation Authority shows that it was indeed a robust process that handled a design load of 0.5 kg NH_4-N/m^3/d with N removal efficiency of 80% without any additional chemical requirement. The loading was even successfully increased to 0.8 NH_4-N/m^3/d without causing a process upset. This removal performance would translate into significant cost savings on energy and chemicals in a full-scale system. Startup was relatively rapid in comparison to a number of other studies that reported problems during start up for up to 350 days (Imajo et al., 2004). This study also provided a unique perspective on the DEMON process control that was required to overcome potential process upset based on operational experience. This could help in the future operation of either pilots or full-scale DEMON processes. We believe that there is still a room to define a clear and consistent strategy for partial ammonia oxidation to nitrite (nitritation) and whether the anammox process should be combined with nitritation in the same reactor, such as with the DEMON, or whether the two processes should be separated. We believe that a single-sludge system such as DEMON has an advantage of nitrite control over the multisludge system. Intermittent aeration allows a limited nitrite production (by AerAOBs) which is directly

consumed (by the AnAOBs). This mutual beneficial relationship allows both AerAOBs and AnAOBs to be subjected to nitrite inhibition. However, the multi-sludge system allows more flexibility in nitritation control and allows a high enrichment in the anammox process. As a result, the nitrogen removal per unit volume is higher compared to a single-sludge system.

This study provides a broader discussion on the most worrying subject about the anammox process, which is that the anammox bacteria have a very low growth rate and therefore will take a significant amount of time to grow. As our study consistently showed, the anammox bacteria were not limiting in the DEMON process at Alexandria Sanitation Authority, based on the fact that no nitrite ever accumulated over the period of study. Instead, ammonia oxidation inhibition seemed to be the primary challenge. A laboratory experiment was conducted to estimate the maximum specific growth rate of anammox bacteria and it was estimated to be 0.017 h^{-1}. Other studies have come to the same conclusion that the anammox bacteria can actually grow faster than some literature suggest. This means that there is good news for the future of this process as the process limitation is not related to the intrinsic property of the microorganisms, but instead to the optimal growth conditions that we provide for them. This implies that it is appropriate to continue research that improves our understanding about the process needs in engineered systems, especially with regard to toxicity and inhibition issues. This study is, to our knowledge, the first to use the high F:M protocol to study the growth rate of the anammox process. The consistency of the data from four different experiments is a good sign that the protocol could be successfully used to study the specific growth rate of anammox bacteria the same way it is used for nitrifying and denitrifying organisms.

Given the interest in nitrite inhibition of anammox that previous studies have highlighted, we have attempted to better understand the nitrite inhibition problem as it relates to anammox. This study suggests that the DEMON sludge can recover from nitrite concentrations as high as 400 mg/L provided that the concentration stress is not sustained. This is also an encouraging finding given that the process can actually be resilient and recover if temporary nitrite inhibition was to happen in a full-scale system. The study also suggests that the DEMON process could withstand a sustained nitrite-N concentration up to 50 mg N/L.

The study also highlights the importance of understanding inhibition issues that might happen in any separate centrate treatment reactor with a long solids retention time. Our suggestion was that any compound that has potential inhibiting effects might not impact the nitrogen removal process because of the dilution effect that happens when centrate is brought back to the biological reactors to be mixed with the rest of wastewater. This might not be the case for a separate centrate treatment reactor where such dilution does not happen. Some chemicals used in the process that may be subject of this discussion include, but are not limited to, a conditioning polymer, a defoaming agent and iron coagulant. Our study has shown that all these chemicals have inhibiting properties at the concentrations tested. We believe that enhanced solids removal by lamella settlers would reduce potential AerAOB inhibition.

In the end, we hope that this work contains helpful information to the designers and operators of the anammox process in general and DEMON process in particular.

References

Imajo U., Tokutomi T., Furukawa K. (2004), Granulation of anammox microorganisms in up-flow reactors, *Water Science and Technology*, 49:5-6

APPENDICES

Appendix A: Data for Chapter 3

Table A: DEMON pilot ammonia, orthophosphate and alkalinity inventory

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
10/9/2006									10/10/2006		450	290	809	2420	1800
10/10/2006	7.7		35	29	0	15	0.1	52	10/11/2006	2794	335	215	843	2740	2040
10/11/2006	7.9		53	41	0	58	0.7	86	10/12/2006		528	324		2710	2060
10/12/2006	7.9	316	35	26	0	59	0.9	113	10/13/2006	2790	82	48	933	3960	2860
10/15/2006	7.8		125	94	0	51	2.4	95	10/16/2006	2350	78	46	921	6140	4420
10/16/2006	7.9		93	71	0	90	3.1	130	10/17/2006		85	57.6		4760	3504
10/17/2006	7.9		88	64	0	106	3.5	123	10/18/2006	3460	98	62	811	5500	4060
10/18/2006	7.9		98	74	0	99	3.3	139	10/19/2006		96	60.8	827	4512	3408
10/22/2006	7.3	270	10	9	0	44	1.8	96	10/23/2006	3450	103	65.6	928	4176	2768
10/23/2006	7.8		24	18	0	105	4.2	125	10/24/2006		126	78	759	5540	3720
10/24/2006	7.7		20	18	0	120	4.8	120	10/25/2006	3570	158	88	773	5700	3920
10/25/2006	7.8		12	10	0	140	5.1	131	10/26/2006		122	90	840	3980	2780
10/26/2006	7.7	233	31	20	0	183	4.3	176	10/27/2006	3220	86	44	784	2960	1980
10/29/2006			50	39	0	145	3.4	161	10/30/2006		210	160	737	3120	2040
10/30/2006	7.6	242	38	32	1	189	3.2	182	10/31/2006	3600	44	30		5140	3640
10/31/2006	7.5		71	57	6	216	2.7	212	11/1/2006	3110	52	34	695	2100	1540
11/1/2006	7.6	240	75	61	28	220	2.4	228	11/2/2006		42	32		4900	3680
11/2/2006	7.6	322	66	54	105	204	2.1	218	11/3/2006	3480	74	68	904	2540	1860
11/5/2006	7.6		49	40	290	287	2.1	321	11/6/2006	3500	42	34	972	4120	3040
11/6/2006	7.7		80	65	324	288	1.4	336	11/7/2006		70	52	978	2400	1720
11/7/2006	8		87	74	140	140	1.8	265	11/8/2006	3580	46	32	888	4100	3060
11/8/2006	8.2		174	143	125	127	1.7	267	11/9/2006		28	18		6100	4480
11/9/2006	8.3	1250	160	128	3	0	3.5	409	11/10/2006	3510	26	20	845	4580	3360
11/12/2006	7.9		64	52	138	138	1.1	215	11/13/2006	2440	22	16	955	2480	1640
11/13/2006	7.7		96	76	166	175	0.7	207	11/14/2006		25	19		2200	1680
11/14/2006	7.7	340	68	60	189	199	0.4	215	11/15/2006	3570	121	82	1063	2460	1900
11/15/2006	7.8		40	28	232	231	0.6	246	11/16/2006		108	80		2720	2120
11/16/2006	7.8	318	118	102	232	204	0.5	197	11/17/2006	3650	68	42		3080	2400
11/19/2006	7.8		86	72	137	135	1.9	173	11/20/2006	3300	24	12	907	5300	4080

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
11/20/2006	7.6	232	46	40	127	141	1.7	177	11/21/2006		72	48		3500	2640
11/21/2006	7.8		72	60	182	119	2.0	152	11/22/2006	3890	74	46	861	3880	3020
11/26/2006	7.9	414	62	38	192	196	1.1	244	11/27/2006	3630	68	30	875	4110	3180
11/27/2006	7.7		80	68	119	117	1.1	134	11/28/2006		102	60	984	3680	3340
11/28/2006	7.8		40	26	91	86	1.1	123	11/29/2006	4110	72	42	1005	4050	3240
11/30/2006	7.8	326	36	34	134	131	0.8	167	12/1/2006	4080	30	16	1015	3980	3180
12/3/2006	7.8	380	30	28	70	67	0.9	101	12/4/2006	3370	30	12	850	3960	3120
12/4/2006	7.5	388	635	540	40	39	0.8	44	12/5/2006	3654	76	52		4460	3620
12/5/2006	7.7	450	88	74	51	50	0.6	63	12/6/2006	4160	72	48	932	4520	3660
12/6/2006	7.8		65	60	70	71	0.6	93	12/7/2006		45	36		4450	3520
12/7/2006	7.8	354	70	64	79	78	0.6	89	12/8/2006	3840	56	38	931	3760	3480
12/10/2006	7.8	360	67	58	58	56	0.8	58	12/11/2006	3700	94	64	1034	4320	3440
12/11/2006	7.8	400	75	64	42	43	1.2	53	12/12/2006	3850	54	34		4433	3600
12/12/2006	7.6	380	102	94	80	74	1.0	130	12/13/2006	3650	49	24.7		4700	3880
12/13/2006	7.5		72	60	79	78	0.9	67	12/14/2006		50	26	762	4600	3660
12/14/2006			102	98	77	80	0.9	96	12/15/2006		52	38	1186	4680	3620
12/17/2006	7.8	368	15	12	25	26	0.7	46	12/18/2006	2900	51	35	704	4460	3600
12/18/2006	7.8		31	26	5	5	0.8	42	12/19/2006		45	30		4490	3590
12/19/2006	7.9	360	53	49	9	10	0.7	31	12/20/2006	2740	25	19	622	4380	3540
12/20/2006	7.7	270	83	67	8	10	0.9	27	12/21/2006	2560	28	15		4380	3500
12/25/2006	8.1	284	77	65	7	7	1.7	17	12/26/2006	3020	66	34		4480	3500
12/26/2006	8.2		59	50	2	2		135	12/27/2006	3520	69	42		4160	3180
12/27/2006	7.6		83	73	80	71	2.0	97	12/28/2006		63	33	803	4140	3300
12/28/2006	7.5	246	70	58	109	108	1.7	105	12/29/2006	3200	60	44	956	4220	3360
1/1/2007	7.6		54	49	60	61	0.9	54	1/2/2007	2900	58	38	908	4280	3480
1/2/2007	7.7		80	68	53	53	0.7	41	1/3/2007	3480	54	30	865	4460	3540
1/3/2007	7.8		154	132	26	23	0.9	36	1/4/2007		40	28	826	4220	3380
1/4/2007	7.6	330	40	28	44	44	0.8	44	1/5/2007	3220	46	28	771	4240	3400
1/7/2007	7.7		76	60	11	11	1.5	47	1/8/2007	2300	44	34	542	6500	5000
1/8/2007	7.6		3948	3812	0	5	1.8	41	1/9/2007		28	22		5880	3812
1/9/2007	7.6		76	54	0	28	1.7	67	1/10/2007	2360	26	16	670	5580	4120
1/10/2007	7.8		138	108	0	18	2.1	81	1/11/2007		30	24		5840	4400
1/11/2007	7.8	538	2960	2240	0	9	2.6	91	1/12/2007	2480	60	40	568	4800	2240
1/15/2007	7.9		47	39	4	19	7.5	142	1/16/2007		69	19	529	4020	3720

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
1/16/2007	7.8		256	192	12	19	7.6	139	1/17/2007	2600	36	16	562	4260	3500
1/17/2007	7.8		35	33	13	17	7.6	131	1/18/2007		39	22	519	4840	3780
1/18/2007			68	58	3	3	8.3	145	1/19/2007		144	99	1019	5220	4040
1/21/2007	7.9	780	92	76	5	5	6.6	146	1/22/2007	3420	54	16	922	4860	3560
1/22/2007	8.1	96	350	260		9	4.0		1/23/2007	3440	64	28	845	4980	3800
1/23/2007									1/24/2007	3240	80	40		4940	3740
1/24/2007	7.8	380	635	475			3.1		1/25/2007	3120	58	26		3740	2780
1/25/2007	7.6	320	104	82	3	77	4.1	87	1/26/2007	3040	62	28	1283	4560	3420
1/28/2007	7.5		55	46	4	90	8.8	132	1/29/2007	3500	86	46	841	5080	3860
1/29/2007	7.7		42	36	2	109	8.1	141	1/30/2007		86	42		5180	3960
1/30/2007	7.6		158	126	4	177	8.1	180	1/31/2007	3170	86	44	876	4460	3260
1/31/2007	7.6		122	102	8	165	0.3	160	2/1/2007		99	60	1012	5400	4160
2/1/2007	7.9	302	54	51	8	213	9.7	219	2/2/2007	3340	92	54		6020	4860
2/4/2007	7.4		38	36	2	79	5.0	115	2/5/2007	3180	74	38	876	4440	3300
2/5/2007	7.7		76	46	2	98	6.5	124	2/6/2007		48	42	764	4320	3240
2/6/2007	7.6		26	22	2	128	8.2	147	2/7/2007	3380	84	56	851	4680	3520
2/7/2007	7.5	520	2800	2140	0	115	10.4	161	2/8/2007	3450	66	42	791	3300	2540
2/8/2007	7.7	490	36	26	0	124	12.5	183	2/9/2007	3220	52	26	847	3680	2760
2/11/2007	7.6	400	290	216	4	195	11.7	226	2/12/2007	3410	94	40	901	2860	2160
2/12/2007	7.7	420	68		50	126	12.2	153	2/13/2007		90			2520	
2/13/2007									2/14/2007	3313	96	44		3240	2780
2/14/2007	7.6	350	76	60	49	52	12.7	65	2/15/2007		58	26	811	2660	2040
2/15/2007	7.7	400	76	64	11	9	12.3	33	2/16/2007	2240	40	30	574	2720	2000
2/19/2007	7.6	420	30	25	13	13	6.8	43	2/20/2007		48	24.8	598	3200	2540
2/20/2007	7.6	400	23	21	11	13	8.2	68	2/21/2007	2460	85	46	782	3360	2660
2/21/2007	7.6	424	17	13	16	18	8.9	61	2/22/2007		132	94.7		3820	3040
2/22/2007	7.7	410	51	43	4	4	13.4	91	2/23/2007	2800	64	44	1022	3580	2880
2/25/2007	7.7	380	128	104		37	9.6	85	2/26/2007	3400	56	26	953	2380	1880
2/26/2007									2/27/2007						
2/27/2007									2/28/2007						
2/28/2007	7.4	280	112	94	1	23	20.1	56	3/1/2007	1960	38	30	517	1250	1090
3/1/2007	7.4	320	21	19	2	14	19.9	48	3/2/2007	3860	113	74.7	864	515	455
3/4/2007	7.3	242	13	11	2	47	13.8	62	3/5/2007	3460	14	8	1221	3400	2840

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
3/5/2007	7.0	224	98	84	1	58	9.4	51	3/6/2007		10	6		7540	5500
3/6/2007	7.3	210	108	90	2	85	9.7	69	3/7/2007	3860	100	60	998	3700	3140
3/7/2007	7.3	215	116	102	1	107	6.0	78	3/8/2007		122	74		3580	2980
3/8/2007	7.3	260	162	132	1	108	4.4	88	3/9/2007	3700	112	66	1175	2660	2220
3/11/2007	7.3	200	144	126	2	138	3.1	100	3/12/2007	3500	48	28	961	3560	2950
3/12/2007	7.3	220	236		1	139	2.5	98	3/13/2007	3460	116		1017	3940	
3/13/2007	7.5	260	260	244	2	147	1.8	106	3/14/2007	3620	108	64	985	3760	3140
3/14/2007	7.4	240	412	340	2	147	1.4	108	3/15/2007	3540	96	50	987	116	92
3/15/2007	7.3	280	464	392	0	181	2.0	145	3/16/2007	1820	94	32	464	3620	2960
3/18/2007	7.2	200	564	464	1	118	1.8	96	3/19/2007	3900	118	86	1022	3220	2720
3/19/2007	7.4	240	76	44	2	136	1.5	121	3/20/2007		130	88		3200	2600
3/20/2007	7.3	260	88	68	1	146	1.3	130	3/21/2007	3820	146	96	1081	2360	2020
3/21/2007	7.4	240	392	320	1	154	1.3	125	3/22/2007		100	65		3260	2740
3/22/2007	7.6	200	88		1	175	1.3	134	3/23/2007	3920	156		1070	4140	
3/27/2007	7.4	280	74	67	0	0	13.0	40	3/28/2007	2000	179	162	533	8075	7438
3/28/2007	7.3	156	150	108	1	9	8.4	29	3/29/2007		37	22	476	3140	2580
3/29/2007	7.3	200	98	87	1	16	5.2	31	3/30/2007	2200	41	28	439	2920	2360
4/1/2007	7.4	280	138	110	2	60	3.7	74	4/2/2007	3540	60	34	943	3200	2620
4/2/2007	7.6	270	116	94	1	76	2.9	72	4/3/2007	3430	52	32			
4/3/2007	7.2	171	1012	794	1	107	2.8	114	4/4/2007	3600	76	46	569	1920	1520
4/4/2007	7.3	160	364	304	1	86	2.4	78	4/5/2007	4080	198	144		1720	1380
4/5/2007	7.4	280	512	412	4	115	1.5	104	4/6/2007	3900	168	120	646	1500	1240
4/8/2007	7.2	282	2860	2280	1	130	0.9	109	4/9/2007	3580	72	48	662	1580	1320
4/9/2007	7.4	200	556	444	3	144	1.0	116	4/10/2007		64	36		1800	1380
4/10/2007	7.4	206	532	420	2	123	0.9	100	4/11/2007	2880	52	32	1507	1760	1420
4/11/2007	7.7	258	136	110	3	148	0.8	138	4/12/2007		172	118		1780	1380
4/12/2007	7.7	279	112	90	0	13	5.6	145	4/13/2007	3920	156	112	1233	2360	1940
4/15/2007	7.8	267	900	700	2	116	3.3	105	4/16/2007	3570	107	73	929	2670	2120
4/16/2007	7.3	210	260	215	2	106	3.2	89	4/17/2007		83	67	946	3260	2620
4/17/2007	7.3	238	80		1	104	3.1	96	4/18/2007	3440	74		931	3000	
4/18/2007	7.2	260	300	228	2	65	3.8	59	4/19/2007		84	62	1081	3480	2660
4/19/2007	7.5	310	216	168	3	68	3.5	76	4/20/2007	3740	78	56	1029	3340	2600
4/22/2007	7.3	280	356		3	70	3.2	109	4/23/2007	3600	58		1043	3620	

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
4/23/2007	7.8	472	54	48	3	106	3.1	150	4/24/2007	3500	32	28		3740	2880
4/24/2007	7.6	480	144	120	3	129	2.9	180	4/25/2007	3520	42	38	981	3220	2400
4/25/2007	7.7	460	92	84	2	75	2.7	99	4/26/2007		44	38		3960	3040
4/26/2007	7.7	520	114		4	156	4.2	216	4/27/2007	4160	120		1131	3480	
4/29/2007	7.7	500	424	328	3	201	2.3	270	4/30/2007	3800	69	44	995	3920	3020
4/30/2007	7.7	480	111	85	3	226	1.8	260	5/1/2007		168	35		3920	2980
5/1/2007	7.7	460	200	150	3	219	1.7	245	5/2/2007	3660	56	36	913	3660	2820
5/2/2007	7.7	427	220	167	2	167	1.5	262	5/3/2007		85	50		3660	2860
5/3/2007	7.6	450	156	124	4	228	1.1	255	5/4/2007	3600	102	56	926	3800	2940
5/6/2007	7.7	430	640	496	2	252	1.0	278	5/7/2007	3800	66	36	985	3920	2960
5/7/2007	7.6	370	256	204	3	239	1.0	232	5/8/2007	3660	66	34		3800	2940
5/8/2007	7.6	380	80	70	5	239	1.3	254	5/9/2007	3320	46	26	910	3340	2660
5/9/2007	7.5	360	544	428	5	241	1.3	266	5/10/2007	4220	192	140		4260	3300
5/10/2007	7.5	390	51		2	249	0.8	281	5/11/2007	4220	94		1287	3860	
5/13/2007	7.4	304	89	73	3	311	0.7	300	5/14/2007	3650	99	63	1140	3820	2960
5/14/2007	7.3	246	127	100	3	326	0.7	312	5/15/2007	3510	92	61		3760	2980
5/15/2007	7.3	230	76	60	4	263	0.9	257	5/16/2007	3660	108	76	832	4240	3320
5/16/2007	7.3	265	58	50	5	311	0.7	303	5/17/2007	3680	96	66		4440	3460
5/17/2007	7.3	250	42	36	4	312	0.6	290	5/18/2007	3600	92	60	1109	4380	3420
5/20/2007	7.4	300	35		3	286	0.7	278	5/21/2007	3260	82		856	4280	
5/21/2007	7.3	380	68	64	2	259	0.8	288	5/22/2007	4240	116	76	1108	4300	3320
5/22/2007	7.5	400	58	64	3	269	0.6	283	5/23/2007	4360	116	80	1168	4250	3560
5/23/2007	7.5	380	106	88	2	295	0.5	305	5/24/2007	4200	140	86	1125	4100	3160
5/24/2007	7.5	340	90	72	3	319	0.5	322	5/25/2007	3960	106	66	1151	4640	3620
5/28/2007	7.5	520	254	170	3	244	0.9	358	5/29/2007	3670	106	74	1096	4560	3780
5/29/2007	7.5	480	46		2	253	1.0	343	5/30/2007	3800	92		998	4440	
5/30/2007	7.6	860	60	58	1	238	1.1	377	5/31/2007	4460	114	104		5380	4200
5/31/2007	7.5	560	248	198	2	282	0.9	319	6/1/2007	4420	96	68	1193	4260	3180
6/3/2007	7.5	520	744	580	2	290	0.8	315	6/4/2007	4220	114	70	1035	4880	3760
6/4/2007	7.5	460	100	81	3	277	0.8	287	6/5/2007		91	57		2257	1771
6/5/2007	7.5	480	113	95	2	271	0.8	303	6/6/2007	4400	135	96	1282	4500	3533
6/6/2007	7.6	396	73	57	2	256	0.8	269	6/7/2007		109	77		4580	3580
6/7/2007	7.5	400	24	21	2	243	0.9	254	6/8/2007	4300	87	60	1147	4080	3220

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
6/10/2007	7.5	320	84	70	3	223	1.1	215	6/11/2007	3550	80	52	1013	4050	3120
6/11/2007	7.5	420	76	60	1	199	1.3	189	6/12/2007	3670	100	58		4280	3300
6/12/2007	7.5	295	32		1	215	1.6	205	6/13/2007	4320	88		1016	4680	
6/13/2007	7.5	315	50		2	246	1.5	246	6/14/2007		76		1070	4360	
6/14/2007	7.5	300	42	38	2	246	1.5	248	6/15/2007	4020	78	52	939	4300	3360
6/17/2007	7.6	291	49	42	2	306	1.7	293	6/18/2007	3870	70	46	906	4800	3780
6/18/2007	7.3	298	23	19	1	247	1.6	243	6/19/2007		69	42		4480	3520
6/19/2007	7.5	310	57	44	1	256	1.8	266	6/20/2007	4480	152	106	1288	4660	3640
6/20/2007	7.3	400	34	30	1	361	1.4	385	6/21/2007		132	92	1196	3980	3080
6/21/2007	7.5	370	30	28	1	370	1.2	383	6/22/2007	4430	126	88	1111	3240	2500
6/24/2007	7.5	340	84	60	2	354	1.2	357	6/25/2007	3660	88	50	1006	4530	3500
6/25/2007	7.5	420	90	68	2	392	1.3	420	6/26/2007		172	114	1103	4620	3600
6/26/2007	7.5	400	88	70	3	351	1.1	370	6/27/2007	4320	154	112	1023	4640	3720
6/27/2007	7.6	480	154	130	3	351	1.0	394	6/28/2007		116	84	1058	4280	3400
6/28/2007	7.6	500	96	78	3	381	1.2	408	6/29/2007	4100	154	130	662	4160	3300
7/1/2007	7.8	590	90	66	3	328	1.5	381	7/2/2007	4100	116	74	1147	4460	3340
7/2/2007	7.6	500	96	70	1	301	1.4	348	7/3/2007	4020	158	132		4600	3760
7/4/2007	7.7	540	94	78	2	300	1.2	349	7/5/2007	4340	126	90	1194	4540	3580
7/5/2007	7.7	500	56	52	3	345	1.1	337	7/6/2007	4500	80	60	1119	4380	3560
7/8/2007	7.5	410	64	55	2	283	1.2	318	7/9/2007	3910	57	36	946	5140	4140
7/9/2007	7.5	480	48		2	315	1.3	342	7/10/2007	4380	86		1226	5380	
7/10/2007	7.6	490	60	52	2	300	1.2	329	7/11/2007	4200	92	62	1181	5340	4320
7/11/2007	7.6		104		2	319	1.2	335	7/12/2007		84		1106	5020	
7/12/2007			80	70	2	295	1.2	352	7/13/2007		88	54	1267	4600	3640
7/15/2007	7.5	490	34	26	2	296	1.1	360	7/16/2007	4620	140	97	730	4940	3940
7/16/2007	7.6	560	25	24	1	307	1.0	356	7/17/2007	4600	164	110		4780	3820
7/17/2007	7.5	515	20	19	1	303	0.8	340	7/18/2007	4360	59	34	1310	6800	5180
7/18/2007	7.6	530	108	90	3	312	0.8	331	7/19/2007	4220	59	35		4580	3640
7/19/2007	7.6	530	21		0	271	0.9	300	7/20/2007	4060	50		1062	4640	
7/22/2007	7.4	720	54		0	257	1.0	351	7/23/2007	4530	44		1250	5700	
7/23/2007	7.5	710	122		0	270	1.2	337	7/24/2007	4510	92			4360	
7/24/2007	7.4	610	126	108	1	256	1.0	331	7/25/2007	4900	82	64	1232	5260	4220
7/25/2007	7.5	600	90		3	267	1.0	300	7/26/2007	4660	86			4780	
7/26/2007	7.3	566	92	82	1	268	1.2	326	7/27/2007	2920	130	96	1288	3780	2980

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
7/29/2007	7.6	720	135	112	3	232	1.5	298	7/30/2007	4780	150	106	1554	4500	3620
7/30/2007	7.6	700	66	58	2	194	1.4	274	7/31/2007	4680	166	116		4160	3420
7/31/2007	7.7	660	*		4	244	1.7	300	8/1/2007	4780	*		1260	*	
8/1/2007	7.6	620	62	56	1	252	1.7	267	8/2/2007		126	88		5340	4230
8/2/2007	7.6	420	124	104	3	263	1.7	252	8/3/2007	4460	148	88	1294	5180	4200
8/5/2007	7.6	390	114	100	2	284	1.9	252	8/6/2007	4240	120	78	1250	5440	4360
8/6/2007	7.5	400	104	94	3	271	2.0	242	8/7/2007		126	68		4720	3760
8/7/2007	7.6	360	98	88	2	274	2.0	240	8/8/2007	4820	70	54	1243	4440	3540
8/8/2007	7.5	340	76	64	2	289	2.2	262	8/9/2007		78	50		4200	4010
8/9/2007	7.6	360	67	53	2	301	2.4	269	8/10/2007	4580	65	41	1384	5020	3980
8/12/2007	7.5	280	92	61	1	240	2.1	204	8/13/2007	4600	64	49	827	4610	3630
8/13/2007	7.5	240	93	75	1	301	2.5	264	8/14/2007		88	61		4340	3480
8/14/2007	7.5	280	108	92	2	301	2.5	263	8/15/2007	3800	100	74	1028	3680	2980
8/15/2007	7.4	290	100	80	2	312	2.5	272	8/16/2007	4150	102	68		3920	3120
8/16/2007	7.5	280	132	82	1	308	2.3	282	8/17/2007	4000	LA	LA	1248	3920	3200
8/19/2007	7.5	260	108	98	2	251	2.2	228	8/20/2007	4300	82	70	934	3820	3140
8/20/2007	7.5	290	78	68	2	229	2.1	213	8/21/2007		86	72	892	4780	3860
8/21/2007	7.6	336	58	52	2	206	2.0	193	8/22/2007	3100	78	68	954	4140	3420
8/22/2007	7.5	340	52	48	2	200	2.0	185	8/23/2007		86	76	935	2720	2280
8/23/2007	7.5	272	70	60	1	192	2.2	184	8/24/2007	3540	80	60	912	3800	3120
8/26/2007	7.4	310	20	16	1	142	3.0	136	8/27/2007	3360	108	78	986	3900	3220
8/27/2007	7.2	284	23	21	2	215	3.0	186	8/28/2007		149	103		3960	3960
8/28/2007	7.4	268	102	88	1	126	3.2	116	8/29/2007	4360	92	66	440	3980	3280
8/29/2007	7.3	256	58	42	2	193	3.6	161	8/30/2007		100	70		3880	3120
8/30/2007	7.2	286	44	40	3	236	3.7	214	8/31/2007	4020	192	146	1101	3860	3120
9/3/2007	7.4	280	28	14	1	248	4.8	192	9/4/2007	3800	120	84	1213	3800	2840
9/4/2007	7.2	240	66	54	1	277	4.7	230	9/5/2007	4380	96	68	1074	3660	2960
9/5/2007	7.3	214	530	434	1	230	5.1	171	9/6/2007		106	78		1620	1340
9/6/2007	7.2	222	154	126	1	221	5.1	168	9/7/2007	4040	120	86	1095	3640	2920
9/9/2007	7.3	224	52	44	1	205	5.3	142	9/10/2007	3420	108	90	1280	3020	2500
9/10/2007	7.4	246	120	110	3	203	5.0	138	9/11/2007		118	80		3000	2520
9/11/2007	7.4	278	454		3	193	5.1	144	9/12/2007	4160	184		1152	1700	
9/12/2007	7.3	270	92	74	3	194	5.8	143	9/13/2007		120	88		3100	2460
9/13/2007	7.4	260	356	294	3	193	6.4	148	9/14/2007	3800	164	132	1000	3040	2520

Pilot Effluent (Comp sample)									Pilot FEED (Grab sample)						
Comp date									Grab date						
9/16/2007	7.3	248	45	41	2	202	7.8	146	9/17/2007	3100	150	120	1096	2240	1830
9/17/2007	7.3	242	405	332	2	201	8.4	140	9/18/2007		109	87		1827	1460
9/18/2007	7.3	156	177	47	2	211	8.5	141	9/19/2007	3120	109	88	1089	3610	2910
9/19/2007	7.3	230	196	160	2	197	8.7	144	9/20/2007		88	72		2830	2320
9/20/2007	7.3	240	131	109	2	202	9.1	148	9/21/2007	3600	158	126	1012	3390	2770
9/23/2007	7.2	220	128		1	207	9.7	164	9/24/2007	2900	86		975	3200	
9/24/2007	7.4	240	108	100	1	196	9.6	156	9/25/2007		94	74	1038	3760	3040
9/25/2007	7.3	220	112	96	2	197	9.6	154	9/26/2007	3800	108	80	995	3540	2900
9/26/2007	7.3	202	112	94	2	199	9.8	141	9/27/2007		84	62		3340	2660
9/27/2007	7.4	170	102	82	2	196	9.5	145	9/28/2007	3520	76	48	1142	2840	2280
9/30/2007	7.4	222	28		2	211	11.0	156	10/1/2007	4160	90		1231	3720	
10/1/2007	7.2	180	174	142	3	226	11.2	165	10/2/2007		80	60		3740	3060
10/2/2007	7.1	164	120	98	1	254	11.4	195	10/3/2007	4180	90	62	1020	3800	3060
10/3/2007	7.2	186	130	104	2	254	11.8	192	10/4/2007		96	80	1112	3940	3160
10/4/2007	7.2	132	70	62	2	256	11.9		10/5/2007	3340	112	90		4100	3300
10/8/2007	7.1	120	74	58	2	245	10.6	172	10/9/2007	3320	136	108	1096	4180	3540
10/9/2007	7.0	120	102	94	3	337		236	10/10/2007	3580	94	88	537		
10/10/2007	7.1	100	166	144	2	237		199	10/11/2007		102	84		4080	3380

Appendix B: Nitrogen Balance for DEMON reactor

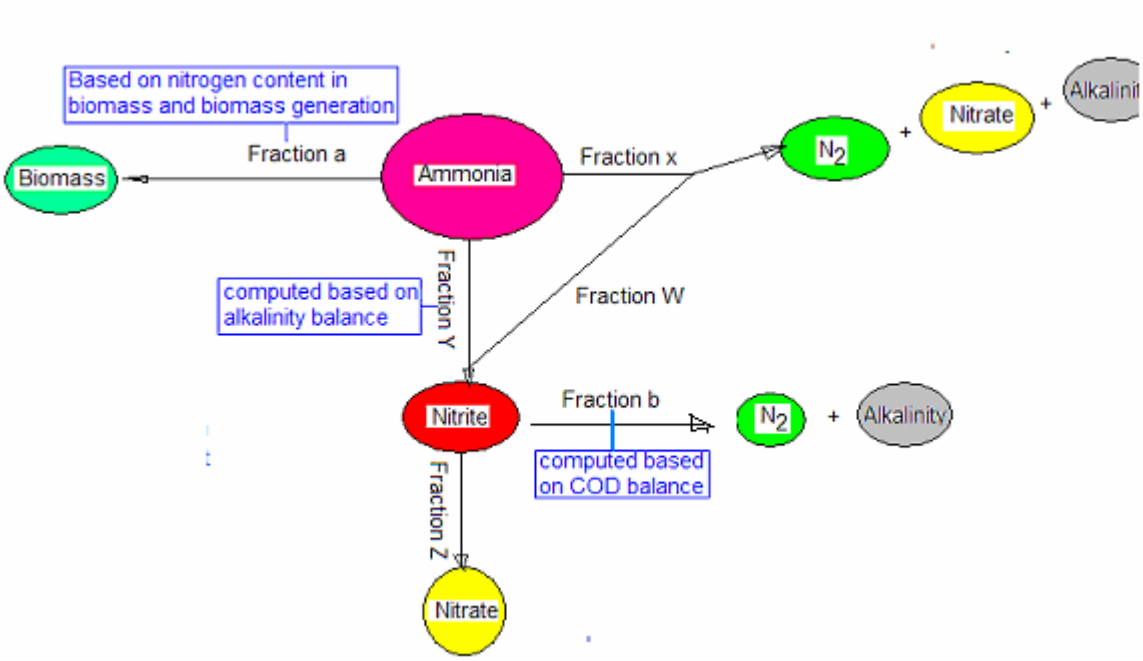

Figure B: Nitrogen mass balance model in DEMON process

The model assumed that Denitrifiers prefer nitrite over nitrate,

Equations

$$a + x + y + [NH_4 - N]_e = [NH_4 - N]_{in}$$
$$y - z - w + [NO_2]_{in} - b = [N$$
$$z + 0.26x = [NO3 - N]_{out}$$
$$[Alk]_{in} - 7.14y + 3.56b = [Alk]_{out}$$
$$b = \frac{(CODs_{in} - CODs_{out})}{2.9}$$

Appendix C: Derivation of Equation 4.4

Mass balance on a biological reactor:

Accumulation = inflow – outflow +generation

- Mass balance on Anammox bacteria biomass concentration X_{An}

$$V\frac{dX_{An}}{dt} = 0 - X_{An} \times Q + (\mu_{An} - b_{An})X_{An}V \qquad \text{(C1)}$$

where

V = Volume [L^3]

μ_{An} = the anammox growth rate [T^{-1}]

b_{An} = Anammox endogeneous decay [T^{-1}]

x_{An} = the anammox biomass [$M\ L^{-3}$]

Q = the inflow and outflow rate [$L^3\ T^{-1}$]

t = time [t]

Q = Discharge [M^3T]

Dividing by V on both sides, we have

$$\frac{dX_{An}}{dt} = 0 - X_{An} \times \frac{Q}{V} + (\mu_{An} - b_{An})X_{An} = X_{An} \times (\mu_{An} - b_{An} - \frac{Q}{V})$$

$$\frac{dX_{An}}{X_{An}} = (\mu_{An} - b_{An} - \frac{Q}{V})\partial t$$

$$\int_{X_{An,0}}^{X_{An,t}} \frac{dX_{An}}{X_{An}} = \int_0^t (\mu_{An} - b_{An} - \frac{Q}{V})dt \qquad \text{(C2)}$$

Integrating equation (3b), we get the nitrifying bacteria biomass concentration with respect to time

$$X_{An,t} = x_{An,o}.e^{(\mu_{An} - b_{An} - \frac{Q}{V})t} \qquad \text{(C3)}$$

For a batch reactor, Q = 0, and equation (C3) becomes

$$X_{An,t} = x_{An,o}.e^{(\mu_{An} - b_{An})t} \qquad \text{(C4)}$$

- Basic equation for nitrite loss during the anammox batch experiment:

$$V\frac{d[NO_2^-]_t}{dt} = -\frac{\mu_{An}X_{An,t}}{Y_{An}}V$$

where Y_{An} is the tield coefficient, mg biomass/mg-N consumed

$$\frac{d[NO_2^-]_t}{dt} = -\frac{\mu_{An}X_{An,t}}{Y_{An}} \qquad \text{(C5)}$$

Substituting equation (C4) into (C5), we get

$$\frac{d[NO_2^-]_t}{dt} = -\frac{\mu_{An}X_{An,0}}{Y_{An}}e^{(\mu_{An} - b_{An})t}$$

$$\int d[NO_2^-]_t = -\int \frac{\mu_{An}X_{An,0}}{Y_{An}}e^{(\mu_{An} - b_{An})t}dt$$

$$\left[[NO_2^-]_t\right]_0^t = -\left[(\frac{1}{\mu_{An} - b_{An}})\frac{\mu_{An}X_{An,0}}{Y_{An}}e^{(\mu_{An} - b_{An})t}\right]_0^t$$

$$[NO_2^-,_t] - [NO_2^-,_0] = -[\frac{\mu_{An}X_{An,o}}{(\mu_{An} - b_{An})Y_{An}}e^{(\mu_{An} - b_{An})t} - (\frac{1}{\mu_{An} - b_{An}})\frac{\mu_{An}X_{An,0}}{Y_{An}}e^{(\mu_{An} - b_{An}).0}]$$

$$\boxed{[NO_2^-,_t] = [NO_2^-,_0] - \frac{\mu_{An}X_{An,o}}{Y_{An}(\mu_{An} - b_{An})}[e^{(\mu_{An} - b_{An})t} - 1]} \qquad \text{(C6)} \equiv \text{Equation 4.4}$$

Appendix D: Most Common Growth Models Used in Microbiology

1. Baranyi Model

The Baranyi Model considers that the biomass growth can be estimated by the equation

$$\mu(t) = \frac{dx}{x(t)dt} = \mu_{\max}\alpha(t) f(t) \qquad \text{(D1)}$$

in which:

x is the biomass density,

α(t) is an adjustment function describing the adaptation of the biomass to its new environment and

f(x) is an inhibition function describing the end-of-growth substrate limitation.

The adjustment function α(t) was defined as

$$\alpha(t) = \frac{q(t)}{1+q(t)} \quad \text{with } \frac{dq}{dt} = vq \qquad \text{(D2)}$$

where

q(t) represents the physiological state of the biomass at any time,

qo is the physiological state of the inoculum. The coefficient

ν is the coefficient which is estimated to be equal to μmax.

In the absence of inhibition, as is in high F: M conditions, equation (D1) becomes

$$\frac{dx}{xdt} = (\mu_{\max})\frac{q_o e^{\mu_{\max} t}}{1+q_o e^{\mu_{\max} t}}. \qquad \text{(D3)}$$

The solution is of equation (c) is $x = xo\left[\frac{1+q_o e^{\mu_{\max} t}}{1+q_o}\right]^{\frac{\mu_{\max}-b}{\mu_{\max}}}$ (D4)

Combining equation (D4) and equation (C5), we get a system of equations describing the nitrite consumption profile under Baranyi Model.

2 Hills and Wright Model

This is a cell-structured model assuming that there is a minimum biomass required for the cell to start the chromosomal replication. The chromosomal replication ν and non-chromosomal synthesis μ_{max} are both constant, defined by the system of equations:

$$\begin{cases} \frac{dm}{dt} = \mu_{\max} m, \text{ with } m(t=0) = x_o & \text{(D5)} \\ \frac{dx}{dt} = vx\left(\frac{m-x}{x}\right) \text{ with } x(t=0) = x_o & \text{(D6)} \end{cases}$$

where

m is the total biomass concentration per cell,
x is the cell concentration and t the time

The system solution is given in equation (D7). Equation (C5) and (D7) describe the theoretical nitrite consumption over time.

$$x = \frac{x_o}{\mu_{\max} + \nu}(\nu e^{\mu_{\max} t} + \frac{\mu_{\max}}{e^{\nu t}}) \qquad \text{(D7)}$$

3. McKellar Model

McKellar's model is structured-population model assuming that within the inoculum, part of biomass x_G will grow exponentially right away without delay while the other, x_{NG}, will never grow. This model was represented by the system of equations

$$\begin{cases} \frac{dx_G}{dt} = x_G \mu_{\max} \text{ with } x_G(t=0) = x_{G0} = \alpha_o x_o \\ \frac{dx_{NG}}{dt} = 0 \quad \text{with } x_{NG}(t=0) = x_{NGo} = (1-\alpha_0)x_o \\ x(t) = x_G(t) + x_{NG}(t) \end{cases} \qquad \text{(D8)}$$

where
xG is the number of immediate growing cells,
xNG is the number of non growing cells,
xo is the initial total cell concentration
αo is the proportion of growing cells in the population and
x is the total number of population

Appendix E: Data for Chapter 4

Table E1: Estimation of the maximum growth rate using the high F:M growth model, Initial nitrite: 103 mg/L as N

Nov 2-7, 2007			NO_2-N, o	103	mg/L			
			μ_{max}	0.39	d^{-1}			
			$X_{An,o}$	2	mg/L			
			b_{An}	0.019	d^{-1}			
			Yield	0.114				
			RSS	57				
		Measured	measured	calculated	Delta	Estimated Mass of nitrifiers		
	Time (days)	Ammonia	NO2-N	NO2-N			F/M	Total N
1	0.0	321	103	103	0	2	49	424
2	0.6	336	99	98	1	3	38	434
3	1.1	333	97	93	11	3	31	430
4	1.7	293	86	86	1	4	22	380
5	2.1	313	86	81	22	4	19	398
6	2.6	273	70	71	2	5	13	343
7	3.2	257	57	59	4	7	8	314
8	3.7	230	45	48	10	8	6	275
9	4.3	203	29	29	1	10	3	232
10	4.7	194	16	14	5	12	1	211
11	5.2	172	2	-8	99	14	0	174

Table E2: Estimation of the maximum growth rate using the high F:M growth model, Initial nitrite: 93 mg/L as N

Nov 8-12, 2007			NOx-N, o	93	mg/L			
			µmax	0.36				
			Xn,o	8				
			kd	0.019				
			Yield	0.114				
		Measured	measured	calculated	Delta	Estimated Mass of nitrifiers		
	Time (days)	Ammonia	NO2-N	NO2-N	0.6		F/M	Total N
1	0.0	321	93	93	0.1	8	41	414
2	0.7	336	74	73	0.3	10	34	409
3	1.8	333	34	34	0.3	14	23	367
4	2.3	293	10	10	0.1	17	17	303

Table E3: Estimation of the maximum growth rate using the high F:M growth model, Initial nitrite: 184 mg/L as N

Nov 19-27, 2007			μmax	0.23				
			NOx-N, o	178	mg/L			
			Xn,o	3				
			kd	0.019				
			Yield	0.114				
						Estimated Mass of nitrifiers		
		Measured	measured	calculated	Delta			
	Time (days)	Ammonia	NO2-N	NO2-N	247.5		F/M	Total N
1	0.0	671	184	178	40.3	3	203	855
2	1.0	645	173	170	5.8	4	158	818
3	1.4	614	164	167	11.8	4	139	777
4	1.9	596	153	162	85.3	5	121	749
5	3.0	576	146	150	13.9	6	93	723
6	4.4	555	137	129	61.6	8	66	691
7	4.9	537	122	121	1.2	9	58	658
8	5.4	535	114	110	14.1	10	52	649
9	5.9	525	97	99	3.6	12	45	622
10	6.3	501	84	88	10.0	13	39	585

Table E4: Estimation of the maximum growth rate using the high F:M growth model, Initial nitrite: 128 mg/L as N

Nov 19-24, 2007		NO_2-$N_{,o}$	128.4097	mg/L		
		μ_{max}	0.42			
		$X_{An,o}$	2.034087			
		b_{An}	0.019			
		Yield	0.114			
					Estimated Mass of nitrifiers	
		measured	calculated	Delta		
Sample	Time (days)	NO2-N	NO2-N	845.8		F/M
1	0.0	126	128	6.0	2	62
2	0.4	128	125	7.8	2	52
3	0.9	120	120	0.0	3	40
4	1.4	110	114	12.4	4	30
5	1.8	100	108	66.6	4	24
6	2.4	106	97	75.6	5	20
7	3.0	102	86	278.4	7	15
8	3.3	75	75	0.0	8	10
9	4.0	35	54	369.8	10	3
10	5.0	13	8	29.1	15	1
11	6.0	2	-61	3859.5	23	0

Table E5: Estimation of the maximum growth rate using the high F:M growth model, Initial nitrite: 113 mg/L as N

Feb 2-13, 2008			NOx-N, o	113	mg/L			
			µmax	0.40	d^{-1}			
			Xn,o	3	mg/L			
			kd	0.019	d^{-1}			
			Yield	0.114	gCOD/gN			
					Estimated Mass of anammox biomass			
	Measured	measured	calculated	Delta				
Time (days)	Ammonia	NO2-N	NO2-N	120.2		F/M	Total N	Time, hr
0.0	213	107	113	30.4	3	41	320	0
0.6	183	107	107	0.1	3	33	290	14.417
1.0	164	106	102	18.2	4	28	270	24.083
1.7	144	95	91	17.9	5	19	239	41.250
2.7	99	71	70	0.5	7	10	170	64.750
3.5	82	44	47	8.8	10	4	125	83.917
4.0	63	24	29	27.0	12	2	88	95.083
4.5	45	8	3	17.3	14	1	52	108.967
5.3	37	0	-38	1433.8	19	0	37	126.050

Table E6: Estimation of maximum specific growth rate using common growth models used in Microbiology for November 2-7, 2007 data -Baranyi and Robert Model-

Baranyi and Robert Model			Init NO2		103	
			Max growth	µmax	0.22	
			physiol state	q	0.14	
			Start Xo	Xo	4	
			Yield	Yan	0.114	
			Decay	bAn	0.019	
			RSE		3.24	
Time	Measured	Measured	Measured	Calculated	RSS	Xn(t)
	ammonia	nitrate	nitrite	nitrite		
0.0	321	16	103	103	0.0	4.0
0.6	336	19	99	97	1.3	4.3
1.1	333	16	97	93	13.7	4.6
1.7	293	18	86	85	1.3	5.1
2.1	283	23	86	81	22.6	5.4
2.6	273	25	70	72	2.7	6.1
3.2	257	27	57	60	8.2	6.8
3.7	230	28	45	49	17.7	7.6
4.3	203	29	29	29	0.0	8.8
4.7	194	27	16	14	5.7	9.7
5.2	172	27	2	-10	154.0	11.2

Table E7: Estimation of maximum specific growth rate using common growth models used in Microbiology for November 2-7, 2007 data

- Hills and Wright Model-

Hills and Wright Model			Init NO2		103
			Max growth	µmax	0.25
			chromos repr	v	1
			Start Xo	Xo	3.6
			Assumed Yield	Yan	0.114
			Decay	bAn	0.019
			RSE		2.8
Time	**Measured**	**Calculated**	**RSS**	**Xn(t)**	
0.0	103	103	0.0	3.6	
0.6	99	98	1.0	3.8	
1.1	97	93	11.4	4.0	
1.7	86	86	0.6	4.5	
2.1	86	81	20.2	4.8	
2.6	70	72	2.3	5.4	
3.2	57	59	5.2	6.2	
3.7	45	48	11.6	6.9	
4.3	29	29	0.2	7.9	
4.7	16	15	3.0	8.6	
5.2	2	-7	76.5	9.7	

Table E8: Estimation of maximum specific growth rate using common growth models used in Microbiology for November 2-7, 2007 Data

-McKellar Model-

McKellar		Init NO2		102.734
		Max growth	µmax	0.242978
		XG portion	v	1
		Start Xo	Xo	3.107851
		Assumed Yield	Yan	0.114
		Decay	bAn	0.019
		RSE		2.656435
	Measured	Calculated		
Time	nitrite	nitrite	RSS	Xn(t)
0.0	103	103	0.0	3.1
0.6	99	98	0.4	3.6
1.1	97	94	9.1	4.0
1.7	86	86	0.4	4.6
2.1	86	81	19.7	4.9
2.6	70	71	2.0	5.6
3.2	57	59	4.4	6.4

3.7	45	48	10.5	7.0
4.3	29	29	0.3	8.1
4.7	16	15	2.6	8.8
5.2	2	-6	68.8	9.9

Appendix F: Data for Chapter 5

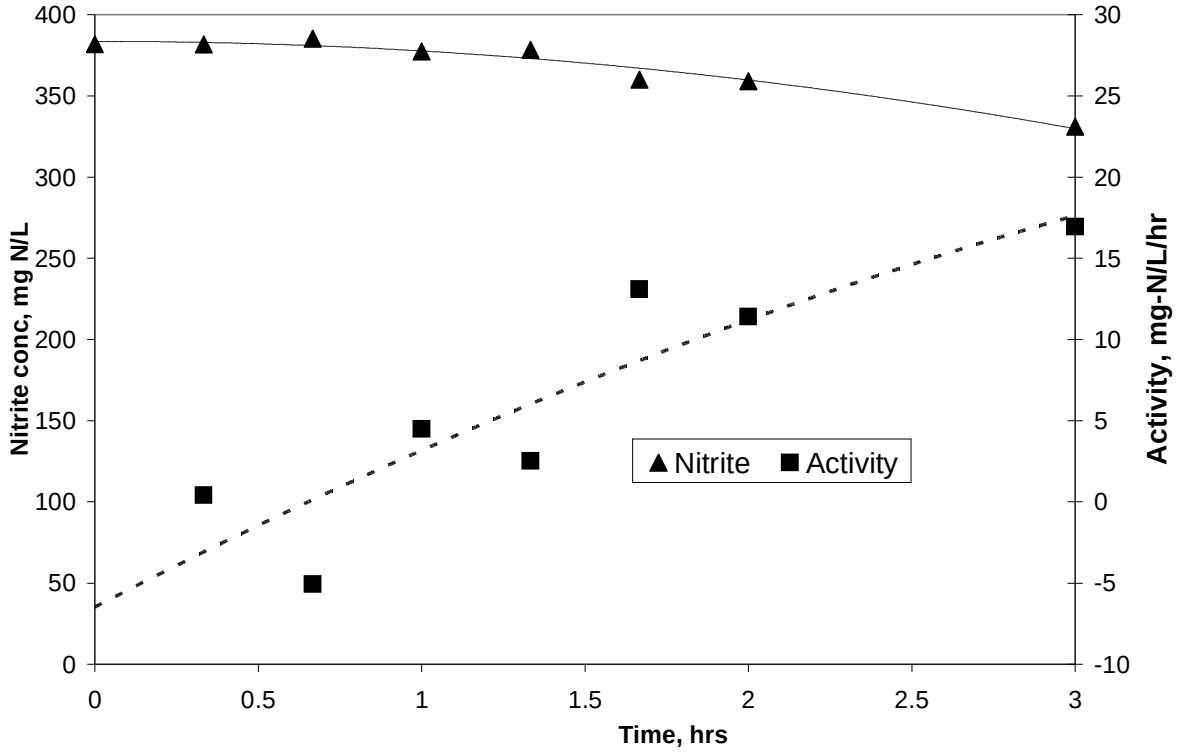

Figure F1: Anammox process response to nigh nitrite loading in a batch test. Activity increases for the first three hours.

Table F1: Data for Figure 5.2; AnAOB activity in DEMON biomass as a function of nitrite concentration as determined with the short-term batch experiment protocol. Experiment 1

		Data For Figure 5.2						
Reactor 1								
		Cumulative time		**NOx**	**NH3**	**NO2**	**NO3**	**TIN**
		minutes	hr					
11/9/2007 18:15	0	0	0.00	102	215	45	57	317
11/9/2007 18:35	20	20	0.33	96	221	37	59	317
11/9/2007 19:10	35	55	0.92	85	208	24	61	293
11/9/2007 19:35	25	80	1.33	74	203	11	63	278
11/9/2007 19:58	23	103	1.72	66	198	3	62	264
Reactor 2								
		Cumulative time		**NOx**	**NH3**	**NO2**	**NO3**	**TIN**
		minutes	hr					
11/9/2007 18:16	0	0	0.00	221	344	156	65	565
11/9/2007 18:36	20	20	0.33	216	304	144	73	521
11/9/2007 19:11	35	55	0.92	205	301	135	70	506
11/9/2007 19:36	25	80	1.33	195	288	121	75	483
11/9/2007 19:59	23	103	1.72	188	290	106	82	478
11/9/2007 20:16	17	120	2.00	185	285	104	81	470
11/9/2007 22:31	135	255	4.25	127	231	47	79	358
Reactor 3								
		Cumulative time		**NOx**	**NH3**	**NO2**	**NO3**	**TIN**
		minutes	hr					

11/9/2007 18:17	0	0	0.00	390	409	332	58	799
11/9/2007 18:37	20	20	0.33	394	441	335	59	835
11/9/2007 19:12	35	55	0.92	378	405	318	60	783
11/9/2007 19:37	25	80	1.33	376	421	295	80	797
11/9/2007 20:00	23	103	1.72	387	448	304	83	836
11/9/2007 20:17	17	120	2.00	359	403	296	63	762
11/9/2007 22:32	135	255	4.25	411	361	291	120	773
11/10/2007 9:45	673	928	15.47	246	201	117	129	447
11/10/2007 11:20	95	1023	17.05	126	169	8	118	295

Table F2: Data for Figure 5.2; AnAOB activity in DEMON biomass as a function of nitrite concentration as determined with the short-term batch experiment protocol. Experiment 2

		Elapsed					Activity
R1	**Time**	**Time (min)**	**Time (hrs)**	**NO2 Reading**	**Dilution**	**Conc**	
	10:00	0	0.0	0.2636	50	13	
	10:05	5	0.1	0.1932	50	10	42.2
	10:10	10	0.2	0.1578	50	8	31.7
	10:15	15	0.3	0.1294	50	6	26.8
	10:20	20	0.3	0.0744	50	4	28.4
	10:25	25	0.4	0.0633	50	3	24.0
	10:30	30	0.5	0.0072	50	0	25.6
	10:35	35	0.6	0.0023	50	0	22.4
	10:40	40	0.7	0.0054	50	0	19.4
	Avrg Activity						27.6
R2	10:02	0	0.0	0.2231	200	45	

	10:23	21	0.4	0.1569	200	31	37.8
	10:43	41	0.7	0.1007	200	20	35.8
	11:00	58	1.0	0.093	200	19	26.9
	11:20	78	1.3	0.0432	200	9	27.7
	11:40	98	1.6	0.019	200	4	25.0
	12:00	118	2.0	0.0031	200	1	22.4
	Avrg Activity						29.3
R3	10:03	0	0.0	0.4522	200	90	
	10:24	21	0.4	0.4013	200	80	29.1
	10:44	41	0.7	0.3862	200	77	19.3
	11:02	59	1.0	0.3517	200	70	20.4
	11:22	79	1.3	0.2911	200	58	24.5
	11:43	100	1.7	0.2723	200	54	21.6
	12:01	118	2.0	0.2407	200	48	21.5
	Avrg Activity						22.7
R4	10:04	0	0.0	0.4771	400	191	
	10:23	19	0.3	0.489	400	196	-15.0
	10:43	39	0.7	0.4773	400	191	-0.1
	11:04	60	1.0	0.4705	400	188	2.6
	11:23	79	1.3	0.4419	400	177	10.7
	11:44	100	1.7	0.4288	400	172	11.6

	12:04	120	2.0	0.4231	400	169	10.8
	Activity						8.9

Table F3: Data for Figure 5.2-5.3; AnAOB activity in DEMON biomass as a function of nitrite concentration as determined with the short-term batch experiment protocol. Experiment 3

	Data for Figure 5.2and 5.3							
				Reactor 1				
Sample ID								
	Time	Time elapsed)		NOx-N	NH4-N	NO2-N	NO3	TIN
		min	hr	mg/L	mg/L	mg/L	mg/L	mg/L
1	19:00	0	0.0	324	217	95	230	542
2	19:20	20	0.3	328	215	88	241	543
3	19:40	40	0.7	323	207	77	246	531
4	20:00	60	1.0	311	199	72	239	511
5	20:20	80	1.3	303	194	51	252	497
6	20:40	100	1.7	296	185	38	258	481
7	21:00	120	2.0	289	178	27	262	468
8	22:00	180	3.0	268	158	1	267	427
Reactor 2								
	Time			NOx-N	NH4-N	NO2-N	NO3	TIN
1	19:00	0	0.0	451	313	220	231	764
2	19:20	20	0.3	467	321	224	243	788

3	19:40	40	0.7	452	308	210	243	761
4	20:00	60	1.0	453	308	208	245	761
5	20:20	80	1.3	444	301	197	247	745
6	20:40	100	1.7	450	295	205	246	746
7	21:00	120	2.0	424	283	178	246	707
8	22:00	180	3.0	410	268	155	254	677
9	8:45	825	13.8	281	137	8	273	418
Reactor 3								
	Time			NOx-N	NH4-N	NO2-N	NO3	TIN
0	19:00	0	0.0	601	439	382	219	1040
0.333333	19:20	20	0.3	603	465	382	221	1068
0.666667	19:40	40	0.7	620	445	385	235	1065
1	20:00	60	1.0	616	456	377	238	1072
1.333333	20:20	80	1.3	625	439	379	246	1064
1.666667	20:40	100	1.7	602	435	360	242	1037
2	21:00	120	2.0	603	435	359	244	1038
3	22:00	180	3.0	573	407	331	242	980
13.75	8:45	825	13.8	404	256	122	282	660
16	11:00	960	16.0	332	175	45	287	508
18	1:00	1080	18.0	314	181	4	310	495
Control								
1	9:45	0	0.0	234	138	1	233	372
2	10:00	15	0.3	245	143	1	244	389
3	10:15	30	0.5	248	144	1	247	392

4	10:30	45	0.8	237	137	0	237	374
5	10:45	60	1.0	247	143	1	246	390
6	11:00	75	1.3	242	140	1	241	381
Second spike of R3								
				NOx-N	NH4-N	NO2-N	NO3	TIN
20	9:45	0	0.0	318	163	42	276	481
20.25	10:00	15	0.3	301	147	37	264	448
20.5	10:15	30	0.5	293	153	28	265	446
20.75	10:30	45	0.8	292	144	24	267	436
21	10:45	60	1.0	285	148	14	271	433
21.25	11:00	75	1.3	284	134	6	278	419

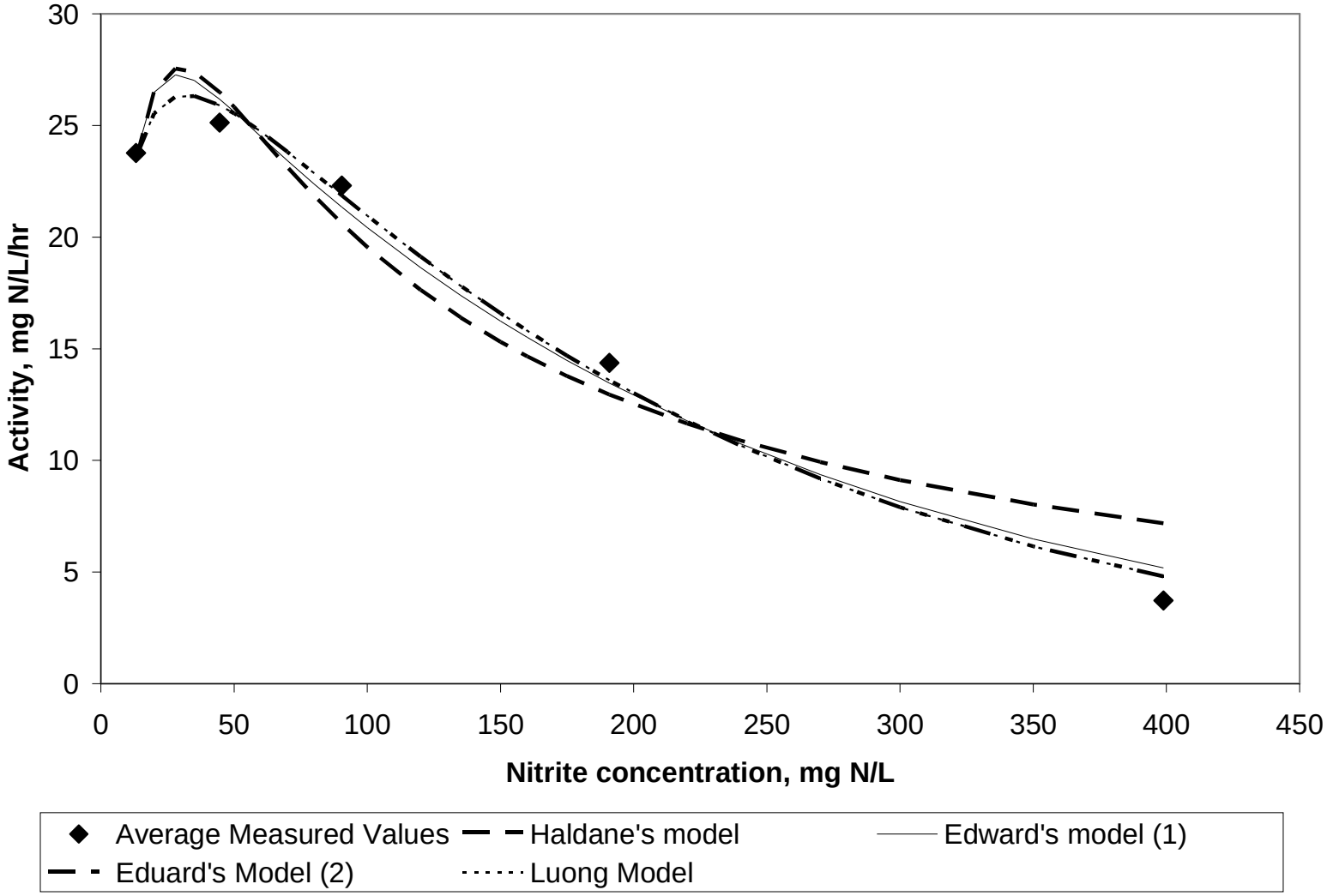

Figure F2: Growth models fit to activity versus nitrite exposure data collected from short-term batch experiment inhibition experiments.

Table F4: Effect of sustained nitrite concentration on AnAOB activity in a long-term, fed batch experiment., Experiment 1

			Feed Conc		21	mg/ml of Nitrite			
			Time	Time	NO2	Nox	NH4	NO3	Vol added
	Time		min	hrs	mg/L	mg/L	mg/L	mg/L	mL
1	9/12/2007 20:45	0	0	0	0.32	51	120	51	0
2	9/12/2007 21:45	60	60	1	0.58	49	116	49	0
3	9/12/2007 22:50	65	125	2	0.20	44	112	44	0
4	9/12/2007 23:50	60	185	3	0.12	40	106	40	0
5	9/13/2007 0:50	60	245	4	0.06	38	104	38	0
6	9/13/2007 1:50	60	305	5	0.25	37	103	36	0
7	9/13/2007 2:50	60	365	6	0.07	34	99	34	0
8	9/13/2007 3:50	60	425	7	0.08	32	96	32	0
9	9/13/2007 4:50	60	485	8	0.11	29	94	28	0
10	9/13/2007 5:50	60	545	9	0.00	27	90	27	0
11	9/13/2007 7:00	70	615	10	0.00	24	84	24	0
	Time				NO2	Nox	NH4	NO3	Vol added
1	9/12/2007 20:46	0	0	0	43	95	164	52	8
2	9/12/2007 21:46	60	60	1	27	87	155	60	6
3	9/12/2007 22:51	65	125	2	47	113	183	66	9
4	9/12/2007 23:51	60	185	3	57	134	202	76	10
5	9/13/2007 0:51	60	245	4	57	142	216	85	10

6	9/13/2007 1:51	60	305	5	62	160	235	98	10
7	9/13/2007 2:51	60	365	6	48	154	231	106	7
8	9/13/2007 3:51	60	425	7	61	176	255	115	9
9	9/13/2007 4:51	60	485	8	48	169	246	121	7
10	9/13/2007 5:51	60	545	9	43	172	253	130	7
11	9/13/2007 7:01	70	615	10	0	134	214	134	0
	Average				50	145	220	95	
	Time				NO2	Nox	NH4	NO3	Vol added
1	9/12/2007 20:47	0	0	0	91	144	208	52	15
2	9/12/2007 21:47	60	60	1	39	106	179	67	6
3	9/12/2007 22:52	65	125	2	52	127	201	76	10
4	9/12/2007 23:52	60	185	3	107	187	254	79	20
5	9/13/2007 0:52	60	245	4	107	209	287	101	10
6	9/13/2007 1:52	60	305	5	118	225	303	107	10
7	9/13/2007 2:52	60	365	6	108	225	299	117	7
8	9/13/2007 3:52	60	425	7	113	237	317	124	9
9	9/13/2007 4:52	60	485	8	107	240	321	133	7
10	9/13/2007 5:52	60	545	9	100	239	321	140	7
11	9/13/2007 7:02	70	615	10	48	193	280	145	0
	Average				95	199	276	105	
					NO2	Nox	NH4	NO3	Vol added

1	9/12/2007 20:48	0	0	0	186	244	313	58	31
2	9/12/2007 21:48	60	60	1	144	213	287	70	6
3	9/12/2007 22:53	65	125	2	195	281	355	86	25
4	9/12/2007 23:53	60	185	3	167	263	344	96	6
5	9/13/2007 0:53	60	245	4	204	309	396	105	15
6	9/13/2007 1:53	60	305	5	218	336	420	118	10
7	9/13/2007 2:53	60	365	6	209	334	421	125	8
8	9/13/2007 3:53	60	425	7	211	345	436	134	7
9	9/13/2007 4:53	60	485	8	214	353	445	139	7
10	9/13/2007 5:53	60	545	9	212	353	449	141	7
11	9/13/2007 7:03	70	615	10	167	313	403	146	0
	Average				197	310	395	113	

Table F5: Effect of sustained nitrite concentration on AnAOB activity in a long-term, Fed- batch experiment., Experiment 3

Reactor 1							
		Dilution	Conc	Cylinder vol	Added Vol	Added mass	NO2 Activity
			mg N/L				
0	0.0214	50	1.1	100	0	0.0	
1	0.0802	50	4.0	95.5	4.5	25.4	22.4
2	0.0034	50	0.2	91	9	50.8	25.8
3	0.0925	50	4.6	85	15	84.6	27.0
4	0.0248	50	1.2	82	18	101.5	25.3
5	0.0631	50	3.2	76	24	135.4	26.7
6	0.0592	50	3.0	70	30	169.2	27.9
7	0.0084	50	0.4	66	34	191.8	27.5
8	0.1207	50	6.0	63	37	208.7	25.5
	Average		3				
Reactor 2							
		Dil	Conc	Cylinder vol	Added Vol	Added mass	
0	0.5036	100	50	100	0	0.0	
1	0.3633	100	36	95	5	28.2	42.2
2	0.3084	200	62	90.5	9.5	53.6	21.1
3	0.2629	200	53	89	11	62.0	19.9
4	0.2461	200	49	85	15	84.6	21.4
5	0.2643	200	53	79.5	20.5	115.6	22.6
6	0.2268	200	45	76	24	135.4	23.4
7	0.3025	200	61	70.5	29.5	166.4	22.3
8	0.2735	200	55	69	31	174.8	21.3
	Average		52				
Reactor 3							
		Dil	Conc	Cylinder vol	Added Vol	Added mass	
0	0.2259	400	90	100	0	0.0	
1	0.2068	400	83	95	5	28.2	35.8
2	0.2438	400	98	90	10	56.4	24.6
3	0.235	400	94	86	14	79.0	25.1
4	0.2229	400	89	86	14	79.0	20.0

5	0.2832	400	113	81	19	107.2	16.8
6	0.2633	400	105	80	20	112.8	16.3
7	0.2849	400	114	78	22	124.1	14.4
8	0.2473	400	99	73.5	26.5	149.5	17.6
	Average		98				
Reactor 4							
		Dil	Conc				
0	0.2312	800	185	100	0	0.0	
1	0.2731	800	218	96.5	3.5	19.7	-13.8
2	0.2136	800	171	94.5	5.5	31.0	22.6
3	0.2482	800	199	90.5	9.5	53.6	13.3
4	0.2603	800	208	86.5	13.5	76.1	13.2
5	0.2489	800	199	85.5	14.5	81.8	13.5
6	0.26739	800	214	85	15	84.6	9.3
7	0.255	800	204	83	17	95.9	11.0
8	0.2734	800	219	83	17	95.9	7.8

Table F6: Source Data for Table 5.4: Correlation between nitrite Concentration and activity in a fed-batch experiment, Example 1

			Average Nitrite:2.63 mg N/L		
			θ	**0.68**	
			n	**0.07**	
			r_{max}	**35**	mg [NO_2^--N]/L/h
			RSE	**1.3**	
			RSS	10.1	
Time (h)	Nitrite	Activity	Calculated	RS	
0	1.1				
1	4.0	22.4	23.8	1.8	
2	0.2	25.8	24.9	0.9	
3	4.6	27.0	25.6	2.0	
4	1.2	25.3	26.1	0.6	
5	3.2	26.7	26.5	0.0	
6	3.0	27.9	26.8	1.1	

7	0.4	27.5	27.1	0.1	
8	6.0	25.5	27.3	3.5	

			Average Nitrite:51		mg N/L
			θ	**0.57**	
			n	**0.06**	
			$\mathbf{r_{max}}$	**35**	mg [NO_2^--N]/L/h
			RSE	**1.0**	
			RSS	**5.4**	
Time (h)	Nitrite	Activity	Calculated	RS	
0	50				
1	36	42	20	499	
2	62	21	21	0	
3	53	20	21	2	
4	49	21	22	0	
5	53	23	22	1	
6	45	23	22	2	
7	61	22	22	0	
8	55	21	22	1	

			Average Nitrite:98		mg N/L
			θ	**0.96**	
			n	**-0.38**	
			$\mathbf{r_{max}}$	**35**	mg [NO_2^--N]/L/h
			RSE	**2.1**	
			RSS	**21.1**	
Time (h)	Nitrite	Activity	Calculated	RS	
0	90				
1	83	36	34	5	
2	98	25	26	2	
3	94	25	22	8	
4	89	20	20	0	

5	113	17	18	2	
6	105	16	17	1	
7	114	14	16	3	
8	99	18	15	5	

			Average Nitrite:202		mg N/L
			θ	**0.96**	
			n	**-0.66**	
			r_{max}	**35**	mg [NO_2^--N]/L/h
			RSE	**1.9**	
			RSS	**18.4**	
Time (h)	Nitrite	Activity	Calculated	RS	
0	185				
1	218	-14	34	2247	
2	171	23	21	2	
3	199	13	16	8	
4	208	13	13	0	
5	199	14	12	4	
6	214	9	10	1	
7	204	11	9	3	
8	219	8	8	1	

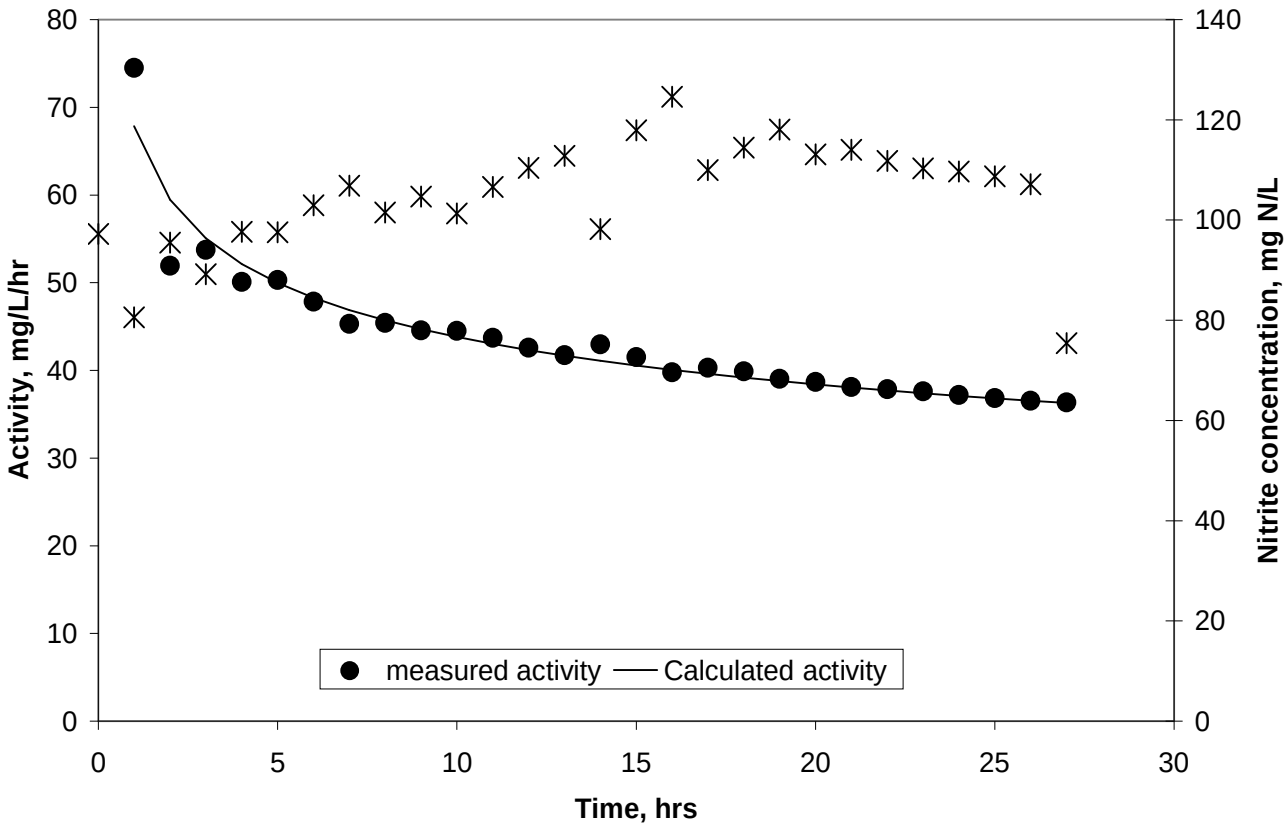

Figure F3: Effect of sustained nitrite concentration on AnAOB activity in a long-term, fed batch experimentAverage Nitrite concentration was 104 mg/L

Table F7: Source Data for Table 5.4: Correlation between nitrite Concentration and activity in a fed-batch experiment, example 2

	rmax	70	mg/L/hr			rmax	70	mg/L/hr	
	θ	0.75				θ	0.97		
	n	-0.08				n	-0.19		
	RSS	86.0				RSS	116.7		
		meaasured	Calculated		Time				
Time	Nitrite	Activity	Activity	RS		Nitrite	Activity	Calculated	RS
0	45.8				0	97.2			
1	49.3	47.5	52.6	26.1	1	80.6	74.5	67.8	44.4
2	49.1	49.4	49.9	0.2	2	95.5	51.9	59.5	56.8
3	55.6	47.8	48.3	0.3	3	89.2	53.7	55.1	1.8
4	56.2	48.4	47.2	1.5	4	97.7	50.1	52.1	4.2
5	54.3	49.4	46.4	8.6	5	97.6	50.3	50.0	0.1
6	58.8	48.3	45.8	6.5	6	103.0	47.8	48.3	0.2
7	56.5	48.6	45.2	11.2	7	106.9	45.3	46.9	2.5
8	64.3	46.6	44.7	3.5	8	101.5	45.4	45.7	0.1
9	62.3	46.2	44.3	3.4	9	104.7	44.6	44.7	0.0
10	62.1	45.7	44.0	2.9	10	101.3	44.5	43.8	0.5
11	65.8	44.3	43.6	0.4	11	106.6	43.7	43.0	0.5
12	61.2	44.4	43.4	1.1	12	110.4	42.6	42.3	0.1
13	67.8	43.1	43.1	0.0	13	112.8	41.7	41.7	0.0
14	54.8	44.1	42.8	1.6	14	98.2	43.0	41.1	3.5
15	67.8	43.0	42.6	0.2	15	117.9	41.5	40.6	0.9
16	71.8	41.3	42.4	1.1	16	124.6	39.8	40.1	0.1
17	51.4	42.7	42.2	0.3	17	110.0	40.3	39.6	0.5
18	63.9	41.2	42.0	0.7	18	114.4	39.9	39.2	0.5
19	55.4	41.2	41.8	0.4	19	118.1	39.0	38.8	0.1
20	54.5	40.9	41.7	0.6	20	113.1	38.7	38.4	0.1
21	56.2	40.5	41.5	1.0	21	114.0	38.1	38.1	0.0
22	53.3	40.3	41.4	1.0	22	111.8	37.9	37.7	0.0
23	54.2	40.3	41.2	0.8	23	110.3	37.6	37.4	0.0
24	62.1	39.3	41.1	3.1	24	109.7	37.2	37.1	0.0
25	52.8	39.3	40.9	2.6	25	108.7	36.8	36.8	0.0
26	54.0	39.0	40.8	3.5	26	107.2	36.5	36.5	0.0
27	20.7	38.7	40.7	3.8	27	75.4	36.4	36.3	0.0

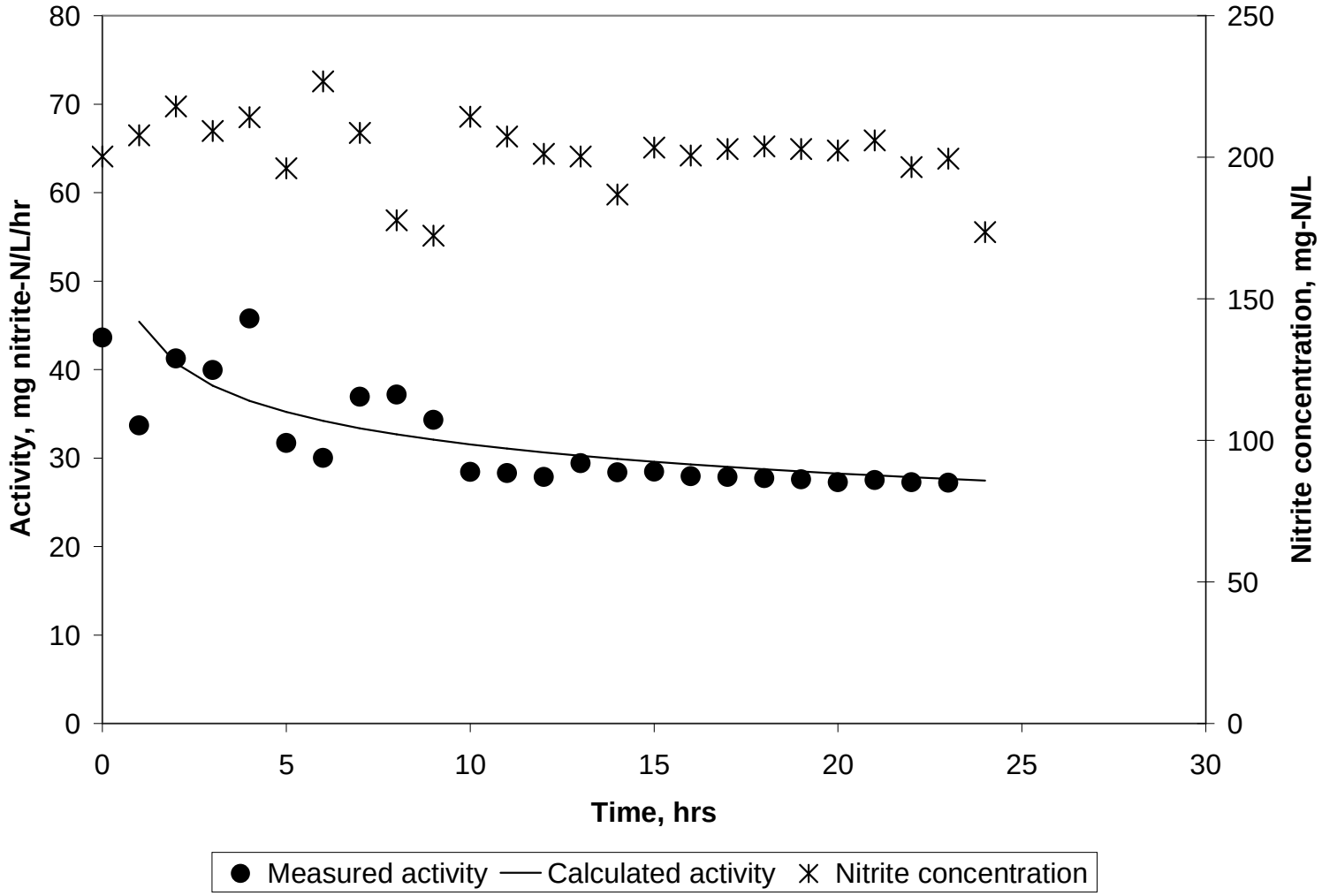

Figure F5: Effect of sustained nitrite concentration on AnAOB activity in a long-term, fed batch experiment
Average Nitrite concentration was 56 mg/L

Table F8: Data for Figure 5.6: Effect of pH on anammox activity

						Slope
pH 6.8	7:00	0	0.0	0.2269	45	
	7:20	20	0.3	0.2381	48	0
	7:40	40	0.7	0.2179	44	
	8:00	60	1.0	0.2276	46	
pH 7.2	7:00	0	0.0	0.2307	46	
	7:20	20	0.3	0.2022	40	
	7:40	40	0.7	0.1509	30	24.93
	8:00	60	1.0	0.1093	22	
pH 7.6	7:00	0	0.0	0.2409	48	
	7:20	20	0.3	0.2107	42	23.74
	7:40	40	0.7	0.1679	34	
	8:00	60	1.0	0.1233	25	
pH 8.0	7:00	0	0.0	0.2358	47	
	7:20	20	0.3	0.2151	43	11.97
	7:40	40	0.7	0.2034	41	
	8:00	60	1.0	0.1732	35	

Appendix G: Data for Chapter 6

Table G1: Data for Figure 6.4, NOx concentration over time in presence of ferrous

	Nitrate	Nitrite	Nox
5/3/2008 11:54	8.8	58	67
5/3/2008 11:57	5.1	54	59
5/3/2008 12:04	6.2	48	54
5/3/2008 12:20	7.0	52	59
5/3/2008 13:00	7.7	51	59
5/3/2008 18:20	13.2	34	47
5/4/2008 9:07	19.6	10	29
5/4/2008 18:00	28.8	1	30

Table G2: Data for Figure 6.5; Evaluation of aerobic ammonia oxidation inhibition using the respirometry

Cell A-3	100 mg/L nitrite 200 mg/L Ferrous						
Cell A-4	Ferrous control: 200 mg/L ferrous						
Cell A-6	Nitrite control: 100 mg/L nitrite						
Cell A-7	Abiotic control: 200 mg/L ferrous, No biomass.						
Cell A-8	Control: No Nitrite or Ferrous						
Save Interval:	1 minute						
Calibration Information:							
	1	1	1	1	1		
Elapse T (hours)	Cell A-3	Cell A-4	Cell A-6	Cell A-7	Cell A-8	Date	Time
0.00	0	0	0	0	0	1/28/2008	15:52:16
0.02	0	0	0	0	0	1/28/2008	15:53:16
0.03	0	0	0	0	0	1/28/2008	15:54:16
0.05	0	0	0	0	0	1/28/2008	15:55:16
0.07	0	5	0	0	0	1/28/2008	15:56:16
0.08	0	15	0	0	0	1/28/2008	15:57:16
0.10	0	23	0	10	6	1/28/2008	15:58:16

Elapse T (hours)	Cell A-3	Cell A-4	Cell A-6	Cell A-7	Cell A-8	Date	Time
0.12	0	34	0	10	17	1/28/2008	15:59:16
0.13	0	45	0	10	28	1/28/2008	16:00:16
0.15	0	56	0	10	39	1/28/2008	16:01:16
0.17	0	66	0	10	50	1/28/2008	16:02:16
0.18	1	78	0	10	62	1/28/2008	16:03:16
0.20	1	90	0	10	72	1/28/2008	16:04:16
0.22	1	102	0	10	82	1/28/2008	16:05:16
0.23	1	113	0	10	93	1/28/2008	16:06:16
0.25	1	126	0	10	103	1/28/2008	16:07:16
0.27	1	137	0	10	113	1/28/2008	16:08:16
0.30	1	161	5	10	132	1/28/2008	16:10:16
0.32	1	173	11	10	141	1/28/2008	16:11:16
0.33	1	184	18	10	150	1/28/2008	16:12:16
0.35	1	196	25	10	155	1/28/2008	16:13:16
0.37	1	206	31	10	158	1/28/2008	16:14:16
0.38	1	218	38	10	162	1/28/2008	16:15:16
0.40	1	229	45	10	162	1/28/2008	16:16:16
0.42	4	240	51	10	166	1/28/2008	16:17:16
0.43	9	251	58	10	169	1/28/2008	16:18:16
0.45	15	262	63	10	171	1/28/2008	16:19:16
0.47	21	273	70	10	171	1/28/2008	16:20:16
0.48	31	288	79	10	181	1/28/2008	16:21:16
0.50	38	299	87	10	188	1/28/2008	16:22:16
0.52	42	307	93	10	194	1/28/2008	16:23:16
0.53	48	316	99	10	201	1/28/2008	16:24:16
0.55	54	322	102	10	207	1/28/2008	16:25:16
0.57	58	328	106	10	212	1/28/2008	16:26:16
0.58	63	334	110	10	218	1/28/2008	16:27:16
0.60	67	339	113	10	223	1/28/2008	16:28:16
0.62	73	346	118	10	230	1/28/2008	16:29:16
0.63	76	350	121	10	233	1/28/2008	16:30:16
0.65	80	355	124	10	236	1/28/2008	16:31:16
0.67	83	359	126	10	238	1/28/2008	16:32:16
0.68	87	363	129	10	240	1/28/2008	16:33:16
0.70	91	368	131	10	242	1/28/2008	16:34:16
0.72	95	373	132	10	243	1/28/2008	16:35:16
0.73	99	378	134	10	245	1/28/2008	16:36:16
0.75	103	383	136	10	247	1/28/2008	16:37:16
0.77	106	388	137	10	249	1/28/2008	16:38:16
0.78	110	393	139	10	250	1/28/2008	16:39:16
0.80	113	398	141	10	252	1/28/2008	16:40:16
0.82	117	403	142	10	254	1/28/2008	16:41:16

Elapse T (hours)	Cell A-3	Cell A-4	Cell A-6	Cell A-7	Cell A-8	Date	Time
0.83	121	409	143	10	255	1/28/2008	16:42:16
0.85	124	414	144	10	257	1/28/2008	16:43:16
0.87	128	419	145	10	260	1/28/2008	16:44:16
0.88	132	425	146	10	261	1/28/2008	16:45:16
0.90	135	430	147	10	262	1/28/2008	16:46:16
0.92	139	436	148	10	264	1/28/2008	16:47:16
0.93	143	442	149	10	265	1/28/2008	16:48:16
0.95	146	447	150	10	266	1/28/2008	16:49:16
0.97	150	452	151	10	267	1/28/2008	16:50:16
0.98	154	458	152	10	269	1/28/2008	16:51:16
1.00	157	464	153	10	270	1/28/2008	16:52:16

Table G3: Data for Figure 6.6; Defoamant inhibition experiment

(2L effluent+ 50 mL Deformant+ 2L RAS sludge)						
Sample ID	**Time**		**Ammonia**	**Nitrite**	**Nitrate**	**Nox**
O1	11:01	0	539	0.0	0.2	0.2
O2	11:31	30	531	0.0	0.1	0.1
O3	12:01	60	532	0.0	0.1	0.1
O4	12:31	90	532	0.0	0.1	0.1
O4	1:01	120	532	0.0	0.1	0.1
O6	2:45	206.4	531	0.0	0.2	0.2
	Blue	**(2L effluent + 2L RAS)**				
Sample ID	**Time**		**Ammonia**	**Nitrite**	**Nitrate**	**Nox**
B1	11:02	0	574	0	3	3
B2	11:32	30	554	1	11	13
B3	12:02	60	544	1	21	22
B4	12:32	90	531	1	32	33
B5	1:02	120	526	1	39	40
B6	2:46	206.4	508	2	68	70

	Blue	**2L centrate + 2 L RAS)**				
Sample ID	**Time**		**Ammonia**	**Nitrite**	**Nitrate**	**Nox**
B1	11:03	0	573	0	1	0
B2	11:33	30	550	2	5	1
B3	12:03	60	542	3	6	9
B4	12:33	90	537	5	9	14
B5	1:03	120	539	5	11	16
B6	2:45	206.4	527	7	17	25

Table G4: Data for Figure 6.7; Polymer Inhibition experiment

Reactor 1	(2L effluent + 2L RAS+ 50 mL Polymer)		
Sample ID	Time		Ammonia
B1	9:40	0	56
B2	10:10	30	53
B3	10:40	60	51
B4	11:10	90	49
B5	11:40	120	43
B6	12:10	150	39
Reactor 2			
Sample ID	Time		Ammonia
O1	9:40	0	57
O2	10:10	30	53
O3	10:40	60	50
O4	11:10	90	46
O5	11:40	120	46
O6	12:10	150	45

Reactor 3	(2L effluent + 2L RAS)		
Sample ID	Time		Ammonia
R1	9:40	0	58
R2	10:10	30	49
R3	10:40	60	43
R4	11:10	90	40
R5	11:40	120	38
R6	12:10	150	35

Table G5: Figure 6.8; Oxygen uptake rate with two different dilutions (4X and 8X)

	Date	7/24/2007				
	Bottle Vol	300	mL			
4x dilution			**8x dilution**			OUR
time (min)	DO		time (min)	DO		
0	3.49	153.6	0			
0.5	3.17	225.6	0.5	6.2		192
1	2.7	216	1	6		172.8
1.5	2.25	220.8	1.5	5.82		134.4
2	1.79	216	2	5.68		163.2
2.5	1.34	211.2	2.5	5.51		144
3	0.9	177.6	3	5.36		153.6
3.5	0.53	139.2	3.5	5.2		153.6
4	0.24	57.6	4	5.04		144
4.5	0.12	19.2	4.5	4.89		153.6
5	0.08	4.8	5	4.73		144
5.5	0.07	0	5.5	4.58		144
6	0.07	0	6	4.43		144
6.5	0.07	0	6.5	4.28		144
7	0.07	0	7	4.13		144
7.5	0.07	0	7.5	3.98		134.4
8	0.07	0	8	3.84		144
8.5	0.07	0	8.5	3.69		134.4
9	0.07	0	9	3.55		144

4x dilution			8x dilution			OUR
9.5	0.07	0	9.5	3.4		134.4
10	0.07	0	10	3.26		144
10.5	0.07		10.5	3.11		134.4
			11	2.97		124.8
			11.5	2.84		134.4
			12	2.7		134.4
			12.5	2.56		134.4
			13	2.42		124.8
			13.5	2.29		134.4
			14	2.15		134.4
			14.5	2.01		124.8
			15	1.88		124.8
			15.5	1.75		124.8
			16	1.62		124.8
			16.5	1.49		124.8
			17	1.36		124.8
			17.5	1.23		124.8
			18	1.1		115.2
			18.5	0.98		115.2
			19.5	0.74		115.2
			20	0.62		115.2
			20.5	0.5		105.6
			21	0.39		96
			21.5	0.29		76.8
			22	0.21		48
			22.5	0.16		38.4
			23	0.12		19.2
			23.5	0.1		9.6
			24	0.09		9.6
			24.5	0.08		0
			25	0.08		0
			25.5	0.08		0
			26	0.08		0
			26.5	0.08		0
			27	0.08		0
			27.5	0.08		0
			28	0.08		0
			28.5	0.08		0
			29	0.08		0
			29.5	0.08		0
			30	0.08		0
			30.5	0.08		0
			31	0.08		0
			31.5	0.08		0
			32	0.08		0
			32.5	0.08		0
			33	0.08		